

Direct application of compound-specific radiocarbon analysis of leaf waxes to establish
lacustrine sediment chronology.

Joji Uchikawa¹, Brian N. Popp², Jane E. Schoonmaker¹, Li Xu³

uchikawa@hawaii.edu
popp@hawaii.edu
jane@soest.hawaii.edu
lxu@whoi.edu

¹ Department of Oceanography, SOEST, University of Hawai'i at Manoa.

1000 Pope Road, Honolulu, HI 96822, U.S.A.

² Department of Geology and Geophysics, SOEST, University of Hawai'i at Manoa

1680 East-West Road, Honolulu, HI 96822, U.S.A.

³ National Ocean Sciences AMS Facility, Department of Geology and Geophysics,

Woods Hole Oceanographic Institution.

266 Woods Hole Road, Woods Hole, MA 02543-1539, U.S.A.

Keywords: Compound-specific radiocarbon analysis; Compound-specific isotopic analysis;
Lacustrine sediment; *N*-alkanes; Leaf waxes; Radiocarbon dating.

Draft Manuscript for *Journal of Paleolimnology*

Corresponding Author: Joji Uchikawa (uchikawa@hawaii.edu)

Tel. (808) 956-5963

Fax. (808) 956-7112

Abstract

This study demonstrates use of compound-specific radiocarbon analysis (CSRA) for dating Holocene lacustrine sediments from carbonate-hosted Ordy Pond, O‘ahu, Hawai‘i. Long-chain odd-numbered normal alkanes (*n*-alkanes), biomarkers characteristic of terrestrial higher plants, were ubiquitous in Ordy Pond sediments. The $\delta^{13}\text{C}$ of individual *n*-alkanes ranged from -29.9 to -25.5‰, within the expected range for *n*-alkanes synthesized by land plants using the C_3 carbon fixation pathway. The ^{14}C ages of *n*-alkanes determined by CSRA showed remarkably good agreement with ^{14}C dates of rare plant macrofossils obtained from nearby sedimentary horizons. In general, CSRA of *n*-alkanes successfully refined the age-control of the sediments. The sum of *n*-alkanes in each sample produced 70 to 170 μg of carbon (C), however, greater age errors were confirmed for samples containing less than 80 μg of C. The ^{14}C age of *n*-alkanes from one particular sedimentary horizon was 4,250 yr older than the value expected from the refined age-control, resulting in an apparent and arguable age discrepancy. Several lines of evidence suggest that this particular sample was contaminated by introduction of ^{14}C -free C during preparative capillary gas chromatography (PCGC). This study simultaneously highlighted the promising potential of CSRA for paleo-applications and the risks of contamination associated with micro-scale ^{14}C measurement of individual organic compounds.

Introduction

Establishment of reliable sediment chronology is one of the most fundamental requirements for paleolimnological investigations on lacustrine sedimentary records; yet, sometimes it can be the most difficult task to accomplish. Until the late 1980's, the majority of radiocarbon dating was conducted on bulk sediments in order to meet relatively large sample size requirements (> 100g of C) of the conventional radiometric β -counting methods (Bradley 1999; Walker et al. 2001).

But it has been well characterized that, in many limnic systems, bulk sediments often record the dilution of ^{14}C activities in the water column due to leaching of fossil calcareous bedrock and incorporation of soils, clays, mineral particles and relict terrestrial organic matter (OM) from watersheds (Björck et al. 1998; Vance and Telka, 1998; Turney et al. 2000; Lowe and Walker, 2000; Walker et al. 2001). These local reservoir effects typically lead to anomalously old ^{14}C ages of bulk sediments. Development of accelerator mass spectrometry (AMS) has greatly reduced the sample size requirements for accurate ^{14}C dating. The use of AMS techniques on well-preserved macrofossils of terrestrial plants such as wood fragments and seeds is now recognized as the preferable strategy to construct reliable sediment chronology (Törnqvist et al. 1992; Turney et al. 2000; Lowe and Walker 2000; Walker et al. 2001). Because terrestrial plants directly utilize atmospheric CO_2 , this method can circumvent the confusion caused by the intractable nature of local reservoir effects encountered in radiocarbon dating of lacustrine bulk sediment.

Modern high-precision AMS systems extend the possibility of ^{14}C dating even further. Eglinton et al. (1996) demonstrated that ^{14}C dating at the molecular-level, so-called compound-specific radiocarbon analysis (CSRA), using AMS in conjunction with automated preparative capillary gas chromatography (PCGC) is a reliable age-dating technique. PCGC allows isolation of ample quantities of target compounds at sufficient purity for AMS ^{14}C measurements. Ideally, target compounds should produce approximately 100 μg of C for accurate CSRA (Eglinton et al. 1996), but CSRA is theoretically possible for samples as small as 15-20 μg of C (von Reden et al. 1998; Pearson et al. 1998). Since the first application of CSRA on environmental samples by Eglinton et al. (1997), the method has been applied mainly to elucidate the sources and residence time of OM in sedimentary reservoirs (e.g. Pearson et al. 2000; Pearson and Eglinton 2000; Pearson et al. 2001; Petsch et al. 2001; Mollenhauer et al. 2005). More recent studies (e.g. Ohkouchi et al. 2003; Smittenberg et al. 2004; Uchida et al. 2005), however, have attempted to directly utilize CSRA for establishment of marine sediment chronology. These studies have demonstrated the promising capability of CSRA with properly chosen target biomarkers as an alternative dating option for sediments. The greatest advantage of the CSRA enabled by the dual use of high-precision AMS and the PCGC is its extremely small sample size requirements. Accurate micro-scale ^{14}C measurements on source-selective biomarkers can overcome the issues associated with dating of bulk sediments as well as the lack of suitable macrofossils for more conventional AMS approaches that are commonly practiced by many paleolimnologists.

In this study, we use CSRA of plant-derived long-chain normal alkanes (*n*-alkanes) to refine the age-control of lacustrine sediments from a tropical coastal pond on a Pleistocene carbonate platform in Hawai‘i. This is the first direct application of this novel method to develop sediment chronology in a lacustrine environment. Sediment cores collected from Ordy Pond on the island of O‘ahu (Fig. 1), Hawai‘i, appear to have recorded continuous paleoclimatic and paleoenvironmental histories of the area throughout the Holocene. This is the only known high-resolution, laminated, aquatic sedimentary record from the Central Pacific, and is thus valuable in the context of tropical/subtropical paleoclimatology. Interpretation of this Quaternary proxy, however, has been limited by lack of a rigorous sediment chronology due to the presence of ¹⁴C-depleted C in the system and the rareness of macroscopic substrates for AMS ¹⁴C dating.

Use of land-derived biomarkers for chronological purposes is known to be problematic for marine sediments. Age-offsets between land-derived biomarkers and marine counterparts can be significant (e.g. Pearson and Eglinton 2000; Pearson et al. 2001; Smittenberg et al. 2004; Uchida et al. 2005) due to the long residence time of terrestrial biomarkers in other reservoirs on land and during physical transport before their incorporation into marine sediments. But the impact of aged biomarkers may be less significant in lacustrine systems. Due to tighter coupling between terrestrial and lacustrine environments, transfer of materials between these systems should be more rapid. Constant turnover of nearby vegetative communities should result in dominance of fresh materials in the overall inventory of terrestrial OM inputs. Additionally, the

use of land-plant biomarkers is particularly beneficial for construction of age-control for lacustrine sediments. Because *n*-alkanes are photosynthetically-fixed atmospheric CO₂ exclusively by terrestrial higher plants, these biomarkers do not require the consideration of a site-specific lake reservoir-effect.

To improve age-control of Ordy Pond sediments, CSRA was conducted on plant-derived *n*-alkanes. In order to assess the reliability of CSRA, one sample was prepared from a depth close to a sedimentary horizon previously dated by AMS using plant seeds. This study is designed to test the applicability of CSRA using terrestrial biomarkers and to enhance the resolution of the age-control for lacustrine sediments where popularly used ¹⁴C dating methods are conceptually impractical.

Study Site, Materials and Background

Geological and environmental settings

Ordy Pond occupies a roughly 22.5 m-deep karstic sinkhole in a Pleistocene limestone reef complex on the 'Ewa Plain of O'ahu (Fig. 1). This fossil-reef platform was established during the interglacial Waimanalo highstand, roughly 135 to 120 kya (Ku et al. 1974; Sherman et al. 1993; Szabo et al. 1994). The pond was formed approximately 10 kya as groundwater inundated the sinkhole during the most recent post-glacial sea-level rise. The circular rim of the pond is

densely vegetated by American mangrove (*Rhizophora mangle*) within a mixed shrub of kiawe trees (*Prosopis pallida*) and sourbush (*Pluchea symphytifolia*) (Athens et al. 1999).

Hydrological studies by Ogden (1999) indicate that the pond is largely isolated from the surrounding aquifer and there is neither surface inflow nor outflow. The system is fed only by very weak intrusion of groundwater and seasonal local rainfall events concentrated during winters. Thus, the pond can be considered as a hydrologically closed system. Today, the pond has a 5 m brackish water column that is eutrophic, highly productive, and supersaturated with respect to calcite and aragonite (Garrison 2002). The water column below ~1 m remains anoxic all year due to extensive primary production in the surface euphotic zone and remineralization of OM at depth.

Description of the sediment core

Sediments from Ordy Pond record the environmental history of the pond since its formation in the sinkhole. Athens et al. (1999; 2002) and Tribble et al. (1998) recovered multiple cores with a cumulative depth extending to 17.5 m. These individual cores were correlated stratigraphically based on the high-resolution laminations. Three distinct stratigraphic units were found in the core: a basal non-aquatic unit, and the middle and top aquatic units (see Fig. 2-c). The basal unit contains fossils of terrestrial snails and pebbles of reef rock in a carbonate-mud matrix. A thin layer of peat-type sediment that separates the basal non-aquatic unit from the overlying aquatic

units represents the terrestrial-aquatic transition of the sinkhole due to the initial intrusion of groundwater as sea-level rose. The middle aquatic unit possesses fine-scale (sub-mm to cm scale) laminations with alternating layers of carbonate and OM with variable amounts of diatomaceous material. The uppermost sedimentary unit, representing the most recent 120 years of depositional history, contains 5 m of organic-rich, subtly-banded sapropelic sediment. Although there is perhaps a hiatus at the transition from the bottom non-aquatic unit to the overlying aquatic units, there is no sedimentological evidence of any hiatus or unconformities in the middle and top limnic sediments. This suggests that the aquatic sediments have been continuously deposited throughout the Holocene epoch.

Previous sediment chronology

From the investigation of Athens et al. (1999; 2002), four reliable ages are available for the upper 8 m of Ordy Pond sediments (Fig. 2-a). The shallowest horizon (horizon A) was dated by the first appearance of pollen from historically introduced species to Hawai'i. Ages of the remaining horizons (horizons B, C and D) were based on AMS ^{14}C dating using rare plant macrofossils (wood fragments and seeds). The ^{14}C age of the base of the aquatic sediments, horizon E, was determined by conventional radiometric ^{14}C techniques using shells of terrestrial snails by Tribble et al. (1999).

The chronology of Ordy Pond aquatic sediments based on these dates and linear

interpolation clearly indicates that the sediment accumulation rate in this pond has been variable (Fig. 2-a). The rate generally increased from 0.05 to 4.33 cm/yr over the course of pond history. This implies that simple point-to-point interpolation may over/underestimate the true sediment accumulation rate. Particularly problematic is the interpolation between horizon E and horizon D, where not a single reliable date is available for more than 5 m that are equivalent to more than 9,800 years of sedimentation history. In addition, validity of the age of horizon E is rather questionable due to the fact that snails from limestone areas often provide anomalous ^{14}C ages (Goodfriend and Stipp 1983; Dye 1994; Goodfriend et al. 1999); this issue will be further addressed below.

The scarcity of ^{14}C dates in Ordy Pond sediments is due to the lack of macroscopic datable materials. Attempts by Athens et al. (1999; 2002) to date bulk sediment, which is comprised mostly of algal materials, documented apparent stratigraphic reversals (Fig 2-b). It is most likely that the pond DIC incorporates ^{14}C -depleted C liberated from the surrounding Pleistocene limestone. Consequently, any autochthonous material directly produced from the pond water, such as OM and carbonates, must be avoided for ^{14}C dating. Thus terrestrial plants are the only option for accurate ^{14}C dating. Unfortunately, macroscopic plant material such as those dated by Athens et al. (1999; 2002) are rarely found in the sediment column due to the hydrologically-closed nature of the pond system.

Methods

Lipid extraction and separation

Methods for extraction and separation of *n*-alkanes from bulk sediment samples were modified from Kenig et al. (2000) and Hoering and Freeman (1984). Sediment samples were taken from the core of Tribble et al. (1998). Oven-dried (60°C) and homogenized sediment samples were soxhlet-extracted with dichloromethane. Samples used for extraction ranged from 10 to 17 g, depending on the *n*-alkane contents. Elemental sulfur was removed from the extracts using an acid-activated copper granular column (20-30 mesh size). The hydrocarbon fraction in the extracts was separated by silica-gel (12 g, 70-230 mesh size, deactivated with 2 wt.% of H₂O) column chromatography with 40 mL of petroleum ether. Silicalite molecular sieve was used to further purify *n*-alkanes. A small column of powdered silicalite was combusted at 325°C. The hydrocarbon fraction was slowly percolated through the column, and the column was flushed with 12 mL of clean pentane to collect a non-adduct fraction consisting of branched/cyclic hydrocarbons. Silicalite was digested with 3 mL of 49% hydrofluoric acid, which was extracted with pentane to recover adducted *n*-alkanes. Finally, as a final purification step, the *n*-alkane fraction was eluted through a pre-combusted silica-gel column with pentane.

Extractions were repeated using new sediment until sufficient amounts of the compounds were collected. Samples from separate extracts were combined once their terrestrial origin was confirmed by compound-specific isotopic analysis (CSIA). Solvents used during the

sample preparations were not distilled; however, best available grade (HPLC quality or higher) was selected.

GC and irm-GC/MS analysis

Gas chromatography (GC) and isotope-ratio-monitoring gas chromatography/mass spectrometry (irm-GC/MS) analyses were conducted in the Stable Isotope Biogeochemistry Laboratories at the University of Hawai'i. Samples were dissolved in cyclohexane for both GC and irm-GC/MS analyses.

Identification and quantification of *n*-alkanes were conducted using a Varian 3400 GC equipped with an SPI on-column injector and flame ionization detector (FID). An HP-Ultra 1 column (50 m length, 0.32 mm i.d., 0.17 μm film thickness) was used in the GC with helium as a carrier gas. Samples were injected at 50 °C and temperature was programmed to 320 °C at a rate of 4 °C/min and held for 20 min thereafter. Amounts of individual *n*-alkanes were quantified by comparing the integrated peak area of each compound to that of a co-injected internal standard (deuterated *n*-C₃₆ hydrocarbon) of known concentration.

In order to confirm the terrestrial origin of *n*-alkanes, CSIA was conducted using irm-GC/MS (Finnigan MAT252 connected to Finnigan GC/C-III interface). The temperature program of the GC (ThermoFinnigan Trace) was same as described above. The GC was equipped with a J&W DB-1 capillary column (60 m length, 0.32 mm i.d., 0.25 μm film

thickness). C isotopic compositions of individual *n*-alkanes ($\delta^{13}\text{C}_{N\text{-Alks}}$) were standardized to the Vienna PDB standard (VPDB) and reported in the δ -notation. An internal standard containing a series of deuterated *n*-alkanes (*n*-C₂₄, *n*-C₃₆ and *n*-C₄₀) was co-injected with each sample in order to identify the series of compounds and to assess the accuracy of the isotopic analysis (better than $\pm 0.4\%$).

PCGC and CSRA by AMS

Prepared *n*-alkane samples were sent to the National Ocean Sciences Accelerator Mass Spectrometry facility (NOSAMS) at the Woods Hole Oceanographic Institution for PCGC and CSRA.

Details on PCGC are described elsewhere (Eglinton et al. 1996; Reddy et al. 2002; 2003). Briefly, samples were repeatedly injected on a PCGC system until sufficient amounts of target *n*-alkanes (\sum *n*-C₂₇, *n*-C₂₉, *n*-C₃₁ and *n*-C₃₃) were collected. Compounds were separated on a Chrompak CP-Sil 5 CB capillary column (50 m length, 0.53 mm i.d., 1 μm film thickness). About 1% of the eluate was diverted to the FID for signal monitoring. The rest of the material was collected in the glass U-tubes. The target *n*-alkanes were rinsed with solvent to vials. A fraction (about 5%) of the isolated biomarkers was taken for purity check in a high resolution GC. The rest of the target compounds were transferred to pre-baked quartz tubes and solvent was removed. After the addition of copper (II) oxide, the quartz tubes were sealed under vacuum

and combusted at 850 °C for 5 hours. 10% of the liberated CO₂ was utilized for δ¹³C analysis and the remainder was reduced to graphite for CSRA by AMS as described by Pearson et al. (1998). ¹⁴C analyses were conducted on the graphite placed in a designated AMS target. CSRA by AMS was conducted according to a method developed for the ¹⁴C dating of micro-scale samples (von Reden et al. 1998).

Two types of analytical error for each sample were determined at NOSAMS. The internal statistical error was calculated based on the total number of AMS ¹⁴C counts measured for samples, standards and instrument blanks, whereas the external error was derived as the standard deviations of multiple analyses on a AMS target over a certain time period (McNichol et al. 2001). The larger of the internal or external error was reported as the final sample error and was propagated to age/error calculations.

Reporting of radiocarbon results

Conversions of AMS data to radiocarbon ages (i.e. yrBP unit) are described in detail elsewhere (McNichol et al. 2001; Reddy et al. 2002; 2003; Smittenberg et al. 2004). The ¹⁴C contents of combined target *n*-alkanes are expressed in terms of “fraction modern (*Fm*)” as:

$$Fm = \frac{(^{14}C/^{12}C)_S - (^{14}C/^{12}C)_B}{(^{14}C/^{12}C)_M - (^{14}C/^{12}C)_B} \quad (1)$$

where subscripts S, B and M denote sample, blank and modern reference material, respectively.

Fm is a measure of the deviation of ¹⁴C activity of a sample from that of the “modern” value,

which is defined as 95% of the ^{14}C concentration of contemporary reference material (NIST SRM 4990B *Oxalic Acid* with $\delta^{13}\text{C}_{\text{VPDB}} = -19\text{‰}$) as in 1950 AD (Stuiver and Polach 1977).

Then Fm is corrected for the isotopic fractionation by normalizing to a conventional value of $\delta^{13}\text{C}_{\text{VPDB}} = -25\text{‰}$:

$$Fm_{\text{Corrected}} = Fm \times \left[\frac{(1 - 25/1000)}{(1 + \delta^{13}\text{C}_{\text{Sample}}/1000)} \right]^2 \quad (2)$$

Based on the ^{14}C half-life of 5568 years, the ^{14}C age (yrBP) of a sample is computed as:

$$^{14}\text{C Age} = -8033 \times \ln (Fm_{\text{Corrected}}) \quad (3)$$

Finally, ^{14}C ages were calibrated to calendar years (cal.yrBP) using CALIB 5.0 program (Stuiver and Reimer 1993; Stuiver et al. 2005) with the IntCal 04 calibration dataset of Reimer et al. (2004).

During *n*-alkane preparations, a wood fragment was found from a horizon bracketed by two of the samples. This wood fragment was also sent to NOSAMS for ^{14}C dating by AMS using a more conventional AMS method (McNichol et al. 1995; Pearson et al. 1998).

Results

Hydrocarbon fractions from Ordy Pond sediments consist of a series of well-resolved *n*-alkanes and minor amounts of branched/cyclic isomeric hydrocarbons. By co-eluting with target *n*-alkanes, these non-aliphatic compounds could affect subsequent CSIA and CSRA. The

silicalite treatment, however, was successful in separating *n*-alkanes from the non-aliphatic hydrocarbons. Resolved compounds are almost exclusively dominated by a long-chain homologous ($C > 25$) series up to *n*-C₃₅ with a maximum at either *n*-C₂₉ or *n*-C₃₁. In addition, resolved *n*-alkanes show strong odd-over-even C-number predominance.

$\delta^{13}C_{N-Alks}$ values range from -30 to -25‰ (Table 1). In most samples, *n*-C₂₇ *n*-alkanes are more enriched in ¹³C relative to the rest of the *n*-alkanes. Reproducibility determined from each compound in the internal standard (deuterated *n*-C₂₄, *n*-C₃₆ and *n*-C₄₀) are $\pm 0.34\%$, $\pm 0.36\%$ and $\pm 0.64\%$ ($n=14$), respectively. Slightly lower reproducibility observed for the *n*-C₄₀ internal standard can be explained by elevated background level due to minor column bleed of the GC stationary phase at high oven temperature and broader and lower peak shape.

Results of CSRA of *n*-alkanes and AMS ¹⁴C dating of the wood fragment are summarized in Table 2. Generally, age errors are greater for samples containing less than 80 μg of C. This trend agrees with a consensus that analytical uncertainties associated with blank corrections become relatively large when C contents in samples are less than 100 μg (e.g. McNichol et al. 2001; Smittenberg et al. 2004). Nonetheless, these age errors are in an acceptable range for CSRA for samples with such limited C yields. ¹⁴C ages increase with depth from D3 to D5. In contrast, ages of deeper samples (D6, D7, D9 and wood fragment) cluster at roughly 8,000 cal.yrBP. These dates showed a trivial extent of age reversals with neighboring sedimentary horizons.

Discussion

Origin of *n*-alkanes

Long-chain odd-numbered *n*-alkanes are derived from epicuticular waxes of terrestrial higher plants (Eglinton and Hamilton 1967; Rieley et al. 1991) and are ubiquitous in aquatic sediments (Pearson and Eglinton 2000). Terrestrial *n*-alkanes possess unique molecular and isotopic diagnostic features that are easily distinguishable from those derived from other precursor sources. These compounds are highly resistant to post-depositional diagenetic alteration (e.g. Cranwell 1981; Sun and Wakeham 1994; Hoefs et al. 2002) and thus persist in sedimentary reservoirs for long periods of time (e.g. Kuypers et al. 1999). Furthermore, progressive biodegradation of *n*-alkanes involves negligible isotopic fractionation (Huang et al. 1997; Mazeas et al. 2002; Sun et al. 2005). Sedimentary *n*-alkanes, therefore, are popular for use as a tracer of terrestrial OM transport (Rieley et al. 1991; Ishiwatari et al. 1994; Pearson and Eglinton 2000; Pearson et al. 2001) and as a molecular proxy to reconstruct paleoenvironmental changes in watersheds (e.g. Bird et al. 1995; Bourbonniere and Meyers 1996; Yamada and Ishiwatari 1999; Brincat et al. 2000; Menzel et al. 2004; Muri et al. 2004).

The most abundant *n*-alkanes in Ordy Pond sediment are odd-numbered compounds from *n*-C₂₅ to *n*-C₃₃, which suggests derivation from epicuticular waxes of terrestrial higher plants. A unimodal *n*-alkane distribution with a maximum at either *n*-C₂₉ or *n*-C₃₁ with

odd-over-even C predominance is a characteristic feature of leaf-wax *n*-alkanes (Eglinton and Hamilton 1967; Collister et al. 1994). The interpretation of terrestrial plant origin is also supported by $\delta^{13}\text{C}$ values of individual *n*-alkanes. $\delta^{13}\text{C}_{N\text{-Alks}}$ values reflect the unique isotope fractionations involved in different carbon fixation pathways (C_3 , C_4 and CAM fixation) utilized by plants. The *n*-alkanes derived from terrestrial plants conducting C_3 C-fixation typically have $\delta^{13}\text{C}_{\text{VPDB}}$ values ranging from -30 to -40‰, whereas those synthesized by C_4 land plants tend to be isotopically heavier and average from -17 to -25‰ (Collister et al. 1994; Reiley et al. 1991; Kuypers et al. 1999; Chikaraishi and Naraoka 2003). $\delta^{13}\text{C}_{N\text{-Alks}}$ values from Ordy Pond sediments range from -30 to -25‰, which implies that these compounds were derived from both C_3 and C_4 land plants.

The name of Ordy Pond presumably comes from an abbreviation of “Ordnance Pond”, referring to the disposal of ordnance during the World War II period (Athens et al. 1999). Although there is no direct evidence or reports of the disposal of ordnance into the pond (Athens et al. 1999), these facts raised a slight concern that the site could be contaminated by petroleum-derived materials. Chromatograms of both hydrocarbon and *n*-alkane fractions from Ordy Pond sediments reveal an overwhelming dominance of odd-numbered compounds and an absence of a baseline hump, which is caused by unresolved complex mixtures comprised of structurally complex isomers and branched/cyclic hydrocarbons. Lack of these diagnostic characteristics of petroleum incorporation (Ishiwatari et al. 1994; Bouloubassi et al. 2001; Wu et al. 2001) suggests that the sediments in Ordy Pond are not contaminated by petroleum-derived

materials.

Ordy Pond sediments contain abundant *n*-alkanes (Table1), despite the absence of riverine inputs of terrigenous OM. This suggests that the main transport pathways of *n*-alkanes into the pond are aeolian transport from the vegetation in the vicinity and perhaps minor contributions (considering the rareness of macro-remains of plants) from direct fall-off of leaves. Epicuticular waxes of plants minimize water loss and serve as a protection barrier against ultraviolet radiation (Eglinton and Hamilton 1967; Kunst and Samuels 2003). Baker (1974) and Wirthensohn and Sedgley (1996) found a positive correlation between the quantity of wax secretions and solar irradiance and aridity, perhaps as an adaptive strategy to minimize water loss and damage caused by solar radiation. In response to the characteristic aridity in the 'Ewa region and the strong incoming solar radiation of the subtropics, the local vegetation might have evolved abundant leaf waxes. Waxy coatings of the leaves are routinely eroded from the surface by wind abrasion as well as by a sandblasting effect, and become airborne (Wirthensohn and Sedgley 1996; Schefuß et al. 2003; Conte et al. 2003). Those waxes physically removed from the surface of the leaves are promptly replaced with newly regenerated waxes to compensate for the losses within just a few days (Hallam 1970; Wirthensohn and Sedgley 1996). The combination of persistent trade-winds and wax-rich vegetation that is adapted to the local climate explains well the abundance of terrestrial plant *n*-alkanes in Ordy Pond sediments.

Horizon D3 was intentionally selected from a depth reasonably close (roughly 20 cm apart) to horizon C dated by AMS using a plant macrofossil (Athens et al. 1999; 2002). The wood sample from horizon W is bracketed between horizons D7 and D9, from which *n*-alkane samples were prepared (Fig. 3). This three sample sequence spans a depth interval of about 160 cm and is also useful in validation of the CSRA method.

In general, ^{14}C ages of *n*-alkanes determined by CSRA show good agreement with those of plant macrofossils dated by AMS. The ^{14}C ages of horizon C and D3, determined by two different methods, are in excellent agreement (Fig. 3). Horizon D5 extends the age-depth trend established by horizons A to D3, which further supports the reliability of the CSRA method (Fig. 3). The ^{14}C ages of horizons W, D7 and D9, however, are not significantly different, although these horizons are contained within a nearly 160 cm interval of laminated sediment. The clustering of these three horizons may be attributed to greater analytical uncertainties associated with CSRA of older materials with limited sample quantities (McNichol et al. 2001). Indeed, the highest analytical uncertainties are associated with *n*-alkanes from horizon D7 and D9, which had two of the lowest C yields among all of the samples (Table 2). Although the age-ranges (2σ probability ranges) of these three samples overlap (Fig. 3), it should be noted that the “true age” of each sample can be anywhere between the upper and lower limits of the 2σ probability ranges. Therefore, the trivial extent of age reversals revealed by the operationally assigned dates (the mid-points of 2σ probable age-ranges of the samples) may not exist in reality.

Despite the lack of the expected trend of increasing age with depth, the similar *n*-alkane ^{14}C dates for these three closely-spaced samples further demonstrate that CSRA using *n*-alkanes can reasonably reproduce ^{14}C ages predicted by AMS dating of plant macrofossils. The results from this study, therefore, clearly show a promising potential of CSRA to serve as an alternative dating technique for paleo-applications.

The *n*-alkane ^{14}C date for horizon D6 is clearly older than anticipated (Fig. 3). The ^{14}C age of D6 deviated from the trend of age progression with depth defined by the other dates, implying anomalous depletion of ^{14}C in the *n*-alkanes from horizon D6. In fact, the ^{14}C age of the *n*-alkanes from D6 was determined to be approximately 4,155 years older than the age predicted by the newly constructed sediment chronology (details will be discussed below).

There are two lines of evidence suggesting that D6 was contaminated during the operation of PCGC. The CO_2 yield from the *n*-alkanes in the D6 sediment sample was significantly higher than from the other samples, although GC quantification confirmed that there were no major differences in *n*-alkane contents among the prepared samples (Table 3). CO_2 recovery from D6 *n*-alkanes by PCGC was slightly greater than 100%, in comparison to roughly 40~60% recovery for the rest of the samples. Furthermore, the CO_2 yield from D6 *n*-alkanes was greater than the maximum theoretical yield based on stoichiometric calculations. Reproducibility of the GC was not monitored in this study, thus quantification of *n*-alkanes could be overestimated. Nevertheless, a recovery of nearly 100% is suspect. Trapping efficiency of PCGC at NOSAMS was reported to be about 80% for compounds with boiling points below

320°C (Eglinton et al. 1996). Meanwhile, Pearson (1999) reported that the PCGC trapping efficiency at NOSAMS ranged from 42 to 78% and averaged 58%, which is in good agreement with this study (except for D6). In addition, Eglinton et al. (1996) reported that when the target compounds are isolated and collected into a designated U-tube via PCGC, there are two potential sources of contaminants: (1) column bleed as a result of thermal degradation of the stationary phase of the capillary column used and (2) incomplete removal of solvent, which is used for transfer of the target compounds from the U-tube to a separate quartz tube for the subsequent procedures. Even though samples after PCGC separation are typically eluted over a silica-gel column as a precautionary step, background contamination due to the column bleed is nevertheless possible. Compounds with high molecular weight (such as long-chain *n*-alkanes) tend to form a layer of viscous “skin” above the solvent during N₂ blow-down, which could potentially lead to incomplete removal of the solvent (Eglinton et al. 1996). In fact, Eglinton et al. (1996) concluded that incomplete removal of solvent prior to combustion is the major potential source of contamination. Both of these sources are likely to add ¹⁴C-depleted C to the sample, because the chromatographic stationary phase and solvents are ultimately derived from petroleum products. Incorporation of impurities could also arise due to the contamination of PCGC traps with residual compounds (i.e. from previous runs).

The possibility of contamination of the D6 sample during PCGC is also demonstrated by simple isotopic mass-balance calculations. The PCGC system separates and traps the target compounds (*n*-C₂₇, *n*-C₂₉, *n*-C₃₁ and *n*-C₃₃) from an admixture of *n*-alkanes in the samples.

These four target compounds collected in the PCGC traps were combined and combusted to yield CO₂ gas, approximately 10% of which was used for the determination of δ¹³C values (δ¹³C_{CO2/NOSAMS}) at NOSAMS. It is noteworthy that, before sample submission to NOSAMS, quantities and δ¹³C values of these individual *n*-alkanes were determined by GC and CSIA. As described earlier, two to three separate lipid extracts were prepared from each horizon in order to meet the sample size requirement. *N*-alkane fractions from separate extracts were independently analyzed by GC and irm-GCMS. Therefore, theoretical δ¹³C values of the CO₂ gas (δ¹³C_{CO2/UH}) evolved from the *n*-alkanes in each sample can be estimated using a simple multi-component isotopic mass-balance calculation. Assuming complete combustion of these *n*-alkanes to the CO₂ gas, δ¹³C values of the post-PCGC CO₂ gas should be equivalent to the weighted averages of the δ¹³C_{*N-Alk*} values. If the trapping of the compounds via PCGC and the subsequent combustion were operated completely without contamination, then technically, post-PCGC δ¹³C values of the CO₂ gas determined in NOSAMS and pre-PCGC values calculated from the isotopic mass-balance model based on the results from GC and CSIA at the University of Hawai‘i should be reasonably close. This hypothesis can be summarized by a set of simple mathematical expressions:

$$\delta^{13}\text{C}_{\text{CO2/NOSAMS}} \approx \delta^{13}\text{C}_{\text{CO2/UH}} \quad (4)$$

$$\begin{aligned} \delta^{13}\text{C}_{\text{CO2/UH}} = & [\text{F}_{27}\delta_{27} + \text{F}_{29}\delta_{29} + \text{F}_{31}\delta_{31} + \text{F}_{33}\delta_{33}]_{\text{Ext. 1}} \\ & + [\text{F}_{27}\delta_{27} + \text{F}_{29}\delta_{29} + \text{F}_{31}\delta_{31} + \text{F}_{33}\delta_{33}]_{\text{Ext. 2}} \end{aligned} \quad (5)$$

$$1 = [\text{F}_{27} + \text{F}_{29} + \text{F}_{31} + \text{F}_{33}]_{\text{Ext. 1}} + [\text{F}_{27} + \text{F}_{29} + \text{F}_{31} + \text{F}_{33}]_{\text{Ext. 2}} \quad (6)$$

where δ and F in Eq. 4 and Eq. 5 denote the $\delta^{13}\text{C}_{N-Alk}$ values and the theoretical molar fractional abundances of CO_2 gas evolved from each compound (denoted by numerical subscripts) with respect to the total CO_2 collected from the combustion of all target n -alkanes, respectively. The isotopic mass-balance model for the D5 sample requires extra terms (“Ext. 3”) because the sample represents the sum of three separate extractions.

The results of the isotopic mass-balance calculations (Table 4) clearly demonstrate agreement between the $\delta^{13}\text{C}_{\text{CO}_2/\text{UH}}$ and the $\delta^{13}\text{C}_{\text{CO}_2/\text{NOSAMS}}$ value for every sample except D6. Observed differences between the theoretical and the actual $\delta^{13}\text{C}$ values of CO_2 gas liberated from the target n -alkanes are all within $\sim 1.0\%$ (the differences for the D3, D7 and D9 sample are within 0.2%). These minor differences between the $\delta^{13}\text{C}_{\text{CO}_2/\text{UH}}$ and the $\delta^{13}\text{C}_{\text{CO}_2/\text{NOSAMS}}$ values can be explained by a small isotopic fractionation due to the nature of chromatographic separation, in which GC columns facilitate earlier elution of molecules containing the heavier isotope (Eglinton et al. 1996). In other words, during GC separation, the leading edge of a peak is more enriched with the heavy isotope (i.e. ^{13}C -rich) and the tailing end of the same peak is more enriched with the light isotope (i.e. ^{12}C -rich). Therefore, minor fractionation could occur if the compounds separated on the GC column were not fully collected by the PCGC system, particularly if the trapping window did not bracket the full width of the peaks of the compounds (Eglinton et al. 1996). A study by Eglinton et al. (1996) showed that the $\delta^{13}\text{C}$ compositions of a number of biomarkers, including long-chain n -alkanes and some volatile compounds with low molecular weight, before and after PCGC generally agreed within a range of $1.0 - 2.0\%$. In

addition, Pearson (1999) suggested that the extent of isotopic fractionation is less than 1.0 to 1.5‰ at most, based on PCGC operations with an analytical standard of a known $\delta^{13}\text{C}$ value. Consequently, the observed 2.2‰ deviation of the $\delta^{13}\text{C}_{\text{CO}_2/\text{UH}}$ and the $\delta^{13}\text{C}_{\text{CO}_2/\text{NOSAMS}}$ values for the D6 sample clearly exceeds the level of uncertainty that can be explained by the potential fractionation effect of the PCGC system. Observed disagreement between theoretical and the actual $\delta^{13}\text{C}_{\text{CO}_2}$ values as well as suspiciously high CO_2 yields from D6 *n*-alkanes, (and the resultant “too-old” ^{14}C age) strongly suggest the introduction of contaminants during PCGC operations.

Alternatively, but less likely, the D6 sample may have been contaminated prior to the PCGC separation. A useful way to assess the age discrepancy is to calculate the ^{14}C content of a sample at the time of deposition. Initial ^{14}C content of a material can be calculated according to the following equation:

$$\text{Initial } \Delta^{14}\text{C} = (Fm_{\text{Corrected}} \times e^{(\lambda \times \text{yrBP})} - 1) \times 1000 \text{ ‰} \quad (7)$$

where, λ and yrBP represent the ^{14}C decay constant ($1 / 8267 \text{ years}^{-1}$), and the year of deposition, respectively. This equation corrects for the losses of ^{14}C through radioactive decay in a material since its sedimentation to the year of AMS measurement, and therefore provides the abundance of ^{14}C at the time of deposition (Smittenberg et al. 2004). The ^{14}C age of the D6 *n*-alkanes determined by CSRA then translates to an initial $\Delta^{14}\text{C}$ value of -25.4‰.

But if the sediment chronology is indeed valid, the age of the D6 *n*-alkanes would be approximately 4,050 cal.yrBP. De-calibration using the IntCal 04 calibration curve by Reimer et

al. (2004) indicates that this calendar age roughly corresponds to a ^{14}C age of 3,680 yrBP and an initial $\Delta^{14}\text{C}$ of -12.9‰. This estimate implies that ^{14}C in the D6 *n*-alkanes is somehow depleted by 12.5‰. The extent of the mixing between true and ^{14}C -depleted *n*-alkanes can also be modeled by an isotopic mass-balance approach:

$$\Delta^{14}\text{C}_{\Sigma} = \{ F_{\text{T}} \times \Delta^{14}\text{C}_{\text{T}} \} + \{ F_{\text{C}} \times \Delta^{14}\text{C}_{\text{C}} \} \quad (8)$$

$$F_{\text{T}} + F_{\text{C}} = 1 \quad (9)$$

where $\Delta^{14}\text{C}$ and F represent the initial ^{14}C content in *n*-alkanes and the fraction of *n*-alkanes from distinct sources in the total combined *n*-alkanes (collective D6 *n*-alkanes). Subscripts Σ , T, and C refer to total combined *n*-alkanes, true *n*-alkanes, and contaminants (^{14}C -depleted *n*-alkanes contributed from different sources), respectively. In this case, the values for $\Delta^{14}\text{C}_{\Sigma}$ and $\Delta^{14}\text{C}_{\text{T}}$ are -25.4‰ and -12.9‰.

If *n*-alkanes from fossil sources such as oils and greases are the sources of contaminants, conventionally, $\Delta^{14}\text{C}_{\text{D}}$ is -1,000‰ (Pearson and Eglinton 2000; Pearson et al. 2001). Then, the isotope mass-balance calculation suggests that the observed age offset of 3,680 years (dilution of $\Delta^{14}\text{C}$ from -25.4 to -12.9‰) from the age-depth curve (Fig. 3) could result from contamination amounting to only 1.3% of the total *n*-alkanes collected from horizon D6.

As discussed earlier, it seems unlikely that the pond and the sediments prior to coring were contaminated by petroleum-related materials. This conclusion is supported by the results from GC and irm-GC/MS analysis of both hydrocarbon and *n*-alkane fractions. The fact that *n*-alkanes from other horizons successfully produced reasonable ^{14}C ages further supports this

interpretation. However, abnormal and accidental contamination by fossil *n*-alkanes in a particular sample is possible during sample preparation and analysis or coring operations in the field. Such trivial amounts (i.e. 1.3% of the total *n*-alkanes) of artificial contaminants are virtually impossible to detect even by GC and irm-GC/MS analysis, particularly in a situation where the signals of terrestrial *n*-alkanes are dominant.

Sediment Chronology

The new sediment chronology constructed from all ^{14}C dates available is shown in Fig. 3. This depth-age curve allows the derivation of specific sediment dates for any interval of the core. All estimated ages, however, must be understood to have an unspecified error range similar to the ^{14}C determinations on which they are based. ^{14}C dates from Athens et al. (1999; 2002) and Tribble et al. (1999) have been re-calibrated using CALIB 5.0 (Stuiver and Reimer 1993; Stuiver et al. 2005) with IntCal 04 of Reimer et al. (2004) (Table 5). The ^{14}C date of horizon D6 was not included in this age-depth model due to contamination of the sample. The ^{14}C date of terrigenous snails by Tribble et al. (1999) was also removed from the model. Earlier studies have shown that ^{14}C dating of snail-shells from limestone areas often causes significant age discrepancy (Goodfriend and Stipp 1983; Dye 1994; Goodfriend et al. 1999). ^{14}C -free C from limestone can be incorporated into snail shells by direct ingestion of limestone or uptake of limestone dissolved by foot secretions (Goodfriend and Hood 1983); however, the magnitude of

such incorporation is quite variable. For example, the age anomalies of snail-shells reported in the literatures range from ~600 to ~3,100 years (e.g. Goodfriend and Stipp 1983; Dye 1994; Goodfriend et al. 1999). Goodfriend and Stipp (1983) concluded that it is impossible to assign a systematic correction factor for ^{14}C -depletion in snail-shells due to the considerable variability in the extent of limestone incorporation. These authors suggested that a reasonable upper limit of dating error caused by the effects of limestone on terrestrial snails is probably on the order of 3,000 years. In order to deal with the unknown level of limestone-effect, a conceptual error of 3,000 years is assigned to the ^{14}C age of terrestrial snails from Ordy Pond sediment, besides the true statistical error associated with the analysis and calibration.

The most recent sedimentation history, phase I in Fig. 3, is estimated by a linear interpolation between the sediment surface and horizon A. Linear interpolation should be suitable for such a short time span (~120 cal.yrBP) relative to the whole sedimentation history of the pond. Sedimentation rates for deeper portions are calculated by first order linear regression with least squares fitting from horizons A to D5 (phase II) and horizons D5 through D9 (phase III). The depth-age curve results in a relatively good statistical fit, $R^2 = 0.96$ for phase II and $R^2 = 0.86$ for phase III. The slightly lower R^2 value for phase III is a result of the clustering of three horizons (D7, W and D9) at very similar 2σ age ranges. The newly constructed age-control suggests that the sedimentation rate in Ordy Pond increased significantly over time. Estimated sedimentation rates according to the age-depth curve are approximately 0.06 (Phase III), 0.18 (Phase II) and 4.33 cm/yr (Phase I).

Conclusions

Terrestrial biomarkers (*n*-alkanes) are ubiquitous and abundant in Ordy Pond sediments. The predominance of long-chain odd-numbered (*n*-C₂₇, *n*-C₂₉, *n*-C₃₁, and *n*-C₃₃) *n*-alkanes and the characteristic carbon isotopic composition of individual compounds indicate that these *n*-alkanes are of terrestrial plant origin. The ¹⁴C age of *n*-alkanes obtained from CSRA on a calibration sample (D3) is in excellent agreement with the age of a sedimentary horizon dated by AMS using plant macrofossils. A series of dates obtained from *n*-alkanes generally match well with independently determined ¹⁴C ages using plant macrofossils. This study thus demonstrated that CSRA using biomarkers is capable of providing reliable ¹⁴C dates for construction of sediment chronology for paleo-applications.

Results of this study also documented a few negative aspects associated with CSRA. Analytical uncertainty becomes larger when the C content in a sample is less than 80 µg. Susceptibility of a sample to artificial contamination can be high during any phase of preparation and analysis. And most of all, sample preparations are time-consuming and CSRA by AMS is inarguably still an expensive and non-routine option. Nonetheless, ¹⁴C dating of individual biomarkers and subsequent construction of sediment chronology is in fact possible in lacustrine sediments. In this particular study, the lack of surface inflow and the poor soil development on the surrounding barren carbonate platform prevented the interference from

relict *n*-alkanes aged in soil reservoirs. But this issue may become a concern when drainage basins of a given lacustrine system have well-developed soil structures which facilitate long-term accumulation of refractory OM (e.g. Smittenberg et al. 2006). But from an opposite point of view, CSRA on *n*-alkanes can be used to differentiate the sedimentary horizons deposited mainly with contemporaneous terrestrial OM and the horizons with significant contributions from inwashed relict terrestrial materials.

The site where this study was conducted is an unusual system in terms of its geological, hydrological and environmental setting and therefore results from this study alone can not draw broad generalization to support reliability of CSRA on *n*-alkanes in other areas. However, this study demonstrates that CSRA with appropriate selection of target biomarkers should be considered as a powerful tool for construction of sediment chronology if more conventional means of ^{14}C dating such as the standard radiometric method are inapplicable. This method can be applied to sediment cores collected from carbonate-hosted lakes and sinkholes or offshore cores from fairly large lakes (e.g. Vance and Telka, 1998) in order to overcome the reservoir effect or rareness of macrofossils.

Acknowledgements

The authors appreciate Dr. J. M. Hayes for his critical advice on the method development and data interpretations as well as the staff at the NOSAMS facility for analyzing the samples. We

truly appreciate the kind assistance and technical support from R. J. Wallsgrove, T. Rust and J. K. Tanimoto in the laboratory. We are grateful to Dr. C. I. Measures, M. Brown and K. Kime, who hospitably allowed us to use their laboratory for sample preparations. We thank significant contribution from Dr. S. J. Athens from International Archaeological Research Institute Inc. We would like to thank Dr. Eric J. Hochberg for his assistance on computer graphics. And lastly, constructive comments from the reviewers and editors tremendously improved the overall quality of our manuscript. This project was funded by Petroleum Research Fund (PRF # 40088-ACS) and in part by Sigma Xi, The Scientific Research Society (Grants in aid of research, 2003). This is SOEST contribution number #####.

References

- Athens JS, Ward JV, Tuggle HD, Welch DJ, Blinn DW, Cowie R, Murakami GM, Sherman C (1999) Environment, vegetation change, and early human settlement on the 'Ewa Plain: A cultural resource inventory of Naval Air Station, Barbers Point, O'ahu, Hawai'i, Part III: Paleoenvironmental investigations. International Archeological Research Institute, Inc., Honolulu, HI.
- Athens JS, Tuggle HD, Ward JV, Welch DJ (2002) Avifaunal extinctions, vegetation change, and Polynesian impacts in prehistoric Hawai'i. *Archaeol. Ocean.* 37: 57-78.
- Baker, EA (1974) The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. *New Phytol.* 73: 955-966.
- Bird MI, Summons RE, Gagan MK, Roksandic Z, Dowling L, Head J, Fifield LK, Cresswell RG, Johnson DP (1995) Terrestrial vegetation change inferred from *n*-alkane $\delta^{13}\text{C}$ analysis in the marine environment. *Geochim. Cosmochim. Acta.* 59:2853-2857.

- Björck S, Bennike O, Possnert G, Wohlfarth B, Digerfeldt G (1998) A high-resolution ^{14}C dated sediment sequence from southwest Sweden: age comparisons between different components of the sediment. *J. Quat. Sci.* 13: 85-89.
- Bouloubassi I, Fillaux J, Saliot A (2001) Hydrocarbons in surface sediments from the Changjiang (Yangtze River) estuary, East China Sea. *Mar. Pollut. Bull.* 42: 1335-1346.
- Bourbonniere RA, Meyers PA (1996) Sedimentary geolipid records of historical changes in the watersheds and productivities of Lakes Ontario and Erie. *Limnol. Oceanogr.* 41: 352-359.
- Bradley RS (1999) *Paleoclimatology: Reconstructing climates of the Quaternary*, 2nd Ed. Academic Press, San Diego, CA.
- Brincat D, Yamada K, Ishiwatari R, Uemura H, Naraoka H (2000) Molecular-isotopic stratigraphy of long-chain *n*-alkanes in Lake Baikal Holocene and glacial sediments. *Org. Geochem.* 31: 287-294.
- Chikaraishi Y, Naraoka H (2003) Compound-specific δD - $\delta^{13}\text{C}$ analyses of *n*-alkanes extracted from terrestrial and aquatic plants. *Phytochemistry.* 63: 361-371.
- Collister JW, Rieley G, Stern B, Eglinton G, Fry B (1994) Compound-specific $\delta^{13}\text{C}$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms. *Org. Geochem.* 21: 619-627.
- Conte MH, Weber JC, Carlson PJ, Flanagan LB (2003) Molecular and carbon isotopic composition of leaf wax in vegetation and aerosols in a northern prairie ecosystem. *Oecologia.* 135: 67-77.
- Cranwell PA (1981) Diagenesis of free and bound lipids in terrestrial detritus deposited in a lacustrine sediment. *Org. Geochem.* 14: 1-14.
- Dye T (1994) Apparent ages of marine shells: Implications for archaeological dating in Hawai'i. *Radiocarbon.* 36: 51-57.
- Eglinton G, Hamilton RJ (1967) Leaf epicuticular waxes. *Science*, 156, 1322-1335.
- Eglinton TI, Aluwihare LI, Bauer JE, Druffel ERM, McNichol AP (1996) Gas chromatographic isolation of individual compounds from complex matrices for radiocarbon dating. *Anal. Chem.* 68: 904-912.
- Eglinton TI, Benitez-Nelson BC, Pearson A, McNichol AP, Bauer JE, Druffel ERM (1997)

- Variability in radiocarbon ages of individual organic compounds from marine sediments. *Science*. 277: 796-799.
- Garrison GH (2002) Holocene sedimentary and aquatic biogeochemical responses reflected in Ordy Pond, Oah‘u, Hawai‘i and Contemporary modeling of submarine groundwater discharge in Kahana Bay, Oah‘u, Hawai‘i. (Ph.D. Dissertation) Department of Geology and Geophysics, University of Hawai‘i at Mānoa.
- Goodfriend GA, Hood DG (1983) Carbon isotope analysis of land snail shells: Implications for carbon sources and radiocarbon dating. *Radiocarbon*. 25: 810-830.
- Goodfriend GA, Stipp JJ (1983) Limestone and the problem of radiocarbon dating of land-snail shell carbonate. *Geology*. 11: 575-577.
- Goodfriend GA, Ellis GL, Toolin LJ (1999) Radiocarbon age anomalies in land snail shells from Texas: Ontogenetic, individual, and geographic patterns of variation. *Radiocarbon*. 41: 149-156.
- Hallam ND (1970) Growth and regeneration of waxes on the leaves of *Eucalyptus*. *Planta*. 93: 257-268.
- Hoefs MJL, Rijpstra WIC, Sinninghe Damsté JS (2002) The influence of oxic degradation on the sedimentary biomarker record I: Evidence from Madeira Abyssal Plain turbidites. *Geochim. Cosmochim. Acta*. 66: 2719-2735.
- Hoering TC, Freeman DH (1984) Shape-selective sorption of monomethylalkanes by silicalite, a zeolite form of silica. *J. Chromatogr.* 316: 333-341.
- Huang Y, Eglinton G, Ineson P, Latter PM, Bol R, Harkness DD (1997) Absence of carbon isotope fractionation of individual *n*-alkanes in a 23-year field decomposition experiment with *Calluna vulgaris*. *Org. Geochem.* 26: 497-501.
- Ishiwatari R, Uzaki M, Yamada K (1994) Carbon isotope compositions of individual *n*-alkanes in recent sediments. *Org. Geochem.* 21: 801-808.
- Kenig F, Popp BN, Summons RE (2000) Preparative HPLC with ultrastable-Y zeolite for compound-specific carbon isotopic analyses. *Org. Geochem.* 31: 1087-1094.
- Ku T-L, Kimmel MA, Easton WH, O’Neil TJ (1974) Eustatic sea level 120,000 years ago on O‘ahu, Hawai‘i. *Science*. 183: 95a.

Kunst A, Samuels AL (2003) Biosynthesis and secretion of plant cuticular wax. *Prog. Lipid Res.* 42: 51-80.

Kuypers MMM, Pancost RD, Sinninghe Damsté JS (1999) A large and abrupt fall in atmospheric CO₂ concentrations during Cretaceous times. *Nature.* 399: 342-345.

Lowe JJ, Walker MJC (2000) Radiocarbon dating the last glacial-interglacial transition (Ca. 14-9 ¹⁴C ka BP) in terrestrial and marine records: The need for new quality assurance protocols. *Radiocarbon.* 42: 53-68.

Mazeas L, Budzinski H, Raymond N (2002) Absence of stable carbon isotope fractionation of saturated and polycyclic aromatic hydrocarbons during aerobic bacterial biodegradation. *Org. Geochem.* 33: 1259-1272.

McNichol AP, Gagnon AR, Osborne EA, Hutton DL, von Reden KF, Schneider RJ (1995) Improvements in procedural blanks at NOSAMS: Reflection of improvements in sample preparation and accelerator operation. *Radiocarbon.* 37: 683-691.

McNichol AP, Jull AJT, Burr GS (2001) Converting AMS data to radiocarbon values: Considerations and conventions. *Radiocarbon.* 43: 313-320.

Menzel D, Schouten S, van Bergen PF, Sinninghe Damsté JS (2004) Higher plant vegetation changes during Pliocene sapropel formation. *Org. Geochem.* 35: 1343-1353.

Mollenhauer G, Kienast M, Lamy F, Meggers H, Schneider RR, Hayes JM, Eglinton TI (2005) An evaluation of ¹⁴C age relationships between co-occurring foraminifera, alkenones, and total organic carbon in continental margin sediments. *Paleoceanography*, 20, PA1016, doi:10.1029/2004PA001103.

Muri G, Wakeham SG, Pease TK, Faganeli J (2004) Evaluation of lipid biomarkers as indicators of changes in organic matter delivery to sediments from Lake Planina, a remote mountain lake in NW Slovenia. *Org. Geochem.* 35: 1083-1093.

Ogden (1999) Remedial investigation report for BRAC – Related activities, regional ground-water system, Naval Air Station Barbers Point, O‘ahu, Hawai‘i. 3 Volumes. Ogden Environmental and Energy Services Company, Inc. Honolulu, Hawai‘i.

Ohkouchi N, Eglinton TI, Hayes JM (2003) Radiocarbon dating of individual fatty acids as a tool for refining Antarctic margin sediment chronologies. *Radiocarbon.* 45: 17-24.

- Pearson A (1999) Biogeochemical applications of compound-specific radiocarbon analysis. (Ph.D. Dissertation) Joint program in oceanography / Applied ocean science and engineering. Massachusetts Institute of Technology / Woods Hole Oceanographic Institution.
- Pearson A, Eglinton TI (2000) The origin of *n*-alkanes in Santa Monica Basin surface sediment: a model based on compound-specific $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ data. *Org. Geochem.* 31: 1103-1116.
- Pearson A, McNichol AP, Schneider RJ, von Reden KF, Zheng Y (1998) Microscale AMS ^{14}C measurement at NOSAMS. *Radiocarbon.* 40: 61-75.
- Pearson A, Eglinton TI, McNichol AP. (2000) An organic tracer for surface ocean radiocarbon. *Paleoceanography.* 15: 541-550.
- Pearson A, McNichol AP, Benitez-Nelson BC, Hayes JM, Eglinton TI (2001) Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific $\Delta^{14}\text{C}$ analysis. *Geochim. Cosmochim. Acta.* 65: 3123-3137.
- Petsch ST, Eglinton TI, Edwards KJ (2001) ^{14}C -dead living biomass: Evidence for microbial assimilation of ancient organic carbon during shale weathering. *Science.* 292: 1127-1131.
- Reddy CM, Pearson A, Xu L, McNichol AP, Benner Jr BA, Wise SA, Klouda GA, Currie LA, Eglinton TI (2002) Radiocarbon as a tool to apportion the sources of polycyclic aromatic hydrocarbons and black carbon in environmental samples. *Environ. Sci. Technol.* 36: 1774-1782.
- Reddy CM, Xu L, O'Connor R (2003) Using radiocarbon to apportion sources of polycyclic aromatic hydrocarbons in household soot. *Environmental Forensics.* 4: 191-197.
- Reimer PJ, Baillie MGL, Bard E, Bayliss A, Beck JW, Bertrand CJH, Blackwell PG, Buck CE, Burr GS, Cutler KB, Damon PE, Edwards RL, Fairbanks RG, Friedrich M, Guilderson TP, Hogg AG, Hughen KA, Kromer B, McCormac FG, Manning SW, Ramsey CB, Reimer RW, Remmele S, Southon JR, Stuiver M, Talamo S, Taylor FW, van der Plicht J, Weyhenmeyer CE (2004) IntCal04 terrestrial radiocarbon age calibration, 26 - 0 ka BP. *Radiocarbon.* 46: 1029-1058.
- Rieley G, Collier RJ, Jones DM, Eglinton G, Eakin PA, Fallick AE (1991) Sources of sedimentary lipids deduced from stable carbon-isotope analyses of individual compounds. *Nature.* 352: 425-427.
- Schefuß E, Ratmeyer V, Stutt J-BW, Jansen JHF, Sinninghe Damsté JS (2003) Carbon isotope

- analyses of *n*-alkanes in dust from the lower atmosphere over the central eastern Atlantic. *Geochim. Cosmochim. Acta.* 67: 1757-1767.
- Sherman CE, Glenn CR, Jones AT, Burnett WC, Schwarcz HP (1993) New evidence for two highstands of the sea during the last interglacial, oxygen isotope substage 5e. *Geology.* 21: 1079-1082.
- Smittenberg RH, Hopmans EC, Schouten S, Hayes JM, Eglinton TI, Sinninghe Damsté JS (2004) Compound-specific radiocarbon dating of the varved Holocene sedimentary record of Saanich Inlet, Canada. *Paleoceanography*, 19, PA2012, doi:10.1029/2003PA000927.
- Smittenberg RH, Eglinton TI, Schouten S, Sinninghe Damsté JS (2006) Ongoing buildup of refractory organic carbon in boreal soils during the Holocene. *Science.* 314: 1283-1286.
- Stuiver M, Polach HA (1977) Discussion: Reporting of ^{14}C data. *Radiocarbon.* 19: 355-363.
- Stuiver M, Reimer PJ (1993) Extended ^{14}C database and revised CALIB radiocarbon calibration program. *Radiocarbon.* 35: 215-230.
- Stuiver M, Reimer PJ, Reimer RW (2005) CALIB 5.0. [WWW program and documentation].
- Sun MY, Wakeham SG (1994) Molecular evidence for degradation and preservation of organic matter in the anoxic Black Sea Basin. *Geochim. Cosmochim. Acta.* 58: 3395-3406.
- Sun Y, Chen Z, Xu S, Cai P (2005) Stable carbon and hydrogen isotopic fractionation of individual *n*-alkanes accompanying biodegradation: evidence from a group of progressively biodegraded oils. *Org. Geochem.* 36: 225-238.
- Szabo BJ, Ludwig KR, Muhs DR, Simmons KR (1994) Thorium-230 ages of corals and duration of the last interglacial sea-level high stand on O'ahu, Hawai'i. *Science.* 266: 93-96.
- Törnqvist TE, de Jong AFM, Oosterbaan WA, van der Borg, K (1992) Accurate dating of organic deposit by AMS ^{14}C measurement of macrofossils. *Radiocarbon.* 34: 566-577.
- Tribble JS, Athens JS, Allen CB, Frankel E, Skilbeck CG (1998) A high resolution sedimentary record of O'ahu Holocene history. *GSA Abstracts with programs*, 1998 Annual Meeting, A-162.
- Tribble JS, Garrison GH, Athens JS, Allen CB, Skilbeck CG, Frankel E (1999) Evidence for Early-Mid Holocene sea-level on O'ahu, Hawai'i from coastal pond sediments. In: Fletcher CH, Matthews JV. (Eds) *The non-steady state of the inner shelf and shoreline: Coastal change on the*

time scale of decades to millennia in the late Quaternary. Inaugural meeting of IGCP Project #437 "Coastal Environmental Change During Sea Level Highstands," Abstracts with Program. University of Hawai'i, Honolulu, U.S.A., Nov. 9012, 215-218.

Turney CSM, Coope GR, Harkness DD, Lowe JJ, Walker MJC (2000) Implications for the dating of Wisconsinan (Weichselian) late-glacial events of systematic radiocarbon age differences between terrestrial plant macrofossils from a site in SW Ireland. *Quat. Res.* 53: 114-121.

Uchida M, Shibata Y, Ohkushi K, Yoneda M, Kawamura K, Morita M (2005) Age discrepancy between molecular biomarkers and calcareous foraminifera isolated from the same horizons of Northwest Pacific sediments. *Chem. Geol.* 218: 73-89.

Vance RE, Telka AM (1998) Accelerator mass spectrometry radiocarbon dating of 1994 Lake Winnipeg cores. *J. Paleolimnol.* 19: 329-334.

von Reden KF, McNichol AP, Pearson A, Schneider RJ (1998) ^{14}C AMS measurements of <100 μg samples with a high-current system. *Radiocarbon.* 40: 247-253.

Walker MJC, Bryant C, Coope GR, Harkness DD, Lowe JJ, Scott EM (2001) Towards a radiocarbon chronology of the late-glacial: Sample selection strategies. *Radiocarbon*, 43: 1007-1019.

Wirthensohn MG, Sedgley M (1996) Epicuticular wax structure and regeneration on developing juvenile *Eucalyptus* leaves. *Aust. J. Bot.* 44: 691-704.

Wu Y, Zhang J, Mi T, Li B (2001) Occurrence of *n*-alkanes and polycyclic aromatic hydrocarbons in the core sediments of the Yellow Sea. *Mar. Chem.* 76: 1-15.

Yamada K, Ishiwatari R (1999) Carbon isotopic compositions of long-chain *n*-alkanes in the Japan Sea sediments: implications for paleoenvironmental changes over the past 85 kyr. *Org. Geochem.* 30: 367-377.

Table 1. Summary of gas chromatography (GC) analysis and compound-specific stable carbon isotopic analysis by irm-GC/MS on target *n*-alkanes.

Horizon	Depth (cm)	<i>N</i> -alkane Abundance ^a ($\mu\text{g/g}$) ^b	CPI ^c	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)			
				<i>n</i> -C ₂₇	<i>n</i> -C ₂₉	<i>n</i> -C ₃₁	<i>n</i> -C ₃₃
D3	700 - 705	17.8	8.6 ± 0.9	-28.2 ± 0.1	-29.6 ± 0.3	-26.6 ± 0.1	-25.5 ± 0.0
D5	925 - 930	12.7	8.0 ± 4.8	-26.5 ± 1.7	-28.3 ± 1.0	-26.6 ± 1.2	-27.1 ± 1.0
D6	1015 - 1020	9.7	9.0 ± 0.2	-27.4 ± 0.5	-28.3 ± 0.4	-28.1 ± 0.7	-28.9 ± 0.6
D7	1170 - 1180	27.4	11.7 ± 2.5	-27.0 ± 0.9	-29.0 ± 1.2	-29.8 ± 1.5	-28.5 ± 1.2
D9	1320 - 1330	21.8	11.1 ± 0.6	-27.2 ± 0.7	-28.9 ± 0.5	-29.9 ± 0.9	-29.2 ± 0.6

Extractions were continued with new sediment sub-samples until sufficient *n*-alkanes were collected from each horizon. Once GC and irm-GCMS were completed, and thus the terrestrial plant origin of the target compounds was confirmed, *n*-alkane fractions from separate extracts were combined. All of the values reported in this table represent averages and standard deviations of individually prepared sub-samples from each horizon (n = 2 or 3).

^a Quantity of *n*-alkanes was estimated by comparing the integrated peak areas of target *n*-alkanes to that of a co-injected internal standard (deuterated *n*-C₃₆) with a known concentration. *N*-alkane abundance refers to combined quantity of *n*-C₂₇, *n*-C₂₉, *n*-C₃₁ and *n*-C₃₃ alkanes.

^b Unit is “quantity (μg) of target compounds per unit mass (g) of dried sediment”.

^c Calculated as $\text{CPI} = \sum(\text{C}_{25}, \text{C}_{27}, \text{C}_{29}, \text{C}_{31}, \text{C}_{33}) / \sum(\text{C}_{24}, \text{C}_{26}, \text{C}_{28}, \text{C}_{30}, \text{C}_{32})$ in terms of *n*-alkane quantity (μg) from Pearson and Eglinton (2000).

Table 2. Summary of preparative capillary gas chromatography (PCGC) and compound-specific radiocarbon analysis (CSRA) data on *n*-alkanes extracted from Ordy Pond sediment, and the result of AMS ^{14}C dating of wood fragments found in the sediment. All of the analyses were conducted in NOSAMS facility at Woods Hole Oceanographic Institution. ^{14}C ages (yrBP) were calibrated to calendar years (cal.yrBP) using CALIB 5.0 program.

Horizon (Sample)	Depth (cm)	CO_2 ^a (μmol)	$\delta^{13}\text{C}_{\text{VPDB}}$ ^b (‰)	C ^c (μg)	Fm	$\Delta^{14}\text{C}$ (‰)	^{14}C Age (yrBP)	Calendar Age ^d (cal.yrBP)
D3	700 - 705	6.0	-27.7	78.3	0.853	-152.7	1,280 ± 95	1,162.5 ± 183.5
D5	925 - 930	8.3	-28.0	107.4	0.729	-275.6	2,540 ± 85	2,563.0 ± 204.0
D6	1015 - 1020	12.8	-30.4	166.4	0.398	-604.5	7,400 ± 110	8,204.0 ± 196.0
D7	1170 - 1180	5.5	-29.7	72.0	0.402	-601.0	7,330 ± 200	8,147.5 ± 390.5
D9	1320 - 1330	5.3	-29.3	69.0	0.413	-589.5	7,100 ± 160	7,952.0 ± 338.0
Wood	1267.2	170.5	-26.7	2048.0	0.398	-605.0	7,410 ± 60	8,211.5 ± 161.5

^a Quantity of CO_2 evolved by combustion of target *n*-alkanes (*n*- C_{27} , *n*- C_{29} , *n*- C_{31} and *n*- C_{33}) purified via PCGC system.

^b Stable carbon isotopic composition of CO_2 described above. Values are reported against the VPDB standard material.

^c Quantity of carbon (C) after graphitization of CO_2 evolved from the target *n*-alkanes.

^d Calendar ages represent the mid-points of the pair of probable dates encompassing 2σ probability age ranges (95% confidence interval) calculated by CALIB 5.0 program. Errors are the age difference from the mid-points to the end of 2σ probability ranges.

Table 3. Summary of changes in the quantities of target *n*-alkanes before and after PCGC in terms of CO₂ equivalence.

Horizon	<i>N</i> -alkane	Quantity ^a 10 ⁻² μmol	Theo. CO ₂ Y. ^b μmol	Act. CO ₂ Y. ^d μmol	PCGC Efficiency ^e %
D3	<i>n</i> -C ₂₇	5.6	1.5		
	<i>n</i> -C ₂₉	18.3	5.3		
	<i>n</i> -C ₃₁	18.4	5.7		
	<i>n</i> -C ₃₃	8.0	2.7		
	Σ ^c	50.3	15.2	6.0	39.5
D5	<i>n</i> -C ₂₇	8.1	2.2		
	<i>n</i> -C ₂₉	13.4	3.9		
	<i>n</i> -C ₃₁	12.8	4.0		
	<i>n</i> -C ₃₃	9.3	3.1		
	Σ	43.6	13.2	8.3	62.9
D6	<i>n</i> -C ₂₇	5.2	1.4		
	<i>n</i> -C ₂₉	14.5	4.2		
	<i>n</i> -C ₃₁	13.2	4.1		
	<i>n</i> -C ₃₃	6.9	2.3		
	Σ	39.8	12.0	12.8	106.7
D7	<i>n</i> -C ₂₇	5.5	1.5		
	<i>n</i> -C ₂₉	14.0	4.1		
	<i>n</i> -C ₃₁	16.9	5.2		
	<i>n</i> -C ₃₃	5.5	1.8		
	Σ	41.9	12.6	5.5	43.7
D9	<i>n</i> -C ₂₇	6.4	1.7		
	<i>n</i> -C ₂₉	16.1	4.7		
	<i>n</i> -C ₃₁	15.6	4.8		
	<i>n</i> -C ₃₃	5.6	1.8		
	Σ	43.7	13.0	5.3	40.8

^a Molar quantity of *n*-alkanes estimated via GC analyses.

^b Theoretical CO₂ yield from individual *n*-alkanes based on the GC quantification of *n*-alkanes and elemental stoichiometry. C₂₇H₅₆, C₂₉H₆₀, C₃₁H₆₄ and C₃₃H₆₈ were used as the elemental structure of each target compound, and complete reaction was assumed for calculation.

^c Sum of *n*-C₂₇, *n*-C₂₉, *n*-C₃₁ and *n*-C₃₃ alkanes for GC quantity and theoretical CO₂ yield.

^d Actual CO₂ yield from the collective *n*-alkanes trapped by PCGC system.

^e PCGC Efficiency = (Actual CO₂ yield) / (Σ Theoretical CO₂ yield) * 100%

Table 4. Comparisons of the $\delta^{13}\text{C}$ values of CO_2 gas that are predicted by the isotopic mass-balance calculations based on the results of GC and CSIA and that are analyzed at the NOSAMS facility after the combustion of the four target *n*-alkanes trapped by PCGC.

Horizon	$\delta^{13}\text{C}_{\text{CO}_2/\text{UH}}$ Theoretical Estimate ‰	$\delta^{13}\text{C}_{\text{CO}_2/\text{NOSAMS}}$ Actual Measurement ‰	Difference ‰
D3	-27.6	-27.7	0.1
D5	-27.0	-28.0	1.0
D6	-28.2	-30.4	2.2
D7	-29.5	-29.7	0.2
D9	-29.1	-29.3	0.2

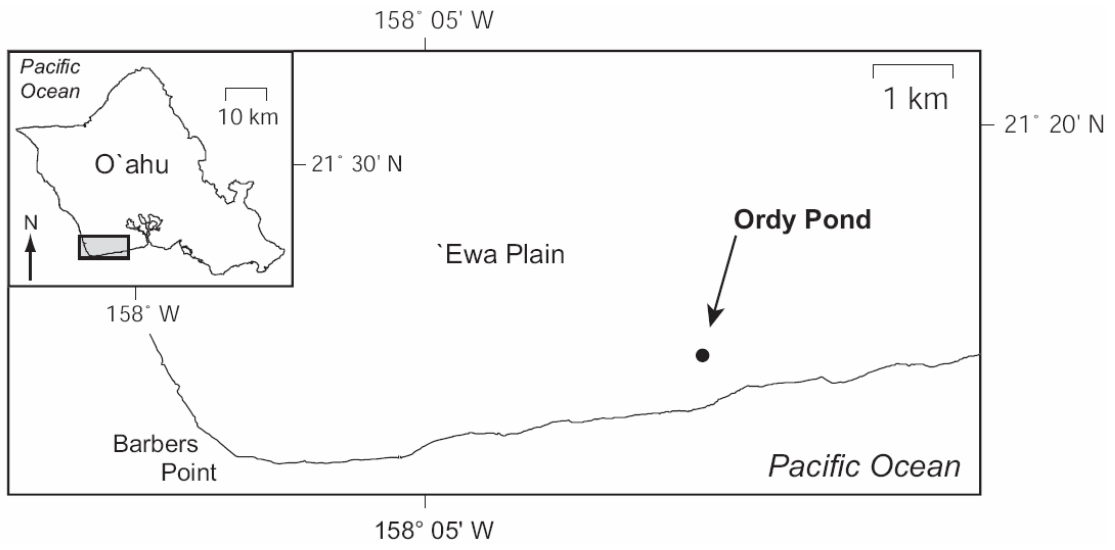
Table 5. Recalibration of the ^{14}C dates used in the previous Ordy Pond sediment chronology.

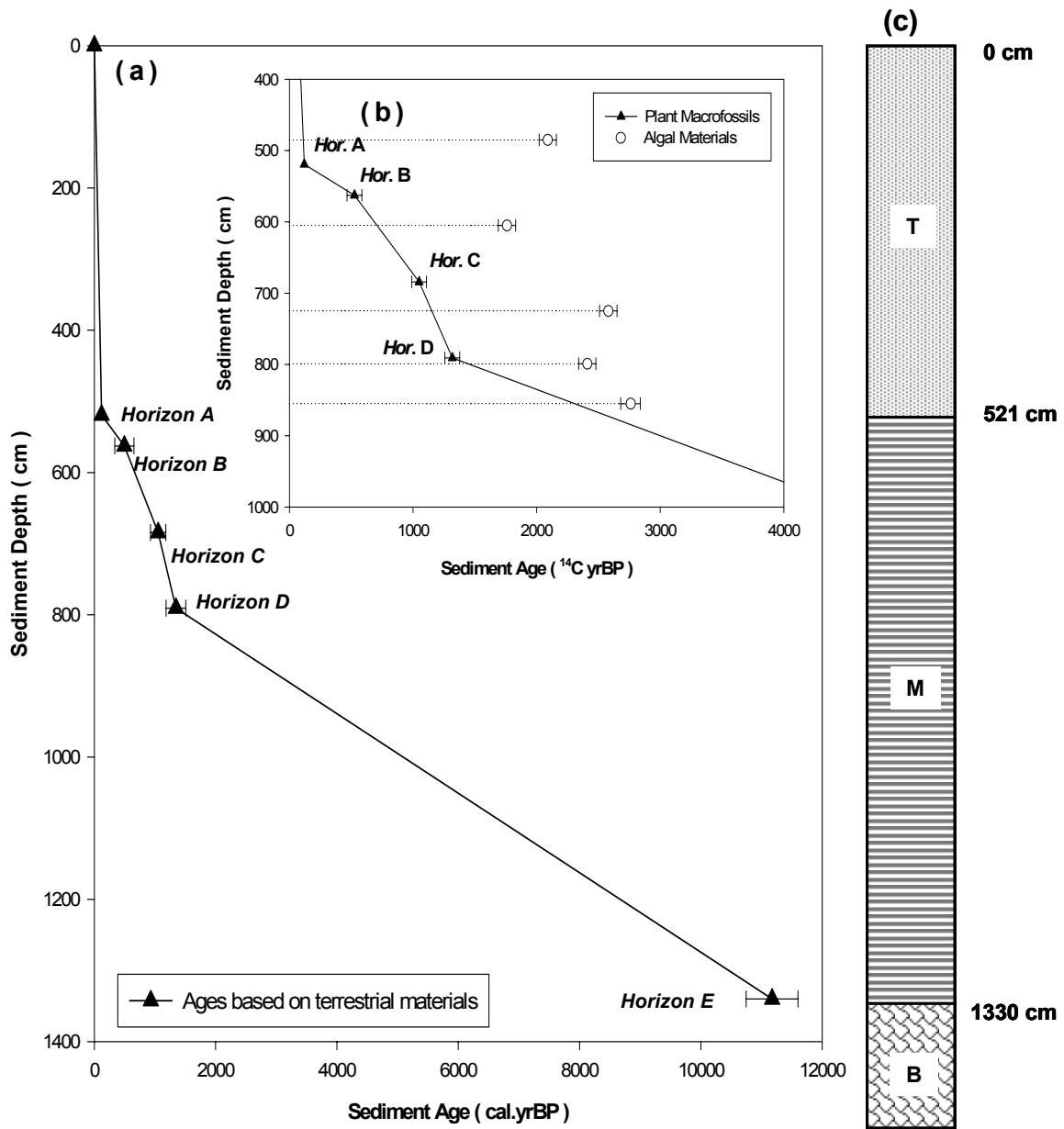
Horizon	Source	Method / Sample Type	Radiocarbon Age ^a (yrBP)	Calendar Age ^b (cal.yrBP)
Horizon A	<i>Athens et al.</i> ('99 & '02)	Historic Pollen	120.0	120
Horizon B	<i>Athens et al.</i> ('99 & '02)	AMS / Plant macrofossils	510 ± 60	496.5 ± 156.5
Horizon C	<i>Athens et al.</i> ('99 & '02)	AMS / Plant macrofossils	1120 ± 60	1053.0 ± 125.0
Horizon D	<i>Athens et al.</i> ('99 & '02)	AMS / Plant macrofossils	1420 ± 60	1345.5 ± 161.5
Horizon E	<i>Tribble et al.</i> ('99)	C.R.M * / Land snails	9780 ± 110.0	11175.0 ± 426.0

^a Uncalibrated radiocarbon ages that were originally reported in earlier studies.

^b Original radiocarbon dates reported by Athens et al. (1999, 2002) and Tribble et al. (1999) were calibrated to calendar age (cal.yrBP) using CALIB 5.0 program (Stuiver and Reimer 1993; Stuiver et al. 2005) with the IntCal 04 calibration dataset (Reimer et al. 2004).

* Conventional radiometric method.





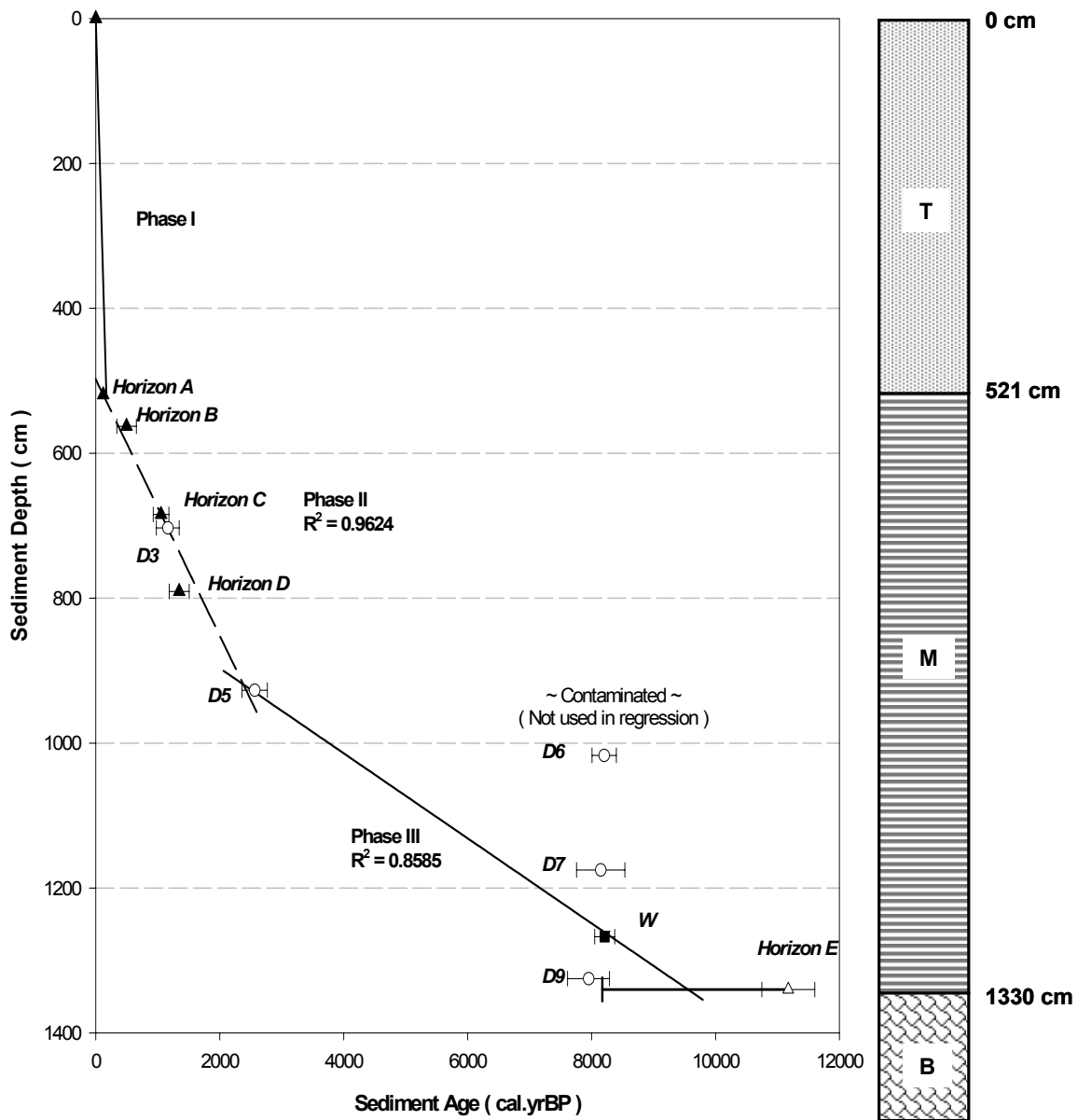


Figure captions

Fig. 1. Location of Ordy Pond in the 'Ewa Plain of O'ahu. The pond is approximately 750 m from the coast and is situated 1.5 m above mean sea-level. The area of the pond is roughly 0.5 ha.

Fig. 2. Depth-age plot of ^{14}C dates of aquatic sediment from Ordy Pond. (a) Sediment chronology based on AMS ^{14}C dates derived from terrestrial plant materials (horizon B, C and D) by Athens et al. (1999; 2000). The age model is also supplemented by ages of horizon A (Athens et al. 1999; 2000) and E (Tribble et al. 1999) based on the first appearance of historic pollen and conventional ^{14}C dating using shells of terrestrial snails, respectively. (b) Comparison of ^{14}C dates obtained from terrestrial materials (filled triangles) from horizons A, B, C and D, and algal materials (open circles) by Athens et al. (1999; 2002). The radiocarbon age scale (yrBP) was used for the comparison because the algal dates could not to be calibrated to calendar ages (cal.yrBP) due to an unknown correction factor for the reservoir effect of the pond water. (c) Schematic representation of core stratigraphy. Labels B, M and T refer to basal non-aquatic sedimentary unit, middle laminated aquatic sedimentary unit and top sapropelic sedimentary unit, respectively. The actual base of the core extends to 17.5 m.

Fig. 3. Complete chronology of Ordy Pond sediment. Dates of horizons A, B, C and D (closed triangles) are from Athens et al. (1999; 2000) and that of horizon E (open triangle) is from Tribble et al. (1999). Dates of *n*-alkanes from horizon D3, D5, D6, D7 and D9 (open circles) were determined by CSRA and that of wood fragments from horizon W (closed square) was from AMS. The two types of error bars on the snail date represent an analytical and statistical error, and an arbitrary error of 3,000 cal.yrBP due to limestone-effect. Three distinct phases of sedimentation (phase I, II and III) were modeled from a series of dates. The phase I (upper solid

line) is based on linear interpolation from the sediment surface to horizon A. Linear regression is used to model Phase II (middle dashed line), from horizon A through D5, and phase III (lower solid line) from horizon D5 through D9. Horizons D6 and E were not used in the regression for phase III due to possible contamination (D6) and the unknown extent of limestone-effect (E).