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3 de *diciembre* de 2009.

CENTRO DE INVESTIGACIÓN CIENTÍFICA Y DE EDUCACIÓN SUPERIOR DE ENSENADA

PROGRAMA DE POSGRADO EN CIENCIAS
EN ECOLOGÍA MARINA

Evaluation of Trophic Levels and Feeding Grounds of Northeastern Pacific Sharks as a function of Ontogeny Based on Stable Isotope Analysis

TESIS

que para cubrir parcialmente los requisitos necesarios para obtener el grado de
MAESTRO EN CIENCIAS

Presenta:

Luis Malpica Cruz

Ensenada, Baja California, México, Diciembre de 2009.

RESUMEN de la tesis de **Luis Malpica Cruz,** presentada como requisito parcial para la obtención del grado de MAESTRO EN CIENCIAS en Ecología Marina. Ensenada, Baja California. Diciembre 2009.

Análisis del nivel trófico y zonas de alimentación de tiburones del noreste del Pacífico en función de la ontogenia por medio del análisis de isótopos estables

Resumen aprobado por:

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Para entender el papel ecológico de los tiburones durante la ontogenia, es necesario caracterizar sus habitats alimentarios y estimar sus niveles tróficos en función de la talla. Los isótopos estables atraviesan procesos de fraccionamiento y mezcla que permiten estimar niveles tróficos (con base en $^{15}N/^{14}N$) e identificar áreas de alimentación sustentadas por diferentes fuentes de producción primaria (con base en $^{13}C/^{12}C$). El objetivo general de este estudio es evaluar la composición isotópica de carbono y nitrógeno ($\delta^{15}N$ y $\delta^{13}C$) en sangre, hígado y músculo en el tiburón blanco (*Carcharodon carcharias*), mako (*Isurus oxyrinchus*) y azul (*Prionace glauca*) en función de la talla para inferir variaciones en la alimentación en función de la talla. Recolecté muestras de juveniles, sub-adultos y adultos de tiburón azul (86 - 295.9 cm), blanco (149.5 - 550 cm) y mako (75 - 193 cm) entre junio y noviembre de 2008 en Bahía Vizcaíno e Isla Guadalupe, B.C., México, y en el Southern California Bight, EUA. Para estimar valores de enriquecimiento trófico y la contribución relativa del crecimiento y recambio metabólico al recambio isotópico, hice un experimento en laboratorio usando al tiburón leopardo como modelo. Para estimar el tiempo en que una nueva señal isotópica se reflejará en diversos tejidos después de un cambio en la fuente de alimento, modelé las tasas de recambio isotópico con base en tasas de crecimiento obtenidas de la literatura y los resultados del experimento en laboratorio. El recambio metabólico contribuyó substancialmente al recambio isotópico de los tejidos de *T. semifasciata*. Hígado y sangre tuvieron una tasa de recambio más rápida que el músculo, cartílago y aletas. Hubo diferencias significativas en los valores de fraccionamiento isotópico entre tejidos, tanto para $\delta^{13}C$ como $\delta^{15}N$. Las tasas de recambio isotópico estimadas indican que los tejidos de tiburones juveniles pueden integrar un periodo de alimentación de meses, mientras que los tejidos de tiburones subadultos y adultos pueden tardar años en alcanzar el equilibrio isotópico con una nueva dieta. Valores $\delta^{15}N$ enriquecidos en función de la talla en muestras de sangre y músculo de tiburones mako y blanco, son indicativos de un incremento en el nivel trófico durante la ontogenia. Valores $\delta^{13}C$ enriquecidos en tiburones blanco juveniles, pueden indicar un hábitat de alimentación bentónico durante esta etapa. Valores $\delta^{13}C$ más negativos en el plasma de tiburones blanco adultos pueden estar relacionados con migración reciente de zonas costeras a oceánicas.

Palabras clave: Ecología trófica, isótopos estables, ontogenia, tiburones, Pacífico mexicano

ABSTRACT of the thesis presented by **Luis Malpica Cruz** as a partial requirement to obtain the MASTERS OF SCIENCE degree in Marine Ecology. Ensenada, Baja California, México December 2009.

Evaluation of Trophic Levels and Feeding Grounds of Northeastern Pacific Sharks as a function of Ontogeny Based on Stable Isotope Analysis

In sharks, ontogenetic changes in feeding habits have been related to an increase in size and as well as the habitat occupied (inshore vs. offshore). Juvenile sharks have feeding habits and dietary preferences that differ from those of adult conspecifics. Characterizing foraging habitats and estimating trophic level during different ontogenetic stages are important steps toward understanding the ecological role of sharks throughout their life cycle. The analysis of the stable isotopes of light elements such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) has greatly improved the understanding of complex food webs in marine systems. Due to fractionation and mixing processes, nitrogen isotope ratios ($\delta^{15}N$) can be used to estimate trophic level, while carbon isotope ratios ($\delta^{13}C$) can be used to discriminate among sources of primary production. My objective was to estimate trophic level and infer feeding grounds of different size classes of white sharks (*Carcharodon carcharias*), mako sharks (*Isurus oxyrinchus*), and blue sharks (*Prionace glauca*) by evaluating $\delta^{15}N$ and $\delta^{13}C$ values of whole blood, liver and muscle. I sampled juvenile (including age 0), sub-adult and adults blue sharks (86-295.9 cm), juveniles (including age 0) and adults (149.5-550 cm) white sharks and juvenile (including age 0) and sub-adult (75-193 cm) mako sharks. Sampling occurred between June and November in 2008 in Vizcaino Bay and Guadalupe Island off Baja California, México, and in the Southern California Bight, USA. To aid in the interpretation of field data, a laboratory experiment was conducted to calculate trophic fractionation values for various tissues and estimate the relative contribution of growth and metabolic turnover to isotopic turnover using leopard sharks (*Triakis semifasciata*) as a model species. I developed tissue specific isotopic turnover rate models using species-specific growth rates from the literature and my laboratory results. Metabolic turnover contributed substantially to the isotopic turnover of leopard shark tissues. Liver and blood had a faster turnover rate than muscle, cartilage and fin tissue. There were significant differences in trophic fractionation values among tissues for both carbon and nitrogen isotope ratios (2.36 - 4.16 ‰ and 1.08 - 1.76 ‰, respectively). Based on the isotopic turnover rate model, I predict juvenile tissues should integrate the isotopic composition of prey consumed over a period of months, while subadult and adult tissues may take years to reach isotopic equilibrium to a new diet. Blood and muscle samples from mako and white sharks exhibited enrichment in ^{15}N as a function of size, while no difference was found among blue sharks of different size classes. Juvenile white sharks exhibited enriched $\delta^{13}C$ values in blood and muscle that is consistent with a benthic foraging preference. Light blood plasma $\delta^{13}C$ values compared to muscle $\delta^{13}C$ values found in adult white sharks could be related to a recent migration to an offshore pelagic feeding ground from coastal webs enriched in ^{13}C.

Keywords: Trophic ecology, stable isotopes, ontogeny, sharks, Mexican Pacific

A mi padre y madre, mis dos grandes ejemplos

A mi hermana, Andrew y a mi pequeño sobrino mis inpiraciones

A mi querida, amada y adorada Boo, el amor de mi vida

A los tiburones leopardo que se sacrificaron durante la realización de este proyecto

Aknowledgments

Resumen ejecutivo

I. Introducción

Los tiburones son considerados depredadores tope durante su etapa adulta. Pueden ejercer una importante influencia en la estructura y funcionamiento del ecosistema marino. Sin embargo, dentro de una misma especie, los tiburones jóvenes suelen tener hábitos y preferencias alimentarias diferentes a los adultos. Los cambios ontogénicos están asociados con aumentos en la talla de los tiburones y sus presas, asi como cambios en sus zonas de alimentación (costera *vs.* oceánica). Para entender la importancia ecológica de los tiburones dentro de las tramas tróficas, es necesario caracterizar sus zonas de alimentación y estimar el nivel trófico durante sus diferentes etapas ontogénicas. El análisis de las razones de los isótopos estables de elementos ligeros como el carbono ($^{13}C/^{12}C$) y el nitrógeno ($^{15}N/^{14}N$) ha contribuido sustancialmente al entendimiento de estructuras tróficas. Los isótopos estables atraviesan procesos de fraccionamiento y mezcla que permiten discriminar niveles tróficos ($^{15}N/^{14}N$) y áreas con diferentes fuentes de producción primaria ($^{13}C/^{12}C$). El objetivo de este trabajo es evaluar la composición isotópica de carbono y nitrógeno ($\delta^{15}N$ y $\delta^{13}C$) en sangre, hígado y músculo en función de la talla en el tiburón blanco (*Carcharodon carcharias*), mako (*Isurus oxyrinchus*) y azul (*Prionace glauca*).

II. Hipótesis

El análisis de isótopos estables ($\delta^{13}C$ y $\delta^{15}N$) de tejidos metabólicamente activos de tiburones puede usarse para evaluar cambios alimentarios a través de la ontogenia.

III. Métodos

Se realizó un experimento en laboratorio usando a *Triakis semifasciata* como especie modelo para conocer los valores de enriquecimiento trófico y tasas de recambio isotópico en tiburones. Durante 196 días, se alimentaron a 15 crías de tiburón leopardo con una dieta comercial, la cual presentó una composición isotópica ($\delta^{13}C$ y $\delta^{15}N$) diferente a la de la sangre y el músculo de una de las crías. Antes del inicio del experimento, y antes de cada muestreo, las crías se identificaron individualmente, se pesaron y midieron. Se muestreó sangre, músculo, hígado, cartílago y aleta de las crías con base en su incremento relativo en peso a lo largo del experimento.

Recolecté muestras de juveniles (incluyendo edad 0), sub-adultos y adultos de *C. carcharias* (149.5 - 550 cm) y *P. glauca* (86 - 295.9cm). Para *I. oxyrinchus* muestreamos juveniles (incluyendo edad 0) y subadultos (75 - 193 cm). En adultos y subadultos de *C. carcharias* únicamente obtuve muestras de plasma y músculo. El muestreo se realizó entre junio y noviembre de 2008 en campos pesqueros de Bahía Vizcaíno e Isla Guadalupe en Baja California y dentro del Southern California Bight en EUA. Los niveles tróficos se discriminaron usando valores de fraccionamiento trófico obtenidos del experimento en laboratorio y de la literatura. Las señales isotópicas costera *vs.* oceánica se discriminaron

tomando como señal isotópica de la fuente de producción primaria datos de literatura. Para estimar en cuanto tiempo una nueva señal isotópica puede reflejarse en los tejidos después de un cambio de fuente de alimento (nivel trófico y/o zona de alimentación), modelé las tasas de recambio isotópico con base en tasas de crecimiento de la literatura, y la contribución relativa del metabolismo estimada a partir de tasas de recambio obtenidas en el experimento en laboratorio con tiburón leopardo.

IV. Resultados y Discusión

Durante el experimento en laboratorio, el recambio metabólico contribuyó substancialmente al recambio isotópico de los tejidos de tiburón leopardo. Tejidos con una alta tasa metabólica, como el hígado y la sangre, tuvieron una tasa de recambio más rápida que el músculo, el cartílago y las aletas. Diferencias significativas existieron en los valores de fraccionamiento isotópico entre tejidos, para valores $\delta^{13}C$ (hígado 2.36 ‰; sangre y músculo 3.27 ‰; aletas y cartílago 4.16 ‰), y para valores $\delta^{15}N$ (hígado, sangre, músculo y aletas 1.76 ‰; cartílago 1.08 ‰). La estimación de las tasas de recambio indicaron que los tejidos de tiburones juveniles pueden integrar un periodo de alimentación de meses, mientras que los tejidos de tiburones subadultos y adultos pueden tardar años en alcanzar equilibrio isotópico a una nueva dieta. Debido a su lento incremento de biomasa, el modelo indicó que los tiburones azules adultos nunca alcanzarían el equilibrio isotópico a una nueva dieta. Encontré un enriquecimiento en ^{15}N en función de la talla en sangre y músculo de tiburones mako y blanco, no se encontraron diferencias entre clases de talla de tiburón azul. La consistente diferencia en valores $\delta^{15}N$ entre tejidos con diferentes tasas de recambio es evidencia de un incremento en el nivel trófico de los tiburones mako y blanco relacionado con la ontogenia. Sangre y músculo con valores $\delta^{13}C$ enriquecidos en tiburones blanco juveniles indican una posible preferencia de los juveniles de tiburón blanco por hábitats bentónicos. Valores $\delta^{13}C$ más negativos en plasma que en músculo en tiburones blanco adultos pueden estar relacionados con una reciente migración de zonas costeras con cadenas alimentarias enriquecidas en ^{13}C a hábitats de alimentación oceánicos.

V. Conclusiones

El análisis de isótopos estables puede ser usado para estudiar las preferencias alimentarias y de hábitats de diferentes clases de talla de tiburones. Sin embargo, se debe de tener precaución al seleccionar el tejido a analizar (tasas de recambio rápidas o lentas). También es importante considerar la especie, fisiología, estadio de desarrollo y tasa de crecimiento. Para interpretar apropiadamente la información isotópica obtenida de tiburones pelágicos, es necesario compararla con estudios en que se hayan usado otras metodologías, tales como análisis de contenido estomacal y marcaje con telemetría satelital. A pesar de esto, el análisis de las razones isotópicas puede ayudar para ampliar el entendimiento de las preferencias alimentarias y de hábitats de las diferentes clases de talla de los tiburones pelágicos

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I. Introduction

Sharks are typically considered top predators in marine systems. Cortés (1999) estimated trophic levels for 8 orders and 23 shark families based mainly on published data on stomach content analyzes, and found that on average sharks exhibit trophic levels higher than those of sea birds and equal to those of marine mammals. He also concluded that food webs in which sharks are found are considerably long, with at least four trophic levels, and that sharks occupy the highest trophic positions. This analysis, however, did not consider the trophic role of sharks across different ontogenetic stages.

Throughout ontogeny, sharks have been shown to migrate to different feeding grounds (*e.g.* inshore *vs.* offshore) depending on predation risk and resource availability (*i.e.* prey abundance) (Bouskila *et al.* 1998). The size changes that occur throughout ontogeny can influence prey capture strategies. For example, juvenile white sharks prey mainly on teleosts using a fast swimming tactic, while adults that feed on marine mammals use a stealth tactic (Tricas & McCosker, 1984). Shifts in prey preference as a function of ontogeny presumably produce changes in the trophic position of these organisms as they grow.

Sharks generally exhibit k-selected life history strategies (late sexual maturity, low reproductive rates, low growth rates and longevity), which make them vulnerable to fishing pressure (Cortés, 2004). Based in the analysis of a database on fishing captures in the Northwest Atlantic, Baum *et al.* (2003) estimated a 75% decline in scalloped hammerhead (*Sphyrna lewini*), white (*Carcharodon carcharias*) and thresher (*Alopias vulpinus*) shark populations between 1986 and 2000. The loss of top predators (*e.g.* marine mammals, sharks and piscivorous fishes) has direct effects on ecosystem structure, including an exponential increase in prey numbers and mesopredator abundance, as well as changes in the population size of organisms at low trophic levels (Baum & Worm, 2009). Heithaus *et al.* (2008) also found that top predators have indirect or no consumptive effects on the

function of marine ecosystems through mechanisms such as predator avoidance and changes in prey behavior.

Baum & Worm (2009) state that obtaining ecological data on marine trophic webs and understanding the relative importance of top-down and bottom-up controls on food web structure are crucial to appropriately manage and conserve marine ecosystems. In order to fully understand the ecological role of specific shark species in food webs and assess their role in mediating top-down effects in marine communities, it is necessary to characterize their feeding grounds and estimate their trophic levels as a function of ontogenetic stage.

Trophic studies on sharks are based mainly on stomach content analysis, which provides an instantaneous assessment of a predator's last feeding event (Pinnegar & Polunin, 1999). However, stomach contents may not be representative of the diet and trophic position of a predator over time. To increase the temporal resolution yielded by analysis of stomach contents, intensive temporal sampling is required (*e.g.* year round sampling). This approach has logistical limitations, particularly for sharks (Cortés, 1999). Cortés (1999) suggested that the stable isotope analysis of metabolically active tissues may be useful in the study of shark feeding ecology and may aid in determining their trophic position (TP) in food webs, at least as a means to validate the results obtained with conventional stomach content studies. The use of stable isotope analysis, mainly carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), has substantially broadened the understanding of food web form and function and energy fluxes in marine and aquatic ecosystems (Vander Zanden & Rasmussen, 2001). In this work, I apply the analysis of stable isotope ratios (SIR) ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) to shark tissues to evaluate whether changes in trophic level and/or feeding grounds throughout ontogeny can be assessed with this approach. I used mako (*Isurus oxyrinchus*), blue (*Prionace glauca*) and white sharks as model species.

I.1 Target Species

Mako sharks belong to the Order Lamniformes and Family Lamnidae. This species has a cosmopolitan distribution in tropical and temperate oceans. Mako sharks inhabit coastal and oceanic regions (Compagno, 2001), and can generally be found in temperatures

of 17-22°C. They are extremely active and fast and can dive as deep as 152 m. It feeds mainly on teleost fishes, although mako sharks can capture and feed on a wide spectrum of prey (Compagno, 2001). Off the California coast, mako sharks feed on some species of teleost fishes (*e.g.* mackerel, bonito and tuna), as well as sharks and squid (Hanan *et al.* 1993). Stomach content analysis of mako sharks captured off southern Baja California coast indicated that 72 % of their preys were teleost fishes, and the remaining 27 % were cephalopods (Velasco-Tarelo, 2005).

Tagging studies in the Southern California Bight (SCB), indicate that mako sharks can move as far north as Point Arena in northern California, as far south as Acapulco, Mexico and to the west all the way to Hawaii. However, additional data and analyses are still needed to assess migratory patterns (Hanan *et al.* 1993; California Department of Fish and Game 1999b, 2000; HMS FMP - Appendix F).

Due to its high market value, mako sharks are an important targeted species of the commercial fisheries in Mexico and other countries (Compagno 2001; Holts *et al.* 1994; Sosa-Nishizaki *et al.* 2007). The International Union for Conservation of Nature (IUCN) classifies this species as "lower risk/near threatened" due to its wide distribution and relatively fast growth rate (Stevens, 2000).

The blue shark belongs to the Family Carcharhinidae. This species has a cosmopolitan distribution and can be found in tropical and temperate oceans. Compared to other shark species the blue shark has high growth rates and high fecundity, and is considered one of the most abundant sharks (Compagno, 2001). Despite its life history traits, the IUCN classifies this species as "lower risk/threatened" because it is heavily fished throughout the world's oceans either through a direct fishery or as bycatch (Stevens, 2000). This species feeds mainly on squid, although it can also feed on other invertebrates such as octopus, lobster and crabs. Its diet can also include bony fishes and sea birds, as well as carrion (Compagno, 2001; Harvey, 1989). Kubodera & Watanabe (2007) report that blue shark in the western North Pacific feeds on a large variety of cephalopod and fish species. A stomach content study of blue sharks caught off Ensenada, Baja California indicated that they prey on cephalopods, crabs and teleost fishes (Markaida & Sosa-Nishizaki in press).

Blue sharks may make transoceanic migrations: Nakano (1994) proposed a blue shark migration model for the North Pacific that incorporated gender and ontogenetic differences in which parturition, nursery and mating grounds were identified across a latitudinal gradient (see Nakano, 1994 for a detailed figure). Tagging studies on blue sharks caught off southern California have shown that they can move as far south as Acapulco, Mexico, as far north as the coast of Oregon, and as far west as Hawaii and Midway in the central Pacific (California Department of Fish and Game 1999b, 2000).

The white shark (Order Lamniformes and Family Lamnidae), is also a cosmopolitan species that inhabits coastal and pelagic zones of nearlyy all oceans, but has also been found in near coastal systems *e.g.* within enclosed bays and coastal lagoons (Compagno, 2001). It feeds on a wide prey spectrum, including invertebrates such as crustaceans and cephalopods, bony fishes, other cartilaginous fishes, sea birds, marine mammals and carrion (Compagno, 2001). Dietary studies on white sharks suggest juvenile white sharks prey mainly on bony and cartilaginous fishes, and that at 300-350 cm total length (TL), they start including marine mammals in their diet (Compagno 2001; Klimley 1985).

Recent studies have shown that white sharks can travel long distances, such as from the coast of California to Hawaii (Le Boeuf 2004; Weng *et al.* 2007a) and from Guadalupe Island in the Mexican Pacific to Hawaii (Domeier & Nasby-Lucas, 2008), over a time period of months. Based on data obtained through satellite telemetry, Weng *et al.* (2007b) suggest the white shark uses the coast of California and Baja California as nursery grounds. Domeier & Nasby-Lucas (2007) reported that Guadalupe Island serves as an important aggregation site for sub-adult and adult white sharks.

Despite the low value of its meat, the fins and jaws of white sharks have a high commercial value (Compagno, 2001). In Mexico, there is also a growing touristic interest in this species. Guadalupe Island has been target of white shark cage diving activities since 2005 (CONANP-SEMARNAT, 2007). The IUCN classifies this species as "vulnerable" and in some countries it is protected, although the effectiveness of its protection is questionable where enforcement is weak (Fergusson *et al.* 2000). In Mexico, white sharks are considered an endangered species. They are protected by the Norma Oficial Mexicana,

Secretaría de Medio Ambiente y Recursos Naturales 059 (NOM-059-SEMARNAT-2001) and the Norma Oficial Mexicana, Pesca 029 (NOM-029-Pesca-2007) forbids its capture.

Mako, blue and white sharks inhabit coastal and pelagic waters off the west coast of California and Baja California (Holts *et al*. 1994). In the Mexican Pacific there is legal fishing effort on different shark species. This fishery activity is mainly artisanal and its effects on shark populations are unknown due to a lack of information on fishing effort and species composition of the target and incidental capture (Holts *et al*. 1994; Sosa-Nishizaki *et al*. 2007; Smith *et al*. 2009). Cartamil *et al*. (2007) and Santana-Morales (2008) reported that blue and mako sharks are targets of the artisanal elasmobranch fisheries in the northwestern coast of Baja California comprising more than 50% of the total catch. A large percentage of the catch are juveniles. White sharks are not targeted by fisheries off the west coast of California and Baja California, although juvenile white sharks are captured as bycatch in fisheries targeting other teleost fishes and elasmobranchs such as rays (Dewar *et al*. 2004; Weng *et al*. 2007b; Cartamil *et al*. 2007; Santana-Morales 2008). The Norma Oficial Mexicana, Pesca 029 (NOM-029-PESC-2007), regulates elasmobranch fisheries in Mexico.

I.2 Stable Isotope Analysis

Stable isotopes are atoms of the same element that have the same number of protons and electrons but differ in the number of neutrons. Stable isotopes do not exhibit radioactive decay. The difference in atomic mass between two isotopes of the same element produces differences in their reaction speed and the strength of their chemical bonds. This leads to differences in the ratio of heavy to light isotope between substrates and products, which is known as isotopic fractionation (Sulzman, 2007).

The relative abundance of the stable isotopes of an element is reported relative to international standards using the following formula:

$$\delta \ (‰) = [(R_{sample} / R_{standard} - 1)] * 1000 \ (1)$$

where δ is expressed in *per mil* and R represents the ratio of the abundance of heavy to light isotope ratio (Sulzman, 2007). The international standards are reference materials

established by the National Institute of Standards and Technology (NIST). The standard used to report $\delta^{13}C$ values is limestone from the Peedee Belemnite formation in South Carolina (PDB). The standard used to report $\delta^{15}N$ values is atmospheric nitrogen.

During the transfer of biomass between trophic levels, metabolic processes produce differences in the isotopes values of a consumer relative to its prey. This is known as trophic fractionation or trophic enrichment (DeNiro & Epstein, 1979; DeNiro & Epstein, 1981; Vander Zanden & Rasmussen, 2001). Carbon isotope fractionation is produced by the discrimination of ^{12}C during respiration, which leads to enrichment in ^{13}C in a consumer's tissues (Rau *et al*. 1983; DeNiro & Epstein, 1978). For $\delta^{13}C$, there is a 0.5-1‰ tropic enrichment value (DeNiro & Epstein, 1978; Post, 2002). This low fractionation value makes it difficult to estimate trophic level based on $\delta^{13}C$ values alone. Carbon isotope ratios however, can be useful in distinguishing between different primary producers (Fry & Sherr, 1984). Marine phytoplankton has depleted $\delta^{13}C$ values when compared to marine benthic algae, while oceanic phytoplankton has lower $\delta^{13}C$ values than coastal phytoplankton (France, 1995; Perry *et al*. 1999). These isotopic differences are reflected at higher trophic levels, rendering it possible to differentiate between feeding grounds (Hobson *et al*. 1994; Perry *et al.* 1999).

Nitrogen isotopes fractionation occurs during deamination and transamination of amino acids. Hence, through metabolic processes animals eliminate nitrogenous waste enriched in ^{14}N while ^{15}N-enriched nitrogen is preferentially retained into their tissues (Shoeninger & DeNiro, 1984). Nitrogen isotope ratios in consumer tissues are 3-4 ‰ more positive than nitrogen isotope ratios of the diet (DeNiro & Epstein 1981; Shoeninger & DeNiro, 1984; Post, 2002). Studies on marine food webs have shown that on average there is 3 - 4 ‰ enrichment in $\delta^{15}N$ values between trophic levels (Michener & Kaufman, 2007).

Post (2002) states that the average fractionation values reported in his studies, obtained for different taxa (0.4‰ for $\delta^{13}C$ and 3.4 ‰ for $\delta^{15}N$) must be used cautiously and preferably applied to community level studies involving several trophic links, since variability in the average values is expected between single trophic transfers. Vander Zanden & Rasmussen (2001) assessed the problem of trophic fractionation variability

between taxonomic groups, and noted that differences in trophic fractionation values might affect or produce errors when estimating trophic position of individual species.

The assimilation and metabolic pathways that lead to fractionation differ among animals. In order to obtain accurate trophic fractionation values for a species of interest, it is recommended that controlled feeding experiments be performed under laboratory conditions (Gannes *et al*. 1997). To estimate fractionation factors, (1) the isotopic composition of diet must remain constant, (2) the new diet must have an isotopic composition as different as possible to the initial composition of the organisms of study, and (3) the experiment must be conducted until isotopic equilibrium to the new diet is reached. Finally, the isotopic composition of different tissues must be measured, since they can vary significantly (Fry & Arnold, 1982; Tieszen *et al*. 1983; Hobson & Clark, 1992). To my knowledge, there are no published fractionation values for elasmobranch tissues.

An additional requirement for applying stable isotope analysis to the study of trophic relationships is to estimate the time integrated by the isotopic composition of an organism's tissues (Phillips & Eldridge, 2006). When a predator switches to a prey source with a different isotopic composition, the isotopic composition of its tissues will change over time to reflect the isotope value of the new diet, reaching a new isotopic equilibrium. The underlying process by which an organism's tissue reflects that of its food source is termed isotopic turnover. The isotopic turnover rate varies as a function of metabolic activity and the growth rates of each tissue (Fig. 1). Tissues that are metabolically more active, such as liver and blood, tend to have higher isotopic turnover rates than tissues like muscle and bone collagen (Tieszen *et al*. 1983; Hobson & Clark, 1992). In addition, fast growing organisms tend to reach equilibrium to new food sources faster (Herzka, 2005).

Analyzing tissues with different turnover rates might elucidate seasonal diet switching not evident from single tissue analysis, resulting in a more complete trophic study (Phillips & Eldridge, 2006). For example, MacNeil *et al*. (2005) analyzed the carbon and nitrogen SIR of different tissues (muscle, liver and blood) from blue, thresher and mako sharks. By comparing their isotopic composition, they identified a seasonal switch in the diet of mako shark. Blue and thresher sharks did not show variation in the isotopic

composition of their tissues over the course of a one-year period, suggesting diet switching did not occur.

Ideally, turnover rates should be obtained from controlled feeding experiments on the taxonomic group of interest (*e.g.* mammals, birds, teleost fishes). If controlled experiments are not possible for the life stage of interest, then laboratory data can be coupled with growth rate data as a first approximation for estimating turnover rates (Herzka, 2005). Isotopic turnover models can be used to estimate timing of dietary shifts or migratory movements (Fry & Arnold, 1982; Herzka & Holt, 2000; Philipps & Eldridge, 2006).

Turnover experiments have been done on different taxa: shrimp (Fry & Arnold (1982)), quails (Hobson & Clark (1992)), gerbils (Tieszen *et al*. (1983)), among others. MacNeil *et al*. (2006) is the only published study assessing isotopic turnover rates in elasmobranchs. They analyzed the isotopic composition of different tissues to estimate isotopic turnover rates of a fresh water stingray (*Potamotrygon motoro*) under laboratory conditions. They found that the liver reflected a dietary switch faster than muscle and cartilage. To date, there are no published isotopic turnover rates for marine elasmobranchs or in sharks.
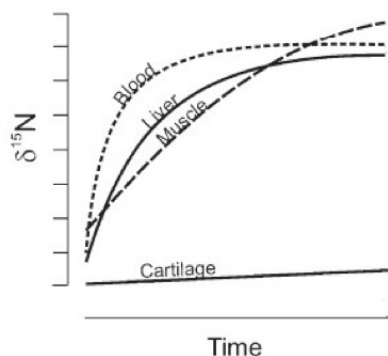
Figure 1. Conceptual model of tissue-specific isotopic turnover rates after a switch to a diet of differing isotopic composition. Modified from MacNeil *et al.* (2006).
Figura 1. Modelo conceptual de la tasa de recambio isotópico de diferentes tejidos luego de un cambio a una dieta de composición isotópica distinta. Modificado de MacNeil *et al*. (2006).

Despite the lack of studies deriving fractionation values and turnover rates for sharks, the application of stable isotopes analysis has been successfully applied to the study

of trophic position and ontogenetic patterns in sharks. Based on δ^{15}N and δ^{13}C values of different sections of the vertebrae of white sharks captured in the North Atlantic, Estrada *et al*. (2006) concluded that there is a relationship between size and trophic level. In particular, they reported a change in trophic level at total lengths >341cm, which is consistent with a switch from a diet based mainly on fishes to one dependent on marine mammals. Estrada *et al*. (2006) also detected a shift in the isotopic composition of the vertebrae corresponding to the time right after birth, suggesting a possible shift in diet from yolk (intrauterine food) to fishes. In sharks with a viviparous reproductive strategy (*i.e.* oophagy, placental analogues and placental viviparity), nourishment to embryos comes directly from the maternal energy supply (Carrier *et al*. 2004). In these types of embryonic development, it is possible that the isotopic composition of neonate shark tissues could be similar to that of the female, and that an isotopic shift will occur as neonates start feeding.

For blue and mako sharks, there are few trophic analyses based on stable isotopes ratios. Estrada *et al*. (2003) estimated the trophic position of blue, mako, basking (*Cetorhinus maximus*) and thresher sharks in the North Atlantic Ocean. They found basking sharks have the lowest trophic position, followed by the blue shark, and that the thresher shark had the highest trophic position. Based on comparisons with dietary reports from the literature, Estrada *et al*. (2003) concluded that the trophic levels they estimated were comparable to those from studies based on stomach content analysis. The trophic level estimated for the mako shark varied, which could be attributed to changes in feeding grounds (inshore *vs*. offshore).

Changes in foraging grounds have also been reported for white sharks, Kerr *et al.* (2006) conducted stable isotopes analysis of nitrogen and carbon on the vertebrae of white shark captured off the central and southern California coast. They suggested a possible shift from inshore to offshore feeding habitat that was related to size.

Domi *et al.* (2005) used SIR to analyze the feeding ecology of five shark species of commercial importance in the northeastern Atlantic (*Galeorhinus galeus*, *Galeus melastomus*, *Mustelus asterias*, *Squalus acanthias* and *Scyliorhinus canicula*). They found differences in δ^{15}N values that suggested *G. galeus* fed at a higher trophic level than the other species studied. Low δ^{15}N values in *S. acanthias* reflected a lower trophic level diet

or migratory behavior. Domi *et al.* (2005) suggested that due to the lack of trophic fractionation values for elasmobranchs, it is necessary to complement the information obtained from stable isotope analysis with a more traditional stomach content analysis. This was also discussed by Fisk *et al.* (2002), whom obtained a lower trophic value than expected when compared to stomach contents analysis and anthropogenic contaminant dietary analysis (*i.e.* organochlorine contaminants as biomagnifying tracers) for the Greenland shark *(Somniosus microcephalus)*.

The main objective of this work is to evaluate the use of the stable isotope ratio (SIR) ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) measurements in blood, muscle and liver for three shark species, the mako shark (*Isurus oxyrinchus*), the blue shark (*Prionace glauca*) and the white shark (*Carcharodon carcharias*) to determine whether there is evidence of changes in trophic level and/or feeding grounds throughout ontogeny. I also compared the carbon and nitrogen isotopic composition between a pregnant female and its near-term embryos to determine whether neonates exhibit an isotopic composition reflective of maternal tissues. To estimate trophic fractionation factors and isotopic turnover rates, I conducted an experiment under controlled laboratory conditions using the leopard shark (*Triakis semifasciata*) as model species. Finally, using growth rates from the literature, and the results of the turnover rate experiment, I estimated the isotopic turnover rates for white, blue and mako sharks across their ontogenetic stages.

II. Hypothesis

The stable isotope analysis (δ^{13}C and δ^{15}N) of metabolically active tissues of sharks can be used to evaluate dietary changes throughout ontogeny.

III. Objectives

Evaluate the use of the stable isotope ratio (SIR) ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) measurements in blood, muscle and liver for three shark species, the mako shark (*Isurus oxyrinchus*), the blue shark (*Prionace glauca*) and the white shark (*Carcharodon carcharias*) to determine whether there is evidence of changes in trophic level and/or foraging grounds throughout ontogeny.

Determine whether neonate sharks exhibit an isotopic composition reflective of maternal tissues.

Estimate trophic fractionation factors and isotopic turnover rates in sharks, using the leopard shark (*Triakis semifasciata*) as model species.

Estimate the isotopic turnover rates for white, blue and mako sharks across their ontogenetic stages.

IV. Methods

IV.1 Study Area

To obtain samples of juveniles, subadults and adults of blue, mako and white sharks, I sampled in different sites of the Northeastern Pacific based on reports of the presence of the target species and size classes (Fig. 2). All three sites were located inside the area of influence of the California Current (CC). The CC is a southward flowing deep current that is 0-500 m, ~1000 km wide and that runs alongshore from Canada to Southern Baja California, Mexico (Batteen *et al*. 2003). The southern part of this current system extends from south of Point Conception, USA, to Southern Baja California. Along the coast of California and Baja California, seasonal alongshore wind stress and bathymetrical irregularities produce upwelling that transport nutrient-rich deep waters to the surface (Batteen *et al*. 2003; Marchesielo & Estrade, 2006). These nutrient rich waters generate seasonal as well as year-round high productivity areas that support a large biomass of mesoconsumers (*e.g*. sardines) (Lluch-Belda *et al*. 2003; Palacios *et al*. 2006), which serve as potential prey for higher trophic level predators such as sharks.

I performed the first sampling in the Southern California Bight (SCB), where juvenile, sub-adult and adult blue and mako sharks are present (Holts & Bedford, 1993; Holts *et al*. 1994). The SCB encompasses a 78,000 km^2 body of water 1000 km in length with a maximum width from shore of 300 km and depth range from 600 to 3000 m. It ranges from Point Conception, north of the Santa Barbara Channel, to San Quintin Bay, about 240 km south of the United States-Mexico border, depths range from 600 to over 3000 m (Dailey *et al*. 1993).

Sampling was also conducted in fishing camps located inside Vizcaino Bay, off the central Baja California peninsula. Juvenile and sub-adult blue, mako and white sharks are caught by artisanal fisheries along the northwestern coast of Baja California (Cartamil *et al*. 2007; Santana-Morales, 2008). Vizcaino Bay is semicircular with 110 km in diameter. It has an area approximately of 11,500 km^2 with an average depth of about 76 m, and has an

ample connection with the Pacific Ocean through a channel that runs between Cedros Island and Punta María (Mancilla-Peraza *et al*. 1993).

The third sampling area was Guadalupe Island, which provides a seasonal aggregation site for adult and sub-adult white sharks (Domeier & Nasby-Lucas, 2007). Guadalupe Island is a 225 km^2 volcanic island located 250 km offshore the central Baja California peninsula. The island hosts a large diversity of terrestrial and marine species, some of which are endemic to the island (García *et al.* 2005).
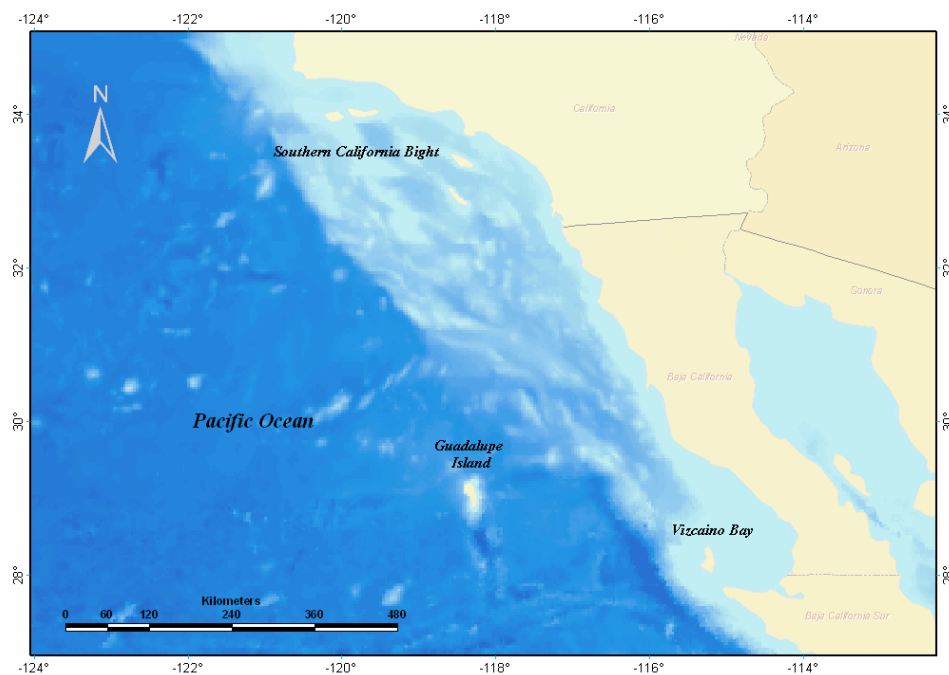
Figure 2. Map of the study area indicating the three sampling sites.
Figura 2. Mapa del área de estudio indicando los tres sitios de muestreo.

IV.2 Laboratory turnover rate experiment

The first component of this study was to conduct a controlled feeding experiment to estimate turnover rates and isotopic fractionation values in shark tissues. Sixteen newborn (2 weeks old) leopard shark pups were donated to CICESE by Dr. Jeff Graham from Scripps Institution of Oceanography of the University of California San Diego (SIO-UCSD). The 16 pups were born from the same female and shared the same tank and food

type during their two weeks at SIO. The 16 pups were transported to the Aquaculture Department at CICESE and placed in three 550 L tanks that shared a closed seawater recirculation system. Seawater was passed throughout a biofilter with polyethylene beads and UV filtration. Temperature, salinity, dissolved oxygen (DO) and ammonium concentrations were held constant, and were monitored daily in each tank during the first two months of the acclimation period and every other day thereafter and throughout the experiment (Table I).

Table I. Mean ± standard deviation of physicochemical parameters measured in three 550 L tanks containing 16 shark pups. Parameters were measured daily during the first two months and every other day thereafter during the turnover rate experiment.
Tabla I. Valores promedio ± desviaciones estándar de parámetros medidos en tres tanques de 550 L que contenían 16 crías de tiburón. Los parámetros se midieron diario durante los primeros dos meses y posteriormente cada segundo día durante el experimento de tasas de recambio isotópico.

Parameter	Mean value ± SD
Temperature (°C)	19.30 ± 0.90
Salinity (psu)	33 ± 1.50
DO (mg/l)	5.80 ± 0.60
Ammonium concentration (mg/l)	0

To identify a diet that differed in $\delta^{13}C$ and $\delta^{15}N$ values to those of the leopard pup tissue, I determined the isotopic composition of whole blood, muscle, liver, and cartilage of one pup and various potential food sources before starting the turnover rate experiment. The $\delta^{13}C$ and $\delta^{15}N$ values of the different pup tissues were compared to those of potential diets: squid mantle, sardine muscle and two commercial fish pellet diets (Table II). The diet with the most different $\delta^{13}C$ and $\delta^{15}N$ values from those of the leopard shark tissues was the commercial diet II (Burris Tilapia Food, Burris Mill and Feed, Franklinton, Louisiana, USA) (4.99 ‰ and 8.56 ‰ respectively). To verify that the isotopic composition of the diet remained constant throughout the experiment, samples were taken at days 0, 90 and 140 (Table II).

Table II. δ^{13}C and δ^{15}N values of leopard shark tissues and potential diets to be used for an isotopic turnover rate experiment. Isotopic values of commercial diet II, which was selected for the experiment (shown in bold). The mean and standard deviation of three samples analyzed at the beginning (day 0), middle (day 90) and near to the end of the experiment (day 140) are reported for commercial diet II.

Tabla II. Valores de δ^{13}C y δ^{15}N de tejidos de tiburón leopardo y dietas potenciales para ser usadas en un experimento para estimar tasas de recambio isotópico. La dieta comercial II, se seleccionó para el experimento (en negritas). Para esta dieta, se muestra el valor promedio y desviación estándar de tres muestras analizadas al principio (día 0), durante (día 90) y cerca al termino del experimento (día 140).

Sample and tissue	δ^{15}N (‰)	δ^{13}C (‰)
Blood leopard shark	15.10	-16.09
Muscle leopard shark	15.28	-16.52
Mantle jumbo squid (*Dosidicus gigas*)	14±0.83	-19.2±0.50
Muscle sardine (*Sardinops sagax*)	13.70	-18.56
Commercial diet I	9.35	-20.46
Commercial diet II	**6.63±0.16**	**-21.3±0.26**

Before beginning the experiment, I weighed and measured the total length (TL) of all leopard shark pups (n=15). The length was measured in centimeters using an ichthyometer. All sharks were weighed 24 hrs after their last feeding in a plastic container with 1.5 L of seawater using a digital scale (AND SK-2000WP). They had similar wet weight at the beginning of the experiment (t_i) (98.5±10.5 gr). I took photographs of their heads and photo-identified them using their individual pigmentation patterns. At the beginning of the experiment, I randomly selected and sacrificed two sharks and sampled blood from the caudal artery, muscle from the dorsal area below the dorsal fin, a piece of liver from the right lobe, cartilage from the pelvic girdle and the lower lobe of caudal fin. Sharks were fed 3% of their body weight daily. I evaluated the growth (total length and weight) of the 13 remaining sharks every 15-20 days. Random selection for shark sampling was done using the "randbetween" function in Excel (Microsoft Excel 2008 for Mac Version 12.1.0) and by assigning numbers to the sharks using the photo-Id archive. During the first three months of experiment (August - October 2008) I sampled blood from three randomly selected sharks (Table III). After 28, 56, 133 and 192 days after the change in diet, I randomly selected and sacrificed two sharks based in the average increase in relative

weight (W_r = weight at time "t"/ initial weight at time "0") of remaining pups throughout the experiment. From these sharks, I prepared the same tissues sampled at the beginning of the experiment for isotopic analysis (Table III).

Table III. Dates of sampling, days after change in diet, tissues sampled, average weight, and relative weight increase during the laboratory experiment designed to evaluate isotopic turnover. Average W_r (W_r = weight at time "t"/ initial weight at time "0") was used to decide when to sample and type of tissues to sample. Only when substantial increase in weight was gained samples from all tissues were collected.
Tabla III. Fechas de muestreo, días después del cambio de dieta, tejidos muestreados, peso promedio e incremento relativo en peso durante el experimento en laboratorio. El valor W_r (W_r = peso al tiempo "t" / peso inicial al tiempo "0") promedio fue usado para decidir cuando muestrear y que tipo de tejidos muestrear. Solo cuando existieron incrementos substanciales en peso se recolectaron muestras de todos los tejidos.

Date	Days of feeding new diet	Tissues sampled	Average weight (gr)	Average W_r (W_t/W_i)
08-14-08	0	All tissues	98.5	1
09-02-08	19	Blood only	114.2	1.16
09-11-08	28	All tissues	124.9	1.27
09-16-08	33	Blood only	133.6	1.36
10-9-08	56	All tissues	144.5	1.47
10-24-08	71	Blood only	162.1	1.65
11-13-08	91	Blood only	178.6	1.81
12-25-08	133	All tissues	194.4	1.97
02-4-09	143	Blood only	203.9	2.07
02-27-09	168	Blood only	236.7	2.40
03-23-09	192	All tissues	239.6	2.43

The isotopic values for different tissues of shark pups' are reported relative to W_r values. I needed to evaluate whether isotopic equilibrium to new food had been reached in the pups' tissues. Isotopic equilibrium was evaluated by a paired-sample t-test to determine if isotopic differences existed between the last two sharks sampled.

Once I confirmed that isotopic equilibrium had been reached at the end of the experiment, I obtained average isotopic values from tissues that were no statistically

different and used these values to estimate trophic fractionation values (Δ) with the following formula,

$$\Delta = \delta_{consumer} - \delta_{diet} \ (2)$$

$\delta_{consumer}$ is the average isotopic composition ($\delta^{13}C$ or $\delta^{15}N$ values) of tissues that did not differ statistically and δ_{diet} is the average isotopic composition ($\delta^{13}C$ or $\delta^{15}N$ values) of the diet. I evaluated if tissue-specific fractionation value differed among tissues by conducting a one-way ANOVA ($\alpha=0.05$) to test for differences in the carbon and nitrogen SIR between tissues of the first and last two sharks sampled. Tukey's honestly significant difference (HSD-post-hoc) test was used to determine which tissues differed in their isotopic values.

Isotopic Turnover models were used to estimate the relative metabolic turnover and growth contribution of isotopic turnover of each tissue sampled in the laboratory experiment. Fry & Arnold (1982) and Hesslein *et al.* (1993) proposed empirical equations as turnover models. I chose to use Fry & Arnold (1982) model because it includes the relative weight gain (W_r) without the need to estimate a separate growth rate as needed in the model of Hesslein *et al.* (1993). This allows for subsequent modeling of isotopic turnover rates using species and stage-specific models. Power functions of the form $Y=a+bx^c$ were fitted with SigmaPlot for Windows Version 10.0 Build 10.0.0.54 to laboratory data using the empirical equation proposed by Fry & Arnold (1982),

$$\delta_t = \delta_f + \left(\delta_i - \delta_f\right) \cdot \left(w_t / w_i\right)^c \ (3)$$

δ_i is the initial isotopic value prior to the switch in diet, w_i is a shark's weight immediately prior to the dietary switch, w_t is weight at time t, δ_f are asymptotic $\delta^{13}C$ or $\delta^{15}N$ values achieved at equilibrium to the new diet, δ_t is the $\delta^{13}C$ or $\delta^{15}N$ value at w_t and c is the coefficient of metabolic decay. When c = -1, the isotopic turnover is attributed solely to weight gain and the metabolic effect is not detectable. When c < -1, the isotopic turnover can be attributed both to growth and metabolism. The relative contribution of metabolic turnover to isotopic change estimated with these models was then used to model turnover rates for wild sharks, across sizes and species.

IV.3 Field sampling

IV.3.1 Sampling in the Southern California Bight

I participated in the mako and blue juvenile shark-tagging cruise held by NOAA-SWFSC in the Southern California Bight from June 8 to 17 2008 onboard the "Ventura II". A total of 17 longline sets were made using "J" type 9/0 hooks during the cruise. Each mainline had approximately 200 hooks placed 15 m apart baited with mackerel.

Blood was sampled from sharks caught alive (n=22). Using a 38x16.5 mm needle with a 3 ml syringe, 1-1.5 ml were taken from the caudal artery. Blood samples were placed in 2.5 ml eppendorf tubes over ice and frozen in a standard freezer at -4°C at the end of the day. After taking each sample, the needle and syringe were washed with distilled water, alcohol, and kim-wipes.

Sharks that died during capture (n=4) were also sampled for blood. In addition, samples of muscle (dorsal area below dorsal fin) and liver were taken by dissecting approximately 1 cm^3 of tissue. Tissue samples were collected using a clean scalpel and dissection pincers. Tissue samples were placed in whirl-pak bags, stored in an ice chest and frozen at the end of the day in a standard freezer at -4°C. I registered the sex of all sharks and measured fork length with a tape measure in centimeters (Table IV).

Table IV. Size range of sharks sampled in the Southern California Bight.
Tabla IV. Rango de tallas de tiburones muestreados en el Southern California Bight.

Specie	Sampling date	Size range TL (cm)	Number of samples
Blue shark	June 2008	99.2-295.9	21
Mako shark	June 2008	103.3-139.6	5

IV.3.2 Sampling in Vizcaino Bay

During the five field trips to fishing camps held between June and August 2008, I collected samples from blue, mako and white sharks. Samples were obtained at "Laguna Manuela" and "Casitas" artisanal fishing camps inside Vizcaino Bay. Fishermen from these

fishing camps, which are members of the fishing cooperatives located in the nearby "Ejido Jesús María" and "Guerrero Negro" towns, caught all sharks. Samples for stable isotope analysis were collected upon arrival of fishing boats to the fishing camps. Sharks were caught by either longline or bottom gill nets.

All sharks were measured for total length (TL, cm) and their sex recorded. Blood (1-1.5 ml from caudal artery), muscle (dorsal area of the head) and liver samples were obtained from mako, blue, white sharks, one *Galeorhinus galeus* pregnant female and two of her 10 near-term embryos as described before. Samples were placed in whirl-pak bags and stored in ice using an ice-chest until arrival to the laboratory.

To avoid the contamination of samples with sand or other organic material in the fishing camps, I took larger samples than those collected during the tagging cruise (approximately 4 cm^3). After collection and prior to storage, these samples were rinsed with distilled water.

IV.3.3 Sampling in Guadalupe Island

For the purpose of sampling adult white sharks, I conducted a sampling trip to the Guadalupe Island Biosphere Reserve in November 2008 onboard the shark cage diving boat "Islander", under the sampling permit issued by "Subsecretaría de Gestión para la Protección Ambiental" (September 10, 2008 number SGPA/DGVS/06103/08). At Guadalupe Island, a small outboard 18ft fishing boat was hired from the local fishing cooperative. White shark adults were baited with tuna, yellowtail or mackerel chunks tied to a natural fiber rope (ixtle). When the sharks approached the boat, I took skin and muscle samples from the dorsal area below the dorsal fin with a stainless steel hole puncher attached to a pole spear and bamboo stick. Before and after taking each sample, I rinsed the hole puncher with hydrogen peroxide, ethanol and distilled water. The skin and muscle samples were placed in whirl-pack bags and stored over ice. At the end of the day, the samples were stored in a standard freezer at -4°C. The total length of each shark was estimated by comparison with the length of the boat. I identified and differentiated

individuals using external characteristics, such as skin color patterns, scars and tags placed at the base of the dorsal fin (acoustic and satellite telemetry tags) (Table V).

In addition, Dr. Michael Domeier from the Marine Conservation and Science Institute in San Diego, CA, USA, donated four white shark plasma samples for SIR analyses. These samples were collected in Guadalupe Island on November of 2008. Blood samples were placed in vials containing lithium heparin and centrifuged to isolate red blood cells from plasma. Samples were kept frozen pending analysis.

Table V. Size range of sharks obtained in the Mexican Pacific (Vizcaino Bay and Guadalupe Island).
Tabla V. Rango de tallas de tiburones muestreados en el Pacífico mexicano (Bahía Vizcaíno e Isla Guadalupe).

Specie	Sampling date	Size range (cm)	Number of samples
Blue shark	July-August 2008	86-240	8
Mako shark	June-August 2008	80-193	23
White Shark	June-November 2008	149.5-550	25

IV.3.4 Size-class determination

For comparison with other studies, all shark fork length (FL) measurements were transformed to TL. No published fork length - total length relationships were found for mako and blue sharks in our study area. Hence, to generate equations to transform fork length to total length for blue and mako sharks, I used a database derived from measurements made on sharks captured inside Vizcaino Bay (Cartamil *et al*. unpublished data). The equations were derived using a linear adjustment of the form y=ax+b using SigmaPlot for Windows Version 10.0 Build 10.0.0.54 (Table VI). After total length was obtained for all sharks, shark data were divided into size classes based on life history traits for male sharks, as described in the HMS FMP - Appendix F, and by Compagno (2001) (Table VII & VIII).

Table VI. Total length (TL) - Fork length (FL) relationships for mako and blue sharks captured in Vizcaino Bay. Linear fit coefficients are presented as values ± standard error.
Tabla VI. Relaciones Longitud total (LT) - longitud furcal (LF) para tiburones mako y azul capturados en Bahía Vizcaíno. Los coeficientes de ajuste linear se presentan como valores ± errores estándar.

Shark species	N	Mean TL (cm)	TL range (cm)	Mean FL (cm)	FL range (cm)	TL=(a)FL+b		
						a	b	r²
Blue	341	141.97	97.50-207	117.89	74-173	1.17±0.01	4.44±1.81	0.94
Mako	44	118.73	78-170.50	106.79	64-150.50	1.08±0.03	3.49±3.59	0.96

Table VII. Stage, size range (total length) and number of samples of specific tissues obtained for the Mexican Pacific (Vizcaino Bay and Guadalupe Island). *Plasma samples.
Tabla VII. Estadio, rango de tallas (longitud total) y número de muestras de tejidos específicos obtenidas en el Pacífico mexicano (Bahía Vizcaíno e Isla Guadalupe). *Muestras de plasma.

Species	Stage	Size range TL (cm)	N		
			Blood	Muscle	Liver
Blue shark	Juveniles	<100	1	1	1
	Subadults	100-200	4	4	4
	Adults	>200	3	3	3
Mako shark	Juveniles	<112	6	6	6
	Subadults	112-196	17	17	17
	Adults	>196	-	-	-
White shark	Juveniles	<200	6	14	7
	Subadults	200-350	-	-	-
	Adults	>350	4*	7	-

Table VIII. Stage, size range and number of samples obtained of specific tissues in the SCB.
Tabla VIII. Estadio, rango de tallas (longitud total) y número de muestras de tejidos específicos obtenidas en el SCB.

Species	Stage	Size range TL (cm)	N		
			Blood	**Muscle**	**Liver**
Blue shark	Juveniles	<100	2	2	2
	Subadults	100-200	14	1	1
	Adults	>200	5	-	-
Mako shark	Juveniles	<112	2	1	1
	Subadults	112-196	3	-	-
	Adults	>196	-	-	-

IV.4 Trophic level estimates

Since I wanted to estimate the differences in trophic level (TL) between size-classes of a given shark specie to do this, the following formula was used:

$$TL_{dif} = \frac{\delta^{15}N_{adult\,/\,subadult} - \delta^{15}N_{subadult\,/\,juvenile}}{\Delta_n} \quad (4)$$

where TL_{dif} is the difference in trophic level, $\delta^{15}N_{adult/subadult}$ is the average nitrogen isotopic ratio for adults or subadults, $\delta^{15}N_{subadult/juvenile}$ is the average nitrogen isotopic ratio for subadults or juveniles, Δ_n is the trophic fractionation factor for $\delta^{15}N$ values for blood and muscle (1.76) found in this study, and assumed that the isotopic composition of the trophic baseline is the same for all species and size classes. For comparative purposes, I also obtained the trophic level differences between size-classes of sharks using the average trophic fractionation value (3.4) derived from the literature for all species (Post, 2002).

IV.5 Samples preparation for stable isotope analysis

All samples were handled using powder-free latex gloves. Using scalpel and pincers cleaned with alcohol and kim-wipes, a small portion (2-3 gr) of inner tissue was dissected

and rinsed with distilled water. I placed samples in pre-cleaned tin boats and dried them at 60°C for 24-48 hours.

I lipid-extracted liver samples using a Labconco Goldfish fat extractor using the continuous solvent extraction Goldfish method extracted with petroleum ether. Samples were placed in Whatman 47mm Ø glass microfibre filters (GFF) during the extraction, then filters where combusted at 500°C in a Lindberg/Blue Box furnace prior to lipid extraction. The extraction time was determined by extracting 5 shark liver samples for different amounts of time (30 and 90 min) and weighting the redried samples. Extraction times of 60 min were selected because dry weights remained constant following 30 and 60 min extraction (Table IX).

After drying, samples were ground to a fine powder using either a porcelain mortar and pestle for muscle, cartilage and fins samples, or by using an agate mortar and pestle for liver and blood samples. The mortar and pestle were cleaned using hydrogen peroxide, alcohol and distilled water and Kim-wipes between grindings to avoid cross-contamination. Once ground, the samples were stored in eppendorf tubes in a desiccator.

Table IX. Evaluation of lipid extraction times for sharks liver. The average (±SD) difference in weight before and after the second extraction was 0.0003±0.0005 gr.
Tabla IX. Evaluación de tiempos de extracción de lípidos para hígados de tiburón. La diferencia promedio (±SD) en peso antes y después de la segunda extracción fue 0.0003±0.0005 gr.

Species	Weight before extraction (gr)	Weight after 30 min extraction (gr)	Weight after 90 min extraction (gr)
Leopard shark	0.1365	0.0320	0.0315
Leopard shark	0.1405	0.0318	0.0323
Leopard shark	0.1374	0.0302	0.0297
Blue shark	0.1838	0.0538	0.0531

I weighed 0.5-1.0 mg of each ground sample and placed it in tin capsules. Samples were sent to UC Davis Stable Isotope Facility where $\delta^{13}C$ and $\delta^{15}N$ values were analyzed using a PDZ Europe ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20

isotope ratio mass spectrometer. The standard deviation of secondary internal standards was ±0.04 for $\delta^{13}C$ values and ±0.16 for $\delta^{15}N$ values.

IV.6 Modelling of isotopic turnover rates

Using the tissue-specific coefficients of metabolic decay obtained from laboratory data, I estimated the time of isotopic change in the three different shark size classes of blue, mako and white sharks following a hypothetic diet shift. These models are used only as a first approximation to estimate the timing of isotopic turnover, since the coefficient of metabolic decay is not specific for the species or life stage of the shark. To do this, I applied Fry & Arnold's (1982) isotope turnover model, and simulated a diet shift. To estimate the relative gain in weight over time (W_r) following a dietary shift I used a von Bertalanffy growth model that includes an empirical estimate of length at birth (Romine *et al.* 2006):

$$L(t) = L_\infty - (L_\infty - L_0)e^{-kt} \quad (5)$$

where t is time in years, L(t) is the total length at time t, L_∞ is the maximum total length, L_0 is the total length at birth and k is the specific growth rate. Solving for t yields:

$$t = -\frac{1}{k} \cdot \ln\left(-\frac{L(t) - L_\infty}{L_\infty - L_0}\right) \quad (6)$$

t was used to estimate time after the diet shift. Von Bertalanffy growth function (VBGF) parameter values were obtained from previous studies in the Pacific Ocean on my target species (Table X). L_0 was obtained from studies of the target species, although due to lack of published data they were not necessarily from my study area, L_0 used in my models are average values of the size range reported in the literature (Table XI). After obtaining t, L(t) was transformed to w_t and L_0 to w_i, to obtain W_r (w_t/w_i). Length-weight relationships were taken from previous studies on my target species in the north Pacific Ocean and from the North Atlantic Ocean when no data were found for the species in the study area (Table

XII). Finally, I estimated the percentage of isotopic change at time t using the following equation:

$$\% = \frac{(\delta_t - \delta_i)}{2} * 100 \quad (7)$$

δ_t is the isotopic composition at time t and δ_i the initial isotopic composition of the tissues. Isotopic turnover models were run until the isotopic composition of the tissues reached 90 % of the isotopic change required to reach equilibrium, because the relationship is asymptotic.

Table X. Von Bertalanffy growth function parameters for the three target species. Lengths are total lengths.
Tabla X. Parámetros de la ecuación de von Bertalanffy para las tres especies objetivo. Los datos de longitud son longitudes totales.

Species	Region	L∞ TL (cm)	k	Study
Blue shark	E-NP	265.5	0.223	Cailliet & Bedford, (1983)
Mako shark	E-NP	375.4	0.05	Ribot-Carballal *et al*. 2005
White shark	NP	767.37	0.058	Cailliet *et al*. 1985

Table XI. Estimated lengths at birth (L_0) of the three target species. L_0 used in my models are average values of the size range reported in the literature. Lengths are total lengths. E-NP: Eastern North Pacific, NP: North Pacific.
Tabla XI. Longitudes de nacimiento (L_0) estimada para las tres especies objetivo. El valor L_0 usado en mi modelo es un promedio del intervalo de tallas reportado en la literatura. Los datos de longitud son longitudes totales. E-NP: Pacífico Noroeste, NP: Pacífico Norte.

Species	L_0 TL (cm)	Study
Blue shark	30-50	Nakano, (1994); Castro & Mejuto, (1995), Snelson *et al*. (2008)
Mako shark	74	Shoou-Jeng Joung & Hua-Hsun Hsu, (2005); Mollet *et al*. (2000)
White shark	120-150	Bruce, (2008)

Table XII. Length - weight relationships for the target species reported in previous studies. For the turnover models, values in pounds were transformed to kilograms and fork lengths were transformed to total lengths.

Tabla XII. Relaciones longitud - peso para las especies objetivo reportadas en estudios previos. Para los modelos de recambio, los valores en libras se transformaron a kilogramos y las longitudes furcales se transformaron a longitudes totales.

Species	Region	r^2	L-W relationship	Study
Blue shark	NP	0.997	WT (lbs) = 4.018×10^{-6} TL$^{3.134}$	Strasburg, 1958
Mako shark	W-NP	0.98	WT (kg) = 1.1×10^{-5} TL$^{2.95}$	Shoou-Jeng & Hua-Hsun, 2005
White shark	NA	0.98	WT (kg) = 7.5763×10^{-6} FL$^{3.0848}$	Kohler *et al*. 1996

V. Results

V.1 Laboratory turnover rate experiments

During the laboratory experiment, all sharks grew consistently but there were variations in growth rate among individuals during the experiment. At the end of the experiment, prior to last sampling, remaining sharks (n=5) had a mean weight of 239.60 gr (±77.2 SD).

$\delta^{13}C$ and $\delta^{15}N$ values of shark tissues changed rapidly after the shift to the new diet. Carbon isotope values consistently showed differences between tissues throughout the experimental period. Final $\delta^{13}C$ values showed a >3 ‰ change from the initial isotopic composition. Final nitrogen isotope ratios showed a change close to 8 ‰ from the initial isotopic composition.

Tissue carbon and nitrogen SIR approximated equilibrium to the new diet at W_r=2.5 (Figs. 3 and 4). The last two sharks sampled had a relative biomass gain (W_r) 2.6 and 3.3, respectively. Based on the paired sample t-test, the isotopic values of each tissue were not statistically different between the last two sharks sacrificed (p=0.33 for $\delta^{15}N$ values and p=0.072 for $\delta^{13}C$ values, at α=0.05). This indicates that despite the difference in weight gain between the last two sharks sacrificed in the experiment, they had both reached an isotopic equilibrium to the new diet.
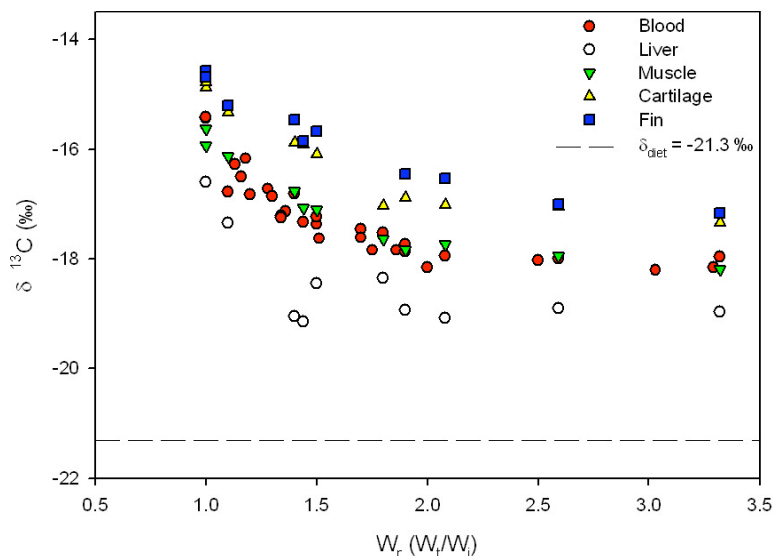
Figure 3. $\delta^{13}C$ values of different leopard shark tissues. Individuals where subjected to a controlled dietary shift under laboratory conditions. The dashed line represents the $\delta^{13}C$ value of diet.
Figura 3. Valores $\delta^{13}C$ de diferentes tejidos de tiburón leopardo. Los individuos se sometieron a un cambio de dieta controlado bajo condiciones de laboratorio. La línea punteada representa el valor $\delta^{13}C$ de la dieta.
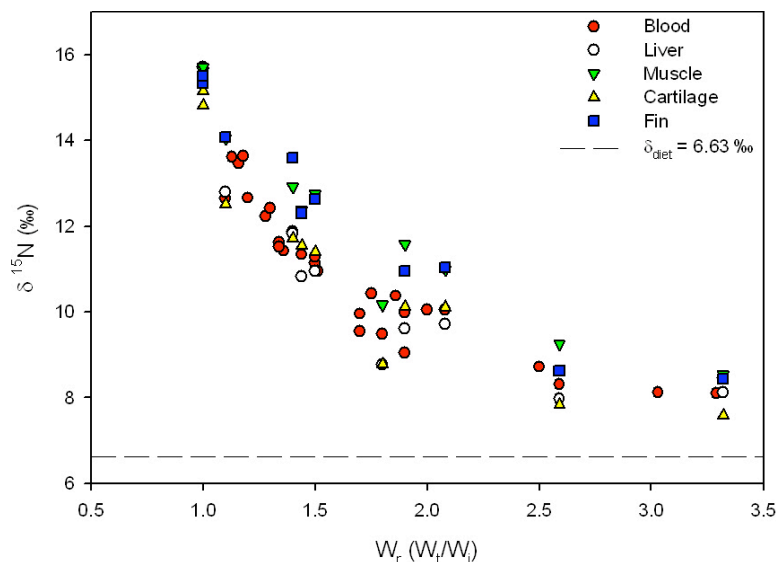
Figure 4. $\delta^{15}N$ values of different leopard shark tissues. Individuals where subjected to a controlled dietary shift under laboratory conditions. the dashed line represents the $\delta^{15}N$ value of diet.
Figura 4. Valores $\delta^{15}N$ de diferentes tejidos de tiburón leopardo. Los individuos se sometieron a un cambio de dieta controlado bajo condiciones de laboratorio. La línea punteada representa el valor $\delta^{15}N$ de la dieta.

There were significant differences between asymptotic $\delta^{13}C$ values of the different leopard shark tissues (one-way ANOVA: t_i p=0.002, t_f p=0.0002) (Fig. 3). Tukey´s HSD test indicated that differences in asymptotic $\delta^{13}C$ values of tissues could be placed in three groups: 1) cartilage and fin, 2) muscle and blood, and 3) liver. Statistical differences were also found between asymptotic $\delta^{15}N$ values (one-way ANOVA: t_i p=0.03, t_f p=0.03) (Fig. 4). Tukey´s HSD test indicated that the asymptotic $\delta^{15}N$ values for cartilage was different to that of muscle (at t_i and t_f) and to blood (at t_i), while no difference was found between muscle, blood, fin and liver. Results from these tests were used to calculate the trophic fractionation (TF) values for the various tissues that are listed in Table XIII. TF values of carbon isotope ratios were different between tissues groups (range of values 2.36 to 4.16 ‰). TF values of nitrogen isotope ratios had differences < 1 ‰ between tissues groups (range of values 1.08 to 1.76 ‰).
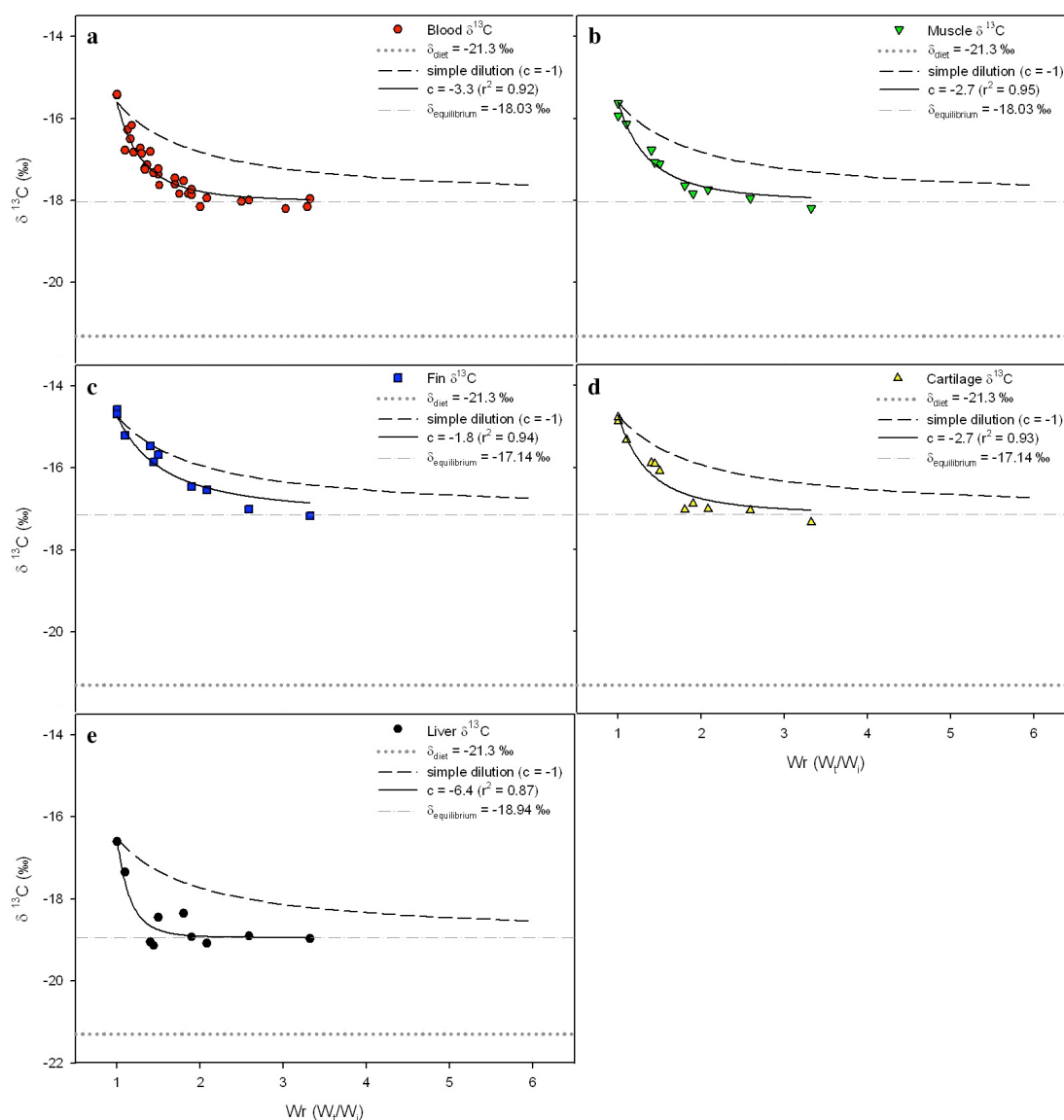
Figure 5. $\delta^{13}C$ values of leopard shark blood (a), muscle (b), fin (c), cartilage (d), and liver (e) of individuals subjected to a controlled dietary shift under laboratory conditions. Dotted lines represent the $\delta^{13}C$ value of the diet. Dashed lines represent Fry & Arnold's (1982) simple dilution (c = -1) isotope turnover model. Solid lines represent non-linear curve fit to Fry & Arnold (1982) isotope turnover model, and c is the fitted value of the coefficients of metabolic turnover for each tissue. Dash-dot lines represent the $\delta^{13}C$ value achieved after reaching isotopic equilibrium to the new diet.
Figura 5. Valores $\delta^{13}C$ de sangre (a), músculo (b), aleta (c), cartílago (d) e hígado (e) de tiburones leopardo sujetos a un cambio de dieta controlado en condiciones de laboratorio. Las líneas punteadas representan los valores $\delta^{13}C$ de la dieta. Las líneas con quiebre representan el modelo de recambio isotópico de Fry & Arnold (1982) bajo condiciones de dilución simple (c = -1). Las líneas continuas representan el ajuste no lineal al modelo de recambio isotópico de Fry & Arnold (1982), y c es el valor ajustado de los coeficientes de recambio metabólico para cada tejido. Las líneas con quiebre y punto representan el valor alcanzado después de llegar al equilibrio isotópico con la nueva dieta.
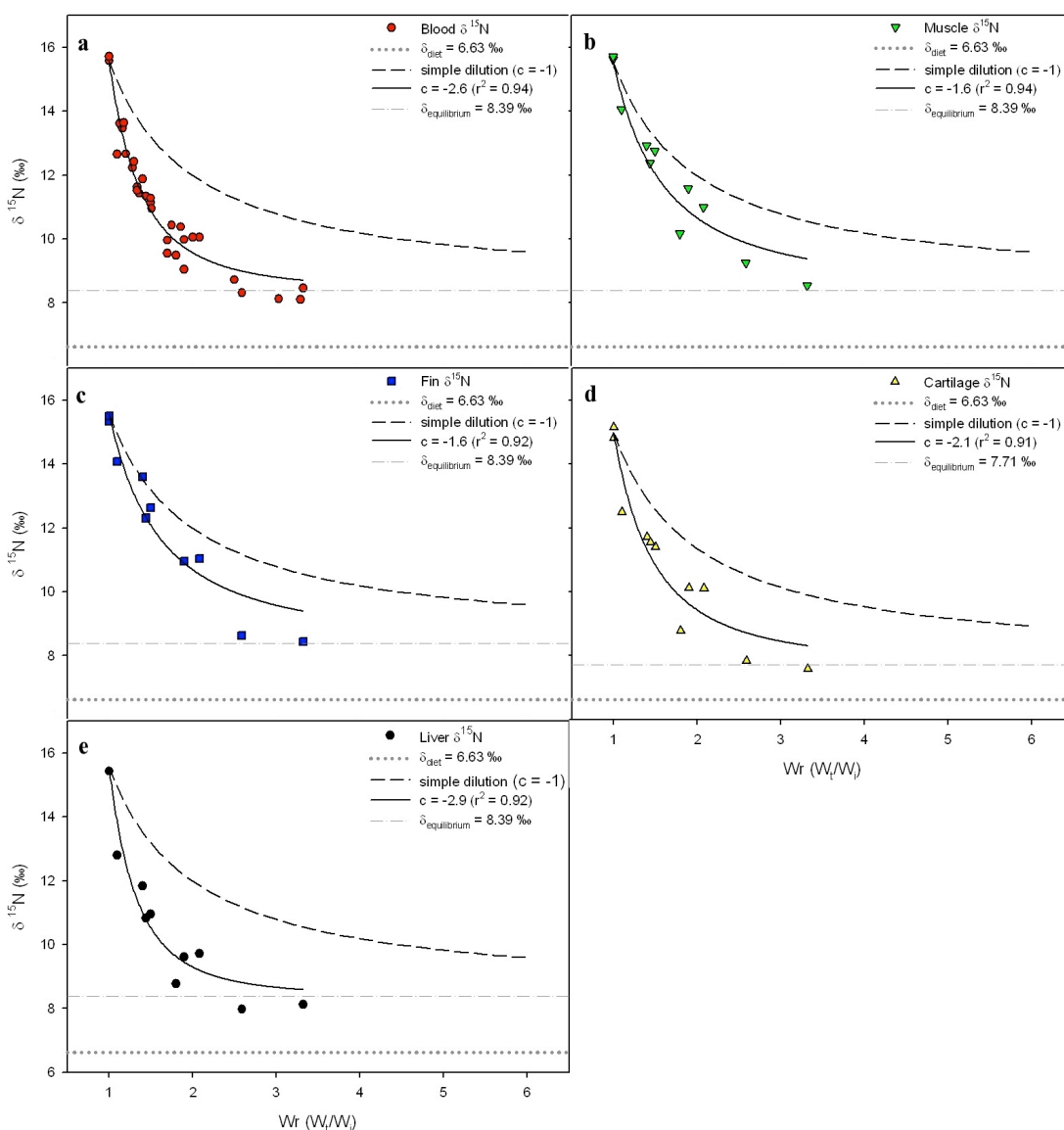
Figure 6. $\delta^{15}N$ values of leopard shark blood (a), liver (b), muscle (c), fin (d), and cartilage (e) of individuals subjected to a controlled dietary shift under laboratory conditions. Dotted lines represent the $\delta^{15}N$ value of the diet. Dashed lines represent Fry & Arnold's (1982) simple dilution (c = -1) isotope turnover model. Solid lines represent non-linear curve fit to Fry & Arnold (1982) isotope turnover model, and c is the fitted value of the coefficients of metabolic turnover for each tissue. Dash-dot lines represent the $\delta^{15}N$ value achieved after reaching isotopic equilibrium to the new diet. Figura 6. Valores $\delta^{15}N$ de sangre (a), músculo (b), aleta (c), cartílago (d) e hígado (e) de tiburones leopardo sujetos a un cambio de dieta controlado en condiciones de laboratorio. Las líneas punteadas representan los valores $\delta^{15}N$ de la dieta. Las líneas con quiebre representan el modelo de recambio isotópico de Fry & Arnold (1982) bajo condiciones de dilución simple (c = -1). Las líneas continuas representan el ajuste no lineal al modelo de recambio isotópico de Fry & Arnold (1982), y c es el valor ajustado de los coeficientes de recambio metabólico para cada tejido. Las líneas con quiebre y punto representan el valor alcanzado después de llegar al equilibrio isotópico con la nueva dieta.

Figures 5 and 6 show tissue-specific carbon and nitrogen SIR values as a function of relative biomass increase (W_r) throughout the experiment. Non-linear curve fits of Fry & Arnold's (1982) isotope turnover model to the empirical data indicated a faster turnover rate than that predicted with a simple dilution model for both carbon and nitrogen. Isotopic equilibrium was reached by $W_r = 3.5$ for all tissues; in contrast, using the simple dilution model (c= -1) equilibrium is reached by $W_r = 6$. In Table XIII, I present the values of the coefficient of metabolic decay for carbon and nitrogen SIR yielded from non-linear curve fits to the experimental data. All values are $c < -1$, lower than the used in a simple dilution model ($c = -1$), indicating that the isotopic turnover cannot be attributed only to biomass gain and that there was metabolic effect. The $\delta^{13}C$ values in the five tissues analyzed showed a faster turnover rate than $\delta^{15}N$ values. Liver and blood showed the fastest turnover rate for carbon and nitrogen, while fin tissue had the slowest turnover rate of the five tissues analyzed.

Table XIII. Curve-fitted values of the coefficient of metabolic decay (c) and trophic fractionation (TF) values for each tissue using final asymptotic $\delta^{13}C$ values or $\delta^{15}N$ (δ_f) derived from laboratory data curve-fitted with Fry & Arnold's (1982) isotope turnover model.
Tabla XIII. Valores de coeficiente de decaimiento metabólico (c) y valores de fraccionamiento trófico (TF) para cada tejido usando los valores asintóticos finales para $\delta^{13}C$ y $\delta^{15}N$ (δ_f) derivados de datos de laboratorio ajustados al modelo de recambio isotópico.

Tissue	$\delta^{13}C$ TF value (‰)	$\delta^{15}N$ TF value (‰)	$\delta^{13}C$ c value	$\delta^{15}N$ c value
Liver	2.36	1.76	-6.4	-2.9
Blood	3.27	1.76	-3.3	-2.6
Muscle	3.27	1.76	-2.7	-1.6
Fins	4.16	1.76	-1.8	-1.6
Cartilage	4.16	1.08	-2.7	-2.1

V.2 Field data analysis

The $\delta^{15}N$ and $\delta^{13}C$ values of muscle, blood and liver of mako and blue shark sampled in Bahia Vizcaino were more enriched (around 1 ‰ and 0.5 ‰, respectively) than those sampled in the Southern California Bight (Figure 7 shows isotopic data range for blood only). For this reason, comparison of isotopic values between size ranges and species were made separately for each sampling area. Vizcaino Bay and Guadalupe Island will be referred hereafter as the Mexican Pacific off Baja California.
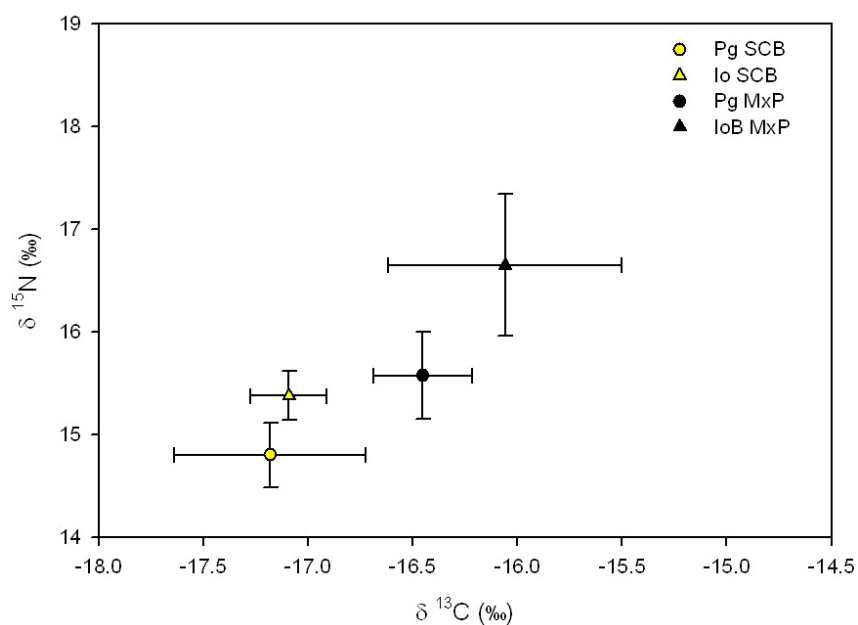
Figure 7. Mean carbon and nitrogen stable isotope ratios (±SD) measured in blue (Pg) and mako (Io) shark blood. Individuals were captured in the Southern California Bight (SCB) in June 2008 and from July through November 2008 in the Mexican Pacific off Baja California (MxP). Size range (TL) of individuals captured in the SCB is Pg: 99-296 cm; Io: 103-140 cm. Size range (TL) of individuals captured in the MxP is Pg: 86-240 cm; Io: 80-193 cm.

Figura 7. Razones isotópicas promedio de carbono y nitrógeno (SD) medidas en sangre de tiburones azul (Pg) y mako (Io). Los tiburones se capturaron en el Southern California Bight (SCB) en junio de 2008 y entre julio y noviembre de 2008 en el Pacífico mexicano (MxP). Rango de tallas (TL) de tiburones capturados en el SCB es Pg: 99-296 cm; Io: 103-140 cm. Rango de tallas (TL) de tiburones capturados en el PMx es Pg: 86-240 cm; Io: 80-193 cm.

V.2.1 Female, embryo and juvenile SIR variability

There was no consistent pattern of isotopic enrichment or depletion between the embryos and the female's tissues. There was <1 ‰ difference in $\delta^{15}N$ values between female and embryo blood, and the same difference was found for muscle. There was 1.3 ‰ difference in $\delta^{15}N$ values between female and embryo liver. The smallest difference in $\delta^{13}C$ values, <0.3 ‰, was found between female and embryo blood. A difference ~1 ‰ in $\delta^{13}C$ values existed between female and embryos liver and muscle (Table XIV).

The nitrogen isotopic composition of muscle and blood of juvenile mako and white sharks showed greater variability than that of subadult and adult sharks. During the juvenile stage (<112 cm TL for mako and <200 cm TL for white shark), small increases in size were accompanied by large increases in $\delta^{15}N$ values for both species (Figs. 8 and 9).

Table XIV. Stable isotope ratios of blood, muscle and liver of two near-term embryos and a pregnant female *of Galeorhinus galeus*. Values are mean ± standard deviation for near-term embryos.
Tabla XIV. Razones isotópicas de sangre, músculo e hígado de dos embriones terminales y una hembra *Galeorhinus galeus* preñada. Los valores se presentan como promedio ± desviación estándar para los embriones terminales.

Source	Tissue	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Female	Blood	16.04	-15.28
Embryos		16.65±0.14	-15.48±0.17
Female	Liver	15.33	-17.88
Embryos		16.59±0.37	-18.91±0.61
Female	Muscle	16.80	-16.28
Embryos		16.03±0.05	-17.61±0.05
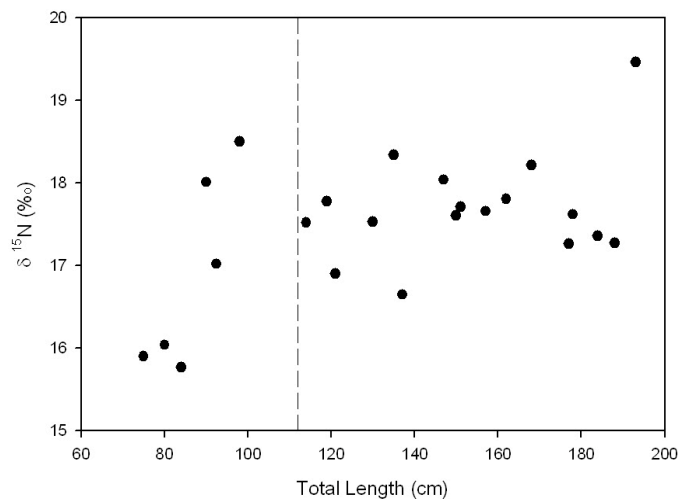
Figure 8. δ^{15}N values as a function of size (TL) measured in mako shark muscle sampled from individuals captured in the Mexican Pacific off Baja California. The dashed line represents division between juvenile and subadult size classes.

Figura 8. Valores δ^{15}N medidos en músculo de tiburones mako capturados en el Pacífico mexicano en función de la talla (TL). La línea con quiebre representa la división entre estadios juveniles y subadultos.
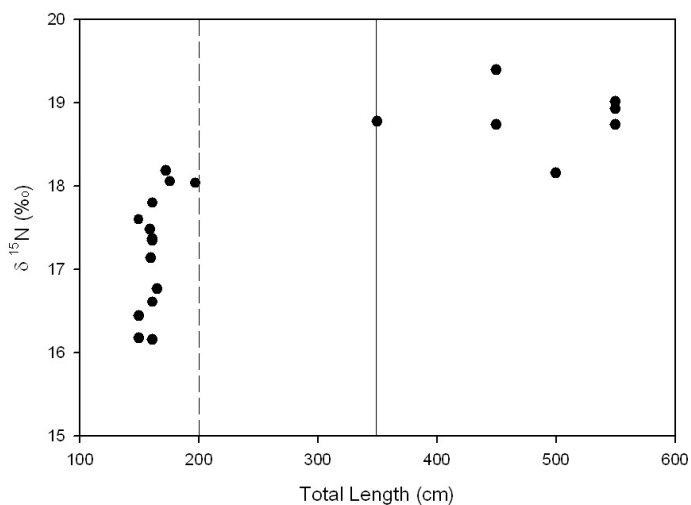
Figure 9. δ^{15}N values as a function of size (TL) measured in white shark muscle sampled from individuals captured in the Mexican Pacific off Baja California. The dashed line represents division between juvenile and subadult size classes. Solid line represents division between subadult and adult size classes.

Figura 9. Valores δ^{15}N medidos en músculo de tiburones mako capturados en el Pacífico mexicano en función de la talla (TL). La línea con quiebre representa la división entre estadios juveniles y subadultos. La línea sólida representa la división entre estadios subadultos y adultos.

V.2.2 Southern California Bight

Mean δ^{13}C values for mako and blue shark muscle sampled in the SCB were close to -18 ‰ (Fig. 10 c). Muscle δ^{15}N mean values were similar (< 0.4 ‰ difference) among size classes and species. Tissues of blue sharks had lower δ^{15}N values than mako sharks, and juvenile blue sharks had the lowest δ^{15}N measured in the region. δ^{13}C values were similar for all shark tissues, with differences < 1 ‰. Juvenile mako shark liver δ^{15}N values were around 1 ‰ more enriched than juvenile and subadult blue sharks, but δ^{13}C values were similar (-21 to -20 ‰). Between juvenile and subadult blue shark liver, there was < 0.5 ‰ difference in δ^{15}N values (Fig. 10 a). The mean nitrogen isotopic composition of blood showed enrichment as a function of increasing size class for blue and mako sharks. However, the absolute differences between size classes were small (0.2 - 0.4 ‰). There was no clear pattern in mean carbon isotopic composition of blood, and the range of δ^{13}C values was -18 to -16 ‰ (Fig. 10 b). Mean muscle carbon isotopic ratios from sharks sampled in the SCB were enriched close to 2 ‰ compared to liver samples while blood samples were enriched near to 1‰ compared to muscle samples (Fig. 10 a-c).
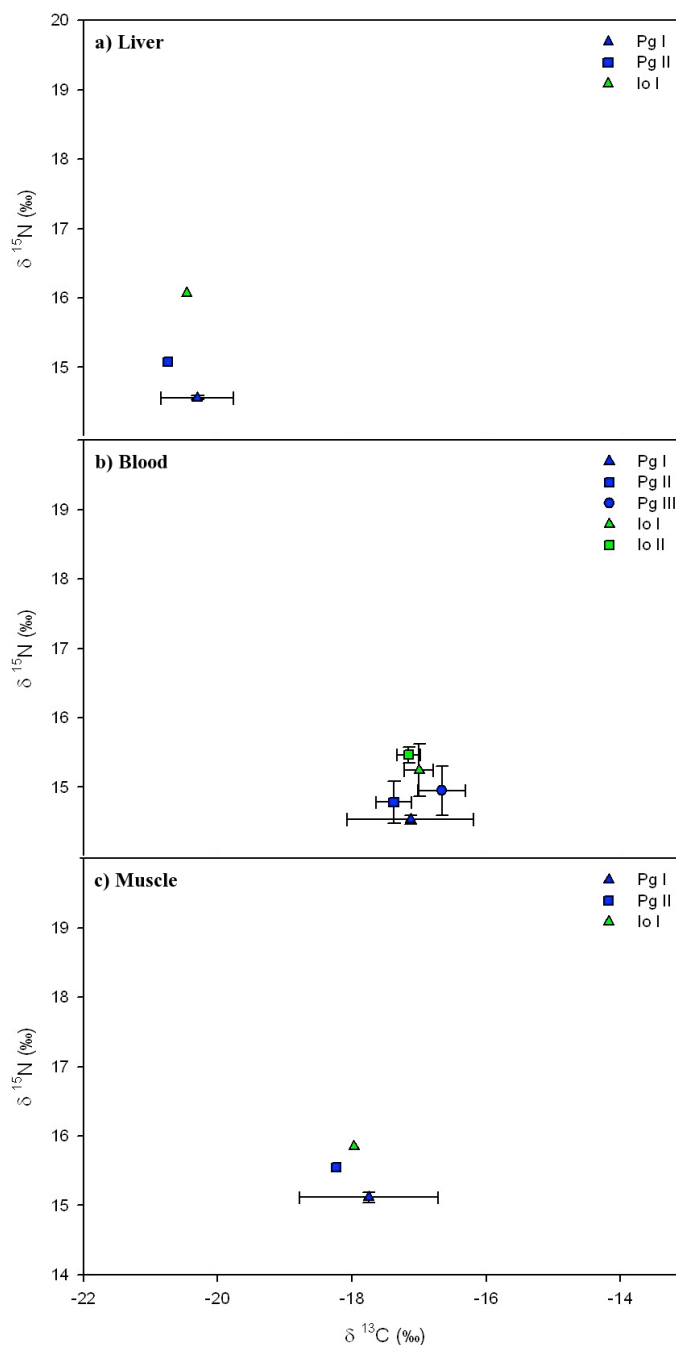
Figure 10. Mean carbon and nitrogen stable isotope ratios (±SD) measured in blue (Pg) and mako (Io) shark liver (a), blood (b) and muscle (c) sampled from individuals captured in the Southern California Bight. Isotopic values are reported as a function of size class: PgI: <100cm TL; PgII: 100-200cm TL; PgIII: >200cm; IoI: <112cm TL; IoII: 112-196cm.

Figura 10. Razones isotópicas promedio (±SD) de carbono y nitrógeno medidas en hígado (a), sangre (b) y músculo (c) de tiburones azul (Pg) y mako (Io) capturados en el Southern California Bight. Valores isotópicos se reportan en función de clases de talla: PgI: <100cm TL; PgII: 100-200cm TL; PgIII: >200cm; IoI: <112cm TL; IoII: 112-196cm.

V.2.3 Mexican Pacific off Baja California

Carbon and nitrogen stable isotope ratios of liver of the sharks sampled in the Mexican Pacific off Baja California overlapped among species. Isotopic differences between size classes were small. Adult and subadult blue sharks had similar mean nitrogen and carbon isotopic values and were slightly enriched (~0.4‰ for $\delta^{15}N$ values) and depleted (~0.5‰ for $\delta^{13}C$ values) compared to juvenile sharks. The mean stable isotope ratios showed enriched $\delta^{15}N$ values subadults relative to juvenile mako shark liver, although differences were small (0.28‰). Mean carbon stable isotope ratios exhibited larger (1.35‰) differences between juvenile and subadult mako sharks. Juvenile white sharks had $\delta^{15}N$ values enriched by approximately 1‰ compared to blue sharks, but depleted ~0.4‰ and ~0.6‰ compared to juvenile and subadult mako sharks, respectively. $\delta^{13}C$ values of juvenile white shark livers were variable and overlapped with the other species and no clear pattern was evident (Fig. 11).

Figure 11. Mean carbon and nitrogen stable isotope ratios (±SD) measured in blue (Pg), mako (Io) and white (Cc) shark liver from individuals captured in the Mexican Pacific off Baja California. Isotopic values are reported as a function of size class: PgI: <100 cm TL; PgII: 100-200 cm TL; PgIII: >200 cm; IoI: <112 cm TL: IoII: 112-196 cm; CcI: <200 cm TL.
Figura 11. Razones isotópicas promedio (±SD) de carbono y nitrógeno medidas en hígado de tiburones azul (Pg), mako (Io) y blanco (Cc) capturados en el Pacífico mexicano. Valores isotópicos se reportan en función de clases de talla: PgI: <100cm TL; PgII: 100-200cm TL; PgIII: >200cm; IoI: <112cm TL; IoII: 112-196cm; CcI: <200 cm TL.

For blood, the three size classes of blue shark had lighter mean $\delta^{15}N$ and $\delta^{13}C$ values than those of the other two target species. Adult blue sharks had mean $\delta^{15}N$ values of blood that were more enriched than those of juveniles but more depleted than for subadults. However, differences among size classes were small (< 0.6 ‰). Mean $\delta^{13}C$ values of blood were very similar, absolute differences were < 0.3 ‰ between size classes. Mean nitrogen stable isotope ratios for mako shark blood was 0.4 ‰ enriched relative to juvenile and subadult sharks. However there was overlap between individual values. There was a 2.17 ‰ difference in $\delta^{15}N$ mean blood and plasma values of juvenile and adult white sharks, thus these size classes showed the greatest differences in SIR. Juvenile white sharks had mean nitrogen stable isotope ratios between those of juvenile and subadult mako sharks (16.46‰), as well as the most enriched $\delta^{13}C$ mean values (-15.27‰) (Fig. 12).
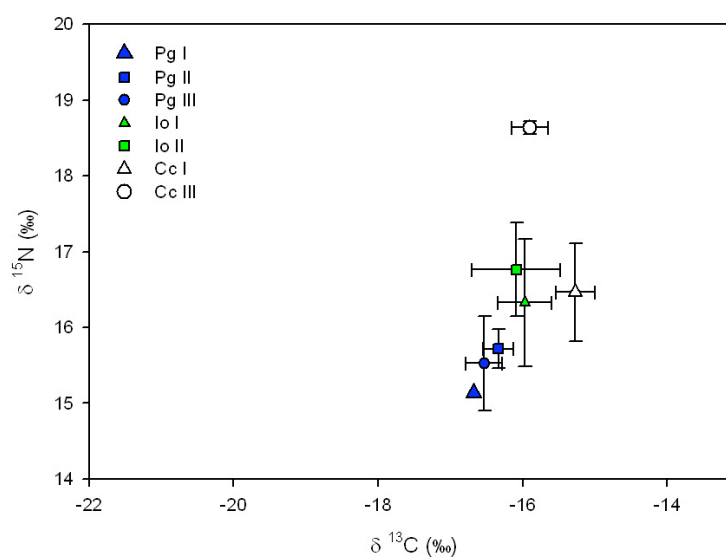
Figure 12. Mean carbon and nitrogen stable isotope ratios (±SD) measured in blue (Pg), mako (Io) and white (Cc) shark. PgI-III, IoI-IoII and CcI are blood samples, CcIII are plasma samples from individuals captured in the Mexican Pacific off Baja California. Isotopic values are reported as a function of size class: PgI: <100 cm TL; PgII: 100-200 cm TL; PgIII: >200 cm; IoI: <112 cm TL: IoII: 112-196 cm; CcI: <200 cm TL; CcIII: >350 cm TL.
Figura 12. Razones isotópicas promedio (±SD) de carbono y nitrógeno medidas en sangre de tiburones azul (Pg), mako (Io) y blanco (Cc). PgI-III, IoI-IoII y CcI son muestras de sangre, CcIII son muestras de plasma de tiburones capturados en el Pacífico mexicano. Valores isotópicos se reportan en función de clases de talla: PgI: <100cm TL; PgII: 100-200cm TL; PgIII: >200cm; IoI: <112cm TL; IoII: 112-196cm; CcI: <200 cm TL; CcIII: >350 cm TL.

Isotopic differences in muscle carbon and nitrogen isotope ratios between size classes of blue sharks were small (<0.20 ‰ for δ^{13}C values and <0.30 ‰ for δ^{15}N values). Likewise, different size classes of mako shark had similar mean muscle δ^{13}C values (differences < 0.10 ‰). Mean muscle nitrogen isotope ratios between size classes of mako and white shark had the greatest differences (0.80 ‰ and 1.59 ‰, respectively). With a mean isotopic value of -14.28 ‰ for δ^{13}C values, adult white shark muscle was the most enriched carbon isotope ratio of the samples collected in the Mexican Pacific off Baja California. For muscle, blue sharks had low δ^{15}N and δ^{13}C values compared to mako and white sharks (mean δ^{15}N values of juvenile mako sharks were enriched ~0.80 ‰ compared to the mean value for blue sharks). Subadult mako sharks were depleted ~1.13 ‰ in δ^{15}N values compared to adult white sharks. Juvenile white sharks had nitrogen stable isotope ratios between juveniles and adult mako sharks (17.22 ‰) but showed enriched δ^{13}C mean values (-16.35 ‰) (Fig. 13).

The stable isotope ratios of shark muscle showed a very similar pattern to that observed with blood and plasma samples. Mean muscle nitrogen stable isotopes ratios were enriched compared to mean blood and plasma δ^{15}N values. The greatest difference in δ^{15}N values was found between blood and muscle of subadult mako sharks (0.93 ‰) and the smallest for adult white sharks (0.19 ‰). δ^{13}C values of muscle were depleted compared to blood δ^{13}C values (from 0.59 ‰ up to 1.12 ‰ for subadult mako and blue sharks, respectively). Adult white shark muscle δ^{13}C values were enriched 1.62 ‰ compared to plasma δ^{13}C values (Figs. 12 & 13).
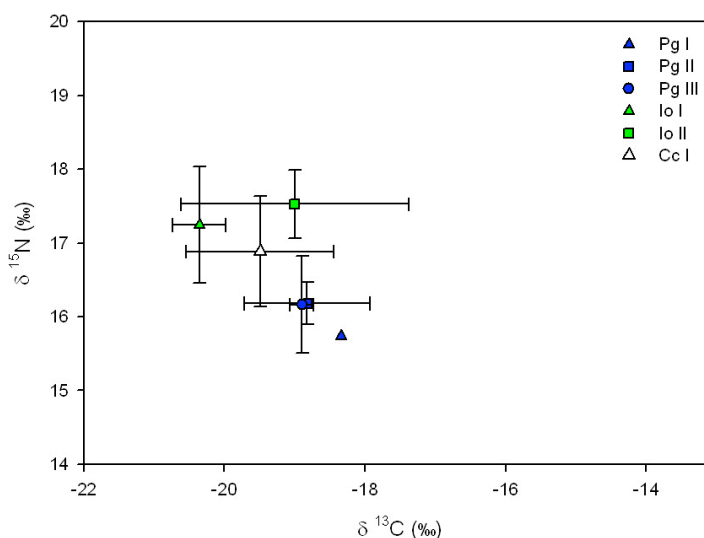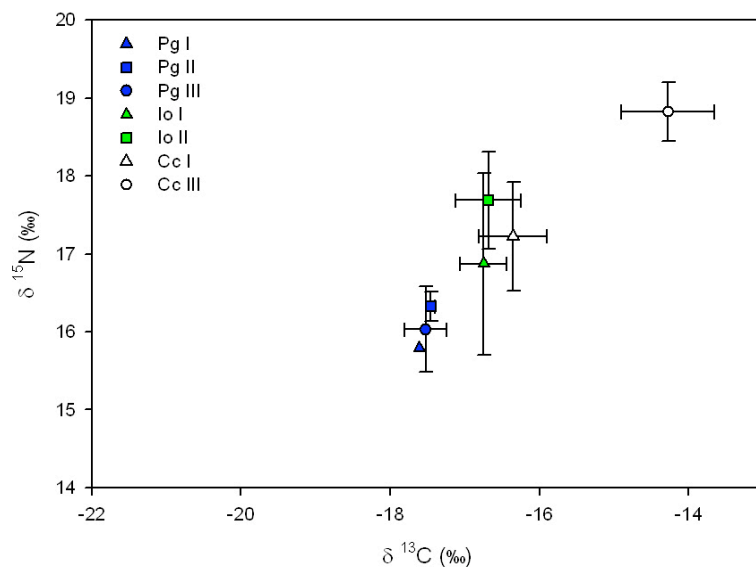
Figure 13. Mean carbon and nitrogen stable isotope ratios (±SD) measured in blue (Pg), mako (Io) and white (Cc) shark muscle sampled from individuals captured in the Mexican Pacific off Baja California. Isotopic values are reported as a function of size class: PgI: <100 cm TL; PgII: 100-200 cm TL; PgIII: >200 cm; IoI: <112 cm TL: IoII: 112-196 cm; CcI: <200 cm TL; CcIII: >350 cm TL. Figura 13. Razones isotópicas promedio (±SD) de carbono y nitrógeno medidas en músculo de tiburones azul (Pg), mako (Io) y blanco (Cc) capturados en el Pacífico mexicano. Valores isotópicos se reportan en función de clases de talla: PgI: <100cm TL; PgII: 100-200cm TL; PgIII: >200cm; IoI: <112cm TL; IoII: 112-196cm; CcI: <200 cm TL; CcIII: >350 cm TL.

V.2.4 Trophic level estimates

The greatest differences in relative trophic level was found between white shark size classes (0.9 - 1.2 TL), followed by mako shark (0.3 - 0.5). Relative trophic level differences between blue shark size classes were < 0.3 TL. Differences of 0.1 - 0.6 TL were found between relative trophic levels estimated using the average trophic fractionation value (3.4 ‰) from the literature and the trophic level values estimated using the fractionation value's derived from my laboratory experiment with sharks (Table XV).

Table XV. Trophic level (TL) differences estimated between target shark size classes calculated using the average trophic fractionation value (TF$_{average}$) derived from the literature and the fractionation value found for sharks (TF$_{shark}$) in a controlled laboratory experiment using leopard sharks as model species. Pg: blue shark; Io: mako shark; Cc: white shark. I: juveniles; II: subadults; III: adults.

Tabla XV. Diferencias en niveles tróficos para diferentes clases de talla de tiburones objetivo calculadas usando el valor de fraccionamiento trófico promedio (TF$_{promedio}$) de la literatura y el valor de fraccionamiento encontrado para tiburones (TF$_{tiburón}$) en un experimento de laboratorio bajo condiciones controladas. Pg: tiburón azul; Io: tiburón mako; Cc: tiburón blanco. I: juveniles; II: subadultos; III: adultos.

Shark species / size class comparison	TF$_{average}$ (Blood)	TF$_{average}$ (Muscle)	TF$_{shark}$ (Blood)	TF$_{shark}$ (Muscle)
PgI - PgII	0.2	0.2	0.3	0.3
PgII - PgIII	0.1	0.1	0.1	0.2
IoI - IoII	0.1	0.2	0.3	0.5
CcI - CcIII	0.6	0.5	1.2	0.9

V.3 Modelling of isotopic turnover rates

Figure 14 a-c shows the weight-age relationships reported in previous studies for blue, mako and white sharks. Blue shark showed the greatest gain in weight during the juvenile stage, while mako and white sharks showed a more consistent rate of growth during their life.

Figure 15 represents the % of carbon isotopic change estimated for the three shark species and size classes as a function of time using the coefficient of metabolic decay of muscle. Juvenile blue sharks could approximate equilibrium after less than 4 months following a switch in diet. White sharks could approximate equilibrium after 1.2 years, while mako sharks could reach equilibrium after 1.6 years (Fig. 15 a). After becoming subadults, blue sharks could approximate equilibrium after only one year, white sharks after two years and mako sharks after almost three years (Fig. 15 b). A dietary shift at the adult stage could take blue sharks up to 10 years to reach equilibrium, while white sharks would take close to six years and mako sharks almost eight years (Fig. 15 c).

The rate of isotopic turnover of $\delta^{15}N$ estimated for the three shark species and size classes using the coefficient of metabolic decay for muscle was slower than that for $\delta^{13}C$ values (Fig. 16). After birth and the initiation of feeding, the isotopic composition of juvenile blue sharks muscle would approximate equilibrium to a new food source (90% of isotopic change) in less than 0.5 yrs, while for juvenile white and mako sharks it could take more than 2 yrs (Fig. 16 a). If a change in diet occurs as the sharks enter the subadult stage, it could take several years to approximate equilibrium (up to two years for blue sharks, around four years for white sharks and more than five years for mako sharks) (Fig. 16 b). If adult white and mako sharks shift to a diet of different isotopic composition, up to 10 years could be needed to approximate equilibrium. After 10 years, adult blue sharks would have achieved less than 75% of the total change to equilibrium (Fig. 16 c).

Turnover rate estimates for the three target species and size classes are summarized in table XVI and XVII. The different c values used correspond to those of the three tissues sampled (liver, muscle and blood), and to the coefficient of metabolic decay used in the simple dilution model (c = -1). For some scenarios, the final percentage of isotopic change is different to 90 %, because due to different growth rates and metabolic turnover sharks will reach equilibrium either too fast or they are predicted never to reach equilibrium.

Coefficients of metabolic decay from carbon stable isotope ratios produced faster isotopic turnover times than nitrogen stable isotope ratios c values. For all c values, juvenile and subadult blue sharks had a faster isotopic turnover compared to juvenile and subadult mako sharks. When blue sharks reach the adult stage, its rate of isotopic turnover is predicted to be similar or slower than in mako and white adult sharks. For all size classes and c values, white shark showed faster turnover speeds than mako sharks. Excluding the blue shark adult size class due to its slow isotopic turnover, there was at least a twofold difference between the time needed to reach isotopic equilibrium based on the simple dilution model and empirical values of c. This difference was also observed for mako sharks (the slowest growing shark in terms of weight gain) (Tables XVI and XVII).
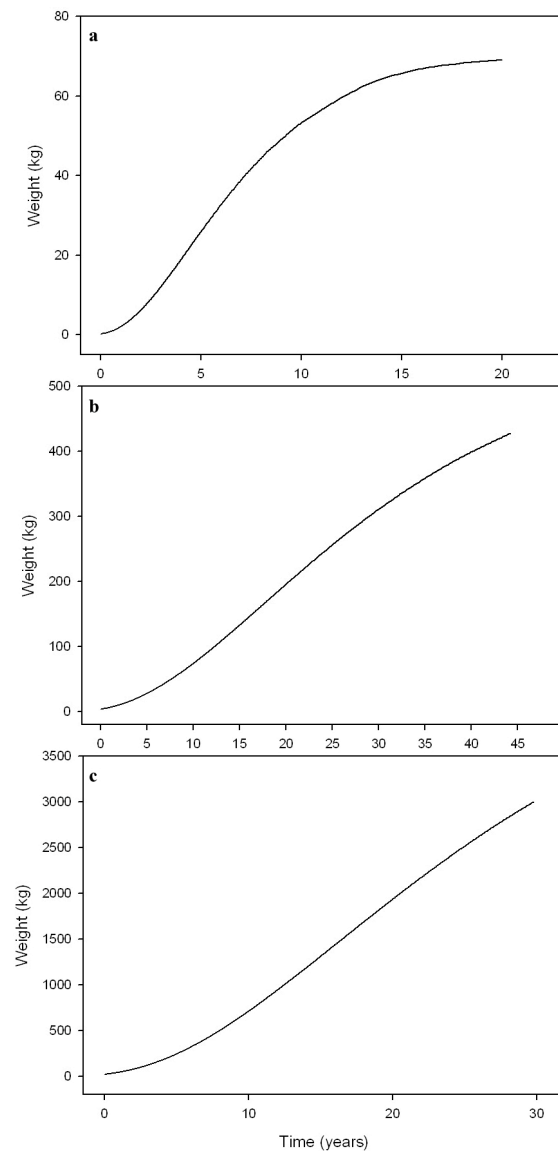
Figure 14. a) Age-weight relationship for blue sharks calculated using Cailliet & Bedford's (1983) von Bertalanffy growth function parameters and the weight-length relationship reported by Strasburg (1958). b) Age-weight relationship for mako sharks calculated using Ribot-Carballal *et al*.'s (2005) von Bertalanffy growth function parameters and the weight-length relationship reported by Shoou-Jeng & Hua-Hsun 2005. c) Age-weight relationship for white sharks calculated using Cailliet *et al*.'s (1985) von Bertalanffy growth function parameters and the weight-length relationship reported by Kohler *et al*. (1996).

Figura 14 a) Relación edad-peso para tiburón azul calculada usando parámetros de la función de crecimiento de von Bertalanffy de Cailliet & Bedford (1983), y la relación peso-longitud reportada por Strasburg (1958). b) Relación edad-peso para tiburón mako calculada usando parámetros de la función de crecimiento de von Bertalanffy de Ribot-Carballal *et al*. (2005), y la relación peso-longitud reportada por Shoou-Jeng & Hua-Hsun 2005. c) Relación edad-peso para tiburón mako calculada usando parámetros de la función de crecimiento de von Bertalanffy de Cailliet *et al*. (1985), y la relación peso-longitud reportada por Kohler *et al*. (1996).
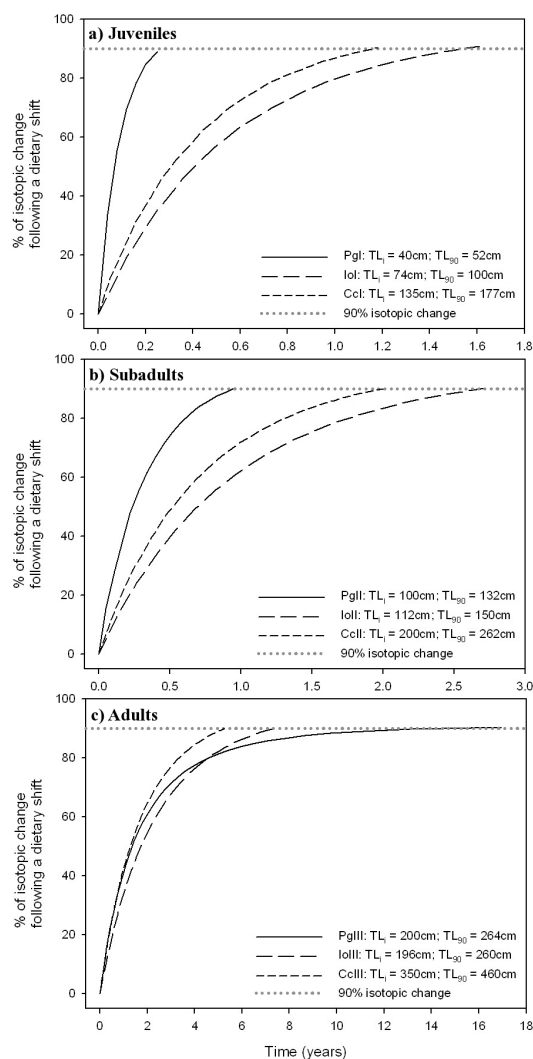
Figure 15. Percentage of isotopic change in δ^{13}C values predicted for different shark size classes following a simulated dietary shift to an isotopically distinct food source. The percentage of isotopic change was calculated using a laboratory-derived coefficient of metabolic decay (c = -2.7) and arbitrary δ^{13}C values. Isotopic turnover rates were calculated for I: juveniles (a); II: subadults (b); III: adults (c). Pg: blue shark; Io: mako shark; Cc: white shark. TL$_i$: total length when change in diet occurs; TL$_{90}$: total length when 90% of equilibrium has been reached. The dotted line indicates 90% of isotopic change. Growth rates were calculated using von Bertalanffy equations and weight-length relationships parameters from previous studies.

Figura 15. Porcentaje de cambio isotópico en valores δ^{13}C para diferentes clases de talla de tiburones posteriores a un cambio simulado a una fuente de alimento con composición isotópica diferente. El porcentaje de cambio isotópico fue calculado usando un coeficiente de decaimiento metabólico (c = -2.7) obtenido en laboratorio y valores δ^{13}C arbitrarios. El recambio isotópico fue simulado para: I: juveniles (a); II: subadultos (b); III: adultos (c). Pg: tiburón azul; Io: tiburón mako; Cc: tiburón blanco. TL$_i$: longitud total cuando ocurre el cambio en la dieta; TL$_{90}$: longitud total cuando los tejidos han alcanzado 90% de la nueva señal isotópica. Las líneas punteadas representan 90% de recambio isotópico. El crecimiento fue calculado usando la ecuación de von Bertalanffy y parámetros de relaciones peso-longitud de estudios previos.
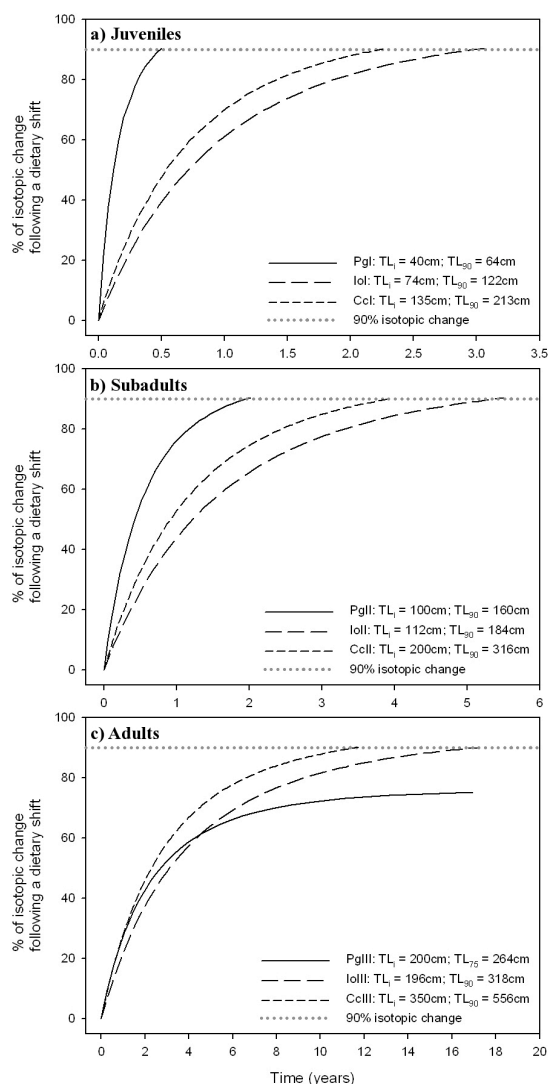
Figure 16. Percentage of isotopic change in δ^{15}N values predicted for different shark size classes following a simulated dietary shift to an isotopically distinct food source. The percentage of isotopic change was calculated using a laboratory-derived coefficient of metabolic decay (c = -1.6) and arbitrary δ^{15}N values. Isotopic turnover rates were calculated for I: juveniles (a); II: subadults (b); III: adults (c). Pg: blue shark; Io: mako shark; Cc: white shark. TL_i: total length when change in diet occurs; $TL_{75,90}$: total length when 75 or 90% of equilibrium has been reached. The dotted line represents 90% of isotopic change. Growth was calculated using von Bertalanffy equation and weight-length relationships parameters from previous studies.

Figura 16. Porcentaje de cambio isotópico en valores δ^{15}N para diferentes clases de talla de tiburones posteriores a un cambio simulado a una fuente de alimento con composición isotópica diferente. El porcentaje de cambio isotópico fue calculado usando un coeficiente de decaimiento metabólico (c = -1.6) obtenido en laboratorio y valores δ^{15}N arbitrarios. El recambio isotópico fue simulado para: I: juveniles (a); II: subadultos (b); III: adultos (c). Pg: tiburón azul; Io: tiburón mako; Cc: tiburón blanco. TL_i: longitud total cuando ocurre el cambio en la dieta; TL_{90}: longitud total cuando los tejidos han alcanzado 90% de la nueva señal isotópica. Las líneas punteadas representan 90% de recambio isotópico. El crecimiento fue calculado usando la ecuación de von Bertalanffy y parámetros de relaciones peso-longitud de estudios previos.

Table XIV. Estimated amount of time required for $\delta^{13}C$ values of juvenile (size class I), subadult (size class II) and adult (size class III) blue, mako and white shark to approximate isotopic equilibrium (90% of change) following a shift to an isotopically distinct food source. Laboratory derived coefficients of metabolic decay for liver, blood and muscle and published growth rates were used to model isotopic turnover rates. For comparative purposes, turnover rates calculated using a simple dilution model (no metabolic turnover) are also presented. TL_i: total length at the time of the dietary change; TL_f: total length when tissues have reached 90% of the new isotopic signal, unless otherwise noted due to limited weight gain for some size classes. Growth was calculated using von Bertalanffy equation and weight-length relationships parameters from previous studies.

Tabla XIV. Tiempo estimado requerido para valores $\delta^{13}C$ de tiburones azul, mako y blanco juveniles, subadultos y adultos posteriores a un cambio simulado a una fuente de alimento con composición isotópica diferente. Coeficientes de decaimiento metabólico para hígado, sangre y músculo obtenidos de un experimento en laboratorio y tasas de crecimiento de la literatura se usaron para modelar tasas de recambio isotópico. Para propósitos comparativos se presentan los resultados obtenidos usando valores c de un modelo de dilución simple (no existe recambio metabólico). TL_i: longitud total cuando ocurre el cambio en la dieta; TL_f: longitud total cuando los tejidos han alcanzado 90% de la nueva señal isotópica, o el valor indicado debido al bajo aumento de biomasa en algunas clases de talla. El crecimiento fue calculado usando la ecuación de von Bertalanffy y parámetros de relaciones peso-longitud de estudios previos.

Species	Size class	TL_i (cm)	c = -1 Simple dilution		c = -6.4 Liver		c = -3.3 Blood		c = -2.7 Muscle	
			TL_f (cm)	t (years)	TL_f (cm)	t (years)	TL_f (cm)	t (years)	TL_f (cm)	t (years)
Blue shark	I	40	85	1.4	46*	0.1	50	0.2	52	0.2
	II	100	230	6.6	112	0.3	126	0.7	132	1.0
	III	200	265†	21	224	2.0	250	6.5	264	16.9
Mako shark	I	74	164	6.2	84	0.6	94	1.2	100	1.6
	II	112	154	12.7	128	1.0	142	2.1	184	5.5
	III	196	370‡	30.5	220	2.5	248	5.8	260	7.3
White shark	I	135	285	4.6	151	0.4	151	0.4	177	1.2
	II	200	435	9.0	224	0.7	250	1.6	316	3.9
	III	350	655‡	22.4	394	1.9	438	4.0	460	5.3

* 93% of isotopic change
† 50% of isotopic change
‡ 85% of isotopic change

Table XV. Estimated time required for $\delta^{15}N$ values of juvenile (size class I), subadult (size class II) and adult (size class III) blue, mako and white shark to approximate isotopic equilibrium (90% of change) following a shift to an isotopically distinct food source. Laboratory derived coefficients of metabolic decay for liver, blood and muscle and published growth rates were used to model isotopic turnover rates. For comparative purposes, turnover rates calculated using a simple dilution model (no metabolic turnover) are also presented. TL_i: total length at the time of the dietary change; TL_f: total length when tissues have reached 90% of the new isotopic signal, unless otherwise noted due to limited weight gain for some size classes. Growth was calculated using von Bertalanffy equation and weight-length relationships parameters from previous studies.

Tabla XV. Tiempo estimado requerido para valores $\delta^{15}N$ de tiburones azul, mako y blanco juveniles, subadultos y adultos posteriores a un cambio simulado a una fuente de alimento con composición isotópica diferente. Coeficientes de decaimiento metabólico para hígado, sangre y músculo obtenidos de un experimento en laboratorio y tasas de crecimiento de la literatura se usaron para modelar tasas de recambio isotópico. Para propósitos comparativos se presentan los resultados obtenidos usando valores c de un modelo de dilución simple (no existe recambio metabólico). TL_i: longitud total cuando ocurre el cambio en la dieta; TL_f: longitud total cuando los tejidos han alcanzado 90% de la nueva señal isotópica, o el valor indicado debido al bajo aumento de biomasa en algunas clases de talla. El crecimiento fue calculado usando la ecuación de von Bertalanffy y parámetros de relaciones peso-longitud de estudios previos.

Species	Size class	TL_i (cm)	c = -1 Simple dilution		c = -2.9 Liver		c = -2.6 Blood		c = -1.6 Muscle	
			TL_f (cm)	t (years)	TL_f (cm)	t (years)	TL_f (cm)	t (years)	TL_f (cm)	t (years)
Blue shark	I	40	85	1.4	52	0.2	54	0.2	64	0.5
	II	100	230	6.6	130	0.9	134	1.0	160	2.0
	III	200	265†	21	224	2.0	264	17	264*	16.9
Mako shark	I	74	164	6.2	98	1.4	100	1.6	122	3.0
	II	112	154	12.7	148	2.5	152	2.8	184	5.5
	III	196	370‡	30.5	258	6.8	264	7.8	318	17.2
White shark	I	135	285	4.6	173	1.0	179	1.2	213	2.2
	II	200	435	9.0	258	1.8	266	2.1	316	3.9
	III	350	655‡	22.4	452	4.8	466	5.6	556	11.7

* 75% of isotopic change
† 50% of isotopic change
‡ 85% of isotopic change

VI. Discussion

VI.1 Laboratory experiment

There were significant differences in isotopic fractionation values among tissues, for both $\delta^{13}C$ (liver 2.36 ‰, blood and muscle 3.27 ‰, fin and cartilage 4.16 ‰) and $\delta^{15}N$ values (liver, blood, muscle and fin 1.76 ‰, cartilage 1.08 ‰). Similarly, Tieszen *et al*. (1983) reported differences in $\delta^{13}C$ fractionation values for different gerbil tissues (+1 ‰ to -3 ‰ for hair and lipids, respectively). Pinnegard & Polunin (1999) reported differences in $\delta^{13}C$ and $\delta^{15}N$ fractionation values of different tissues in rainbow trout. They attributed differences in ^{15}N fractionation among tissues to variations in the composition of essential and non-essential amino acids, while differences in ^{13}C fractionation was related to differences in lipid content. Miller (2006) suggested that differential allocation of specific amino acids to different proteins among tissues was responsible for variable fractionation of ^{15}N in Pacific herring.

To my knowledge, this is the first study to report $\delta^{15}N$ and $\delta^{13}C$ fractionation values for elasmobranchs, particularly for sharks, based on a controlled laboratory experiment. $\delta^{15}N$ trophic enrichment values were lower (1.08 - 1.76 ‰) than the mean value Post (2002) suggested could be used to estimate trophic level (3.4 ‰ for $\delta^{15}N$) based on the analysis of food webs in freshwater systems. Likewise, the $\delta^{13}C$ trophic fractionation factor I estimated (2.36 - 4.16 ‰) was higher that the mean value reported by Post (2002; 0.4 ‰ for $\delta^{13}C$). Post's (2002) values have been used to estimate trophic level in previous studies with sharks (Fisk *et al*. 2002; Estrada *et al*. 2003), although Post (2002) states that the average fractionation values reported in his studies must be used cautiously and preferably used in community level studies involving several trophic links, since variability in the average values is expected between single trophic transfers. Given that small errors in trophic fractionation factors can lead to the under or overestimation of an individual's trophic level (Post 2002), it is preferable to use taxa-specific values rather than mean values obtained from the literature (Gannes *et al*. 1997).

As hypothesized by Fisk *et al*. (2002), relatively low δ^{15}N trophic enrichment values in sharks may be due to the maintenance of high urea and trimethylamine-N-oxide (TMAO) levels in their tissues for osmoregulatory purposes. Instead of excreting urea, the shark renal system reabsorbs up to 95% into the body fluids (Evans *et al.* 2004; Hammerschlag, 2006). During protein catabolism, discrimination between amino acids containing ^{15}N and ^{14}N results in isotopically light urinary nitrogen relative to an animal's tissues (Macko & Estep, 1984; Minagawa & Wada, 1984). If there is limited loss of urea as an excretory product, it would be retained within the tissues. This implies less isotopic discrimination against the heavy isotope (^{15}N) compared to other ureotelic organisms, which would lead to the lower fractionation values observed in this study. In future studies, it would be useful to compare the isotopic composition of urea-extracted *versus* non-urea extracted tissues to evaluate the role of urea retention in δ^{15}N values of shark tissues.

Studies that have applied δ^{15}N analysis to estimate trophic level in sharks by using average fractionation values have yielded lower than expected trophic level estimates. For example, Fisk *et al.* (2002) used Hobson *et al.*'s (1995) average fractionation value of 3.8 ‰ for δ^{15}N, and estimated that Greenland sharks (*Somniosus microcephalus*) had the same trophic level as that of turbot (*Reinhardtius hippoglossoides*) and ringed seals (*Phoca hispida*), despite the prevalence of these species as prey in the stomach contents of the 14 sharks studied. Similarly, Ostrom *et al.* (1993) found that the basking shark (*Cetorhinus maximus*) had lower δ^{15}N values than that of the blue whale (*Balaenoptera musculus*), although they share dietary preferences. Thus, it is possible that trophic level estimates of sharks may be underestimated if "average" values derived from other taxonomic groups are used. When I compared trophic levels estimates using literature-derived average fractionation values to those I estimated using the values estimated experimentally, I found that trophic levels of sharks could potentially be underestimated.

The carbon trophic enrichment values I estimated were greater than the average values reported by Post (2002) and Vander Zanden & Rasmussen (2001) for various taxa. Carbon isotope fractionation is produced by discrimination of ^{12}C during respiration, which leads to enrichment in ^{13}C in consumer tissues (DeNiro & Epstein, 1978; Rau *et al*. 1983).

Further studies are needed to understand the metabolism responsible for the high trophic fractionation observed in leopard sharks.

With regard to among-tissue variations in trophic fractionation, it is important to consider the potential differential assimilation of major biochemical components of a diet to specific tissues, a process called isotopic routing (DeNiro & Epstein, 1978; Gannes *et al.* 1997). For example, Gaye-Siessegger *et al.* (2004) reported that Nile tilapia fed diets with different protein levels exhibited variations nitrogen and carbon fractionation among tissues. Since we used a commercial diet with a formulation that differs from that of the natural diet of sharks, trophic fractionation may differ from that observed under natural conditions.

During the laboratory experiment, the growth of early juvenile leopard sharks was consistent and there was no evidence of starvation, which has been shown to lead to high fractionation of nitrogen isotopes in some taxa. For example, for birds, Hobson *et al.* (1993) reported that after a fasting period, catabolism of existing tissues resulted in the enrichment of all body tissues in ^{15}N, while no difference was found for carbon isotope ratios. Given that there was no evidence of starvation during my experiment, I can attribute the changes in isotopic composition observed for all tissues solely to isotopic turnover resulting from the shift to an isotopically distinct diet.

The isotopic turnover of sharks kept under laboratory conditions was more rapid than expected based on a simple dilution (growth only) model. Based on the values of the coefficient of metabolic decay ($c \ll -1$), metabolic turnover contributed substantially to the isotopic turnover observed in leopard sharks. My findings differ from other studies done with juvenile poikilothermic teleosts, in which isotopic turnover has been attributed primarily to growth (Hesslein *et al.* 1993; Herzka & Holt, 2000; Herzka, 2005; Miller, 2006). My results are more similar to studies that have examined turnover rates in endothermic adults with low growth rates, in which a higher contribution of metabolism to isotopic turnover has been found (Tieszen *et al.* 1983, Hobson & Clark, 1992).

I found differences in tissue-specific isotopic turnover rates. Tissues with higher metabolic rates, namely liver and blood, had a faster turnover rate than muscle, cartilage

and fin tissue. This is similar to what has been reported in previous isotopic turnover studies done with gerbils (Tieszen *et al*. 1983), Japanese quail (Hobson & Clark, 1992), and Pacific herring (Miller, 2006), as well as in the only other laboratory study performed on an elasmobranch, the freshwater ocellate river stingray (*Potamotrygon motoro*; MacNeil *et al*. 2006).

VI.2 Female, embryo and juvenile SIR variability

Due to the small number of samples obtained in this study, I was unable to conduct statistical analyses to determine whether there were significant differences between embryonic and maternal shark tissues of *G. galeus*. Nevertheless, the differences I found in the isotopic composition of maternal and embryonic tissues suggests that intrauterine fractionation may occur between mother and embryos, and that differences in fractionation values might exist between tissues, although these differences were limited (< 1.5 ‰). Since sharks have different intrauterine feeding strategies (Carrier *et al*. 2004), variations in fractionation values between a mother and the embryos may occur as a function of reproductive strategy.

VI.3 Modeling of isotopic turnover rates for sharks

The shark species evaluated in this study are capable of extensive transoceanic migrations (Weng *et al*. 2007a; Domeier & Nasby-Lucas, 2008). Hence, it is important to consider the time integrated by stable isotope measurements conducted on wild-caught organisms to be able to to discriminate between migratory individuals and residents (Herzka, 2005). Further, if sharks move among areas in which food sources differ in isotopic composition, isotopic turnover rate estimate are needed to evaluate whether an isotopic signature corresponds to local feeding. Herzka (2005) used a simple dilution model and various estimates of natural growth rates to estimate the amount of time needed to approximate isotopic equilibrium following a dietary switch for larval, juvenile and adult teleost fishes, and predicted it could take years for equilibrium to be reached in older fish with low growth rates.

I estimated the time required for various shark tissues to reflect a shift in diet to a differing isotopic composition using published growth rates and by taking into account the relative contribution of metabolic turnover derived from the laboratory experiments on leopard sharks. For comparative purposes, I also estimated rates of isotopic turnover using a simple dilution (growth only) model.

When using a simple dilution model, shark tissues of all three target species were estimated to need years to reach isotopic equilibrium to a new diet (1.4 - 21 years for blue sharks, 6.2 - 30.5 years for mako sharks and 4.6 - 22.4 years for white sharks, depending on the life stage. Even the fastest growing of the three shark species and size classes studied (juvenile blue sharks) were predicted to need more than one year to approximate equilibrium. This would imply that the isotopic composition of juvenile and subadult tissues will not reach isotopic equilibrium to a new diet before entering the next size class, and that adult sharks would probably reach their maximum estimated longevity before reaching an isotopic equilibrium (longevity is 20 years for blue sharks Nakano & Stevens (2008), 45 years for mako sharks Cailliet *et al*. (1983) and 40-50 years for white sharks Bruce (2008)).

When both growth and metabolic turnover were incorporated into the $\delta^{13}C$ and $\delta^{15}N$ turnover rate model by using the coefficients of metabolic decay estimated for liver, blood and muscle based on the laboratory experiment, the times required for tissues to approximate equilibrium were reduced drastically relative to the simple dilution model. This was the case even for the slower growing shark species (mako and white shark) and life stages (subadults and adults). When metabolic turnover was accounted for, I found that the isotopic composition of juvenile sharks should integrate over a relative short time period (months), particularly for tissues with fast turnover rates, such as blood and liver. In contrast, the isotopic composition of tissues with lower metabolic turnover (*e.g.* muscle) of subadults and adults could integrate a dietary period of a few years.

Juvenile sharks should reflect the isotopic composition of the post-birth diet well before entering the subadult stage. Subadult mako and white shark tissues should also reflect changes in the isotopic composition of their diet well before entering the adult stage, while adults would reflect the change before reaching their maximum estimated longevity.

However, due to their slow rate of biomass gain, adult blue sharks are predicted to need more time than adult mako and white sharks to reach isotopic equilibrium to a new diet. In contrast, adult mako and white sharks should approximate equilibrium to a new food source within their lifetime if the dietary switch occurs during the beginning of the adult stage (196 cm TL for mako and 350 cm TL or white sharks).

Some teleost and cartilaginous fishes posses a network of blood vessels known as *retia mirabilis*, which allows them to preserve the heat produced as a byproduct of metabolism and maintain some parts of their body at a higher temperature than the external environment. This is the case for sharks of the Alopiidae and Lamnidae families, which includes the white and mako sharks. Due to this characteristic, these types of sharks have a higher metabolic rate than sharks lacking heat retention mechanisms (Carlson *et al*. 2004). In sharks with a higher metabolic rate such as the mako and white sharks, a more rapid turnover might occur than in fully poikilothermic sharks, such as the leopard sharks used in my laboratory experiment to estimate coefficients of metabolic decay. Hence, the predicted isotopic turnover rates derived in this study for mako and white sharks may be conservative estimates (*i.e.* the rate of isotopic turnover may be faster).

In addition, given that the relative weight gain used in the isotopic turnover models is not tissue-specific, there could be differences in the rate of isotopic turnover under natural conditions. Tissues with high renewal rates, such as the liver, will have faster isotopic turnover rates than slow renewal tissues like cartilage. Despite this consideration, it is evident that turnover times in tissues of large and slow growing sharks is not as fast as those found in endothermic animals (*e.g.* mammals: Tieszen, 1983; birds: Hobson & Clark, 1992) or fast-growing poikilothermic animals (*e.g.* Fry & Arnold, 1982; Hesslein *et al*. 1993; Herzka, 2005; Miller, 2006).

VI.4 Ontogenetic shifts in trophic level

Due to their life history strategies, ontogenetic changes in sharks are likely to be common. They have been previously reported for a variety of species, including the spiny dogfish (Alonso *et al*. 2002), tiger shark (Lowe *et al*. 1996), sandbar shark (McElroy *et al*.

2006), and others. These studies have been based on stomach content analyses. To my knowledge, the use of stable isotope ratios to assess ontogenetic changes in feeding for sharks has only relied on the analysis of shark vertebrae (Kerr *et al*. 2006; Estrada *et al*. 2006).

The samples I collected in the SCB were limited in number, and it was not always possible to sample various tissues from the same shark. This limited my ability to perform a thorough evaluation of ontogenetic changes in feeding habits and between-species comparisons using those data. However, the samples obtained in the Mexican Pacific allowed for a more robust comparison between size classes and species. Considering the data from all tissues samples from the Mexican Pacific, I documented enrichment in δ^{15}N as a function of size class as well as variations between species.

Differences in nitrogen isotope ratios between size classes were found for mako and white shark based on blood and muscle; larger size classes exhibited enrichment in ^{15}N. The consistent difference in the nitrogen isotope ratios between tissues with fast (blood) and slow (muscle) turnover is strong evidence of an increase in trophic level related to ontogeny.

I found juvenile white sharks' blood δ^{15}N values to be enriched by 2.17 ‰ and muscle 1.59 ‰ to adults, which corresponds to a difference in trophic level of 1.2 - 0.9, respectively. An ontogenetic shift has been described for white sharks based on stomach content analysis. Juveniles feed mainly on fishes, while adults (> 340cm TL) include marine mammals in their diet (Klimley, 1985; Compagno, 2001). An ontogenetic shift in feeding has also been reported for great white sharks based on stable isotope analysis of vertebrae, a tissue with a low metabolic rate (Estrada *et al*. 2006). Estrada *et al*. (2006) also found evidence of a shift from a yolk to a fish-based diet between neonates and early juvenile white sharks in the north Atlantic. However, a study using the same approach to examine feeding in white sharks in the northeastern Pacific did not detect evidence of trophic differences between juvenile and adult sharks (Kerr *et al*. 2006). A comparison including subadult size class samples is needed to assess differences between the three size classes.

Juvenile mako shark blood and muscle δ^{15}N values were enriched to subadults by 0.4 ‰ and 0.8 ‰ respectively, which would correspond to a difference in trophic levels of 0.3 and 0.5 respectively. Dietary studies of mako sharks in the Eastern Pacific are scarce. Off the coast of California, they have been reported to feed on several teleost species and squid (Hanan *et al.* 1993). Based on a stomach content analysis of two adult makos, this size class feeds on larger teleosts like billfishes and even marine mammals (Holts pers. comm. as reported in the HMS-FMP-Appendix F). If this is a consistent feeding behavior, the enrichment in ^{15}N I observed in subadults relative to juveniles would be consistent with a dietary shift related to ontogeny. Samples from adult mako sharks for stable isotope analysis would be needed to further test this hypothesis.

Blood, muscle and liver of juvenile blue sharks had lower δ^{15}N values (0.2-0.5 ‰) than adult and subadult sharks, while nitrogen isotope ratios of subadult and adult blue sharks exhibited a smaller difference (< 0.3 ‰). This led to a < 0.3 difference in trophic levels among size classes. Dietary studies indicate blue sharks feed on small teleost fishes, squid and occasionally on some small shark species (Compagno, 2001; Harvey, 1989). Based on what has been observed for other shark species, it is unlikely that dietary preferences will be similar among size classes. To my knowledge, there are no published studies reporting an ontogenetic diet shift in blue sharks.

Accordingly to my turnover rate model, the slow growth of subadult and adult blue sharks should limit the rate of isotopic turnover, especially in muscle. A slow rate of isotopic turnover could explain the apparent lack of enrichment between adult, subadult and juvenile sharks, even if a shift in diet does occur during the subadult or adult stage. More generally, for sharks with low growth rates during the subadult and adult stage, the interpretation of stable isotope ratios must be conducted cautiously, and the evaluation of dietary shifts should be preferably conducted in tissues with a fast metabolic and turnover rate (*i.e.* blood and liver). The fast turnover rate expected in neonates might produce the step increase observed in δ^{15}N at small sizes as the yolk diet isotopic signature is diluted by growth, as proposed by Estrada *et al.* (2006) for juvenile white sharks based on SIR analysis of vertebrae.

VI.5 Foraging grounds

Carbon isotope ratios have been useful for differentiating between offshore pelagic, inshore and benthic feeding habits. Several studies have shown that food webs in offshore regions tend to have lower $\delta^{13}C$ values than more coastal areas (Hobson *et al*. 1994; Sydeman *et al*. 1997). In general, I did not find evidence of differences in feeding grounds associated with $\delta^{13}C$ values of the three size classes I examined. The greatest difference I found in my analysis was between juvenile and adult white shark muscle (2.07 ‰ absolute difference in $\delta^{13}C$ values). However, those differences may be related to the high trophic fractionation value found for muscle $\delta^{13}C$ values rather than variations in isotopic composition of the base of the food web. Nevertheless, a thorough characterization of $\delta^{13}C$ values of lower trophic levels in my study area is needed to be able to identify the causes of the limited variation in $\delta^{13}C$ values I found.

I found a difference between average plasma and muscle carbon isotope ratios of adult white sharks sampled in Guadalupe Island. Given that blood plasma has been shown to have very fast turnover rates in some taxa (Hobson & Clark, 1993), relatively light plasma $\delta^{13}C$ values could be related to a recent migration to an offshore pelagic feeding ground from a more coastal area characterized by food webs more enriched in ^{13}C. Adult white sharks found in Guadalupe Island and California have been reported to migrate to potential offshore foraging areas in the eastern Pacific (Weng *et al*. 2007a; Domeier & Nasby-Lucas, 2008).

In addition, I found enriched blood and muscle $\delta^{13}C$ values in juvenile white sharks compared to blue and mako sharks captured in Vizcaino Bay, which might indicate a preference for benthic prey. The artisanal fishing technique in the region primarily uses gill-nets set on the bottom. Juvenile white sharks are predominantly captured as bycatch with this type of gear (Santana-Morales, 2008), which would be consistent with the use of a benthic habitat. Also, Sosa-Nishizaki (personal communication) conducted stomach content analysis in juvenile white sharks caught in the Gulf of California, and found a high percentage of benthic prey such as the bat ray (*Myliobatis californica*). The potential preference from benthic resources found in this study are also consistent with satellite tagging studies; Weng *et al*. (2007b) suggest that there is resource partitioning between

mako and juvenile white sharks, and that the later prefers demersal resources, while mako shark feeds on epipelagic prey.

Carbon isotope ratios of blood and muscle obtained from blood and muscle in field samples had similar isotopic differences compared to those found in the laboratory experiment ($\delta^{13}C$ values enriched 1 ‰ compared to liver). Liver samples of sharks from both study areas were depleted by 1 - 3 ‰ compared to blood and muscle $\delta^{13}C$ values. This finding is consistent with the fractionation values estimated from the laboratory experiment. The faster isotopic turnover rate observed for liver tissue in the laboratory might indicate it is more sensitive to changes in diet and or feeding grounds, which could explain the high variability found in liver $\delta^{13}C$ values compared to the more limited variability found in blood and muscle carbon isotope ratios.

VI.6 Isotopic differences between SCB and the Mexican Pacific

I found differences in the isotopic composition ($\delta^{15}N$ and $\delta^{13}C$ values) of shark tissues from the same species and size-classes sampled in two different areas of the eastern north Pacific. This could be attributed to differences in trophic level, although this must be considered cautiously because the base of the food web was not sampled in this study. Wallace *et al.* (2006) found that $\delta^{15}N$ values of leatherback turtles sampled in the eastern Pacific and north Atlantic Oceans differed substantially, despite their known similar feeding preferences. They concluded that differences in the isotopic compositions of turtles among ocean basins were caused by differences in nitrogen cycling regimes that led to variations in the isotopic composition at the base of the food web. Marine primary producers use different forms of nitrogen (*e.g.* NO_3^-, NH_3), and their availability depends upon oceanographic (*e.g.* upwelling) and biological processes (*e.g.* bacterial denitrification and nitrogen fixation) (Tyrrell, 1999; Deutsch *et al.* 2007). For the North Pacific, Mullin *et al.* (1984) analyzed the isotopic composition of zooplankton samples and related enriched $\delta^{15}N$ values to the exchange between deep water (^{15}N enriched water) and shallow water (^{14}N enriched water). Lower $\delta^{15}N$ values were found in areas where nitrogen fixation was

high. The isotopic differences I observed between SCB and Mexican Pacific sharks are more likely to be attributed to nutrient sourcing processes than to differences in diet.

It is interesting to note that isotopic differences were found between SCB and Mexican Pacific sharks despite the fact that several shark species have been reported to migrate between California and Baja California waters (Hanan *et al.* 1993; California Department of Fish and Game 1999b, 2000; Medellin-Ortiz, 2008; Weng *et al.* 2007b). Further stomach content studies of sharks studied in our sampling areas are needed as well as an ecosystem study to assess whether the same isotopic differences can be found in lower trophic levels.

VII. Conclusions

Metabolic turnover contributed substantially to the isotopic turnover observed in leopard sharks. Tissues with higher metabolic rates, namely liver and blood, had a faster turnover rate than muscle, cartilage and fin tissue. There were significant differences in isotopic fractionation values among tissues for $\delta^{13}C$ values (liver 2.36 ‰, blood and muscle 3.27 ‰, fins and cartilage 4.16 ‰) as well as $\delta^{15}N$ values (liver, blood, muscle and fins 1.76 ‰, cartilage 1.08 ‰). The trophic enrichment estimates found for $\delta^{15}N$ and $\delta^{13}C$ values were different to the average values reported in the literature, implying that using average fractionation values derived for other taxa would lead to incorrect estimates of TL or the incorrect identification of potential carbon sources.

It is expected that in natural shark populations, both growth and metabolic turnover would influence isotopic turnover, thus juvenile shark tissues, and fast turnover adult tissues would integrate a relative short time period (months). The isotopic composition of tissues with lower turnover, such as subadult and adult tissues, such as muscle, could integrate a dietary period of years.

Differences in nitrogen isotope ratios between size classes were found for blood and muscle of mako and white sharks sampled in the Mexican Pacific; larger size classes exhibited enrichment in ^{15}N. The consistent difference in the nitrogen isotope ratios between size classes in tissues with fast (blood) and slow (muscle) turnover is strong evidence of an increase in trophic level related to ontogeny.

The relatively high trophic fractionation value found for $\delta^{13}C$ values of muscle and blood made it difficult to assess variations in the isotopic composition at the base of the food web (*i.e.* different foraging grounds), among size classes. However, SIR of sharks captured in the same region suggest that juvenile white sharks preferentially feed from a benthic food web, and the comparison in the SIR of a fast and a slow turnover rate tissue suggested a recent feeding migration in adult white sharks.

The analysis of stable isotopes can be used to study dietary and habitat preferences of the different size classes of sharks. However, caution must be used when selecting the

tissue to analyze (fast or slow turnover rates). It is important also to consider the species, physiology, life stage and growth rate. In order to appropriately interpret isotopic data obtained from sampling of large pelagic sharks, it is necessary to compare it to studies applying other methodologies, such as stomach content analysis and satellite tagging studies. Nevertheless, SIR can help to broaden the understanding of dietary and habitat preferences of the different size classes of large pelagic sharks.

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