UTILITY OF LEAF WAX NORMAL ALKANES FOR LACUSTRINE SEDIMENT CHRONOLOGY AND FOR RECONSTRUCTION OF HOLOCENE PALEOVEGETATIVE CHANGES IN HAWAI'I: AN APPLICATION OF ROBUST MOLECULAR RADIOCARBON AND STABLE CARBON ISOTOPE TECHNIQUES

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CHAPTER I

Background and Introduction
1.1. Introduction

Understanding of the consequences and impacts of past climate changes provides valuable information in prediction of how the Earth system will respond to future climate changes. Because most historical records of climate change extend back to roughly 150 years at most, studies of earlier climate changes and variability on longer time-scales require proxy records from corals, tree rings, ice cores and sediments. Small reservoir sizes and rapid sedimentation rates (and hypoxic/anoxic water columns and sediments in some cases) of many lakes facilitate development and preservation of high-resolution paleoclimatic proxies in sediments. Numerous paleoclimatic reconstructions have relied on a variety of proxy records extracted from lacustrine sediments (e.g., *Brauer et al.*, 1999; *Karabanov et al.*, 2000; *Seltzer et al.*, 2000; *Moy et al.*, 2002; *Huang et al.*, 2004).

Proxy records from the tropical/subtropical oceanic region of the Pacific are extremely rare, except for several coral records (e.g., *Beck et al.*, 1997; *Corrège et al.*, 2000; *Corrège et al.*, 2004), even though this area plays a critical role in modulating global climate systems by distributing incoming solar energy to the rest of the globe via oceanic and atmospheric circulation and in driving oscillatory climatic variability such as El Niño/Southern Oscillation (ENSO) events. The rareness of proxy records, particularly from sediments, in the tropical/subtropical oceanic regions arises from the absence of distinct seasons. Climates in these regions are typically homogeneously warm/hot throughout the year, and signals of seasonality (i.e., changes in temperature) are buffered by the vast oceans.
A series of sediment cores recovered from Ordy Pond, O‘ahu, Hawai‘i, however, provides a complete Holocene record of high-resolution varved sediments. Fine-scale laminations (a few millimeters to centimeters in thickness) reflect the compositional differences in the deposits produced within the water column in each season. Given such exceptional continuity and resolution, the sedimentary record from Ordy Pond is expected to provide valuable paleoclimatic proxies for the Holocene from a low-latitude coastal location.

This introductory chapter first describes the study site, Ordy Pond, in terms of the geological characteristics of the area, hydrology of the water column, contemporary climate patterns and several important biogeochemical reactions occurring in the water column that provide the fundamental basis for interpretations of the proxy records from the sediments. Secondly, previous efforts to extract and interpret paleoenvironmental information from the sediment cores collected from Ordy Pond are summarized. And finally the motivations and specific goals of this investigation are addressed.

1.2. Geology and Hydrology of Ordy Pond

Ordy Pond is located on the former Naval Air Station Barbers Point in the Kalaeloa district of the ‘Ewa Plain, O‘ahu, Hawai‘i (Figure 1.1). The ‘Ewa Plain is an exposed Pleistocene fossil-reef terrain established during the Waimanalo interglacial highstand of sea-level roughly from 135 to 120 kyr (Ku et al., 1974; Sherman et al., 1993; Szabo et al., 1994). The pond occupies one of the numerous karstic sinkholes (~20m deep) developed throughout the limestone platform, and currently is the only permanent aquatic feature in the area. The initial transformation of the open sinkhole into
Figure 1.1. Location of Ordy Pond in the ‘Ewa Plain of O’ahu (from Garrison, 2002). The pond is approximately 750 m from the coast and is situated at 1.5 m above mean sea-level. The area of the pond is 0.5 ha.

A pond was probably triggered by intrusion of groundwater in response to the most recent post-glacial sea-level rise around 10 kya. Today, the pond is approximately 0.5 ha in area, and contains roughly 5 m of water and 13.5 m of aquatic sediments. The pond possesses neither surface inflow nor outflow. Furthermore, the pond water appears to be largely isolated from the surrounding groundwater aquifer. Hydrological and tidal surveys conducted by Ogden (1999) showed that the water level of Ordy Pond is usually lower than that of the local groundwater system; furthermore, vertical fluctuations of the surrounding aquifer resulting from oceanic tidal oscillations were barely reflected in the pond water level. These results suggest that, although there is a possibility of intrusion of groundwater into the pond (because of the vertically higher groundwater table), a very limited connection between the pond and the surrounding aquifer exists due to the accumulation of fine-grained sediments in the pond. Thus the pond is largely a
hydrologically closed system. The only significant source of water to the pond is meteoric water via rainfall.

Figure 1.2. Average monthly temperature records in Honolulu and average monthly rainfall measured at Honolulu International Airport from 1971 to 2000. Data were taken from NOAA National Climatic Data Center.

The ‘Ewa Plain is a leeward low-elevation coastal flat, and the area is dominated by relatively hot and arid climate. Nonetheless, there are two distinct seasons; a hot and dry summer, and a cooler and wetter winter. Annual rainfall in the ‘Ewa Plain averages only about 50 cm, and most of the precipitation is concentrated during the winter months (Figure 1.2) when southward shifts of the North Pacific subtropical high pressure system allow passage of tropical storm systems closer to the islands. The abundance of precipitation also shows interannual variability. Years of limited precipitation correlate
well with the occurrences of ENSO events over the past 100 years (Schroeder, 1993).

ENSO events typically result in anomalously low levels of precipitation during winters as the North Pacific high pressure system migrates closer to the main island chain of Hawai'i (Lyons, 1982; Chu, 1989; Chu, 1995). Characteristically dry contemporary climate supports sporadic communities of mainly grasses and shrubs, with a few dominant species such as kiawe trees (Prosopis pallida) and sourbush (Pluchea symphytifolia), in the area. Today, the circular rim of the pond is densely populated by American Mangrove (Rhizophora mangle) (Athens et al., 1999).

1.3. Modern Biogeochemical Processes in the Water Column

A one year time-series analysis of physical and biogeochemical properties of the water column was conducted from April 2000 by Garrison (2002). As a baseline condition, the water of Ordy Pond can be described as brackish, eutrophic and highly productive. Salinity of the water typically fluctuates from 22 to 25 under the influence of the seasonal precipitation pattern. Primary productivity, cycling of nutrients and particulate formation seem to be largely influenced by the extent of seasonal water column stratification.

The water column develops a thermal stratification during the summer months from early May to September (see Figure 1.3 a, b). The combination of increased incoming solar radiation and the density stratification, which helps phytoplankton communities to remain in the shallow euphotic zone, stimulates a massive phytoplankton bloom during the summer. Elevated levels of photosynthetic production during the summer are clearly revealed by the super-saturation of the dissolved O₂ concentrations in
Figure 1.3. One year time-series profiles of the water column of Ordy Pond in terms of (a) density, (b) temperature, (c) saturation state of dissolved O₂ and (d) carbon isotopic composition of the DIC. Each measurement is represented by ○ (from Garrison, 2002).
the epilimnion (see Figure 1.3 c). Enhanced photosynthetic activity also triggers the inorganic precipitation of carbonate minerals. Precipitation of carbonate minerals (in terms of calcium carbonate) in the water column can be modeled by a set of equations:

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} &= \text{CH}_2\text{O} + \text{O}_2 \\
2\text{HCO}_3^{-} &= \text{CO}_2 + \text{H}_2\text{O} + \text{CO}_3^{2-} \\
\text{Ca}^{2+} + \text{CO}_3^{2-} &= \text{CaCO}_3
\end{align*}
\]

Drawdown of CO\textsubscript{2} during photosynthesis (Eq. 1.1) lowers the acidity of the residual water and subsequently shifts the speciation of dissolved inorganic carbon (DIC) toward CO\textsubscript{3}\textsuperscript{2-} (Eq. 1.2). A rise in the saturation states of carbonate minerals, owing to build-up of CO\textsubscript{3}\textsuperscript{2-} ions, prompts the inorganic precipitation of carbonates. The net equation, which summarizes the effect of photosynthesis and biologically induced inorganic precipitation of carbonate minerals, can be expressed as:

\[
\text{Ca}^{2+} + 2\text{HCO}_3^{-} = \text{CH}_2\text{O} + \text{O}_2 + \text{CaCO}_3
\]

As the equation suggests, particulates formed during the summer have large quantities of both organic matter (OM) and carbonate minerals, and thus result in low organic carbon to inorganic carbon carbon (OC:IC) ratios relative to those in winter particulates (see Figure 1.4).

Stratification weakens during the winter as the ambient temperature starts to cool. Termination of stratification results in the vertical mixing of the water column, which is shown by the absence of density and temperature gradients (Figure 1.3 a, b) starting from
late autumn. Despite the vertical mixing of the water column, which should aerate the O₂-depleted deeper water column, the water body below 1m depth remains anoxic during the winter. This year-round anoxia reflects the high O₂ consumption by heterotrophic organisms that are actively respiring the OM. Production of OM within the epilimnion continues at a reduced rate, but in contrast to summer, precipitation of carbonate minerals is substantially diminished during the winter most likely due to weakened biological CO₂ drawdown. This leads to a dramatic increase in the OC:IC ratios at least by a factor of 3 (see Figure 1.4).

Figure 1.4. Fluxes of total particulates (red) and organic carbon to inorganic carbon ratios (OC:IC) of the particulates (blue) from July 2000 to February 2001 (modified from Garrison, 2002).
Seasonal changes in sedimentation processes are also recorded in the isotopic compositions of the precipitated carbonate minerals. Oxygen isotopic composition of the carbonates ($\delta^{18}O_{\text{Carb}}$), in general, is a function of the reaction temperature and the $\delta^{18}O$ values of the water body from which the minerals are precipitated (Talbot & Kelts, 1990). The isotopic fractionation associated with the precipitation of carbonates from a given water body ($\alpha_{\text{Calcite-H2O}}$) shows weak temperature dependence. The magnitude of $\alpha_{\text{Calcite-H2O}}$ decreases with an increase in water temperature (Kim and O’Neil, 1997; Zeebe and Wolf-Gladrow, 2001). The temperature dependence of the isotopic fractionation between calcite and water at low temperatures is given as:

$$\ln(\alpha_{\text{Calcite-H2O}}) = (18.03 / T) - (32.42 \times 10^{-3})$$

(Eq. 1.5)

where $T$ is temperature in Kelvin (Kim and O’Neil, 1997). The effect of temperature on $\delta^{18}O_{\text{Carb}}$ values, however, is much smaller than typical variations in the $\delta^{18}O$ values of water bodies, which are largely determined by the balance of water inputs and evaporative losses. In the case of Ordy Pond, where meteoric water is virtually the only source of water, $\delta^{18}O$ values of precipitated carbonates largely reflect the net hydrological balance between precipitation and evaporation. Stable carbon isotopic compositions of the carbonates ($\delta^{13}C_{\text{Carb}}$) are assumed to record the isotopic composition of the DIC in the upper water column (Talbot & Kelts, 1990). The $\delta^{13}C$ value of the DIC is a function of the rate of atmospheric CO$_2$ exchange, the $\delta^{13}C$ of the incoming water, photosynthetic activity by primary producers, and decomposition of OM (Yu et al., 1997). Variability in the $\delta^{13}C$ values of the DIC in Ordy Pond seem to be largely controlled by photosynthesis.
Discriminatory uptake of $^{12}$CO$_2$ during photosynthesis results in progressive enrichment of $^{13}$C in the residual DIC. Considerably heavy isotopic values (enriched with $^{13}$C) of the DIC in the upper water column (see Figure 1.3 d) coincide with the period of dissolved O$_2$ supersaturation, which is indicative of the summer phytoplankton bloom. Much of the $^{12}$C fixed in OM is eventually released as DIC into the water column and accumulates at depth as a result of remineralization.

Particulates collected by sediment traps, as a part of the one year time-series study by Garrison (2002), showed the highest $\delta^{18}$O$_{Carb}$ value (VPDB) of +2.9‰ during summer reflecting the enrichment of pond water with $^{18}$O due to evaporation. The lowest value of +0.9‰ was recorded during the winter due to increased rainfall. $\delta^{13}$C$_{Carb}$ (VPDB) was the highest in July at -1.8‰ and the lowest in March at -3.3‰. Enrichment of $^{13}$C in carbonate formed during the summer clearly indicates the effects of photosynthesis. The negative shift in the $\delta^{13}$C$_{Carb}$ during the winter is caused by the intrusion of deep water enriched with $^{12}$C into the shallower water column as a result of vertical mixing.

Table 1.1 compares the characteristics of the water column properties and particulates in the summer and winter. In summary, the summer particulates have a characteristic light-color because of the relatively large quantity of carbonates that are rich in both $^{13}$C and $^{18}$O (heavier isotopes). Winter particulates tend to be much darker in color due to a substantial decrease in carbonate content. Winter carbonates also typically have much lighter isotopic compositions ($^{12}$C and $^{16}$O rich). Therefore, differences in $\delta^{18}$O$_{Carb}$ and $\delta^{13}$C$_{Carb}$ between light-colored and dark-colored layers are thought to reflect the degree of seasonality.
Table 1.1. A summary and comparison of the physical and biogeochemical characteristics of the contemporary water column and particulates during the summer and winter in Ordy Pond.

<table>
<thead>
<tr>
<th>Water Properties</th>
<th>Summer</th>
<th>Winter</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>Hot</td>
<td>Cooler</td>
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<tr>
<td>Hydrologic Balance</td>
<td>Net Evaporation</td>
<td>Net Precipitation</td>
</tr>
<tr>
<td>Water Column</td>
<td>Stratification</td>
<td>Well-mixed</td>
</tr>
<tr>
<td>Primary Productivity</td>
<td>High</td>
<td>Lower</td>
</tr>
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<table>
<thead>
<tr>
<th>Particulates</th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Inorganic C (Carbonates)</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Organic C</td>
<td>High</td>
<td>Lower</td>
</tr>
<tr>
<td>OC : IC</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Appearance</td>
<td>Light-Color</td>
<td>Dark-Color</td>
</tr>
<tr>
<td>$\delta^{18}$O of Carbonates</td>
<td>More Positive ($^{18}$O-rich)</td>
<td>More Negative ($^{18}$O-rich)</td>
</tr>
<tr>
<td>$\delta^{13}$C of Carbonates</td>
<td>More Positive ($^{13}$C-rich)</td>
<td>More Negative ($^{12}$C-rich)</td>
</tr>
</tbody>
</table>

1.4. Sediment Record of Ordy Pond

The sediments of Ordy Pond were initially cored to a depth of 8.7 m by Athens et al. (1999, 2002) in 1994. Subsequent coring by Tribble et al. (1998) using a vibracore and a piston core system extended the cumulative core length to 17.5 m. Three separate stratigraphic units were identified: a basal non-aquatic unit and the middle and top aquatic units (Figure 1.5). The basal unit is made of approximately 4 m of fine-grained carbonate matrix containing debris of reef-rocks and abundant hash of shell-remains of terrigenous snails. The absence of aquatic forms in this unit implies that these sediments were sub-aerially deposited and the sinkhole was dry at this stage.

The boundary separating the bottom-most terrigenous unit and the overlying aquatic sediments is a thin peaty layer of sediment, probably deposited in a marsh-type of environment. The upper 13.5 m of aquatic sediments have no visible signs of hiatuses or
Figure 1.5. Schematic diagram of the sediment core collected from Ordy Pond (modified from Tribble et al., 1998). The 17.5 m long core has three distinct stratigraphic units. Horizon E separates the overlying aquatic sediments from non-aquatic sediments and was dated as 11,175 cal.yrBP using shells of land snails. Horizon D (7.91 m), C (6.84 m) and B (5.63 m) were dated as 1,345.5, 1,053 and 496.5 cal.yrBP, respectively based on AMS $^{14}$C dating on terrestrial plant materials (Athens et al., 1999, 2000). Horizon A at 5.21 m separates the top sapropelic unit from underlying laminated middle unit. Horizon A is dated as 120 cal.yrBP by the first appearance of historic pollen by Athens et al. (1999, 2002).
unconformities. The sedimentary sequence, therefore, records the continuous environmental history of the pond since the time of its initial transformation from an open sinkhole to an aquatic feature. Aquatic sediments have three main compositional components: authigenic carbonates (combination of calcite and aragonite), organic matter (mostly autochthonous), and amorphous silica (diatom tests). Roughly 8.5 m of the middle aquatic unit comprises finely laminated couplets of light-colored (yellow to beige) sediments rich in carbonates and dark (olive to brownish green) organic-rich sediments. Layers of pure diatomaceous sediments are occasionally detected in this sediment interval. The 5 m of the top aquatic unit are dominated by highly organic-rich dark sapropelic sediments that are gelatinous to soupy in texture. Apparent and distinguishable lamination patterns observed in the deeper unit are replaced by rather subtle bandings in the top sapropelic unit.

1.5. Summary of Previous Studies at Ordy Pond

A series of studies have been conducted at Ordy Pond in the past. Paleoenvironmental and archaeological investigations including the analyses of pollen and diatom assemblages were conducted by Athens et al. (1999, 2002) on the upper 8.7 m of sediment. Studies by Tribble et al. (1998, 1999a, 1999b, 2001), Schoonmaker et al. (2002, 2003), Garrison (2002), Garrison and Schoonmaker (2002), Garrison et al. (2000), Uchikawa (2002) and Uchikawa et al. (2005) include geochemical, mineralogical and isotopic investigation of the sediment and a time-series biogeochemical monitoring of the water column.
Athens et al. (1999) also examined the distribution of pollen from sediment cores collected from a nearby evaporate pan and extended the reconstruction back to about 7,200 cal.yrBP, but those cores were of low temporal resolution and had missing time-intervals. Detailed pollen reconstructions by Athens et al. (2002) documented changes in the adjacent terrestrial ecosystem since approximately 1,500 cal.yrBP. One of the most critical findings from their study was the rapid disappearance of native dry forests around 930 cal.yrBP. The authors suggested the main cause of the rapid forest decline was the Polynesian rat (Rattus exulans), which was introduced to the Hawaiian Islands by the ancestral Polynesian voyagers.

Tribble et al. (1999a) focused on the carbonate mineralogy in the sediments and tried to link the fluctuations in the relative abundance of calcite and aragonite to the climate and/or sea-level changes during the early to mid-Holocene. Throughout much of the pond’s history, precipitated carbonates have been dominated by calcite. Precipitation of aragonite, however, also occurred for discrete intervals of time, including the present day. According to the preliminary chronology, it also appeared that carbonate precipitation was dominated by aragonite during the mid-Holocene climatic optimum (e.g., Grossman & Fletcher, 1998) and the Medieval Warm Phase. The authors hypothesized that a combination of increased temperature and salinity of the water column altered the state of carbonate geochemistry. Although there is no direct evidence, elevation of the groundwater table in response to the rising sea-level during the mid-Holocene, and perhaps during the Medieval Warm Phase, potentially could have lead to the intrusion of saline groundwater in the pond and subsequent chemical and physical changes in the water properties. Alternatively, greater net evaporation during these
Holocene warm climatic events may have resulted in higher carbonate saturation states in the pond water and in the precipitation of aragonite.

*Garrison* (2002) and *Garrison et al.* (2000, 2002) utilized a wide array of sedimentary analyses such as carbonate mineralogy, and elemental and stable isotopic analysis both on carbonates and OM in order to document both short-time (seasonal to interannual) and long-term paleoenvironmental changes in the area, and relatively recent human impacts on the regional ecosystem. $\delta^{18}O_{\text{Carb}}$ and $\delta^{13}C_{\text{Carb}}$ values in Ordy Pond seem to indicate both long-term changes in the pond environment and the extremes of seasonal variations. $\delta^{18}O_{\text{Carb}}$ values fluctuate widely with sediment depth (Figure 1.6). In general, $\delta^{18}O_{\text{Carb}}$ values of the carbonate-rich sediments are heavier than those from organic-rich sediment, in agreement with the proposed seasonal cycles of modern particulate formation (see Table 1.1). Temporal variations in the spread of $\delta^{18}O_{\text{Carb}}$ values between consecutive sequences of light and dark layers could reflect variations in the strength of the seasonal rainfall signals (*Schoonmaker et al.*, 2003). There are also a few exceptional negative excursions of $\delta^{18}O_{\text{Carb}}$ shortly after inundation of the sinkhole (~13.4 m) and the decline of native forest (~6.7 m) revealed by pollen analyses of *Athens et al.* (1999, 2002). *Garrison* (2002) suggested that these anomalous excursions may be related to considerable changes in the isotopic composition of the pond water perhaps due to intrusion of groundwater. The observed behavior of $\delta^{13}C_{\text{Carb}}$, on the other hand, is not clearly explained by the modern sedimentation model. $\delta^{13}C_{\text{Carb}}$ records from carbonate-rich and organic-rich sediments either show no seasonal differences or even display the opposite trend (summer carbonate-rich layers having lighter $\delta^{13}C_{\text{Carb}}$ values than the winter organic-rich layers). Lack of consistency in the $\delta^{13}C_{\text{Carb}}$ records may suggest
Figure 1.6. Oxygen (left panel) and carbon (right panel) isotopic compositions of sedimentary carbonate from Ordy Pond. Data are sorted by the light-colored layers (blue) and dark-colored layers (red) in both panels. Data are from Garrison (2002).
alteration of primary isotopic signals due to carbonate diagenesis. Therefore, paleohydrological and paleoclimatological reconstructions based on δ^{18}O_{Carb} and δ^{13}C_{Carb} are challenging because isotopic signatures of the carbonate reflect mixed signals that are combinations of the extremes of seasonality, interannual to decadal climatic oscillations, groundwater intrusions, and perhaps diagenetic alterations of the carbonates.

Cross-comparison of elemental carbon to nitrogen ratios (C:N ratios) and δ^{13}C_{org} values can elucidate the main source of sedimentary bulk OM (Figure 1.7). Algae typically have C:N ratios between 4 and 15 due to the relative richness of N-containing organic molecules such as protein, whereas vascular land plants have C:N ratios of 20 and greater owing to the abundant cellulose required for structural support (Meyers, 1997). δ^{13}C_{org} values of land-derived OM can be used to distinguish C₃ from C₄ plants because of the unique isotopic discrimination against ^13C associated with C₃ vs. C₄ photosynthesis (Collister et al., 1994; Farquhar et al., 1989; Hayes, 2001). The isotopic difference found between marine and lacustrine algae reflects the distinct baseline isotopic compositions of the DIC utilized by algae in the two environments (Meyers, 1997).

The results of elemental and isotopic analyses of bulk OM by Garrison (2002) and Ibara (unpublished data) suggest that autochthonous algal materials are the main sources of OM to the sediments in Ordy Pond (Figure 1.7). Furthermore, δ^{13}C signals seem to indicate the lacustrine algal origin of the bulk OM. Observed C:N ratios of bulk OM, however, are slightly higher than the typical algal C:N signature of 4 to 10. This can be explained by the preferential degradation of N-rich components of OM in the water column and sediments and subsequent increase in the C:N ratio (Meyers, 1997).
Figure 1.7. Typical distributions of major components (derived from lacustrine and marine algae, and terrestrial C_3 and C_4 plants) of bulk organic matter in aquatic sediments in terms of elemental C:N ratios and carbon isotopic compositions (modified from Meyers, 1997). Data points represent the results of elemental and stable carbon isotopic analyses of the Ordy Pond bulk sedimentary organic matter determined by Garrison (2002), shown in red upward triangles, and by Ibara (unpublished data), shown by blue downward triangles.

Alternatively, C:N ratios higher than those of typical algal values may suggest that bulk OM represents an admixture of autochthonous (primarily algal) and allochthonous (terrestrial plants) components. The few exceptional data-points with C:N values well above 20 in Figure 1.7 may reflect occasions of anomalous inputs of terrestrial OM, perhaps via runoff in response to heavy rainfall events. The possibility of allochthonous contributions to bulk OM may also explain the relatively large spread of $\delta^{13}C_{org}$ values towards more negative values. Attempts to use $\delta^{13}C$ values of bulk OM as a
paleoenvironmental and paleoclimatic proxy, therefore, can be biased by possible impacts of diagenetic alteration and/or concurrent deposition of allochthonous materials.

1.6. **Chronology of Ordy Pond Sediments**

A more fundamental problem associated with the investigation of Ordy Pond sediments is the lack of rigorous sediment chronology. Prior to this study, reliable dates from the sediments were limited to one sedimentary horizon dated by the first appearance of pollen from historically introduced species to Hawai‘i (Athens et al., 1999, 2002), three horizons dated by AMS radiocarbon ($^{14}C$) dating of rare plant macroscopic fossils (wood fragments and seeds) (Athens et al., 1999, 2002) and an additional horizon dated by the conventional radiometric method using shells of terrestrial snails (Tribble et al., 1999). Sediment chronology was constructed based on linear interpolation of these 5 tie-in points for over 13 m of sediments (Figure 1.8 a). The age-depth model showed that the sedimentation rate increased from 0.05 to 4.33 cm/yr over the course of pond history.

Given such changes in the estimated sedimentation rate over time, the limited age-control was deemed unreliable as it could potentially over/underestimate the true sediment accumulation rate during each time interval. This model was particularly problematic for the segment between horizon E and horizon D. There were no $^{14}C$ dates for more than 5 m of sediment that are equivalent to more than 9,800 years of sedimentation history.

Furthermore, this model directly relied on the $^{14}C$ date derived from terrestrial snail shells as an end-point of the interpolation. Earlier studies have clearly demonstrated that $^{14}C$ dating of snail shells from limestone areas can cause significant age discrepancies (Goodfriend and Stipp, 1983; Dye, 1994; Goodfriend et al., 1999). Snails tend to
Figure 1.8. 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, 2002). The age model is also supplemented by ages of horizon A (Athens et al., 1999, 2002) and horizon 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 (blue triangles) from horizon A, B, C and D, and algal materials (red diamonds) by Athens et al. (1999, 2002). The radiocarbon age scale (yrBP) was used for the comparison because the algal dates could not be calibrated to calendar ages (cal.yrBP) due to an unknown correction factor ($\Delta$R) for the reservoir effect of the pond water.
incorporate $^{14}\text{C}$-free carbon into their shells by direct ingestion of limestone, or uptake carbonate ions dissolved from limestone by foot secretions (Goodfriend and Hood, 1983). Age anomalies found from the $^{14}\text{C}$ dating of shells of contemporary land snails were as large as 3,120 years in Jamaica (Goodfriend and Stipp, 1983).

The limited number of $^{14}\text{C}$ dates for the Ordy Pond sediment arose from the lack of datable materials. $^{14}\text{C}$ dating of bulk OM, which is mostly made of algal materials, by Athens et al. (1999 & 2002) documented arguable age discrepancies and stratigraphic reversals with respect to the dates obtained from terrestrial plant materials (Figure 1.8 b). It is most likely that the DIC pool in the pond water contains $^{14}\text{C}$-depleted C from the surrounding limestone platform of Pleistocene origin. Consequently, any autochthonous material, whether it is OM or authigenic carbonate, retains an anomalous $^{14}\text{C}$ signature and is unsuitable for $^{14}\text{C}$ dating. In order to avoid contamination from the limestone effect on the pond DIC, reliable $^{14}\text{C}$ dating in this particular environment requires terrestrial plant materials. Unfortunately however, macroscopic terrestrial plant materials such as those dated in the investigation by Athens et al. (1999, 2002) are rarely found in the sediments due to the hydrologically-closed nature of the pond system.

1.7. **Foci of This Project**

The primary goals of this investigation were (1) to improve the age-control of Ordy Pond sediments by the novel molecular radiocarbon technique and (2) to assess paleo-vegetative changes by utilizing terrestrial biomarkers as a new proxy that is, unlike carbonates and bulk OM, independent of biogeochemical cycles within the pond water.
1.7.1 Improvement of Sediment Chronology

Chapter II of this thesis describes improvements to the age-control of Ordy Pond sediments using compound-specific radiocarbon analysis (CSRA) by accelerator mass spectrometry (AMS) on normal alkanes (n-alkanes) derived from epicuticular waxes of terrestrial higher plants. Modern high-precision AMS in conjunction with preparative capillary gas chromatography, which allows isolation of ample quantities of target compounds from organic matrices at sufficient purity (Eglinton et al., 1996), enables reliable microscale CSRA of biomarkers even when sample sizes are limited to less than 100 μg of C equivalence (Eglinton et al., 1996; Pearson et al., 1998; von Reden et al., 1998; Pearson et al., 2001; Smittenberg et al., 2004). Recent studies, which directly employed this method, demonstrated encouraging results and obtained reasonable chronologies from marine sediments (e.g., Ohkouchi et al., 2003; Smittenberg et al., 2004; Uchida et al., 2005) as well as soil (e.g., Huang et al., 1999). Long-chain odd-C-numbered n-alkanes (namely n-C27, n-C29, n-C31 and n-C33) are exclusively derived from the epicuticular waxy coatings secreted by terrestrial higher plants (Eglinton and Hamilton, 1967; Riley et al., 1991) and are known to be ubiquitous in aquatic sediments (Pearson and Eglinton, 2000). Unlike autochthonous OM and carbonates, 14C ages of the n-alkanes derived from leaf waxes of land plants are unaffected by 14C-depleted C known to be present in the DIC of Ordy Pond, because these compounds are ultimately synthesized in equilibrium with atmospheric 14C. Therefore, CSRA of plant-derived n-alkanes was used to refine the resolution of the current chronology of Ordy Pond sediment. Because this is the first direct application of CSRA to obtain a sediment chronology in a lacustrine environment, not only the outcomes of the study but also the
reliability and applicability of the method and its potential as an alternative option for age-determination of non-marine sediments are discussed in detail. A draft manuscript based on the materials presented in Chapter II was submitted to the Journal of Paleolimnology in June 2006.

1.7.2 Assessment of Paleovegetative Changes in the ‘Ewa Plain

In Chapter III, additional analyses of the n-alkane biomarkers are used to reveal changes in the vegetative structure in the area throughout the Holocene epoch. Natural ecological successions of plants in a given location are largely controlled by climatic conditions. Each plant species has a tolerance range and optimum condition with respect to various climatic factors including ambient temperature and amount of precipitation. As a broad generalization, plants conducting C4-photosynthesis (C4 plants), mainly composed of grasses and shrubs, are dominant in relatively hot and arid/dry environments such as tropical/subtropical savannas (Ehleringer et al., 1997; Schefuß et al., 2003). On the other hand, plants using the C3-photosynthetic pathway (C3 plants) prefer much milder climates with relatively abundant precipitation. Thus, changes in the fractional abundances of C3 and C4 plants in a given plant community over time can be considered as an indirect barometer of past climate changes if anthropogenic impacts are negligible. Carbon isotopic compositions of individual n-alkanes (δ13Cn-Alk) originating from C3 vs. C4 plants differ (compounds from C3-plants are isotopically more depleted than those from C4 plants) because of the different magnitudes of intrinsic kinetic isotope effects in each photosynthetic pathway (Collister et al., 1994; Farquhar et al., 1989; Hayes, 2001). Measurements of δ13Cn-Alk, with the use of compound-specific carbon isotopic analysis,
allow one to distinguish the precursor plant groups, and subsequent isotope mass-balance calculations further enable one to estimate quantitatively the relative dominance of plants from C₃ and C₄ functional groups within a given vegetative community. N-alkanes are essentially independent of biogeochemical processes occurring in the water column and sediments of Ordy Pond because these compounds are synthesized outside of the pond system and are resistant to diagenetic alterations (Huang et al., 1997; Hoef et al., 2002). δ¹³C_N-Alks values are completely independent of seasonal oscillations of the biogeochemical state of the water column and diagenetic alterations of the primary signals. Molecular stratigraphy based on δ¹³C_N-Alks should strictly reflect the successions of local plant communities over time in response to either gradual or abrupt environmental and climatic changes of the Holocene, and perhaps some ecosystem modifications caused by historic anthropogenic activities during the late Holocene.
1.8. References


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CHAPTER II

Direct Application of Compound-Specific Radiocarbon Analysis of Leaf Waxes for Establishing Lacustrine Sediment Chronology
Abstract

Compound-specific radiocarbon analysis (CSRA) of terrestrial biomarkers was used to construct sediment chronology. This study demonstrated an encouraging novel method for radiocarbon ($^{14}$C) dating of Holocene lacustrine sediments from carbonate-hosted Ordy Pond, O'ahu, Hawai‘i, where conventional dating approaches are impractical. Long-chain odd-numbered normal alkanes ($n$-alkanes), biomarkers characteristic of terrestrial higher plants, were found to be ubiquitous in Ordy Pond sediments. The $\delta^{13}$C of these 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 $\mu$g of carbon (C), however, greater age errors were confirmed for samples containing less than 80 $\mu$g C. The $^{14}$C age of $n$-alkanes from one particular sedimentary horizon was 4,250 years older than the value expected from the refined age-control, resulting in an apparent and arguable age discrepancy. The corresponding sediment interval consists of continuous mm-scale laminations without visible signs of hiatus or unconformities. Stratigraphic evidence and a suspiciously high CO$_2$ yield by preparative capillary gas chromatography (PCGC) associated with the sample from this particular horizon suggest possible introduction of $^{14}$C-free C by artificial contamination. This study simultaneously highlighted the promising potential of CSRA for paleo-applications and the risks of contamination associated with micro-scale $^{14}$C measurement.
2.1. Introduction

Interpretations of sedimentary signals for paleoenvironmental reconstructions must be accompanied by reliable sediment chronology. Radiocarbon (\(^{14}\text{C}\)) analysis has proven to be a powerful tool for determining the age of a variety of environmental samples in the fields of geology, archeology and oceanography. Conventional radiometric methods, which determine the \(^{14}\text{C}\) contents of samples by counting \(\beta\)-particle emissions, are practical only when samples are sufficiently large to yield at least 100 g of carbon (C) for analysis (Bradley, 1999). Bulk organic matter (OM) and sedimentary carbonates are commonly used for such methods in order to meet such a large sample size requirement. \(^{14}\text{C}\) dating of carbonate minerals, however, can be problematic if the dissolved inorganic carbon (DIC) pool from which carbonate minerals are precipitated is not in equilibrium with \(^{14}\text{C}\) in the atmosphere or if carbonates have been diagenetically altered. Carbonate dissolution in metabolically active sediments (Wenzhöfer et al., 2001) or in acidic environments may also prevent sufficient accumulation. Dating of bulk OM can potentially produce misleading sediment chronology if sub-fractions of OM with different origins and \(^{14}\text{C}\) signatures are concurrently deposited (e.g., Eglinton et al., 1997; Pearson et al., 2001). Therefore, the “age” of bulk OM simply represents the weighted average of \(^{14}\text{C}\) contents of individual constituents. Incorporation of terrestrial OM previously aged in other reservoirs such as soil (e.g., Raymond and Bauer, 2001) or \(^{14}\text{C}\)-depleted carbon from petroleum sources (e.g., Eglinton et al., 1997) into sedimentary bulk OM, for example, causes a significant age discrepancy.

Development of accelerator mass spectrometry (AMS) since the 1980s has greatly reduced sample size requirements for accurate \(^{14}\text{C}\) dating. Furthermore, Eglinton
et al. (1996) showed that $^{14}$C dating at the molecular-level, so-called compound-specific radiocarbon analysis (CSRA), was possible using AMS in conjunction with automated preparative capillary gas chromatography (PCGC). PCGC allows isolation of ample quantities of target compounds at sufficient purity required for AMS $^{14}$C measurements. Ideally, target compounds should produce approximately $100 \mu g$ C for accurate CSRA (Eglinton et al., 1996), but CSRA is theoretically possible for samples as small as 15-20 $\mu g$ 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 of OM and the residence time of biomarkers 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).

Ohkouchi et al. (2003) tested the validity of CSRA using short-chain fatty acids (C$_{14}$, C$_{16}$ and C$_{18}$) as a technique for refining sediment chronologies of coastal margin sediments off Antarctica. These compounds, ultimately derived from surface ocean DIC via photosynthesis by phytoplankton, provided a useful sediment chronology. Smittenberg et al. (2004) and Uchida et al. (2005) further investigated the potential of CSRA for dating of marine sediments by expanding the selections of target biomarkers. Uchida et al. (2005) concluded that CSRA of short-chain fatty acids, synthesized by phytoplankton and marine bacteria, and alkenones can be useful in establishing the chronology of sediments on the continental slope of the Northwest Pacific off Japan. A study by Smittenberg et al. (2004) using sediments from Saanich Inlet, a fjord at the southeast coast of Vancouver Island, Canada, however, documented significant variability in $^{14}$C ages of phytoplankton-derived biomarkers presumably due to
considerable fluctuations of the $^{14}$C contents in surface seawater DIC resulting from stratification, air-sea CO$_2$ exchange and freshwater input with hard-water effects. In this particular environmental setting, the most reliable type of biomarkers for sediment chronology was crenarchaeol derived from pelagic archea. These prokaryotic organisms are typically concentrated in the water column near the bottom of the euphotic zone and are less influenced by $^{14}$C variability in the surface water DIC. These studies indicate the capability of CSRA with properly chosen target compounds to serve as an alternative dating option for sediments where more traditional approaches are unsuitable.

This study uses that CSRA of land-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 construction of sediment chronology in a lacustrine environment.

Sediment cores collected from the study site, Ordy Pond on the island of O’ahu, Hawai‘i, consist of an aquatic sedimentary sequence which seems to have recorded continuous paleoclimatic and paleoenvironmental histories of the area throughout the Holocene epoch. This is the only known high-resolution, varved, aquatic sedimentary record found in the Central Pacific. Interpretations of Quaternary proxies using Ordy Pond sediments, however, have been challenged by lack of a rigorous sediment chronology. Because $^{14}$C ages of carbonate and algal OM reflect the presence of $^{14}$C-depleted C of Pleistocene origin dissolved in the pond water column, autochthonous materials in the sediments are unsuitable for $^{14}$C analysis. Macroscopic terrestrial materials such as wood fragments and seeds are rarely found in the sediment due to the hydrologically-closed nature of the pond.
Use of land-derived biomarkers (such as long-chain \( n \)-alkanes and fatty acids) for chronological purposes can be problematic for sediments taken from marine environments. Age-offsets between land-derived biomarkers and marine counterparts can be significant (e.g., Pearson and Eglington, 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 organic materials within the overall inventory of terrestrial OM inputs. Additionally, the use of land-plant biomarkers seems to be particularly beneficial for construction of age-control for lacustrine sediments. Quantitative estimation of the lake reservoir-effect (commonly denoted as \( \Delta R \)) can be difficult due to a complex combination of various factors, such as the hardwater-effect and geochemical constituents of the surrounding basin that cause decoupling of \(^{14}\text{C}\) levels between the atmosphere and lake water. Smaller reservoir size and rapid response time of lacustrine systems may result in significant fluctuations in the extent of the reservoir-effect over time (e.g., Geyh et al., 1998), which is even harder to model. Biomarkers from terrestrial plants are ultimately synthesized from C that are directly fixed from atmospheric \( \text{CO}_2 \), and thus do not require such site-specific \( \Delta R \) correction.

To improve age-control of Ordy Pond sediments, CSRA was conducted on plant-derived sedimentary \( n \)-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 $^{14}$C-dating methods are conceptually impractical.

Figure 2.1 Location of Ordy Pond in the 'Ewa Plain of O'ahu (from Garrison, 2002). The pond is approximately 750 m from the coast and is situated at 1.5 m above mean sea-level. The area of the pond is 0.5 ha.

2.2. Study Site, Materials and Background

2.2.1 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 (Figure 2.1). This fossil-reef platform was established during the interglacial Waimanalo sea-level highstand (oxygen isotope sub-stage 5e), 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 rainfalls concentrated during winters. Thus, the pond can be considered a hydrologically closed system.

Garrison (2002) conducted time-series monitoring of Ordy Pond in order to study the biogeochemistry of the system. Today, the pond has a 5 m brackish water column. The waters are eutrophic, highly productive, and supersaturated with respect to calcite and/or aragonite. The water column develops climate-dependent thermal stratification during the summer. Summer blooms of photoautotrophs within the epilimnion produce organic particulates and simultaneously trigger the inorganic precipitation of carbonate minerals in response to the drawdown of DIC. The extent of stratification weakens during autumn and winter which results in enhanced vertical mixing of the epilimnion and hypolimnion. Nonetheless, the hypolimnion remains anoxic all year due to extensive remineralization processes. Particulates formed during autumn and winter are dominated by algal OM as the magnitude of carbonate precipitation decreases.

2.2.2 Descriptions of the Sediment Core

Sediments from Ordy Pond record the Holocene 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. Three
Figure 2.2. Schematic diagram of the sediment core collected from Ordy Pond (modified from Tribble et al., 1998). The core has three stratigraphic units: the lowermost non-aquatic unit, middle laminated aquatic unit and uppermost sapropelic unit. Horizon E (17.5 m; 11,175 cal.yrBP) was dated by the conventional $^{14}$C technique using shells of terrestrial snails. $^{14}$C ages of horizon D (7.91 m; 1,345.5 cal.yrBP), C (6.84 m; 1,053 cal.yrBP) and B (5.63 m; 496.5 cal.yrBP) were determined by Athens et al. (1999, 2002) using AMS $^{14}$C dating on terrestrial plant materials. Horizon A at 5.21 m was dated as ~120 cal.yrBP by the first appearance of historic pollen by Athens et al. (1999, 2002). $^{14}$C dates of horizon E,D,C and B reported in this figure represent recalibrated values of the original $^{14}$C dates (reported in yrBP) from the publications using CALIB 5.0 program (Stuiver and Reimer, 1993; Stuiver et al., 2005) with InCal 04 dataset (Reimer et al., 2004).
distinct stratigraphic units were found in the core: a basal non-aquatic unit, and the middle and top aquatic units (Figure 2.2). The basal unit contains fossils of terrestrial snails and pebbles of reef rocks in a carbonate 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. The aquatic history of this pond probably started with a swamp/marsh type of environment as the open sinkhole was inundated with water as sea-level rose. The middle aquatic unit possesses fine-scale (sub-millimeter to centimeter scale) laminations with alternating layers dominated by carbonate and OM. The uppermost sedimentary unit, representing the most recent 120 years of depositional history, contains 5 m of organic-rich subtly-banded sapropelic sediment.

Sediment cores from Ordy Pond provide the only known continuous lacustrine sedimentary sequence with exceptionally high-resolution in Hawai‘i, and probably even from the entire central tropical/sub-tropical Pacific. Proxies imbedded in the pond sediments are thus valuable in the context of tropical/subtropical paleoclimatology and regional paleoenvironmental history.

2.2.3 Previous Sediment Chronology

From the archaeological investigation of Athens et al. (1999, 2002), four reliable ages are available for the upper 8 m of Ordy Pond aquatic sediments (Figure 2.2 and Figure 2.3 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 (horizon B, C and D) were based on AMS $^{14}$C determinations using rare plant macrofossils (wood fragments and seeds). The $^{14}$C age of the base of the aquatic
Figure 2.3. 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 (blue triangles) from horizons A, B, C and D, and algal materials (red diamonds) 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 ($\Delta R$) for the reservoir effect of the pond water.
sediments, horizon E, was determined by conventional radiometric $^{14}$C techniques using shells of terrestrial snails by Tribble et al. (1999). These snails are all native to the main Hawaiian Islands, and belong to the family Helcinidae, Achatinellidae, Amastridae, Vertiginidae, Succineidae and Endodontidae (Cowie et al., 1995).

The chronology of Ordy Pond aquatic sediments based on these five dates and linear interpolation clearly indicates that the sediment accumulation rate in this pond has been variable through time (see Figure 2.3 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 applied to this chronological model may potentially over/underestimate the true sediment accumulation rate during each time interval. Particularly problematic is the interpolation between horizon E and horizon D, where not a single reliable date is available for more than 5 m section that are equivalent to more than 9,800 years of sedimentation history. In addition, validity of the age of horizon E is rather questionable because snails from limestone areas often provide anomalous (typically too old) $^{14}$C ages (Goodfriend and Stipp, 1983; Dye, 1994; Goodfriend et al., 1999); this issue will be further addressed.

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 and age discrepancies (see Figure 2.3, b). It is most likely that the pond DIC incorporates $^{14}$C-depleted C liberated from the surrounding Pleistocene limestone terrain. Consequently, any autochthnous material directly produced from the pond water, such as OM and carbonates, must be avoided for $^{14}$C dating. This leaves
terrestrial plants as the only option for accurate $^{14}$C dating. However, macroscopic terrestrial plant materials such as those dated in the investigation by Athens et al. (1999 & 2002) are rarely found in the sediment column due to the hydrologically-closed nature of the pond system.

2.3 Methods

2.3.1 Lipid Extraction and Separation

Methods for extraction and separation of $n$-alkanes from bulk sediment samples were modified from Kenig et al., (2000), Hoering and Freeman, (1984) and Yamada et al., (1994). Sediment samples were oven-dried at 60 °C for 48 to 72 hours and homogenized with a solvent-rinsed agate mortar and pestle. Total free lipids were soxhlet-extracted from sediments with dichloromethane. Samples of sediment for extraction ranged from 10 to 17 g, depending on the $n$-alkane contents. Elemental sulfur was removed from the extracts using acid-activated copper granular (20-30 mesh size). The hydrocarbon fraction in the total free lipids was separated by silica-gel (12 g, 70-230 mesh size, deactivated with 2 wt.% of H$_2$O) column chromatography. The hydrocarbon fraction was eluted with 40 mL of petroleum ether, which was subsequently exchanged with pentane. Silicalite molecular sieve was used to further purify $n$-alkanes. A small column of powdered silicalite (~150 µm grain size) was combusted at 325 °C for a minimum of 24 hours. The hydrocarbon fraction was slowly percolated through the column, and the column was flushed with roughly 12 mL of clean pentane to collect a non-adduct fraction consisting of branched and cyclic hydrocarbons. The non-adduct fraction was archived. Silicalite was digested by 3 mL of 49% hydrofluoric acid, which
was extracted with pentane to recover adducted $n$-alkanes. Finally, the $n$-alkane fraction was eluted through a pre-combusted silica-gel column with pentane.

Extractions were repeated using newly prepared sediments until sufficient quantities of target $n$-alkanes were collected. Samples from separate extractions were combined once their terrestrial origin was confirmed by compound-specific $\delta^{13}C$ analysis.

Solvents used during the extraction and purification were not distilled. Best available grade (HPLC quality or higher) for each solvent was selected.

2.3.2 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 Lab at the University of Hawai‘i. Samples were dissolved in cyclohexane for both GC and irm-GC/MS analyses.

Identification and quantification of individual $n$-alkanes were conducted using a Varian 3400 GC 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 $n$-C$_{36}$ hydrocarbon) of known concentration.

In order to confirm the terrestrial origin of $n$-alkanes, compound-specific stable isotopic analyses (CSIA) were conducted using irm-GC/MS (Finnigan MAT252
connected to Finnigan GC/C-III interface). The temperature program of the GC interface was same as for the GC analysis. 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). \(^{12}\)C/\(^{13}\)C ratios for the peaks of target \(n\)-alkanes (\(n-C_{27}, n-C_{29}, n-C_{31}\) and \(n-C_{33}\)) were calculated against a CO\(_2\) reference gas. Finally, carbon isotopic compositions of individual \(n\)-alkanes were standardized to the Vienna PDB standard (VPDB) and expressed in terms of the conventional δ-notation (δ\(^{13}\)C). An internal standard containing a series of deuterated \(n\)-alkanes (\(n-C_{24}, n-C_{36}\) and \(n-C_{40}\)) of known carbon isotopic compositions was co-injected with each sample in order to identify the series of compounds and to assess the accuracy of the isotopic analysis (generally better than ± 0.4‰).

2.3.3 PCGC and CSRA by AMS

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

Details on purification of biomarker compounds using 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 (\(\Sigma n-C_{27}, n-C_{29}, n-C_{31}\) and \(n-C_{33}\)) 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 remainder of the target compounds was transferred to pre-baked quartz tubes and solvent was subsequently 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 stable carbon isotopic 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 obtained graphite placed in a designated AMS target. CSRA by AMS was conducted according to a method specifically developed for the accurate measurement of ¹⁴C with 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.

2.3.4 Reporting of Radiocarbon Dates

Conversions of raw data from AMS to radiocarbon ages (i.e., yrBP unit) are described in detail elsewhere (McNichol et al., 2001; Matsumoto et al., 2002; Reddy et al., 2002, 2003; Smittenberg et al., 2004). First, the ¹⁴C contents of combined target n-alkanes are expressed in terms of “fraction modern (Fm)”. 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 ¹⁴C concentration of contemporary reference material for ¹⁴C dating (NIST).
SRM 4990B Oxalic Acid with $\delta^{13}C_{VPDB} = -19\%o$ as in 1950 AD (Stuiver and Polach, 1977). Fm is calculated from the following equation:

$$Fm = \frac{\left(\frac{^{14}C}{^{12}C}\right)_S - \left(\frac{^{14}C}{^{12}C}\right)_B}{\left(\frac{^{14}C}{^{12}C}\right)_{M} - \left(\frac{^{14}C}{^{12}C}\right)_B}$$

(Eq. 2.1)

where subscripts S, B and M denote sample, blank and modern reference material, respectively. Then Fm is corrected for the isotope fractionation effect by normalizing to a conventional value of $\delta^{13}C_{VPDB} = -25\%o$:

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

(Eq. 2.2)

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

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

(Eq. 2.3)

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

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

A typical chromatogram of the hydrocarbon fraction from Ordy Pond sediments is shown in Figure 2.4 (a). Hydrocarbon fractions consist of a series of well-resolved \( n \)-alkanes and minor amounts of either branched or cyclic isomeric hydrocarbons. By co-eluting with target \( n \)-alkanes, these non-aliphatic hydrocarbons could affect subsequent CSIA and CSRA. A representative chromatogram of \( n \)-alkane fractions (see Figure 2.4 b), however, indicates that the silicalite molecular sieve treatment was successful in separating straight-chain \( n \)-alkanes from the non-aliphatic hydrocarbons. Resolved compounds were almost exclusively dominated by a long-chain homologous (C > 25) series of \( n \)-alkanes up to \( n \)-C\(_{35}\) with a maximum at either \( n \)-C\(_{29}\) or \( n \)-C\(_{31}\), whereas the short-chain counterparts (C < 24) were barely detectable in all samples. In addition, resolved \( n \)-alkanes show strong odd-over-even C-number predominance. \( \delta^{13}C \) values of the target \( n \)-alkanes (Table 2.1) roughly ranged from -30 to -25‰. In most samples, \( n \)-C\(_{27}\) \( n \)-alkanes are more enriched with \( ^{13}C \) relative to the rest of the \( n \)-alkanes.

Duplicate analyses were not conducted. Reliability of the instrument, however, was monitored via a co-injected internal standard (deuterated \( n \)-C\(_{24}\), \( n \)-C\(_{36}\) and \( n \)-C\(_{40}\) \( n \)-alkanes). Reproducibility determined from each compound in the internal standard was ± 0.34‰, ± 0.36‰ and ± 0.64‰ (n = 14), respectively. Slightly lower reproducibility observed for the \( n \)-C\(_{40}\) internal standard can be explained by elevated background level, perhaps due to a 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 \(^{14}C \) dating of the wood fragment are summarized in Table 2.2. Generally, age errors tend to be greater for samples containing
Figure 2.4. Typical gas chromatograms of (a) hydrocarbon fraction and (b) \( n \)-alkane fraction extracted from Ordy Pond sediment.
Table 2.1  Summary of gas chromatography (GC) and compound-specific stable carbon isotopic analysis on target n-alkanes.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Abundance $^a$ (µg/g) $^b$</th>
<th>CPI $^c$</th>
<th>$\delta^{13}$C VPDB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n-C$_{27}$</td>
</tr>
<tr>
<td>D3</td>
<td>700 - 705</td>
<td>17.82</td>
<td>8.60 ± 0.89</td>
<td>-28.2 ± 0.1</td>
</tr>
<tr>
<td>D5</td>
<td>925 - 930</td>
<td>12.72</td>
<td>7.97 ± 4.76</td>
<td>-26.5 ± 1.7</td>
</tr>
<tr>
<td>D6</td>
<td>1015 - 1020</td>
<td>9.67</td>
<td>8.99 ± 0.23</td>
<td>-27.4 ± 0.5</td>
</tr>
<tr>
<td>D7</td>
<td>1170 - 1180</td>
<td>27.39</td>
<td>11.74 ± 2.52</td>
<td>-27.0 ± 0.9</td>
</tr>
<tr>
<td>D9</td>
<td>1320 - 1330</td>
<td>21.78</td>
<td>11.14 ± 0.55</td>
<td>-27.2 ± 0.7</td>
</tr>
</tbody>
</table>

$^a$ 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 $3$).

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

$^c$ Carbon preference index was calculated as CPI = $\Sigma$(C$_{25}$, C$_{27}$, C$_{29}$, C$_{31}$, C$_{33}$) / $\Sigma$(C$_{24}$, C$_{26}$, C$_{28}$, C$_{30}$, C$_{32}$) in terms of n-alkane quantity (µg) from Pearson and Eglinton (2000).
Table 2.2. 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 at the NOSAMS facility at Woods Hole Oceanographic Institution. $^{14}$C ages (yrBP) were calibrated to calendar years (cal.yrBP) using CALIB 5.0 program.

<table>
<thead>
<tr>
<th>Horizon (Sample)</th>
<th>Depth (cm)</th>
<th>CO$_2$ (umol)</th>
<th>$\delta^{13}$C$_{VPDB}$ (%)</th>
<th>C (µg)</th>
<th>Fm (%)</th>
<th>$\Delta^{14}$C (%)</th>
<th>$^{14}$C Age (yrBP)</th>
<th>Calender Age (cal.yrBP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3 700 - 705</td>
<td>6.02</td>
<td>-27.7</td>
<td>78.26</td>
<td>0.853</td>
<td>-152.7</td>
<td>1280 ± 95</td>
<td>1162.5 ± 183.5</td>
<td></td>
</tr>
<tr>
<td>D5 925 - 930</td>
<td>8.26</td>
<td>-28.0</td>
<td>107.38</td>
<td>0.729</td>
<td>-275.6</td>
<td>2540 ± 85</td>
<td>2563.0 ± 204.0</td>
<td></td>
</tr>
<tr>
<td>D6 1015 - 1020</td>
<td>12.80</td>
<td>-30.4</td>
<td>166.40</td>
<td>0.398</td>
<td>-604.5</td>
<td>7400 ± 110</td>
<td>8204.0 ± 196.0</td>
<td></td>
</tr>
<tr>
<td>D7 1170 - 1180</td>
<td>5.54</td>
<td>-29.7</td>
<td>72.02</td>
<td>0.402</td>
<td>-601.0</td>
<td>7330 ± 200</td>
<td>8147.5 ± 390.5</td>
<td></td>
</tr>
<tr>
<td>D9 1320 - 1330</td>
<td>5.31</td>
<td>-29.3</td>
<td>69.03</td>
<td>0.413</td>
<td>-589.5</td>
<td>7100 ± 160</td>
<td>7952.0 ± 338.0</td>
<td></td>
</tr>
<tr>
<td>Wood 1267.2</td>
<td>nr</td>
<td>-26.7</td>
<td>nr</td>
<td>0.398</td>
<td>-605.0</td>
<td>7410 ± 60</td>
<td>8211.5 ± 161.5</td>
<td></td>
</tr>
</tbody>
</table>

nr: Data not reported.

$^{a}$ 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 evolved CO$_2$. 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.
less than 80 µg C. This trend is consistent with a consensus that analytical uncertainties associated with blank corrections become relatively large when C contents in samples are less than 100 µg (i.e., McNichol et al., 2001; Smittenberg et al., 2004). Nonetheless, these age errors are in an acceptable range for CSRA on samples with such limited C yields. 

$^{14}$C ages increased with depth from D3 to D5. In contrast, ages of deeper samples (D6, D7, D9 and wood fragment) clustered at roughly 8,000 cal.yrBP. These dates showed a trivial extent of age-reversals with neighboring sedimentary horizons.

2.5. Discussion

2.5.1 Origin of $n$-Alkanes

Long-chain odd-C-numbered $n$-alkanes are derived from epicuticular waxes of terrestrial higher plants (Eglinton and Hamilton, 1967; Rieley et al., 1991) and are ubiquitously found in aquatic sediments (Pearson and Eglinton, 2000). Terrestrial $n$-alkanes possess unique molecular and isotopic diagnostic features that are easily distinguishable from those of $n$-alkanes derived from other precursor sources. In addition, terrestrial $n$-alkanes 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, Huang et al. (1997), Mazeas et al. (2002) and Sun et al. (2005) reported that progressive biodegradation of individual $n$-alkanes involves negligible isotopic fractionation. Sedimentary $n$-alkanes, therefore, are popular for use as a tracer for OM transport from terrestrial environments (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; Kuypers et al., 1999; Yamada and Ishiwatari, 1999; Brincat et al., 2000; Huang et al., 2001; Menzel et al., 2004).

The most abundant n-alkanes in Ordy Pond sediment are odd-numbered compounds ranging from n-C25 to n-C33. This distribution 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 the characteristic feature of n-alkanes originating from plant waxes (Eglinton and Hamilton, 1967; Collister et al., 1994). On the other hand, shorter-chain counterparts, n-C15 ~ n-C19 n-alkanes, that are major components of algal lipids, were virtually absent in the free lipid extracts from Ordy Pond sediments. The interpretation of terrestrial plant origin is also supported by δ13C values of individual n-alkanes. δ13C values of n-alkanes reflect the unique isotope fractionations involved in different C-fixation pathways (C3, C4 and CAM fixation) utilized by plants. The n-alkanes derived from terrestrial plants conducting C3 C-fixation typically have δ13CVPDB values that range from -30 to -40‰, whereas those synthesized by C4 land plants tend to be isotopically heavier and vary anywhere from -17 to -25‰ (Collister et al., 1994; Reiley et al., 1991; Kuypers et al., 1999; Chikaraishi and Naraoka, 2003). δ13C values of individual n-alkanes from Ordy Pond ranged from -30 to -25‰, which implies that these compounds were derived from terrestrial higher plants.

The name of Ordy Pond presumably comes from an abbreviation of “Ordnance Pond”, referring to the possible disposal of ordnance during the World War II period (Athens et al., 1999). The area is still under the jurisdiction of the U.S. Navy and the site is located on the former Naval Air Station Barbers Point. Although there is no direct
evidence or reports of the disposal of ordnance into the pond, these facts raised a slight concern that the site could be contaminated by petroleum-derived materials. Hydrocarbons, including \( n \)-alkanes, contained in petroleum are virtually \(^{14} \text{C}\)-free. Therefore, contamination of sediments with petroleum exerts detrimental effects on CSRA when the method is applied for sediment chronology. However, geochemical evidence indicates contamination of the sediment by petroleum-related hydrocarbons is unlikely for Ordy Pond. Aliphatic hydrocarbons originating from petroleum-related sources are known to contain roughly equal amounts of even and odd \( n \)-alkanes (Ishiwatari et al., 1994). In addition, petroleum-derived hydrocarbons contain unresolved complex mixtures comprised of structurally complex isomers as well as branched and cyclic hydrocarbons (Ishiwatari et al., 1994; Bouloubassi et al., 2001; Wu et al., 2001). These compounds cannot be well-separated by conventional GC columns, and consequently appear as a baseline hump on GC chromatograms (Ishiwatari et al., 1994; Pearson and Eglinton, 2000; Wakeham et al., 2004). Absence of these diagnostic characteristics on GC chromatograms of the hydrocarbon and \( n \)-alkane fractions extracted from Ordy Pond sediments (see Figure 2.4 b) suggests that the study site is not likely contaminated by petroleum-derived materials.

Ordy Pond sediments contain abundant \( n \)-alkanes (Table 2.1), despite the absence of riverine inputs of terrigeneous organic matter. This suggests that the main transport pathways of \( n \)-alkanes into the pond are direct fall-off of leaves or leaf-litter and aeolian transport from the vegetation in the vicinity. Epicuticular waxes of plants minimize water loss through evapotranspiration and serve as a protection barrier against ultraviolet radiation (Eglinton and Hamilton, 1967; Kunst and Samuels, 2003). An experiment by
Baker (1974) demonstrated that quantities of epicuticular waxes secreted from the leaf-surface of *Brassica oleracea var. gemmifera* (Brussels sprout) grown under controlled environments are positively correlated with the solar irradiance level and aridity.

Wirthensohn and Sedgley (1996) also reported that, among the *Eucalyptus* group, species distributed in areas with low rainfall had the greatest wax coverage on the leaf surface in comparison to other species from wetter environments, perhaps as an adaptive strategy to minimize water loss and protection against solar radiation. In response to the characteristic leeward aridity in the ‘Ewa region and 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 the 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 juvenile leaves are promptly replaced with newly regenerated waxes to compensate for the losses within just a few days (Hallam, 1970; Wirthensohn and Sedgley, 1996). Combination of these effects presumably explains the abundant deposition of plant *n*-alkanes in the Ordy Pond sediments.

### 2.5.2 Validation of CSRA / Error Assessment

Horizon D3 was intentionally selected from a depth close to horizon C that was dated by AMS using a plant macrofossil (Athens et al., 1999, 2002) in order to determine the reliability of the CSRA method. In addition, the wood sample from horizon W is bracketed in depth by *n*-alkane samples from horizons D7 and D9 (see Figure 2.5). This
Figure 2.5 Complete chronology of Ordy Pond sediment. Two types of error bars on the snail date from horizon E represent an analytical and error and an estimated error of 3,000 cal. yrBP, which accounts for possible incorporations of $^{14}$C-depleted C from the limestone. The sedimentation history was modeled by linear interpolation for the phase I (black line: sediment surface ~ horizon A) and first order linear regression with least squares fitting for the Phase II (blue line: horizons A, B, C, D3, D and D5) and for the phase III (red line: horizons D5, D7, W and D9). Horizon D6 and E were not used in the regression for phase III due to a possible contamination and unknown extent of limestone-effect, respectively.
three sample sequence spans a depth interval of about 160cm 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 (Figure 2.5). Horizon D5 extends the age-depth trend established by horizons A to D3, which further supports the reliability of the CSRA method (Figure 2.5). The $^{14}C$ ages of horizons W, D7 and D9, however, are not significantly different, even though these horizons are contained within a nearly 160 cm-long 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, two of the highest analytical uncertainties are associated with $n$-alkanes from horizon D7 and D9, which had two of the lowest C yields (Table 2.2). Although the age-ranges ($2\sigma$ probability ranges) of these three samples overlap (Figure 2.5), it should be noted that the "true age" of each sample can be anywhere between the upper and lower limits of the $2\sigma$ probability ranges. Therefore, the trivial extent of age reversals revealed by the operationally assigned dates (which are simply the mid-points of $2\sigma$ probable age-ranges of the samples) may not exist in terms of the true ages. 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 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 (Figure 2.5). The $^{14}$C age of D6 deviated from the trend of age progression with depth defined by the other dates, suggesting 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 in Section 2.6.3).

Several natural mechanisms could cause such age discrepancy when n-alkanes that are released immediately after death of plants are concurrently deposited into sedimentary reservoirs with materials that have already aged. Pre-aged materials can be introduced to the pond from nearby soil reservoirs or other remote sources through runoff or aeolian transport. The residence time of organic matter in soil can extend as long as centuries to millennia (Trumbore, 2000) due to very slow post-depositional biodegradation and encapsulation within soil matrices such as humus by weak noncovalant bonding (Lichtfouse et al., 1998; Huang et al., 1999). Current surface topography in the vicinity of the pond, however, is characterized as an exposed fossil limestone with sporadic dry shrubs and little surface soil development. Thus, the current infertile environment is unfavorable for long-term storage of organic materials in the top soil. Pollen assemblages of sediment cores from a nearby salt-flat suggest that the area sustained relatively well-developed open canopy forests during the period roughly from 7,000 to 1,000 cal.yrBP (Athens et al., 1999). Nonetheless, the relatively hot and dry regional climate probably limited the development of thick surface soil cover in this area. Therefore, significant deposition of n-alkanes that have been stored in soil reservoirs on a geologic time-scale is not likely in this particular environment.
N-alkanes from remote locations could be introduced to any given area via dry/wet deposition of atmospheric particles. It has been reported that plant \( n \)-alkanes from the terrestrial environment are incorporated into aerosols and transported for considerable distances (Kawamura and Ishimura, 2003; Schefuβ et al., 2003; Conte et al., 2003). Plant waxes are routinely sloughed off plant surfaces or decaying plant OM in soils, and become airborne in strong wind (Schefuβ et al., 2003; Conte et al., 2003). The study area is located on the leeward side of the island. Prevailing trade-winds could potentially deliver pre-aged \( n \)-alkanes via atmospheric transport from remote locations on the windward side of the island. But trade-winds are a fairly steady and continuous phenomenon in Hawai‘i. The fact that all of the samples except for D6 produced reasonable ages indicates it is unlikely that eolian transport of older \( n \)-alkanes is responsible for the anomalous date. Therefore, it is difficult to logically explain the anomalous \(^{14}\text{C} \) age of the \( n \)-alkanes from horizon D6.

Another plausible, and perhaps more realistic, explanation for this error is mechanical or operational contamination of the sample during PCGC. The \( \text{CO}_2 \) yield from the \( n \)-alkanes in the D6 sediment sample was significantly higher than from the other samples, even though GC quantification confirmed that there were not significant differences in \( n \)-alkane contents among the prepared samples (Table 2.3). \( \text{CO}_2 \) recovery from D6 \( n \)-alkanes by PCGC was nearly 100%, in comparison to roughly 40–60% recovery for the rest of the samples. Furthermore, the \( \text{CO}_2 \) 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.
Table 2.3. Summary of changes in the quantities of target n-alkanes before and after PCGC in terms of CO$_2$ equivalence.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>n-alkane</th>
<th>Quantity $^a$</th>
<th>Theo. CO$_2$ Y. $^b$</th>
<th>Act. CO$_2$ Y. $^d$</th>
<th>PCGC Efficiency $^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10$^{-2}$ μmol</td>
<td>μmol</td>
<td>μmol</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>n-C$_{27}$</td>
<td>5.62</td>
<td>1.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{29}$</td>
<td>18.25</td>
<td>5.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{31}$</td>
<td>18.35</td>
<td>5.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{33}$</td>
<td>8.02</td>
<td>2.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ $^c$</td>
<td>50.24</td>
<td>15.15</td>
<td>6.02</td>
<td>39.74</td>
</tr>
<tr>
<td>D5</td>
<td>n-C$_{27}$</td>
<td>8.09</td>
<td>2.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{29}$</td>
<td>13.43</td>
<td>3.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{31}$</td>
<td>12.78</td>
<td>3.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{33}$</td>
<td>9.33</td>
<td>3.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ $^c$</td>
<td>43.63</td>
<td>13.12</td>
<td>8.26</td>
<td>62.95</td>
</tr>
<tr>
<td>D6</td>
<td>n-C$_{27}$</td>
<td>5.21</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{29}$</td>
<td>14.53</td>
<td>4.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{31}$</td>
<td>13.15</td>
<td>4.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{33}$</td>
<td>6.90</td>
<td>2.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ $^c$</td>
<td>42.79</td>
<td>11.98</td>
<td>12.8</td>
<td>106.84</td>
</tr>
<tr>
<td>D7</td>
<td>n-C$_{27}$</td>
<td>5.50</td>
<td>1.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{29}$</td>
<td>13.89</td>
<td>4.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{31}$</td>
<td>16.89</td>
<td>5.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{33}$</td>
<td>5.53</td>
<td>1.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ $^c$</td>
<td>41.91</td>
<td>12.60</td>
<td>5.54</td>
<td>43.96</td>
</tr>
<tr>
<td>D9</td>
<td>n-C$_{27}$</td>
<td>6.36</td>
<td>1.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{29}$</td>
<td>16.07</td>
<td>4.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{31}$</td>
<td>15.59</td>
<td>4.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C$_{33}$</td>
<td>5.58</td>
<td>1.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Σ $^c$</td>
<td>43.60</td>
<td>13.05</td>
<td>5.31</td>
<td>40.68</td>
</tr>
</tbody>
</table>

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

$^b$ Theoretical CO$_2$ yield from individual n-alkanes based on the GC quantification of n-alkanes and elemental stoichiometry. C$_{27}$H$_{56}$, C$_{29}$H$_{60}$, C$_{31}$H$_{64}$ and C$_{33}$H$_{68}$ were used as the elemental structure of each target compound, and complete reaction was assumed for calculation.

$^c$ Sum of n-C$_{27}$, n-C$_{29}$, n-C$_{31}$ and n-C$_{33}$ alkanes for GC quantity and theoretical CO$_2$ yield.

$^d$ Actual CO$_2$ yield from the collective n-alkanes trapped by PCGC system.

$^e$ PCGC Efficiency = (Actual CO$_2$ yield) / (Σ Theoretical CO$_2$ yield) * 100%.
Trapping recovery efficiency (i.e., the amount of compounds recovered from the PCGC trap relative to the total amount injected) of the PCGC at the NOSAMS facility was reported to be about 80% for compounds with boiling points below 320°C (Eglinton et al., 1996). On the other hand, Pearson (1999) reported that the recoveries of compounds through PCGC at the NOSAMS facility ranged from 42 to 78% and averaged 58%, which is in good agreement with this study. Pearson (1999) suggests that most of the ~40% loss occurred within the cooled injection system of the PCGC system, with much more minor losses at the FID split of PCGC. 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 chromatographic stationary phase and (2) incomplete removal of solvent (usually dichloromethane or diethyl ether), which is used for transfer of the target compounds from the U-tube to a separate pre-combusted quartz tube for the subsequent procedures. Even though samples after PCGC separation are typically eluted over a pre-combusted mini silica-gel column to remove possible column bleed materials 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 the PCGC traps with residual compounds (i.e., from previous runs).

Possible introduction of contaminants to the D6 sample from unknown sources during PCGC is also demonstrated by simple isotopic mass-balance calculations. The PCGC system separated and traps the target compounds (n-C_{27}, n-C_{29}, n-C_{31} and n-C_{33}) from an admixture of n-alkanes in the samples. These four target compounds collected in the PCGC traps were subsequently combined and combusted to yield CO_{2} gas, approximately 10% of which was directed to the determination of δ^{13}C values (δ^{13}C_{CO_{2}/NOSAMS, hereafter}) at the NOSAMS facility. It is noteworthy that, before the sample submission to the NOSAMS facility, quantities and δ^{13}C values of these individual n-alkanes were determined by GC and CSIA in the Stable Isotope Biogeochemistry Lab at the University of Hawai‘i. As described earlier, generally two separate lipid extracts (the D5 sample, an exception, was obtained by three rounds of extractions) were prepared in order to meet the sample size requirement from each sedimentary horizon. N-alkane fractions from separate extracts were independently analyzed by GC and irm-GCMS, and they were finally combined once terrestrial-origins of these compounds were confirmed by the δ^{13}C_{N-Alk} values. Therefore, theoretical δ^{13}C values of the CO_{2} gas (δ^{13}C_{CO_{2}/UH, hereafter}) evolved from n-alkanes in each sample can be precisely estimated by a multi-component isotopic mass-balance model. If the complete combustion of these n-alkanes to the CO_{2} gas is assumed, then δ^{13}C values of the resultant CO_{2} gas determined in the NOSAMS facility should be equivalent to the weighted averages of the δ^{13}C values of the target n-alkanes. And if the trapping of the compounds via PCGC and the subsequent combustion were precisely operated without
contaminations, then technically, post-PCGC $\delta^{13}C$ values of the CO$_2$ gas determined in the NOSAMS facility 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:

\[
\delta^{13}C_{CO2/NOSAMS} = \delta^{13}C_{CO2/UH} \quad (Eq. 2.4)
\]

\[
\delta^{13}C_{CO2/UH} = [F_{27}\delta_{27} + F_{29}\delta_{29} + F_{31}\delta_{31} + F_{33}\delta_{33}] \text{ Extract-1}
\]
\[
+ [F_{27}\delta_{27} + F_{29}\delta_{29} + F_{31}\delta_{31} + F_{33}\delta_{33}] \text{ Extract-2} \quad (Eq. 2.5)
\]

and

\[
l = [F_{27} + F_{29} + F_{31} + F_{33}] \text{ Extract-1} + [F_{27} + F_{29} + F_{31} + F_{33}] \text{ Extract-2} \quad (Eq. 2.6)
\]

where $\delta$ and $F$ in Eq. 2.4 and Eq. 2.5 denote the $\delta^{13}C$ compositions of the $n$-alkanes and the theoretical molar fractional abundances of CO$_2$ gas that are evolved from each compound in the separate extracts 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 ("Extract-3") because the sample represents the sum of three separate extractions.

The results of the isotopic mass-balance calculations (Table 2.4) clearly demonstrate that every sample, except the D6 sample has good agreement between the
Table 2.4. Comparisons of the $\delta^{13}$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.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>$\delta^{13}$C$_{CO_2}$ [UH] Theoretical Estimate</th>
<th>$\delta^{13}$C$_{CO_2}$ [NOSAMS] Actual Measurement</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td>-27.6%</td>
<td>-27.7%</td>
<td>0.1%</td>
</tr>
<tr>
<td>D5</td>
<td>-27.0%</td>
<td>-28.0%</td>
<td>1.0%</td>
</tr>
<tr>
<td>D6</td>
<td>-28.2%</td>
<td>-30.4%</td>
<td>2.2%</td>
</tr>
<tr>
<td>D7</td>
<td>-29.5%</td>
<td>-29.7%</td>
<td>0.2%</td>
</tr>
<tr>
<td>D9</td>
<td>-29.1%</td>
<td>-29.3%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

$\delta^{13}$C$_{CO_2/UH}$ and the $\delta^{13}$C$_{CO_2/NOSAMS}$ values. Observed differences in the theoretical and the actual $\delta^{13}$C value of CO$_2$ gas liberated from the target n-alkanes are all within ~1.0% (the difference for the D3, D7 and D9 sample are even within 0.2%). These minor difference between the $\delta^{13}$C$_{CO_2/UH}$ and the $\delta^{13}$C$_{CO_2/NOSAMS}$ 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 fractionations could occur if the compounds separated on the GC column were not fully collected by 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 $\delta^{13}$C compositions of a number of biomarkers, including long-chain n-alkanes and some volatile compounds with low molecular weight, before and after PCGC purification

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generally agreed within $1.0 - 2.0\%$. In addition, *Pearson* (1999) suggests 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 $\delta^{13}C$ value. Consequently, observed deviation of the $\delta^{13}C_{\text{CO2/UE}}$ and the $\delta^{13}C_{\text{CO2/NSAMS}}$ values for the D6 sample, which is as large as $2.2\%$, clearly exceeds the level of uncertainty that can be explained by the fractionation effect of PCGC system. Observed disagreement between theoretical and the actual $\delta^{13}C_{\text{CO2}}$ 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:

\[
\text{Initial } \Delta^{14}C = ( Fm \times e^{(\lambda \times \text{yrBP})} - 1 ) \times 1000 \% \\
\text{(Eq. 2.7)}
\]

where $Fm$, $\lambda$, and yrBP represent the “fraction modern”, $^{14}C$ decay constant (1 / 8267 years$^{-1}$), and the timing of deposition in calendar years, respectively. The above 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, 7400yrBP, of the D6 $n$-alkanes determined by CSRA then translates to an initial $\Delta^{14}C$ value of $-25.4\%$.  

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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, thus, an initial $\Delta^{14}$C of -12.9‰. This estimate implies that $^{14}$C in the D6 n-alkanes is somehow depleted by 12.5‰ due to the incorporation of $^{14}$C-depleted C. The extent of the mixing between true and $^{14}$C-depleted n-alkanes can also be modeled by an isotopic mass-balance approach:

$$\Delta^{14}C_{\Sigma} = \{ F_T \times \Delta^{14}C_T \} + \{ F_C \times \Delta^{14}C_C \}$$  \hspace{1cm} (Eq. 2.8)

and

$$F_T + F_C = 1$$  \hspace{1cm} (Eq. 2.9)

where $\Delta^{14}$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 $\Sigma$, T, and C refer to total combined n-alkanes, true n-alkanes, and $^{14}$C-depleted n-alkanes contributed from different sources (or contaminants), respectively. In this case, the values for $\Delta^{14}C_{\Sigma}$ and $\Delta^{14}C_T$ are -25.4‰ and -12.9‰, respectively.

If n-alkanes from fossil sources such as oils and greases contributed to the $^{14}$C-depleted n-alkanes, $\Delta^{14}C_D$ is -1,000‰ according to the conventional definition (Pearson & Eglinton, 2000; Pearson et al., 2001). Subsequently, the isotope mass-balance calculation suggests that the observed age offset of 3,680 years from the sedimentation model (dilution of $\Delta^{14}$C from -25.4 to -12.9‰) 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 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 support 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.

2.5.3 New Sediment Chronology

Figure 2.5 shows the new sediment chronology constructed from previous $^{14}$C dates obtained by Athens et al. (1999, 2002) and a series of new $^{14}$C dates from this study. $^{14}$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 2.5). The $^{14}$C date of horizon D6 was not included in this age-depth model due to presumed contamination of the sample. The $^{14}$C date of terrigeneous snails by Tribble et al. (1999) was also removed from the model. Several 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 by snails or uptake of limestone dissolved by foot secretions (Goodfriend and Hood, 1983); however, the magnitude of such incorporation is quite
Table 2.5. Recalibration of the $^{14}$C dates used in the previous Ordy Pond sediment chronology.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Source</th>
<th>Method / Sample Type</th>
<th>Previously Reported Age</th>
<th>Adjusted Age $^a$ (cal.yrBP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon A</td>
<td>Athens et al., ('99, '02)</td>
<td>Historic Pollen</td>
<td>120.0 cal.yrBP</td>
<td>120</td>
</tr>
<tr>
<td>Horizon B</td>
<td>Athens et al., ('99, '02)</td>
<td>AMS / Plant macrofossils</td>
<td>553.5 ± 68.5 (cal.yrBP) $^b$</td>
<td>496.5 ± 156.5</td>
</tr>
<tr>
<td>Horizon C</td>
<td>Athens et al., ('99, '02)</td>
<td>AMS / Plant macrofossils</td>
<td>1059.5 ± 106.5 (cal.yrBP) $^b$</td>
<td>1053.0 ± 125.0</td>
</tr>
<tr>
<td>Horizon D</td>
<td>Athens et al., ('99, '02)</td>
<td>AMS / Plant macrofossils</td>
<td>1324.5 ± 60.5 (cal.yrBP) $^b$</td>
<td>1345.5 ± 161.5</td>
</tr>
<tr>
<td>Horizon E</td>
<td>Tribble et al., ('99)</td>
<td>C.R.M * / Land snails</td>
<td>9780 ± 110.0 (yrBP) $^c$</td>
<td>11175.0 ± 426.0</td>
</tr>
</tbody>
</table>

$^a$ Dates were calibrated to "calendar years before present (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).

$^b$ Dates were calibrated using CALIB 3.0.3 program by the authors in the original publications.

$^c$ Date was reported in terms of $^{14}$C age (yrBP) and was not calibrated to calendar years (cal.yrBP) by the authors in the original publication.

* Conventional radiometric method.
variable. For example, the maximum age-offset of 620 years was observed for shells of 3 shallow-marine species from the 'Ewa region of O'ahu by Dye (1994). The age anomaly of land snails was on average 700 ± 180 years in Texas (Goodfriend et al., 1999), and was as large as 3,120 years in Jamaica (Goodfriend and Stipp, 1983). Goodfriend and Stipp (1983) concluded that it is impossible to assign a systematic correction factor for 14C-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 this "limestone-effect", a conceptual error of 3,000 years is assigned to the 14C age of terrestrial snails from Ordy Pond sediment, besides the true statistical error associated solely with the analysis and calibration.

The most recent sedimentation history, phase I in Figure 2.5, is modeled by a simple linear interpolation between the sediment surface and horizon A. Linear interpolation would be appropriate for such a short time span (~120 cal.yrBP) relative to the whole sedimentation history of the pond. Sedimentation rates for deeper sediments are modeled 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 model results in a relatively high 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, which seems to reflect higher analytical uncertainties associated with CSRA of older materials with micro-scale sample quantities (McNichol et al., 2001). The newly constructed age-control suggests that sedimentation
rates in Ordy Pond increased over time in a stepwise manner. Predicted sedimentation rates according to the age-depth model are approximately 0.06 (Phase III), 0.17 (Phase II) and 4.33 cm/yr (Phase I).

2.6. Conclusion

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 the D3 and D5 n-alkanes determined by CSRA is in excellent agreement with the age of the horizon C dated by AMS using plant macrofossils. A series of dates obtained from n-alkanes (horizons D7 and D9) generally matched well with independently determined 14C ages using plant macrofossils (horizon W). This study thus demonstrated that CSRA using biomarkers is capable of providing reliable 14C dates for construction of sediment chronology for paleo-applications. Chronology of Ordy Pond sediments was refined with the addition of a series of new 14C dates collected in this study.

Results of this study also documented a few negative aspects associated with CSRA. Analytical uncertainty becomes an issue when the C content in a sample is less than 100 μg. Susceptibility of a sample to artificial contamination is rather high during any phase of preparation and analysis, especially for n-alkanes which are found in petroleum-derived greases and oils. Incorporation of pre-aged materials, such as soil n-alkanes, may not be resolved even at the molecular-level. And most of all, sample preparations are time-consuming and CSRA by AMS is inarguably still an expensive and
non-routine option. Nonetheless, $^{14}$C dating of individual biomarkers and subsequent construction of sediment chronology is in fact possible in lacustrine sediments. CSRA with appropriate selection of target biomarkers is certainly a powerful tool for construction of sediment chronology if more conventional means of $^{14}$C dating such as the standard radiometric method are inapplicable.
2.7. References


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CHAPTER III

Holocene Paleovegetative Reconstruction from Ordy Pond, O‘ahu, Based on Compound-Specific $\delta^{13}$C Analysis of Sedimentary Leaf Waxes
Abstract

Paleoclimatic records from the vast north subtropical Pacific are rare and are largely limited to coral records. This study presents a paleobotanical record from a continuous Holocene sedimentary sequence from Hawai‘i. Normal alkanes (n-alkanes) derived from the epicuticular waxes of terrestrial higher plants were extracted from Holocene lacustrine sediments from Ordy Pond, O‘ahu, Hawai‘i. Carbon isotopic (δ¹³C) compositions of individual n-alkanes (n-C₂₇, n-C₂₉, n-C₃₁ and n-C₃₃) were determined by compound-specific isotopic analysis (CSIA) in order to assess changes in paleovegetation. Observed δ¹³C values of n-alkanes ranged from -33.3 to -20.5‰, indicating large variability in the relative ratio of the leaf waxes originating from C₃ and C₄ plants. Measured δ¹³C values were translated into the abundance of C₄ plants relative to C₃ plants, referred to as % C₄ abundance, using a two end-member isotopic mass-balance approach. Comparisons with several pollen records from O‘ahu indicate that the trends observed in the isotopically-derived % C₄ abundance agree well with the pollen data, supporting the validity of this proxy in elucidating changes in paleovegetation. Further comparisons with some key pollen groups that are diagnostic of specific climatic regimes, mainly in terms of rainfall, demonstrate that % C₄ abundance can be considered as a paleo-aridity index in tropics and subtropics. The % C₄ abundance combined with previously reported pollen spectra from multiple locations on O‘ahu reveal intriguing fluctuations in the extent of aridity on O‘ahu during the Holocene. A nearly 40% increase in the % C₄ abundance from ~10,000 to ~4,800 cal.yrBP suggests significant and continuous aridification during this period. A subsequent rapid decrease by about 25% within less than 1,000 years perhaps infers a large scale climate change toward wetter
climate. The time interval from \(~4,000\) to \(~1,600\) cal.yrBP is characterized climatically as a relatively stable but wetter condition. Changes in vegetation recorded after \(~1,000\) cal.yrBP appear to be under the influence of large scale ecosystem alterations due to anthropogenic activities such as land clearing, agriculture and cattle ranching, by the early Polynesian settlers as well as later European settlers.
3.1. Introduction

Lacustrine sediments provide valuable information to assess the impacts of past climate changes and anthropogenic activities on local watersheds and regional ecosystems. Owing to relatively small reservoir sizes and close couplings with terrestrial ecosystems, typical lake systems respond more rapidly and sensitively to environmental changes than the ocean does (Meyers, 2003). Elemental and isotopic analyses on lacustrine organic matter (OM) are widely used to extract the fingerprints of past environmental histories embedded in sediments.

Bulk OM is made of complex mixtures of both autochthonous and allochthonous fractions, mainly derived from primary production by aquatic microorganisms and remains of terrestrial biota introduced to sediments, respectively. Analyses of bulk OM thus reflect the miscellaneous origins of sub-components as well as alterations of the primary signals caused by diagenesis. Analyses of biomarkers, which are unique organic molecules synthesized by restricted ranges of precursor organisms (Meyers, 1997, 2003; Meyers & Ishiwatari, 1995), minimize the biases caused by mixed signals from various subfractions of OM. Modern isotope-ratio-monitoring gas chromatography/mass spectrometry (irm-GC/MS) allows precise compound-specific isotopic analysis (CSIA) of individual biomarkers present in trace quantities in complex organic mixtures (Hayes et al., 1990; Lichtfouse, 2000). CSIA of individual lipids contained in the epicuticular waxes of terrestrial higher plants, especially long-chain odd-carbon (C)-numbered normal alkanes (n-alkanes), from sediments is frequently used to infer paleoenvironmental and paleoclimatological changes in the watersheds (i.e., Rieley et al., 1991; Bird et al., 1995; Bourbonniere & Meyers, 1996, Kuypers et al., 1999; Menzel et al., 2004) because these
compounds are ubiquitous in aquatic sediments (Pearson & Eglinton, 2000) and are exceptionally resistant to post-depositional degradation (i.e., Sun and Wakeham, 1994; Huang et al., 1997; Hoef et al., 2002; Mazeas et al., 2002; Sun et al., 2005).

Plants have certain ranges of optimum climatic conditions that are functions of a variety of factors including temperature, precipitation and light availability (Teeri & Stowe, 1976; Keeley & Rundel, 2003). As a broad generalization, plants utilizing C₄-photosynthesis, which include grasses, shrubs and some sedges mainly flourishing in the tropics and subtropics, are more tolerant of hot and dry environments than most typical woody plants relying on C₃-photosynthesis (Schefuß et al., 2003). Each mode of photosynthesis results in a different level of C isotope discrimination (Farquhar et al., 1989), which is propagated to the isotopic compositions of biomolecules (including leaf wax n-alkanes) that are ultimately derived from the precursor photosynthetic intermediate molecules (Hayes, 1993, 2001). Therefore, CSIA of individual n-alkanes and subsequent isotopic mass-balance calculations allow quantitative estimates of relative abundances of C₃ and C₄ plants in the watershed at a given time. Changes in the vegetative community structure, in turn, may be directly or indirectly linked to past climate change or anthropogenic activities. For example, Menzel et al. (2004) revealed an expansion of C₄ plants on the continents surrounding the Mediterranean Basin during the Pliocene as a result of transformations of barren desert areas into grass-dominated savannas due to a wetter climate. Bird et al. (1995) concluded that C isotopic (δ¹³C) compositions of sedimentary n-alkanes at the mouth of Johnstone River, Australia, reflect the history of land burning by indigenous aborigines and clearing of forests for sugarcane agriculture and pastures by the European settlers in the late 19th century.
In this study, past vegetative changes on the leeward coastal flat in the ‘Ewa Plain of O‘ahu, Hawai‘i (Figure 3.1), were assessed based on $\delta^{13}$C compositions of plant $n$-alkanes in order to infer Holocene climate changes and the effects of historic human activities on the local ecosystems. Ordy Pond, a brackish water body filling one of the numerous karstic sinkholes in the area, offers a 13.5 m high-resolution sedimentary record covering the past 10,000 years. Pollen reconstruction by Athens et al. (1999, 2002) for the last 1,500 years of the Ordy Pond sedimentary record documented significant changes in the local vegetation over time. The authors concluded that the arrival of Polynesians, as well as alien species brought by the early settlers, resulted in remarkable alterations of the native Hawaiian ecosystems. The main purpose of this study is to investigate possible paleovegetative changes that are due to climatic and other natural causes during the entire Holocene period, however, anthropogenic activities that occurred after the arrivals of Europeans are also revealed. Only a limited number of pollen records (i.e., Burney et al., 1995; Athens et al., 1992, 1995, 1999, 2002; Hotchkiss and Juvik, 1999) for discrete intervals of the Holocene has been reported from Hawai‘i in the past. Thus the record of $\delta^{13}$C compositions of the sedimentary $n$-alkanes obtained from this study is valuable due to the fact that it is the first continuous paleoenvironmental proxy extending throughout the entire Holocene period from Hawai‘i.

3.2. Regional settings and Sediment cores

Ordy Pond fills one of the numerous karstic sinkholes (~20 m deep) in the Kalaeloa district on the ‘Ewa Plain of Oahu, Hawai‘i (Figure 3.1). The ‘Ewa Plain is formed by an exposed Pleistocene limestone terrain developed during the interglacial
Figure 3.1 Location of Ordy Pond in the 'Ewa Plain of O'ahu (from Garrison, 2002). The pond is approximately 750 m from the coast and is situated at 1.5 m above mean sea-level. The area of the pond is 0.5 ha.

Figure 3.2. Average monthly temperature records in Honolulu and average monthly rainfall measured at Honolulu International Airport from 1971 to 2000. Data were taken from NOAA National Climatic Data Center.
Waimanalo sea-level highstand (+8 m above current mean sea-level) that spanned roughly from 135 to 120 thousand years ago (Ku et al., 1974; Sherman et al., 1993; Szabo et al., 1994). The pond was formed in response to the most recent post-glacial sea-level rise as the open sinkhole was inundated with groundwater approximately 10,000 years ago. The pond possesses neither surface inflow nor outflow, and appears to be largely isolated from the surrounding aquifer today according to a hydrological survey conducted by Ogden (1999). The system is only fed by weak intrusion of groundwater and seasonal local rainfall concentrated during winters. Thus the pond can be considered as a hydrologically closed system. The local climate in the ‘Ewa Plain has two distinct seasons; a hot and dry summer, and a cooler and wetter winter (Figure 3.2). A few dominant species such as Prosopis pallida (kiawe tree) and Pluchea symphytifolia (sourbush) form sporadic shrubs in the area. The circular rim of the pond, however, is densely vegetated by Rhizophora mangle (American mangrove) (Athens et al., 1999). The pond water column is 5 m deep. The brackish waters are highly eutrophic and productive. A time-series biogeochemical monitoring of the pond water column by Garrison (2002) revealed that the water column develops climate-driven thermal stratification during the summer. The extent of stratification weakens during autumn and winter which results in enhanced vertical mixing of the epilimnion and hypolimnion. Nonetheless, the hypolimnion remains anoxic all year due most likely to extensive remineralization processes.

Sediments from Ordy Pond record the continuous environmental history of the pond since its formation in the sinkhole. Initial coring by Athens et al. (1999, 2002) in 1994 recovered 8.7 m of sediment, which dates back to 1,500 calendar yr before present
(cal.yrBP). Tribble et al. (1998) in 1997 used a vibracore system to collect the upper 8 m of sediments, and a piston core system to extend the cumulative core length up to 17.5 m. Sub-samples for this study were taken from the portion of the sediments collected by the piston core system.

Three distinct stratigraphic units were identified in the core: a basal terrigenous unit, and the middle and top aquatic units (Figure 3.3). The basal unit contains fossils of terrestrial snails and pebbles of reef rock in a fine-grained carbonate matrix, which suggests that this interval was sub-aerially deposited and that Ordy Pond was a dry sinkhole at this stage. The base of this sedimentary unit dates up to 25,750 yrBP based on radiocarbon ($^{14}$C) dating on shells of terrestrial snails (Tribble et al., 1999). A thin layer of blackish peat-type sediment that separates the basal terrigenous unit from the overlying aquatic unit marks the terrestrial-aquatic transition. Aquatic history of this pond probably started from a swamp/marsh type of environment as the open sinkhole was inundated with water in response to sea-level rise. The middle stratigraphic unit consists of 8.5 m of undisrupted aquatic sediment with apparent laminae (sub-millimeter to centimeter scale) and bandings. The color of each layer reflects variations in the relative richness of organic matter and carbonates. Layers rich in OM are typically dark green to brownish in color, whereas layers with high carbonate contents tend to be much lighter (whitish to beige). The uppermost unit of sediment, representing the most recent 120 years of depositional history, contains 5 m of organic-rich, subtly-banded sapropelic sediment.
Figure 3.3  Schematic diagram of the sediment core collected from Ordy Pond (modified from Tribble et al., 1998). The core has three stratigraphic units. Horizon E marks the transition from non-aquatic to aquatic sediments. The age of horizon E was estimated to be roughly 9,550 cal.yrBP based on the age-depth model by Uchikawa et al. (2006). $^{14}$C ages of horizon D (7.91 m; 1,345.5 cal.yrBP), C (6.84 m; 1,053 cal.yrBP) and B (5.63 m; 496.5 cal.yrBP) were determined by Athens et al. (1999, 2002) from terrestrial plant materials. Horizon A at 5.21 m separates the top stratigraphic unit with sapropelic sediment from underlying laminated middle unit. This horizon is dated as 120 cal.yrBP by the first appearance of historic pollen by Athens et al. (1999, 2002).
3.3. Methods

3.3.1 Lipid Extraction and Separation

Methods for extraction and separation of n-alkanes from bulk sediment samples were modified from Kenig et al., (2000), Hoering and Freeman, (1984) and Yamada et al., (1994). Sediment samples were oven-dried at 60 °C and homogenized with a solvent-rinsed agate mortar and pestle. Total free lipids in the sediments were soxhlet-extracted with dichloromethane for a minimum of 48 hours. The extracts were percolated through a column of acid-activated and solvent-rinsed copper-granular (20-30 mesh size) in order to remove elemental sulfur (S). The hydrocarbon fraction in the total free lipids was separated by silica-gel (12 g, 70-230 mesh size) column chromatography. Silica-gel was pre-extracted with dichloromethane, activated at 500 °C overnight, and finally de-activated with 2 wt.% of solvent-extracted deionized H2O. The hydrocarbon fraction was eluted with 40 mL of petroleum ether. Petroleum ether was subsequently evaporated under a stream of N2 gas, and the residues were re-dissolved in pentane. N-alkanes in the hydrocarbon fractions were adducted onto a silicalite molecular sieve. The silicalite (powdered to 150 μm grain size) column was combusted at 325 °C overnight in order to eliminate possible organic contaminants. The hydrocarbon fraction was slowly percolated through the silicalite column, and the column was flushed with approximately 12 mL of clean pentane to collect non-adduct fractions, which were archived. The silicalite molecular sieve was digested by approximately 3 mL of 49% hydrofluoric acid, which was subsequently extracted with pentane to recover adducted n-alkanes. Finally, possible impurities were removed by eluting the n-alkane fraction through a pre-combusted silica-
gel column with pentane. Solvents used during the extraction and purification were not distilled. Best available grade (HPLC quality or higher) for each solvent was selected.

3.3.2 GC and irm-GC/MS Analysis

Gas chromatography (GC) and irm-GC/MS analyses were conducted in the Stable Isotope Biogeochemistry Lab at the University of Hawai‘i. Samples were dissolved in cyclohexane and helium was used as a carrier gas for both GC and irm-GC/MS analyses.

Identification of individual n-alkanes was conducted using a Varian 3400 GC with SPI on-column injector and flame ionization detector. A HP-Ultra 1 column (50 m length, 0.32 mm i.d., 0.17 µm film thickness) was used in the GC. 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.

$\delta^{13}C$ compositions of individual n-alkanes ($\delta^{13}C_{n-Alk}$ hereafter) were determined by CSIA using irm-GC/MS (Finnigan MAT 252 connected to Finnigan GC/C-III interface). The temperature program of the GC interface was same as for the GC analysis. A J&W DB-1 capillary column (60 m length, 0.32 mm i.d., 0.25 µm film thickness) was used in the GC interface. $^{12}C/^{13}C$ ratios for the peaks of target n-alkanes (n-C$_{27}$, n-C$_{29}$, n-C$_{31}$ and n-C$_{33}$) were calculated against a CO$_2$ reference gas. Finally, carbon isotopic compositions of individual n-alkanes were standardized to the Vienna PDB (VPDB) standard and expressed in terms of the conventional $\delta$ notation:

$$\delta^{13}C = \left( \frac{^{13}R_{Sample}}{^{13}R_{VPDB}} - 1 \right) \times 1000$$

(Eq. 3.1)
where

\[
{^{13}R} = \frac{{^{13}C}}{{^{12}C}}
\]  
(Eq. 3.2)

An internal standard (deuterated n-C\textsubscript{36}) of known carbon isotopic composition was also co-injected with samples for each run in order to identify the series of compounds and to monitor the operating conditions of the instrument.

3.3.3 Sediment Chronology

Depth-scales (cm below the surface) of the samples were converted to age-scales (cal.yrBP) according to the sedimentation model proposed by Uchikawa et al. (2006). The model compiles a series of \(^{14}C\) dates obtained from AMS radiocarbon dating of rare terrestrial macrofossils by Athens et al. (1999, 2002) and Uchikawa et al. (2006), as well as several \(^{14}C\) dates based on the compound-specific \(^{14}C\) analysis of n-alkanes originating from plant leaf-waxes by Uchikawa et al. (2006).

3.4. Results

Hydrocarbon fractions extracted from Ordy Pond sediments contained a series of n-alkanes and minor amounts of either branched or cyclic isomers (Figure 3.4 a). Presence of these isomers is somewhat problematic for irm-GC/MS analysis as they could potentially co-elute with target n-alkanes. But silicalite molecular sieve treatment successfully removed the non-aliphatic isomers, and n-alkanes were purified to a sufficient level for accurate CSIA. A typical chromatogram of the n-alkane fraction (Figure 3.4 b) clearly shows that compounds with odd-C-numbers overwhelmed the abundance of even-C-numbered counterparts. Resolved n-alkanes were largely
Figure 3.4. Typical gas chromatograms of (a) hydrocarbon fraction and (b) $n$-alkane fraction extracted from Ordy Pond sediment.
dominated by a long-chain homologous series ranging from \( n-C_{25} \) to \( n-C_{35} \), with a maximum at either \( n-C_{29} \) or \( n-C_{31} \). Shorter-chain \( n \)-alkanes (\( C < 24 \)) were virtually undetected in all samples.

\( \delta^{13}C \) values of \( n-C_{27}, n-C_{29}, n-C_{31} \) and \( n-C_{33} \) \( n \)-alkanes were determined by irm-GC/MS. The operating condition of the instrument was monitored by an internal standard (deuterated \( n-C_{36} \)), and thus duplicate analyses were not always conducted. The analytical error of the CSIA was determined from the standard deviation of the isotopic compositions of the co-injected internal standard on a daily basis. The analytical error of CSIA was typically better than \( \pm 0.3 \% \). Seven samples that were analyzed on two particular days, however, possessed analytical errors greater than \( \pm 0.6 \% \). Exceptionally large analytical errors that are unique to these particular samples were most likely caused by system failures. Operating conditions of the instrument on these two days were affected by reduced sensitivity due to unexpectedly low flow rate of the carrier gas as well as minor system failure associated with the oxidation of the GC column. The results of CSIA of individual \( n \)-alkanes are summarized in Table 3.1 and plotted in Figure 3.5. Observed \( \delta^{13}C \) values of \( n \)-alkanes (\( \delta^{13}C_{N-Alk} \)) ranged from -33.3 to -20.5\%. For most of the samples analyzed, \( \delta^{13}C \) values of \( n-C_{27} \) were least depleted. Despite the small differences in the \( \delta^{13}C_{N-Alk} \) among the four target \( n \)-alkanes, individual \( n \)-alkanes demonstrate very similar trends of change in the \( \delta^{13}C \) compositions (see Figure 3.5). The similarity suggests that these individual compounds are equally reliable in revealing past changes in the \( \delta^{13}C \) values of leaf waxes.
Table 3.1. Results of the compound-specific carbon isotopic analysis on individual n-alkanes extracted from Ordy Pond sediments.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Age (cal. yr BP)</th>
<th>δ(^{13})C(_{VPDB}) (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n-C(_{27})</td>
</tr>
<tr>
<td>492</td>
<td>114</td>
<td>-23.5</td>
</tr>
<tr>
<td>525</td>
<td>133</td>
<td>-21.3</td>
</tr>
<tr>
<td>564</td>
<td>356</td>
<td>-20.5</td>
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</tr>
<tr>
<td>1362.5</td>
<td>9,935</td>
<td>-29.5</td>
</tr>
</tbody>
</table>

† The ages of the lowermost two samples could be significantly underestimated. These samples were collected from the non-aquatic stratigraphic unit (see Figure 3.3), for which the chronology by Uchikawa et al., (2006) is not valid (due to lack of \(^{14}\)C dates and the possibilities of unconformities and hiatuses at the terrestrial/aquatic transition).

\(a\) Mid-points of the sediment intervals sampled for the lipid extractions.

\(b\) Ages based on the sediment chronology proposed by Uchikawa et al., (2006).

\(c\) Daily error report of the irm-GC/MS system based on the standard deviation of the δ\(^{13}\)C\(_{VPDB}\) values of the co-injected internal standard.
Figure 3.5. Variations in the $\delta^{13}$C values of individual $n$-alkanes ($n$-C$_{27}$, $n$-C$_{29}$, $n$-C$_{31}$ and $n$-C$_{33}$) extracted from Ordy Pond sediments. Error bars in the plots indicate daily analytical errors of the irm-GC/MS determined from the internal standard.
3.5. Discussion

3.5.1 Sources of n-Alkanes

Contributions of n-alkanes from different classes of organisms can be distinguished by the chain-length of the compounds. Short-chain n-alkanes, specifically \(n-C_{17}\) and \(n-C_{19}\), are predominantly synthesized by algae and cyanobacteria (Cranwell et al., 1987; Meyers, 1997), whereas aquatic macrophytes are known to produce mid-chain homologous compounds ranging from \(n-C_{23}\) to \(n-C_{25}\) (Ficken et al., 2000). In contrast, long-chain odd-C-numbered n-alkanes from \(n-C_{27}\) to \(n-C_{33}\) are synthesized by terrestrial higher plants as the main component of epicuticular leaf waxes (Eglinton & Hamilton, 1967; Meyers, 1997). Furthermore, n-alkanes of terrestrial plant-origin commonly possess a characteristic unimodal distribution with a maximum at \(n-C_{29}\) or \(n-C_{31}\) (Eglinton & Hamilton, 1967; Collister et al., 1994). The n-alkanes isolated from Ordy Pond sediment contained overwhelming abundances of odd-C-numbered compounds ranging from \(n-C_{25}\) to \(n-C_{33}\) (Figure 3.4 b). The most abundant alkanes were either \(n-C_{29}\) or \(n-C_{31}\) in every sample. This suggests that these compounds were derived from epicuticular waxes of terrestrial higher plants.

On average, organic C accounts for 7.4 wt.% (with a maximum of 22.9%) of the bulk sediment from Ordy Pond (Garrison, 2002) and most of it is believed to be produced by unicellular primary producers in the water column. Garrison (2002) also determined that the depth-integrated net production rate in the water column was more than 170 g C/yr. Nonetheless, shorter-chain n-alkanes (\(n-C_{15} - n-C_{19}\)), which are primarily derived from algal lipids, were virtually undetectable in the n-alkane fractions obtained by solvent extraction of the pond sediments. This discrepancy can be explained
by the transformation within the water column of free (solvent extractable) algal lipids into "bound" form, which can not be recovered by simple solvent extraction. Extraction of bound-lipids usually requires base-hydrolysis (saponification) of sediment under basic conditions (Cranwell, 1978). The presence of bound algal lipids such as shorter-chain n-alkanes, n-alkanoic acids and phytols in association with high-molecular-weight organic substances and humic acids has been reported both from marine (i.e., Sun et al., 1998) and lacustrine systems (i.e., Cranwell, 1978; Albaiges et al., 1984; Ogura et al., 1989; Chikaraishi and Naraoka, 2005). In addition, some lipids can interact with reduced S species such as H₂S and convert to S-bound lipids or form organic sulfur compounds via sulfurization (Kohnen et al., 1991; Grice et al., 1996). Kohnen et al. (1991) suggest that algal lipid biomarkers are present only at low levels as the “free” form, but they can be highly prevalent in the S-bound form. Year-long hypoxia/anoxia and high concentrations of SO₄²⁻ (> 7 μM) in the hypolimnion of Ordy Pond (Garrison, 2002) indicate the possible importance of microbial sulfate reduction as a part of the early diagenetic alteration of OM. In fact, a time-series study of the water column by Garrison (2002) shows hypolimnion S²⁻ concentrations up to 0.48 μM, clearly suggesting the occurrence of sulfate reduction in the water column and most likely in the sediments as well. Therefore, it is possible that a significant portion of algal n-alkanes was either bound to organic compounds with high molecular weight or present as S-bound forms, and thus was not retrieved by simple solvent extraction.

Additional losses of free algal lipids can be caused by selective degradation by heterotrophic activities in the water column and progressive early diagenesis in the sediments. Algal lipids are more labile relative to terrestrial lipids, and thus are rapidly
and preferentially metabolized by heterotrophic organisms (Cranwell, 1981; Sun and Wakeham, 1994; Meyers and Ishiwatari, 1995). It is also worthwhile to mention that the dearth of shorter-chain algal-derived compounds in the n-alkane fractions from Ordy Pond could be in part caused by laboratory artifacts. Given their smaller molecular weights, shorter-chain algal n-alkanes are more easily vaporized than the long-chain plant n-alkanes. Therefore, some portions of algal lipids could have been volatized and lost during sample preparations (i.e., use of rotary evaporator and evaporation of solvents).

3.5.2 Transport Mechanisms

Leaf-waxes can be incorporated into lacustrine sediments in conjunction with bulk OM, such as leaf litter and other forms of dead plant debris, from the terrestrial ecosystems via fluvial transport or runoff. Direct fall-off of leaves (or relatively fresh leaf litter) into water bodies from neighboring vegetative cover and eolian transport of airborne plant-waxes are other plausible transport mechanisms. The latter two seem more likely to be the main transport pathways of n-alkanes into Ordy Pond.

Fluvial transport of considerable amount of terrestrial OM should be negligible in Ordy Pond, considering the absence of incoming rivers and streams. Based on C:N ratios of the bulk OM of Ordy Pond sediment, Garrison (2002) concluded that autochthonous algal-derived OM is the main contributor to the sediment. Rarity of plant macrofossils in the sediment cores (Tribble et al., 1998, 1999; Athens et al., 1999, 2002; Garrison, 2002; Uchikawa et al., 2006) provides further evidence that substantial inputs of allochthonous OM, particularly in the form of coarse debris of terrestrial plants, are uncommon. The lack of quantitatively significant plant-derived OM accordingly implies
that the main physical transport mechanisms of leaf-waxes into the pond is the direct fall-off of leaves and eolian transport. Epicuticular waxes of land plants function as a protection barrier against water loss via evapotranspiration and harmful ultraviolet radiation (Eglinton & Hamilton, 1967; Kunst & Samuels, 2003). Baker (1974) found strong positive correlations between radiant energy and aridity with the quantity of wax deposits on the surface of Brassica oleracea var. gemmifera (Brussels sprout) leaves. Wirthensohn and Sedgley (1996) also confirmed that, among the Eucalyptus group, species from shrubland or heathland areas with low rainfall had the greatest wax coverage on the leaf surface. Because Ordy Pond is located on the arid leeward side of the island in a low latitude region, local plant communities may secrete significant quantities of epicuticular waxes in order to adapt to the characteristic dry climate and strong incoming solar irradiance of the subtropics. Waxy coatings of the leaves are easily and routinely eroded from the leaf surface by wind abrasion as well as by the sandblasting effect, and become airborne (Wirthensohn and Sedgley, 1996; Schefuß et al., 2003; Conte et al., 2003). Meanwhile, leaf surfaces are known to regenerate and re-secrete epicuticular waxes quite rapidly in order to compensate for the loss of waxes due to such physical removal processes (Hallam, 1970; Wirthensohn and Sedgley, 1996; Jetter and Schäffer, 2001). For example, an experiment by Hallam (1970) showed that surfaces of Eucalyptus leaves, from which epicuticular waxes were artificially removed, regenerated quantities of waxes equivalent to those on control leaves (without wax removal) in 24 hours. A similar experiment by Jetter and Schäffer (2001) using the leaves of Prunus laurocerasus (English laurel) also documented complete restoration of surface wax-coatings within 3 days after artificial removal. The prevalence of n-alkanes
in Ordy Pond sediments, despite the absence of continuous fluvial transport of plant debris, thus can be explained by abundant leaf wax contents of the surrounding vegetation, constant aeolian transport of leaf waxes over the pond aided by the prevailing trade-winds, as well as simultaneous and rapid wax regeneration at leaf surfaces.

3.5.3 Biochemistry and Isotope Effects of Photosynthesis

Figure 3.6 schematically describes the fundamental biochemical reactions involved in the different photosynthetic pathways (C₃, C₄ and CAM-photosynthesis) and lipid synthesis. $\delta^{13}C_{\text{N-Alex}}$ values reflect the unique isotope effects involved in each mode of photosynthesis. C₃ plants utilize the Calvin-Benson cycle for fixation of CO₂ during photosynthesis, in which CO₂ is fixed by carboxylation of ribulose-1, 5-bisphosphate (RuBP). The reaction is catalyzed by an enzyme, ribulose-1, 5-bisphosphate carboxylase oxygenase (rubisco). The name C₃ photosynthesis originates from the fact that the first stable metabolic intermediate molecules in the Calvin-Benson cycle are the three-C compounds (Furbank & Taylor, 1995; Hayes, 2001). CO₂ fixation in C₄-photosynthesis, on the other hand, is a two-step process. In the mesophyll cells, carboxylation of phosphoenolpyruvate (REP), with the aid of the phosphoenolpyruvate carboxylase (REPCase) enzyme, fixes CO₂ into oxaloacetate (a four-C compound), which is converted to malate. Malate is subsequently transported to the bundle-sheath cells, where de-carboxylated CO₂ from malate is re-fixed by rubisco (O'Leary, 1981; Furbank & Taylor, 1995; Keeley & Rundel, 2003). CO₂ fixation by CAM (Crassulacean Acid Metabolism) plants is both spatially and temporarily segregated. During the night time, when the stomata are opened for gas-exchange, CO₂ is fixed by REPCase to malic acid,
Figure 3.6. Schematic diagram of C<sub>3</sub>, C<sub>4</sub> and CAM photosynthetic pathways and lipid synthesis via acetogenic pathway (modified from Furbank & Taylor, 1995; Hayes, 2001; Keeley & Rundel, 2003; Chikaraishi et al., 2004).

Abbreviations
RuBP (Ribulose-1, 5-bisphosphate)
Rubisco (Ribulose-1, 5-bisphosphate carboxylase oxygenase)
REP (Phosphoenolpyruvate)
REPCase (Phosphoenolpyruvate carboxylase)
PGA (3-Phosphoglyceric acid)
G3P (Glyceraldehyde 3-phosphate)
which is temporally stored in the vacuole. CO₂ regenerated from this organic acid is re-
fixed by rubisco during the following day time when stomata are closed (O'Leary, 1981; 
Keeley & Rundel, 2003). Overall kinetic isotope effects of the different photosynthetic 
pathways largely reflect the distinct levels of isotopic discrimination against ¹³C by 
rubisco and REPcase (O'Leary, 1981; Farquhar et al., 1989).

A study by Collister et al. (1994) reported that δ¹³C values of bulk leaves of C₃ 
and C₄ plants that ranged from -27.6 to -30.5‰ and -10.7 to -11.9‰, respectively. CAM 
plants could potentially show greater variability in isotopic composition as they are 
capable of both C₃ and C₄ modes of photosynthesis. O'Leary (1981) suggested that, 
theoretically, CAM plants have δ¹³C values near those of C₃ plants (~ -27‰) when they 
function strictly in the C₃ mode, or those of C₄ plants (~ -13‰) when they are engaging 
only in dark fixation.

It is well known that lipids have significant ¹³C depletion relative to the “bulk” 
isotopic compositions of organisms (DeNiro & Epstein, 1977; Hayes, 1993). The initial 
step of lipid synthesis in plants takes place at a branch-point in the Calvin-Benson cycle, 
where glyceraldehyde 3-phosphate (G3P) is transformed to pyruvate (see Figure 3.6). 
Pyruvate is subsequently converted to acetyl coenzyme A (acetyl-CoA) from which n-
alkyl lipids such as n-alkanes are synthesized by oxidative decarboxylation. This reaction 
is catalyzed by an enzyme, pyruvate-dehydrogenase, that is known to result in significant 
kinetic isotope effects leading to ¹³C depletion (Hayes, 1993, 2001). Offsets between δ¹³C 
values of n-alkyl lipids and those of bulk leaf tissue, therefore, largely reflect the ¹³C 
depletion associated with formation of acetyl-CoA from pyruvate. In addition, further 
isotopic shifts may occur at many branch-points during elongation of the C-chain in the
acetogenic pathway (Lockheart et al., 1997; Chikaraishi et al., 2004). The extent of $^{13}$C depletion in n-alkanes relative to total leaf tissue ranges from 1.6 to 13.8‰ with an average of 7.6‰ for the plants analyzed by Collister et al., (1994). $\delta^{13}$C analyses of leaf waxes from a variety of terrestrial higher plants from previous studies (Collister et al., 1994; Chikaraishi & Naraoka, 2003; Chikaraishi et al., 2004; Bi et al., 2005) have shown that, on average, $\delta^{13}$C values of n-alkanes derived from C$_3$, C$_4$ and CAM plants range from -31.9 to -35.5‰, -20.1 to -21.5‰ and -24.9 to -27.4‰, respectively.

### 3.5.4 C-Fixation and Plant Ecology

Most land plants fix CO$_2$ via C$_3$-photosynthesis, and thus the overall abundance of C$_3$ plants outnumbers those of both C$_4$ and CAM plants (Furbank & Taylor, 1995). Virtually all trees and some cool-season grasses are C$_3$ plants (Schefuß et al., 2003). In C$_3$-photosynthesis, rubisco catalyzes the reaction between CO$_2$ and RuBP for C-fixation (see Figure 3.6). But rubisco also catalyzes the fixation of O$_2$ in a process known as photorespiration (Furbank & Taylor, 1995; Hayes, 2001). When the internal O$_2$ concentrations of plants are high, as they are likely to be under high light and temperature conditions, photorespiration competes for rubisco with carboxylation of CO$_2$ (Hayes, 2001). Decline in photosynthetic efficiency caused by photorespiration can be exacerbated in hot dry environments where the gas exchange through stomata is minimized to reduce water loss by evapotranspiration. Stomatal closures result in O$_2$ accumulation in the leaves and reduced CO$_2$ intake into the cell.

The C$_4$-photosynthetic pathway represents an evolutionary advancement from the ancestral C$_3$-photosynthesis. C-concentrating mechanisms in C$_4$ plants reduce the
effect of photorespiration (Ehleringer et al., 1997). C₄ plants initially fix CO₂ into malate, which is de-carboxylated to CO₂ in the bundle-sheath cells. Activity of REPCase, which catalyzes the initial C fixation in C₄-photosynthesis, is not inhibited by O₂, and thus it can act as a strong CO₂ pump into the bundle-sheath cells (Keeley & Rundel, 2003).

Impermeability of the bundle-sheath cells allows the accumulation of CO₂ to a level as high as 1 mM (~700 times higher than the internal CO₂ concentration that is in equilibrium with atmospheric CO₂) around rubisco (Hayes, 2001). Shrubs, grasses and some sedges (Cerling et al., 1993; Ehleringer et al., 1997; Schefuß et al., 2003), as well as a few important agricultural crops such as maize, sugarcane and sorghum, are a few examples of C₄ plants (Furbank & Taylor, 1995).

On a geologic timescale (i.e., on the order of a million years), atmospheric CO₂ concentration is an important selective factor determining the successions of C₃ and C₄ plants (Ehleringer et al., 1997). C-concentrating mechanisms provide significant ecological advantages to C₄ plants over C₃ plants under low atmospheric CO₂ concentrations (Kuypers et al., 1999). Considerable expansion of C₄ plants at the Cenomanian/Turonian boundary (~90 million years ago) of the Cretaceous period (Kuypers et al., 1999) and during the late Miocene around 7 million years ago (Cerling et al., 1993) coincide with significant reductions in the atmospheric CO₂ concentration. Due to the high energy requirement of the C-concentrating mechanisms of C₄-photosynthesis, however, C₄ plants have lower photosynthetic efficiency relative to C₃ plants (Kuypers et al., 1999). Therefore, under high atmospheric CO₂ concentrations, C₃ plants are at disadvantage owing to high photosynthetic efficiency, and ecosystems tend to be dominated by C₃ plants (Kuypers et al., 1999). For example, abrupt vegetation changes
have been reported for the last deglaciation, which was accompanied by an increase of
the atmospheric CO$_2$ concentration by as much as 80 ~ 100 ppmv in 8,000 years (Anklin
et al., 1997; Smith et al., 1999). Cole and Monger (1994) observed rapid replacement of a
preexisting C$_4$-predominant ecosystem by C$_3$ plants during the last deglaciation based on
C and O isotopic analyses on a paleosol from south-central New Mexico. Huang et al.
(2006) similarly reported substantially greater abundance of C$_4$ plants during the Last
Glacial Maximum (LGM) than the Holocene based on the CSIA of sedimentary leaf
waxes of a sinkhole lake in central Florida. Both studies attributed the transition from C$_4$-
dominated flora to a C$_3$-dominated system during the last deglaciation to the large
increase in atmospheric CO$_2$ concentration. Model simulation by Collatz et al. (1998)
also indicates substantial global expansion of C$_4$ grasses under the atmospheric CO$_2$ level
of the last glacial maximum relative to their modern global distribution. Furthermore,
Polley et al. (1993) reported a positive correlation between overall biomass production of
C$_3$ plants and an increase in the ambient CO$_2$ concentration in their laboratory
experiments.

The increase in atmospheric CO$_2$ concentration from the LGM to the beginning
of the Holocene (see Figure 3.7), which resulted in significant alterations in the relative
dominance of C$_3$ and C$_4$ vegetations (i.e., Cole and Monger, 1994; Huang et al., 2006),
was on the order of 80 ppmv (Smith et al., 1999). Reconstruction based on air bubbles
trapped in ice cores, however, does not reveal such significant changes in the atmospheric
CO$_2$ concentration for the Holocene. For example, the Taylor Dome ice core, Antarctica,
shows changes in the atmospheric CO$_2$ level of as little as roughly 20 ppmv during the
Holocene (Indermühle et al., 1999; Smith et al., 1999), as shown in Figure 3.7.
Consequently, it is questionable if the magnitude of Holocene atmospheric CO₂ change was significant enough to be an important selective factor modulating the ecological success of C₃ and C₄ plants. Given relative stability in the atmospheric CO₂ concentrations, climatic factors largely governed by temperature and precipitation seem to be the essential factors influencing the natural abundance of C₃ and C₄ plants for the Holocene. Today, C₄ plants outperform C₃ plants in hot and dry environments due to the advantage of the C-concentrating mechanism and better water use efficiency (Epstein et al., 1997), and thus they are widely distributed in a variety of environments including savannas, semi-desert and grasslands in the tropics and subtropics (Schefuß et al., 2003).
3.5.5 Sedimentary $n$-Alkane Records from Ordy Pond

When the climate shifts toward arid conditions, one would expect to see an increase in $\delta^{13}C$ compositions of plant organic matter, including $n$-alkanes. This change in isotopic composition can be due to reduced isotope effect associated with C$_3$-photosynthesis and/or an increase in the relative abundance of C$_4$ plants in the vegetative community. Under a water stress, C$_3$ plants tend to reduce stomatal conductance in order to minimize evaporative losses of intercellular water, which enhances water use efficiency (Ehleringer et al., 1997; Winner et al., 2004). Reduced stomatal aperture, however, simultaneously decreases the intercellular CO$_2$ level in C$_3$ plants. It is known that the extent of overall isotope effect associated with C$_3$-photosynthesis (largely reflecting the discrimination against $^{13}CO_2$ by rubisco) is reduced linearly with decreasing intercellular CO$_2$ level (i.e., Farquhar and Richards, 1984; Farquhar et al., 1989; Martin and Thorstenson, 1988; Winner et al., 2004). In other words, when intercellular CO$_2$ is low, the leaf assimilates more $^{13}CO_2$ relative to $^{12}CO_2$ than when intercellular CO$_2$ is high, leading to increase in $\delta^{13}C$ values.

C$_4$ plants have better water use efficiency owing to the C-concentrating mechanism, which allows them to maintain high intercellular CO$_2$ levels even upon stomatal closure. Therefore, under arid conditions (where C$_3$ plants are at a disadvantage because of reduced photosynthesis owing to the reduced stomatal conductance), C$_4$ plants are favored over C$_3$ plants in ecosystem. It is not possible from $\delta^{13}C$ data alone to distinguish between reduced stomatal conductance of C$_3$ plants and a shift from C$_3$ to C$_4$ dominated vegetation. In both case, however, an increase in $\delta^{13}C_{N-Alks}$ values would reflect increased aridity. In this study, variations in the $\delta^{13}C_{N-Alks}$ in Ordy Pond sediments
are interpreted to reflect changes in vegetative structure in terms of C3 and C4 plants in response to climatic changes, although a contribution of reduced stomatal conductance in C3 plants would lead to the same conclusions concerning climatic change.

Figure 3.5 shows the changes in $\delta^{13}C_{\text{n-alk}}$ ($n$-C$_{27} - n$-C$_{33}$) from Ordy Pond sediments over time. $\delta^{13}C_{\text{n-alk}}$ of Ordy Pond sediments varied between -33.3 to -20.5%, suggesting varying degrees of the mixing of $n$-alkanes originating from C$_3$ (isotopically more depleted) and C$_4$ plants (isotopically more enriched).

The isotopic mass-balance approach allows one to assess quantitatively relative abundances of each end-member in a given sample. Fractional contributions of $n$-alkanes from C$_3$ and C$_4$ plants to the total sedimentary $n$-alkanes in Ordy Pond can be partitioned by a simple two end-member mixing model:

$$\delta_T = F_{C3} \delta_{C3} + F_{C4} \delta_{C4}$$  (Eq. 3.3)

and

$$F_{C3} + F_{C4} = 1$$  (Eq. 3.4)

where $\delta$ represents the isotopic composition of $n$-alkanes and $F$ denotes the fractional abundance of each component. Subscripts $T$, $C3$ and $C4$ symbolize total sample $n$-alkanes (extracted from the sediments and analyzed by irm-GC/MS), and $n$-alkanes from C$_3$ plants and C$_4$ plants, respectively. $F_{C4}$ provides a measure of the fraction of total $n$-alkanes originating from C$_4$ plants, and can be interpreted as the abundance of C$_4$ plants within the local vegetative community at a given time.
The isotopic compositions of the C\textsubscript{3} and C\textsubscript{4} end-members (\(\delta_{\text{C3}}\) and \(\delta_{\text{C4}}\), respectively) were determined based on the averages of \(\delta^{13}\text{C}_{\text{N-alk}}\) values for a variety of living specimens of C\textsubscript{3} and C\textsubscript{4} plants reported in the literature (Collister et al., 1994; Chikaraishi & Naraoka, 2003; Bi et al., 2005). Given the considerable geographic isolation of Hawai‘i and the resultant high degree of endemism, however, specimens that are characteristic of or unique to much higher latitudes, such as those belonging to the Aceraceae family (maple group) and Fagaceae family (oak group), were eliminated. In addition, specimens showing strong endemism to certain geographic locations other than Hawai‘i were also removed. The plants database filed by the United States Department of Agriculture (http://www.plants.usda.gov) was used to search common distributions and ecological characteristics of the specimens examined in the literature referenced above. \(\delta^{13}\text{C}_{\text{N-alk}}\) values of the specimens that satisfy these criteria were averaged to obtain the isotopic signature of the C\textsubscript{3} and C\textsubscript{4} end-members (Table 3.2). Mass-balance calculations were separately conducted on each individual n-alkane (n-C\textsubscript{27}, n-C\textsubscript{29}, n-C\textsubscript{31} and n-C\textsubscript{33}) to account for potential isotopic variations of n-alkanes of different C chain-length.

Contributions of n-alkanes from CAM plants are neglected in the model. CAM plants are commonly found in desert environments and mainly include species such as Cactaceae family (cacti), Agavaceae family (yucca and agaves), and Ananas comosus (pineapple), or a few ornamental species including some orchids (Orchidaceae family) (Cushman, 2001). Some of these species, particularly pineapple which has been one of the most important local commercial crops, are commonly found in Hawai‘i today due to post-colonial introduction by European and American settlers. But these species are not originally native to Hawai‘i. The only known species possibly capable of CAM C-
fixation among the native Hawaiian flora is *Sesuvium portulacastrum*, commonly known as Sea Purslane, that mainly occupies coastal sand dunes (*Clifford W. Morden*, personal communication). Thus the influence of n-alkanes derived from CAM plants in Ordy Pond sediments should be negligible prior to the late 1800's.

Table 3.2. $\delta^{13}$C compositions of the individual n-alkanes from C3 and C4 plants end-members.

<table>
<thead>
<tr>
<th></th>
<th>$\delta^{13}$C</th>
<th>C3 Plants</th>
<th></th>
<th>$\delta^{13}$C</th>
<th>C4 Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$-C25</td>
<td>-34.8 ±3.4</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$-C27</td>
<td>-35.9 ±2.2</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$-C29</td>
<td>-36.4 ±1.8</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$-C31</td>
<td>-36.6 ±2.3</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2. $\delta^{13}$C compositions of the individual n-alkanes from C3 and C4 plants end-members.

† Values are the average $\delta^{13}$C compositions of n-alkanes derived from leaf waxes by C3 and C4 plant specimens reported by *Collister et al.*, (1994), *Chikaraishi and Naraoka*, (2003) and *Bi et al.*, (2005).

‡ $\delta^{13}$C$_{N-alk}$ values obtained from some specimens that are unique to higher latitudes or that show strong endemism to certain geographic areas other than Hawai‘i were removed.

a Average $\delta^{13}$C values
b Standard Deviation
c Number of specimens

Variations in the $\delta^{13}$C$_{N-alk}$ values between individual species are relatively large within plant groups possessing the same C fixation mechanism (*Collister et al.*, 1994). Furthermore, considerable isotopic variations are known to occur even for a single species depending on the seasons and a variety of environmental factors (*Lockheart et al.*, 1997). Not surprisingly, standard deviations of $\delta^{13}$C$_{N-alk}$ values from selected C3 and C4 specimens from the literature ranged from 1.8 to 3.5‰; this range frequently exceeds the analytical uncertainties of irm-GC/MS analyses (see Table 3.1). The standard deviations associated with the $\delta^{13}$C values of the end-members are not accounted for by the isotopic
mass-balance model itself. Therefore, in order to report realistic ranges of uncertainties, the intrinsic variability of n-alkanes synthesized by plants (uncertainties involved in the $\delta^{13}C_{N\text{-alks}}$ of each end-member), as well as analytical uncertainties, need to be properly propagated through the process of isotopic mass-balance calculations. The general scheme of error propagation was modified from the model developed for blank correction for isotope measurements by Hayes (1983). Detailed descriptions are also found in Phillips & Gregg (2001).

First, Eq. 3.3 and 3.4 can be rearranged to:

$$F_{C_4} = \frac{\delta_T}{\delta_{C_4} - \delta_{C_3}} - \frac{\delta_{C_3}}{\delta_{C_4} - \delta_{C_3}} \quad \text{(Eq. 3.5)}$$

Then, propagation of errors can be performed based on the standard deviations of the terms contributing to $F_{C_4}$ using partial derivatives as:

$$\sigma^2_{F_{C_4}} = \left( \frac{\partial F_{C_4}}{\partial \delta_T} \right)^2 \sigma^2_{\delta_T} + \left( \frac{\partial F_{C_4}}{\partial \delta_{C_3}} \right)^2 \sigma^2_{\delta_{C_3}} + \left( \frac{\partial F_{C_4}}{\partial \delta_{C_4}} \right)^2 \sigma^2_{\delta_{C_4}} \quad \text{(Eq. 3.6)}$$

where lower case Greek sigmas ($\sigma$) are used to denote standard deviations and the square of a standard deviation ($\sigma^2$) is referred to as a variance. Because duplicate irm-GC/MS analyses were not conducted for the majority of the samples, reproducibility of the instrument (reported on a daily basis) determined from the co-injected internal standard is used to calculate $\sigma^2_{\delta_T}$. Numerical values for $\sigma^2_{\delta_{C_3}}$ and $\sigma^2_{\delta_{C_4}}$ are based on the standard deviations calculated from $\delta^{13}C_{N\text{-alks}}$ values from selected C$_3$ and C$_4$ specimens (Table 3.2). Eq. 3.6 can be expanded to (see Appendix):
FC4 (Eq. 3.3 and 3.4, or Eq. 3.5) and $\sigma^2_{FC4}$ (Eq. 3.7) values determined for each individual $n$-alkane ($n$-C$_{27}$, $n$-C$_{29}$, $n$-C$_{31}$ and $n$-C$_{33}$) were utilized to obtain weighted mean values of FC4. The weighted means and accompanying standard deviations are calculated as:

$$\text{Weighted Mean} = \frac{\sum \left( \frac{F_{C4}}{\sigma^2_{FC4}} \right)_{n-C_{27}, n-C_{29}, n-C_{31}, n-C_{33}}}{\sum \left( \frac{1}{\sigma^2_{FC4}} \right)_{n-C_{27}, n-C_{29}, n-C_{31}, n-C_{33}}}$$

(Eq. 3.8)

and

$$\text{Std. Dev} = \left[ \sum \left( \frac{1}{\sigma^2_{FC4}} \right)_{n-C_{27}, n-C_{29}, n-C_{31}, n-C_{33}} \right]^{-0.5}$$

(Eq. 3.9)

Finally, the weighted mean and standard deviation, calculated as fractions, are reported as percentages and are referred to as "% C$_4$ abundance" hereafter.

Figure 3.8 shows changes in % C$_4$ abundance throughout the Holocene period. Before interpretation of these data, it is appropriate to address one fundamental question: does the % C$_4$ abundance estimated from the methods and the isotopic mass-balance approach applied in this study faithfully record the changes in vegetative community over time? Or more simply, is this a valid proxy to elucidate paleovegetative changes?

Calibration tests such as a comparison of $\delta^{13}$C values of $n$-alkanes extracted from the surface sediments or organic particulates within the water column and $\delta^{13}$C
Figure 3.8. Variations in the % C₄ abundance during the Holocene. The % C₄ abundance was calculated as the weighted mean of the four separate estimates based on δ¹³C values of the individual n-alkanes (n-C₂₇, n-C₂₉, n-C₃₁ and n-C₃₃). The red error bars indicate the probable ranges of errors in the estimates caused by the analytical errors associated with the irm-GC/MS. The blue error bars denote combined errors (both analytical errors and the uncertainties in the end-member δ¹³C values) that were propagated through the isotopic mass-balance calculations. Four distinct Zones were established based on the trends observed in the % C₄ abundance: Zone A (~9,940 to ~9,300 cal.yrBP), Zone B (~9,300 to ~4,800 cal.yrBP), Zone C (~4,800 to ~1,250 cal.yrBP) and Zone D (~1,250 cal.yrBP and onward).
values of \( n \)-alkanes sampled from the modern locally-representative \( C_3 \) and \( C_4 \) plants found in the surrounding areas were beyond the scope of this study. Nonetheless, close examination of the \% \( C_4 \) contribution for the sediments younger than \( \sim 1,250 \) cal.yrBP (Zone D in Figure 3.8) should be considered as a reasonable substitute for a modern calibration test due to (1) comparatively dense sample-coverage (see Figure 3.8), (2) highly reliable chronology for the corresponding portion of sediments (Uchikawa et al., 2006) and (3) availability of archaeological, paleoenvironmental and historical data for the corresponding period. The timing of initial arrival of the Polynesians to Hawai‘i, as well as significant changes in the pristine island ecosystem during the post-Polynesian era, have been of great interest to archaeologists. Consequently, reliable archaeological and paleoenvironmental studies (including pollen reconstructions) are available.

Zone D in Figure 3.8, which was deposited after the initial arrival of the Polynesians to Hawai‘i (after \( \sim 1,250 \) cal.yrBP), is further sub-divided into Zones 1, 2 and 3 (Figure 3.9). These zones correspond to (1) the probable age range of the first arrival of the Polynesians (Zone 1: \( \sim 1,250 \) to \( \sim 950 \) cal.yrBP), (2) post-Polynesian era (Zone 2: \( \sim 950 \) to \( \sim 130 \) cal.yrBP) and (3) post-European era (Zone 3: \( \sim 130 \) cal.yrBP and onward), respectively.

Many archaeological surveys report that the first settlement of the Hawaiian Archipelago by the Polynesians occurred sometime between 1,250 and 1,150 cal.yrBP (e.g., Athens et al., 1992 and 2002; Vitousek et al., 2004). But there are also other lines of archaeological evidence that suggest initial Polynesian arrival from 1,050 to as late as 950 cal.yrBP (Stephen J. Athens, personal communication). Therefore, the period from 1,250 to 950 cal.yrBP was assigned as the probable age range for the first arrival of the
Figure 3.9. Variations in the % C₄ abundance after ~1,250 cal.yrBP (Zone D). This portion of the record is further divided into three separate periods. Zone 1 (from ~1,250 to ~950 cal.yrBP) corresponds to the possible age range of the first arrival of the Polynesians to Hawai'ī. Zone 2 extends from ~950 to ~130 cal.yrBP and represents the post-Polynesian era. Finally, Zone 3 starting from ~130 cal.yrBP covers the post-European contact era.
Polynesians to Hawai‘i (Zone 1). During this time interval, the % C₄ abundance ranged from 61.8 to 67.3%. Also, the estimates of the % C₄ abundance in Zone 1 are not significantly different from the estimates at the end of Zone C and at the beginning of Zone 2 (these four estimates cluster in the 60 to 70% range), which implies that the extent of anthropogenic influence on the local environment was relatively minor in the early stage of Polynesian settlement. Pollen records from Ordy Pond sediments (Athens et al., 1999, 2002) indicate the existence of a mixed dryland forest in the area during the corresponding period (see Figure 3.10). Table 3.3 shows the list of pollen groups identified from Ordy Pond sediments by Athens et al. (1999, 2002) and the family names to which these pollen groups belong. Also, Table 3.4 lists the plant families that are known to contain C₄ taxa. The pollen reconstructions show dominant signals of woody plants such as the *Pritchardia* group (Palm group: C₃), *Dodonaea viscosa* (Florida Hopbush: C₃) and *Kanaloa kahoolawensis* (Kanaloa: C₃) as well as signals of weedy grasses from the *Poaceae* and *Cheno-am* group. The *Poaceae* family includes common grass species, whereas the *Cheno-am* pollen type is defined as a morphologically similar group of pollen grains belonging to the *Chenopodiaceae* and/or *Amaranthaceae* families that include a variety of weedy herbaceous plants (both C₃ and C₄ plants; see Table 3.3 and Table 3.4). These pollen assemblages imply that the woody plants formed the main canopy of the forest, the understory of which was abundantly occupied by a variety of grasses and herbaceous weeds. It is reported that about 40% of species belonging to the *Poaceae* family worldwide utilize the C₄ pathway, and among the grass species native to Hawai‘i, 62% of the species in the *Poaceae* family belong to C₄ taxa (Rundel, 1980). Thus, the % C₄ abundance of 60-70% estimated from the n-alkane measurement seems to
Figure 3.10. Vertical profiles of several pollen groups from Ordy Pond sediments analyzed by Athens et al., 2002. Cheno-am and Poaceae groups can be considered as the pollen-based representatives of C₄ taxa, whereas Dodonaea viscosa, Kanaloa kahoolawensis and Pritchardia are the most abundant pollen groups representing C₃ vegetation (see Table 3.3 and 3.4) in the sediment. Three sub-zones (1, 2 and 3 on the right) correspond to the ones shown in Figure 3.9. Pollen diagrams are plotted in silhouette style and the hatching indicates the ten-fold exaggeration (see Athens et al., 2002 for details).
Table 3.3. List of pollen types identified from Ordy Pond sediments by Athens et al. (1999, 2002).

<table>
<thead>
<tr>
<th>Pollen from Ordy Pond sediments</th>
<th>Type</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhizophora</strong></td>
<td>Mangrove</td>
<td>Rhizophoraceae</td>
</tr>
<tr>
<td>Acacia Koa</td>
<td>Dry - Mesic Forest</td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Albizia</td>
<td></td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Aleurites moluccana</td>
<td></td>
<td>Euphorbiaceae</td>
</tr>
<tr>
<td>Asteraceae</td>
<td></td>
<td>Asteraceae</td>
</tr>
<tr>
<td>Chamaesyce</td>
<td></td>
<td>Euphorbiaceae</td>
</tr>
<tr>
<td>Cheno-am</td>
<td></td>
<td>Chenopodiaceae</td>
</tr>
<tr>
<td>Achyranthes splendens</td>
<td></td>
<td>Amaranthaceae</td>
</tr>
<tr>
<td>Cocos nucifera</td>
<td></td>
<td>Arecaceae</td>
</tr>
<tr>
<td>Dodonaea viscosa</td>
<td></td>
<td>Sapindaceae</td>
</tr>
<tr>
<td>Kanaioa kahoolawensis</td>
<td></td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Leucaena leucocephala</td>
<td></td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td></td>
<td>Myrtaceae</td>
</tr>
<tr>
<td>Nestegis sandwicensis</td>
<td></td>
<td>Oleaceae</td>
</tr>
<tr>
<td>Pouteria</td>
<td></td>
<td>Sapotaceae</td>
</tr>
<tr>
<td>Pritchardia</td>
<td></td>
<td>Arecaceae</td>
</tr>
<tr>
<td>Prosopis pallida</td>
<td></td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Sesbania tomentosa</td>
<td></td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Solanum</td>
<td></td>
<td>Solanaceae</td>
</tr>
<tr>
<td>Waltheria indica</td>
<td></td>
<td>Sterculiaceae</td>
</tr>
<tr>
<td>Myrsine</td>
<td>Mesic Forest</td>
<td>Myrsinaceae</td>
</tr>
<tr>
<td>Rubiaceae (Triporate Type)</td>
<td></td>
<td>Rubiaceae</td>
</tr>
<tr>
<td>Tetraplasandra comp</td>
<td></td>
<td>Araliaceae</td>
</tr>
<tr>
<td>Ambrosia artemisifolia</td>
<td>Herbs</td>
<td>Asteraceae</td>
</tr>
<tr>
<td>Asteraceae (Lactuceae)</td>
<td></td>
<td>Asteraceae</td>
</tr>
<tr>
<td>Cyperaceae (Sedge)</td>
<td></td>
<td>Cyperaceae</td>
</tr>
<tr>
<td>Cyperaceae (reticulate type)</td>
<td></td>
<td>Cyperaceae</td>
</tr>
<tr>
<td>Poaceae (Grass)</td>
<td></td>
<td>Poaceae</td>
</tr>
<tr>
<td>Ruppia maritima</td>
<td></td>
<td>Ruppiaceae</td>
</tr>
</tbody>
</table>

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Table 3.3. Continued.

<table>
<thead>
<tr>
<th>Pollens from Ordy Pond sediments</th>
<th>(Athens et al., 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pollen</strong></td>
<td><strong>Type</strong></td>
</tr>
<tr>
<td><em>Euphorbiaceae</em> (Unknown Type)</td>
<td>Unknowns</td>
</tr>
<tr>
<td><em>Fabaceae</em> (Unknown Type)</td>
<td></td>
</tr>
<tr>
<td><em>Monolete granulate</em></td>
<td></td>
</tr>
<tr>
<td><em>Monolete psilate</em></td>
<td></td>
</tr>
<tr>
<td><em>Trilete echinate</em></td>
<td></td>
</tr>
<tr>
<td><em>Trilete psilate</em></td>
<td></td>
</tr>
</tbody>
</table>

† *Cheno-am* pollen type refers to morphologically similar (and thus indistinguishable) pollen grains belonging to the *Chenopodiaceae* and/or *Amaranthaceae* families.
Table 3.4. List of plant families that are known to contain C₄ taxa (from Ehleringer et al., 1997).

<table>
<thead>
<tr>
<th>C₄ distribution</th>
<th>Family Name</th>
<th>Order</th>
<th>Examples (Genera)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aizoaceae</td>
<td>Caryophyllanae</td>
<td>Cypselea, Gisekia, Trianthema, Zealety, Acanthochiton, Aerva, Alteranthera, Amaranthus, Bryunina, Froelichia, Gomphrena, Gossypianthus, Lithophila, Tidestromia.</td>
</tr>
<tr>
<td></td>
<td>Caryophyllaceae</td>
<td>Polycarpae</td>
<td>Glinis, Mollugo.</td>
</tr>
<tr>
<td></td>
<td>Chenopodiaceae</td>
<td>Anabinae</td>
<td>Allionia, Boerhaavia, Okenia.</td>
</tr>
<tr>
<td></td>
<td>Portulaceae</td>
<td>Polygonales</td>
<td>Polygona, Portulaca.</td>
</tr>
<tr>
<td></td>
<td>Polygonaceae</td>
<td>Euphniaceae</td>
<td>Chamaesyce, Euphorbia.</td>
</tr>
<tr>
<td></td>
<td>Capparaceae</td>
<td>Brassicales</td>
<td>Gynandroptis.</td>
</tr>
<tr>
<td></td>
<td>Zygophyllaceae</td>
<td>Linales</td>
<td>Kalstroemia, Tribul, Zygophyllum.</td>
</tr>
<tr>
<td></td>
<td>Asteraceae</td>
<td>Asterales</td>
<td>Fiaveria, Glossocordia, Glossogyne, Isostigma, Pectis.</td>
</tr>
<tr>
<td></td>
<td>Boraginaceae</td>
<td>Solanales</td>
<td>Heliotropium</td>
</tr>
<tr>
<td></td>
<td>Convolvulaceae</td>
<td>Solanales</td>
<td>Evolurus</td>
</tr>
<tr>
<td></td>
<td>Acanthaceae</td>
<td>Scrophulariales</td>
<td>Belpharis</td>
</tr>
</tbody>
</table>

121
Table 3.4. Continued.

<table>
<thead>
<tr>
<th>Family Name</th>
<th>Order</th>
<th>Examples (Genera)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyperaceae</td>
<td>Juncales</td>
<td>Ascolepis, Bulbostyli s, Crosslandia, Cyperus, Eleocharis, Fimbristyli s, Kyllinga, Lipocarpha, Mariscus, Pecraus, Rhynchospora.</td>
</tr>
<tr>
<td>Poaceae</td>
<td>Poales</td>
<td>Alloteropsis, Andropogon, Arundinella, Bouteloua, Cynodon, Echinochloa, Leptochloa, Microstegium, Panicum, Paspalum, Setaria, Sorghum, Spartina, Sporobolus, Zea And many more genera.</td>
</tr>
</tbody>
</table>

agree reasonably with the descriptive features of the dryland forest based on the pollen records.

During the post-Polynesian era (Zone 2: ~950 to ~130 cal.yrBP), the % C₄ abundance surged roughly from 60 to 90%. The pollen records (Athens et al., 1999, 2002) show abrupt declines in trees and shrubs, including Pritchardia, Dodonaea viscosa and Kanaloa kahoolawensis, at approximately 930 cal.yrBP. The dryland forest that flourished before and during the early phase of Polynesian arrivals to Hawai‘i seems to have been quickly replaced by grass communities as suggested by a coincident increase in the Cheno-am and Poaceae pollens after ~930 cal.yrBP (see Figure 3.10). Dramatic change in the local vegetative structure during this period can be attributed to significant human settlements in the area and subsequent anthropogenic activities such as land
cultivation. The first appearance of pollen from the *Cocos nucifera* (Coconut: C₃), an anthropogenic indicator, also around 930 cal.yrBP is direct evidence of significant human settlements in the area. Concurrent emergence of charcoal in the sediments suggests land clearing activities by the initial settlers with the use of fire. The shorter life-span or turnover time of common grasses enables them to respond to physical disturbance such as wildfire much faster than woody plants (*Collatz et al.*, 1998). Consequently, land disturbances caused by human activities seem to cause the expansion of grass species. These paleovegetative changes inferred from pollen and archaeological evidence are consistent with the observed trend of increase in % C₄ abundance in Zone 2.

C₄ plants continued to dominate (> 80%) the local vegetation during the post-European era (Zone 3: since ~130 cal.yrBP). Even before the establishment of well-organized formal cattle ranching in the 'Ewa Plain in the 1870s (~130 cal.yrBP), the area was under the influence of widespread wild cattle in the beginning of 19th century (~200 cal.yrBP) (*Athens et al.*, 1999). The pollen record by *Athens et al.* (1999, 2002) illustrates dominance of the Poaceae family from ~150 to ~90 cal.yrBP, after which these grasses decreased dramatically (see Figure 3.10). The domination of the area by grasses during this era was most likely due to physical disturbances of the surface soil by the cattle; however, sporadic occurrences of charcoal in the sediments also indicate additional disturbances by either anthropogenic land clearing or natural wildfire. Estimates of the abundance of C₄ plants based on isotopic analyses, again, show a reasonable consistency with the pollen records in Zone 3.

In summary, examination of the % C₄ abundance in Zone D, which spans from the initial Polynesian arrival to the post-European era, illustrates remarkable agreement
with the pollen records as well as other archaeological evidence. Observed consistency with the pollen records consequently supports the capability of the % C₄ abundance established in this study to elucidate paleovegetative changes.

It is important to note that the % C₄ abundance in Zone D is not a perfect indicator of the natural history of vegetation changes due to imprints of anthropogenic activities, especially in Zones 1 and 2. But the record prior to the arrival of humans in Hawai‘i (Zones A ~ C in Figure 3.8) should strictly reflect the natural successions of C₄ plants. Zone A (from ~9,940 to ~9,300 cal.yrBP) can be characterized as a period of dominance of C₃ plants. Proportions of C₄ plants were less than ~30%. Zone B (from ~9,300 to ~4,800 cal.yrBP) reveals the most significant change in the local vegetation. The % C₄ abundance steadily escalated to almost 80% (the highest level observed for the pre-human part of the record) at ~4,800 cal.yrBP, even though there is a sign of retreat around 7,000 cal.yrBP. The dominance of C₄ plants, however, suddenly crashed to approximately 50% sometime between 4,800 and 4,050 cal.yrBP. This abrupt decline of C₄ plants marks the beginning of Zone C (from ~4,800 to ~1,250 cal.yrBP), in which C₄ plants generally plateaued for almost 2,000 years in the range from 50 to 60%.

As previously discussed, the effect of atmospheric CO₂ concentrations on the ecology of C₃ and C₄ plants seems to be negligible for the Holocene. The reconstructed changes in the atmospheric CO₂ concentrations by Indermühle et al. (1999) show nearly a linear increase by about 20 ppmv throughout the Holocene (see Figure 3.7). When C₃ plants are not stressed by low CO₂ availability, they should outcompete C₄ plants owing to greater photosynthetic efficiency (Kuypers et al., 1999). Thus, a continuous increase in atmospheric CO₂ during the Holocene should favor C₃ plants, if the atmospheric CO₂ is
indeed acting as a significant selective force. The Holocene vegetation in the ‘Ewa Plain, however, generally switched from a C₃-dominated system to a C₄-dominated system with time. Consequently, it suggests that the Holocene paleovegetative changes were largely under the influence of local climatic conditions (mainly a function of temperature and precipitation), rather than atmospheric CO₂ concentrations.

*Teeri and Stowe* (1976) demonstrated a strong correlation between the distribution of C₄ grasses in North America and the minimum temperature of the growing seasons. The authors suggested that the warmer the temperature of the growing season the greater the success of C₄ taxa in North America. More recently, *Epstein et al.* (1997) likewise conducted a statistical study and found a strong correlation between temperature and the success of C₄ grasses in the Great Plains. These observations led to recognition that temperature is the most important climatic parameter governing the natural distribution of C₃ and C₄ plants (*Ehleringer et al.*, 1997; *Cowling and Sykes*, 1999). Nevertheless, the amount of precipitation (or conversely, the extent of aridity) can also be important in affecting the distribution of C₄ plants. For instance, *Clark et al.* (2001) suggested that abundance of C₄ plants increases with a decrease in moisture because of their superior water use efficiency aided by C-concentrating mechanisms. *Paruelo and Lauenroth* (1996) concluded that precipitation and its seasonal distribution are important controls on the relative abundance of C₃ and C₄ grasses in North America. But it is perhaps most realistic to assume that the relative dominance of C₃ versus C₄ plants is dynamic in nature and their sensitivities to temperature and/or precipitation have considerable local variability, as suggested by *Huang et al.* (2006). Different ecosystems should have site-specific, flora-specific and species-specific threshold levels of
temperature and precipitation, which ultimately shape the unique vegetative community in a given geographic area.

Paleovegetative changes in the 'Ewa Plain are more likely to be controlled by changes in the amount of precipitation (or the extent of aridity) than by temperature. A nearly uniform day-length throughout the year and the buffering capacity of the vast Pacific Ocean surrounding the islands result in low seasonal variation of temperature in Hawai'i (Gavenda, 1992), as shown in Figure 3.2. In contrast, rainfall in Hawai'i shows considerable seasonal (i.e., Giambelluca et al., 1986), interannual (i.e., Chu, 1995; Chu and Wang, 1997) and decadal variability (i.e, Chu and Clark, 1999; Lukas, 2001; Chu, 2002). A survey of the distribution of C4 and C3 grasses in Hawai'i by Rundel (1980) illustrates an extreme transition from C4 to C3-dominated flora with increase in elevation. Because the elevation gain in the main Hawaiian Islands typically results in greater precipitation and less evaporation (Giambelluca et al., 1986; Lohse et al., 1995), the survey by Rundel (1980) implies the underlying importance of the extent of aridity for the ecological success of C4 grasses in Hawai'i. Furthermore, based on an observation of the local vegetation on Mauna Loa, Hawai'i, Lohse et al. (1995) suggested that occasional severe drought could serve as a possible mechanism to cause a shift from dominance by woody species to dominance by herbaceous species in the area. These arguments strongly favor aridity as a main factor influencing the ecological success of C4 plants in the 'Ewa Plain.

Further evidence for the importance of aridity as a selective control on the dominance of C4 plants is provided by an intermediate complexity global climate model, ECBILT/CLIO (version 3) described by Timmermann et al. (2004). The model was used
Figure 3.11. Changes in the aridity index (measured as evaporation minus precipitation) and surface air temperature in Hawai`i for the last 20,000 years, shown in blue dots, simulated by the ECBILT/CLIO global climate model (see Timmermann et al., 2004). Results of the model simulation shown in the plots were extracted from a grid (resolution of approximately 5.6° both in latitude and longitude) encompassing Hawai`i. Outputs from the model were filtered with a 500 year running mean, shown by thick red curves, in order to observe general trends of the change in the aridity index and temperature.

to simulate past changes in the surface temperature and evaporation minus precipitation for an area of the Pacific including Hawai`i. The latter parameter simulated by the model (evaporation minus precipitation) can be considered as the aridity index. Figure 3.11 shows reconstructed surface temperature and annual net evaporation for the last 20 kyr. The model simulation shows considerable noise due to internal variability that is intrinsic to the model, as shown by numerous extreme excursions in Figure 3.11. But when the
results of the simulation are filtered by a 500 yr running mean, the general trends of changes in the temperature and aridity become apparent. Reconstructed temperature for the last 20 kyr generally fluctuates within a range of only 0.7 °C. For the Holocene, the model shows a reduction of temperature by only about 0.2 °C for the first 4,000 followed by an irregular increase of about 0.1 °C from -6,000 to 0 yr. The model simulation clearly demonstrates long-term stability in the temperature for the Holocene, supporting the idea that temperature is unlikely to cause significant changes in the vegetative structure. Moreover, the % C₄ abundance demonstrates a steady increase of nearly 50% from -10,000 to -5,000 cal.yrBP. If the increase in temperature was assumed to be the leading factor the expansion of C₄ species, then the trend of the % C₄ abundance and the simulated temperature evidently contradict each other. This decoupling between the % C₄ abundance and temperature strongly suggests that temperature is not the main forcing for the vegetation change during the Holocene.

The aridity index (evaporation minus precipitation) simulated by the model demonstrates that evaporative loss was always in excess of rainfall for the last 20,000 years (see Figure 3.11). The magnitude of the fluctuations of the aridity index for the last 20,000 years is roughly within 4 cm/yr. The aridity index demonstrates an almost continuous increase by about 3 cm/yr from 10,000 to 5,000 years ago, with a noticeable break in the trend roughly from 7,500 to 6,500 years ago. The simulation for the most recent 5,000 years subsequently reveals a steady reduction in the aridity index by 4 cm/yr. The atmospheric component (ECBILT) of the model has an approximate resolution of 5.6° in both latitude and longitude (Timmermann et al., 2004). Therefore, the simulated aridity index may not accurately characterize the extent of aridity on land areas because
considerable geographic variations in the rainfall patterns on land due to orographic
effect that are unique to Hawai‘i (Gavenda, 1992) can not be accounted by the given
resolution of the model. Nonetheless, the model-simulated aridity index is a good
measure of the baseline climatic conditions in the vicinity of Hawai‘i in terms of the net
balance between evaporation and rainfall. The trends of the % C4 abundance generally
match with those of the simulated aridity index (i.e., expansion of C4 plants occurred
contemporaneously with the increase in the aridity index, and vice versa) except for the
record in Zone D which has overwhelming influences of anthropogenic activities.
Although the continuous decrease in the aridity index from the mid to late Holocene
simulated by the model is not perfectly reflected, the % C4 abundance shows slightly
reduced abundance of C4 plants after ~4,800 cal.yrBP. Positive correlation between the %
C4 abundance and simulated aridity index seems to support the dominant role of the
extent of aridity in modulating the vegetative compositions in the vicinity of the study
site. Therefore, it is most probable that the increase in C4 plants is indicative of prolonged
aridity whereas the reduction in the relative abundance of C4 plants suggests the
mitigation of aridity.

Terrestrial records from Hawai‘i that are comparable to the paleovegetative
reconstruction from Ordy Pond sediments are limited to only a few pollen records
covering discrete intervals of the Holocene. Pollen records from Ka‘au Crater, an
explosive volcanic vent in southeastern O‘ahu, (Hotchkiss and Juvik, 1999) were
constructed mainly in order to examine the vegetation changes during the LGM, however,
the records also extend approximately to 7,000 cal.yrBP. A noticeable increase in the
pollen of the Poaceae and Pritchardia and a considerable reduction in the
Grammitidaceae (Fern) spores suggest possible aridification from ~9,700 to ~7,000 cal.yrBP. The corresponding part of the pollen data, however, also contains abundant pollen from species that can be found in relatively moist areas such as the Myrtaceae (Metrosideros-type: 'Ohi'a Lehua: C₃), Myrsine (Colicwood: C₃) and Freycinetia arborea ('ie'ie: C₃). This is perhaps due to a relatively wet baseline climatic condition in the vicinity of the study site. Ka'au Crater is situated on the leeward slope of the Ko'olau Mountain Range at elevation of 303 m, and receives annual rainfall of 340 cm/yr (Hotchikiss and Juvik, 1999). Because the hydrologic patterns in Hawai‘i are largely controlled by orographic effects, the geographic distribution of rainfall on O'ahu is largely determined by the elevation and distance from mountain ridges (Giambelluca et al., 1986, Athens et al., 1995).

During the paleoenvironmental investigation of the 'Ewa Plain, Athens et al. (1999) also collected sediment cores from an evaporate pan near Ordy Pond. The evaporite pan is situated within a slight topographic depression, and occupies a surface area of approximately 0.8 ha (Athens et al., 1999). Ordy Pond and the evaporite pan are separated by approximately 1.2 km. The cumulative depth of the cores extends to 3.3 m below the surface, translating to almost 7,200 years worth of sedimentation history. Comprehensive interpretations of sedimentary records from the evaporite pan are difficult because of the incomplete chronology, which is supported by only two ¹⁴C dates. Nevertheless, the pollen spectra by Athens et al. (1999) for the relatively well-dated section of the core (the bottommost 30 cm of the core: ~7,230 to 6,750 cal.yrBP) interestingly demonstrate a strong signal of the Cyperaceae (Sedges: include both C₃ and C₄ taxa) pollen, perhaps indicating the prevalence of a pond or marshy environment.
during this period. Sedge pollens are most abundant at the base of this section and continuously diminish upcore. The signals are hardly seen after 6,750 cal.yrBP.

Moreover, the decline in the sedge pollen is accompanied by increases in *Cheno-am* and *Pritchardia* pollen. Such pollen evidence perhaps illustrates the presence of wetter climate no later than ~7,230 cal.yrBP as well as an onset of aridification sometime between ~7,230 and ~6,750 cal.yrBP.

Although some sedimentary sequences are unfortunately unavailable due to unconformities (at ~2,200 cal.yrBP and somewhere between ~7,500 and ~2,500 cal.yrBP and between ~450 and ~300 cal.yrBP), a relatively well-dated 27 m core taken from a site in the district of Liliha in downtown Honolulu contains valuable paleoenvironmental records spanning from ~8,800 to ~2,000 cal.yrBP (Athens et al., 1997). Abundant silt, loam and clay as well as biological debris including fragments of marine shells and corals imply a lagoonal origin of the deposits. The authors relate the presence of basalt pebbles and gravels in sediments to the fluvial transport of materials by the nearby Nu‘uanu Stream. Therefore, the depositional environment at this particular site is considerably different from that of Ordy Pond. Pollen records from the Liliha core (Athens et al., 1997) demonstrate relatively abundant *Pteridophytes* (Fern) spores throughout, probably reflecting abundant water supplies in the area. Lagoonal deposits in the core, the proximity to the Nu'uanu Stream and archaeological evidence for the existence of ancient fishponds and taro patches in the core agree with such speculation. Nevertheless, changes in the fern spores and some other pollen groups including the *Cheno-am* and *Pritchardia* appear to reflect a few climatic shifts during the Holocene. Increases in the *Cheno-am* pollen and dominance of the *Pritchardia* pollen perhaps reflect aridification from ~8,800
to \(-6,500\) cal.yrBP, which is followed by a brief mitigation period as indicated by small retreats in these pollens as well as increases in the spores of some *Pteridophytes* from \(-6,500\) to \(-6,100\) cal.yrBP. In a similar fashion, these pollen groups seem to record alternating periods of aridification (from \(-6,100\) to \(-4,400\) cal.yrBP and from \(-3,100\) to \(-2,000\) cal.yrBP) and mitigation (from \(-4,400\) to \(-3,100\) cal.yrBP).

Pollen diagrams constructed from ‘Uko’a Pond sediments by *Athens et al.* (1995) provide a record of vegetation changes from \(-6,000\) to \(-500\) cal.yrBP. ‘Uko’a Pond is located near the town of Haleiwa on the north coast of O‘ahu at elevation of approximately 2.4 m (*Athens et al.*, 1995). Considerable fluctuations of a variety of pollen groups provide evidence for numerous large-scale floristic changes. There is an apparent trend of aridification from \(-5,800\) to \(-5,200\) cal.yrBP characterized by substantial increases in the *Cheno-am* and *Pritchardia* pollens and also by a reduction in *Pteridophyte* spores. Increase in the signals of the *Pteridophytes*, *Cyperaceae* and *Kanaloa kahoolawensis* accompanied by declines in the *Cheno-am* and *Pritchardia* from \(-5,200\) to \(-3,500\) cal.yrBP seem to indicate a shift to much wetter climate. The pollen records from \(-3,500\) to \(-1,000\) cal.yrBP suggest the presence of a relatively stable vegetative structure, however, the abundance of the *Pteridophyte* spores and the pollens from woody plants, such as the *Kanaloa kahoolawensis* and *Myrtaceae*, as well as noticeably low levels of the *Cheno-am* and *Pritchardia* most likely imply wetter climatic conditions. The pollen records after \(-1,000\) cal.yrBP show declines in *Kanaloa kahoolawensis*, *Dodonaea viscosa* and *Myrtaceae*. Observed reductions in the pollen from these woody species are accompanied by the expansion of grasses such as the *Poaceae* and *Cheno-am*. Significant changes in the vegetation during this era, however,
seems to be driven by land modification by anthropogenic activities, rather than aridification of the climate, considering the concurrent surges in the Cyperaceae and Pteridophytes (indicating wetter conditions) as well as abundant charcoal deposits.

Figure 3.12 summarizes the climatic trends revealed by available pollen records from O'ahu and compares those with % C₄ abundance constructed from Ordy Pond sediments. As Figure 3.12 shows, durations of increase (or decrease) in the % C₄ abundance match nicely with the incidents of arid climate or intervals of aridification (or mitigation) demonstrated by the pollen records from O'ahu. Observed synchronization between the pollen records and the record based on leaf wax n-alkanes strongly supports the use of the % C₄ abundance as a proxy for the Holocene paleo-aridity index.

Based on the % C₄ abundance record established in this study and rigorous reviews and comparisons of pollen records from O'ahu, changes in the aridity during the Holocene on O'ahu can be summarized as follows (see Figure 3.12): (1) wet climatic conditions before ~10,000 cal.yrBP, (2) nearly continuous trend of aridification from ~10,000 to ~5,000 cal.yrBP, perhaps interrupted by a relatively short (on the order of a few hundred years) period of increased moisture around 7,000 cal.yrBP, (3) an abrupt and strong climatic shift toward wetter conditions sometime between ~5,000 and ~4,000 cal.yrBP and (4) almost 2,000 years of relatively stable but slightly wet climatic conditions after ~3,500 cal.yrBP.

The most dramatic change in the aridity regime during the Holocene is the termination of nearly 5,000 years of aridification by abrupt mitigation sometime between ~4,800 and ~4,000 cal.yrBP (see Figure 3.8 or 3.12). The abrupt nature and the magnitude of the transition from a long-lasting aridification to a wetter climate most
Figure 3.11. Comparisons of the % C₄ abundance established in this study with climate changes during the Holocene inferred from the four O‘ahu pollen records: Ka‘au Crater (Hotchkiss and Juvik, 1999), Liliha (Athens et al., 1997), Evaporite Pan (Athens et al., 1999) and ‘Uko’a Pond (Athens et al., 1995). The red bars for the pollen records denote either arid climatic conditions or phases of aridification, and the blue bars represent relatively wetter periods or phases of mitigation. The red and blue arrows on the Evaporite Pan record indicate the direction of change in % C₄ abundance consistent with aridification and mitigation, respectively.
likely suggest a significant climatic shift. The pollen records from Liliha and ‘Uko‘a Pond similarly show noticeable changes in the average pollen compositions before and after 5,000 cal.yrBP. The overall abundance of the Cheno-am pollens in the Liliha sediment cores is greater before 5,000 cal.yrBP (Athens et al., 1997). The ‘Uko‘a Pond pollen records (Athens et al., 1995) also document greater abundance of the Cheno-am pollens, as well as the Pritchardia pollens, prior to 5,000 cal.yrBP. Moreover, abundances of the Cyperaceae pollens and the Pteridophytes spores become much greater after 5,000 cal.yrBP. Consequently, these pollen records likewise indicate the significance of this time interval as an important transition point for the Holocene climate in Hawai‘i.

Sediment cores from multiple locations widely dispersed on the island of Kaua‘i may also provide evidence of a significant climate change around 5,000 cal.yrBP. A number of these cores display a characteristic increase in charcoal abundance between ~4,300 and ~4,000 cal.yrBP followed by a sudden decline to the low background levels (Burney and Burney, 2003). The authors proposed a multi-decadal drought combined with an increase in dry lightning as the leading cause for the occurrences of charcoal deposits before human arrivals on the volcanically non-active island of Kaua‘i. The synchronous charcoal deposits may be related to the climate change deduced from the O‘ahu records.

Additional evidence of Holocene climate change is provided by records of reef accretion in Hawai‘i. Rooney et al. (2004) and Engels et al. (2004) cored reefs from several sites in Kaua‘i, O‘ahu and Moloka‘i. Reefs in Hawai‘i had been continuously accreted during the first half of the Holocene and then terminated around 5,000 cal.yrBP.
The primary factor responsible for curtailing the healthy reef growth on the time scale of years to as much as a few centuries is exposure to destructive wave energy particularly caused by large winter North Pacific swells and occasional tropical storms or hurricanes (Rooney et al., 2004). Considering the close link between atmospheric forcing and the development of oceanic waves, the termination of reef accretion at ~5,000 cal.yrBP at many sites in Kaua‘i, O‘ahu and Moloka‘i could also be an indicator of large scale climate change.

Based on statistical analysis, Rooney et al. (2004) found intensification of wave energy in Hawai‘i owing to the North Pacific swells during El Niño years. In addition, more frequent tropical storms and hurricanes are known to occur in the vicinity of Hawai‘i during El Niño years (Chu and Wang, 1997). Thus, Rooney et al. (2004) and Engels et al. (2004) hypothesized that the termination of Holocene reef accretion was related to the enhancement of El Niño activity beginning at ~5,000 cal.yrBP. Indeed, numerous studies report the possible onset or intensification of climate variability similar to that associated with modern El Niño/Southern Oscillation (ENSO) during the mid-Holocene based on a variety of proxy records (i.e., Sandweiss et al., 1996; Rodbell et al., 1999; Moy et al., 2002; Haberle and Ledru, 2001; Tudhope et al., 2001; Corrège et al., 2000; Friddell et al., 2003). Droughts in Hawai‘i are known to correlate with El Niño events on the interannual time scale (Chu, 1989, 1995; Chu and Chen, 2005). It is tempting, therefore, to attribute the exceptionally intense aridification at ~5,000 cal.yrBP, archived in Ordy Pond as well as a variety of other terrestrial paleo-records from Hawai‘i to an ENSO-related climate change. Holocene ENSO variability, however, still remains poorly resolved and highly controversial. For example, some authors strongly question
the reliability of certain paleoclimatic records that were previously proposed (referenced above) as evidence for the mid-Holocene onset and/or intensification of ENSO (i.e., Koutavas et al., 2002; Rodó and Rodríguez-Arias, 2004). Furthermore, proxy records from the Galápagos Islands (Riedinger et al., 2002) and Christmas Island (Woodroffe and Beech, 2003), both located in the core region of modern ENSO activity, indicate a much later increase in the frequency and intensity signals of ENSO, from ~3,100 cal.yrBP and from ~3,800 cal.yrBP, respectively. Considering such large unknowns for the Holocene ENSO variability, the cause of the climate change in Hawai‘i during the mid-Holocene remains uncertain.

Unfortunately, the approach used in this study is perhaps incapable of reflecting the high frequency signals of the ENSO. Modern ENSO frequency is on the order of 2 to 8 years (Chu and Chen, 2005). Each sediment sub-sample that was extracted to obtain n-alkanes in this study, however, contained multiple couplets of annually-deposited laminae (i.e., Garrison, 2002; Tribble et al., 1998). This means that the signals of C₄ plants given by individual samples are broadly averaged, probably on the order of decades, a time period which exceeds the periodicity of ENSO. Pacific Decadal Oscillation (PDO) is an ENSO-like oscillatory climatic phenomenon characterized by positive (or warm) and negative (or cold) regimes on a multi-decadal (typically 20 ~ 30 years) time scale (Mantua et al., 1997; Viles and Goudie, 2003). Although the fingerprints of the PDO are more prominent in higher latitudes (Viles and Goudie, 2003), the PDO signals could potentially be archived in the tropics and subtropics through its influence on ENSO activity. It is known that the PDO modulates ENSO activity such that the positive phase of the PDO induces more prolonged and intense El Niño events (Mantua et al., 1997;
Viles and Goudie, 2003; Chu and Chen, 2005; Rooney and Fletcher, 2005). Long-term meteorological observations in Hawai‘i demonstrate that the positive phase of the PDO tends to result in below-average rainfall (Chu and Chen, 2005). In addition, a strong correlation is found between the PDO and Kona storm activity. Atmospheric conditions associated with a positive phase of the PDO tend to suppress the activity of Kona storms (or subtropical cyclones) that typically develop in the vicinity of Hawai‘i, and which deliver a significant portion of the annual rainfall to the leeward coasts of the main Hawaiian Islands (Rooney and Fletcher, 2005). These combined effects could result in severe droughts that extend to a decadal to multi-decadal time scale in the leeward areas of Hawai‘i. Considering the location of Ordy Pond and the decadal time-resolution covered by individual \( n \)-alkane samples, there could be a potential link between the observed climate changes and the PDO. This hypothesis is, however, entirely speculative because the nature of the PDO beyond the historic past has not been characterized yet. Enhancement of the sampling resolution would certainly improve the recognition and characterization of the Holocene climate changes. Meanwhile, new sets of proxies (including biomarker-based proxies) are necessary in order to further investigate direct causes and/or mechanisms of such climate changes.
3.6. Conclusion

Characterization of sedimentary n-alkanes extracted from Ordy Pond sediments by GC and irm-GC/MS analyses confirmed their terrestrial higher plant leaf wax origin. The results of CSIA revealed considerable fluctuations in the relative abundance of C\textsubscript{3} and C\textsubscript{4} plants throughout the Holocene. With the subsequent use of a two end-member isotopic mass-balance approach, the results from this study produced the first continuous Holocene paleovegetative history (% C\textsubscript{4} abundance) in Hawai‘i. Comparisons with previously published pollen records from Ordy Pond sediments showed remarkable agreement with the trend of fluctuations in the relative abundance of C\textsubscript{4} plants. The % C\textsubscript{4} abundance revealed a considerable expansion of C\textsubscript{4} plants, mainly weedy grasses, in response to ecosystem alterations by anthropogenic activities of both the early Polynesian settlers and the European settlers in the late 18th century. Further comparisons of the % C\textsubscript{4} abundance constructed in this study with a few pollen records covering discrete intervals of the Holocene from O‘ahu demonstrated that the advent and/or decline of C\textsubscript{4} plants with time matched well with the changes in the aridity inferred from several key diagnostic pollen groups. These observations verify that the % C\textsubscript{4} abundance can faithfully reflect the vegetation changes and can be interpreted as an index for aridity changes during the Holocene. Based on the results from this study as well as rigorous reviews of the previous pollen records from O‘ahu, the following climatic episodes of the Holocene in Hawaii were detected; (1) wet climate before \(\sim10,000\) cal.yrBP, (2) continuous and significant aridification from \(\sim10,000\) to \(\sim5,000\) cal.yrBP, (3) an abrupt climate change toward wetter climate sometime between \(\sim5,000\) and \(\sim4,000\) cal.yrBP, (4) relatively stable but slightly wet climate from \(\sim3,500\) to \(\sim1,500\) cal.yrBP. The timing of
the dramatic change in the aridification/mitigation trend at ~4,800 cal.yrBP seems to be caused by a significant climate change that could be potentially influenced by the combination of the PDO and ENSO.
3.7 Appendix

The general expression of the isotope mass-balance model (Eq. 3.3 and 3.4) can be solved for $F_{C4}$ (Eq. 3.5) as:

$$F_{C4} = \frac{\delta_T}{\delta_{C4} - \delta_{C3}} - \frac{\delta_{C3}}{\delta_{C4} - \delta_{C3}}$$  \hspace{1cm} (Eq. 3.5)

Then, the propagation of error based on the uncertainties, in terms of standard deviation ($\sigma$), of the terms contributing to the estimation of $F_{C4}$ can be performed by:

$$\sigma^2_{F_{C4}} = \left( \frac{\partial F_{C4}}{\partial \delta_T} \right)^2 \sigma^2_{\delta_T} + \left( \frac{\partial F_{C4}}{\partial \delta_{C3}} \right)^2 \sigma^2_{\delta_{C3}} + \left( \frac{\partial F_{C4}}{\partial \delta_{C4}} \right)^2 \sigma^2_{\delta_{C4}}$$  \hspace{1cm} (Eq. 3.10)

The partial derivatives required for the above equation are:

$$\frac{\partial F_{C4}}{\partial \delta_T} = \frac{1}{\delta_{C4} - \delta_{C3}}$$  \hspace{1cm} (Eq. 3.11)

$$\frac{\partial F_{C4}}{\partial \delta_{C3}} = \frac{\delta_T}{(\delta_{C4} - \delta_{C3})^2} - \frac{\delta_{C4}}{(\delta_{C4} - \delta_{C3})^2} = \frac{\delta_T - \delta_{C3}}{(\delta_{C4} - \delta_{C3})^2}$$  \hspace{1cm} (Eq. 3.12)

and

$$\frac{\partial F_{C4}}{\partial \delta_{C4}} = \frac{-\delta_T}{(\delta_{C4} - \delta_{C3})^2} - \frac{-\delta_{C3}}{(\delta_{C4} - \delta_{C3})^2} = \frac{\delta_{C3} - \delta_T}{(\delta_{C4} - \delta_{C3})^2}$$  \hspace{1cm} (Eq. 3.13)

Eq. 3.10 can be expanded with these partial derivatives (Eq. 3.11, 3.12 and 3.13) as:
\[ \sigma^2_{F_{C4}} = \left( \frac{1}{\delta_{C4} - \delta_{C3}} \right)^2 \sigma^2_{\delta r} + \left( \frac{\delta_{T} - \delta_{C4}}{(\delta_{C4} - \delta_{C3})^2} \right)^2 \sigma^2_{\delta C3} + \left( \frac{\delta_{C3} - \delta_{C4}}{(\delta_{C4} - \delta_{C3})^2} \right)^2 \sigma^2_{\delta C4} \]  

(Eq. 3.14)

which can be subsequently rearranged to:

\[ \sigma^2_{F_{C4}} = \left( \frac{1}{(\delta_{C4} - \delta_{C3})^2} \right) \left[ \sigma^2_{\delta r} + \left( \frac{\delta_{T} - \delta_{C4}}{\delta_{C4} - \delta_{C3}} \right)^2 \sigma^2_{\delta C3} + \left( \frac{\delta_{C3} - \delta_{C4}}{\delta_{C4} - \delta_{C3}} \right)^2 \sigma^2_{\delta C4} \right] \]  

(Eq. 3.15)

Eq. 3.15 is the general mathematical expression to calculate standard deviation of \( F_{C4} \) as a function of the standard deviations of the factors contributing to \( F_{C4} \).
3.8. References


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specific $\delta^{13}$C analyses of leaf lipids from plants with differing carbon dioxide

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CHAPTER IV

Outcomes of the Project and Future Research Directions
4.1. Summaries and Evaluations

4.1.1 Chapter II: Reviews and Evaluations

The main scope of Chapter II was to improve the age control of Ordy Pond sediments with the use of compound-specific radiocarbon analysis (CSRA). Only a handful of studies have applied the method to establishing sediment chronologies. In addition, such reports have been limited to marine sediments (Ohkouchi et al., 2003; Smittenberg et al., 2004) and soils (Huang et al., 1999). In order to overcome the contamination of the DIC in the water column of Ordy Pond with $^{14}$C-depleted C from the Pleistocene limestone, CSRA was conducted on long-chain odd-C-numbered $n$-alkanes originating from the leaf waxes of terrestrial higher plants. Because this study represents the first direct application of CSRA of $n$-alkanes for lacustrine sediments, significant attention was given to address the reliability of the method. Several samples used for the lipid extraction were picked from core locations that were relatively close to sedimentary horizons that had been $^{14}$C-dated with rare plant macrofossils in the previous studies by Athens et al. (1999, 2002) to examine if CSRA based on $n$-alkanes is capable of producing reasonable $^{14}$C ages.

In general, $^{14}$C ages of $n$-alkanes determined by CSRA demonstrated remarkable agreement with the $^{14}$C ages of nearby horizons based on accelerator mass spectrometry (AMS) $^{14}$C-dating of terrestrial macrofossils. With newly obtained $^{14}$C tie-in points from $n$-alkanes, the age-control of Ordy Pond sediments was significantly improved. Previous studies (i.e., Pearson and Eglinton, 2000; Pearson et al., 2001; Smittenberg et al., 2004) indicated that $n$-alkanes in marine sediments typically yield anomalously too-old $^{14}$C-dates due to incorporation of relict fractions (pre-aged $n$-alkanes due to long-term storage
in other sedimentary environments or due to long-term physical transport before their final deposition into marine sediments). Such interference caused by relict $n$-alkanes seems to be minimal for Ordy Pond sediment probably because of the absence of fluvial transport into the pond system. Furthermore, the use of $n$-alkanes for age-determinations of lacustrine sediments appears to be particularly favorable because these compounds are directly fixed from atmospheric CO$_2$ and thus are independent of the $^{14}$C reservoir effect that can be highly difficult to accurately estimate in lacustrine systems due to considerable site-specific variability.

One of the $n$-alkane samples, however, produced an anomalously old $^{14}$C age. The deviation of the $^{14}$C age from the expected age calculated from the refined chronology was approximately 4,155 years. Careful examination of this particular sample with the use of isotopic mass-balance calculations suggests that the sample was artificially contaminated during preparative capillary gas chromatography (PCGC). Assuming that the contaminant incorporated into the sample is petroleum-derived (such as common organic solvents), and thus having $\Delta^{14}$C of $-1,000\%$, further calculations indicate that the observed $^{14}$C depletion could occur if the contaminant represents only 1.3% of the total quantity of the $n$-alkanes in the sample. This example clearly illustrates the potential risks of contamination associated with sample preparation and micro-scale AMS $^{14}$C dating of CSRA. Nonetheless, the encountered contamination is not intrinsic to the method or the instruments, and thus it is certainly avoidable if proper precautions are taken. In fact, five other samples produced acceptable $^{14}$C ages. Thus the contamination encountered in this study does not disprove the utility of the method.
This study demonstrated that CSRA can serve as an alternative $^{14}$C-dating method for lacustrine sediments when more conventional techniques are impractical or inapplicable. Use of plant $n$-alkanes for CSRA in developing lacustrine sediment chronologies is highly recommended due to (1) no requirement for reservoir corrections, (2) the refractory nature of $n$-alkanes and their persistence in sedimentary environments, (3) their ubiquitous presence in sediments and (4) the relatively easy extraction and purification procedures. CSRA based on $n$-alkanes may particularly be applicable in the tropics and subtropics where plants seem to secrete relatively large quantities of leaf waxes in order to avoid evaporative losses of water and damage from ultraviolet radiation. Ordy Pond sediments indeed contained significant amounts of $n$-alkanes. Moreover, contamination of samples can be rather difficult to detect in conventional $^{14}$C techniques, and thus removing $^{14}$C dates from a chronology can sometimes be subjective. But as shown in this study, CSRA is superior in this respect because subsequent isotopic mass-balance calculations allow objective verification of the results.

Despite its remarkable potential, it is true that the use of CSRA is still a non-routine option because of the very expensive analytical costs and time consuming sample preparation. In addition, only a handful of facilities possess appropriate instruments (such as AMS and PCGC) and protocols for reliable CSRA. It is also important to note that CSRA, enabled by AMS and PCGC, is highly advantageous in regard to its small sample requirement ($\sim 100 \mu g$ of C), but reduced sample sizes simultaneously enhance the susceptibility to contamination. Leaf wax $n$-alkanes may not always be ideal biomarkers for age-determination of lacustrine sediments. If the system receives significant amounts of fluvial materials, relict $n$-alkanes once stored in other sedimentary environments such
as terrestrial soils or riverbanks could dilute the locally-delivered signal. In such case, other biomarkers (such as algal biomarkers) need to be exploited.

4.1.2 Chapter III: Reviews and Evaluations

Previous paleoenvironmental investigations from Ordy Pond sediments based on sediment mineralogy (Tribble et al., 1999) and stable isotope studies on authigenic carbonates and bulk organic matter (Garrison, 2002) have shown that these proxies are impacted by multiple factors (climatological factors, physical and hydrological processes, groundwater intrusion, Holocene sea-level changes, occasional inputs of terrestrial organic matter, diagenesis, other biogeochemical processes, anthropogenic influences etc), which are almost impossible to de-convolute from each other. During the sample preparation for CSRA, compound-specific isotopic analysis (CSIA) surprisingly demonstrated a large variability in the C isotopic compositions of n-alkanes ($\delta^{13}C_{N-Alks}$). These preliminary results from CSIA brought a new scope to this project. Because these leaf wax n-alkanes are highly stable and refractory, isotopic signatures retained in these compounds are primary (solely reflecting precursor vegetation, distinguished as C$_3$ versus C$_4$ plants) and are not affected by other complicated pond processes. Then, $\delta^{13}C_{N-Alks}$ should be able to provide a straightforward record of changes in paleovegetation, which can be interpreted as an indirect archive of Holocene climate.

$\delta^{13}C_{N-Alks}$ analyzed in this study provided the first proxy for Ordy Pond that is completely independent of pond hydrology and biogeochemical processes. Using a two end-member isotopic mass-balance approach, the $\delta^{13}C_{N-Alks}$ record was translated into the relative abundance of C$_4$ plants over C$_3$ (the $\%$ C$_4$ abundance). Comparisons with pollen
records from O‘ahu demonstrated that the % C_4 abundance is indeed capable of reconstructing Holocene vegetation changes, which seem to be predominantly governed by changes in the extent of aridity. In summary, the % C_4 abundance record illustrated a steady aridification from ~10,000 to ~4,800 cal.yrBP, abrupt mitigation in climate sometime between ~4,800 and ~4,000 cal.yrBP and over 2,000 years of relatively stable climate from ~4,000 cal.yrBP. The record also illustrated large-scale environmental alterations after the arrivals of the Polynesians and European settlers. Thus, this study produced the first continuous paleoclimatological and paleoenvironmental reconstruction for the Holocene from Hawai‘i.

Paleoclimatic information inferred from the relative dominance of C_3 and C_4 plants may not be as descriptive as pollen records, however, unlike pollen analysis that requires highly sophisticated diagnostic skills (taxonomical, botanical and ecological expertise), the approach used in this study is a relatively easy and practical method to investigate paleoclimatic and paleoenvironmental information from sedimentary records. This approach should be particularly suitable for paleoclimatological investigations in the tropics and subtropics. Plants in these areas secrete abundant leaf waxes, which will be eventually stored in sedimentary environments. Because seasonal fluctuations in temperature are usually small in low latitude areas, shifts in dominance of C_3 and C_4 plants should be largely controlled by changes in aridity (at least during the Holocene when changes in the atmospheric CO_2 concentration were insignificant). In addition, aridity/precipitation changes in low latitude regions are typically influenced by climatic phenomena such as ENSO, PDO and movement of the intertropical convergence zone.
Vigorous investigations of tropical/subtropical paleovegetative changes during the Holocene could be useful for better characterization of these important climatic features.

4.2. Future Research Directions

This study definitely reveals the great potential and utility of the sedimentary record from Ordy Pond as a paleoclimatic archive. As a concluding remark of this thesis, this section addresses future research directions that are recommended to further improve paleoclimatological reconstructions from Ordy Pond sediments.

4.2.1 Further Improvement of Sediment Chronology

Unexpected contamination in one of the samples prepared for CSRA unfortunately resulted in a relatively large gap in the chronology of Ordy Pond sediments. Nearly 2.5 m of sediment (between 1,175 and 930 cm), which is roughly equivalent to 5,600 years of sedimentation history, still does not have a $^{14}$C tie-in point. The sediment chronology established in this study should ideally be supplemented with a few more extra $^{14}$C dates based on CSRA on leaf wax $n$-alkanes.

4.2.2 Improvement in the Resolution of the $\%$ C$_4$ Abundance Record

The $\%$ C$_4$ abundance based on the $\delta^{13}$C$_{N-Alk}$ records indicates a possible large scale climate change around 4,800 cal.yrBP in Hawai‘i. Although it was not discussed in Chapter II, the record may also indicate the possibility of four distinct periods of mitigation (~9,500, ~7,000, ~4,000 and ~2,000 cal.yrBP) that intriguingly occurred every 3,000 years or so. The pollen records from O‘ahu also appear to suggest climate
mitigation in these time frames (see Figure 3.12). In order to better characterize the apparent climate change at ~4,800 calyrBP and the much more speculative signs of numerous climate mitigations, the resolution of the % C₄ abundance needs to be improved.

The resolution can be improved by increasing the number of samples. Unlike the most recent 2,000 years worth of the sediments, where sample coverage was comparatively dense, the sample frequency for the older sediments was limited to 3 to 4 samples in every 2,000 years or so (see Figure 3.5 or Figure 3.8). Extra samples, particularly in the vicinity of significant changes in the % C₄ abundance record as shown in Figure 3.8, should be able to refine the quality of the proxy record. In addition, the resolution covered by each sample can be improved. The sediment samples for lipid extractions contained multiple layers of annually-deposited sedimentary laminae, therefore each sample in the profile (Figure 3.8) reflects the mean signal averaged over several decades. By adjusting the sample dilution (increasing the concentration of n-alkanes in a given sample using less solvent for the analyses by GC and irm-GC/MS), the sample requirement could be significantly reduced (leading to better resolution covered by each sample). If sufficient resolution (both in terms of sample coverage and possible age-range covered by individual sample) are obtained, this proxy may be able to deduce climate changes associated with relatively high-frequency climate variability such as ENSO and PDO, whose behaviors during the Holocene are still not well-resolved.

Two n-alkane samples analyzed in this study were extracted from non-aquatic sediments (sediments below the transiton to aquatic sediments; see Figure 1.5, Figure 2.2 or Figure 3.3). δ¹³C_N-ALK records from the paleosols should be equally as reliable as the
lacustrine portion of the sediments if the deposits are stratigraphically intact (which seems to be the case for this fairly deep sinkhole). Paleovegetative reconstructions based on $\delta^{13}C_{N-Alk}$ records from paleosols have been successfully performed in several studies (i.e., Freeman and Colarusso, 2001; Zhang et al., 2003, 2006; Liu et al., 2005). Investigations of the leaf waxes in the paleosol in the sediment core from Ordy Pond may elongate the record even to the Pleistocene.

4.2.3 Dual Use of Compound-Specific $\delta^{13}C$ and $\delta D$ Analysis on Lipid Biomarkers

Chapter III concludes that the large-scale vegetation changes during the Holocene revealed by $\delta^{13}C_{N-Alk}$ data from Ordy Pond sediments are related to past climate changes, particularly changes in the aridity. But, technically, $\delta^{13}C_{N-Alk}$ values only apportion the sources of those compounds in terms of vegetation types according to the C-fixation pathways ($C_3$, $C_4$ and CAM plants). Thus, any paleoclimatic information deduced from the $\delta^{13}C_{N-Alk}$ data in this study represents only the indirect speculation of the paleoclimate changes. The same statement also holds true for the pollen-based reconstruction. Certainly this study illustrated vegetation changes that appear to be related to climate changes; however, direct investigations of paleoclimatic changes require a new set of proxies.

Within the last decade or so, much attention has been given to the development of compound-specific hydrogen isotopic ($\delta D$) analysis of biomarkers as an additional tool to reconstruct paleoclimate changes. The Deuterium/hydrogen isotope ($D/H$) ratio of water in a lacustrine system is largely dominated by the balance between $D/H$ ratios of the source water (mainly precipitation) and evaporative loss of water leading to $D$-
enrichment in the residual water body. Therefore, past fluctuations in the δD compositions of the water in lacustrine systems are directly related to paleoclimate changes. Recent investigations by Sauer et al. (2001) and Huang et al. (2004) demonstrate that compound-specific δD analysis of algal-derived lipid biomarkers (i.e., n-C₁₇, n-C₁₉, phytol, palmitic acid, etc) can be useful in reconstructing paleohydrology of lake water. Although algal-derived n-alkanes such as n-C₁₇ and n-C₁₉ were not detected in the free-lipids (lipids that can be obtained from sediments by simple solvent extractions) extracted from Ordy Pond sediments, these biomarkers may be bound in the sediments and be extractable by base-hydrolysis (saponification) of sediments. Or the free-lipids from Ordy Pond sediments may in fact have algal-derived lipids such as phytol and sterols other than short-chain n-alkanes (characterization of lipid biomarkers other than n-alkanes was not performed in this study). More rigorous investigations and identifications of lipid biomarkers in the sediments are recommended prior to compound-specific δD analysis on algal lipids.

Compound-specific δD analysis can be similarly applied to terrestrial biomarkers. Because meteoric water is the dominant source of water to the land plants, δD compositions of terrestrial plant materials should be proportional to the D/H ratios of the meteoric water that in turn are directly related to climatic parameters such as water vapor source, temperature and evaporation (Sachse et al., 2006). Because hydrogen atoms in n-alkanes are tightly bound to C atoms and are non-exchangeable (Schimmelmann et al., 1999), δD compositions of n-alkanes can be preserved on the order of tens of millions of years (Yang and Huang, 2003). Smith and Freeman (2006) found that δD compositions of leaf wax n-alkanes from grasses in the Great Plains reflect modern latitudinal
differences in δD values of precipitation. Sachse et al. (2004) similarly showed that plant
n-alkanes from recent lake sediments record the latitudinal gradient of δD values of
precipitation along a north-south transect in Europe. It is important to notice, however,
that these studies clearly illustrate significant deuterium enrichment in the n-alkanes
relative to the D/H ratios of the meteoric water. Such offsets relative to the δD values of
meteoric water reflect a combination of isotopic fractionations (leading to deuterium
enrichment) during the evaporation of soil water and the evapotranspiration of leaf water
(Sauer et al., 2001; Smith and Freeman, 2006; Sachse et al., 2004, 2006).

The difference between the δD values of algal and terrestrial lipid biomarkers
conceptually can be used as a proxy for paleo-evaporation and paleo-evapotranspiration
(Sachse et al., 2004) as shown in Figure 4.1. Sachse et al. (2004) argued that this proxy
should be particularly useful in small closed lake systems, which are fed by the meteoric
water. Application of this proxy to Ordy Pond is, therefore, quite appealing because the
contemporary hydrology of the pond is indeed predominantly regulated by the balance
between rainfall and evaporation. Characterization of the isotopic fractionations
accompanying lipid biosynthesis (see Figure 4.1) as well as the climatic, physiological
and species specific influences on the isotopic fractionation have been actively
investigated in recent years (i.e., Chikaraishi and Naraoka, 2003; Chikaraishi et al.,
2003; Bi et al., 2005; Smith and Freeman, 2006; Sessions, 2006) and the trend seems to
continue in future. Such information will certainly improve the quality of the proxy based
on δD of lipid biomarkers. Past efforts to characterize paleo-hydrology of the pond based
on δ13C data of authigenic carbonate seem to be rather unrealistic due to miscellaneous
signals carried by the carbonate. Compound-specific δD analysis on restrictive
Figure 4.1. A schematic diagram of the hydrogen isotopic relationship between source water and lipid biomarkers of aquatic and terrestrial origin in lacustrine sediment (from Sachse et al., 2006).
4.3. References


