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ISOTOPIC RECORDS FROM EARLY WHALES AND SEA COWS: CONTRASTING PATTERNS OF ECOLOGICAL TRANSITION

MARK T. CLEMENTZ[1], ANJALI GOSWAMI[2], PHILIP D. GINGERICH[3], and PAUL L. KOCH[4]
[1]Department of Geology and Geophysics, University of Wyoming, Dept. 3006, 1000 E. University Ave., Laramie, Wyoming, 82071, U.S.A., mclemen1@uwyo.edu;
[2]School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS U.K., a.goswami@nhm.ac.uk;
[3]Geological Sciences and Museum of Paleontology, University of Michigan, 1109 Geddes Road, Ann Arbor, Michigan, 48109-1079 U.S.A., gingeric@umich.edu;
[4]Department of Earth Sciences, University of California-Santa Cruz, A250 Earth and Marine Sciences, 1156 High Street, Santa Cruz, California 95064 U.S.A., pkoch@es.ucsc.edu;

ABSTRACT—Recent fossil discoveries of early cetaceans and sirenians document the functional transitions that occurred as each group adapted to a completely aquatic existence, but the timing and path of their ecological transition remain uncertain. We analyzed the stable-isotope composition of tooth enamel from several early members of each group to reconstruct the dietary, foraging, and habitat preferences of basal taxa. Carbon isotope ($\delta^{13}C$) values provided evidence of foraging within freshwater, terrestrial, and marine environments, including seagrass beds, whereas oxygen isotope ($\delta^{18}O$) variation was used as a measure of commitment to aquatic habitats. Enamel samples were collected from four regions (south Asia, north and west Africa, and southern Europe) spanning the late early Eocene (ca. 53.5 Ma) to the late Eocene (ca. 36 Ma). Sirenian and cetacean taxa included species that were likely capable of some terrestrial locomotion, and more specialized forms that were morphologically fully aquatic. Cetacean $\delta^{13}C$ and $\delta^{18}O$ values indicate that some early members of this group (some pakicetids) inhabited freshwater environments, but later members (e.g., remingtonocetids, protocetids, and basilosaurids) moved quickly into estuarine and marine environments. Low $\delta^{18}O$ variation confirms that all of these early forms were primarily aquatic, but $\delta^{13}C$ and $\delta^{18}O$ values for early sirenians indicate an early transition to a marine seagrass diet without any evidence of an intermediate connection to freshwater habitats.

INTRODUCTION

Cetaceans (whales, dolphins, and porpoises) and sirenians (manatees and dugongs) are the only mammals completely committed to an aquatic existence. Fossil evidence suggests that the transition from terrestrial ancestors to fully aquatic descendants was relatively rapid within both groups, possibly occurring over a period of only a few million years during the early and middle Eocene (Domning, 2002; Fordyce, 2002; Fig. 1). The exact timing of the invasion of aquatic ecosystems remains unclear, however, and many questions about the diet, behavior, and habitat preferences of early, transitional forms are unanswered.

Nearly complete specimens of the earliest sirenian groups (prorastomids and protosirenids) have been recovered from early middle Eocene deposits (Savage, 1977; Domning et al., 1982; Domning, 2001). Although the morphology of the limbs and pelvis attest to their capacity for terrestrial locomotion, these early sirenians also had clear adaptations for aquatic environments (e.g., pachyosteosclerotic bones, dorsally placed nasal openings). By the middle to late Eocene, more specialized sirenians (e.g., *Eotheroides*, *Eosiren*, *Halitherium*) were incapable of supporting their bodies on land and had become obligatorily aquatic mammals. Yet, while strong evidence of the aquatic affinities of early sirenians can be drawn from the fossil record, the diet and habitat preferences of early species remain ambiguous. Today, sirenians are one of the few vertebrate groups that subsist largely on marine seagrasses, a dietary specialization speculated to have arisen early within this lineage, possibly by the middle Eocene (Domning, 1981; Ivany et al., 1990). The capacity for terrestrial locomotion in early forms, however, suggests that vegetation from other habitats (i.e., terrestrial or freshwater) could have been consumed by these semi-aquatic species, much as modern semi-aquatic hippopotamus eat largely terrestrial grasses. The tight link between sirenians and seagrasses seen today (Preen, 1995) could have arisen after sirenians had invaded aquatic habitats. Reconstructions of Eocene seagrass communities based on sirenian fossil occurrences must be viewed with caution until the timing of the sirenian–seagrass connection can be confirmed by other lines of evidence.

Cetaceans display an evolutionary pattern of aquatic adaptation similar to that of sirenians, but their record includes a larger representation of transitional forms (Fig. 1). The earliest cetaceans, the pakicetids (ca. 53.5 to 47 Ma) are known from incomplete skeletal remains, mainly cranial and dental elements, which have made for uncertain paleoecological interpretations (Gingerich and Russell, 1990; Bajpai and Gingerich, 1998). Although craniodental characters indicate an affinity for aquatic foraging (Gingerich and Russell, 1990; O'Leary and Uhen, 1999), examination of the inner ear bones and isolated postcranial material has suggested to some workers that pakicetids were in fact cursorial, terrestrial carnivores (Thewissen et al., 2001; Spoor et al., 2002). If so, then the morphological characters that define the clade Cetacea pre-date their transition to aquatic environments. The debate surrounding interpretations of the ecologies of pakicetids and later, unmistakably aquatic archaeocete species based on limb structure highlights the need for methods of paleoecological reconstruction that are independent of morphology.

Stable isotope analysis of fossil material is one such method of

Corresponding Author: Mark T. Clementz, Department of Geology and Geophysics, University of Wyoming, Dept. 3006, 1000 E. University Ave., Laramie, Wyoming 82071 U.S.A., mclemen1@uwyo.edu.

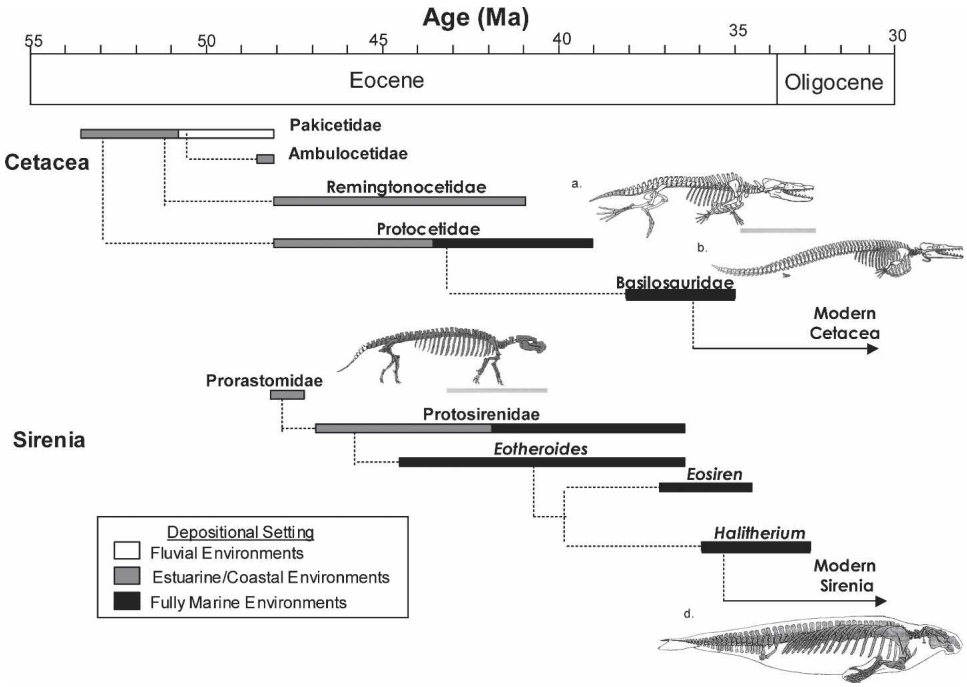

FIGURE 1. Stratigraphic ranges and depositional environments for Eocene Cetacea and Sirenia, modified from Fordyce (2002) and Domning (2001). Skeletal reconstructions include a) the protocetid *Rodhocetus balochistanensis* (Gingerich et al., 2001), b) the basilosaurid *Dorudon atrox* (Uhen, 2004), c) the prorastomid *Pezosiren portelli* (Domning, 2001), and d) the dugongid *Dusisiren jordani* (Domning, 1999). Light gray bars beneath images represent a length of 1 m.

paleoecological inquiry that has already been applied to some questions of archaeocete ecology. Roe et al. (1998) analyzed bone and tooth material from several species of early archaeocetes and compared carbon isotope ($\delta^{13}C$) and oxygen isotope ($\delta^{18}O$) values to those obtained from modern cetaceans. Although they found compelling evidence for exploitation of marine resources early in cetacean evolution (~47 Ma), there are some concerns regarding their interpretations. First, for many taxa, analysis was limited to just one or two specimens, too few to provide an accurate estimate of the mean $\delta^{13}C$ and $\delta^{18}O$ values for a population (Clementz and Koch, 2001). Second, as noted previously, the earliest archaeocetes and sirenians may not have been wholly aquatic, suggesting that modern aquatically specialized cetaceans may not provide a complete isotopic comparison set and that stable isotope values for other semi-aquatic taxa (i.e., pinnipeds and mustelids) should be included. Third and most importantly, Roe et al. (1998) provided no information on the tooth type (e.g., canines, molars, incisors, etc.) analyzed in their study. Teeth formed shortly after birth retain an isotopic signature of the mother's milk (i.e., nursing signal), which introduces additional fractionation factors that can significantly offset the enamel isotope values from those of weaned animals and confound dietary interpretations. Stable isotope analysis for paleoecological reconstruction must be restricted to teeth forming later in life (i.e., permanent canines and first premolars for archaeocetes and third molars for sirenians). Without knowledge of the tooth type sampled, ecological interpretations based on the results of Roe et al. (1998) are of unclear significance.

Stable isotope analysis of additional specimens could provide further insights into the aquatic habits and foraging preferences of early archaeocetes and sirenians. Here, we present stable isotope data from tooth enamel carbonate to reconstruct the early ecological preferences and transitions within each clade. Using differences in the variation and mean of carbon and oxygen isotope values, we examine the aquatic habits (i.e., amount of time spent in water), diets, and salinity preferences of early sirenians and cetaceans. We also analyzed several different teeth from the early archaeocete *Pakicetus* to assess the impact of tooth type on stable isotope analyses and paleoecological interpretation.

MATERIALS AND METHODS

Teeth as Isotopic Recorders of Ecological Information

The stable-isotope composition of tooth enamel bioapatite has been widely used as a recorder of environmental and dietary information (Koch et al., 1994; Koch, 1998; Kohn and Cerling, 2002). The usefulness of enamel stems from its formation via accretion over a limited interval during an animal's life. Once formed, enamel does not turn over and its stable-isotope composition remains fixed, providing a nearly continuous record that may cover a period of months to years and can be retained for millions of years after fossilization (Fricke and O'Neil, 1996; Passey and Cerling, 2002). The oxygen- and carbon-isotope composition of the carbonate fraction of the enamel bioapatite can be measured as a proxy for habitat and dietary preferences, respectively.

The $\delta^{18}O$ value of carbonate in bioapatite is controlled by the $\delta^{18}O$ value of a mammal's body water, which is influenced by several environmental sources (e.g., atmospheric O_2, drinking water, and dietary water) as well as temperature and physiology (Luz and Kolodny, 1985; Huertas et al., 1995; Kohn, 1996). Because most mammals are homeothermic, the temperature-dependent fractionation of oxygen isotopes in bioapatite is constant, removing a potential source of variation. Physiology, on the other hand, can introduce a large amount of variation by altering the quantity and fractionation of oxygen entering and leaving the body during temperature regulation (sweating/panting), waste excretion (urination/defecation), and respiration (Bryant and Froelich, 1995; Kohn, 1996; Hoppe et al., 2004).

For terrestrial mammals, the dominant sources of oxygen are dietary and drinking water, the isotopic values of which can fluc-

tuate widely on temporal and spatial scales. Physiological factors further enhance the disparity in body-water $\delta^{18}O$ values within populations, which in turn causes extreme variation in enamel $\delta^{18}O$ values of terrestrial populations, producing standard deviations for populations that are typically $\geq 1.0\%$ (Clementz and Koch, 2001). In contrast, fully aquatic species living in isotopically homogeneous waters (i.e., seawater) typically show very small differences in $\delta^{18}O$ values among individuals, with standard deviations for populations that are typically $\leq 0.5\%$ (Yoshida and Miyazaki, 1991; Clementz and Koch, 2001; Fig. 3). Because ~98% of the oxygen entering and leaving an aquatic animal's body comes from the water in which it lives (Hui, 1981; Andersen and Nielsen, 1983), body-water and therefore enamel $\delta^{18}O$ values are tightly controlled by environmental water $\delta^{18}O$ values (Fig. 2). Because freshwater environments are typically ^{18}O-depleted relative to seawater as a result of fractionation during evaporation of seawater and precipitation of meteoric water (Dansgaard, 1964; Gat, 1996), mean enamel $\delta^{18}O$ values for aquatic animals can be used to discriminate between marine (high mean $\delta^{18}O$) and freshwater (low mean $\delta^{18}O$) species (Roe et al., 1998).

The carbon isotope composition of bioapatite is directly controlled by the $\delta^{13}C$ value of an animal's diet (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). Before dietary reconstructions can be drawn from these analyses, however, two factors must be considered. First, the magnitude of fractionation between diet and enamel must be known. Second, the range in $\delta^{13}C$ values of dietary resources available to an animal must be gauged.

^{13}C-enrichment in tooth enamel relative to diet ($\delta^{13}C_{enamel} - \delta^{13}C_{diet} = \Delta^{13}C_{enamel-diet}$) varies among mammals. Modern cetacean bone and tooth enamel is fractionated by ~9% to 10% relative to diet (Toperoff, 2002), a value comparable to that of terrestrial carnivores (Lee-Thorp et al., 1989). Because dental evidence indicates that even the earliest archaeocetes were carnivores (Gingerich and Russell, 1990; Bajpai and Gingerich, 1998), they likely possessed a similar $\delta^{13}C_{enamel-diet}$ value. For early sirenians, defining the $\delta^{13}C_{enamel-diet}$ value is more complicated, since the range of values observed for modern sirenians (~10% to 14%) is much greater than that reported for ungulate herbivores (14.1% ± 0.5%; Cerling and Harris, 1998; Clementz, 2002). We assumed a standard fractionation of 12% for early sirenians. To facilitate comparisons across taxa, we converted all enamel $\delta^{13}C$ values to 'ecosystem $\delta^{13}C$ values' by adjusting for $\delta_{13}C_{enamel-diet}$ for carnivores (9.5%), terrestrial herbivores (14%) and sirenians (12%) and by compensating for any additional fractionation associated with each trophic step above primary consumer (0.8% per trophic step; Vander Zanden and Rasmussen, 2001). Herbivores (i.e., sirenians, terrestrial taxa) were assigned a trophic level of zero, terrestrial carnivores were assigned a trophic level of one, and, because marine food chains are typically longer than terrestrial food chains, marine piscivores were assigned a trophic level of two (Pauly et al., 1998). Because pakicetids, remingtonocetids, and protocetids were still capable of coming ashore or foraging in freshwater environments, these groups were assigned a trophic level of 1.5 to allow for any possible mix of terrestrial/freshwater and marine resources. Basilosaurids were assigned a trophic level of two, since fossilized stomach contents for *Dorudon* and *Basilosaurus* indicate a marine, fish-based diet (Swift and Barnes, 1996; Uhen, 1996).

Ecosystem $\delta^{13}C$ values directly reflect the $\delta^{13}C$ value of primary producers at the base of a food web, labeling consumers that forage within particular food webs (Fig. 3). The chief factors controlling $\delta^{13}C$ differences among primary producers include

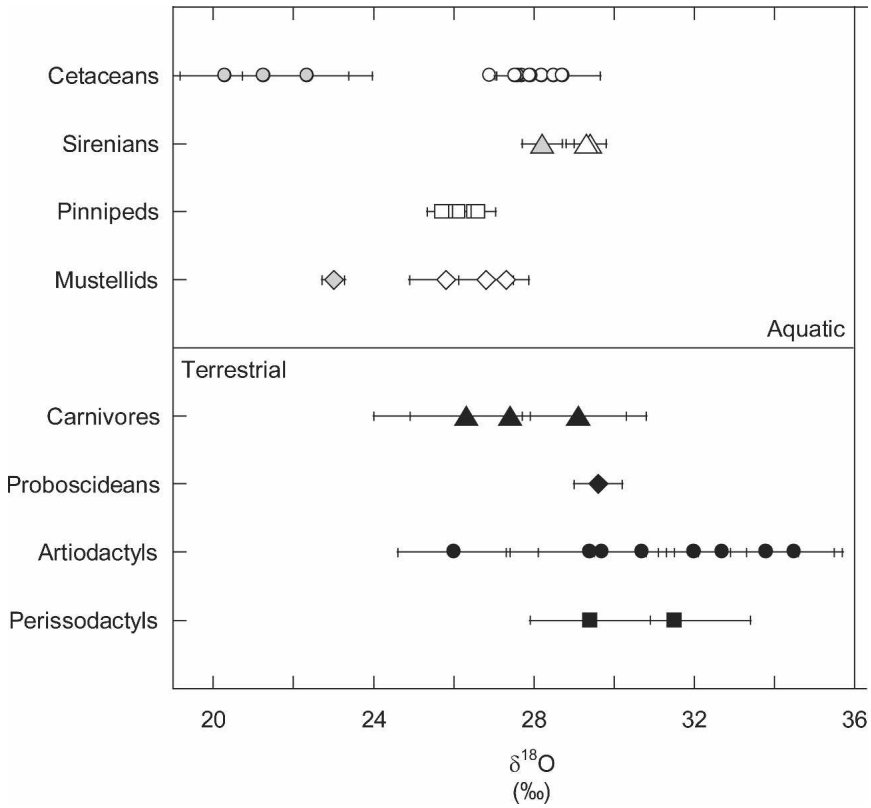

FIGURE 2. Graph of mean enamel $\delta^{18}O$ values for modern aquatic and terrestrial mammals. Error bars represent ±1 *s* from the mean value. Black symbols represent terrestrial mammal values, open symbols represent marine mammal values, and gray symbols represent freshwater mammal values. Terrestrial mammals were sampled from California and South and East Africa. Isotopic values were taken from Bocherens et al. (1996), Clementz and Koch (2001), Sponheimer and Lee-Thorp (2001), MacFadden et al. (2004), and Clementz and Koch (unpublished).

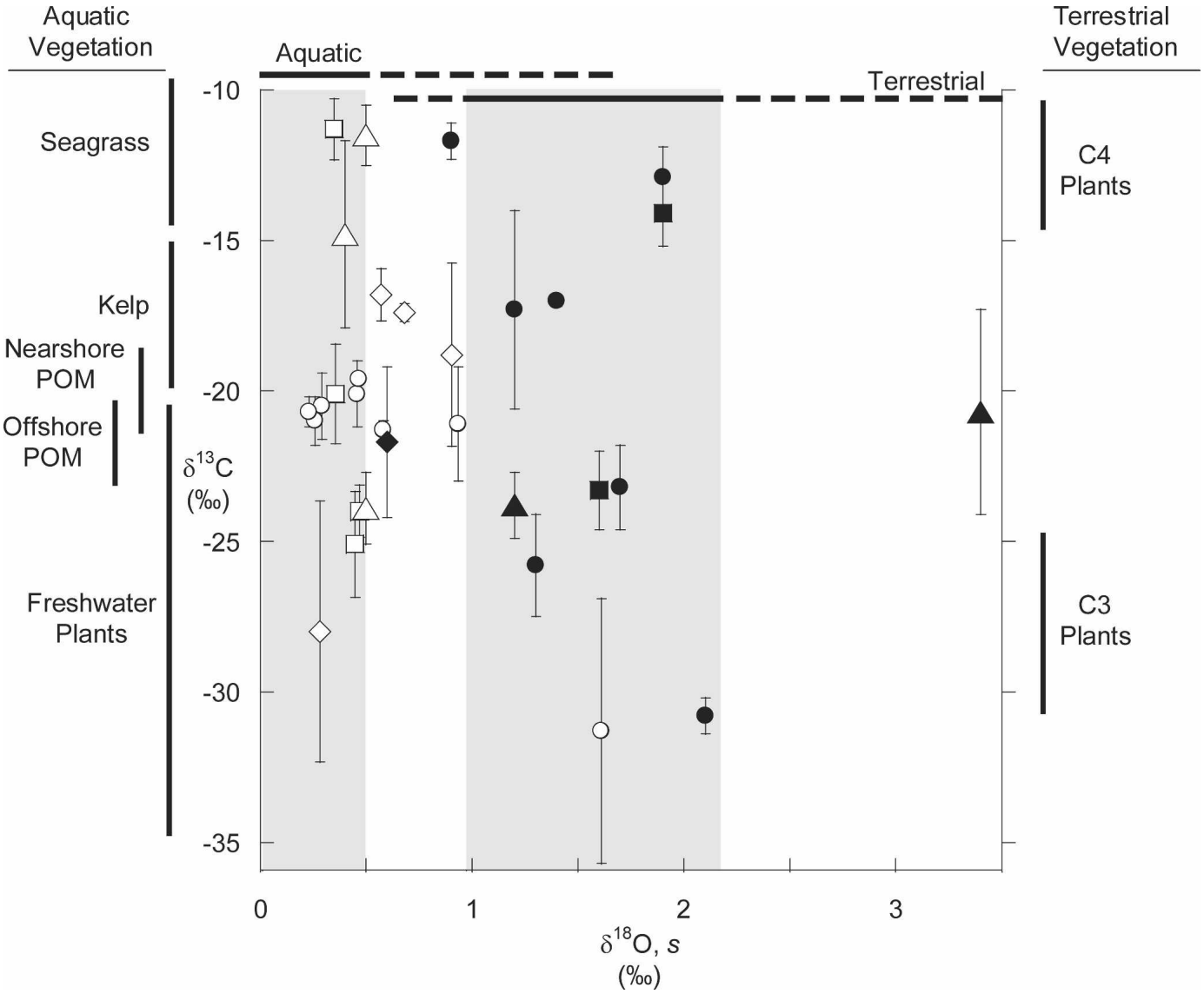

FIGURE 3. Graph of $\delta^{18}O$ variability (standard deviation, *s*) versus mean $\delta^{13}C$ values for modern species of aquatic and terrestrial mammals. Enamel $\delta^{13}C$ values have been converted to ecosystem $\delta^{13}C$ values by removing dietary and trophic-level fractionations (see text) and each symbol represents the mean value for a single species with error bars representing ± 1 *s* from the mean. Filled symbols represent terrestrial species; open symbols represent fully aquatic and semi-aquatic species. Enamel isotopic values were taken from Bocherens et al. (1996), Clementz and Koch (2001), Sponheimer and Lee-Thorp (2001), MacFadden et al. (2004), and Clementz and Koch (unpublished). Thick vertical bars represent ranges in $\delta^{13}C$ values for terrestrial and aquatic vegetation (Osmond et al., 1981; O'Leary, 1988; Hemminga and Mateo, 1996; Raven et al., 2002).

the type of photosynthetic pathway employed (i.e., C3, C4, or CAM; O'Leary, 1988) and environmental conditions (e.g., high vs. low wave energy, atmospheric vs. respired CO_2; Osmond et al., 1981; Duggins et al., 1989; Hemminga and Mateo, 1996; Raven et al., 2002). These factors generate distinct differences in the carbon-isotope composition of vegetation growing in the five habitats available to early marine mammals: freshwater ecosystems (lowest $\delta^{13}C$), terrestrial ecosystems (low $\delta^{13}C$), estuarine ecosystems (intermediate $\delta^{13}C$), nearshore marine ecosystems (high $\delta^{13}C$), and marine seagrass ecosystems (highest $\delta^{13}C$; Fig. 3).

Figures 2 and 3 illustrate how enamel $\delta^{18}O$ and $\delta^{13}C$ values can be used in tandem to discriminate among modern consumers within the five ecosystems listed above. Marine and freshwater aquatic species cluster along the x-axis with most 1*s* $\delta^{18}O$ values $\leq 0.5\%$, whereas terrestrial mammals typically yield 1*s* $\geq 1.0\%$ (Fig. 3). The few exceptions to this pattern include aquatic species (i.e., river otter, river dolphin) sampled from ecosystems with highly variable water $\delta^{18}O$ values (i.e., estuaries, multiple river systems) and very large-bodied terrestrial species (i.e., African elephant) that may obtain a large percentage of their oxygen from drinking water, which tends to dampen inter-individual variation (Bryant and Froelich, 1995). Ecosystem $\delta^{13}C$ values show a broad range in values, but clearly label aquatic consumers foraging within seagrass, nearshore marine, offshore marine, and freshwater environments, and terrestrial consumers foraging in C3 (low $\delta^{13}C$) and C4 (high $\delta^{13}C$) habitats. Finally, comparison of mean $\delta^{18}O$ values among these same species illustrates that expected differences in enamel $\delta^{18}O$ values between freshwater and marine species can be detected (Fig. 2), providing a proxy for salinity and habitat preferences of early marine mammals. Furthermore, the wide range in mean enamel $\delta^{18}O$ values for terrestrial mammals, which overlaps those for some marine and freshwater mammals, highlights the importance of applying this proxy only to species that were previously defined as aquatic.

Based on these patterns observed for modern consumers, a model of expected differences in enamel $\delta^{13}C$ and $\delta^{18}O$ values can be constructed to aid in paleoecological interpretation (Fig. 4). First, differences in $\delta^{18}O$ variability are used to discriminate between aquatic ($1\ s\ \delta^{18}O \leq 0.5\%$) and terrestrial ($1s\ \delta^{18}O \geq 1.0\%$) mammals; taxa with values falling between these extremes will be interpreted as terrestrial unless morphological evidence suggests otherwise. Second, among aquatic taxa, mean enamel $\delta^{18}O$ values are then used as a proxy for freshwater versus marine habitats. Third, ecosystem $\delta^{13}C$ values are calculated for each taxon and used to define the foraging preferences within both aquatic and terrestrial environments. Because the $\delta^{13}C$ values of Earth surface carbon reservoirs are known to have fluctuated over time (Koch et al., 1995), relative differences among taxa, rather than absolute values, are more useful when making ecological interpretations, and terrestrial consumer $\delta^{13}C$ values serve as the baseline for evaluation of archaeocete and sirenian values. Together, these three measures provide a powerful tool for quantifying the habitat and dietary preferences of early marine mammals.

Assessment of Diagenetic Alteration

Enamel's density and large crystal size make it more resistant to diagenetic alteration of stable isotope ratios than other mineralized tissues (e.g., bone, dentine) (Lee-Thorp and van der Merwe, 1987; Wang and Cerling, 1994; Koch et al., 1997; Zazzo et al., 2004), but enamel apatite alteration does still occur (Lee-Thorp and van der Merwe, 1987; Zazzo et al., 2004). We will use expected patterns in isotopic composition and variation among taxa as a monitor of the extent of diagenetic alteration. We assume that the main environmental controls on the carbon- and oxygen-isotope composition in the Eocene were similar to those today, such that fossilized terrestrial taxa are expected to show high variation in $\delta^{18}O$ values and $\delta^{13}C$ values similar to those of modern terrestrial C3 consumers, whereas if present, taxa restricted to aquatic habitats (based on inferences from morphology) should show lower $\delta^{18}O$ variability than terrestrial taxa. Retention of consistent differences in mean and variability in terrestrial and morphologically obligate aquatic taxa (i.e., basilosaurids and derived sirenians) provides support for the assumption that in-vivo isotopic patterns in our intermediate archaeocete and sirenian specimens are preserved as well.

Specimen Selection and Locality Information

Teeth were collected from eight archaeocete species in four families: Pakicetidae (*Himalayacetus subathuensis*, *Pakicetus inachus*), Remingtonocetidae (*Dalanistes ahmedi*), Protocetidae

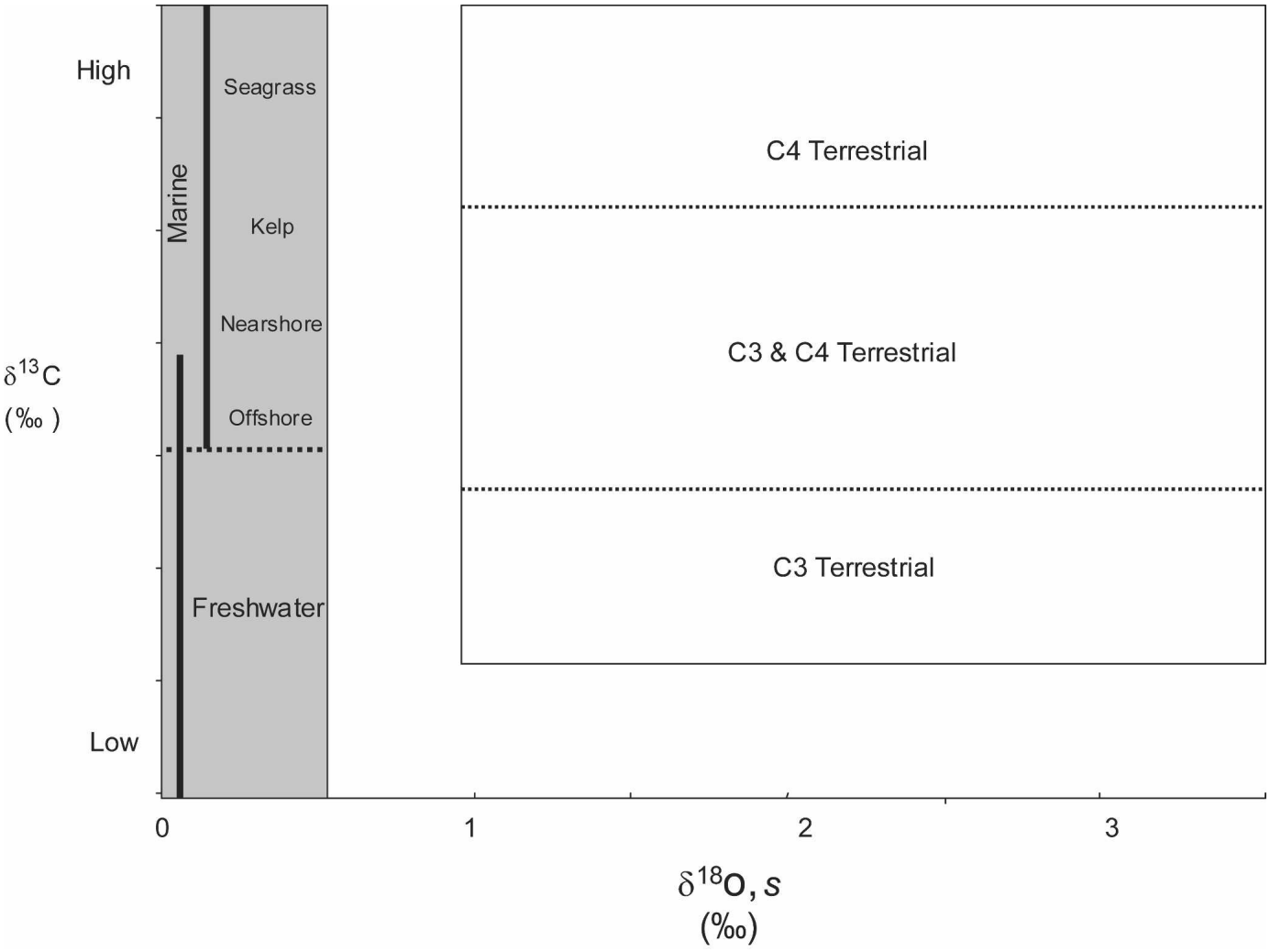

FIGURE 4. Paleoecological model comparing 1 *s* for enamel $\delta^{18}O$ values versus ecosystem $\delta^{13}C$ values for terrestrial and aquatic consumers. The dark gray area represents the field occupied by aquatic consumers and the large, open rectangle is that occupied by terrestrial consumers. Thick vertical bars represent the range in expected ecosystem $\delta^{13}C$ values for consumers in different aquatic foodwebs.

(*Babiacetus indicus*, *Rodhocetus kasrani*, and an undescribed new genus and species), and Basilosauridae (*Basilosaurus isis* and *Dorudon atrox*) (Fig. 1). Archaeocete material was collected from several localities in south Asia and Africa, spanning a considerable range of ages (early to late Eocene) and depositional environments (Fig. 5; Appendix 1). Teeth were also collected from four species of early sirenians in two families: Protosirenidae (*Protosiren smithae*) and Dugongidae (*Eosiren libyca*, *Eotheroides sp.*, and *Halitherium taulannense*) (Fig. 1). Fossil material was obtained from localities in North Africa and southern Europe, ranging from middle to late Eocene in age (Fig. 5; Appendix 1). When available, tooth samples from contemporary terrestrial mammals were sampled. Two localities yielded terrestrial faunas that were either directly associated or roughly coeval with the earliest archaeocete remains—the Ghazij and Kuldana Formations in Pakistan (early Lutetian, ca. 48 Ma) and the Qasr el-Sagha and Gebel Qatrani Formations in Egypt (Priabonian to Rupelian age) (Fig. 5; Appendix 1). Although not directly associated with marine fossils, the Qasr el-Sagha and Gebel Qatrani formations overlie the marine-mammal bearing Gehannam Formation and probably experienced similar post-burial conditions, so comparison of marine and terrestrial fossils from these different levels can still provide a valuable constraint on diagenetic alteration.

Analytical Methods

Approximately 10 mg of enamel powder were collected from the base of each sampled tooth. To remove organic contaminants, samples were treated with 0.5 mL of 2–3% NaOHCl solution, agitated, and allowed to soak overnight. Samples were then rinsed 5 times with de-ionized water, aspirated dry, and treated with 0.5 mL of 1.0 N acetic acid buffered to pH = 5.3 with calcium acetate to remove any diagenetic or non-lattice-bound carbonate. After rinsing 5 times with de-ionized water, samples were lyophylized overnight to dryness (Koch et al., 1997).

All stable-isotope analyses were done at the UCSC Stable Isotope Laboratory using a Micromass Optima gas source mass spectrometer linked to an ISOCARB preparation system. Approximately 1.5 mg of bone/tooth powder were dissolved in 100% phosphoric acid at 90°C for 8 minutes. After dissolution, CO_2 was cryogenically distilled and then introduced to the mass spectrometer for measurement.

All isotope values are reported in standard delta notation, where $\delta = ((R_{sample}/R_{standard}) - 1) \times 1000$ and R is $^{13}C/^{12}C$ for carbon and $^{18}O/^{16}O$ for oxygen. Carbon values are reported relative to the V-PDB standard and $\delta^{18}O$ values are reported relative to standard mean ocean water (V-SMOW). Precision of isotopic analysis was assessed via multiple analyses of an in-house elephant enamel standard ($\delta^{13}C$: standard deviation s = 0.1%; $\delta^{18}O$: s = 0.2%; n = 30 for both).

Statistical Methods

To assess the significance of differences in mean values among multiple groups of samples, we used a parametric, one-factor analysis of variance (ANOVA) followed by a post-hoc Tukey test for pair-wise comparisons to determine which groups were statistically distinct. When the assumptions of ANOVA were violated (i.e., unequal variance among populations, non-normal distribution), we used a non-parametric, Kruskal-Wallis ANOVA by Ranks (KWAR), followed by a post-hoc Dunn's Method for pair-wise comparisons. For comparisons between only two sample populations, we used a student *t* test; comparisons of variance between populations were assessed using a simple F-test. Statistical significance of correlation between values was evaluated using the Spearman rank correlation test. Statistical analyses were conducted using either SigmaStat v. 2.03 or Microsoft Excel 2000.

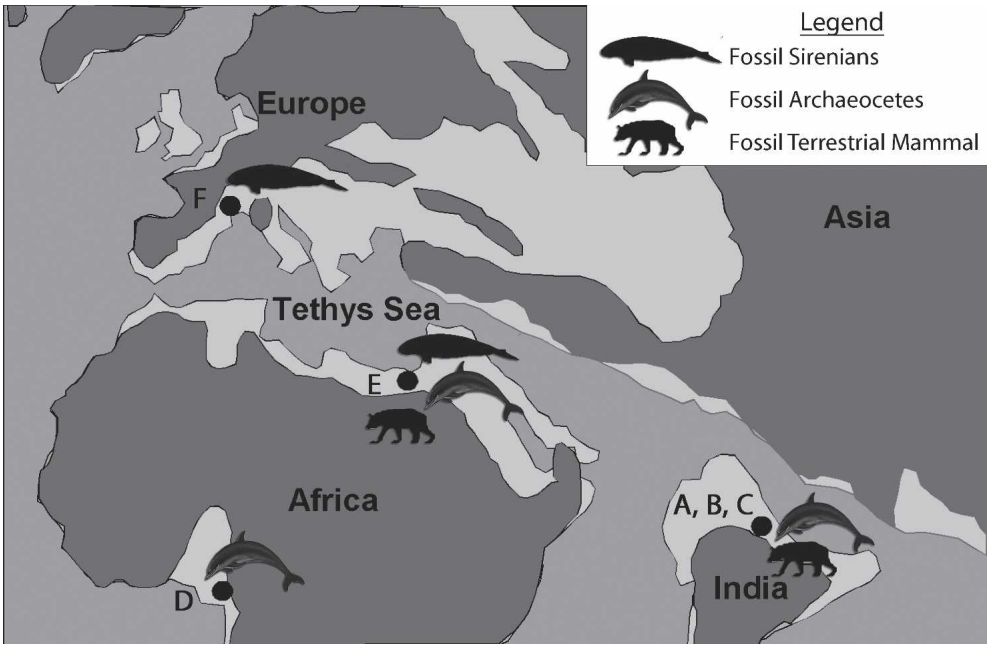

FIGURE 5. Paleogeographic reconstruction of the Tethys Sea in the Middle Eocene ~45 Ma (modified from Scotese [2001]). Sites from which fossils were obtained are represented by black dots. **Abbreviations**: **A**, Subathu Formation, northern India, early Eocene (*Himalayacetus*); **B**, Kuldana Formation, Pakistan, early middle Eocene (*Pakicetus*); **C**, Domanda Formation, Pakistan, middle Eocene (*Dalanistes, Rodhocetus*) and Drazinda Formation, Pakistan, middle Eocene (*Babiacetus*); **D**, Kpogame-Hahotoe basin, Togo, W. Africa, middle Eocene (undescribed protocetid); **E**, Birket Qarun and Gehannam Formation, Egypt, middle to late Eocene (*Eosiren, Eotheroides, Protosiren, Basilosaurus, Dorudon*); **F**, Alps-de-Haute-Provence, France, late Eocene (*Halitherium*).

RESULTS

For initial comparisons, specimens were grouped as archaeocetes, sirenians, and terrestrial mammals (Fig. 6). These groups exhibited statistically significant differences in isotopic variation—the $\delta^{18}O$ standard deviation for sirenians (0.6%) was much lower than that for land mammals (1.9%) and archaeocetes (2.3%) (F-test, $P < .01$), whereas the $\delta^{13}C$ standard deviation for archaeocetes (2.0%) was much greater than that for either sirenians (1.4%; F-test, $P = .056$) or land mammals (1.3%; F-test, $P < .01$). Statistically significant differences in mean $\delta^{13}C$ (one-way ANOVA, $F = 192.270$, $P < .01$) and mean $\delta^{18}O$ values (KWAR, $H = 10.35$, $P < .01$) were also observed. Sirenians consistently had the highest $\delta^{13}C$ values (mean = –0.2 ± 1.4%; Tukey test, $P < .01$), whereas mean values for archaeocetes (–9.6 ± 2.0%) and terrestrial mammals (-10.8 ± 1.3%) were not significantly different (Tukey test, $P > 0.71$). No statistically significant difference in mean $\delta^{18}O$ values was detected among archaeocetes (26.1 ± 2.3%), terrestrial mammals (26.6 ± 1.9%), and sirenians (27.6 ± 0.6%) (one-way ANOVA, $F = 2.641$, $P = .08$).

Archaeocetes

Samples from several different tooth types of *P. inachus* were analyzed, ranging from deciduous premolars to permanent molars (Fig. 7; Appendix 1). Deciduous and permanent teeth did not differ significantly in mean $\delta^{13}C$ values (student *t* test, $t = –0.050$, $P = .96$) or in mean $\delta^{18}O$ values (student *t* test, $t = –2.069$, $P = .07$). Variation in $\delta^{13}C$ and $\delta^{18}O$ values was also found not to differ significantly ($\delta^{13}C$: F-test, $P = .48$; $\delta^{18}O$: F-test, $P = .06$), though low sample size may be obscuring statistical significance. No statistically significant correlation was detected between $\delta^{13}C$ and $\delta^{18}O$ values among teeth (Spearman rank correlation, $P = .57$). Enamel isotope values of *P. inachus* were also compared based on timing of eruption, following the sequence determined for *D. atrox* by Uhen (2000) (Table 1). Samples were grouped into three categories: eruption while in utero, early eruption, and late eruption. Mean $\delta^{13}C$ values did not differ significantly among the groups (one-way ANOVA, $F = 0.881$, $P = .46$), but differences in mean $\delta^{18}O$ values were significant (one-way ANOVA, $F = 15.240$, $P < .01$). Pair-wise comparison showed that the mean $\delta^{18}O$ value of early erupting teeth (25.6 ± 1.2%) was significantly higher than that of teeth that had erupted in utero (22.7 ± 0.3%) or teeth that had erupted later in life (23.4 ± 0.3%) (Tukey test, $P < .01$). For comparisons made among taxa (including those presented for major groups above), the mean values and standard deviations for *P. inachus* are based on $\delta^{13}C$ and $\delta^{18}O$ values for the 6 teeth that formed in utero or erupted later in life (Table 1).

Among archaeocetes, only three species (*P. inachus, D. atrox*, and *B. isis*) had enough samples ($n \geq 3$) to estimate mean and standard deviation for $\delta^{18}O$ and $\delta^{13}C$ values. Variation in $\delta^{18}O$ values did not differ significantly among these genera (F-test, $p \geq 0.55$), but the low variation in $\delta^{13}C$ values for *B. isis* was statistically distinct from that of *P. inachus* (F-test, $P = .03$) and *D. atrox* (F-test, $P = .02$) (Table 1). Differences in mean $\delta^{13}C$ values among these species were statistically significant (one-way ANOVA, $F = 15.007$, $p < 0.01$); the low mean value for *P. inachus* (–12.1 ± 1.3%) was statistically distinct from that of *D.*

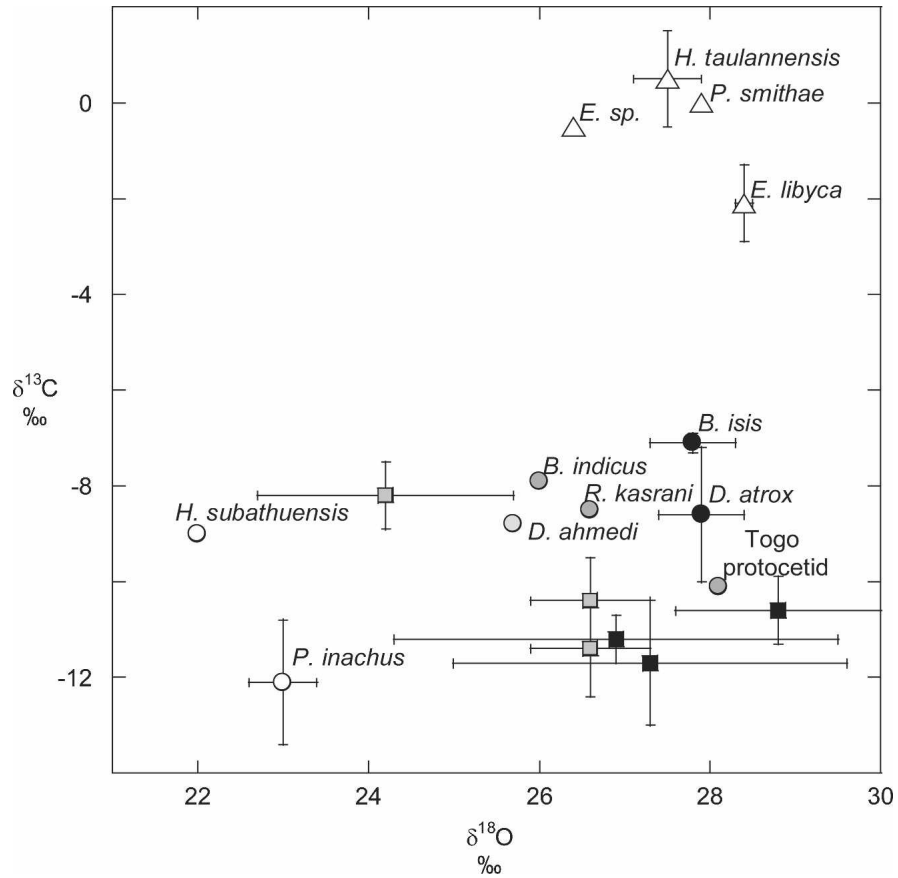

FIGURE 6. Plot of mean enamel $\delta^{18}O$ and $\delta^{13}C$ values for Eocene archaeocetes (circles: white = pakicetids, light gray = remingtonocetids, dark gray = protocetids, black = basilosaurids), sirenians (triangles) and terrestrial mammals (black squares = Egypt; gray squares = Pakistan). Sirenian and archaeocete symbols are labeled with species names. Error bars represent ± 1 *s* from the mean. Note that enamel $\delta^{13}C$ values have not been corrected to ecosystem $\delta^{13}C$ values.

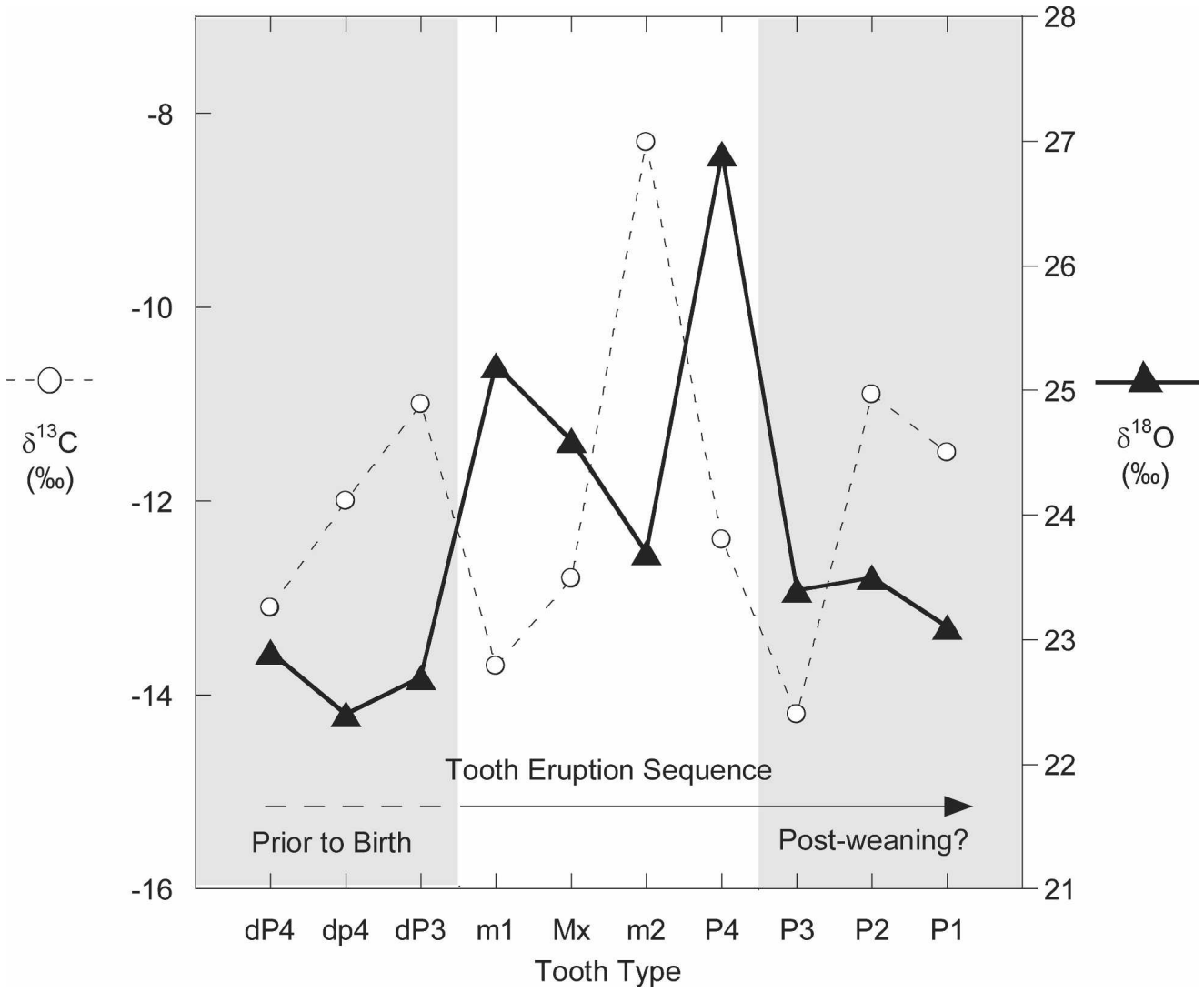

FIGURE 7. Graph of enamel $\delta^{13}C$ and $\delta^{18}O$ values for individual teeth of *Pakicetus*. Upper and lower case letters identify upper and lower teeth, respectively. **Abbreviations**: **d**, deciduous; **p** = premolar; **m** = molar. The estimated sequence of tooth eruption for *P. inachus* is based on that for the later archeocete, *D. atrox* (Uhen, 2000).

atrox (–8.6 ± 1.4%) and *B. isis* (–7.1 ± 0.2%) (Tukey test, $P < .01$). Likewise, differences in mean $\delta^{18}O$ values were statistically significant (One-way ANOVA, $F = 215.934$, $P < .01$) because of the extremely low mean $\delta^{18}O$ value for *P. inachus* (23.0 ± 0.4‰) relative to that of *D. atrox* (27.9 ± 0.5‰) and *B. isis* (27.8 ± 0.5‰) (Tukey test, $P < .01$).

Carbon isotope values for the protocetids, remingtonocetids, and *Himalayacetus* were all high and comparable to mean $\delta^{13}C$ values for basilosaurids (Table 1). Oxygen isotope values, however, were more varied. *Himalayacetus subathuensis* had the lowest $\delta^{18}O$ value for any specimen sampled, most comparable to the mean $\delta^{18}O$ value of *Pakicetus* (Table 1). For the remingtonocetid, *D. ahmedi*, and the protocetids, *R. kasrani* and *B. indicus*, enamel $\delta^{18}O$ values were much higher, but still lower than mean values reported for basilosaurids (Table 1). Only one specimen, the undescribed protocetid genus, had a $\delta^{18}O$ value as high as that reported for the basilosaurids.

A significant positive correlation was detected between archaeocete $\delta^{13}C$ and $\delta^{18}O$ values (Spearman rank correlation coefficient = 0.565, $P < .01$).

Sirenians

Four species of sirenians from the families Protosirenidae (*P. smithae*) and Dugongidae (*E. libyca*, *E. sp.*, and *H. taulannense*) were analyzed and comparisons were made at the generic level. Only two species, *E. libyca* and *H. taulannense*, had large enough sample sizes to generate reliable estimates of the mean and standard deviation in isotope values. No significant differences in variation were detected between genera for either $\delta^{13}C$ or $\delta^{18}O$ values (F-test, $P > .05$). Mean $\delta^{13}C$ values differed significantly between genera (student *t* test, $t = –4.043$, $P < .01$); the mean $\delta^{13}C$ value for *E. libyca* was significantly lower than that for *H. taulannense* (Table 1). The individual $\delta^{13}C$ values for *P. smithae* and *E.* sp. were relatively high and most comparable to the mean $\delta^{13}C$ value for *H. taulannense*. As for $\delta^{18}O$ values, mean values for *E. libyca* and *H. taulannense* were statistically distinct (student *t* test, $t = 3.949$, $P < .01$). The single $\delta^{18}O$ value for *P. smithae* was between mean values for *E. libyca* and *H. taulannense*, but the value for *E.* sp. was significantly lower than values for any other sirenian genus (Table 1). No statistically significant

TABLE 1. Mean $\delta^{13}C$ and $\delta^{18}O$ values (± one standard deviation *s*) for fossil specimens of archaeocetes, sirenians, and terrestrial mammals are grouped by locality

Locality	Taxonomic information			N	$\delta^{13}C$(‰)		$\delta^{18}O$(‰)
	Groups	Family	Genus		Enamel	Ecosystem	
Pakistan, India, and West Africa	Archaeocetes	Pakicetidae	*Himalayacetus*	1	−9.0	−19.7	22.0
			Pakicetus	6	−12.1 ± 1.3	−22.8 ± 1.3	23.0 ± 0.4
		Remingtonocetidae	*Dalanistes*	1	−8.8	−19.5	25.7
		Protocetidae	*Rodhocetus*	1	−8.5	−19.2	26.6
			Babiacetus	1	−7.9	−18.6	26.0
			Undescribed	1	−10.1	−20.8	28.1
	Terrestrial Mammals	Quettacyonidae	*Sorocyon*	6	−10.4 ± 0.9	−24.4 ± 0.9	26.6 ± 0.7
		Tillodontia	Undescribed	3	−11.4 ± 1.0	−25.4 ± 1.0	26.6 ± 0.7
		Artiodactyla	*Khirarthra*	3	−8.2 ± 0.7	−22.2 ± 0.7	24.2 ± 1.5
Egypt	Archaeocetes	Basilosauridae	*Basilosaurus*	3	−7.1 ± 0.2	−17.8 ± 0.2	27.8 ± 0.5
			Dorudon	6	−8.6 ± 1.4	−19.3 ± 1.4	27.9 ± 0.5
	Sirenians	Protosirenidae	*Protosiren*	1	0.0	−12	27.9
		Dugongidae	*Eosiren*	3	−2.1 ± 0.8	−14.1 ± 0.8	28.4 ± 0.1
			Eotheroides	1	−0.5	−12.5	26.4
	Terrestrial Mammals	Anthracotheriidae	*Bothriogenys*	7	−11.2 ± 0.5	−25.2 ± 0.5	26.9 ± 2.6
		Embrithopoda	*Arsinoitherium*	6	−10.6 ± 0.7	−24.6 ± 0.7	28.8 ± 1.2
		Hyracoidea	*Saghatherium*	4	−11.7 ± 1.3	−25.7 ± 1.3	27.3 ± 2.3
France	Sirenia	Dugongidae	*Halitherium*	7	0.5 ± 1.0	−11.5 ± 1.0	27.5 ± 0.4

For sample sizes of only 2 individuals, both values are reported rather than a mean or standard deviation. Individual values and information on specimen identification are presented in Appendix 1.

correlation between $\delta^{13}C$ and $\delta^{18}O$ values was detected (Spearman rank, $P > .05$).

DISCUSSION

Archaeocete and sirenian tooth enamel displayed a wide range in mean $\delta^{13}C$ and $\delta^{18}O$ values (Fig. 6). Before ecological interpretations can be drawn from these results, however, the possibility of diagenetic alteration must be assessed. Our gauge of the extent of diagenetic alteration is based on whether expected ecological patterns are present in the control taxa (i.e., terrestrial and morphologically-obligate aquatic mammals). Mean enamel $\delta^{13}C$ values of terrestrial mammals range from –8.2% to –11.7% (Table 1), fitting the expectation based on independent evidence that Eocene terrestrial ecosystems were dominated by C3 vegetation (Quade et al., 1992; Cerling et al., 1997) (Fig. 8). Furthermore, variations in enamel $\delta^{18}O$ values are high, ranging from 0.7% to 2.6% (Fig. 8; Table 1), and are comparable to values for modern terrestrial mammals (Fig. 3). Because diagenetic alteration would tend to homogenize $\delta^{18}O$ values among samples, the high variation in terrestrial samples suggests that the original isotope signal has been at least partially retained. Furthermore, $\delta^{18}O$ variations in basilosaurids and specialized sirenians are extremely low (0.3% to 0.5%) and within the expected range based on modern aquatic species (Fig. 3). The retention of expected differences between aquatic and terrestrial species supports our use of the isotope values of early cetacean and sirenian tooth enamel for ecological interpretations.

Our results confirm that the timing of tooth formation and eruption is another factor that might confound ecological interpretations based on enamel stable-isotope composition. Various tooth types sampled from the pakicetid *P. inachus* exhibited a wide range in stable isotope values (Fig. 7). While $\delta^{13}C$ values showed no obvious trend with timing of eruption, $\delta^{18}O$ values had a distinct pattern: teeth that formed in utero or toward the end of the eruptive sequence had relatively low $\delta^{18}O$ values, while teeth erupting in between these two stages had much higher values (Fig. 7). Higher $\delta^{18}O$ values suggest ingestion of ^{18}O-enriched water and, considering the timing of eruption of these teeth (Uhen, 2000), mother's milk is the most likely source. Mammalian body water and milk water $\delta^{18}O$ values are typically ^{18}O-enriched relative to drinking water, resulting in enriched enamel $\delta^{18}O$ values for teeth formed while an individual is nursing (Wright and Schwarcz, 1998). Inclusion of enamel $\delta^{18}O$ values from these teeth into our dataset would significantly impact ecological interpretations of habitat use. Consequently, we restricted further consideration of data to teeth that reflect adult diet, which include teeth that form in utero, since no significant isotope differences have been detected between modern cetacean teeth that form in utero and bone or teeth that forms postweaning (Yoshida and Miyazaki, 1991; Barrick et al., 1992; Roe et al., 1998; Clementz and Koch, 2001).

How Aquatic Were Early Archaeocetes and Sirenians?

Only sample sizes for three archaeocete and two sirenian taxa were large enough (i.e., $n \geq 3$) to yield reliable estimates of population mean and standard deviation in $\delta^{18}O$ values. These are the pakicetid *P. inachus*, the two basilosaurids *B. isis* and *D. atrox*, and the early dugongids *E. libyca* and *H. taulannense*. Levels of $\delta^{18}O$ variation within unquestionably aquatic taxa (i.e., basilosaurids and early dugongids) were low (<0.5%; Fig. 8; Table 1); this, combined with retention of high variation in cooccurring or approximately coeval terrestrial mammals, suggests minimal diagenetic modification of original isotopic values. Based on this test, $\delta^{18}O$ variation in taxa with unknown habitat preferences (e.g., *P. inachus*) can be used as a proxy for aquatic affinity.

Variation in adult enamel $\delta^{18}O$ values for *P. inachus* is comparable to that of wholly aquatic taxa, ~0.4%. Thus though the skeleton of this animal may not have been highly modified for an aquatic lifestyle (according to the interpretation of Thewissen et al., 2001), it was deriving an appreciable amount of its body water from a source with homogeneous $\delta^{18}O$ values, implying aquatic immersion (Fig. 8). The small body size of this early archaeocete lends support to this interpretation; small mammals typically experience greater levels of evaporation of water across the skin, a process that can increase variation in $\delta^{18}O$ values among individuals within a population. If *P. inachus* spent most of its time onshore, this relative increase in evaporation would have led to significantly greater levels of $\delta^{18}O$ variation, comparable to that detected for coeval terrestrial mammals ($1\ s \geq 0.7\%$). An aquatic interpretation may also explain the osteosclerotic long bones of pakicetids; increased deposition of compact bone in the limbs may have provided ballast for submersion (Thewissen and Williams, 2002). Though analysis of additional

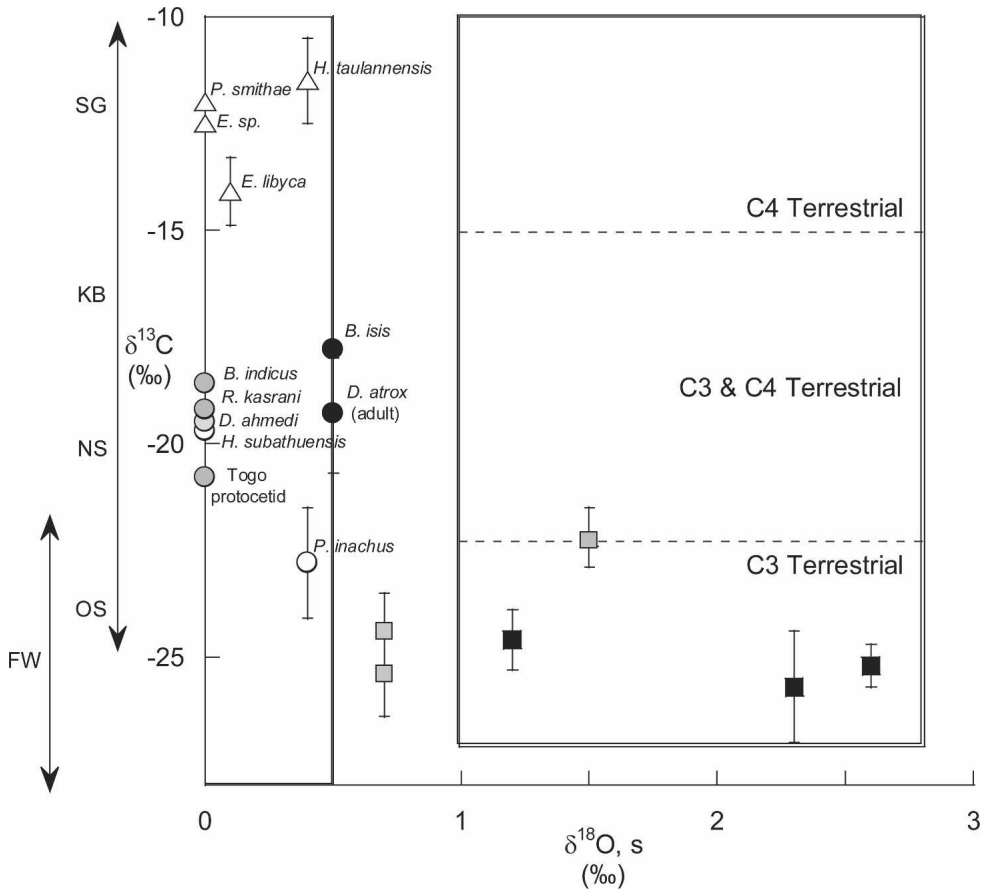

FIGURE 8. Graph of enamel $\delta^{18}O$ variability (in standard deviation, *s*) versus ecosystem $\delta^{13}C$ values for Eocene archaeocetes (circles: white = pakicetids, light gray = remingtonocetids, dark gray = protocetids, black = basilosaurids), sirenians (triangles) and terrestrial mammals (squares: black = Egypt; gray = Pakistan). Sirenian and archaeocete symbols are labeled with species names. Vertical arrows to the left of the y-axis represent the expected $\delta^{13}C$ ranges for different aquatic habitats. **Abbreviations**: **SG**, seagrass meadows; **KB**, kelp beds; **NS**, nearshore marine; **OS**, offshore marine; and **FW**, fresh water. Error bars represent ±1 *s* from the mean. Rectangular fields are the same as those presented in Fig. 4.

species of pakicetids is warranted before our interpretation of their aquatic habits can be confirmed, our results suggest that the aquatic habits of cetaceans were established early within their evolution. Only later, after cetaceans had moved into marine environments, were major modifications to the skeleton made to facilitate swimming.

We had planned to use variation in $\delta^{18}O$ values to interpret the aquatic habits of the earliest sirenians and other archaeocete taxa as well, but were unable to gather sufficient sample sizes. At least for the archaeocete taxa, however, we can use results from *P. inachus* and the basilosaurids to generate interpretations of behavior/habits of related groups based on the premise of phylogenetic bracketing. These taxa represent, respectively, the oldest and youngest families of archaeocetes (O'Leary and Uhen, 1999; Thewissen and Williams, 2002; Uhen, 2004). Assuming parsimonious character transformations, our isotopic evidence for aquatic habits in these bracketing taxa would imply that all intermediate groups were probably aquatic as well. Of course, additional isotopic evidence and other direct forms of information on the habits of these extinct taxa (as well as early sirenians) will be needed to verify this inference. As to the aquatic affinity of the earliest pakicetid, *H. subathuensis,* which is found in marine strata, our single $\delta^{18}O$ value is insufficient to make any firm conclusions, but the similarity in its $\delta^{18}O$ value to the mean value of *P. inachus* could suggest that the ecologies of these animals were similar.

Where Were Early Marine Mammals Feeding?

Examination of ecosystem $\delta^{13}C$ values suggests early archaeocetes and sirenians were exploiting a broad range of habitats that were quite different from those exploited by coeval terrestrial mammals (Fig. 8). Mean ecosystem $\delta^{13}C$ values for the Eocene terrestrial mammals from Pakistan and Egypt were generally consistent with expected values for pure C3 consumers (Cerling and Harris, 1998). Furthermore, mean terrestrial mammal $\delta^{13}C$ values from early middle Eocene Pakistan and late Eocene Egypt were nearly identical, indicating that no major changes in the $\delta^{13}C$ value of Earth surface carbon reservoirs had occurred during this time period. This consistency among the terrestrial taxa supports the assumption that archaeocete and sirenian samples from all sites are directly comparable for ecological interpretations.

Early archaeocetes can be split into two groups based on $\delta^{13}C$ values. The first group consists of the earliest cetaceans (e.g., pakicetids) and is defined by extremely low but highly variable $\delta^{13}C$ values (Fig. 8). Though pakicetid enamel $\delta^{13}C$ values were significantly lower than those for coeval terrestrial mammals (Table 1), ecosystem $\delta^{13}C$ values reconstructed from these samples are nearly identical between these two groups, allowing the possibility that pakicetids were consuming terrestrial resources (Fig. 8). If so, the aquatic *P. inachus* may have ambushed terrestrial prey as they came to drink at the water's edge just as

modern crocodiles do today. This predatory behavior was originally proposed for another early archaeocete, *Ambulocetus* (Thewissen et al., 1996), and has been interpreted for taxa in other tetrapod lineages that have made a similar transition to aquatic habitats (Taylor, 1987). Alternatively, *P. inachus* could have been foraging in freshwater habitats; modern freshwater carnivores have much lower ecosystem $\delta^{13}C$ values (Fig. 3) than obtained for pakicetids, but the magnitude of $\delta^{13}C$ variation is very large, bracketing that exhibited by *P. inachus* (Fig. 8). An interpretation of freshwater foraging is consistent with previous inferences based on tooth morphology (Gingerich and Russell, 1990; O'Leary and Uhen, 1999) and isotope analysis (Roe et al., 1998). Regardless, *P. inachus* $\delta^{13}C$ values are much lower than those reported for other archaeocete taxa, especially confirmed marine consumers (e.g., basilosaurids), indicating that the foraging habits of pakicetids were unique relative to other archaeocetes, whether they were foraging on terrestrial or freshwater resources. This interpretation is complicated by isotope evidence from the earliest pakicetid, *H. subathuensis*, known from a single specimen found in marine deposits (Bajpai and Gingerich, 1998). Though more material is needed for determination of the ecology of this animal, its high ecosystem $\delta^{13}C$ value is a hint that some of the earliest archaeocetes may have invaded the marine realm.

The second group of early archaeocetes consists of more-specialized groups—the remingtonocetids, protocetids, and basilosaurids. The mean ecosystem $\delta^{13}C$ values for these groups are significantly higher than those reported for the pakicetids or ambulocetids (Roe et al., 1998), and are higher than mean $\delta^{13}C$ values reported for terrestrial mammals (Table 1). The high $\delta^{13}C$ values for these groups are indicative of foraging in nearshore marine habitats (Fig. 8). An interpretation of marine diets for these groups is supported by sedimentological data—remains for all three groups have been reported from marine deposits (Gingerich, 1992; Gingerich et al., 1995). The range in $\delta^{13}C$ values observed in remingtonocetids and protocetids is identical to that for the more-specialized basilosaurids, suggesting that major changes in foraging habits were not linked to the origin of these groups. After cetaceans began foraging in marine habitats, they remained restricted to nearshore habitats until at least the late Eocene; offshore foraging may not have arisen until the evolution of more modern forms (i.e., odontocetes and mysticetes).

With mean $\delta^{13}C$ values as much as 11% higher than those for terrestrial mammals, sirenian enamel and ecosystem $\delta^{13}C$ values are extremely distinct (Figs. 6 and 8). Only two dietary resources could generate these high values: terrestrial C4 grasses or aquatic seagrass. Previous fossil and isotopic evidence does not support the occurrence of C4 grasses at this time; and this interpretation is bolstered by the low $\delta^{13}C$ values for terrestrial herbivores. Sirenian values are similar to those for the modern dugong (Fig. 3), a species of sea cow that feeds almost exclusively on seagrass (Marsh et al., 1982). The fact that all early sirenian species, including the four-legged *P. smithae*, had extremely high $\delta^{13}C$ values demonstrates that seagrass was an important component of sirenian diets early in their evolution (Fig. 8). Previously reported fossil associations of sirenian remains with seagrass bed deposits support this interpretation (Domning, 1981; Ivany et al., 1990). The paucity of vertebrate and invertebrate herbivores capable of consuming marine macrophytes throughout the Phanerozoic implies that sirenians faced little competition for this abundant, but low quality, dietary resource (Vermeij and Lindberg, 2000). Their incursion into seagrass meadows must have generated significant changes in ecosystem structure and dynamics. The close herbivore-plant connection between sirenians and seagrasses over the past 50 million years may have led to significant co-evolution. Analysis of earlier sirenians (i.e., the prorastomids from Jamaica and Florida) would reveal the antiquity of this ecological association.

Osmoregulation and Habitat preferences of Early Marine Mammals

The transition from terrestrial to freshwater and marine environments necessitated major changes in the way early archaeocetes and sirenians osmoregulated (i.e., maintained constant internal water and electrolyte concentrations). Living cetaceans and sirenians have adopted several methods to handle this problem, including hormonal regulation and efficient excretory systems (Ortiz, 2001; Costa, 2002). Although preservation of direct evidence for osmoregulatory adaptations in the fossil record is unlikely, isotopic evidence can help constrain when animals first invaded marine environments. Enamel $\delta^{18}O$ values can be used to infer the types of aquatic ecosystems a group inhabited, based on differences in the $\delta^{18}O$ composition of fresh (low $\delta^{18}O$), estuarine (intermediate $\delta^{18}O$), and marine (high $\delta^{18}O$) waters (Fig. 9).

Mean $\delta^{18}O$ values for the earliest archaeocetes, the pakicetids, are all extremely low (Fig. 9), which is a strong indication that these animals spent a significant amount of time in freshwater habitats. Indeed, mean $\delta^{18}O$ values for pakicetids are significantly lower than values for terrestrial mammals, another line of evidence that pakicetids were aquatic. The low $\delta^{18}O$ value for *H. subathuensis* is interesting given the $\delta^{13}C$ evidence of a marine diet, and hints that this early cetacean may have foraged along the coast, but still required fresh water to offset the high salt load of its diet.

More specialized archaeocetes, the remingtonocetids, protocetids and basilosaurids, have higher $\delta^{18}O$ values, which indicate they were moving into marine waters (Fig. 9). Carbon-isotope values show that all were consuming marine resources (Fig. 8), yet mean $\delta^{18}O$ values for *D. ahmedi*, *B. indicus*, and *R. kasrani* are significantly lower than those for the undescribed protocetid and the basilosaurids. The former taxa may not have been fully capable of osmoregulating within seawater and therefore restricted their foraging to estuarine and nearshore habitats with access to low salinity waters. The high $\delta^{18}O$ values for the basilosaurids, *D. atrox*, and *B. isis*, and the undescribed protocetid are nearly identical to mean values reported for modern cetaceans and are indicative of marine habits (Figs. 2 and 9). These values, however, are slightly higher than would be predicted for Eocene marine mammals, since the mean $\delta^{18}O$ value of Eocene seawater is estimated to have been ~1% lower than that of seawater today (Zachos et al., 2001). The most likely explanation for the minor enrichment in enamel $\delta^{18}O$ values is that the $\delta^{18}O$ value of the Tethys Sea may have been slightly higher than that of the global ocean during the Eocene. Although the world's oceans are strongly homogeneous in $\delta^{18}O$ composition, there are slight variations (~1%) due to regional differences in precipitation and evaporation (Craig and Gordon, 1965). During the Eocene, the Tethys Sea likely experienced intense levels of evaporation because of its tropical location; this may have left the waters ^{18}O-enriched by 1‰ or more than global seawater. Tooth enamel from archaeocetes that foraged in these waters and had achieved the ability to osmoregulate within hypersaline environments would possess a similar level of enrichment and enamel $\delta^{18}O$ values similar to modern cetaceans.

Interestingly, the two archaeocete families that show evidence for the capacity to osmoregulate in seawater (i.e., protocetids and basilosaurids) are the only archaeocetes found outside of the Tethys Sea. Protocetids had reached western Africa by the middle Eocene (ca. 45 Ma) and both protocetids and basilosaurids were present in North America shortly thereafter (ca. 40 Ma; Halstead and Middleton, 1974; Gingerich et al., 1992; Hulbert et al., 1998; Uhen and Gingerich, 2001). Thus, the difficulty associated with osmoregulation in the marine environment was undoubtedly a significant factor restricting the dispersal of archaeocetes across open marine waters. Once overcome, archaeocetes

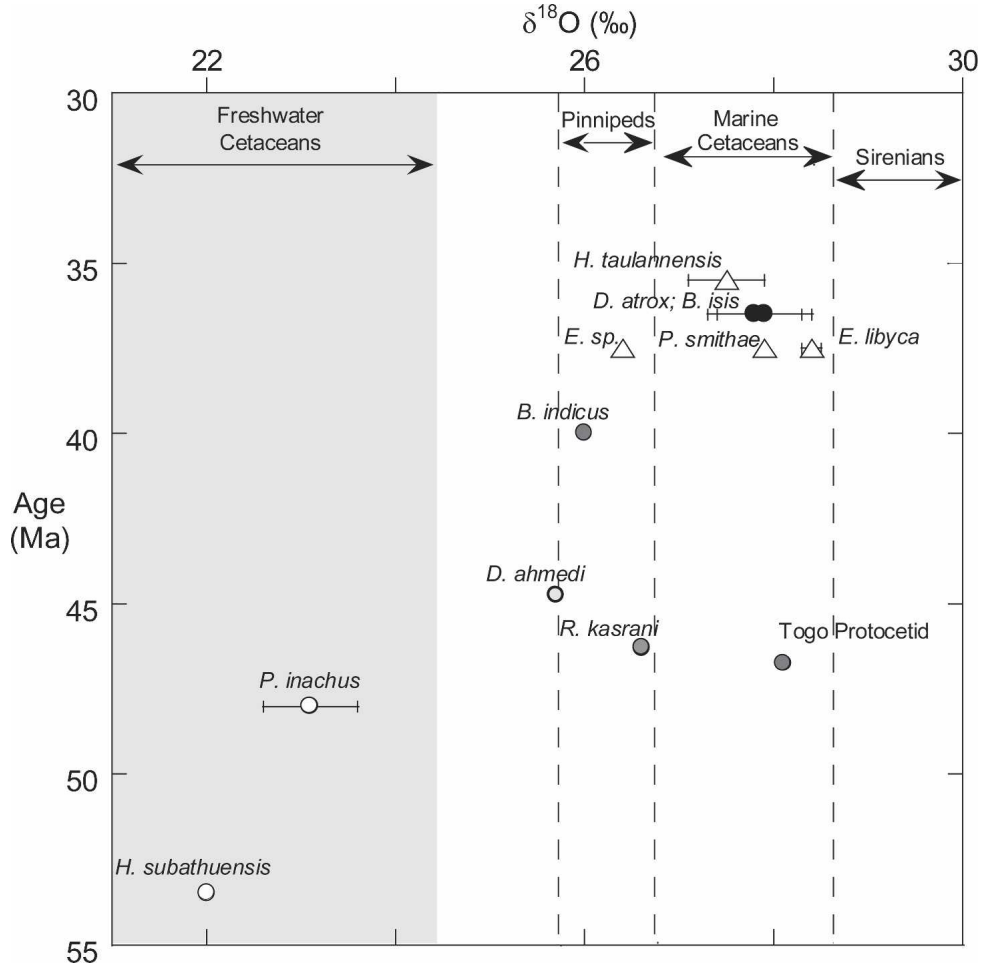

FIGURE 9. Plot of mean enamel $\delta^{18}O$ from Eocene archaeocetes (circles: white = pakicetids, light gray = remingtonocetids, dark gray = protocetids, black = basilosaurids), and sirenians (triangles) versus age. Sirenian and archaeocete symbols are labeled with species names. Error bars represent ±1 *s* from the mean. Gray fields represent mean $\delta^{18}O$ values (± 1 *s*) for extant marine mammals and freshwater cetaceans.

were able to sever their final tie to terrestrial ecosystems and achieve a much broader geographic distribution, ultimately spreading into the Atlantic and Pacific Oceans by the late Eocene (Fordyce, 1985).

Sirenian $\delta^{18}O$ values offer an interesting contrast to the scenario reconstructed for early cetaceans. If the earliest sirenian analyzed, the protosirenid *Protosiren*, was aquatic, then the high $\delta^{18}O$ value for this specimen would imply this animal fed in estuarine or marine ecosystems (Fig. 9). This interpretation is supported by the high ecosystem $\delta^{13}C$ value for this specimen, indicating that *P. smithae* was foraging within marine seagrass meadows (Fig. 8), and by similar isotopic values reported for another species of *P. smithae* from the late Eocene of Florida (MacFadden et al., 2004). Later sirenians, *E. libyca* and *H. taulannensis*, continue this trend, with mean $\delta^{18}O$ values identical to those of the two marine basilosaurids we analyzed. Today, enamel $\delta^{18}O$ values for marine sirenians and cetaceans are similar, differing by <1% (Figs. 2 and 9), and this relationship seems to have been true in the late Eocene. However, not all of the sirenian species we analyzed were solely marine; the single specimen of *E. sp.* had a $\delta^{18}O$ value 2% lower than that of other sirenians, perhaps because this sirenian had ventured into freshwater or estuarine environments at some point in its life. It is certainly true that in Recent and Pleistocene ecosystems, manatees have ventured into freshwater systems to live and forage (Lefebvre et al., 2001; Deutsch et al., 2003; MacFadden et al., 2004).

Our results point to an interesting dichotomy. Archaeocetes appear to have started as freshwater carnivores and gradually moved into marine habitats. Currently available evidence suggests that sirenians were initially marine herbivores, feeding in seagrass beds, and only later moved into freshwater habitats. Given that the earliest sirenians occur in the west Atlantic at least 10 Ma before archaeocetes, it might not be too surprising that sirenians took a more direct route than archaeocetes into the marine environment. Additional analysis of these early sirenians, the prorastomids, and specimens of *E. sp.* will be needed to confirm this pattern.

CONCLUSIONS

Isotopic study of archaeocete and sirenian tooth enamel has revealed important new information about the transition of these groups from terrestrial to aquatic environments. Both groups appear to have adapted to the marine environment early in their evolutionary history. Yet each group may have taken a slightly different path (i.e., archaeocetes: freshwater to marine; sirenians: marine to freshwater), suggesting that dietary differences between the two groups played a role in guiding the transition in each group.

Though the most basal archaeocetes, the pakicetids, do not appear to have been highly modified for aquatic locomotion, isotopic data suggest they were predominantly freshwater carnivores that spent a significant amount of time in the water. Later, more-specialized archaeocetes, remingtonocetids and protocetids, appear to have spent a greater amount of time in marine habitats, mainly within nearshore ecosystems. Basilosaurids maintained this affinity for marine environments and continued to forage predominantly within nearshore ecosystems. The increasing aquatic adaptation in these groups was not necessarily associated with an expansion into offshore habitats.

Sirenians show the opposite pattern, though our interpretations are limited by the lack of information on the earliest known group, the prorastomids. Early in their evolution, sirenians adapted to a predominantly marine, seagrass-based diet. They appear to have been capable of osmoregulating within marine ecosystems without ingestion of fresh water. Low variation in $\delta^{13}C$ values for this group suggests that their dietary preferences were highly focused and only later, with the evolution of trichechids (ca. 37–28 Ma) and hydrodamalines (ca. 16 Ma), did sirenians branch out to other aquatic resources (freshwater vegetation and marine kelp, respectively). The lack of dietary differentiation within this group may help in explaining the low species diversity of sirenians relative to other groups of marine mammals.

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APPENDIX 1

Stable isotope values for all specimens analyzed in this study. Specimens are organized by taxonomic order and family and include collection numbers, species names, locality information, and tooth type when available. Materials were obtained from collections at the Geological Survey of Pakistan—University of Michigan (GSP-UM), the University of Michigan Museum of Paleontology (UM), the University of California Museum of Paleontology (UCMP), and the 'Réserve géologique de haute Provence' (RGHP).

Taxonomy	Locality (Age)	Specimen ID	Tooth type	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
CETACEA					
Pakicetidae					
Himalayacetus subathuensis	Subathu Fm., India (e. Eocene, Ypresian)	RUSB-2003	m3	–9.0	22.0
Pakicetus inachus	Kuldana Fm., Pakistan (m. Eocene, Lutetian)	GSP-UM1938	dP4	–13.1	22.9
		GSP-UM749	dP4	–12.0	22.4
		GSP-UM1722	dP3	–11.0	22.7
		GSP-UM110	p1	–11.5	23.0
		N.A.	Mx	–8.3	23.7
		GSP-UM136	P2	–10.9	23.5
		GSP-UM1937	P3	–14.2	23.3
		GSP-UM751	P4	–12.4	26.8
		GSP-UM113	m1	–13.7	25.1
		GSP-UM135	M2	–12.8	24.6
Remingtonocetidae					
Dalanistes ahmedi	Domanda Fm., Pakistan (m. Eocene, Lutetian)	N.A.	Frag.	–8.8	25.7
Protocetidae					
Babiacetus indicus	Drazinda Fm, Pakistan (m. Eocene, Bartonian)	N.A.	Frag.	–7.9	26.0
Rodhocetus kasrani	Domanda Fm., Pakistan (m. Eocene, Lutetian)	N.A.	Frag.	–8.5	26.6
Gen. et sp. nov.	Kpogame–Hahotoe Basin, Togo, Africa (m. Eocene, Lutetian)	N.A.	Frag.	–10.1	28.1
Basilosauridae					
Dorudon atrox	Gehannam Fm., Egypt (m. – l. Eocene, Bartonian to Priabonian)	N.A.	C1	–8.3	27.5
		N.A.	C1	–10.6	28.5
		UM97506	C1	–7.3	27.8
		UM97512	C1	–8.4	28.1
		UM100146	C1	–9.8	27.3
		N.A.	C1	–7.2	28.3
Basilosaurus isis	Gehannam Fm., Egypt (m. – l. Eocene, Bartonian to Priabonian)	N.A.	N.A.	–7.1	28.3
		UM100141	N.A.	–7.0	28.0
		UM83901	N.A.	–7.3	27.3
SIRENIA					
Protosirenidae					
Protosiren smithae	Gehannam Fm., Egypt (m. – 1. Eocene, Bartonian to Priabonian)	UM101224	Mx	0.0	27.9
Dugongidae					
Eotheroides sp.	Birket Qarun Fm., Egypt (m. – l. Eocene, Bartonian to Priabonian)	N.A.	Px	–0.5	26.4
Eosiren libyca	Gehannam Fm., Egypt (m.– l. Eocene, Bartonian to Priabonian)	UM97540	N.A.	–2.3	28.3
		UM100137	M2	–1.2	28.5
		UM101219	P4	–2.8	28.3
Halitherium taulannensis	Alpes-de-Haute Provence, France (l. Eocene, Priabonian)	N.A.	Frag.	1.46	28.1
		n-RGHP E.9.056	Px	1.6	27.3
		n-RGHP E.5.104	Frag.	–0.3	27.6
		N.A.	Px	–0.9	27.5
		n-RGHP D344	Px	–0.5	
		n-RGHP D347	Mx	1.4	27.7
		n-RGHP C001	Mx	1.0	27.0
		N.A.	Mx	0.3	27.1
CONDYLARTHRA					
Quettacyonidae					
Sororocyon sp.	Ghazij Fm., Pakistan (e. Eocene, Ypresian)	GSP-UM4144	N.A.	–10.1	26.6
		GSP-UM4144	N.A.	–9.1	26.3
		GSP-UM4144	N.A.	–11.1	25.7
		GSP-UM4133	N.A.	–9.6	27.0
		GSP-UM4132	N.A.	–11.7	26.4
		GSP-UM4144	N.A.	–10.9	28.0
		GSP-UM4172	N.A.	–10.4	26.2

APPENDIX 1 *(Continued)*

Taxonomy	Locality (Age)	Specimen ID	Tooth type	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
TILLODONTIA					
Undescribed	Pakistan (e. Eocene, Ypresian)	GSP-UM4147	N.A.	–8.2	24.7
		GSP-UM4131	N.A.	–7.6	22.5
		GSP-UM4155	N.A.	–8.9	25.4
ARTIODACTYLA					
Raoellidae					
Khirarthra dayi	Kuldana Fm, Pakistan (m. Eocene, Lutetian)	GSP-UM694	N.A.	–12.4	25.8
		N.A.	N.A.	–11.1	27.1
		N.A.	N.A.	–10.6	27.1
Anthracotheriidae					
Bothriogenys sp.	Gebel Qatrani Fm, Fayum, Egypt (e. Oligocene)	UCMP 41494	m3	–10.8	23.6
		UCMP 41497	m3	–10.9	27.9
		UCMP 41492	m3	–10.8	27.5
		UCMP 41490	m3	–10.8	31.6
		UCMP 41601	m3	–12.2	27.1
		UCMP 41568	M3	–11.2	25.1
		UCMP 41489	m3	–11.7	25.7
HYRACOIDEA					
Pliohyracidae					
Saghatherium antiquum	Gebel Qatrani Fm, Fayum, Egypt (e. Oligocene)	UCMP 41554	m3	–10.3	30.0
		UCMP 41553	M3	–11.1	28.4
		UCMP 86102	M3	–12.8	25.9
		UCMP 41556	m2	–12.8	24.9
EMBRITHOPODA					
Arsinoitheriidae					
Arsinoitherium sp.	Gebel Qatrani Fm, Fayum, Egypt (e. Oligocene)	UCMP 41507	Frag.	–9.3	30.5
		UCMP 41382	m1	–11.3	28.6
		UCMP 41469	Frag.	–11.4	27.0
		UCMP 41388	Frag.	–10.7	28.6
		UCMP 41462	Frag.	–10.6	28.3
		UCMP 41385	Frag.	–10.6	28.5
		UCMP 41384	Frag.	–10.4	30.0