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Investigations of $\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$ in vertebrae of white shark (*Carcharodon carcharias*) from the eastern North Pacific Ocean .

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Synopsis

The white shark (*Carcharodon carcharias*) has a complex life history that is characterized by large scale movements and a highly variable diet. Estimates of age and growth for the white shark from the eastern North Pacific Ocean indicate they have a slow growth rate and a relatively high longevity. Age, growth, and longevity estimates useful for stock assessment and fishery models, however, require some form of validation. By counting vertebral growth band pairs, ages can be estimated, but because not all sharks deposit annual growth bands and many are not easily discernable, it is necessary to validate growth band periodicity with an independent method. Radiocarbon (^{14}C) age validation uses the discrete ^{14}C signal produced from thermonuclear testing in the 1950s and 1960s that is retained in skeletal structures as a time-specific marker. Growth band pairs in vertebrae, estimated as annual and spanning the 1930s to 1990s, were analyzed for Δ^{14}C and stable carbon and nitrogen isotopes (δ^{13}C and δ^{15}N). The aim of this study was to evaluate the utility of ^{14}C age validation for a wide-ranging species with a complex life history and to use stable isotope measurements in vertebrae as a means of resolving complexity introduced into the ^{14}C chronology by ontogenetic shifts in diet and habitat. Stable isotopes provided useful trophic position information; however, validation of age estimates was confounded by what may have been some combination of the dietary source of carbon to the vertebrae, large-scale movement patterns, and steep ^{14}C gradients with depth in the eastern North Pacific Ocean.

Introduction

The life history of the white shark (*Carcharodon carcharias*) varies seasonally, geographically, and ontogenetically, making it difficult to fully characterize the lifestyle of this animal. The white shark is globally distributed, ranging in habitat from temperate coastal and shelf to pelagic waters (Compagno 2001, Boustany et al. 2002). Recent satellite tagging data revealed that juvenile white sharks occur in nearshore waters, whereas adults are wide-ranging, with extensive periods of oceanic travel and what appears to be distinct oceanic and coastal phases (Boustany et al. 2002, Dewar et al. 2004, Bonfil et al. 2005). Both juvenile and adult white sharks exhibit deep-diving behavior below the ocean's mixed layer, with juveniles documented to dive to depths of 100 m (Dewar et al. 2004) and adults to 980 m (Bonfil et al. 2005). The diet of the white shark is variable and it is often described as a scavenger, feeding upon a wide range of prey taxa that includes marine mammals, teleost fishes, and invertebrates (Compagno 2001). In addition, an ontogenetic shift in diet has been documented for the white shark, with diet mainly composed of fishes (for sharks less than 2 m) to a diet of marine mammals (for sharks > 3 m; Tricas and McCosker 1984, Klimley 1985, Compagno 2001). Although our understanding of the life history of the white shark is advancing, there still exists relatively limited knowledge of the habitat and diet of this animal over its lifetime. In addition, basic demographic information, including reliable age, growth, and longevity estimates, useful for stock assessment and fishery models, are not well defined.

Age determination of sharks is most commonly performed by counting growth band pairs in the vertebral centra that comprise the vertebral column (Ridewood 1921, Cailliet 1990). Vertebral centra are calcified cartilage, composed primarily of the mineral hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ deposited within an organic matrix, of which collagen is the primary component (Urist 1961). In most elasmobranchs, this mineralization process occurs

incrementally, resulting in vertebral growth bands (Cailliet 1990). One translucent and one

opaque band comprise a band pair that is often assumed to represent one year of growth (Cailliet

et al. 1983, Cailliet and Goldman 2004). The only age and growth study for the Pacific coast

white shark population revealed they may have a slow growth rate and be long-lived based on

growth band counts (Cailliet et al. 1985). However, no age validation has been successfully

undertaken to date. Results from the Cailliet et al. (1985) study indicated that white shark may

mature at an age of 9 to 10 years, live up to 27 years (assuming a maximum size of 7.6 m).

Sharks were estimated to grow at a rate of 25-30 cm/year for young animals and 22 cm/year for

older animals. In addition to a relatively late age at maturity and a slow growth rate, life history

characteristics such as low fecundity and hypothesized infrequent reproduction frequency

(Mollet et al. 2000), may make the white shark particularly vulnerable to exploitation (Cailliet et

al. 1985). Validation of age and age estimation procedures is essential because proper

management strategies rely heavily on accurate growth rates, age, and longevity.

Age can be estimated by counting vertebral growth band pairs; however, not all sharks

deposit annual growth bands and many are not easily discernable. Thus, it is necessary to

validate growth band periodicity with an independent method. Traditional age validation

techniques, such as captive rearing, mark-recapture, and tag-recapture, can be difficult or

impractical for these long-lived, pelagic fishes (Cailliet 1990). Marginal increment analysis and

oxytetracycline injection of white sharks was attempted off the coast of South Africa, but results

were inconclusive with regard to determining periodicity of growth band formation (Wintner and

Cliff 1999). Radiometric age validation using lead-210 dating was also explored for this species,

but results were inconclusive (Welden et al. 1987), due to failed assumptions. The irregular

radiometric results were attributed to possible metabolic reworking of the vertebrae (likely the

inorganic component, hydroxyapatite), or the result of an ontogenetic shift in habitat and diet of

individual animals. A recent captive juvenile white shark held at the Monterey Bay Aquarium established the longest time span and record of growth in captivity. Prior to its release, the white shark was shown to have increased from 5 feet (1.52 m) TL and 62 lbs (28.1 kg) to 6 feet 4.5 inches (1.94 m) TL and 162 lbs (73.5 kg) during the 198 days of captivity [1]. This shark had a growth rate more than double that estimated by Cailliet et al. (1985); however, this high growth rate is not reflective of growth rates in the wild due to the sizeable feeding regime of this animal in captivity.

Measurement of the change in radiocarbon levels (Δ^{14}C) produced by atmospheric testing of thermonuclear devices in the 1950s and 1960s has been established as an effective method for validating age estimates in calcified skeletal structures (Campana 2001). The discrete Δ^{14}C signal created by this testing was incorporated into the oceans of the world and has been used as a time-specific marker. Recent studies have correlated the changes in marine ^{14}C over time and used this temporal information to either: 1) make estimates of age and growth where no reliable age estimations were possible (e.g. calcareous algae, invertebrates, and some fishes; Frantz et al. 2000, Ebert and Southon 2003, Andrews et al. 2005, Frantz et al. 2005); or 2) validate estimates of age and growth (e.g. fishes; Kalish 1995, Kerr et al. 2005). For some Δ^{14}C records, age and growth of an organism was validated with another method and used to establish a Δ^{14}C reference time-series (e.g. hermatypic corals and fishes; Guilderson et al. 1998, Kerr et al. 2004, Piner and Wischniowski 2004). The utility of the method is dependent upon the ^{14}C signal retained in the skeletal structure of marine organisms as a permanent record of the ^{14}C present in ambient seawater at the time of formation. In fishes, this application is most commonly applied to the calcified ear bones, or otoliths. Recently, the use of this technique was expanded to shark vertebrae, including the school shark (*Galeorhinus galeus*; Kalish and Johnston 2000) and two

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[1] Monterey Bay Aquarium News Release, 31 March 2005

lamnoid sharks (Campana et al. 2002). Results of Δ^{14}C analyses for porbeagle (*Lamna nasus*) vertebrae indicated the Δ^{14}C signal was conserved across growth bands through time. Therefore, it was concluded that metabolic reworking of the organic or cartilaginous component of the vertebrae was minimal (Campana et al. 2002). This notion was further supported by a preliminary determination of the Δ^{14}C values for four growth bands from a single shortfin mako (*Isurus oxyrinchus*) vertebrae (Campana et al. 2002).

Radiocarbon age validation has been successfully applied to marine teleosts and sharks inhabiting surface waters during the period of life sampled from the growth structure for Δ^{14}C analysis. It has been noted, however, that the application of this technique can be problematic for species inhabiting waters below the mixed layer; due to the dependence of the ^{14}C signal in deeper waters on oceanic circulation and mixing rates (Kalish 1995). Therefore, knowledge of both seasonal and ontogenetic movements of a study species is important for interpreting Δ^{14}C values, especially in the case of elasmobranchs for which we are able to serially sample growth bands from vertebrae over the lifetime of the individual.

Interpretation of vertebral Δ^{14}C values is further complicated by the source of ^{14}C to the shark vertebrae. Unlike fish otoliths, which primarily obtain ^{14}C from ambient seawater (70 – 90 % derived from dissolved inorganic carbon (DIC) in seawater and 10 – 30% is dietary; Kalish 1991, Farrell and Campana 1996), shark vertebrae reflect the ^{14}C composition of their diet (Kalish and Johnston 2000, Campana et al. 2002). Thus, an understanding of a species' diet is also necessary for interpreting shark vertebral Δ^{14}C values. Documented large-scale movements (Boustany et al. 2002, Bonfil et al. 2005) and a variable diet (Tricas and McCosker 1984, Klimley 1985) present complications in the application of ^{14}C age validation to the white shark. We proposed to approach these complications using stable isotopes in concert with ^{14}C

measurements in vertebrae to discern possible changes in life history and diet over the lifetime of animals.

The objectives of this study were to: 1) measure $\Delta^{14}C$ in aged vertebrae as a means of determining the validity of white shark age estimates and the periodicity of growth band formation, and 2) use $\delta^{13}C$ and $\delta^{15}N$ measurements in white shark vertebrae to better understand the trophic position and carbon source to the vertebrae and to aid in the interpretation of $\Delta^{14}C$ values.

Materials and methods

Archived white shark vertebrae collected off the coast of central and southern California (capture years ranging from 1936 to 1994) were obtained from various collections for this study (sources included vertebrae from collections at Moss Landing Marine Laboratories, Los Angeles County Museum, California Academy of Sciences, Sea World San Diego, and from Leonard J.V. Compagno at the Shark Research Center, Iziko-Museums of Cape Town South African Museum, Cape Town, South Africa (Table 1).

Age Estimation

One whole vertebra from each specimen was transversely sectioned, using the thin-section technique, for age estimation purposes. Sectioning was performed on a low-speed saw with two diamond blades separated by a spacer (2-3 mm) and polished with a Buehler® Ecomet III lapping wheel using 600 and 800 grit silicon-carbide wet/dry sandpaper for optimal viewing thickness. Sections were examined and images captured using a Leica dissecting microscope with an attached Spot RT® video camera. Transmitted light was used to make growth bands visible for counting. A growth band pair was defined as one translucent and one opaque growth band. Thin section age estimates were estimated by one reader (3 independent reads) and

compared to whole vertebra age estimates and/or calculated age (for those vertebrae that were not used in the original age and growth study by Cailliet et al. 1985). Calculated age was estimated with the von Bertalanffy growth function (VBGF) determined by Cailliet et al. (1985). Coefficient of Variation (CV) was calculated for thin section age estimates as a measure of ageing precision (Chang 1982).

Radiocarbon analysis

Vertebrae from nine individual white sharks were sectioned (described previously) for $\Delta^{14}C$ analysis. In total, twenty-two white shark vertebra growth band pairs (one opaque and one translucent band), with estimated growth years ranging from the pre-bomb 1930s to the post-bomb mid-1980s, were extracted for $\Delta^{14}C$ analyses. The limited availability of archived vertebrae with known capture years restricted the selection of vertebra and growth band years to be analyzed for $\Delta^{14}C$. Individual growth band pairs were sampled from thin-sections of the corpus calcareum using a New Wave ® micro-milling machine with a small-scale end mill. The width of growth band pairs was used to guide extraction and minimize the amount of older or younger material incorporated in the sample. Hence the amount of material extracted decreased as growth slowed and band width decreased. The first growth band pair past the birth band (estimated as the first year of growth after birth) and one to three subsequent growth band pairs further up the corpus calcareum were extracted for analysis from each vertebrae. The last band pair, corresponding to the last year of growth, was targeted to provide a sample where time of formation was constrained by the collection date. In some cases, the penultimate growth band pair was targeted because the width of the last growth band pair provided insufficient sample size. Extraction of the sample with the micro-mill resulted in a solid sample of material that was weighed to the nearest 0.1 mg.

Pre-treatment of vertebral samples was performed to isolate the organic portion (collagen), by removing the inorganic carbon and maximizing the carbon yield from vertebrae (Brown et al. 1988). A process called demineralization was applied to the vertebral samples by soaking them in 0.25 N HCl at refrigerator temperatures (slows reaction). Treated samples were dried in an oven and placed in clean quartz tubes. CuO (copper oxide, oxidizing agent) and Ag (silver, used to remove impurities: SOx and NOx) were added to the treated organic sample. Three samples from individual vertebrae were replicated and analyzed for $\Delta^{14}C$ without demineralization to evaluate the effect of demineralization on $\Delta^{14}C$ measurements. Quartz tubes were evacuated, sealed, and heated for two hours at 900 °C to convert the organic carbon to CO_2. Sample CO_2 was converted to graphite (Vogel et al., 1984; Vogel et al., 1987) and measured for ^{14}C content using an Accelerator Mass Spectrometer (AMS) at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory. The ^{14}C values were reported as $\Delta^{14}C$ (Stuivier and Polach, 1977).

A qualitative comparison of the white shark $\Delta^{14}C$ record was made with existing marine records including two otolith-based records, the yelloweye rockfish (*Sebastes ruberrimus*; Kerr et al. 2004) and Pacific halibut (*Hypoglossus stenolepis*, Piner and Wischniowski 2004), and three vertebra-based shark records, the western North Atlantic porbeagle (Campana et al. 2002), western North Atlantic shortfin mako (Campana et al. 2002, Ardizzone et al. 2006.), and the western South Pacific school shark (Kalish and Johnstone 2000).

Stable Isotope Analysis

Stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) were measured to provide insight into trophic level and possible ontogenetic diet shift (Michener and Schell 1994, Burton and Koch 1999). Vertebrae from six individual white sharks were sectioned (described previously) for isotopic

analysis (δ^{15}N and δ^{13}C). Four of the six vertebra samples were also analyzed for Δ^{14}C and

many of the growth band pairs analyzed for stable isotopes were targeted to coincide with those

analyzed for Δ^{14}C. In total, twenty-three white shark vertebra growth band pairs (one opaque and

one translucent band) were extracted from the corpus calcareum using a New Wave micro-

milling machine and analyzed for δ^{13}C and δ^{15}N. Vertebral samples were demineralized to

isolate collagen from the vertebrae (Brown et al. 1988). In addition, lipids were extracted from

samples analyzed for δ^{13}C and δ^{15}N using a 2:1:0.8 methanol/chloroform/water mixture. Samples

were analyzed by continuous flow Isotope Ratio Mass Spectrometer (IRMS) at University of

California, Davis. Values are reported as δ^{13}C and δ^{15}N relative to standards of Pee Dee

Belemnite limestone (^{13}C) and atmospheric N_2 (^{15}N).

Trophic position (TP) of the white shark was calculated using the equation:

$$TP = \lambda + \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{base})}{\Delta n}$$

Where λ is the trophic position of the organism employed to estimate the baseline δ^{15}N for the

region, $\delta^{15}N_{consumer}$ is the average value of the consumer, $\delta^{15}N_{base}$ is the average value of the base

organism, and Δn is the average enrichment per trophic level (Post 2002). The northern anchovy

(*Engraulis mordax*) was chosen as a representative secondary consumer (assigned a trophic

position (λ) of 3.0) for estimating $\delta^{15}N_{base}$ of the region and an average trophic enrichment

(Δ^{15}N) of 3.4 was assumed following Estrada et al. (2003).

Results

Age Estimation

The vertebrae from white sharks were relatively difficult to age. The comparison of thin-

section age estimates with the corresponding whole vertebra estimates (Cailliet et al 1985) and

the calculated age estimates based on the VBGF indicated there was good agreement between

thin-section and whole vertebrae age estimates (H_0: no significant difference between techniques; paired t-test: $P = 0.65$, $\alpha = 0.05$; Table 2). Thin section age estimates were, on average, higher than whole vertebrae age estimates. The mean CV for thin section age estimates was 16.0% of the individual age. Age estimates from thin sections deviated from those of whole vertebrae by as much as 2 years.

Radiocarbon Analysis

Radiocarbon values measured in growth band pairs from white sharks had considerable variation over time (Table 3). When $\Delta^{14}C$ values were plotted against estimated growth year, the values produced a $\Delta^{14}C$ time series from 1936 to 1984 (Figure 1). Overall, $\Delta^{14}C$ values increased as growth year progresses into the post-bomb era. However, this increase was not synchronous with the characteristic bomb ^{14}C rise and had considerably more scatter in the post-bomb period than could be explained by $\Delta^{14}C$ measurement uncertainty. Replicate samples from 1956 had very depleted $\Delta^{14}C$ values that were atypical of $\Delta^{14}C$ measured in surface waters of the Pacific Ocean. These replicate samples differed from each other by 87.8‰. Pre-bomb $\Delta^{14}C$ values (1936 to approximately 1959 and excluding the 1956 outliers) had a trend similar to the pre-bomb record from the reference time-series, averaging -90.5 ± 5.1‰ (mean ± SD). The first evidence of an increase due to atmospheric testing of thermonuclear devices was the elevated $\Delta^{14}C$ value measured for the 1966 growth band pair (-72.2 ± 4.0‰). This value was the first to have a $\Delta^{14}C$ value that was significantly above pre- bomb $\Delta^{14}C$ levels using a $+ 2$ SD criteria ($+ 10.2$‰). The rise in $\Delta^{14}C$ continued until 1984 (the last growth year sampled) with a maximum observed $\Delta^{14}C$ value of 79.8‰ with no indication of a post-bomb decline.

Radiocarbon values for the first year of growth from five age-1 (± 1 year) white sharks created a $\Delta^{14}C$ reference time-series for this species composed of essentially known-age

specimens (Figure 1). The $\Delta^{14}C$ values in these vertebrae showed a response to the bomb pulse that is similar in magnitude to the two otolith reference time-series from the Pacific Ocean, but differed by what appears to be a significant phase lag.

Radiocarbon values from vertebral material near the known year of capture (up to minus 2 years from date of collection) revealed a very different pattern relative to the known-age juvenile series (Figure 1). Compared to the juvenile white shark reference time-series $\Delta^{14}C$ values in these vertebrae showed a depleted and/or delayed bomb ^{14}C signal based on knowing the collection year. All other samples, for which age was estimated from growth band pair counts, had $\Delta^{14}C$ values distributed between these two sets of time-constrained samples.

Radiocarbon values for demineralized and untreated vertebral samples (these samples were as close to replicates as we could get based on the position in the corpus calcareum) indicated a difference in the $\Delta^{14}C$ between treated and untreated samples; hence, the inorganic portion of the vertebrae had different $\Delta^{14}C$ levels (Table 3). All untreated samples had elevated $\Delta^{14}C$ values compared to their demineralized counterparts.

Stable Isotope Analysis

Twenty-three growth band pairs, extracted from the vertebra of six individual white sharks, were analyzed for $\delta^{13}C$ and $\delta^{15}N$. A decreasing trend with increased age was exhibited for $\delta^{13}C$ values within individual vertebrae (Figure 3). Growth band pairs $\geq$ age 6 in the vertebrae were used to discriminate between what we have termed juvenile and adult growth, a criterion based on a reported size at transition in diet of 3 meters documented by Compagno (2001) and estimated age at transition (6 years) based on the VBGF estimated from Cailliet et al. (1985). Mean stable isotope values associated with juvenile growth (1–5 years: $\delta^{13}C$: -11.81 ± 0.60‰, $\delta^{15}N$: 19.16 ± 1.01‰) and adult growth (6–18 years: $\delta^{13}C$: -12.73 ± 0.62‰, $\delta^{15}N$: 19.34 ±

0.93‰) showed no significant difference in δ^{15}N (paired t-test: $P = 0.70$) or calculated trophic position (paired t-test:, $P = 0.70$), but did exhibit a significant difference in δ^{13}C values between juvenile and adult growth (paired t-test $P = 0.01$).

Overall, δ^{15}N values ranged from 17.68 to 20.84‰ (Mean: 19.24‰ ± 0.95‰) and δ^{13}C values ranged from -13.31 to -11.10‰ (Mean: -12.24 ± 0.76; Table 4). Across longitudinal sections of individual vertebra, nitrogen values differed by a maximum of 0.51 to 2.85‰ and carbon values differed by a maximum 0.39 to 2.05‰ (Table 4). An approximate increase of 1‰ δ^{13}C and 3–4‰ δ^{15}N is associated with an increase in one trophic level (Michener and Schell 1994). Four individual vertebrae exhibited differences in δ^{13}C across longitudinal sections of vertebra >1‰, however no vertebrae exhibited differences in δ^{15}N values >3‰ across longitudinal sections. White shark Δ^{14}C values indicated feeding at an upper trophic level relative to fish and marine mammal stable isotope values from the eastern North Pacific Ocean (Figure 2). Trophic position calculation of the white shark based on mean δ^{15}N value resulted in an estimated tropic position of 4.57 (range 4.11 to 5.04).

Discussion

Age Estimation

The observation that thin section age estimates were, on average, higher than those from whole vertebrae was expected based on what we know about these two techniques. Currently, thin sectioning is accepted as the more accurate technique (Cailliet and Goldman 2004); and because growth band pairs were isolated from thin sections for Δ^{14}C analyses, we relied on these age estimates in this study.

Radiocarbon Analysis

It is well established that the initial rise in $\Delta^{14}C$ is nearly synchronous in all marine carbonate records, but the white shark record exhibited an asynchronous $\Delta^{14}C$ time series. The uncharacteristic timing of $\Delta^{14}C$ values differed from the nearest chronologies in the region (Kerr et al. 2004, Piner and Wischchniowski 2004). There are three plausible mechanisms for explaining this uncharacteristic trend: 1) an apparent delay of the $\Delta^{14}C$ signal due to age underestimation; 2) metabolic reworking of the vertebral collagen; and 3) a depletion of the $\Delta^{14}C$ levels due to depleted dietary sources of carbon. Each of these factors could explain the observed trend individually, but a combination of some or all of them cannot be ruled out.

Age underestimation of white shark vertebrae would be manifested as a delayed $\Delta^{14}C$ time series. The degree of age underestimation was estimated by tracing estimated growth years back to their appropriate place on the white shark reference curve (known-age juvenile series). Potential age underestimation ranged from 6–11 years (mean 9 ± 3 years). Examination of the residuals (estimated age vs. expected age based on the reference time series) for potential age underestimation indicated there was no systematic ageing bias (no significant trend observed, r^2=0.02). If age underestimation were the sole source of the apparent delay, it would not explain the delay in the time constrained samples taken near the collection year. These samples alone strongly suggest there are other factors responsible for the observed trend.

A more plausible explanation is that there is a metabolic reworking of the vertebrae through the life of the fish. The question of metabolic reworking of vertebrae has been hypothesized for the white shark in relation to radiometric age determination. Analysis of vertebrae for lead-210 indicated this may not be a static or conserved structure with respect to the inorganic portion of the vertebrae (Welden et al. 1987). Unlike cancellous or "true" bone which undergoes remodeling over the lifetime of the animal, hydroxyapatite, the primary component of

shark cartilage, is thought to grow by accretion with little remodeling; therefore, recording conserved information for different periods of the animal's life (Koch et al. 1994).

Because there is no empirical evidence to support or refute the temporal stability of the organic portion of vertebral cartilage, we looked to isotopes for indirect evidence. Differences in $\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$ values preserved across longitudinal sections of vertebrae, with differences in $\Delta^{14}C$ as great as 235‰ (sample #WH 6), support the conclusion that vertebrae are not completely reworked. This is also supported by $\Delta^{14}C$ results for the porbeagle that indicated metabolic reworking was minimal and the ^{14}C signal was not transported across growth bands through time (Campana et al. 2002). These findings suggest that carbon in the vertebral material is metabolically and temporally stable (Campana et al. 2002). However, further research is needed to provide direct evidence to validate the assumption of shark vertebrae functioning as a closed-system with respect to carbon and nitrogen.

A third possibility for the uncharacteristic trend in the white shark $\Delta^{14}C$ signal is that values were depleted due to the dietary source of carbon to the vertebrae. Because the composition of vertebral collagen reflects dietary sources, low $\Delta^{14}C$ values of vertebrae could be the result of prey consumption that has integrated a ^{14}C depleted signal, possibly from a deep sea source (Campana 1999). Incorporation of ^{14}C in the ocean is related to ocean circulation and mixing, with different water masses reflecting different ^{14}C values and values becoming attenuated with depth (Broeker and Peng 1982). Radiocarbon enters the food web through photosynthetic organisms and is transferred to higher trophic levels (Pearcy and Stuvier 1983). Lower mesopelagic, bathypelagic and abyssopelagic animals in the northeastern Pacific Ocean, however, were found to have depleted $\Delta^{14}C$ values relative to surface dwellers (1973-1976), indicating that surface-derived POC was not the major source of organic carbon for deep-sea fish (Pearcy and Stuvier 1983).

A steep gradient in bomb $\Delta^{14}C$ values with depth was documented in the North Pacific Ocean, with $\Delta^{14}C$ values of DIC in the North Central Pacific reaching approximately -100‰ at 500 m (1986), and values of -150‰ in the Santa Monica Basin at about 500 m (1986-1987; Druffel and Williams 1990). Similarly, low DOC $\Delta^{14}C$ values have been documented in the North Pacific Ocean (<-300‰ at 500 m in the North Central Pacific and -300‰ at 100 m in Santa Monica Basin; Druffel and Williams 1990). Druffel and Williams (1990) proposed three possible mechanisms for the incorporation of ^{14}C depleted carbon into the oceanic food chain: 1) uptake of DOC by bacteria in the water column, or adsorption of depleted DOC through webs and mucous of filter feeders (e.g. larvaceans and salps), 2) chemosynthetic production of organic matter from DIC, and 3) resuspension of depleted sedimentary organic carbon. Druffel and Williams (1990) identified this depleted deep sea source of carbon as responsible for depletion of surface water POC $\Delta^{14}C$ values by an average of 93‰ in these regions. This magnitude of depletion was strikingly similar to the average depletion of white shark vertebral values (93‰ ±53‰).

Until recently, white sharks in the Pacific Ocean were thought to reside in waters of the upper continental shelf and were not known to dive below 100 m. Satellite tagging results revealed the white shark are deep diving, with a bimodal depth of occurrence at 0–5 and 300–500 m (Boustany et al. 2002). This tagging study, along with Bonfil et al. (2005), revealed that white sharks are wider ranging than previously thought, with extensive periods of oceanic travel and what appears to be distinctive oceanic and coastal phases. Based on what is now known about its depth of occurrence and extensive movements, consumption of deep-dwelling prey or prey that feed in deep waters is a strong possibility. This depleted source offers a reasonable explanation for the unusually low $\Delta^{14}C$ values for replicate measures of the 1956 white shark samples and attenuated values measured for known collection years during the post-bomb era. In

addition, the degree of variation in depletion could be related to the variable diet of this pelagic

species that inhabits both inshore and offshore habitats and consumes a wide spectrum of prey

over the course of their lifetime.

The reference chronology comprised of known-age juveniles reflected a more rapid

response to the bomb pulse, most likely due to the habitat utilized during the juvenile stage.

Satellite tagging results of a juvenile (young of the year) white shark off the coast of California

supported by evidence of capture locations indicate that juvenile white shark remain in coastal

waters (Klimley, 1985. Dewar et al. 2004). Juveniles inhabiting and feeding nearshore on coastal

fish would reflect a $\Delta^{14}C$ signal typical of coastal, surface waters and would be more

synchronous with the reference time series.

Employing a simple mass balance equation for $\Delta^{14}C$ allowed us to examine the likelihood

the observed values in white shark vertebrae were attributable to the influence of a depleted

deepwater carbon source. The equation describing this deep-water influence is:

$$\Delta^{14}C_{observed} = \Delta^{14}C_{expected} (x) - \Delta^{14}C_{deepwater} (1-x)$$

where $\Delta^{14}C_{observed}$ is the value measured in the vertebrae, $\Delta^{14}C_{expected}$ is the value expected based

on the white shark reference curve, x is the fraction contributed from the "expected" surface

water source, $\Delta^{14}C_{deepwater}$ is a value of a possible deepwater source based on ^{14}C gradients off

the coast of California (Druffel and Williams 1990), and 1-x is the fraction contributed from the

"deepwater" source. Modeling the sensitivity of $\Delta^{14}C$ values in shark vertebrae helped with the

interpretation of the magnitude of depletion possible due to the incorporation of a depleted ^{14}C

source. As a result of this modeling, most observed $\Delta^{14}C$ values for white shark vertebrae were

found to be similar in value to contributions ranging from a small percent of the diet composed

of a highly depleted ^{14}C source (25% contribution of a -300‰ source) to a large percentage of

the diet composed of a slightly depleted ^{14}C source (75% contribution of a -100‰ source). Based

on the highly variable diet of white sharks and this modeling, it is likely that depleted values are attributable to a small contribution of highly depleted ^{14}C sources in the diet.

Factors associated with the sampling method should also be considered. There are two factors that may have influenced the outcome of Δ^{14}C levels measured in white shark vertebrae: 1) the location of sampling within the vertebrae and 2) the preparation of vertebrae for Δ^{14}C analysis (demineralized vs. untreated vertebrae). The variation in replicated demineralized samples (WH 6: 1956 samples) from the same location within the vertebra indicated that small deviations in coring location can result in large deviations in Δ^{14}C (an observed difference of 87.8‰). This can be explained by large variations of ^{14}C in food sources that were variably accreted during the period of formation. In addition, relatively little is known about the growth and accretion of white shark vertebrae. If growth was episodic and accretion was not uniform, but rather focused where material is needed from a structural perspective, this could explain regional variations of the Δ^{14}C signal of the vertebrae.

Results from untreated samples (the inorganic and organic portions) indicated the potential presence of carbonates in the inorganic portion of the vertebrae in two ways. First, we observed bubbling during the acid dissolution of the vertebrae indicating CO_2 was liberated from the inorganic portion of the vertebral matrix. Second, the elevated levels of Δ^{14}C in replicate untreated samples compared to demineralized (the organic portion) samples indicated a ^{14}C source in the inorganic portion of the vertebrae. The depletion of Δ^{14}C values in the organic portion of the vertebrae likely reflects the different sources of ^{14}C to the organic and inorganic portions of the vertebrae. The dominant source of carbon to the organic portion of the vertebrae is known to be metabolic (Kalish and Johnston 2001, Campana et al. 2001). The difference between the demineralized and untreated vertebrae represents the addition of elevated ^{14}C values, most likely from a DIC source. Evidence of a similar trend was identified in the treatment of

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organic (collagen) and inorganic (carbonate) portions of swordfish (*Xiphias gladius*) vertebrae (Kalish 2002). Results from the study revealed the organic component of the swordfish vertebrae was more ^{14}C depleted than the inorganic component (Kalish 2002). This difference was attributed to the dominant source of carbon to the organic portion of the vertebrae being metabolically derived and the source to inorganic portion derived from DIC. Our results indicate this same trend is present in the white shark vertebrae.

A comparison of the white shark Δ^{14}C record with three other shark records indicated the white shark record was most temporally similar to the school shark record from the South Pacific Ocean (Figure 4; Kalish and Johnston 2000). The school shark Δ^{14}C time series was delayed in relation to the nearest marine carbonate chronology and in this case the delay was attributed to age underestimation. A 3-year lag observed in the porbeagle Δ^{14}C time series, based on comparison to a juvenile porbeagle reference time series, was attributed to both the mean age and depth of occupied by prey in the diet of porbeagle (Campana et al. 2002). All of these shark records show, to differing degrees, a nonconforming Δ^{14}C signal compared to their nearest Δ^{14}C chronology. In the case of the white shark, we hypothesize that there is depletion of the Δ^{14}C time series due to the influence of depleted ^{14}C sources to the vertebrae. This nonconformity indicates the increased complexity of interpreting Δ^{14}C values in shark vertebrae for the purpose of validating age and the necessity to interpret values in the context of diet, movement, and regional ^{14}C gradient information.

Stable Isotope Analysis

Stable isotope ratios can provide valuable information relative to trophic level, carbon flow to a consumer, and location of feeding (Vander Zanden and Rasmussen 2001). While gut content analysis provides a snapshot of what was most recently consumed by an animal, stable

isotopes (δ^{13}C and δ^{15}N) can provide time-integrated diet information (Vander Zanden and Rasmussen 2001). This application is based on predictable enrichment factors of both ^{13}C (approximately 1‰ enrichment) and ^{15}N (approximately 3-4‰ enrichment) in consumer bone collagen relative to prey with increasing trophic level, termed trophic fractionation (Deniro and Epstein 1981, Schoeniger and Deniro 1984, Vander Zanden and Rasmussen 1999). Additionally, δ^{13}C can provide information relative to feeding habitat (inshore vs. offshore; France 1995). A caveat to this approach is that spatial and temporal variability in stable isotope ratios must be considered when interpreting stable isotope values.

In general, adults are reported to feed primarily on marine mammals and juveniles on fishes (Le Boeuf et al. 1982, Tricas and McCosker 1984, Klimley 1985, Compagno 2001). Current knowledge of the white shark diet is based on stomach contents from opportunistic nearshore landings and observed feeding events. The main prey items of the white shark include marine cephalopods, crustaceans, ray-finned bony fishes, cartilaginous fishes, mammals, and birds (Klimley 1985, Compagno 2001). In an examination of white sharks captured off the coast of northern and central California, Tricas and McCosker (1984) found stomach contents to be comprised solely of elasmobranchs and teleosts fish that inhabit both pelagic and inshore habitats.

Stable isotope values obtained in this study for white shark vertebrae, when compared with representative prey item values for bony fishes and mammals off the coast of California, indicate that the white shark is an upper trophic level consumer. δ^{13}C values indicate enrichment relative to fish and most marine mammals and δ^{15}N values indicate isotopic enrichment relative to fishes and similar values to mammals in the region. Differences in δ^{13}C and δ^{15}N values across longitudinal sections of individual vertebra were variable over the lifetime of individuals (Table 4).

Calculated trophic position (TP) for the white shark was greater in value than trophic position calculated for the basking shark *Cetorhinus maximus* = 3.1 (primary prey: zooplankton), blue shark *Prionace glauca* = 3.8 (primary prey: fish), and shortfin mako *Isurus oxyrinchus* = 4.0 (primary prey: fish) and similar to the TP calculated for the common thresher shark *Alopias vulpinus* = 4.5 (primary prey: fish and squid). The white shark, however, had similar variability in TP (TP ranged from 4.11 to 5.04) as calculated for the shortfin mako (TP ranged from 3.6 to 4.5), this was attributed to migration and feeding in both inshore and offshore regions (Estrada et al. 2003). Calculated white shark TP for this study agrees with the determination of trophic level for the family Lamnidae by Cortes (1999) based on diet composition (mean = 4.3, range= 4.22 to 4.5) and specifically the trophic level determined for the white shark (4.5) based on diet composition. In this analysis the mean TP of marine mammals was estimated at 4.0, placing the trophic level of white sharks somewhat higher than marine mammals (Cortes 1999).

Estrada et al. (2003) contend that TP values obtained for sharks in their study do not support the idea put forth by Fisk et al. (2002), that retention of urea in elasmobranchs may lower δ^{15}N values and therefore lead to underestimation of trophic position. Based on our limited data, we cannot determine whether urea retention may have affected white shark δ^{15}N values. However, the similarity of calculated TP in this study to the diet analysis TP supports the idea that urea retention was not a problem.

Values of δ^{13}C measured in juvenile growth band pairs were significantly enriched compared to adult growth bands; which may reflect variation of ^{13}C due to feeding location rather than trophic level. Isotopic signatures in mobile animals may reflect feeding in different environments or an actual change in diet (Michener and Schell 1994). Gradients of ^{13}C in the northeast Pacific Ocean demonstrate an enriched nearshore signal (benthic-based food web) compared to the offshore, oceanic signal (phytoplankton-based food web) and a ~2‰ enrichment

in $\delta^{13}C$ has been associated with marine mammals foraging in nearshore versus offshore waters of California (Burton and Koch 1999). In addition, gradients of ^{13}C with depth have been observed in the northeast Pacific Ocean (off the coast of British Columbia, Canada) where the slope/deep ocean food web was determined to be depleted relative to the pelagic food web (4.3‰ depletion in $\delta^{13}C$ based on fish larvae; Perry et al. 1999). Therefore, feeding on prey from offshore and deeper waters will reflect a depleted $\delta^{13}C$ signal relative to nearshore $\delta^{13}C$ values.

The trend in $\delta^{13}C$ across individual longitudinal sections of vertebra tended to decrease with increased age of white shark, thus perhaps reflecting an ontogenetic shift in habitat and foraging location. Evidence suggests that juveniles are residing and feeding in shallow inshore waters and adults spending increased time offshore feeding in deep oceanic waters, incorporating prey reflecting a depleted $\delta^{13}C$ signal relative to nearshore surface waters. However, the decrease observed in $\delta^{13}C$ is a small one (~1.5‰ difference) with respect to potential spatial and temporal variability of $\delta^{13}C$ and cannot be considered as evidence alone for this behavior, but is supported by the tagging literature.

Although an ontogenetic shift in trophic level has been documented in the diet literature and identified through stable isotope analysis of white shark vertebrae from the western North Atlantic (Estrada et al. 2006), we found no significant difference between mean $\delta^{15}N$ or calculated trophic position of juvenile growth and adult growth band pairs (juvenile vs. adult classification is based solely on an ontogenetic shift in diet documented at 3 m length). The lack of evidence for an ontogenetic trophic level shift based on $\delta^{15}N$ values may reflect environmental (spatial and temporal) variation in $\delta^{15}N$ (Vander Zanden & Rasmussen 1999) and/or a variable adult white shark diet with lower trophic items remaining an important component in the adult diet (Estrada et al. 2006). Because white shark migratory movements consist of coastal and pelagic periods (Boustany et al. 2002), lower trophic items, such as pelagic fish, could dominate

the diet during these pelagic phases. Although most diet studies stress the importance of pinnipeds in adult white shark diets, Tricas and McCosker (1984) documented the greater numerical importance of teleosts and elasmobranchs over marine mammals in white shark diet. If no change in $\delta^{15}N$ is attributable to diet, this finding would support an increased diversity and size spectrum of prey consumed as white shark increase in size, as opposed a strict shift from a fish to a marine mammal diet (Compagno 2001). Alternatively, the time period over which growth bands were sampled from white shark vertebrae coincided with the decline in abundance of pinnipeds in the North Pacific Ocean over the last several decades (Springer et al. 2003). Therefore, the relatively low trophic position observed in adult white sharks, relative to juveniles and marine mammals, during this period may reflect the low abundance of higher trophic level prey (Sora Kim, personal communication).

Conclusions

The uncharacteristic trend in $\Delta^{14}C$ in white shark vertebrae is most likely attributable to the influence of a deep-water depleted carbon source to the vertebrae. Conclusive age validation of vertebral age estimates of white shark was confounded by what may have been some combination of the dietary source of carbon to the vertebrae, large-scale movement patterns, and steep $\Delta^{14}C$ gradients with depth in the eastern North Pacific Ocean. Stable isotopes provided us with time-integrated trophic information, reflecting the upper trophic level status of the white shark. No evidence of an ontogenetic shift in diet was detected, although increased movement into offshore, deeper waters with age was supported by a decrease in $\delta^{13}C$ values.

Future application of this technique is best suited to sharks with well-characterized diet and movement patterns, inhabiting surface waters in an area for which there is knowledge of regional $\Delta^{14}C$ gradients. Sampling strategies should include both known age individuals to construct a reference chronology and older individuals with samples taken from growth bands

over the lifetime of the individual. This approach enables indirect confirmation of the stability of the ^{14}C signal within vertebrae. To gain a better understanding of the chemical composition of shark vertebrae, the pathway of elemental uptake, and the metabolic stability of tissue more studies need to be done. Captive rearing studies, feeding small shark species prey with a known ^{14}C content and measuring the Δ^{14}C values in blood, tissue, and vertebrae over time would improve our understanding of carbon pathways and turnover rates. This could be used to provide direct evidence for the assumption of metabolic stability of carbon in shark vertebrae, one of the main assumptions in the application of this technique in elasmobranchs.

Acknowledgments

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Table 1. Summary of data from white shark collected off the coast of California. Sample number identifies the individual white shark with corresponding sex, year of capture, capture location and total length (TL). Application indicates if vertebrae from the individual was used for ageing (Age), stable isotope analysis (SI), and radiocarbon analysis (^{14}C). The number of samples analyzed for stable isotopes or ^{14}C for each individual is noted in parentheses.

Sample #	Sex	Year of Capture	Capture location	TL (cm)	Application
WH 1	M	1978	Moss Landing, CA	393	Age, SI (5), ^{14}C (3)
WH 3	M	1968	Half Moon Bay, CA	234	Age, SI (2), ^{14}C (3)
WH 6	F	1959	Tomales Bay, CA	277.5	Age, SI (3), ^{14}C (4)
WH 7	F	1936	Malibu, CA	167.6	Age, ^{14}C (1)
WH 8	M	1981	southern CA	147.3	Age, ^{14}C (1)
WH 9	M	1981	southern CA	159	Age
WH 12	?	1977	Ventura, CA	210	Age, ^{14}C (1)
WH 17	M	1982	southern CA	460.9	Age, SI (4), ^{14}C (4)
WH 25	?	1984	Half Moon Bay, CA	168 *	Age, ^{14}C (1)
WH 90	M	Unknown	California	471	SI (4)
WH 128	F	1994	California	534.4	SI (5)
WH 26694	?	1959	NE Pacific, CA	225.4	Age, ^{14}C (1)

*TL not recorded and calculated from a total length to centrum diameter regression.

Table 2. Age estimate comparison between values obtained from thin sections in this study, and whole vertebra reads and calculated age based on von Bertalanffy growth function (VGBF) from Cailliet et al. 1985. Age estimation error was the uncertainty associated with the thin section age estimates (individual coefficient of variation (CV); rounded to the nearest whole number). Mean CV = 16 %.

| Sample # | Estimated age | | |
	Thin section (± CV)	Whole vertebrae (Cailliet et al 1985)	Calculated age (VBGF) (Cailliet et al 1985,)
WH 1	7 (± 1)	9	9
WH 3	3 (± 0)	2	3
WH 6	4 (± 1)	2	4
WH 7	1 (± 0)	1	1
WH 9	0 (± 0)	1	0
WH 12	1 (± 1)	2	2
WH 17	18 (± 1)	13	12

Table 3. Summary of vertebra and Δ^{14}C data from white shark collected off the coast of California. Resolved age is the final age estimate. Birth year is collection year minus the resolved age. Ageing error is the uncertainty associated with the age estimate (CV). Radiocarbon values for the extracted samples were expressed as Δ^{14}C with the AMS analytical uncertainty.

Sample #	Resolved age (years ± age error)	Capture Year	Birth Year	Year Sampled for ^{14}C analysis (years)	Δ^{14}C (‰)
WH 7	1 ± 0	1936	1935	1936	-94.2 ± 3.5
WH 26694	2 ± 1	1959	1957	1956 (pre-birth material)	-92.7 ± 3.5
WH6	4 ± 1	1959	1955	1956	-318.2 ± 2.9
WH 6	4 ± 1	1959	1955	1956	-230.4 ± 3.0
WH 6*	4 ± 1	1959	1955	1956	-63.1± 3.6
WH 6	4 ± 1	1959	1955	1957	-83.0 ± 3.5
WH 6	4 ± 1	1959	1955	1959	-92.1 ± 4.0
WH 3	3 ± 0	1968	1965	1966	-72.2 ± 4.0
WH 3	3 ± 0	1968	1965	1967	-21.8 ± 3.7
WH 3	3 ± 0	1968	1965	1968	-98.5 ± 3.7
WH 3*	3 ± 0	1968	1965	1968	-72.9 ± 3.9
WH 17	18 ± 1	1982	1964	1966	-74.1 ± 5.0
WH 17	18 ± 1	1982	1964	1971	-65.6 ± 4.9
WH 17	18 ± 1	1982	1964	1976	-29.2 ± 4.2
WH 17	18 ± 1	1982	1964	1981	34.7 ± 4.5
WH 1	7 ± 1	1978	1971	1972	-59.7 ± 4.2
WH 1	7 ± 0	1978	1971	1975	-58.3 ± 3.5
WH 1	7 ± 1	1978	1971	1978	-55.8 ± 3.6
WH 1*	7 ± 1	1978	1971	1978	1.8 ± 3.8
WH 12	1 ± 1	1977	1975	1975	58.2 ± 4.9
WH 8	1 ± 0	1981	1980	1981	75.0 ± 4.2
WH 25	1 ± 0	1984	1983	1984	79.8 ± 4.1

*: indicates samples that were not demineralized.

Table 4. Summary of vertebra and stable isotope data from white shark collected off the coast of California. Sample number identifies the individual white shark. Isotope values are reported as δ^{13}C and δ^{15}N relative to standards of Pee Dee Belemnite limestone (δ^{13}C) and atmospheric N_2 (δ^{15}N). Overall difference in stable isotope ratios is the maximum difference observed across longitudinal sections of an individual vertebra.

Sample #	δ^{13}C (‰)	δ^{15}N (‰)	Estimated Age (yr)	Overall difference in δ^{13}C (‰)	Overall difference in δ^{15}N (‰)
WH 17	-11.39	19.51	2	1.75	0.51
WH 17	-12.88	20.03	6		
WH 17	-13.14	20.00	11		
WH 17	-13.06	19.81	16		
WH 90	-11.28	17.87	2	1.71	2.16
WH 90	-11.74	19.51	5		
WH 90	-12.19	20.03	10		
WH 90	-12.99	18.67	18		
WH 128	-11.49	19.87	0	2.05	1.69
WH 128	-11.73	19.48	2		
WH 128	-11.26	19.13	6		
WH 128	-12.87	18.18	10		
WH 128	-13.31	19.70	15		
WH 6	-11.92	18.31	1	0.82	0.91
WH 6	-11.87	19.21	2		
WH 6	-11.10	18.86	4		
WH 3	-13.08	17.68	1	0.39	0.53
WH 3	-12.69	18.21	3		
WH 1	-12.20	20.61	1	2.00	2.85
WH 1	-11.20	20.78	4		
WH 1	-12.09	20.84	6		
WH 1	-13.01	18.41	7		
WH 1	-13.20	17.93	7		

Figure Captions

Figure 1. Radiocarbon (Δ^{14}C) values for white shark (*Carcharodon carcharias*, solid symbols) vertebral cores (*n* = 22) in relation to year. The solid line connects values for age estimates from known-age juvenile white sharks, the dashed line connects values for years of formation associated with the known collection years of the sharks, the unconnected black circles are Δ^{14}C values plotted in relation to years based on estimated age, and black triangles are replicate untreated samples that were analyzed for Δ^{14}C. Horizontal error bars represent the age estimate uncertainty from calculated as individual CV, year rounded to the nearest whole number, and vertical error bars represent the 1 σ AMS analytical uncertainty. The white shark ^{14}C time series is plotted with two regional Δ^{14}C chronologies for the yelloweye rockfish (open circles, Kerr et al. 2004) and Pacific halibut otoliths (open squares, Piner and Wischniowski 2004).

Figure 2. Stable carbon isotope ratio (δ^{13}C) for individual white shark (*Carcharodon carcharias*) vertebral cores (open circles; *n* = 23) in relation to stable nitrogen ratios (δ^{15}N) plotted with mean marine mammal (open triangles; harbor seal (*Phoca Vitulina*), elephant seal, California sea lion (*Zalophus californianus*), Stellar sea lion (*Eumetopias jubata*; Burton et al. 2001, Jamon et al. 1996) and fish stable isotope values (open squares; rockfishes (*Sebastes* spp.), white croaker (*Genyonemus lineatus*), shortraker rockfish (*Sebastes brevispinis*), northern anchovy (*Engraulis mordax*), and California market squid (*Loligo opalescens*; Toperoff 1997, Jarman et al. 1996)), and blue shark (crosses, *Prionace glauca*; R. Leaf personal communication). Mean (± SD) isotope ratio for juvenile growth band pairs (closed circle; *n* = 12, growth band pairs 1-5 years) and adult growth band pairs (closed square; *n*=11, growth band

pairs 6-18 years) in white shark vertebrae are shown. This distinction based on ontogenetic shift in diet at size 3m.

Figure 3. Stable carbon ratio (δ^{13}C) for samples (*n* = 23) taken across longitudinal sections of six individual white shark (*Carcharodon carcharias*) vertebrae. Unique symbols represent δ^{13}C results for samples from an individual white shark vertebra.

Figure 4. Radiocarbon (Δ^{14}C) values from vertebral cores of white shark (*Carcharodon carcharias*; closed circles) vertebrae and three vertebra-based shark records, the northwest Atlantic porbeagle (open diamonds, Campana, 2002), northwest Atlantic mako shark (solid triangles, Campana, 2002, Ardizzone et al. 2006), and the southern Pacific school shark (open squares, Kalish and Johnston 2000).
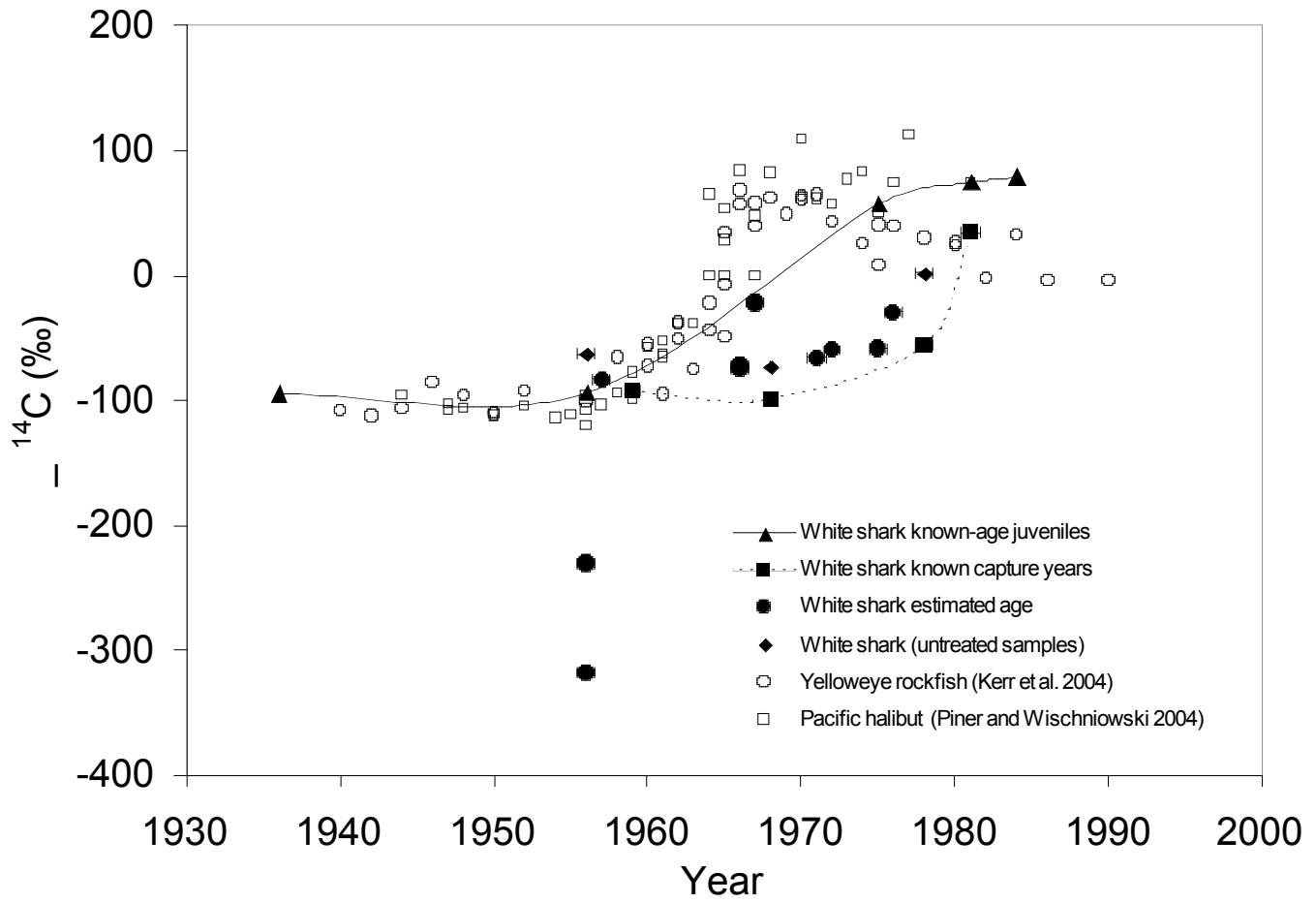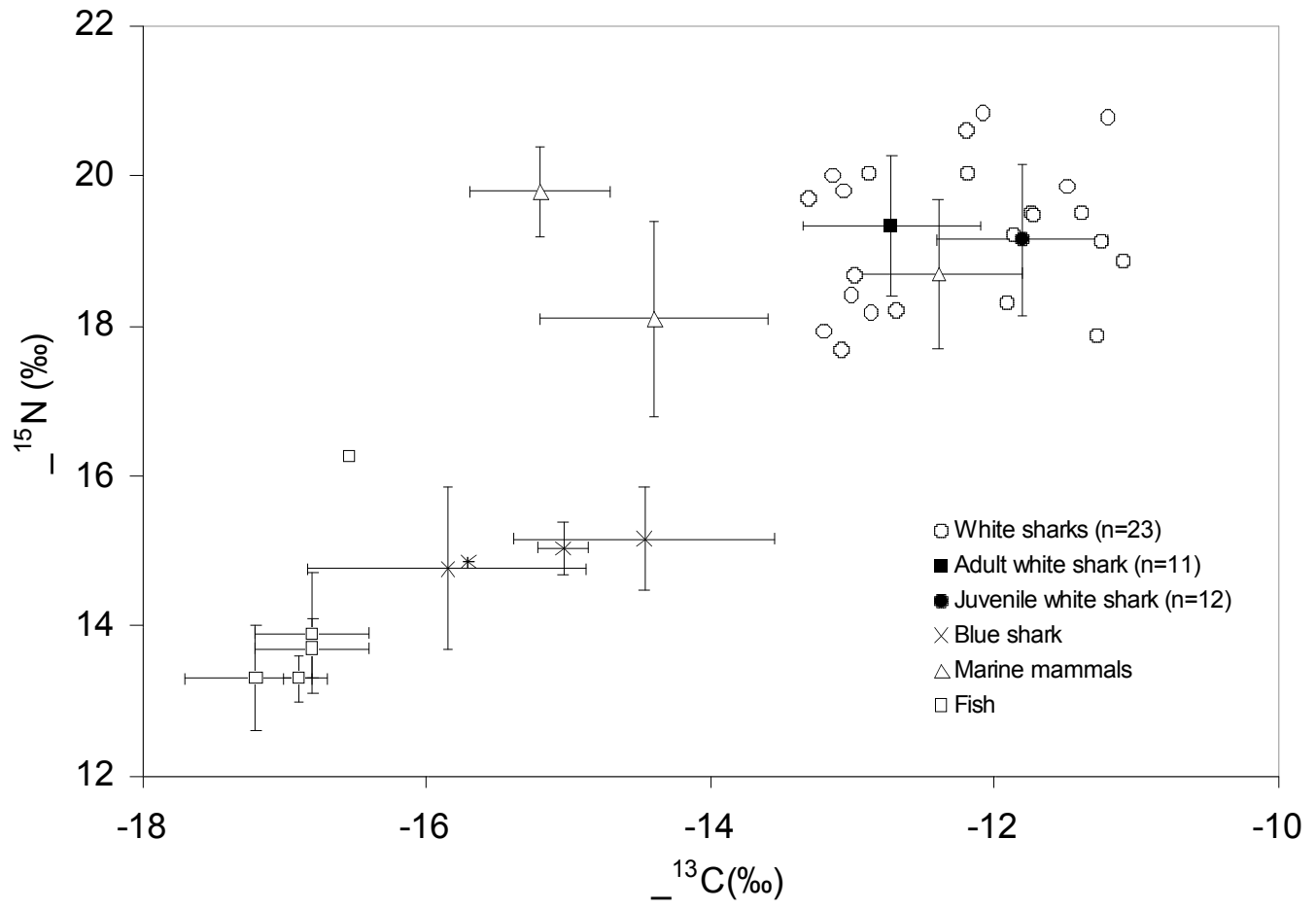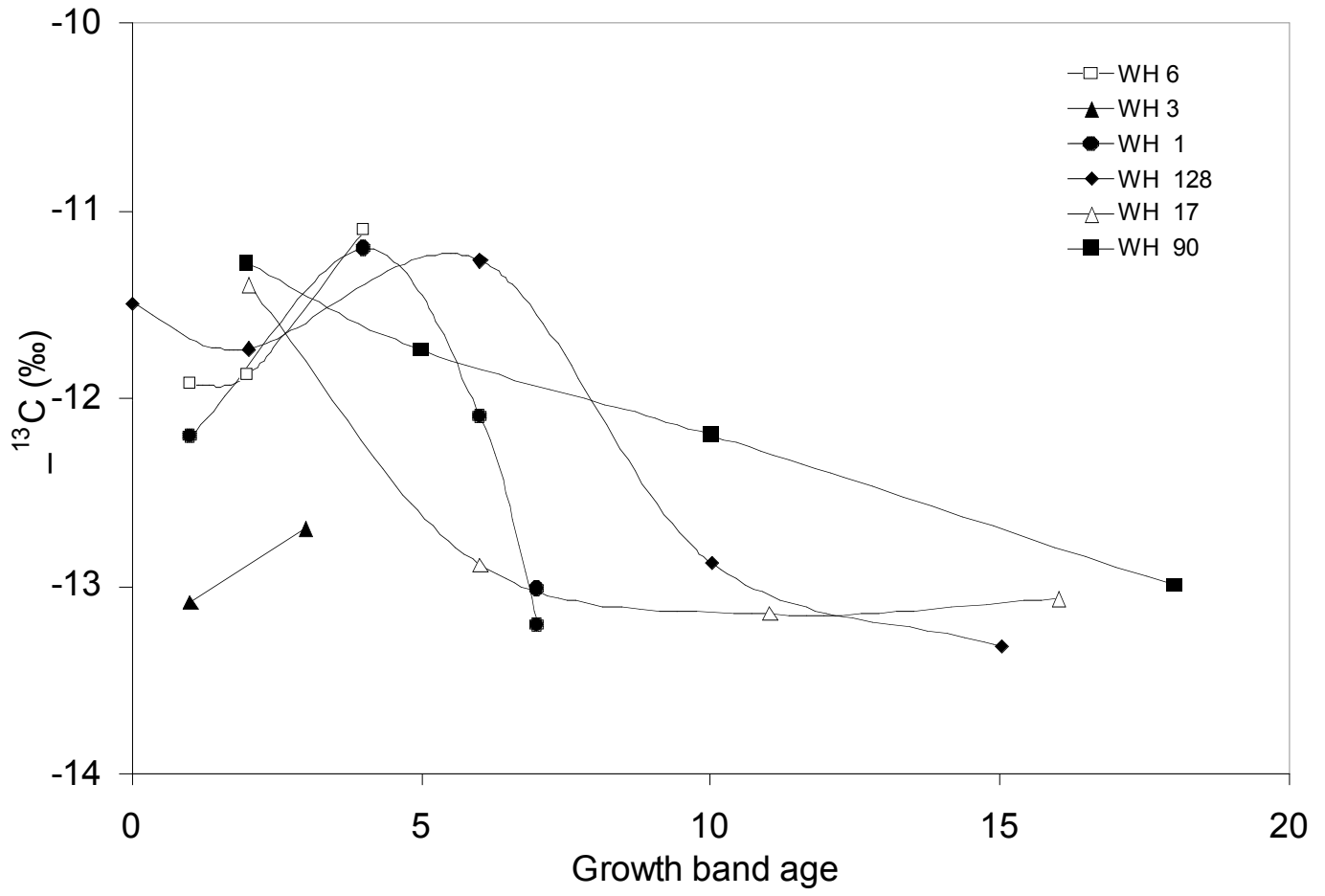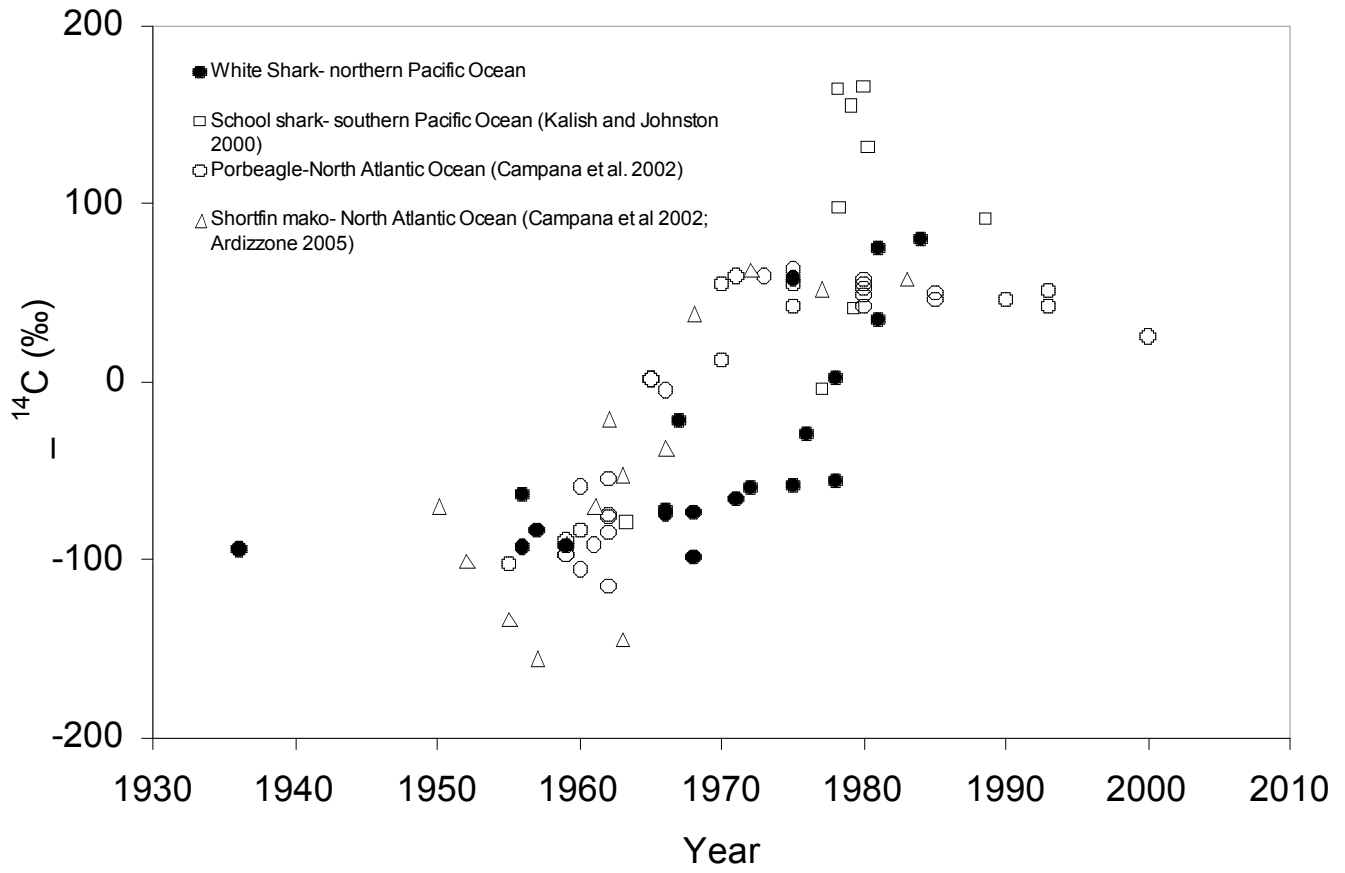
Figure 1

Figure 2

Figure 3

Figure 4