The Bioenergetics and Trophic Ecology of Leatherback Turtles (*Dermochelys coriacea*)

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Leatherback turtles, *Dermochelys coriacea*, are critically endangered, long-lived migratory reptiles and are specialist predators for gelatinous prey. Their unique physiological and life history traits make quantification of their energetic requirements and understanding of their trophic ecology crucial to conservation of the species. Using doubly labeled water (DLW) on adult female leatherbacks I obtained the first field metabolic rates (FMRs; 0.20 - 0.74 W kg\(^{-1}\)) and water turnover rates (16 - 30% Total Body Water d\(^{-1}\)) for free-swimming marine turtles and combined these data with dive information from electronic archival tags to quantify the bioenergetics and diving activity of reproductive adult female leatherback turtles. Internesting leatherback dive durations were consistently shorter than aerobic dive limits calculated from my FMRs, indicating that internesting female leatherbacks spent relatively little energy while active at sea. Energy budget calculations using the FMRs indicated that resource limitation related to the El Niño-Southern Oscillation (ENSO) might lengthen remigration intervals for eastern Pacific leatherbacks as compared to North Atlantic leatherbacks, thus decreasing the eastern Pacific population’s reproductive success and increasing its exposure to risk of incidental fisheries mortality, resulting in plummeting eastern Pacific populations. Analyses of stable carbon and nitrogen stable isotopes ratios (\(\delta^{13}C\) and \(\delta^{15}N\)) of leatherback tissues from eastern Pacific and North Atlantic nesting populations revealed that while \(\delta^{13}C\) signatures were similar between North Atlantic (-19.4\(\%\) ± 1.0\(\%\)) and
eastern Pacific leatherbacks (-19.1‰ ± 0.7‰), reflecting the pelagic foraging strategy of the species, eastern Pacific leatherback $\delta^{15}N$ signatures (15.4‰ ± 1.8‰) was significantly enriched relative to North Atlantic leatherback $\delta^{15}N$ signatures (9.8‰ ± 1.5‰). This $\delta^{15}N$ discrepancy reflects inter-basin differences in nitrogen cycling regimes and their influence on primary production being transferred through several trophic levels.

Combination of FMRs and dive data described the relationship between physiology, environment, and activity, and energy budget calculations and stable isotope analyses demonstrated fundamental differences in marine habitats of eastern Pacific and North Atlantic leatherbacks. Integrated approaches to understanding how physiology, environment, and resource availability interact to constrain animal bioenergetics, and thus, life history can drastically strengthen the efficacy of global initiatives toward conservation of marine biodiversity.
Chapter 1: Introduction to leatherback bioenergetics and trophic ecology

Physiology, environment, and resource limitation are the dominant influences on an organism’s bioenergetics and thus its life history. Tradeoffs with and constraints of an organism’s physiological state and limitations, biophysical environment (Spotila & Standora, 1985), and resource availability, acquisition and assimilation (Congdon et al., 1982) affect both time and energy allocation to different functions (Congdon, 1989). Specifically, animals make differential investments of time into various activities, such as mating or foraging, and differential allocations of assimilated energy to competing operations, such as growth or reproduction, and adjustments of reproductive investment through clutch sizes, egg sizes, or parental care (Dunham et al., 1989). Therefore, studies of animal bioenergetics must focus on understanding allocations animals make between various activities and resource investments in the context of their biotic and abiotic environment.

Leatherback turtles (*Dermochelys coriacea*, Family Dermochelyidae, Vandelli 1761) are critically endangered (Spotila et al., 2000) and inhabit almost every ocean from the sub-arctic to the tropics (Reina et al., 2002a). Each stage of leatherback life history imposes different energetic and ecological challenges that must be met by specific behavioral and physiological adjustments. In a departure from the life histories of other sea turtles species, leatherbacks are pelagic even as adults, instead of migrating from pelagic juvenile nurseries to coastal adult foraging areas (Musick & Limpus, 1997). On the other hand, leatherbacks maintain a connection to the tropics for reproduction, like other sea turtle species (Miller, 1997).
Leatherbacks are prolific divers, with recorded dives to 1,230 m (Hays et al., 2004a) and 67 min in duration (Southwood et al., 1999). In addition, leatherbacks thermoregulate in varied thermal environments (Paladino et al., 1990), make epic migrations every 2-4 yr between nesting beaches in the tropics and foraging grounds in high-latitude to circumpolar regions (Morreale et al., 1996; Eckert & Sarti, 1997; Ferraroli et al., 2004; Hays et al., 2004a and b; James et al., 2005) and occupy a unique ecological niche as a specialist predator for gelatinous prey (e.g., jellyfish, salps) (James & Herman, 2001; Bjorndal, 1997). Leatherbacks have the fastest growth rate and are among the largest living reptile species (200-900 kg), reaching sexual maturity within 9-14 yr (Zug & Parham, 1996), at which point allocation of assimilated energy to reproduction becomes paramount, and they exhibit the largest reproductive output of any turtle (Reina et al., 2002a). These superlative traits require enhanced energy intake and assimilation and make leatherbacks attractive subjects for investigation of interactions between environmental constraints, physiological performance, and life history demands. Moreover, effective conservation strategies to save leatherbacks from extinction require quantification and understanding of the considerable energetic demands of the species’ distinctive physiology, movements, and life history.

Metabolism influences energy requirements and allocation in organisms (Brown et al., 2004). Therefore, measurements of leatherback metabolic rates at sea are key to understanding the bioenergetics of this species. For example, these field metabolic rates (FMRs) would allow for calculation of aerobic dive limits, which would elucidate the physiological diving capacity and performance in these animals (Costa et al., 2001). Also, FMRs of internesting periods, combined with values for other components of the
reproduction, such as nest construction and egg production, would allow for calculation of the energetic costs of reproduction, and thus individual and population reproductive energy budgets (Jones et al., 2004). Additionally, within the constraints of resource availability, metabolic requirements influence reproductive outputs and schedules, as well as growth and morphometrics, which in turn affect overall population demography (Dunham et al., 1989). Therefore, metabolic rate measurements are critical for answering fundamental questions about the biology of leatherbacks as well as other organisms.

Since energetic costs of leatherback physiology and life history demands must be very high, copious resource consumption and efficient assimilation are required (Davenport & Balazs, 1991; Zug & Parham, 1996). Leatherbacks are specialist predators for jellyfish and related gelatinous prey (Lazelle, 1980; Holland et al., 1990; Davenport & Balazs, 1991; James & Herman, 2001), thus limiting their foraging opportunities to areas of high productivity and jellyfish abundance (Bjorndal, 1997). High-latitude oceanic convergence zones and areas of high nutrient upwelling and productivity are marine habitats known to support sufficient biomass of gelatinous prey to sustain the basic metabolic requirements and rapid growth rates of leatherbacks (Musick & Limpus, 1997).

Because of the vast geographic range and depth leatherbacks inhabit, they face two major thermoregulatory challenges; 1) maintaining a core temperature as much as 18°C above ambient temperature (Frair et al., 1972) while in cold (down to 5°C) waters of higher latitudes and great depths, and 2) avoiding overheating while in tropical waters, especially while on land during nesting. Leatherbacks perform this feat by combining large body size with low metabolic rates, blood flow adjustments, and peripheral
insulation, a unique suite of adaptations termed “gigantothermy” (Paladino et al., 1990). Gigantothermy has allowed leatherbacks to exploit a distinctive ecological niche in the marine ecosystem unavailable to other sea turtle species (Davenport, 1997), which is characterized by temporal and spatial heterogeneity, cold ambient temperatures, and a distinct prey type. This is similar to the “thermal niche expansion” hypothesis that explains the multiple origins of endothermic adaptations in the Scombroidei (mackerels, tunas, and billfishes) (Block et al., 1993).

The recent dramatic decline in global leatherback populations is chiefly due to the combined effects of historic egg harvest and incidental fisheries mortality (Eckert & Sarti, 1997; Spotila et al., 2000; Ferraroli et al., 2004; Hays et al., 2004a; Lewison et al., 2004). However, analyses of leatherback population dynamics and characteristics have not considered the effects of climatically altered resource availability on energy acquisition. In order for adult female sea turtles to reproduce, they must harvest and store sufficient energy while foraging to facilitate nest construction, egg production, survival at sea between nesting events, and round-trip migration between foraging and nesting areas (Owens, 1980; Hamann et al., 2002). Climatically influenced variations in oceanographic conditions affect resource availability and thus population trends in sea turtles (Solow et al., 2002). Such oceanographic fluctuations cause abundances and distributions of leatherback prey to vary greatly in time and space (Mills, 2001; Lynam et al., 2004). Therefore, estimates of leatherback bioenergetics and population dynamics must take into account the effects of climate on resource availability.

Additionally, distinct differences exist between leatherback populations from the eastern Pacific and North Atlantic Ocean basins. North Atlantic leatherbacks, on average,
are larger, more massive, have larger clutch sizes, and shorter remigration intervals than their eastern Pacific counterparts (Boulon et al., 1996; Reina et al., 2002a). Resource quality and availability affect morphometrics and reproduction of sea turtles (Limpus & Nicholls, 1988; Hays, 2000; Broderick et al., 2003; Solow et al., 2002) and other reptiles (Congdon, 1989; Wikelski & Thom, 2000). Determining leatherback trophic status and resource availability in both basins could provide insight into the nature of these population differences and to leatherback energy budgets.

Declining leatherback populations are considered indicators of threats to marine biodiversity (Spotila et al., 2000), so understanding the environmental and physiological context in which leatherbacks allocate time and energy that affect their life history would be an important contribution to bioenergetics research generally and to conservation of this species specifically. In Chapter 2, I characterized adult female leatherback internesting activity-energy tradeoffs by combining DLW-derived FMRs and water turnover rates with dive information from archival electronic tags. In this way, I was able to discern temporal patterns in leatherback dive behavior and temperature preferences, and to calculate aerobic dive limits (cADL). Leatherback energy expenditure during activity at sea is relatively low (Chapter 2), which has an important influence on reproductive energy budgets (Chapter 3). This represents the most extensive analysis of leatherback diving physiology and activity to date. Next, I combined the FMRs with literature values to calculate the overall leatherback energy budget and then estimated rates of prey biomass intake required to sustain maintenance physiological requirements and reproduction costs (Chapter 3). I then related these energy budget and intake values to the dual effect of differential resource availability and fisheries interactions on
leatherback populations in the eastern Pacific and North Atlantic. This is the first articulation of a valid hypothesis explaining the striking differences between eastern Pacific and North Atlantic leatherbacks populations. In Chapter 4, I analyzed stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotopic signatures of red blood cells and egg yolk from nesting leatherbacks over two nesting seasons from a nesting population in the eastern Pacific (Playa Grande, Costa Rica) and the North Atlantic (St. Croix, USVI) to compare nutrient sourcing at the base of marine food webs in the North Atlantic and eastern Pacific Oceans and the relative trophic position of leatherbacks in both basins. The significant discrepancy I found between eastern Pacific and North Atlantic leatherback signatures indicate how distinct oceanographic processes that influence primary productivity in each basin are reflected through several trophic levels.

Morphological and population differences between eastern Pacific and North Atlantic leatherbacks are probably due in part to higher productivity rates and thus enhanced resource availability in the Atlantic (Chapter 3). The disparate $\delta^{15}$N signatures I report here provide further support for fundamental oceanographic differences between eastern Pacific and North Atlantic leatherback foraging grounds (Chapter 4), but how can the distinct nitrogen cycling regimes account for differences in productivity between the basins? Primary production using nitrogen derived originally by nitrogen fixing organisms (e.g.: *Trichodesmium* spp.) is considered “new production” because it results in an addition of available nitrogen to producers (Saino & Hattori, 1987). Conversely, denitrification is considered “regenerated production”, and results in a net loss of nitrogen from the euphotic zone and reliance upon further upwelling of benthic nitrogen sources (Saino & Hattori, 1987). Therefore, new production occurs in cold, nutrient-rich
waters of the northwest Atlantic on a broad and relatively consistent scale (from 10°-
50°N; Gruber & Sarmiento, 1997; Karl et al., 2002), thus resulting in consistently high
levels of primary productivity. On the other hand, leatherbacks nesting at PNMB, Costa
Rica, are known to migrate to the denitrification zone within the Humboldt
Current/Equatorial Current/Peruvian upwelling system (Morreale et al., 1996; Eckert &
Sarti, 1997; G.L. Shillinger, B.P. Wallace, J.R. Spotila, F.V. Paladino, & B.A. Block,
unpublished data), which is relatively devoid of nitrogen fixation due to iron-limitation
and thus is a global nitrogen sink (Saino & Hattori, 1987; Gruber & Sarmiento, 1997;
Deutsch et al., 2001). Additionally, the El Niño-Southern Oscillation (ENSO) has
profound effects on the productivity in this region, manifested in transient and irregular
foraging grounds in the ETP, which cause broad-scale movements of apex predators in
response to patchy prey distribution (Lehodey et al., 1997), and declines in seabird
(Scheiber & Scheiber, 1984; Hays, 1986) and pinniped (Trillmich & Limberger, 1985)
populations. Therefore, I conclude that contrasting oceanographic processes affect
resource availability on eastern Pacific and North Atlantic leatherback foraging grounds
and potentially account for observed morphological and population trend disparities.

Oceanographic sampling is often limited to local and relatively instantaneous
assessments of nutrient cycling, productivity, and other factors which are actually
transient and widespread (Michaels et al., 2001; McClelland et al., 2003). Models
derived from such information are unable to characterize complexities of open ocean
ecosystems (Karl et al., 2002). To address this problem, highly migratory pelagic
vertebrates can be employed as oceanographic sampling platforms that collect detailed
oceanographic data integrated over large spatio-temporal scales when equipped with
electronic satellite and/or archival tags (Block et al., 2003). Here I used leatherback
turtles (Dermochelys coriacea) as ocean samplers of internesting habitat and as
oceanographic indicators to characterize physiological and environmental constraints on
activity and energy allocation and to establish differences between nutrient sourcing and
primary production and its influence on leatherback populations in the North Atlantic and
eastern Pacific Oceans. I propose that integration of conventional ocean sampling with
information from tissues, movements, and activity of high-order pelagic consumers can
be utilized to elucidate oceanographic processes and trophic system functioning in global
oceans. This integration will provide new and useful insights into the interaction between
marine organisms and the biophysical parameters of the marine ecosystems they inhabit,
and facilitate creative, effective approaches to bioenergetics of marine organisms and to
conservation of marine biodiversity.
CHAPTER 2: Bioenergetics and Diving Activity of Internesting Leatherback Turtles at Parque Nacional Marino Las Baulas, Costa Rica

ABSTRACT

Physiology, environment and life history demands interact to influence marine turtle bioenergetics and activity. However, metabolism and diving behavior of free-swimming marine turtles never have been measured simultaneously. Using doubly labeled water (DLW), I obtained the first field metabolic rates (FMRs) (0.20-0.74 W kg\(^{-1}\)) and water fluxes (16-30% Total Body Water d\(^{-1}\)) for free-ranging marine turtles and combined these data with dive information from electronic archival tags to quantify the bioenergetics and diving activity of reproductive adult female leatherback turtles (Dermochelys coriacea). Mean dive durations (7.8 ± 2.4 min (mean ± 1 S.D.), bottom times (2.7 ± 0.8 min), and percentage of time spent in water temperatures (T\(_w\)) ≤ 24°C (9.5% ± 5.7%) increased with increasing mean maximum dive depths (22.6 ± 7.1 m; all \(P ≤ 0.001\)). The FMRs increased with longer mean dive durations, bottom times and surface intervals and increased time spent in T\(_w\) ≤ 24°C (all \(r^2 ≥ 0.99\)). This suggests that low FMRs and activity levels combined with shuttling between different water temperatures could allow leatherbacks to avoid overheating while in warm tropical waters. Additionally, internesting dive durations were consistently shorter than aerobic dive limits calculated from my FMRs (11.7 - 44.3 min). My results indicate that internesting female leatherbacks maintained low FMRs and activity levels, thereby spending relatively little energy while active at sea. Future studies should incorporate data on metabolic rate, dive patterns, water temperatures, and body temperatures to
develop further the relationship between physiological, environmental and life history
demands and marine turtle bioenergetics and activity.
INTRODUCTION

Physiology, environment, and life history constrain animal energetics and behavior (Dunham et al., 1989). Marine turtles are attractive subjects for investigation of tradeoffs between activity levels, physiological constraints, and life history demands because they play several important ecological roles (Bjorndal & Jackson 2003), migrate between distinct foraging and reproductive areas (Plotkin, 2003), and are long-lived and iteroparous (Miller, 1997). At-sea metabolic rates for marine turtles are the most critical components in calculating individual and population energy requirements, improving our understanding of physiological limitations on diving and thermoregulation, and for refinement of demographic parameters necessary to estimate population trends (Jones et al., 2004). However, concurrent measurements of metabolism and diving behavior of free-swimming marine turtles have not yet been documented.

Leatherback turtles (Dermochelys coriacea) are critically endangered (Spotila et al., 2000) and range circumglobally from sub-polar to tropical waters (Goff & Lien, 1988; Paladino et al., 1990). Their unique thermoregulatory adaptations (Frair et al., 1972; Greer et al., 1973; Paladino et al., 1990), pan-oceanic migrations (Morreale et al., 1996; Hays et al., 2004a and b; Ferrarioli et al., 2004; James et al., 2005), prodigious growth rate (Zug & Parham, 1996), reproductive output (Reina et al., 2002a), and size (200-900 kg) make quantification and understanding of the energy-activity tradeoffs of the species’ distinctive physiology, movements, and life history crucial to their conservation.

Leatherbacks utilize gigantothermy – a suite of physiological adaptations including low metabolic rate, large thermal inertia, blood flow adjustments and peripheral
insulation – to maintain elevated body temperatures in cold water and avoid overheating in the tropics (Paladino et al., 1990). Such thermal tolerance probably has allowed leatherbacks to exploit an ecological niche unavailable to other marine turtle species, similar to the thermal niche expansion theory proposed by Block et al. (1993) to explain the multiple and diverse origins of endothermy in the Family Scombroidei (tunas, billfish). Leatherback metabolic rate (MR) during nesting is intermediate between reptilian and mammalian resting metabolic rates (RMRs) scaled to leatherback size (Paladino et al., 1990; 1996). However, all metabolic measurements have been on adult leatherbacks during nesting, walking on the beach, or while restrained in nets (Lutcavage et al., 1990; 1992; Paladino et al., 1990; 1996) and not during in-water activities that constitute the vast majority of the lifespan of adult leatherbacks. Therefore, quantification of metabolic rates for free-swimming leatherbacks would provide ecologically relevant measures of energy expenditure during at-sea activity.

The energetic costs of activity and internal physiological processes during the internesting period are unknown. Internesting leatherbacks swim continuously, displaying distinct swim-speed patterns for diving and traveling (Eckert, 2002; Reina et al., 2004; Southwood et al., 2005), in contrast to hypotheses that turtles rest or bask for extended periods at or near the surface (Eckert et al., 1986; 1989; Southwood et al., 1999). Leatherbacks exhibit distinct dive patterns during different activities. For instance, U-shaped dives, during which turtles decrease activity on or near the ocean bottom, are thought to serve a resting or energy conservation purpose, relative to V-shaped dives, which appear to serve mainly a transit purpose (Reina et al., 2004). Southwood et al. (1999) hypothesized that leatherback metabolism at sea might be higher
than during oviposition due to other costs (reproduction, swimming, foraging, etc.). However, swimming is more energetically efficient than walking in other vertebrates (Schmidt-Nielsen, 1972) and elevated water temperatures in the tropics might constrain leatherback metabolic activity due to the possibility of overheating, as reported for giant endothermic bluefin tuna (Blank et al., 2004). Furthermore, given the competing reproductive energy requirements of round-trip migration between foraging and nesting grounds, egg production and nesting, and internesting activity at sea, leatherbacks should conserve energy while at sea during the internesting period in order to enhance their seasonal reproductive success.

Telemetric studies of adult female leatherback diving behavior during the internesting period have revealed some general trends among geographically different nesting areas. Leatherbacks nesting in St. Croix (Eckert et al., 1986; 1989), Malaysia (Eckert et al., 1996), and Parque Nacional Marino Las Baulas (PNMB), Costa Rica (Southwood et al., 1999; Reina et al., 2004) demonstrated patterns of continual diving punctuated by brief surface intervals. Additionally, PNMB leatherbacks decreased dive duration and depth as the internesting interval progressed (Southwood et al., 1999). However, these studies of leatherback dive behavior have included a small sample of turtles or a brief study period. Therefore, increasing the sample size of turtles monitored during complete internesting periods is necessary to elucidate leatherback internesting diving behavior patterns.

Aerobic dive limit (ADL) can provide estimates of physiological and energetic constraints on activity in air-breathing, diving animals (Costa et al., 2001). The ADL concept specifically refers to the dive duration beyond which blood lactate levels increase.
above resting levels (Kooyman et al., 1980). However, direct measurements of post-dive blood lactate concentrations are difficult to obtain from free-swimming animals, so many reports combine data on individual total oxygen stores and at-sea metabolic rates to obtain calculated aerobic dive limits (cADL) (see Costa et al., 2001 for review). Leatherback respiratory and cardiovascular physiology allow for deep and prolonged diving (Lutcavage et al., 1990; Paladino et al., 1996), with the deepest recorded dive to 1,230 m (Hays et al., 2004b) and the longest dive duration in excess of 1 h (Southwood et al., 1999). Lutcavage et al. (1992) combined measurements of nesting leatherback metabolic rates, blood O2 carrying capacity and tissue myoglobin concentration (Lutcavage et al, 1990) with data on blood and lung volumes to calculate total O2 stores of 27 ml kg\(^{-1}\) and estimated that leatherback cADL was between 5 - 70 min. Southwood et al. (1999) recorded the longest dive duration for a leatherback (67.3 min) and refined the cADL estimate to between 33 - 67 min based on heart rates and dive patterns of free-swimming adult female leatherbacks during the internesting period. In order to better estimate the cADL, however, measurements of metabolic rates of free-swimming leatherbacks are necessary.

Using conventional respirometry to measure metabolic rates of free-ranging marine animals is logistically infeasible in most cases. However, the doubly labeled water (DLW) method has proven a useful tool for studying field energetics and diving activity of marine animals (Costa, 1988; Arnould et al., 1996; Costa & Gales, 2000; 2003). The DLW method estimates CO2 production (rCO2) from the divergence between washout curves of hydrogen (deuterium, D or tritium, T) and oxygen (18-oxygen, \(^{18}\)O) isotopes introduced into an animal’s total body water (Fig. 1; Lifson et al., 1955). Disadvantages
of the method include high cost of the isotopes (~$200 - $300 per ml) and the reliance of the method on significant divergence of the isotope washout curves that is created by a relatively higher rCO₂ than water turnover rate (rH₂O). The accuracy of the DLW method decreases considerably as the ratio of rCO₂ to rH₂O decreases (Butler et al., 2004). Although the DLW method has been used to measure the FMR and water turnover of many terrestrial reptilian species (see Speakman, 1997 for review), Booth (2002) concluded that DLW would not work for aquatic turtles because their water turnover rates are too high (approximately 1.6 - 4.3 times Total Body Water d⁻¹). Clusella Trullas et al. (in press) recently reported DLW-derived FMRs and water turnover rates during dispersal in hatchling olive ridley turtles (Lepidochelys olivacea), but there are no published reports of DLW being used to quantify the field metabolic rates (FMR) of free-swimming adult marine turtles. However, since marine turtles face a different osmoregulatory challenge than do freshwater turtles and osmoregulate efficiently (Reina, 2000; Reina et al., 2002b), they should have a lower water turnover rate than their freshwater counterparts and sufficient divergence in the isotopes should occur to allow measurement of FMRs in this species.

Therefore, using highly enriched DLW, I measured the first FMRs and water turnover rates for free-swimming adult marine turtles and used electronic archival tags to record diving activity of 18 adult female leatherbacks during the internesting period. Here I combine metabolic and diving data to examine relationships between physiology, environment and activity in leatherbacks.

MATERIALS AND METHODS
I conducted this study at Playa Grande, Parque Nacional Marino Las Baulas (PNMB), Costa Rica. Since 1988-89, PNMB has been home to one of the most comprehensive marine turtle population studies in the world (Steyermark et al., 1996; Reina et al., 2002a).

**Doubly Labeled Water Experiment**

I performed the DLW experiment on 5 turtles, 2 in 2002 and 3 more in 2003-04. First, I and a team of colleagues weighed turtles (mean = 268 ± 44 kg) using a tripod, winch, cargo net and hanging scale (Chatillon 500 kg capacity ± 2 kg). Next, I took initial blood samples (5-20 ml) from the dorsal cervical sinus (Owens & Ruiz, 1980) for determination of background deuterium (D) and oxygen-18 (¹⁸O) levels. I then intravenously injected between 15 - 30 ml D₂¹⁸O (99 APE (atom percent excess) D₂ and 75 APE ¹⁸O solution) (Isotec, Inc., Miamisburg, OH, USA) into the dorsal cervical sinus (which empties directly into the vena cava), in order to ensure rapid equilibration of the isotopes in the turtles’ body water (Speakman, 1997). I used equation 12.1 from Speakman (1997) to estimate the DLW dosages required for leatherbacks within the range of body sizes we studied. In 2002-2003, I only had approximately 30 g of the DLW available, so I took a conservative approach to the use of our expensive labeled water. Due to the highly exploratory nature of this study, I decided to divide the DLW we had into two doses and attempt the experiment on two turtles, rather than putting all of the DLW into one turtle. This way I avoided the risk that that turtle would not return to nest, in which case, I would have had no possibility of obtaining any results. In the 2003-04 season I adhered closely to the Speakman (1997) equation.
I sampled blood (≤ 5 ml) hourly from a rear flipper to establish equilibration of the isotopes with body water and released the turtles after approximately 4 h. Subsequent analyses confirmed that the injected isotopes had equilibrated with the animals’ body water in this time period, indicated by the stable plateau of isotopic enrichments between 2 - 4 h after injection of isotopes (Fig. 2). A recent DLW study on Atlantic walruses (body mass = 1,310 kg; ~ 5 times the body mass of leatherbacks in this study) reported that the isotopes (intravenous injection) equilibrated in 2.5 - 3 h (Aquarone, 2004).

Because the DLW method requires recapture to measure final plasma isotope levels, and female leatherbacks at PNMB nest on the average 7 - 8 times in a season (Reina et al., 2002a), I selected female turtles which were early in their nesting season (1st - 4th nest) to assure return and recapture upon subsequent nesting. I took a final blood sample (≤ 5 ml) from a rear flipper and albumen samples from shelled albumen gobs (SAGs; Wallace et al., 2004) when the turtles returned to nest in order to measure final isotope concentrations remaining in turtle body water at the end of the study period. All blood and albumen samples were later analyzed for D₂ and ¹⁸O isotope concentrations by Metabolic Solutions, Inc. (Nashua, NH, USA), which assures the accuracy of their analyses to 2% of 1 S.D. for deuterium and 0.4% of 1 S.D. for ¹⁸O.

I calculated total body water (TBW) from oxygen dilution space and water turnover (rH₂O) using TBW derived from deuterium dilution space (Speakman, 1997). I calculated CO₂ production (rCO₂) assuming an RQ of 0.7 for nesting leatherbacks (Paladino et al., 1996) and using a 2-pool equation 7.43 from Speakman (1997) recommended for large animals.
Recording Diving Activity

I used LTD (light-temperature-depth) 2310 archival tags (Lotek Wireless, Inc., Newfoundland, Canada) attached to the pygal process (Morreale, 1999) of 18 turtles—4 of which were also subjects of the DLW experiments—to record their diving activity. The LTDs were programmed to record time, depth, water temperature, and light level data at intervals from 4-60 s (depending on the tag) and had a maximum depth rating of 2000 m, with 1% accuracy to full scale. I analyzed dive data using Surface Adjust and Dive Analysis Programs from Lotek Wireless, Inc. To improve the reliability of classifying true surfacing events for the purposes of dive analysis, the automated Surface Adjust program was arbitrarily limited to search within areas of the data containing readings of < 10 m when referenced to the daily minimum depth value. This assumes that the zero offset error on any given day will be not fluctuating by more than 10 m. Regions of data that met this condition were processed and the median depth values determined as estimates of the zero offset error. The zero offset error for a given dive was then calculated by averaging the median value from the surface events that preceded and followed each dive. Once the depth data were adjusted based on the zero offset, the entire data set was processed by Dive Analysis, which classified surfacing events as those regions of the data where the corrected depth records were exactly zero. I further filtered the adjusted data and accepted only dives > 3 m to increase my confidence that I were only analyzing true diving events. I calculated bottom time as the portion of a dive at or below 85% of maximum depth. A dive was counted as a U-dive if the turtle spent ≥ 1 min on the “bottom” (Reina et al., 2004). Based on video footage of breathing episodes at the surface (Reina et al., 2004), and because extended surface intervals correspond to
traveling periods near the surface, not necessarily breathing or basking (Eckert, 2002), I only included surface events of $> 12$ s and $\leq 20$ min in calculation of post-dive surface intervals. I excluded less than 6% of all surface intervals using these criteria.

I used least squares linear regressions to analyze relationships between mean dive variables, and Student’s t-tests to compare dive variables between treatment groups (DLW vs. LTD turtles) (SPSS 11.5.1, Chicago, USA) and accepted significance at $\alpha = 0.05$ level. I arcsine transformed percentage data and present means ± 1 S.D. unless otherwise noted. I conducted all procedures under permits 288-2002-OFAU and 273-2003-OFAU from the Costa Rican Ministerio del Ambiente y Energía (MINAE) and Drexel University IACUC Approval 02183-01.

RESULTS

Field metabolic rates and water turnover rates

I measured four FMRs for three free-ranging internesting leatherbacks (Table 1). I obtained two FMRs for Female 3, one for the first 3 d of her internesting period, and one for her entire 14 d period. Turtle 3 came ashore 3 d after nesting, and I obtained a blood sample at that point. She then returned to nest successfully 11 d later, or 14 d after her previous nest. The FMRs (range: 0.20 - 0.74 W kg$^{-1}$) were similar to MRs for nesting female leatherbacks and green turtles ($Chelonia mydas$) obtained by analyses of respiratory gases during oviposition (Fig. 3). I was unable to measure FMRs for two of the study turtles (Females 2 and 5).

Calculated Total Body Water (TBW) for the 5 study female leatherbacks (ranging in mass from 196 - 308 kg) was 73.9% ± 5.7% (range: 68.5% - 82.4%) (Table 1). Water
turnover rates (rH2Os) of internesting leatherbacks (including the rH2O during the 3 d period for Turtle 3) ranged from 16% to 30% of TBW d\(^{-1}\) (mean: 24% ± 5.5% TBW d\(^{-1}\)), which were within published rH2Os for leatherbacks and other species of marine turtles (Fig. 4). Female 2 exhibited the highest rH2O for her entire 11 d internesting period (27.2 % TBW d\(^{-1}\)), while Female 3 had a higher rH2O during the first 3 d of her internesting period (29.9 % TBW d\(^{-1}\)).

**Diving activity during the internesting period**

I recorded diving activity of four of five DLW turtles and 14 “control” (LTD) turtles, totaling 23,402 total dives. Individual turtles demonstrated different diving patterns in terms of mean dive variables and T\(_w\) preferences (Table 2). Across all turtles, mean maximum dive depth was 22.6 ± 7.1 m, with mean dive depth 14.6 ± 4.6 m and mean dive duration 7.8 ± 2.4 min. The deepest single dive was 200 m (Turtle 16) and the longest was 44.9 min (Turtle 2). Turtles reached maximum depths of \(\leq\) 20 m on approximately 60% of all dives, and approximately 43% of all dive durations were \(\leq\) 5 min. The mean water temperature leatherbacks experienced was 26.6°C, while the minimum experienced was 13.6°C (Turtle 16).

Including all dives for all turtles, post-dive surface intervals increased as the duration of the preceding dive increased (Spearman Rank Correlation, \(r^2 = 0.159, P < 0.001\)) As mean maximum depth increased for all turtles, mean dive duration (\(r^2 = 0.588, P < 0.001\); Fig. 5A), mean bottom time (\(r^2 = 0.590, P < 0.001\)), and proportion of U-dives (\(r^2 = 0.750, P < 0.001\)) increased while mean dive rate decreased (\(r^2 = 0.469, P = 0.002\); Fig 5B). Additionally, increased mean maximum depth resulted in increased proportion
of time turtles spent in $T_w \leq 24^\circ C$ ($r^2 = 0.424$, $P = 0.003$); a stronger relationship existed with percentage of time spent in $T_w \leq 20^\circ C$ ($r^2 = 0.595$, $P < 0.001$).

Leatherback diving activity varied between the early (~days 1-3, after nesting), middle (~days 4-7) and late (~days 8-10, before re-nesting) phases of the internesting period. Dive rate and proportion of V-shaped dives increased while mean depth, dive duration, bottom time, proportion of U-dives, and percent time spent in $T_w \leq 24^\circ C$ decreased as the internesting period progressed (Repeated Measures ANOVA: all $P < 0.001$).

Turtles that underwent DLW experiments (with LTDs attached) had significantly longer internesting periods than turtles that only had LTDs attached (Student’s t-test: $t_{17} = 7.951$, $P < 0.001$; DLW turtles: $13.1 \pm 1.4$ d, LTD turtles: $9.1 \pm 0.8$ d). Additionally, DLW turtles spent a significantly higher proportion of time in $T_w \leq 24^\circ C$ than LTD turtles ($t_{16} = 3.165$, $P = 0.006$; DLW: $16.0 \pm 2.6\%$, LTD: $7.6 \pm 5.0\%$), especially during the early phase of the internesting period, which directly followed nesting and the restraint portion of the experiment ($t_{16} = 3.508$, $P = 0.003$; DLW: $25.5 \pm 4.5\%$, LTD: $10.4 \pm 6.9\%$). Furthermore, DLW turtles made significantly more total dives ($t_{16} = 3.325$, $P = 0.004$; DLW: $1671 \pm 324$, LTD: $1194 \pm 233$), and particularly more U-dives during the internesting period than LTD turtles ($t_{16} = 5.125$, $P < 0.001$; DLW: $1206 \pm 242$, LTD: $750 \pm 130$).

**FMRs, diving physiology and activity**

Although I had a small sample size, (1 FMR for Turtles 1 and 4, 2 FMRs for Turtle 3), I used statistical analyses (Pearson-Product Moment Correlation) to examine
the relationships between FMR, diving physiology and activity. I found several suggestively strong positive relationships between FMRs and mean dive durations \( (r^2 = 0.991) \), bottom times \( (r^2 = 0.992) \), percentage of time spent in \( T_w \leq 24^\circ C \) \( (r^2 = 0.990) \), and surface interval \( (r^2 = 0.999, P = 0.027) \).

Combining the FMRs we acquired with the value for adult leatherback total O2 stores reported by Lutcavage et al. (1992), cADLs for internesting female leatherbacks were between 11.7 min and 44.3 min (Table 3).

**DISCUSSION**

Free-swimming leatherbacks have low internesting metabolic rates and spend relatively little energy while active at sea between nesting events. The DLW-derived FMRs that we measured for free-swimming, internesting leatherbacks were similar to leatherback MRs measured during restraint on the beach, oviposition, or nest chamber construction, and lower than leatherback MRs obtained by analyses of respiratory gases during vigorous nest-covering and walking on the beach (Paladino et al., 1990; 1996). In addition, our data are consistent with the findings of Southwood et al. (1999), who reported that diving heart rates of leatherbacks were more similar to heart rates during oviposition than relatively higher heart rates measured during active nest covering or walking on the beach. In a broader context, leatherback FMRs were intermediate between allometric expectations of reptilian and mammalian RMRs (Paladino et al., 1990; 1996) (Table 1). The FMRs were slightly higher than the metabolic rates for green turtles during oviposition but lower than the metabolic rates of green turtles during activity (Prange & Jackson, 1976; Jackson, 1985) (Fig. 3). These FMRs also indicate that
swimming is more energetically efficient than walking for leatherbacks as it is for other vertebrates (Schmidt-Nielsen, 1972).

The adult female leatherbacks in this study exhibited high rH2O values (16%-30% TBW), but within the range of values obtained with isotopically-labeled water (Clusella Trullas et al., in press; Ortiz et al. 2001; Jones et al., in press) and by analyses of lachrymal gland secretion rates (Reina, 2000; Reina et al., 2002b) reported for leatherbacks and other marine turtle species (Fig. 4). Turtle 3 exhibited a higher rH2O during the first 3 d of her internesting period than for the entire 14 d period (Table 2), which corresponded to the high frequency of water/prey ingestion events during the first few days after nesting reported by Southwood et al. (2005). The rH2O values for female leatherbacks reported here are reasonable considering that internesting leatherbacks produce massive egg clutches, which contain large amounts of water. Indeed, the female leatherbacks in this study laid subsequent clutches of approximately 3 - 8 kg in mass. Moreover, leatherbacks possess highly effective osmoregulatory capabilities which allow them to drink seawater without incurring negative water and ionic balance (Reina et al., 2002b). Turtle 3 exhibited a higher rH2O during the first 3 d of her internesting period than for the entire 14 d period (Table 2), which corresponded to the high frequency of water/prey ingestion events during the first few days after nesting reported by Southwood et al. (2005).

**Efficacy of DLW method in studies of marine turtle energetics**

While the rH2O values for all turtles in this study were within the range of water fluxes for other marine turtles (Fig. 4), I was unable to calculate FMRs for Females 2 and
5 using DLW. Female 2 turned over 27.2% of her TBW daily, or nearly 3 times during her 11 d internesting period, while Female 5 completely washed out the deuterium isotope, also indicating a high rH₂O. In other studies using isotopically-labeled water to calculate rH₂O values for olive ridley (Lepidochelys olivacea; Clusella Trullas et al., in press), Kemp’s ridley (L. kempii; Ortiz et al., 2001), and green turtles (Jones et al., in press), no animals turned over their TBW more than 2.5 times during the study period. Apparently, there exists a threshold ratio of rCO₂ to rH₂O (where rH₂O must be < 27.2 % TBW d⁻¹) necessary for the DLW method to be able to measure rCO₂ in leatherbacks and perhaps marine turtles in general.

Validation experiments of the accuracy of the DLW method have been performed for several animal species over a wide size range (reviewed in Speakman, 1997). Performing simultaneous metabolic measurements (DLW and respirometry, for example) on adult marine turtles is extremely difficult, due to factors such as their marine lifestyle, large size, endangered status, and the high cost of the large volume of enriched DLW required. In general, validations indicate that although individual variation might account for serious discrepancies between DLW measurements and those acquired by reference methods, the DLW method tends to overestimate rCO₂ by less than 5% among different animal clades (Butler et al., 2004). Only one truly simultaneous validation study has been performed for marine turtles to date, which reported that the global mean of DLW-derived rCO₂ values for juvenile green turtles was not significantly different and only varied by 5.2% from that obtained by gas respirometry for the same period from the same animals (Jones et al., in press).
A ratio of deuterium to oxygen-18 isotopic washout (kD:kO) that exceeds 0.9 implies that ~90% of oxygen elimination is tied to water losses, and therefore the DLW method might not accurately quantify CO2 production (Speakman, 1997). The combination of long study durations, high rH2Os and low metabolic rates resulted in insufficient divergence (Female 2; kD:kO = 1.04) or complete washout (Female 5) of the isotopes, rendering the DLW method unable to calculate rCO2 (Butler et al., 2004) for these two leatherbacks. The other kD:kO ratios I calculated ranged from 0.70-0.93, slightly above or within the recommended range (Speakman, 1997). The relatively high water turnover rates and kD:kO ratios we measured indicate that our results should be interpreted with caution (Speakman, 1997; Butler et al., 2004). However, Jones et al. (in press) reported kD:kO ratios between 0.84 - 0.92 for juvenile green turtles, and the DLW-derived MRs were not significantly different from MRs obtained by respirometry in that study. Considering existing DLW validation information (Jones et al., in press) and the fact that our FMRs fell within the range of measured MRs for leatherbacks during various activities (Paladino et al., 1990; 1996), we conclude that our measurements were accurate and biologically realistic, despite the lack of simultaneous validation data via respirometry.

**Internesting diving activity**

Leatherbacks dived continuously throughout the internesting period. Dives tended to be shorter and shallower for Pacific Costa Rican leatherbacks (mean durations ≈ 7-8 min, mean depths ≈ 15-19 m; Southwood et al., 1999; Southwood et al., 2005; this study) than dives for leatherbacks in the Caribbean near St. Croix (mean durations ≈ 10-15 min,
mean depths ≈ 60-100 m; Eckert et al., 1986; 1989; Eckert, 2002). Dive variables for leatherbacks in the South China Sea off Malaysia were intermediate (mean durations ≈ 8-12 min, mean depths ≈ 26-45 m; Eckert et al., 1996). These differences were probably due to relatively shallower depths available to internesting leatherbacks on the continental shelf near PNMB, Costa Rica, relative to the other sites (Morreale, 1999; Southwood et al., 1999). Increasing mean maximum dive depths resulted in increased mean dive duration and decreased mean dive rates (Fig. 5), similar to trends reported for leatherbacks worldwide (Eckert et al., 1986; 1996; Southwood et al., 1999; Reina et al., 2004). Similar trends were reported for New Zealand sea lions Phocarctos hookeri (Costa & Gales, 2000) and Australian sea lions Neophoca cinerea (Costa & Gales, 2003).

Therefore, while leatherback diving activity patterns appear to be constrained primarily by different depths encountered in different internesting habitats (Eckert et al., 1996; Morreale, 1999; Southwood et al., 1999), some general patterns in dive behavior exist globally between and among taxa of diving animals.

I found that post-dive surface intervals lengthened with increased dive duration for all dives across all turtles, contrary to some findings (Eckert et al., 1989; 1996; Southwood et al., 1999; but see Reina et al., 2004). However, this relationship had a low $r^2$ value (0.159), indicating that > 80% of the variance in post-dive surface interval durations that were not explained simply by preceding dive durations. Given the relatively short dive durations and surface intervals of leatherbacks in this study, turtles were probably not using the post-dive surface interval to recover from CO2 accumulation caused by diving apnea.
Leatherbacks occasionally exhibited extremely long surface intervals (maxima 21.8 - 108.3 min). These long surface intervals represented periods of traveling within the upper few meters of the water column, and not resting or basking behavior, as previously hypothesized (Eckert et al., 1986; 1989; Southwood et al., 1999). This is supported by swim speed and location data off Playa Grande (Southwood et al., 2005) and St. Croix (Eckert, 2002) and video footage (Reina et al., 2004) for internesting leatherbacks. Penick (1996) measured minimal blood flow to the carapace surfaces of nesting leatherbacks, indicating that leatherbacks would have limited ability for heat gain while basking.

Leatherbacks which had undergone the experimental handling required by the DLW methodology had significantly longer internesting periods, made significantly more U-dives, and spent more time in $T_w \leq 24^\circ C$ than leatherbacks to which we only attached data loggers. This was especially evident during the early third of the internesting period. Corticosteroid hormone concentrations increase in response to stress related to prolonged handling (Gregory & Schmid, 2001), and this can inhibit various physiological functions, including egg production (Owens, 1997; Rostal et al., 1998; 2001; Milton & Lutz, 2003). Such hormonal inhibition of reproductive function would account for the extended internesting period of the DLW turtles (13.1 d) relative to the LTD turtles (9.1 d). Turtle 3’s re-emergence 3 d after nesting was probably another manifestation of the hormonal inhibition of the natural egg production process during the internesting period in response to this prolonged stress. This turtle went through the entire nesting process, but did not lay eggs. Although this was a rare occurrence in this population, the turtle’s field metabolic rates (FMRs) were similar to the others we obtained (Table 1) and her diving
behavior was similar to the behavior of other turtles (Table 2). Furthermore, Turtle 3 returned to nest successfully 11 d later (14 d after the previous nesting), which was her fifth and final nest of the season. A season total of five nests is within the normal range for nesting leatherbacks in this population (Reina et al., 2002a).

According to Reina et al. (2004), U-dives chiefly served a resting purpose, and almost 20% of these dives involved the turtles remaining stationary on the ocean bottom for up to 1 min. Furthermore, Southwood et al. (2005) reported decreased $T_b$s during relative inactivity on or near the ocean bottom, indicating physiological heat dissipation. As the number of U-dives that turtles made increased, so did the percentage of time spent in $T_w \leq 24^\circ C$ ($r^2 = 0.311, P = 0.016$; Fig. 6). However, DLW turtles made more U-dives than LTD turtles (Table 2), thus accounting for the increased percentage of time DLW turtles spent in colder waters than LTD turtles. I hypothesize that the prolonged restraint in cargo nets and sequential blood-drawing procedures caused the experimental turtles to incur elevated corticosteroid levels and increased heat loads, which resulted in protracted internesting periods and compensatory thermoregulatory behavior during the first few days at sea after the experiment. What effects, if any, the experimental stress had on the FMR values themselves is unknown, especially since DLW-derived FMRs are integrations of metabolism during the entire study period, not for particular activities. However, DLW turtles performed within the range of dive variables for all turtles (Table 2), indicating that their diving activities (and presumably their FMRs) were representative of internesting leatherbacks in general. It is important to point out that all DLW turtles returned to nest successfully and also resumed normal internesting periods after the experiment. While the prolonged restraint was necessary and the DLW experiment likely
imposed stress on leatherbacks, it did not interfere with their long-term reproduction or behavior. Nonetheless, these factors should be taken into consideration when conducting this type of experiment with marine turtles because of their endangered status and the unavoidably stressful nature of the experiment.

Diving activity trends among leatherbacks in this study varied as the internesting period progressed, supporting previous studies at Playa Grande (Morreale, 1999; Southwood et al., 1999) and other locations (Eckert et al., 1986; 1989; 1996). Turtles exhibited the deepest dives, the longest dive durations, the lowest dive rates, and the highest percentage of time in $T_w \leq 24^\circ C$, during the early phase (~days 1-3) of the internesting period. These patterns indicated that these turtles were swimming away from the nesting beach into deeper waters offshore. Dives were shallowest, shortest, occurred most frequently, and occupied the lowest percentage of time in colder water ($\leq 24^\circ C$) during the late phase, or previous to re-nesting (~days 8-10), which indicated that turtles were constrained by shallower water closer to shore (Morreale, 1999; Southwood et al., 1999).

**FMRs, diving physiology and activity**

Our proposed upper limit on leatherback cADL (44.3 min) corroborates the findings of Hays et al. (2004b), who reported an apparent ceiling on leatherback migratory dive durations at around 40 min. Mean dive durations for all 4 DLW turtles were below the cADL range (11.7 to 44.3 min), and DLW turtles exceeded their cADL on only 0-33% of their dives (Table 3). We then calculated the percentage of dives for all 18 turtles exceeding the lower (11.7 min) and upper bounds (44.3 min) of the FMR-
derived cADLs. All turtles regularly dived below or within the cADL range, and on the average 25% of dives exceeded the lower bound (Table 4 and Fig. 7). Only one dive exceeded the upper bound (44.9 min; Turtle 2), but none exceeded the upper limits proposed by Lutcavage et al. (70 min; 1990) and Southwood et al. (67 min; 1999).

According to Thompson and Fedak (2001), an air-breathing, diving animal should regularly approach and often exceed its ADL when foraging conditions are advantageous and doing so would allow it to better exploit such conditions. Many marine mammals and birds routinely exceed cADLs while actively foraging (Costa et al., 2001). Conversely, if potential foraging success is low, an animal should limit its energy expenditure and dive well within its ADL. The fact that internesting leatherbacks rarely approached their cADLs suggests that prey availability off PNMB was low and turtles were not actively foraging (Thompson & Fedak 2001; Hays et al., 2004b). Considering the weak relationship between dive durations and post-dive surface interval ($r^2 = 0.159$), these analyses further support the conclusion that internesting leatherbacks maintain relatively low activity levels, conserve energy by diving well-within physiological limits, and are rarely, if ever, anaerobic during the internesting period (Jones et al., 2004).

We found positive relationships between FMRs and mean dive durations, bottom times, surface intervals, and the proportion of time turtles spent in $T_w \leq 24^\circ C$ (all $r^2 > 0.99$). These results raise interesting questions about leatherback thermoregulation, diving physiology, and behavior. Leatherbacks might dive more actively, thereby increasing metabolic rates (since increased muscle activity automatically results in higher metabolism), in order to exploit colder waters, presumably to forage. Eckert et al. (1986; 1989; 1996) hypothesized that diurnal differences in dive patterns represented foraging
activity following the deep-scattering layer (DSL). However, Hays et al. (2004b) pointed out that leatherbacks migrate great distances away from nesting grounds to increase foraging success because prey abundance is presumably greater on pelagic foraging grounds than along tropical coasts. In addition, Reina et al. (2004) did not observe any feeding activity in video footage of the first day after nesting, and Southwood et al. (2005) found no relationship between ingestion events and diel dive patterns, which were previously thought to be related to vertical movements of DSL (Eckert et al., 1986; 1989; 1996). If leatherbacks were actively foraging, an increase in FMR over RMR of 10-30% might be expected due to specific dynamic action (SDA) (Withers, 1992). However, the fact that FMRs I obtained were not significantly elevated relative to nesting leatherback MRs (Fig. 3) renders the possibility that leatherbacks were foraging during the internesting period highly unlikely.

According to the gigantothermy model, leatherbacks must maintain low MRs and increase blood flow to peripheral tissues to dissipate heat generated internally to avoid overheating in the tropics (Paladino et al., 1990). Southwood et al. (2005) recorded subcarapace and gastrointestinal tract temperatures of internesting leatherbacks and surmised that their measured gradients between T_b and T_w for leatherbacks in the tropics supported the predictions of the gigantothermy model. While data on blood flow adjustments by leatherbacks at sea are not available, the relatively low FMRs I obtained for internesting leatherbacks also supported the conclusions of Paladino et al. (1990). Furthermore, I found relationships between FMRs, increased activity levels (mean maximum depth, dive duration, bottom time), and proportion of time leatherbacks spent in T_w ≤ 24°C. Southwood et al. (2005) reported leatherback T_b's that were consistently
elevated above $T_w$, but $T_b$ could be affected by modifications in swimming and diving activity and fluctuating $T_w$. In addition, Penick (1996) reported increased perfusion to the skin in the gular/pectoral region when body temperatures were elevated during rigorous terrestrial activity, suggesting that leatherbacks could use this area to dissipate internally-generated heat via blood flow. These findings suggest that leatherbacks with increased activity levels (and perhaps higher metabolic rates) might use both behavioral and physiological means to avoid overheating while in the tropics by increasing the proportion of time spent in cool $T_w$, thus using cooler water as a heat sink to dump (via blood flow) internally generated heat (Fig. 8). Tuna also experience limitations on activity in warm water in the tropics (Blank et al., 2004), and modulate heat transfer both physiologically and behaviorally (Dewar et al., 1994). Leatherbacks in this study spent the highest percentage of time in cooler waters in the early third of the internesting period after nesting, implying that increased heat loads incurred during increased activity associated with nesting necessitated shuttling to colder $T_w$. Southwood et al. (2005) recorded frequent ingestion events during this segment of the internesting period, potentially indicating internal heat dissipation through ingestion of cooler water and/or prey. It is plausible that dive patterns of leatherbacks foraging in cold waters would be opposite to those for internesting leatherbacks, in that turtles might spend more time near the surface in warmer water in response to prolonged periods submerged in colder water, as documented in bigeye (Holland et al., 1992) and bluefin tuna (Gunn & Block, 2001). Additional experiments simultaneously measuring leatherback body temperatures, metabolism, and diving activity in cold water are needed to distinguish between these possibilities.
In order for female leatherbacks to reproduce, they must harvest and store sufficient energy to facilitate nest construction, egg production, and survival at sea between nesting events. Of those components, only energy expenditure during the internesting period can be flexible, since compromises in egg production and nest construction would decrease reproductive success. Despite their capacity for extremely deep (Hays et al., 2004a) and prolonged (Southwood et al., 1999; Hays et al., 2004b) dives, leatherbacks tended to exhibit relatively shallow and short dives during their internesting periods. The relatively low FMRs reported here, apparent thermal constraints on activity imposed by warm tropical water, and almost exclusively aerobic diving exhibited by leatherbacks in this study suggest minimized energy expenditure during the internesting period, which might facilitate increased energy allocation to egg production and nesting, as reported for internesting green turtles (Hays et al., 2000). Future studies should incorporate more data on metabolism, body temperatures, and diving behavior of migrating and foraging turtles in cooler waters in order to understand how environmental and life history demands affect marine turtle energetics and activity.
Table 1. Masses, water turnover rates, and field metabolic rates of adult female leatherback turtles. I obtained 4 FMRs for 3 leatherback turtles during the interesting period (one for the first 3 d interval and one for the entire 14 d interval for turtle 3). Leatherback FMRs are intermediate between allometric predictions of resting metabolic rates (RMRs) for reptiles (RMR = 0.378 · M^{0.71}) and mammals (RMR = 3.35 · M^{0.75}) (equations from Paladino et al., 1990; 1996).

| Turtle | Mass (kg) | Study Duration (d) | N_{ox} (O^{18} dilution space) (mol) | %TBW | k_{H2O} | ml H_{2}O d^{-1} | %TBW d^{-1} | FMR (W kg^{-1}) | RMR (W kg^{-1}) | RMR (W kg^{-1}) |
|--------|-----------|--------------------|---------------------------------|-------|---------|------------------|-------------|----------------|----------------|
| 1      | 270       | 14.7               | 10275                           | 68.5  | 0.70    | 28696            | 15.5        | 0.74           | 0.146          | 0.826          |
| 2      | 196       | 11.2               | 7976                            | 73.3  | 1.04    | 39391            | 27.2        | ND             | 0.154          | 0.895          |
| 3      | 268       | 14.1               | 11036                           | 74.1  | 0.86    | 45825            | 21.5        | 0.40           | 0.146          | 0.828          |
| 3 (3d) | 268       | 3.1                | 11036                           | 74.1  | 0.92    | 62465            | 29.9        | 0.24           | 0.146          | 0.828          |
| 4      | 308       | 12.8               | 14105                           | 82.4  | 0.93    | 58408            | 23.6        | 0.20           | 0.143          | 0.800          |
| 5      | 298       | 12.7               | 11402                           | 68.9  | ND      | ND               | ND          | ND             | 0.144          | 0.806          |
| Mean   | 268       |                    |                                 | 73.9  |         | 23.5             | 0.40        | 0.147          | 0.830          |
| S.D.   | 44        |                    |                                 | 5.7   |         | 5.5              | 0.20        |                |                |


Table 2. Dive variables for internesting female leatherback turtles at Parque Nacional Marino Las Baulas, Costa Rica, 2002-03 and 2003-04. ND: no data; There were no dive data for Turtle 4 because she did not have a data logger. DLW/LTD: turtles used for doubly labeled water/data logger experiments; LTD: turtles with only data loggers attached. Data from the 3 d period for Turtle 3 were not included in means. Significantly different pairs denoted by common superscript: aP < 0.001, bP = 0.004; cP < 0.001; dP = 0.006.

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<th>Total U-Dives</th>
<th>Total V-Dives</th>
<th>Duration (min)</th>
<th>Surface Interval (min)</th>
<th>Depth (m)</th>
<th>Maximum Depth (m)</th>
<th>Rate (dives h⁻¹)</th>
<th>Bottom Time (min)</th>
<th>% Time Tc ≤ 24°C</th>
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Table 3. Calculated aerobic dive limits (cADLs) of leatherback turtles derived from field metabolic rates. Mean dive durations were all lower than cADLs for leatherbacks that underwent the doubly labeled water experiment and turtles rarely exceeded their cADLs. I used data on leatherback total body oxygen stores from Lutcavage et al. (1990; 1992) to calculate cADLs.

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<th>Turtle</th>
<th>Mass (kg)</th>
<th>FMR (ml O₂ min⁻¹)</th>
<th>O₂ stores (ml)</th>
<th>cADL (min)</th>
<th>Mean Duration (min)</th>
<th>Maximum Duration (min)</th>
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Table 4. Calculated aerobic dive limits (cADLs) and mean and maximum dive durations for leatherback turtles. Mean dive durations for all turtles in this study were within ADLs calculated using field metabolic rates (11.7 - 44.3 min). Overall, only one dive duration (44.9 min; Turtle 2) exceeded the maximum cADL.

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<th>Ratio of Mean Duration to cADL=44.3 min</th>
<th>% Dives Exceeding cADL=11.7 min</th>
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Figure 1. The doubly labeled water method (DLW). A known quantity and concentration of hydrogen and oxygen stable isotopes is administered to an animal allowed to reach equilibration with the animal’s body water. After an equilibrium blood sample is taken, the animal is released. During the study period, hydrogen isotopes washout via water losses, while oxygen isotopes washout via water losses as well as carbon dioxide production. Therefore, when the final samples is taken at the conclusion of the study period, the divergence of the two isotopes is an indirect measure of CO₂ production, from which energy expenditure by the animal for the study period can be calculated. See Speakman (1997) for review of the theory and practice of the DLW method.
Figure 2. Log isotopic enrichment values for (A) deuterium and (B) oxygen-18 for 5 leatherback turtles. The filled circle, square and diamond with solid lines represent the isotopic enrichments and washouts for the three turtles for which I was able to calculate FMRs. Open triangles and dotted lines represent the isotopic enrichments and washouts for the two turtles for which I was unable to calculate FMRs. Note the 3 d values for Female 3 which allowed for calculation of an FMR for the first 3 d of her internesting period and an FMR for her entire internesting period.
Figure 3. Mass-specific metabolic rates of adult female leatherback and green turtles. The field metabolic rates reported in this study for internesting leatherbacks (filled circles) were similar to metabolic rates measured during restraint, oviposition or nest construction for leatherbacks (open squares; Paladino et al. 1990; 1996) and slightly higher than those for green turtles (open down triangles; Prange & Jackson 1976; Jackson 1985). These FMRs were lower than metabolic rates measured during vigorous nest covering or walking along the beach for green turtles (filled down triangles; Prange & Jackson 1976; Jackson 1985) and leatherbacks (filled squares; Paladino et al. 1990; 1996). Data shown are mean ± 1 S.E.M.; numbers in parentheses indicate sample size.
Figure 4. Water turnover rates (% of Total calculated Body Water d⁻¹) for marine turtles. Water turnover rates measured by DLW in this study (mean = 24% TBW d⁻¹, range = 16-30% TBW d⁻¹) are within the range of published values for marine turtles. X-axis labels from left to right, with the method by which water turnover rates were derived: L.k.: *Lepidochelys kempii* adults, deuterated water (D₂O) (Ortiz et al., 2001); D.c.: *D. coriacea* hatchlings, lachrymal gland secretions (Reina et al., 2002b); C.m.1: *C. mydas* hatchlings, lachrymal gland secretions (Reina, 2000); C.m.2: *C. mydas* juveniles, DLW (Jones et al., in press); L.o.: *Lepidochelys olivacea* hatchlings, left to right: swimming, crawling, digging, DLW (Clusella Trullas et al., in press); D.c. (striped bar): *D. coriacea* adults, DLW, this study.
Figure 5. Mean maximum dive depth vs. (A) mean dive duration and (B) mean dive rate for internesting leatherback turtles. Increases in mean maximum dive depth resulted in increased (A) mean dive durations ($y = 2.033 + 0.2553x$, $r^2 = 0.588$, $P < 0.001$) and decreases in (B) mean dive rates ($y = 7.727 - 0.1048x$, $r^2 = 0.469$, $P = 0.002$) of 18 adult female leatherbacks.
Figure 6. Total U-shaped dives vs. percentage of time spent in water temperatures ($T_w$) $\leq$ 24°C. The proportion of time turtles spent in colder water was dependent upon the number of U-shaped dives made. Experimental, or DLW turtles (open circles) made more U-dives and thus spent more time in colder water, than “control,” or LTD turtles (filled circles) ($y = -0.0169 + 0.0001x$; $r^2 = 0.311$, $P = 0.016$).
Figure 7. Frequency distribution of dive durations for all turtles in relation to calculated aerobic dive limits (cADLs). Approximately 43% of all dives were ≤ 5 min long, and 25% of all dives exceeded the lower bound of the ADLs (11.7 min) I calculated from field metabolic rates for free-swimming, internesting leatherbacks. Only one dive duration exceeded the upper bound (44.3 min) of cADLs.
Figure 8. Depth vs. temperature diving profiles for (A) 12 h and (B) 1 h. Electronic archival dive data displayed were recorded during day 3 of the internesting period for Turtle 1 and include her deepest dive (120 m), longest dive duration (22.2 min), and coldest water temperature experienced (14.4°C). Water temperatures experienced by Turtle 1 during this 12 h period ranged from 28.1-14.4 °C.
CHAPTER 3: Calculation of the Leatherback Reproductive Energy Budget and Implications for Population Dynamics:

Climate and Fisheries Deliver a One-Two Punch to Eastern Pacific Leatherbacks

ABSTRACT

Leatherback turtles (*Dermochelys coriacea*) are critically endangered and their unique physiological and life history traits make quantification of their energetic requirements crucial to conservation of the species. Using doubly labeled water (DLW) I obtained field metabolic rates (FMRs; 0.20 - 0.74 W kg\(^{-1}\)) for free-swimming adult female leatherbacks and used these FMRs to calculate the leatherback reproductive energy budget. My estimations indicate that resource limitation might lengthen remigration intervals for eastern Pacific leatherbacks as compared to North Atlantic leatherbacks, thus decreasing the eastern Pacific population’s reproductive success and increasing its exposure to risk of pelagic fisheries mortality. Stochastic resource availability related to El Niño-Southern Oscillation (ENSO) is probably exacerbating the effects of high incidental fisheries mortality resulting in plummeting eastern Pacific leatherback populations. Therefore, management strategies for fisheries should allow little, if any, mortality of Pacific leatherbacks if there is to be any reasonable hope for recovery of this population.
INTRODUCTION

Leatherback turtles (*Dermochelys coriacea*) are critically endangered (Spotila et al., 2000) and inhabit almost every ocean from the sub-arctic to the tropics (Reina et al., 2002a). Leatherbacks thermoregulate in varied thermal environments (Paladino et al., 1990), make epic migrations (Morreale et al., 1996; Ferraroli et al., 2004; Hays et al., 2004a), have the fastest growth rate (Zug & Parham, 1996) and largest reproductive output (Reina et al., 2002a) of any turtle, and are among the largest living reptile species (200-900 kg). Effective conservation strategies to save leatherbacks from extinction require quantification and understanding of the considerable energetic demands of the species’ distinctive physiology, movements, and life history. Metabolic rate measurements are critical for calculating individual and population energy requirements and for refinement of demographic parameters necessary to estimate population trends (Ernest et al., 2003; Jones et al., 2004). However, at-sea metabolic rates for marine turtles have not yet been documented.

Field metabolic rates (FMRs) of free-swimming leatherbacks are key to accurate reproductive energy budget calculations and would provide insight into their unique physiological adaptations as well as the biology of large animals. Leatherback metabolic rate (MR) during nesting is intermediate between reptilian resting metabolic rates (RMRs) scaled to leatherback size and mammalian RMRs (Paladino et al., 1990; 1996). However, all metabolic measurements have been on adult leatherbacks during nesting, walking on the beach, or while restrained in nets (Lutcavage et al., 1990; 1992; Paladino et al., 1990; 1996) and not during in-water activities that constitute the vast majority of the lifespan of adult leatherbacks. Moreover, swimming is more energetically efficient
than walking (Schmidt-Nielsen, 1972) and elevated water temperatures in the tropics might constrain leatherback activity due to the possibility of overheating, as reported for endothermic giant bluefin tuna (Blank et al., 2004).

Allocations of time and energy to mutually exclusive functions (i.e.: growth versus reproduction, courtship versus foraging) are constrained by an animal’s physiology, its environment, and by resource limitations (Dunham et al., 1989). In order for female leatherbacks to reproduce, they must harvest and store sufficient energy while foraging to facilitate nest construction, egg production, survival at sea between nesting events, and round-trip migration between foraging and nesting areas (Owens, 1980). Of the energetic costs that reproductive female leatherbacks incur, the extent of internesting activity is the least critical, so leatherbacks should spend relatively little energy while at sea between consecutive nesting events.

The recent dramatic decline in global leatherback populations is chiefly due to fisheries interactions within their extensive range (Spotila et al., 2000; Lewison et al., 2004; Ferraroli et al., 2004; Hays et al., 2004a; James et al., 2005). However, effects of climatically altered resource availability on energy acquisition have not been considered in these analyses, and might also have significant impacts on viability of leatherback populations. Additionally, distinct differences exist between leatherback populations from the eastern Pacific and North Atlantic Ocean basins. North Atlantic leatherbacks are larger, more massive, have larger clutch sizes, and shorter remigration intervals on average than their eastern Pacific counterparts (Table 5; Boulon et al., 1996; Reina et al., 2002a). Such morphometric and reproductive traits are influenced by resource quality and availability in sea turtles (Limpus & Nicholls, 1988; Hays, 2000; Solow et al., 2002;
Broderick et al., 2003) and other reptiles (Congdon, 1989; Wikelski & Thom, 2000). Climatically influenced variations in oceanographic conditions affect resource availability and thus population trends in sea turtles (Limpus & Nicholls, 1988; Solow et al., 2002). Such oceanographic fluctuations cause abundances and distributions of leatherback prey (e.g.: jellyfish, ctenophores and other gelatinous animals) (Bjorndal, 1997; James & Herman, 2001) to vary greatly in time and space (Mills, 2001; Lynam et al., 2004). Therefore, estimates of leatherback bioenergetics and population dynamics must take into account the effects of climate on resource availability.

The doubly labeled water method (DLW) (Lifson et al., 1955; Fig. 1) has become widespread in studies of animal field energetics (see Speakman, 1997 for review). Chapter 2 provides a thorough discussion of the DLW method. I used DLW-derived FMRs for internesting female leatherbacks to calculate the leatherback reproductive energy budget and estimated feeding rates necessary to meet energy costs. I interpret these results in the context of climatically influenced resource availability on population dynamics of this species.

MATERIALS AND METHODS

I conducted this study during the 2002-03 and 2003-04 leatherback nesting seasons at Playa Grande, Parque Nacional Marino Las Baulas (PNMB), Costa Rica.

Doubly Labeled Water Experiment

For a thorough description of materials and methods of the DLW experiment, refer to Chapter 2, Materials and Methods section.
Leatherback energy budget estimations and resource acquisition model

I included internesting FMRs with energetic costs of egg clutches, nesting activity, and migration to estimate the total leatherback reproductive energy budget for both eastern Pacific (Playa Grande, PNMB, Costa Rica) and North Atlantic (St. Croix, USVI) populations. I used mass specific metabolic rates (Paladino et al., 1990; 1996) and durations (Reina et al., 2002a) of each phase of the nesting process, average clutch sizes (Boulon et al., 1996; Reina et al., 2002a), and data on energy content of reptilian eggs (Schmidt-Nielsen, 1997) to estimate energy costs of nesting events and egg clutches for each population. While estimated clutch frequency (ECF) was not reported for St. Croix leatherbacks, I assumed that clutch frequencies between the two populations were similar because Playa Grande leatherback ECFs were consistently 1-2 nests greater than observed clutch frequencies (OCF) (Reina et al., 2002a), and the St. Croix OCF was 5.26 (Boulon et al., 1996). I estimated travel rates, distances, and durations of round-trip migrations for both populations as being approximately 4000 km each way at 70 km d⁻¹ (Morreale et al., 1996; Eckert, 2002; Ferraroli et al., 2004; James et al., 2005). Because leatherbacks are probably more active and spend more time in colder waters during migration and foraging in higher latitude pelagic areas than in coastal, neritic habitats during the nesting season, I assumed that the FMR during migration and foraging is closer to the maximum I recorded (0.74 W kg⁻¹). I calculated daily maintenance costs while on foraging grounds by assuming that the maximum FMR I measured (0.74 W kg⁻¹) included all regulatory processes (e.g., thermoregulation, osmoregulation, digestion, assimilation) and activity costs (e.g., diving, prey handling) (Table 6). I also assumed
leatherbacks do not forage during their nesting season but instead rely on fat stores accumulated while foraging (Owens, 1980; Hamann et al., 2002), an assumption corroborated by video of internesting female leatherbacks at PNMB, which indicated that they swim by small jellyfish without feeding (Reina et al., 2004). I then used the equation

\[ RE = N + E + I + M \]

where \( N \) = nesting activity, \( E \) = egg clutches, \( I \) = internesting periods, and \( M \) = migrations to and from Playa Grande or St. Croix to calculate total reproductive energy (\( RE \)) cost for a female leatherback (see Table 6 for model inputs).

I conducted all procedures under permits 288-2002-OFAU and 273-2003-OFAU from the Costa Rican Ministerio del Ambiente y Energía (MINAE) and Drexel University IACUC Approval 02183-01. Due to the critically endangered status of the leatherback turtle, I limited the number of experimental animals to minimize the impact on the population.

**RESULTS**

**Leatherback Field Metabolic Rates**

I measured four FMRs for three free-ranging internesting leatherbacks (two FMRs for one female; one FMR for the first 3 d of her internesting period, and one FMR for the entire 14 d period). The FMRs (range = 0.20 - 0.74 W kg\(^{-1}\), mean = 0.40 W kg\(^{-1}\)) were less than MRs measured for leatherbacks vigorously throwing sand while covering nests or actively walking on the beach, and similar to leatherback MRs during quiescent nest construction or oviposition (Fig. 9).
Leatherback energy budget estimations and resource acquisition model

With the addition of metabolic cost of activity at sea during the internesting period to the calculated energetic costs of egg clutches, nesting activity, and migration, I estimated the total reproductive energy budget for both Playa Grande and St. Croix leatherbacks. Total energy requirements for all components of reproduction for Playa Grande and St. Croix leatherbacks were 4.9 x 10^6 kJ season^{-1} and 6.3 x 10^6 kJ season^{-1}, respectively (Table 7). The cost of migration dominated the overall reproductive energy budgets of leatherbacks from both populations (Tables 6 and 7).

Leatherbacks occupy a unique ecological niche by being specialist predators for gelatinous prey (Bjorndal & Jackson, 2003). In order to estimate energy intake necessary for reproduction, I calculated total prey biomass necessary for \( RE \), subtracted prey biomass necessary to meet foraging costs (at FMR = 0.74 W kg^{-1}), and divided this by prey biomass remaining at different feeding rates. We conservatively assumed 80% assimilation efficiency, based on measurements for slider turtles, \textit{Trachemys scripta} (Avery \textit{et al.}, 1993) fed a diet of similar protein content to that of pelagic jellyfish (Malej \textit{et al.}, 1993) and given the high digestion rate of gelatinous prey in fish (Arai \textit{et al.}, 2003). Based on caloric content of leatherback prey (248 kJ kg^{-1}) and reported leatherback feeding rates of 200 kg d^{-1} (Davenport & Balazs, 1991), a Playa Grande leatherback would need about 151 d to consume sufficient biomass (~ 20 x 10^3 kg; Table 7) to meet reproductive energy costs (\( RE \)), while a St. Croix leatherback (required biomass ~25 x 10^3 kg) would need about 232 d at the same feeding rate. At a 150 kg d^{-1} feeding rate, required foraging durations would extend to 245 d and 426 d for Playa Grande and St. Croix leatherbacks, respectively. If daily feeding rates were 100 kg d^{-1}, a
Playa Grande leatherback would take 650 d while a St. Croix leatherback would take 2,632 d to acquire sufficient energy to meet $RE$ (Table 7).

**DISCUSSION**

The FMRs I obtained were similar to MRs of nesting female leatherbacks obtained by analyses of respiratory gases during restraint, oviposition, or quiescent nest chamber construction, and lower than MRs for leatherbacks actively walking or throwing sand (Paladino *et al.*, 1990; 1996) (Fig. 9). The FMRs were intermediate between RMRs of reptiles and mammals scaled to leatherback size and support the findings of Paladino *et al.* (1990) that leatherbacks avoid overheating in the tropics by maintaining low metabolic rates and thereby minimizing heat generated internally.

Of the components of the leatherback reproductive energy budget, only energy expenditure during the internesting period can be flexible, since compromises in egg production and nest construction would decrease reproductive success. My results indicate that free-swimming leatherbacks have low internesting metabolic rates (Figs. 4 & 10) and spend relatively little energy while swimming between nesting events (Table 7). The internesting FMRs I obtained were consistent with the findings of Southwood *et al.* (1999), who reported that diving heart rates of leatherbacks were more similar to the relatively low heart rates during oviposition than during active nest covering or walking on the beach. These results also corroborate the hypothesis that FMRs of internesting female leatherbacks are low because swimming is more energetically efficient than walking in other vertebrates (Schmidt-Nielsen, 1972).
Population implications of the resource acquisition model

It is apparent that my estimate of required foraging duration of 151 d dramatically underestimates the 3.7 yr average remigration interval (time period between subsequent nesting seasons) observed for eastern Pacific leatherbacks (Reina et al., 2002a). Several possible explanations exist. First, given the seemingly wide range of FMRs I report here, flaws in my metabolic measurements could be to blame for this discrepancy. However, I am confident in the technical accuracy of my measurements because a validation study comparing metabolic rates measured using DLW and those obtained by respirometry in captive green turtles reported only 5.2% difference between global means of values from the two methods (Jones et al., in press; see Chapter 2 for further discussion). Further, my FMRs (measured by DLW) are low relative to MRs for leatherback turtles strenuously exercising on the beach and similar to MRs for nesting leatherbacks (measured by other methods) (Figs. 4 & 10).

Second, I might be underestimating the metabolic cost of activity and thermoregulation in cold temperate waters during migrations and foraging and this could therefore affect the results of my model. However, as these are the first MRs available for free-swimming marine turtles, they are more appropriate than using nesting MRs. Additionally, according to the gigantothermy model, the FMR I recorded and used in my calculations (0.74 W kg⁻¹) would be adequate – if coupled with appropriate blood flow adjustments – to maintain a body core temperature approximately 20°C warmer than the ambient water temperature (Paladino et al., 1990).

Third, my migratory distance inputs might have been underestimates, as current studies are demonstrating (G.L. Shillinger, B.P. Wallace, J.R. Spotila, F.V. Paladino,
B.A. Block, *unpublished results*). However, none of the leatherback telemetry studies I utilized for input values indicated that leatherbacks traveled farther than approximately 4,000 km to reach presumed foraging grounds (Morreale *et al.*, 1996; Eckert & Sarti, 1997; Ferraroli *et al.*, 2004; Hays *et al.*, 2004; James *et al.*, 2004). Additionally, James *et al.* (2005) reported that leatherbacks traveled total distances (including round-trip migrations and movements on foraging grounds) between nesting seasons of approximately 10,000 km, so my assumption of round-trip migration distance of 8,000 km is reasonable.

Fourth, I did not include the metabolic cost of movement between and among prey patches, which are crucial components of optimal foraging energetics (Mori, 1998). Leatherbacks seem to occur in distinct foraging locations (James & Herman, 2001; Casale *et al.*, 2003) and alter swim speeds depending on whether they are traveling along fronts (probable foraging) or migrating (Ferraroli *et al.*, 2004). Variations in jellyfish abundance and distribution would alter swim speed and travel distance inputs to the model, thereby increasing estimates of foraging energy expenditure if prey organisms were patchily distributed in different areas of the ocean. Experiments combining DLW and records of diving activity on foraging grounds are necessary in order to better quantify leatherback foraging energetics.

Climatically-driven fluctuations in oceanographic conditions (*i.e.*, El Niño-Southern Oscillation, or ENSO) might significantly impact resource abundance and distribution, further increasing the required foraging time beyond the best case estimate I present here. The ENSO events are characterized by the advection of a warm water anomaly across the Pacific toward western South America (Glantz, 2001) depressing the
nutrient-rich, cold upwelling off the South American Pacific coast, resulting in decreased primary productivity, altered marine and terrestrial food webs, and widespread changes in weather patterns (Chavez et al., 1999; Glantz, 2001; Stenseth et al., 2002). Repeated exposure to ENSO causes slower growth rates, delayed maturation and decreased fecundity in fish (Harvey, 2005) and oceanographic variability affects sea turtle reproduction by influencing energy acquisition, assimilation, and allocation (Limpus & Nicholls, 1988; Solow et al., 2002). Furthermore, ENSO episodes result in broad-scale movements of apex predators in the Pacific Ocean following transient foraging grounds (Lehodey et al., 1997), and declines in seabird (Scheiber & Scheiber, 1984; Hays, 1986) and pinniped (Trillmich & Limberger, 1985) populations in the equatorial eastern Pacific. To my knowledge, no data are available on abundances of gelatinous animals in the eastern Pacific and their responses to ENSO-driven climate change. However, jellyfish abundances and distributions in other regions are affected by climatic forcing (Lynam et al., 2004) on broad temporal and geographic scales (Mills, 2001), and productivity in areas where leatherbacks are presumed to forage in the eastern Pacific (Morreale et al., 1996) is patchy and unstable due to ENSO (Glantz, 2001). Such ENSO-related decreases in primary productivity would trigger bottom-up trophic cascade effects resulting in decreased jellyfish abundances, since jellyfish and related organisms are chiefly secondary consumers. Therefore, it is doubtful that sufficiently large jellyfish aggregations consistently exist in relatively small areas to sustain feeding rates necessary (200 kg d⁻¹) for energy acquisition in the 151 d period I estimated for eastern Pacific leatherbacks.
Since ENSO events occur in the equatorial eastern Pacific approximately every 3-7 yr (Chavez et al., 1999), individual leatherbacks will presumably face ENSO-related conditions several times during their reproductive lifespan, which could last > 20 yr (Spotila et al., 1996). Meanwhile, the Atlantic counterpart to ENSO, the North Atlantic Oscillation (NAO), shifts from one extreme to the other over decades (Parsons & Lear, 2001), suggesting more consistent foraging conditions for North Atlantic leatherbacks. Composite chlorophyll \( a \) concentrations over 1978-1986 (Fig. 10A) and 1997-2004 (Fig. 10B) encompassing the two most severe ENSO events of the past century (1982-83 and 1997-98) confirm that North Atlantic leatherback foraging zones were more consistently productive over these time periods than areas where eastern Pacific leatherbacks forage.

Hays (2000) surmised that an individual marine turtle must meet a reproductive energy threshold before returning to her nesting beach to reproduce. Moreover, extended remigration intervals do not result in increased reproductive output for PNMB leatherbacks (Price et al., 2004). Thus foraging ground conditions, not increased allocation of resources to reproduction, should influence remigration interval length. Therefore, I hypothesize that differential resource availability prevents eastern Pacific leatherbacks from acquiring sufficient resources to match the size (carapace length and mass) and reproductive output (clutch size and remigration interval) of North Atlantic leatherbacks (Table 5).

Leatherback bycatch rates across longline fleets in the Atlantic and Mediterranean in 2000 were actually higher than in the Pacific (Lewison et al., 2004), suggesting that fisheries pressures alone cannot explain the difference in population trends between the two oceans. Lengthened remigration intervals and decreased clutch sizes – consequences
of resource limitation – would decrease the lifetime reproductive success of individual
eastern Pacific leatherbacks, thus making eastern Pacific populations more vulnerable to
currently high fisheries bycatch probability (0.63 annually in the Pacific basin; Lewison
et al., 2004). These factors may account for differences between population trends in the
two oceans (Boulon et al., 1996; Spotila et al., 2000; Reina et al., 2002a). Indeed, the
compounded effects of anthropogenic pressures and environmental stochasticity can have
severely detrimental effects on marine animal populations (Trillmich & Limberger, 1985;
Hays, 1986; Stenseth et al., 2002).

To investigate this resource limitation hypothesis, I created a simple model to
estimate the energetic costs incurred and the daily feeding rates necessary to meet
reproductive energy requirements during lengthened remigration intervals for both
eastern Pacific and North Atlantic leatherbacks. First, I added foraging and maintenance
costs to $RE$ and divided this cost per egg laid per reproductive season for a given
remigration interval. I then calculated daily feeding rates necessary to accrue sufficient
energy for reproduction for different remigration interval lengths. By doing so, I
determined how the costs of reproduction and foraging and feeding rates necessary to
meet these costs vary with remigration interval length in both populations. For
clarification, remigration interval lengths included the interval beginning at the end of
nesting season 1 and ending at the beginning of nesting season 2, but excluding the
duration of the round-trip migration (~ 228 d).

In general, North Atlantic leatherbacks have higher absolute energy costs than
eastern Pacific leatherbacks, due mainly to their larger size, but this difference is much
smaller when the two populations are compared relative to reproductive investment,
because North Atlantic turtles lay more eggs (Tables 5-7; Fig. 11). The longer a turtle from either population spends foraging at sea between nesting seasons, the more energy she must spend while harvesting sufficient resources for reproduction. For instance, turtles nesting after 2 and 8 yr intervals at sea have the same seasonal reproductive output, but the 8 yr remigrant spends approximately 4 times more energy than the 2 yr remigrant on foraging activities during the remigration interval (Fig. 11). Therefore, not only does delayed remigration not confer reproductive or growth advantages on individual leatherbacks (Price et al., 2004), lengthened remigration intervals impose enormous energetic costs.

Daily feeding rates decrease as remigration intervals lengthen because the fixed cost of reproduction is distributed over a longer period of time (Fig. 11). Obviously, the driving force in this relationship in nature is actually that feeding rates, which are dictated by resource availability, affect remigration interval length (Hays, 2000). Since I cannot measure that feeding rate in the ocean, the remigration interval becomes a proxy for that measurement. Most importantly, the daily feeding rate required for an eastern Pacific leatherback to achieve a 2 yr remigration interval is about 32 kg d⁻¹ less than that required for a 2 yr North Atlantic remigrant (Table 7; Fig. 11). Moreover, shorter remigration intervals for eastern Pacific leatherbacks (from 3.7 yr to 2 yr) are possible through relatively slight increases (~22 kg d⁻¹) in feeding rates (Table 7). The fact that North Atlantic leatherbacks, despite having higher energy costs and required feeding rates, exhibit remigration intervals half as long as their eastern Pacific counterparts indicates that resources on North Atlantic leatherback foraging grounds are probably more abundant and/or more consistent than those available to eastern Pacific leatherbacks.
Climate driven fluctuations in resource availability would also explain intra-population variation in remigration intervals because of different foraging conditions encountered by different individual turtles (Hays, 2000). If recent climate modeling predictions of increased frequency and intensity of El Niño events due to global warming prove accurate (Timmerman et al., 1999), further declines in jellyfish abundances could result. Based on these results, I conclude that variability in foraging ground conditions in the eastern Pacific related to ENSO can have considerable impacts on leatherback energy acquisition, remigration interval length and reproductive success, and thus population dynamics.

Decreased resource availability between reproductive seasons can lengthen remigration intervals (Hays, 2000), thus decreasing seasonal and lifetime reproductive success of eastern Pacific leatherbacks and thereby increasing this population’s exposure to risk of and sensitivity to fisheries-induced mortality. Given the low metabolic requirements of leatherbacks it is unlikely that starvation has caused recent declines in eastern Pacific populations. Therefore, ENSO-related resource limitation may be decreasing leatherback reproductive success and increasing risk of incidental capture by fisheries between nesting seasons, thus causing recent (Spotila et al., 1996; 2000) and continued declines (Lewison et al., 2004) in eastern Pacific leatherback populations. While fisheries impose the more severe threat to the survival of eastern Pacific leatherbacks, the compounding effects of ENSO should be taken into account in creating conservation strategies to save leatherbacks from extinction. Since the frequency and intensity of ENSO events in the eastern Pacific will probably increase over the next few decades (Timmerman et al., 1999), management strategies for fisheries should be more
conservative than they are presently and should allow little, if any, mortality of leatherbacks if there is to be any reasonable hope for recovery of this species in the Pacific.
Table 5. Comparison of biological parameters of eastern Pacific and North Atlantic leatherback populations. Data are from an eastern Pacific leatherback population at Playa Grande, Parque Nacional Marino Las Baulas, Costa Rica, and a North Atlantic population at Sandy Point National Wildlife Refuge, St. Croix, U.S. Virgin Islands.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Playa Grande Leatherbacks*</th>
<th>St. Croix Leatherbacks†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curved Carapace Length (cm)</td>
<td>range = 123 - 170</td>
<td>range = 131 - 177</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>range = 196 - 308‡</td>
<td>range = 259 - 506</td>
</tr>
<tr>
<td>Clutch Size (# eggs per clutch)</td>
<td>mean = 64.1</td>
<td>mean = 79.7</td>
</tr>
<tr>
<td>Remigration Interval (yr)</td>
<td>mean = 3.7</td>
<td>91.7% remigrant turtles = 2-3</td>
</tr>
<tr>
<td>Population Status</td>
<td>DECREASING</td>
<td>STABLE/INCREASING</td>
</tr>
</tbody>
</table>

*Reina et al. 2002a; †Boulon et al. 1996; ‡Range for leatherbacks weighed during the present study, 2002-04.
Table 6. Reproductive energy expenditures of adult female leatherbacks.
Calculations of energy expenditures for each component of the reproductive energy budget of nesting adult female leatherbacks from (A) Playa Grande, PNMB, Costa Rica (eastern Pacific), and (B) St. Croix, USVI (North Atlantic), using available literature values and the field metabolic rates obtained in this study.

(A)

<table>
<thead>
<tr>
<th>EVENT</th>
<th>Frequency</th>
<th>Duration (min)</th>
<th>MR (W/kg)</th>
<th>Energy expenditure (kJ)/stage</th>
<th>Energy expenditure/stage/season (kJ)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nesting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emerging</td>
<td>7.15</td>
<td>22</td>
<td>1.227</td>
<td>437.30</td>
<td>3126.72</td>
<td>Reina et al., 2002a</td>
</tr>
<tr>
<td>Body Pit</td>
<td>7.15</td>
<td>16.5</td>
<td>1.227</td>
<td>327.98</td>
<td>2345.04</td>
<td>Reina et al., 2002a</td>
</tr>
<tr>
<td>Egg Chamber</td>
<td>7.15</td>
<td>17.4</td>
<td>0.387</td>
<td>109.09</td>
<td>779.98</td>
<td>Reina et al., 2002a</td>
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<tr>
<td>Laying</td>
<td>7.15</td>
<td>12.7</td>
<td>0.33</td>
<td>67.89</td>
<td>485.44</td>
<td>Reina et al., 2002a</td>
</tr>
<tr>
<td>Covering</td>
<td>7.15</td>
<td>47.3</td>
<td>1.227</td>
<td>940.20</td>
<td>6722.44</td>
<td>Reina et al., 2002a</td>
</tr>
<tr>
<td>Returning</td>
<td>7.15</td>
<td>22</td>
<td>1.227</td>
<td>437.30</td>
<td>3126.72</td>
<td>Reina et al., 2002a</td>
</tr>
<tr>
<td>Total</td>
<td>7.15</td>
<td>117.8</td>
<td></td>
<td></td>
<td>2319.77</td>
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Internesting Based on FMR via DLW

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<tr>
<th>EVENT</th>
<th>Frequency</th>
<th>Duration (days)</th>
<th>MR (W/kg)</th>
<th>Energy expenditure (kJ/interval)</th>
<th>Energy expenditure/season (kJ)</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>0.40</td>
<td>87538.32</td>
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Reproductive Investment

<table>
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<tr>
<th>EVENT</th>
<th>Clutches</th>
<th># Eggs</th>
<th>Dry Mass (g)</th>
<th>Energy Content (kJ/g)</th>
<th>Total Energy Content (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eggs-yolk</td>
<td>7.15</td>
<td>64.1</td>
<td>16</td>
<td>39.4</td>
<td>288921.78</td>
</tr>
<tr>
<td>eggs-albumin</td>
<td>7.15</td>
<td>64.1</td>
<td>7.6</td>
<td>18</td>
<td>62697.49</td>
</tr>
<tr>
<td>SAGs</td>
<td>7.15</td>
<td>38.5</td>
<td>7.6</td>
<td>18</td>
<td>37657.62</td>
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Round Trip Migration

<table>
<thead>
<tr>
<th>EVENT</th>
<th>Days</th>
<th>MR (W/kg)</th>
<th>Energy expenditure (kJ/day)</th>
<th>Total Energy Expenditure (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>at 70 km/d</td>
<td>2</td>
<td>0.74</td>
<td>17286.05</td>
<td>4.0 x 10⁶</td>
</tr>
<tr>
<td>4000 km each way</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVENT</td>
<td>Frequency</td>
<td>Duration (min)</td>
<td>MR (W/kg)</td>
<td>Energy expenditure (kJ)/stage</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------</td>
<td>----------------</td>
<td>-----------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Nesting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emerging</td>
<td>7.15</td>
<td>22</td>
<td>1.227</td>
<td>566.87</td>
</tr>
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<td>Body Pit</td>
<td>7.15</td>
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<td>1.227</td>
<td>425.16</td>
</tr>
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<td>Egg Chamber</td>
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</tr>
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<td>Covering</td>
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<td>47.3</td>
<td>1.227</td>
<td>1218.78</td>
</tr>
<tr>
<td>Returning</td>
<td>7.15</td>
<td>22</td>
<td>1.227</td>
<td>566.87</td>
</tr>
<tr>
<td>Total</td>
<td>7.15</td>
<td>117.8</td>
<td></td>
<td>3007.10</td>
</tr>
<tr>
<td>Internesting</td>
<td>Based on FMR via DLW</td>
<td>(days)</td>
<td>6</td>
<td>9.5</td>
</tr>
<tr>
<td>Reproductive Investment</td>
<td>Clutches</td>
<td># Eggs</td>
<td>Dry Mass (g)</td>
<td>Energy Content (kJ/g)</td>
</tr>
<tr>
<td>Eggs-yolk</td>
<td>7.15</td>
<td>79.7</td>
<td>16</td>
<td>39.4</td>
</tr>
<tr>
<td>Eggs-albumin</td>
<td>7.15</td>
<td>79.7</td>
<td>7.6</td>
<td>18</td>
</tr>
<tr>
<td>SAGs</td>
<td>7.15</td>
<td>36.4</td>
<td>7.6</td>
<td>18</td>
</tr>
<tr>
<td>Round Trip Migration</td>
<td>days</td>
<td>MR (W/kg)</td>
<td>Energy expenditure (kJ/day)</td>
<td>Total energy expenditure (kJ)</td>
</tr>
<tr>
<td>Migration</td>
<td>70 km/d and 4000 km each way</td>
<td>2</td>
<td>114</td>
<td>0.74</td>
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</table>
Table 7. Summary of energy and prey biomass requirements for leatherback turtles. Using the results from Table 6, I estimated energetic costs and prey biomass required for components of reproduction (Reproduction Costs), time spent on foraging grounds between nesting seasons (Foraging and Maintenance Costs), and the two combined (Total Costs) for various feeding rates. I also calculated feeding rates necessary for 2 yr (mean for North Atlantic leatherbacks) and 3.7 yr (mean for eastern Pacific leatherbacks) remigration intervals. I made the calculations for Playa Grande (eastern Pacific) and St. Croix (North Atlantic) leatherback populations (see Tables 5 and 6 for parameters) and using prey energy content of 248 kJ kg\(^{-1}\) (wet mass; Davenport & Balazs, 1991; at 80% assimilation efficiency). RI: Remigration Interval.

<table>
<thead>
<tr>
<th>Reproductive Costs</th>
<th>Playa Grande (eastern Pacific)</th>
<th>St. Croix (North Atlantic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nesting Activity (N)</td>
<td>17 x10(^3) kJ</td>
<td>22 x10(^3) kJ</td>
</tr>
<tr>
<td>Egg Clutches (E)</td>
<td>390 x 10(^3) kJ</td>
<td>473 x 10(^3) kJ</td>
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<tr>
<td>Internesting Periods (I)</td>
<td>530 x 10(^3) kJ</td>
<td>681 x 10(^3) kJ</td>
</tr>
<tr>
<td>Round-trip Migration (M)</td>
<td>4.0 x 10(^6) kJ</td>
<td>5.1 x 10(^6) kJ</td>
</tr>
<tr>
<td>Total Reproductive Energy Cost (RE)</td>
<td>4.9 x 10(^6) kJ</td>
<td>6.3 x 10(^6) kJ</td>
</tr>
<tr>
<td>Prey Biomass Required</td>
<td>20 x 10(^3) kg</td>
<td>25 x 10(^3) kg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Foraging + Maintenance Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Foraging + Maintenance Costs</td>
</tr>
<tr>
<td>Required Feeding Rate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Costs and Required Foraging Durations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prey Biomass Remaining (@ 200 kg d(^{-1}))</td>
</tr>
<tr>
<td>Days Required to Meet RE</td>
</tr>
<tr>
<td>Prey Biomass Remaining (@ 150 kg d(^{-1}))</td>
</tr>
<tr>
<td>Days Required to Meet RE</td>
</tr>
<tr>
<td>Prey Biomass Remaining (@ 100 kg d(^{-1}))</td>
</tr>
<tr>
<td>Days Required to Meet RE</td>
</tr>
<tr>
<td>Required Feeding Rate (for 2 yr RI)</td>
</tr>
<tr>
<td>Required Feeding Rate (for 3.7 yr RI)</td>
</tr>
</tbody>
</table>
Figure 9. Allometric comparison of mass-specific metabolic rates of adult female leatherbacks, mammals and other reptiles. Means (± 1 S.E.M.) of metabolic rate (MR) for leatherbacks during oviposition (◇), nest chamber construction and restraint (○), active walking or sand-throwing (□), and internesting FMR reported by this study (●) are compared to predictions from allometric expressions for reptiles (RMR = 0.378M^{-0.17}) and mammals (RMR = 3.35M^{-0.25}) (Paladino et al. 1990; 1996). Internesting FMRs for leatherbacks are similar to leatherback MRs during oviposition, nest construction, and restraint and lower than MRs during vigorous exercise, indicating that leatherbacks spend relatively little energy while at sea during the period between subsequent nesting events.
Figure 10. Chlorophyll $a$ concentrations integrated over 1978-1986 (A) and 1997-2004 (B) and encompassing the two largest El Niño events of the past century (1982-83 & 1997-98) increase from purple to red (Figures: http://oceancolor.gsfc.nasa.gov/SeaWiFS). Primary productivity was consistently higher in areas where North Atlantic leatherbacks foraging than where eastern Pacific leatherbacks forage (areas indicated by circles), probably resulting in delayed remigration in eastern Pacific (remigration interval = 3.7 yr) versus North Atlantic leatherbacks (remigration interval = 2 yr).
Figure 11. Estimates of leatherback turtle energy and feeding rate requirements for various remigration intervals. Bars (energetic cost per egg; black: N. Atlantic population, white: eastern Pacific) = \( RE + \) foraging cost (kJ) for a given remigration interval / egg laid per reproductive season. Lines (feeding rates required to meet reproductive energy cost for each remigration interval; dashed; N. Atlantic, dotted: eastern Pacific) = \( RE + \) foraging cost (kJ) / jellyfish energy content (kJ kg\(^{-1}\)) / remigration interval length (d). Lengthening remigration intervals increases total energetic costs of foraging for female leatherbacks, but decreases required daily feeding rates. Small increases in resource abundance can greatly shorten remigration intervals. See text for details on model inputs and results.
CHAPTER 4: Inter-basin populations of leatherback turtles reveal an ocean-mediated trophic dichotomy

ABSTRACT

Oceanographic sampling is often limited to local and temporally concise assessments of complex, transient and widespread phenomena. Migratory pelagic animals can serve as integrated indicators of broad-scale oceanographic factors that influence marine primary productivity. Leatherback turtles are long-lived migratory reptiles and are specialist predators for gelatinous prey. In addition, differences in morphometrics, reproductive output, and population trends exist between Atlantic and Pacific leatherbacks, and could be due to differential resource availability on leatherback foraging grounds. Therefore, we analyzed stable carbon and nitrogen stable isotopes ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of tissues from a nesting leatherback population from each the Pacific and Atlantic Ocean to establish differences between nutrient sourcing and primary production and its influence on higher trophic level consumers in the both basins. While $\delta^{13}\text{C}$ signatures were similar between Atlantic (-19.4‰ ± 1.0‰) and Pacific leatherbacks (-19.1‰ ± 0.7‰), reflecting the pelagic foraging strategy of the species, Pacific leatherback $\delta^{15}\text{N}$ (15.4‰ ± 1.8‰) was significantly enriched relative to Atlantic leatherback $\delta^{15}\text{N}$ (9.8‰ ± 1.5‰). This $\delta^{15}\text{N}$ discrepancy reflects inter-basin differences in nitrogen cycling regimes and their influence on primary production being transferred through several trophic levels. Therefore, my results demonstrate intriguing potential for combining high-order marine consumer movements, habitat preferences, and stable
isotope signatures with ocean sampling to elucidate interactions between oceanographic processes and marine biodiversity.
INTRODUCTION

Introduction to stable isotope (SI) analyses

Determination of stable nitrogen ($^{15}$N/$^{14}$N: $\delta^{15}$N) and carbon ($^{13}$C/$^{12}$C: $\delta^{13}$C) isotope ratios have been used to reconstruct diets, track movement patterns (Kurle & Worthy, 2002; Rubenstein & Hobson, 2004), assess physical condition (Hobson et al., 1993), and characterize nutrient allocation and turnover rates (Tieszen et al., 1983) in several marine vertebrate animal taxa, including cetaceans (Schell et al., 1989; Ostrom et al., 1993; Abend & Smith, 1995; 1997; Pauly et al., 1998; Ruiz-Cooley et al., 2004), pinnipeds (Hirons et al., 2001; Kurle & Worthy, 2001; 2002), marine birds (Hobson, 1993; Minami et al., 1995; Gould et al., 1997; Minami & Ogi, 1997; Sydeman et al., 1997), marine turtles (Godley et al., 1998; Hatase et al., 2002; Biasatti, 2004), and fish (Satterfield & Finney, 2002). Additionally, stable isotope analyses have been employed to characterize trophic interactions and energy flows and sources within ecosystems (Peterson & Fry 1987; Gannes et al., 1997; 1998; Hobson, 1999; Vander Zanden & Rasmussen, 2001).

The rate at which the isotope ratios of diet items become incorporated in consumer tissues varies with the level of metabolic activity of the tissue in question (isotopic turnover rate) and the effects of biochemical fractionation of isotopes during tissue catabolism (DeNiro & Epstein 1978; 1981; Tieszen et al., 1983; Hobson, 1999). Thus, the appropriate tissue(s) should be selected depending on the length of the time period relevant for a given stable isotope study. For instance, stable isotope ratios of metabolically active tissues (i.e. liver, blood serum) are most appropriate for short-term dietary analyses, whereas less metabolically active tissues (i.e. skin, bone) are appropriate
for studies of an animal’s diet integrated over a longer time period (Hobson, 1999; Rubenstein & Hobson, 2004). Due to selectivity of heavier isotopes during metabolic processes, animal tissues tend to be enriched relative to their diet by 3 - 4‰ for δ¹⁵N and 0-1% for δ¹³C per trophic level (DeNiro & Epstein 1978; 1981; Tieszen et al., 1983; Minagawa & Wada, 1984). Thus, because of their large enrichment factor, δ¹⁵N signatures are useful for identification of trophic level or trophic structure of the organism or system of interest (DeNiro & Epstein 1981; Minagawa & Wada, 1984). On the other hand, because δ¹³C signatures undergo only slight trophic enrichment, they are not necessarily good indicators of trophic levels, but more effectively describe different carbon sources and flow pathways (DeNiro & Epstein, 1978; Peterson & Fry; 1987; Vander Zanden & Rasmussen, 1999).

**Stable isotopes and marine systems**

In the case of marine trophic systems, δ¹³C values indicate the proximity of marine vertebrate consumers foraging habitats to the coast (i.e.: pelagic vs. near-shore foraging habitats; Rau et al., 1983; Rubenstein & Hobson, 2004). Stepwise trophic enrichments in δ¹⁵N and δ¹³C vary considerably in freshwater aquatic systems due to nutrient fluxes and allochthonous input, as well as to different rates of isotopic assimilation at different trophic levels (O’Reilly & Hecky, 2002). Therefore, more variation in stable isotope ratios exists among the producers in aquatic systems, but trophic fractionation stabilizes with successive increases in consumer levels (Vander Zanden & Rasmussen, 1999; 2001). Marine systems, however, exhibit more reliable rates of isotopic enrichment per trophic level because there are not such drastic
fluctuations of nutrients, fewer outside nutrient sources, and because they are much more open systems (Rau et al., 1983; Vander Zanden et al., 2001).

Different oceanographic processes can affect baseline δ^{13}C and δ^{15}N in marine trophic systems (Rau et al., 1982; Saino & Hattori, 1987; Goericke & Fry, 1994; Gruber & Sarmiento, 1997; Minami & Ogi, 1997; Deutsch et al., 2001). Carbon isotope ratios tend to decrease (i.e.: become more negative) from low to high latitudes due to a variety of factors such as water temperature and CO_2 concentration effects on carbon fixation by phytoplankton (Rau et al., 1982; Goericke & Fry, 1994). Nitrogen isotope ratios vary depending on the predominant form of nitrogen cycling and metabolism of a given oceanic region (Wada & Hattori, 1976; Saino & Hattori, 1987; Gruber & Sarmiento, 1997; Deutsch et al., 2001). Denitrification occurs in oxygen-depleted areas of the open ocean, such as the eastern tropical Pacific (ETP), and in hypoxic-anoxic marine sediments, such as the Bering Sea (Gruber & Sarmiento, 1997), and results in ^{15}N enrichment of the nitrogen source available for production and thus elevated baseline δ^{15}N values (Saino & Hattori, 1987). Alternatively, fixation of ^{15}N-depleted nitrogen gas (N_2) generates new nitrogen available for production, and baseline δ^{15}N values in areas of high N_2-fixation are generally low (Saino & Hattori, 1987; Montoya et al., 2002; McClelland et al., 2003). Nitrogen fixation is iron limited, and therefore occurs in areas of high aeolian flux of dust or riverine input, such as the North Atlantic, which receives consistent delivery of airborne Saharan dust (Falkowski et al., 1998; Berman-Frank et al., 2001; Karl et al., 2002).

While analyses of stable isotope ratios of animal tissues are employed commonly to investigate migratory animal origins and movement patterns and to distinguish
between nutrient sources (i.e.: C4 vs. C3 and CAM plants, aquatic vs. terrestrial systems; see Hobson, 1999 for review), few studies have invoked effects of oceanographic factors on trophic dynamics in marine systems to explain geographic variation in stable isotope ratio patterns, particularly $\delta^{15}N$ values, and are usually confined to a single ocean basin or region within one basin (Minami & Ogi, 1997; Hirons et al., 2001; Takai et al., 2000).

Minami and Ogi (1997) reported that differences in baseline $\delta^{15}N$ between eastern (high denitrification and $^{15}N$-enriched) and western Pacific (high N$_2$-fixation and $^{15}N$-depleted due to dust input from Asia and Australia) foraging and migratory habitats resulted in higher $\delta^{15}N$ in sooty shearwaters (*Puffinus griseus*) that had followed eastern Pacific migratory trajectories than their western Pacific counterparts. These intra-Pacific baseline $\delta^{15}N$ differences were also reflected in $\delta^{15}N$ signatures from ten squid species from several areas of the Pacific and surrounding seas (Takai et al., 2000). Stable isotope analyses of inter-basin (i.e.: Pacific vs. Atlantic Oceans) conspecific taxa would be crucial to expanding our understanding of how oceanography influences the trophic ecology of high-order marine consumers.

**Leatherback turtles: model organisms for SI analyses of distinct marine systems**

Leatherback turtles (*Dermochelys coriacea*) are critically endangered (Spotila et al., 2000) and range throughout the worlds’ oceans from circumpolar to tropical regions. Leatherbacks are long-lived and undertake long-distance migrations every 2-4 yr between nesting beaches in the tropics and foraging grounds in high-latitude to circumpolar regions (Morreale et al., 1996; Eckert & Sarti, 1997; Hays et al., 2004a and b; Ferraroli et al., 2004; James et al., 2005). Leatherbacks maintain elevated body temperatures in cold
water (Paladino et al., 1990), have the highest reproductive output of any reptile (Reina et al., 2002a), and have the fastest growth rate of any turtle (Zug & Parham, 1996). These superlative traits must require enhanced energy intake and assimilation. Additionally, distinct differences exist between leatherback populations from the eastern Pacific and North Atlantic Ocean basins. North Atlantic leatherbacks, on average, are larger, more massive, have larger clutch sizes, and shorter remigration intervals than their eastern Pacific counterparts (Table 5; Boulon et al., 1996; Reina et al., 2002a). Such morphometric and reproductive traits are influenced by resource quality and availability in sea turtles (Limpus & Nicholls, 1988; Hays, 2000; Solow et al., 2002; Broderick et al., 2003) and other reptiles (Congdon, 1989; Wikelski & Thom, 2000). Determining trophic status and sources of energy available to leatherbacks in both basins could provide insight into the nature of these population differences and to leatherback energy budgets.

Leatherbacks forage primarily on jellyfish and related prey (Davenport et al., 1990; Holland et al., 1990; Davenport & Balazs, 1991; James & Herman, 2001) in high-latitude, cool water convergence zones and areas of nutrient upwelling (Lazelle, 1980; Goff & Lien, 1988; Bjorndal, 1997; Ferraroli et al., 2004). Leatherbacks might have to consume daily prey biomass equivalent to their body mass to meet the high energy demands of their physiology and life history (Lutcavage & Lutz, 1986; Davenport & Balazs, 1991). However, gelatinous prey items appear to be highly digestible (Arai et al., 2003) and have C:N:P nutrient ratios that are favorable to rapid and efficient nutrient assimilation (Malej et al., 1993), so should be good energy sources for leatherbacks when consumed in large masses. Indeed, anecdotal reports of feeding rates of 200 kg d⁻¹ exist (Davenport & Balazs, 1991).
Stable isotope analyses of sea turtles have revealed interspecies differences in trophic ecology (Godley et al., 1998), variation in foraging grounds (Hatase et al., 2002), and diving physiology (Biasatti, 2004). Godley et al. (1998) reported δ^{15}N signatures increasing from green (Chelonia mydas) to leatherback to loggerhead turtles (Caretta caretta) turtles, reflecting the continuum from herbivory to carnivory in these species, and δ^{13}C values representative of the distinct marine domains in which each species is known to forage. Combining δ^{15}N and δ^{13}C signatures of egg yolk with post-nesting satellite telemetry, Hatase et al. (2002) discerned size-related increases in both isotopes which revealed differences in foraging strategies and habitats among female loggerheads. In addition, the influence of CO₂ accumulation during breath-hold diving on bone δ^{13}C confirmed differences in diving physiology between deep-diving leatherbacks, shallow-diving green turtles, and olive ridley turtles (Lepidochelys olivacea), whose dive performance is intermediate between the two (Biasatti, 2004).

Stable isotope ratios of diet become incorporated into egg yolk within 8-15 d in birds (Hobson, 1995; Hobson et al., 2000), but for reptiles this process requires more time, due to differences in metabolism. Vitellogenesis in leatherbacks and other sea turtle species probably lasts 3-6 months and is complete upon arrival at nesting grounds before mating begins (Rostal et al., 1996; 1998). Likewise, while mammalian red blood cells turn over on the order of weeks, the longevity of nucleated red blood cells in reptiles is ≥ 200 d (Kirkland & Altland, 1955). Therefore, egg yolk and RBC stable isotope signatures should represent integration of dietary resources consumed on foraging grounds before migration to the nesting beach.
Declining leatherback populations are considered indicators of threats to marine biodiversity (Spotila et al., 2000), but their longevity and pan-oceanic migrations could also make them indicators of oceanographic processes that affect their nutrient intake and processing via analyses of stable isotope signatures of their tissues. Therefore, I analyzed stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotopic signatures of red blood cells and egg yolk from nesting leatherbacks over two nesting seasons from a nesting population in the eastern Pacific (Playa Grande, Costa Rica) and the North Atlantic (St. Croix, USVI) to compare nutrient sourcing at the base of marine food webs in the Atlantic and Pacific Oceans and the relative trophic position of leatherbacks in both basins. My results demonstrate the intriguing potential for combining short-term, high resolution oceanographic sampling with stable isotope analyses of marine consumer tissues, which provide long-term and broad-scale integration of diet, and thus oceanographic processes, to elucidate the influence of physical, chemical and biological factors on marine trophic systems.

**MATERIALS AND METHODS**

I and colleagues analyzed tissue samples collected from female leatherbacks nesting at Playa Grande, Parque Nacional Marino Las Baulas (PNMB), Guanacaste Province, Costa Rica, during the 2003-04 and 2004-05 nesting seasons, and at Sandy Point National Wildlife Refuge, St. Croix, US Virgin Islands, during the 2003 and 2004 nesting seasons. These two leatherback nesting populations have been studied extensively for the past 15 - 20+ yr (Boulon et al., 1996; Steyermark et al., 1996; Reina
et al., 2002a). We collected blood samples from the dorsal cervical sinus or a rear flipper during oviposition to avoid disturbing the nesting females. We separated blood components and isolated red blood cells (RBCs) by centrifugation.

Because intra-clutch variation in stable isotope ratios is low for loggerhead turtles (Caretta caretta) (0.1-0.2‰ δ¹³C and 0.1-0.3‰ δ¹⁵N; Hatase et al., 2002), I collected a yolk sample from only one egg per clutch at Playa Grande and colleagues did the same at St. Croix. Playa Grande eggs were non-viable, unhatched dead eggs excavated following hatchling emergence, while St. Croix eggs were viable eggs. We collected Playa Grande samples under appropriate institutional protocols, Costa Rican Ministerio del Ambiente y Energía (MINAE) permits 273-2003-OFAU and ACT-OR-056 and St. Croix samples under USFWS permit 04US844694/9 and transported samples using Costa Rican CITES export permits and USFWS CITES import permits. We stored blood and yolk samples at -10°C until they could be processed at NMFS Southwest Fisheries Science Center, La Jolla, CA, USA.

Sample preparation and analysis

We dried blood samples (RBCs) at 60°C for 24 h and then powdered the samples with a mortar and pestle. We removed lipids from yolk samples using a Soxhlet apparatus with a 1:1 solvent mixture of petroleum ether and ethyl ether for at least two 10-hour cycles and then dried the samples at 60°C for 24 h to remove any residual solvent. We loaded between 0.1 - 1.8 mg tissue subsamples into sterilized tin capsules and analyzed them with a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Laboratory at Scripps Institution of Oceanography, La Jolla, CA, USA. We used a
Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT, Bremen, Germany) to a Deltaplus gas isotope-ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

I expressed stable isotope ratios of samples relative to isotope standards in the following conventional delta (δ) notation in parts per thousand (‰):

\[ \delta = (\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1) \times 1000 \]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the corresponding ratios of heavy to light isotopes (\(^{13}\)C/\(^{12}\)C: \( \delta^{13}\)C and \(^{15}\)N/\(^{14}\)N: \( \delta^{15}\)N) in the sample and standard, respectively. \( R_{\text{standard}} \) for \(^{13}\)C was Baker Acetanilide (C\(_8\)H\(_9\)NO; \( \delta^{13}\)C = -10.4) calibrated monthly against NBS 19 and NBS 18 +20 and \( R_{\text{standard}} \) for \(^{15}\)N was IAEA N1 Ammonium Sulfate ((NH\(_4\))\(_2\)SO\(_4\); \( \delta^{15}\)N = +0.4) calibrated against Atmospheric N\(_2\) and USGS Nitrogen standards. All analytical runs included samples of standard materials inserted every 6 to 7 samples to calibrate the system and compensate for any drift in the mass spectrophotometer over time. Hundreds of replicate assays of standard materials indicated measurement errors of 0.05‰ and 0.095‰ for carbon and nitrogen, respectively.

We also measured %C and %N for each tissue sample. Samples were combusted in pure oxygen in the elemental analyzer. Resultant CO\(_2\) and N\(_2\) gasses were passed through a series of thermal conductivity detectors and element traps to determine percent compositions. Acetanilide standards (10.36%N, 71.09%C) were used for calibration.

**Statistical analyses**

I used SPSS 11.5.1 (Chicago, USA) to analyze the stable isotope and sample element composition data. I tested for normality using Shapiro-Wilkes W test and arcsine
transformed percentage data. I used Student’s t-tests to compare mean stable isotope and element composition data between tissue types and locations. I accepted significance at $\alpha = 0.05$ level and present means ± 1 S.D. unless otherwise noted.

RESULTS

Playa Grande samples

Playa Grande yolk %N was slightly lower than red blood cell %N ($t = -2.232$, $P = 0.032$), but the two tissues had similar %C values ($t = 0.401$, $P = 0.691$).

Yolk $\delta^{15}$N was significantly lower than RBC $\delta^{15}$N values ($t = -2.694$, $P = 0.011$), while there was no difference between $\delta^{13}$C signatures of the two tissues ($t = 1.126$, $P = 0.268$; Table 8A).

St. Croix samples

St. Croix yolk %N and %C were significantly lower than RBC %N ($t = -13.649$, $P < 0.001$) and %C ($t = -3.408$, $P = 0.001$).

Yolk $\delta^{15}$N was significantly higher than RBC $\delta^{15}$N ($t = 3.608$, $P = 0.001$), while yolk $\delta^{13}$C was significantly lower than RBC $\delta^{13}$C ($t = -9.665$, $P < 0.001$; Table 8B).

Yolk versus red blood cells

Yolk samples pooled between the two locations had significantly lower %N than RBC samples ($t = -7.770$, $P < 0.001$), but %C did not differ significantly between the two tissues ($t = -0.840$, $P = 0.405$).
Yolk and RBC samples had similar $\delta^{15}$N signatures across locations ($t = -1.211, P = 0.231$), while yolk $\delta^{13}$C was significantly lower than RBC $\delta^{13}$C ($t = -7.497, P < 0.001$; Table 9).

**Playa Grande versus St. Croix samples**

Yolk % N and %C were similar between locations (%N: $t = 1.123, P = 0.273$). Playa Grande yolk $\delta^{15}$N and $\delta^{13}$C were higher than St. Croix yolk $\delta^{15}$N and $\delta^{13}$C ($\delta^{15}$N: $t = -11.118, P < 0.001$; $\delta^{13}$C: $t = -4.132, P < 0.001$).

St. Croix RBC %N was significantly higher than Playa Grande RBC %N ($t = 3.040, P = 0.006$). However, RBC %C values did not differ between locations ($t = 0.834, P = 0.414$). Playa Grande RBC $\delta^{15}$N was significantly higher than St. Croix RBC $\delta^{15}$N ($t = -16.346, P < 0.001$), but Playa Grande RBC $\delta^{13}$C was lower than that from St. Croix ($t = 2.950, P = 0.006$; Table 9).

In order to compare results between Playa Grande and St. Croix, I pooled yolk and RBC elemental composition and stable isotope values within locations. Neither nitrogen nor carbon composition differed between locations (%N: $t = -0.142, P = 0.887$; %C: $t = 0.227, P = 0.821$).

I found no difference in $\delta^{13}$C signatures between locations ($t = 1.650, P = 0.102$). However, Playa Grande $\delta^{15}$N was significantly higher than St. Croix $\delta^{15}$N ($t = 17.393, P < 0.001$; Table 9; Fig. 12).

**DISCUSSION**

Differences between yolk and red blood cells and within locations
Blood and blood components contain higher amounts of protein than lipid (Nelson & Cox, 2005) while yolk contains a high proportion of lipid (Hobson, 1995). These differences potentially explain the higher %N in RBC than yolk samples from leatherback turtles. However, this difference was relatively small (1.5%) and thus probably biologically irrelevant. More importantly, comparable δ^{15}N values for the two tissues indicated that the incorporation of dietary nitrogen isotopes occurred similarly for yolk and RBCs (Table 9).

Egg yolk contains high amounts of lipids, which are relatively depleted in ^{13}C (DeNiro & Epstein, 1978; Tieszen et al., 1983). Although we lipid-extracted our yolk samples, persistence of residual lipids could have decreased the δ^{13}C signature of yolk relative to RBC δ^{13}C. Furthermore, the higher RBC δ^{13}C might reflect more rapid turnover than yolk δ^{13}C, thus reflecting the influence of coastal nesting areas on the RBC δ^{13}C signature, but I cannot substantiate this possibility.

Differences between elemental compositions between tissues could also be due to heterogeneity of carbon and nitrogen in the samples used in mass spectrophotometry analyses. Since only 0.1 - 1.8 mg of samples were used to analyze stable isotope ratios, it is possible that the small subsample analyzed was not a homogeneous representation of the %C or %N of the entire tissue sample. In general, when elemental composition data were combined across locations, %N and %C values were similar for both tissues and both sites (Table 9).

Yolk δ^{15}N was higher than RBC δ^{15}N from Playa Grande, but the reverse was true for tissues from St. Croix. These differences could be due to incorporation of dietary isotopic signatures on different timescales or differential fractionation during synthesis of
the two tissues (DeNiro & Epstein, 1978; Peterson & Fry, 1987). In general, while
differences existed within locations and within tissues, directions of the significant
differences were not consistent, so no discernible patterns explain all results. Therefore,
because both tissues reflect long-term incorporation of dietary signatures, the most
meaningful comparisons were between Playa Grande leatherback tissues and St. Croix
leatherback tissues.

**Playa Grande versus St. Croix samples**

Nitrogen and carbon composition values were similar between Playa Grande and
St. Croix when data were pooled between tissues, indicating that samples taken from both
sites contained similar %N and %C (Table 9). However, while stable carbon ($\delta^{13}C$)
isotopic signatures were similar for both populations, supporting the well-established
high-latitude, pelagic foraging strategy of this species (Bjorndal, 1997), Playa Grande
$\delta^{15}N$ signatures were 5.6‰ higher than St. Croix $\delta^{15}N$ signatures (Table 9; Fig. 12).

Invoking the accepted 3-4‰ stepwise trophic enrichment in $\delta^{15}N$ (DeNiro &
Epstein, 1981; Minagawa & Wada, 1984) suggests that eastern Pacific leatherbacks are
foraging at almost 2 trophic levels above North Atlantic leatherbacks. This would imply
that while North Atlantic leatherbacks were consuming jellyfish, eastern Pacific
leatherbacks were consuming top trophic level predators, such as orcas and sharks. This
is highly unlikely because leatherbacks are specialist predators for gelatinous prey
regardless of geographical location (Lazelle, 1980; Holland *et al.*, 1990; Davenport &
Balazs, 1991; Bjorndal, 1997) and orcas and sharks are known predators of leatherbacks,
not the reverse. While jellyfish as a group exhibit diverse diets ranging from herbivorous
planktivores (e.g., *Aurelia aurita*) to at least partial piscivores (e.g., *Stomolophus* spp., *Chrysaora* spp.), differences in species-specific prey consumed by leatherbacks between basins could not, by itself, explain the large $\delta^{15}N$ difference between populations.

Another possible explanation for the inter-basin $\delta^{15}N$ difference could be that food chain length increases result in higher $\delta^{15}N$ signatures for conspecific populations of high-order consumers in larger, more species-rich lakes (Vander Zanden *et al.*, 1999). However, application of such relationships to open marine systems is inappropriate given the transient spatio-temporal nature of marine primary production (Berman-Frank *et al.*, 2001; Montoya *et al.*, 2002) and high degree of movement of marine consumers (Minami *et al.*, 1995; Minami & Ogi, 1997; Kurle & Worthy, 2002) such as leatherbacks (Morreale *et al.*, 1996; Ferraroli *et al.*, 2004; Hays *et al.*, 2004a and b).

Leatherbacks nesting at PNMB, Costa Rica, are known to migrate to the highly denitrified upwelling zone off Peru and Chile (Morreale *et al.*, 1996; Eckert & Sarti, 1997; G.L. Shillinger, B.P. Wallace, J.R. Spotila, F.V. Paladino, & B.A. Block, *unpublished data*), where they presumably forage. Meanwhile, leatherbacks nesting in the Caribbean and western tropical Atlantic migrate throughout the North Atlantic and forage in various locations, from the northwest Atlantic on the North American continental shelf to the UK and west African waters (Ferraroli *et al.*, 2004; Hays *et al.*, 2004a and b; James *et al.*, 2005). If high rates of N$_2$-fixation influence primary production throughout the North Atlantic, as suggested by several field and laboratory studies (Gruber & Sarmiento, 1997; McClelland & Montoya, 2002; Montoya *et al.*, 2002; McClelland *et al.*, 2003), North Atlantic baseline $\delta^{15}N$ values should be close to 0, since atmospheric N$_2$ is typically $^{15}N$-depleted and N$_2$-fixation involves very little, if any,
isotopic fractionation (Karl et al., 2002). Meanwhile oceanic nitrate, which is utilized in denitrification processes, has a $\delta^{15}N$ of $\sim$5‰ (Karl et al., 2002). In fact, zooplankton $\delta^{15}N$ signatures from the North Atlantic range from 0.3 to 2.2‰ (Montoya et al., 2002), while particulate organic nitrogen (PON) $\delta^{15}N$ in the eastern equatorial Pacific ranges between 6.6 to 14.4‰ (Saino & Hattori, 1987). Since leatherbacks consuming jellyfish would be three trophic steps from baseline primary producers (primary producers $\rightarrow$ zooplankton $\rightarrow$ jellyfish $\rightarrow$ leatherbacks), leatherback tissues theoretically should reflect 3 trophic level enrichments in $\delta^{15}N$ (approximately 3 - 4‰ per trophic level; DeNiro & Epstein, 1981; Minagawa & Wada, 1984). Given the above ranges of baseline $\delta^{15}N$, I can assume leatherback tissue $\delta^{15}N$ should be roughly 7 - 11‰ in the North Atlantic and $>$14‰ in the eastern Pacific. North Atlantic leatherback tissues in this study had $\delta^{15}N$ signatures of 8.9‰ for RBC and 10.2‰ for yolk (mean $\delta^{15}N = 9.8‰$), while $\delta^{15}N$ values of the same two tissues from eastern Pacific leatherbacks were 16.1‰ and 14.7‰ (mean $\delta^{15}N = 15.4‰$). Therefore, the discrepancy between eastern Pacific (Playa Grande) and North Atlantic (St. Croix) $\delta^{15}N$ values reveal the influence of the markedly higher levels denitrification in the ETP and of elevated N$_2$-fixation by diazotrophic phytoplankton in the North Atlantic (Saino & Hattori, 1987; Gruber & Sarmiento, 1997; Deutsch et al., 2001; Karl et al., 2002) supported by large inputs of terrestrial dust and riverine outflow (Falkowski et al., 1998; Berman-Frank et al., 2001) on baseline $\delta^{15}N$ being conserved through several trophic levels. Interestingly, populations of sperm whales (Physeter macrocephalus) from the ETNP (19.6‰; Ruiz-Cooley et al., 2004) and the northwest Atlantic (11.1‰; Ostrom et al., 1993) also reflect this inter-basin $\delta^{15}N$ dichotomy.
To investigate further the influence of differential primary producer nitrogen metabolism on marine trophic dynamics globally, I compiled δ¹⁵N signatures from the literature representing several diverse taxa from cetaceans to invertebrates (Appendix A). First, I grouped consumers across taxa according to trophic level following Pauly et al. (1998) for marine mammals, Cortes (1999) for sharks, and Sydeman et al. (1997) for seabirds. Trophic level 4 and above (TL > 4) included apex predators (e.g.: odontocetes, sharks), owing to their predominantly piscivorous diets. Trophic level 3 - 4 (TL 3 - 4) included predators such as humpback whales (*Megaptera novaeangliae*) and minke whales (*Balaenoptera acutorostrata*) with mixed carnivorous diets comprising fish as well as small invertebrates such as krill). I then grouped remaining adult sized invertebrate animals according to taxonomical relationships (i.e.: squid spp., jellyfish spp.). Finally, I divided the consumer groups according to the predominant nitrogen regime in the foraging habitat from which the samples came, either “denitrification” or “nitrogen fixation” (Table 10). Marine regions of high denitrification included the eastern tropical Pacific (ETP) and the Bering Sea (NE Pacific), while marine regions of high N₂-fixation included the North Atlantic and the western Pacific (off Japan; S. China Sea) (Wada & Hattori, 1976; Saino & Hattori, 1987; Gruber & Sarmiento, 1997; Takai et al., 2000; Berman-Frank et al., 2001; Deutsch et al., 2001; Karl et al., 2002; Montoya et al., 2002). I expressed the leatherback and sperm whale δ¹⁵N data in separate categories because those were the only data for inter-basin conspecific populations, and I calculated the overall mean with and without them because they were for single species, not taxa or entire trophic level groupings.
Categorically, consumer tissues from ocean regions of high denitrification were higher than those from marine areas of high N₂-fixation (Fig. 13) with an overall average difference of 3.4‰ δ¹⁵N (range: 1.9‰ for squid spp. to 4.1‰ for TL 3 - 4; Table 10). Including the leatherback and sperm whale data increased the average difference to 4.6‰. It is important to point out that stepwise trophic enrichment values cannot be inferred from these results since the values for each trophic level represent several geographically distinct ocean regions. Stepwise δ¹⁵N enrichment values within trophic systems must be calculated within their known geographic location (Minagawa & Wada, 1984; Peterson & Fry, 1984; Hobson, 1999). Instead, these analyses demonstrate that the global effects of distinct nitrogen cycling processes on baseline δ¹⁵N signatures are conserved through several trophic levels, which previously had only been suggested by a few studies (Minami & Ogi, 1997; Takai et al., 2000; Hatase et al., 2002) and not completely understood (McClelland & Montoya, 2002).

Morphological and population differences between eastern Pacific and North Atlantic leatherbacks are probably due in part to differential resource availability between the two basins (Chapter 3; Fig. 10). The disparate δ¹⁵N signatures I report here provide further support for fundamental oceanographic differences between eastern Pacific and North Atlantic leatherback foraging grounds, but how can the distinct nitrogen cycling regimes account for differences in productivity between the basins? Primary production using nitrogen derived originally by nitrogen fixing organisms (e.g.: *Trichodesmium* spp.) is considered “new production” because it results in an addition of available nitrogen to producers (Saino & Hattori, 1987). Conversely, denitrification is considered “regenerated production”, and results in a net loss of nitrogen from the euphotic zone and
reliance upon further upwelling of benthic nitrogen sources (Saino & Hattori, 1987).
Therefore, new production occurs in the North Atlantic Ocean on a broad and relatively consistent scale, thus making this region a global nitrogen source by adding new nitrogen to subsurface water which is then transported throughout the basin by advection (Gruber & Sarmiento, 1997). This process could affect leatherback foraging grounds via utilization of remineralized, nitrogen-rich organic matter originally derived from diazotrophic organisms (Gruber & Sarmiento, 1997) in colder, nutrient-rich waters of the northwest Atlantic, thus resulting in consistently high levels of primary productivity. On the other hand, leatherbacks that nest at PNMB, Costa Rica, forage in the oxygen-depleted denitrifying zone within the Humboldt Current/Equatorial Current/Peruvian upwelling system, which is relatively devoid of nitrogen fixation due to iron-limitation and thus a global nitrogen sink (Saino & Hattori, 1987; Gruber & Sarmiento, 1997; Deutsch et al., 2001). Additionally, the El Niño-Southern Oscillation (ENSO) has profound effects on the productivity in this region because the warm water anomaly associated with it depresses the thermocline, thus prohibiting the upwelling of crucial nutrients – including dissolved subsurface organic nitrogen – from reaching the euphotic zone (Glantz, 2001). Furthermore, ENSO episodes result in transient and irregular foraging grounds in the ETP, which cause broad-scale movements of apex predators in the Pacific Ocean in response to patchy prey distribution (Lehodey et al. 1997), and declines in seabird (Scheiber & Scheiber 1984; Hays 1986) and pinniped (Trillmich & Limberger, 1985) populations. Therefore, contrasting oceanographic processes affect eastern Pacific and North Atlantic leatherbacks differentially and potentially account for
observed morphological and population trend disparities (Boulon et al., 1996; Reina et al., 2002a; Table 5).

Oceanographic sampling is often confined to local “snapshots” of processes that are actually widespread and transient, and models derived from such information are unable to fully characterize complexities of open ocean ecosystems (Michaels et al., 2001; Karl et al., 2002). To address this problem, highly migratory pelagic vertebrates can be employed as “ocean samplers” to collect detailed and integrated oceanographic data over large spatio-temporal scales (Block et al., 2001; 2003). Here I used leatherback turtles (*Dermochelys coriacea*) as oceanographic indicators to establish differences between nutrient sourcing at the base of marine food chains and its influence on higher trophic level consumers in the North Atlantic and eastern Pacific Oceans. High-order pelagic consumers, combined with conventional ocean sampling, can be utilized as integrated oceanographic indicators of spatial and temporal shifts in nutrient production and trophic system functioning.
Table 8. Nitrogen and carbon compositions and stable nitrogen and carbon isotope values for yolk and red blood cells (RBC) from adult female leatherback turtles nesting at (A) Playa Grande, Parque Nacional Marino Las Baulas, Costa Rica (PG), during the 2003-04 and 2004-05 seasons and (B) Sandy Point National Wildlife Refuge, St. Croix, USVI (STX), during the 2003 and 2004 seasons. Mean shown ± 1 S.D.

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Table 9. Nitrogen and carbon compositions and stable nitrogen and carbon isotope values for both tissues (yolk and red blood cells (RBC)) from adult female leatherback turtles nesting at Playa Grande, Parque Nacional Marino Las Baulas, Costa Rica (PG) and Sandy Point National Wildlife Refuge, St. Croix, USVI (STX). Means shown ± 1 S.D.

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<td>PG</td>
<td>RBC</td>
<td>18</td>
<td>12.9 ± 1.2</td>
<td>46.4 ± 6.1</td>
<td>16.1 ± 1.2</td>
<td>-19.0 ± 0.7</td>
</tr>
<tr>
<td>BOTH</td>
<td>RBC</td>
<td>38</td>
<td>13.4 ± 1.1</td>
<td>47.1 ± 4.3</td>
<td>12.3 ± 3.9</td>
<td>-18.6 ± 0.8</td>
</tr>
<tr>
<td>STX</td>
<td>BOTH</td>
<td>67</td>
<td>12.4 ± 1.1</td>
<td>46.7 ± 1.7</td>
<td>9.8 ± 1.5</td>
<td>-19.4 ± 1.0</td>
</tr>
<tr>
<td>PG</td>
<td>BOTH</td>
<td>37</td>
<td>12.5 ± 1.1</td>
<td>46.8 ± 4.9</td>
<td>15.4 ± 1.8</td>
<td>-19.1 ± 0.7</td>
</tr>
</tbody>
</table>
Table 10. Comparison of δ¹⁵N signatures of consumers across trophic levels from foraging regions of high denitrification or high nitrogen fixation rates. I assigned trophic levels (TL) following Pauly et al. (1998) for marine mammals and Cortes (1999) for sharks. Only data for sperm whales and leatherbacks represent inter-basin comparisons. Therefore, mean difference in δ¹⁵N within trophic levels between areas of high denitrification and high nitrogen fixation was calculated with and without data for sperm whales and leatherbacks. Values for particulate organic nitrogen δ¹⁵N represent ranges from literature reports. Raw individual δ¹⁵N signatures from the literature are included in Appendix A. Data shown are means ± 1 S.D. and number of literature values from which means and S.D. are derived are in parentheses.

<table>
<thead>
<tr>
<th>TROPHIC LEVEL or SPECIES</th>
<th>Denitrification</th>
<th>Nitrogen-fixation</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SPECIES</strong></td>
<td>δ¹⁵N (‰)</td>
<td>δ¹⁵N (‰)</td>
<td>δ¹⁵N (‰)</td>
</tr>
<tr>
<td>Sperm whale (Physeter macrocephalus)</td>
<td>19.6 ± 0.6 (1)</td>
<td>11.1 (1)</td>
<td><strong>8.5</strong></td>
</tr>
<tr>
<td>TL &gt; 4</td>
<td>17.0 ± 0.7 (9)</td>
<td>13.3 ± 1.9 (14)</td>
<td><strong>3.7</strong></td>
</tr>
<tr>
<td>TL 3-4</td>
<td>14.5 ± 1.9 (7)</td>
<td>10.4 ± 2.1 (6)</td>
<td><strong>4.1</strong></td>
</tr>
<tr>
<td>Squid spp.</td>
<td>14.0 ± 3.1 (4)</td>
<td>12.1 ± 1.3 (11)</td>
<td><strong>1.9</strong></td>
</tr>
<tr>
<td>Leatherback turtle (Dermochelys coriacea)</td>
<td>15.4 ± 1.8 (1)</td>
<td>9.8 ± 1.5 (1)</td>
<td><strong>5.6</strong></td>
</tr>
<tr>
<td>Jellyfish spp.</td>
<td>14.0 ± 0.9 (2)</td>
<td>10.4 ± 0.4 (2)</td>
<td><strong>3.6</strong></td>
</tr>
<tr>
<td><strong>Mean Difference</strong></td>
<td></td>
<td></td>
<td><strong>4.6‰</strong></td>
</tr>
<tr>
<td>(with leatherbacks and sperm whales)</td>
<td></td>
<td></td>
<td><strong>3.4‰</strong></td>
</tr>
<tr>
<td><strong>Mean Difference</strong></td>
<td></td>
<td></td>
<td><strong>3.4‰</strong></td>
</tr>
<tr>
<td>(without leatherbacks and sperm whales)</td>
<td></td>
<td></td>
<td><strong>3.4‰</strong></td>
</tr>
</tbody>
</table>
Figure 12. Stable nitrogen ($\delta^{15}$N) and carbon ($\delta^{13}$C) isotope ratios for red blood cells (RBC, indicated by circles) and yolk (triangles) and for both red blood cells and yolk combined (squares) from adult female leatherbacks nesting at Playa Grande (PG, filled symbols) and St. Croix (STX, open symbols). Means shown ± 1 S.D.
Figure 13. Global comparison of $\delta^{15}N$ signatures (in ppt) of consumers across trophic levels from foraging regions of high denitrification or high nitrogen fixation rates. Grey bars represent mean values for marine consumers from oceanic areas of high denitrification, and white bars are mean values for consumers from areas of high nitrogen fixation (see Appendix A for raw values and species assigned to each category). Across trophic levels, consumer $\delta^{15}N$ values from denitrified areas are higher than those from regions of high N$_2$-fixation by an average of 3.4‰ $\delta^{15}N$. Leatherback data are from this study. Error bars are ± 1 S.D.
LIST OF REFERENCES


APPENDIX A: Raw data across trophic levels and species groups of global consumer δ15N signatures from areas of high denitrification and high nitrogen-fixation that I used in calculations in Table 10 and Figure 14.

<table>
<thead>
<tr>
<th>Species</th>
<th>Trophic Level</th>
<th>Site 1</th>
<th>δ¹⁵N</th>
<th>Site 2</th>
<th>δ¹⁵N</th>
<th>Site 3</th>
<th>δ¹⁵N</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Globicephala mela* beluga.</td>
<td>&gt;4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Species</td>
<td>Population Center</td>
<td>Age 1</td>
<td>Age 2</td>
<td>Age 3</td>
<td></td>
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<tr>
<td>Northern fur seal, <em>Callorhinus ursinus</em></td>
<td>NE Pacific (Pribilof Islands, Alaska)</td>
<td>17.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harbor seals, <em>Phoca vitulina</em></td>
<td>NE Pacific (Alaska)</td>
<td>17.2</td>
<td>NW Atlantic</td>
<td>16.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steller sea lions, <em>Eumetopias jubatus</em></td>
<td>NE Pacific (Alaska)</td>
<td>18.5</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Sooty shearwaters, <em>Puffinus griseus</em></td>
<td>off Japan, NW Pacific</td>
<td>8.8</td>
<td>NE Pacific</td>
<td>15.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flesh-footed shearwaters, <em>Puffinus carneipes</em></td>
<td>off Japan, NW Pacific</td>
<td>12.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelagic cormorant, <em>Phalacrocorax pelagicus</em></td>
<td>NE Pacific (Central CA)</td>
<td>16.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinoceros auklet, <em>Cerorhinca monocerata</em></td>
<td>NE Pacific (Central CA)</td>
<td>16.9</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon guillemot, <em>Cepphus columba</em></td>
<td>NE Pacific (Central CA)</td>
<td>16.9</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Brandt's cormorant, <em>Phalacrocorax penicillatus</em></td>
<td>NE Pacific (Central CA)</td>
<td>17.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Humpback whale, <em>Megaptera novaeangliae</em></td>
<td>Newfoundland, NW Atlantic</td>
<td>13.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Minke whale, <em>Balaenoptera acutorostrata</em></td>
<td>Newfoundland, NW Atlantic</td>
<td>12.3</td>
<td></td>
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<td></td>
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<tr>
<td>Blue whale, <em>Balaenoptera musculus</em></td>
<td>Newfoundland, NW Atlantic</td>
<td>9.6</td>
<td></td>
<td></td>
<td></td>
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</table>

Reference:
<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>Abundance</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Category</td>
<td>Location</td>
<td>Depth Range</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Illex illecebrosus</td>
<td>Squid spp.</td>
<td>Newfoundland, NW Atlantic</td>
<td>15.1</td>
</tr>
<tr>
<td>Loligo spp.</td>
<td>Squid spp.</td>
<td>NW Atlantic</td>
<td>12.2</td>
</tr>
<tr>
<td>Todarodes pacificus</td>
<td>Squid spp.</td>
<td>off Japan, NW Pacific</td>
<td>10.5</td>
</tr>
<tr>
<td>Gonatus borealis</td>
<td>Squid spp.</td>
<td>(Pribilof Islands, Alaska)</td>
<td>11.1</td>
</tr>
<tr>
<td>Berryteuthis magister</td>
<td>Squid spp.</td>
<td>(Pribilof Islands, Alaska)</td>
<td>11.4</td>
</tr>
<tr>
<td>Squid (large, 40-82cm), Dosidicus gigas</td>
<td>Squid spp.</td>
<td>(Gulf of Calif.)</td>
<td>17.0</td>
</tr>
<tr>
<td>Dermochelys coriacea</td>
<td>Leatherback turtle</td>
<td>E Tropical Pacific (Playa Grande, Costa Rica)</td>
<td>15.4</td>
</tr>
<tr>
<td>Beroe cucumis</td>
<td>Jellyfish spp.</td>
<td>off Japan, NW Pacific</td>
<td>10.1</td>
</tr>
<tr>
<td>Chrysaora melanaster</td>
<td>Jellyfish spp.</td>
<td>off Japan, NW Pacific</td>
<td>10.6</td>
</tr>
<tr>
<td>Unidentified jellyfish, Sea nettle</td>
<td>Jellyfish spp.</td>
<td>NE Pacific (Bering Sea, Alaska)</td>
<td>13.3</td>
</tr>
</tbody>
</table>


Ruiz-Cooley, et al. 2004

**THIS STUDY**
Vita

BRYAN P. WALLACE
National Science Foundation Pre-Doctoral Fellow

Specialization: Ecology, Conservation Biology, Comparative Physiology

Major Advisor: James R. Spotila, Ph.D.

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EDUCATION

B.S., Biology, 2000: University of Dayton, Dayton, Ohio, Summa cum laude
Ph.D., Environmental Science/Ecology, 2001-present: Drexel University

Dissertation research: The Bioenergetics and Trophic Ecology of Leatherback Turtles (Dermochelys coriacea). First field metabolic rates for marine turtles using doubly labeled water, diving physiology using archival electronic tagging, trophic ecology using stable carbon and nitrogen isotopes, and calculated energy budget of leatherbacks. DEFENSE DATE: 3 JUNE 2005

EXPERIENCE

October 2003-February 2004 Co-Principal Investigator, Project Manager and Science Coordinator on Costa Rican Sea Turtles, long-term EARTHWATCH project in Parque Nacional Marino Las Baulas on population monitoring, conservation behavioral and physiological ecology of critically endangered leatherback turtles

October 2001-Present Team Leader/Research Assistant/Co-Principal Investigator on field project with leatherback turtles in Parque Nacional Marino Las Baulas. Conducted research on satellite telemetry of internesting and post-nesting movements of leatherbacks, nest physiology and nesting ecology, egg metabolism, population ecology, beach and sand characteristics

2002-present Executive Assistant, The Leatherback Trust, non-profit organization for the conservation of the endangered leatherback sea turtle through consolidation of Las Baulas National Park, Costa Rica.

May 2004-present Research Assistant, population demography study of state endangered red-bellied turtles at Philadelphia International Airport

June 2002-present Research Assistant, comprehensive floral and herpetological survey and endangered snake radio telemetry project at Air Force National Guard Warren Grove Range, NJ
**July 2002; April-July 2003**  
Research Assistant, population/reproductive biology/behavior study of threatened desert tortoises (*Gopherus agassizii*) in the Mojave Desert in CA and NV

**June 2002-July 2003**  
Research Assistant, population/reproductive biology study of freshwater turtles at John Heinz Wildlife Refuge, Philadelphia

**October 2000- April 2001**  
Research Assistant, conservation project with Mexican non-profit ASUPMATOMA on leatherback turtles in Baja California Sur, México and Research Assistant, captive breeding/reintroduction project with Peruvian non-profit Asociación Cracidae with critically endangered white-winged guans in Lambayeque, Peru

**December 1998- August 2000**  
Research Assistant, behavioral ecology project with northern cardinals in Dayton, OH

**PUBLICATIONS: In Peer-Reviewed Literature**


**PUBLICATIONS: In Preparation (Target Publication)**


ORAL PRESENTATIONS AT INTERNATIONAL SYMPOSIA

- **Climate and fisheries a one-two punch for Pacific leatherbacks.** 25th Annual Symposium on Sea Turtle Conservation and Biology. Savannah, GA, January 2005.
- **First field metabolic rates for marine turtles: Climate and fisheries deliver a one-two punch to Pacific leatherback turtles.** Annual Meeting of the Society of Integrative and Comparative Biology, San Diego, CA, January 2005.

TEACHING EXPERIENCE

- **Evolution,** Dept. of Bioscience, Summer 2004, taught 30% of course
- **Biophysical Ecology,** Dept. of Bioscience, Spring 2003, taught 50% of course
- **Ecology,** Dept. of Bioscience, Fall 2003, taught 30% of course
- **Ecology,** School of Env. Sci., Engineering, and Policy, Spring 2002, taught 50% of course
- **Evolution,** Dept. of Bioscience, Spring 2002, taught 50% of course
- **Boonshoft Museum of Discovery, Dayton Society of Natural History,** 1999-2001, program instructor and curriculum coordinator for classes in life and physical science classes for students K-8.

GRANTS AND FELLOWSHIPS

- National Science Foundation Graduate Research Fellowship Award, 2002-2005: $40,500 annually for stipend and cost-of-education
- Society of Integrative and Comparative Biology Meeting Student Support: $500
- International Sea Turtle Symposium Student Travel Grant, 2005: $250
- Columbus (OH) Zoo and Aquarium Conservation Fund, 2004: $5,000
- International Sea Turtle Symposium Student Travel Grant, 2003: $600
- Chelonian Research Foundation Linnaeus Fund Award, 2003: $1,000
- International Sea Turtle Symposium Student Travel Grant, 2002: $500
- Dayton (OH) Society of Natural History Blincoe Grant, 2001: $2,000
- Dayton (OH) Audubon Society Research Scholarship, 2001: $1,000
AWARDS & SKILLS
• Special Recognition for Conservation Merit from the Costa Rican Ministry of the Environment and Energy, 2004
• Fluency in Spanish (speaking and writing skills)
• School of Environmental Science, Engineering, and Policy 2002 Research Day Poster Award: $500
• Foerste Award for Excellence, Boonshoft Museum of Discovery, 2000

SERVICE
• Co-organizer, Earth Day Celebration, Crofton, MD, April 22, 2005
• 2003 Executive Board, Graduate Student Association, Drexel University
• 2003 Master of Ceremonies for Earth Day, Green’s Idea (student environmental organization)

PROFESSIONAL MEMBERSHIPS
• Society of Conservation Biology (SCB)
• Society for Integrative and Comparative Biology (SICB)
• International Sea Turtle Society (ISTS)
• Society for the Study of Reptiles and Amphibians (SSAR)