UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
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HISTORIA DE VIDA DEL TIBURÓN MARTILLO *Sphyrna lewini* EN EL PACÍFICO MEXICANO

TESIS
(POR ARTÍCULOS CIENTÍFICOS)

QUE PARA OPTAR POR EL GRADO DE:
DOCTORA EN CIENCIAS
BIOLOGÍA MARINA

PRESENTA:
CLAIRE COIRATON

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Mazatlán, México, febrero 2020

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Ilustración “Historia de vida del tiburón martillo en el Pacifico mexicano” por ©*Flora pixelia*

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RESUMEN

La disminución masiva de las poblaciones de tiburón martillo común *Sphyrna lewini* en varias regiones de su área de distribución durante las últimas décadas hace necesario mejorar las medidas de conservación actualmente implementadas en México. Las características de su historia de vida, adaptaciones morfológicas, fisiológicas y de comportamiento limitan drásticamente sus posibilidades de recuperarse de una captura excesiva, por lo que es primordial tener un conocimiento detallado y actualizado de su distribución, estructura de tallas, parámetros de crecimiento, patrones de movimientos y uso de hábitat. El presente trabajo de tesis permitió documentar aspectos importantes de la historia de vida de esta especie en el Pacifico mexicano aún no estudiados, a partir de datos provenientes de las pesquerías artesanal y semi-industrial. Se emplearon diferentes métodos que incluyeron el análisis de tallas de capturas, estimaciones de edad con base al conteo de bandas de crecimiento en vértebras y la validación subsecuente de su ciclo de crecimiento, así como la caracterización de sus patrones migratorios y cambios ontogenéticos y sexuales de uso de hábitat en la región por medio de análisis microquímicos de vértebras. Los resultados mostraron que las medidas de manejo actuales podrían ser insuficientes para el manejo sostenible de la población de *S. lewini* en el Pacífico mexicano debido a que en esta región: (1) es probable que se capturen en mayoría organismos inmaduros, especialmente al finalizar los meses de veda, lo que podría impedirles alcanzar su talla de primera madurez y reclutarse al stock adulto, (2) un solo par de bandas de crecimiento se forma cada año en las vértebras la periodicidad de formación de bandas de crecimiento vertebrales es anual, por lo cual la constante de crecimiento podría ser menor a la estimada previamente, al menos para los organismos juveniles, (3) los organismos adultos, y en particular los machos, ocupan hábitats costeros más tiempo de lo que se suponía antes siendo por lo tanto más susceptibles a ser capturados por las pesquerías que operan en la zona, y (4) las hembras preñadas pueden permanecer cerca de la costa durante su gestación o bien regresarse a la costa antes de que los embriones estén completamente formados donde son más accesibles por las pesquerías, lo cual podría limitar las posibilidades de llevar a cabo su gestación y reducir el potencial reproductivo de la población. Se concluyó que las medidas de manejo actualmente implementadas en el Pacífico mexicano para la pesca responsable de tiburones (NOM-029-PESC-2006) y su prohibición estacional entre mayo y julio (NOM-009-SAG/PESC-2015) podría ser insuficientes para que la población de *S. lewini* en aguas mexicanas se pueda mantener y regenerar teniendo en cuenta sus características de historia de vida y adaptaciones específicas. Una solución de manejo más viable podría ser el establecimiento de zonas de refugio costeras que complementen las limitaciones temporales ya existentes. A pesar de que en la actualidad no se aprecia una reducción inmediata en las capturas y desembarcos de esta especie, una vez que esta se dé, podría ser irreversible como se ha reportado para tres otras especies de su genero (*S. media*, *S. mokarran* and *S. tiburo*) cuyas abundancias disminuyeron considerablemente durante las ultimas décadas en el Pacífico mexicano, e incluso desaparecieron del Golfo de California.

ABSTRACT

Following the massive declines in abundance of the scalloped hammerhead shark *Sphyrna lewini* in several regions of its distribution range during the last decades, and considering that the life history traits and morphological, physiological and behavioral adaptations of this species have drastically limited its chances to recover from an excessive exploitation, obtaining updated and accurate information of its distribution, size structure, growth parameters, movement patterns and habitat use is crucial to help to establish more protection areas and improve the conservation efforts currently implemented in Mexico. The research carried out as part of this thesis allowed to document several novel aspects on the life history of *S. lewini* in the Mexican Pacific not reported in the literature through the use of different methods that included the analysis of catch size data obtained from both the artisanal and semi-industrial fisheries, age estimates based on the vertebral growth band counts and subsequent validation of their growth cycle, as well as the characterization of its migratory patterns and ontogenetic and sexual habitat use changes in the region through microchemical analysis of the vertebrae. The results showed that current management measures may be insufficient for the sustainable management of the population of *S. lewini* because in this region: (1) fisheries mainly capture immature specimens, especially following birthing season between August and November, thus precluding juveniles to reach their reproductive size, (2) vertebral growth band cycle is slower (*i.e.* annual) than previously reported (*i.e.* biannual), at least for the juveniles, (3) adult, and in particular the males, have probable stronger affiliation with coastal habitats than previously assumed, thus being more susceptible to be caught by artisanal fisheries operating in the area, and (4) pregnant females can either remain nearshore during complete gestation or entering coastal nurseries slightly before term, therefore possible limiting their possibilities to complete gestation to the term and reducing the reproductive potential of the population as it is briefly centralized in coastal areas, more accessible by fisheries. Overall, the findings obtained in this study allowed to conclude that current management measures in the Mexican Pacific such as the regulation norm of the commercial shark fishery (NOM-029-PESC-2006) and its seasonal prohibition between May and July (NOM-009-SAG/PESC-2015) may be insufficient for the sustainable management and conservation of this population of *S. lewini,* especially considering the life history traits and highly evolved biological and ecological adaptations of this endangered species. An additional measure could be the creation of more protection and refuge areas that complement the existing temporary limitations since it is possible that, even if at present the population decline may not be yet discernible, consequences could be irreversible, as reported for three other species of its genus (*S. media*, *S. mokarran* and *S. tiburo*) that considerably declined in Mexican Pacific during the last decades and even became extinct from the Gulf of California.

CAPÍTULO 1

INTRODUCCIÓN

Los tiburones son depredadores de los ambientes marinos vulnerables a la explotación pesquera al tener, en general, tasas de crecimiento bajas, madurez tardía y baja fecundidad, lo que resulta en un crecimiento poblacional lento y una baja resiliencia a los cambios rápidos (Musick 1999; Stevens et al. 2000; Frisk et al. 2001; Schindler et al. 2002). Estos parámetros tienen como consecuencia una capacidad limitada de las poblaciones de estas especies en soportar la presión pesquera y recuperarse de una captura excesiva (FAO 1998), debido a que se encuentran cada vez más amenazadas por la intensa explotación a la que están sometidas (Stevens et al. 2000), la degradación de sus hábitats generada por el incremento de la población humana y la demanda en recursos alimentarios subsecuente (Butchart et al. 2010) así como los efectos del cambio climático (Chin et al. 2010; Lasram et al. 2010; Pistevos et al. 2015).

De manera general, la preocupación sobre los impactos de la explotación pesquera en las especies de tiburones aumentó desde que se demostró que varias de sus poblaciones habían disminuido drásticamente en los océanos durante las últimas décadas debido a los efectos directos e indirectos de la pesca (Baum et al. 2003; Shepherd y Myers 2005; Myers et al. 2007; Dulvy et al. 2008; Ferretti et al. 2008; Hayes et al. 2009; Baum y Blanchard 2010), lo cual ha llevado a una mayor focalización de los esfuerzos de conservación en este grupo de peces (Musick et al. 2000; Stevens et al. 2000). Entre las especies cuyas abundancias han más disminuido se encuentran, en orden descendente, las especies de tiburones martillo (*Sphyrna* spp), varias especies de Carcharhinidos costeros (*Carcharhinus falciformis*, *C. limbatus, C. obscurus, C. plumbeus*, *C. signatus* y *C. leucas*) junto con algunas especies de tiburones oceánicos en algunas partes del mundo como por ejemplo el tiburón azul *Prionace glauca*, tiburón azul *Prionace glauca*, el tiburón punta blanca oceánico *Carcharhinus longimanus,* el tiburón zorro *Alopias vulpinus* y el tiburón mako (*Isurus oxyrinchus*) (Myers et al. 2007; Ferretti et al. 2008; Baum y Blanchard 2010). .A pesar de que algunas de estas especies han sido recientemente protegidas por legislaciones nacionales y tratados internacionales debido a su insuficiente estado de conservación (ver Fischer et al. 2012), el incremento de la presión pesquera y la expansión de las áreas explotadas es motivo de preocupación por sus consecuencias en las especies que siguen siendo explotadas y sus ecosistemas.

Más específicamente, la disminución de la abundancia o desaparición de poblaciones de algunas especies de tiburones puede llevar a la desaparición completa de eslabones de la cadena trófica por el incremento de abundancia de especies mesodepredadoras más pequeñas puesto que dichas especies ya no podrían cumplir con su papel ecológico de depredador tope (Dulvy et al. 2000; Shepherd y Myers 2005; Myers et al. 2007; Ferretti et al. 2010). Aunado a lo anterior, algunas especies de tiburones pueden ser más vulnerables de lo esperado a las amenazas humanas, independientemente (pero no exclusivamente), de sus características de historia de vida como resultado de especializaciones ecológicas, comportamentales y funciones altamente evolucionadas (Futuyma y Moreno 1988; Irschick et al. 2005; Gallagher et al. 2012). Esta vulnerabilidad adicional se puede provocar cuando rasgos o comportamientos que presumiblemente eran adaptativos bajo los regímenes de selección históricos (*e.g.* formación de cardúmenes, forma de la cabeza) repentinamente se volvieron una desventaja bajo los cambios antropogénicos recientes (Harcourt et al. 2002; Gallagher et al. 2014). Clásicamente, este tipo de dinámica podrá generar una disminución significativa de una población, y en algunos casos, la extirpación local de una especie (Rodewald et al. 2011). Por lo tanto si bien el aprovechamiento sustentable del tiburón es posible, exige un manejo pesquero estricto que conlleve una disminución de las capturas de acuerdo a las poblaciones remanentes, y el conocimiento detallado de sus características de historia de vida tales como distribución, biología de la reproducción, edad y crecimiento poblacional, uso de hábitat y patrones de movimientos para poder implementar medidas de protección apropiadas y eficientes, esenciales para el mantenimiento o reconstitución de una población (FAO 1998).

El tiburón martillo *Sphyrna lewini* es una especie tropical pelágico-costera ampliamente distribuida y vulnerable a la explotación pesquera durante todas las etapas de su desarrollo debido a sus características de historia de vida (*i.e.* tasas de crecimiento bajas, madurez tardía; *e.g.* Piercy et al. 2007; Torres-Huerta et al. 2008). Otras características relevantes son sus adaptaciones morfológicas (*i.e.* anatomía de la cabeza incrementando susceptibilidad de captura y pequeño tamaño de boca posiblemente limitando asimilación de oxigeno al ser capturado; Gallagher et al. 2014), fisiológicas (*i.e.* tasas de sobrevivencia extremadamente bajas al ser liberado después de una captura incidental; Morgan y Burgess 2007, Gulak et al. 2015) y de comportamiento (*i.e.* formación de cardúmenes, migraciones entre zonas costeras y de mar abierto, y fidelidad hacia zonas de crianza; *e.g.* Klimley y Nelson 1981; Klimley 1985) altamente evolucionadas que han limitado drásticamente sus posibilidades de recuperarse de una captura excesiva (Gallagher et al. 2014). Aunado a lo anterior, el alto valor comercial de sus aletas debido al mayor número de rayos que las componen en comparación con otras especies de tiburones ha contribuido en aumentar significativamente su vulnerabilidad (Rose 1996). Aunque *S. lewini* se evaluó en 2007 en Peligro de Extinción a Nivel Mundial en la Lista Roja de la Unión

Internacional para la Conservación de la Naturaleza (IUCN por sus siglas en inglés; Baum et al. 2007), los países miembros de la Convención del Comercio Internacional de Especies Amenazadas de Fauna y Flora (CITES, por sus siglas en inglés) decidieron incluir a esta especie también en el Apéndice II de esta Convención en 2013 con el fin de controlar el comercio internacional de sus productos derivados, y en especial sus aletas (CITES 2013). Esta categoría de protección no prohíbe la comercialización de la especie, pero ejerce un control más estricto sobre las cantidades que se comercian internacionalmente.

En México, el tiburón martillo *S. lewini* es una especie de alto interés comercial. Su captura es abundante tanto en pesquerías de pequeña escala como semi-industriales en el Pacífico mexicano, y en particular en el sur del Golfo de California y en el Golfo de Tehuantepec donde más se captura (CONAPESCA-INP 2004), pudiendo contribuir hasta el 70% de la biomasa extraída total de tiburón en estas regiones (Ruiz y Madrid 1997; Anislado-Tolentino y Robinson-Mendoza 2001). Aunque las aletas de los tiburones martillo (*Sphyrna* spp) son de alto valor en el mercado internacional (Rose 1996; Clarke et al. 2005), en México *S. lewini* se captura principalmente para el consumo de su carne, la cual representa una fuente de proteína de bajo costo para las comunidades costeras y el resto del país (Bonfil 1994). A pesar de los numerosos estudios de *S. lewini* que se han llevado a cabo en el país, la información sobre la historia de vida de esta especie sigue siendo relativamente escasa, lo cual posiblemente esté relacionado con la dificultad en obtener tamaños de muestras suficientes y/o que abarquen todos los componentes de las poblaciones de estudio, ya que neonatos, juveniles, hembras y machos adultos por lo general ocupan distintos hábitats (Clarke 1971; Klimley 1987). Más específicamente, no existe información en lo que respecta a la comparación de datos de captura de *S. lewini* de las flotas de pequeña escala e industrial entre zonas costeras y de mar abierto en el Pacífico mexicano a pesar de la importancia pesquera de esta especie en la región. Esta información es primordial ya que mediante la utilización de datos de captura y frecuencia de longitudes de tallas es posible predecir la distribución espacial de los diferentes estadios de madurez, así como sus frecuencias de captura a lo largo del año y periodos y zonas de máxima susceptibilidad de captura de los mismos (Maunder y Watters 2003; Furlong-Estrada et al. 2014). Por otro lado, si bien la edad y crecimiento de *S. lewini* se han estimado en varias zonas del Pacífico mexicano (Anislado-Tolentino and Robinson-Mendoza 2001; Anislado-Tolentino et al. 2008), no se ha validado el patrón de crecimiento de la especie (anual o bienal), lo cual es primordial para poder estimar parámetros de crecimiento y productividad de una población e implementar medidas de manejo apropiadas.

En el Pacífico mexicano, las crías de *S. lewini* nacen entre mayo y julio en áreas de crianza de poca profundidad (Torres-Huerta 1999; Torres-Huerta et al. 2008) donde permanecen hasta alcanzar una longitud total de aproximadamente 95-115 cm para las hembras y 160 cm para los machos antes de iniciar migraciones hacia hábitats pelágicos (Klimley 1987; Hoyos-Padilla et al. 2014) y formar cardúmenes cerca de bajos oceánicos e islas volcánicas (Klimley and Nelson 1981; Klimley 1985). Valiosos estudios de marcajes y contenidos estomacales permitieron documentar aspectos importantes de los patrones de movimientos horizontales y verticales y uso de hábitat de *S. lewini* en el Pacifico tropical oriental en general (Klimley et al. 1988; Klimley 1993). Sin embargo, casi no existe información acerca del uso de hábitat de los organismos juveniles de la especie, y en particular después de que estos se hayan alejado de la zona costera. Por otro lado, si bien estudios de reproducción indicaron que *S. lewini* tiene un ciclo de reproducción anual, por lo cual hembras adultas probablemente regresarían a la costa cada año para tener a sus crías, no existe información acerca del comportamiento migratorio de hembras preñadas durante su gestación. Finalmente, si bien valiosos estudios genéticos indicaron que los machos adultos de *S. lewini* aparentemente se dispersan en el mar abierto durante el resto de su vida siendo responsable de la mayor parte del flujo genético de la especie por sus migraciones entre las distintas regiones oceánicas de su rango de distribución (Daly-Engel et al. 2012), se ha propuesto también que algunos machos de esta especie podrían en realidad adoptar una distinta estrategia de dispersión al quedarse en áreas costeras durante su ciclo de vida completo (Harry et al. 2011).

Por lo anterior, el presente trabajo se enfocó en generar información novedosa de la historia de vida de *S. lewini* en el Pacífico mexicano mediante el análisis de muestras provenientes de la pesca de pequeña escala, enfocada en zonas costeras, así como de muestras provenientes de la pesca semi-industrial, la cual se enfoca en zonas pelágicas, a fin de contar con una amplia representatividad de organismos de diferentes tallas, sexos, latitudes y ambientes. Específicamente, se analizaron su distribución y estructura de tallas (Capítulo 2), edad y crecimiento (Capítulo 3), y uso de hábitat y patrones de migración (Capítulos 4 y 5), mediante análisis y técnicas innovadoras como lo son el análisis de Bhattacharya para la descomposición gaussiana de la distribución multimodal, los modelos de Okamura y Semba (2009) y Okamura et al. (2013) para la determinación de periodicidad de formación de bandas de crecimiento en vértebras y el análisis de microquímica vertebral mediante el empleo de un sistema de ablación láser acoplado inductivamente con un espectrómetro plasma de masas (LA-ICP-MS) para inferir cambios ontogenéticos y/o sexuales de uso de hábitat y patrones migratorios.

El tiburón martillo *S. lewini* es una de las especies de tiburones más explotadas, y su manejo y conservación se dificultan al no conocer adecuadamente sus patrones de migración y uso de hábitat. Esta información es esencial ya que mediante la misma se pueden identificar hábitats clave (áreas de crianza y nacimiento, y rutas migratorias por ejemplo), lo que podría ayudar a definir zonas prioritarias de protección o refugio para esta especie (Speed et al. 2010). El conocimiento de sus hábitats clave resulta relevante desde el punto de vista tanto evolutivo como de su explotación sustentable y conservación. Por lo tanto, la información generada por este estudio podría usarse para mejorar las medidas de conservación actuales y llevar a cabo una explotación sustentable de la especie. Más específicamente, estos resultados podrán complementar las medidas contempladas por la NOM-029-PESC 2006 (DOF 2007) en lo que respecta a los patrones de movimiento de *S. lewini* entre costa y mar abierto así como sus zonas de refugio, ya que en la actualidad no queda claro si este documento toma en cuenta también el uso del mar abierto especifico a esta especie, el regreso de hembras preñadas a la costa, y el uso de otras posibles áreas de crianza aún no identificadas en el Pacífico mexicano.

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CAPÍTULO 2

ESTRUCTURA DE TALLAS DE CAPTURA DEL TIBURÓN MARTILLO COMÚN (*Sphyrna lewini*) EN EL PACÍFICO MEXICANO

Trabajo publicado en: Coiraton C, Amezcua F & Salgado-Ugarte I (2017) Estructura de longitudes de las capturas del tiburón martillo común (*Sphyrna lewini*) en el Pacífico mexicano. Ciencia Pesquera. 25(1): 25-40

RESUMEN

El tiburón martillo *Sphyrna lewini* es una de las especies de tiburón más frecuentes en las pesquerías artesanal y semi-industrial en el Pacifico mexicano, sin embargo, existe poca información sobre su distribución y estructura de longitudes a pesar de su importancia pesquera y ecológica, lo que dificulta la implementación de medidas de manejo. Se describe la distribución de longitudes de captura por sexo y pesquería de *S. lewini* en el Pacífico mexicano. Entre agosto 2013 y abril 2016, se recolectaron 240 organismos; 75% fueron capturados por la flota artesanal, con longitudes <100 cm de longitud total (LT), que corresponden a organismos inmaduros. La LT_{min} fue 45 cm y la LT_{max} 302.9 cm. Los resultados coinciden con estudios que indican como zonas de crianza el sur de Sinaloa, norte de Nayarit y de Oaxaca, con nacimiento entre mayo y agosto, y longitud de nacimiento de entre 45 y 47 cm LT. Los organismos inmaduros que se encuentran en la zona costera son más susceptibles a la pesca artesanal, especialmente cuando nacen, mientras que los organismos adultos capturados en altamar son aparentemente menos susceptibles a la captura porque son zonas menos accesibles a las pesquerías. En este estudio se corrobora para una gran parte del Pacífico mexicano que la pesca artesanal captura en su mayoría organismos inmaduros de *S. lewini*, de ambos sexos, lo que les impide alcanzar su longitud de primera madurez y reclutarse al stock adulto. Considerando lo anterior, se recomienda continuar con estudios sobre el uso del hábitat de *S. lewini*, a fin de dar soluciones de manejo más viables, como el establecimiento de zonas de no pesca que complementen las limitaciones temporales ya existentes, a fin de realizar una explotación sustentable.

ABSTRACT

In the Mexican Pacific, the scalloped hammerhead shark *Sphyrna lewini* is one of the most frequent species of shark in both artisanal and industrial fisheries landings. However, there is little information about its distribution and size structure despite its economic and ecological importance, which makes it difficult to implement management measures. This study describes the size structure of catch of *S. lewini* according to sex and fishery in the Mexican Pacific. Between August 2013 and April 2016, 240 sharks were collected. Seventy-five percent of these were caught by the artisanal fishery, with sizes generally <100 cm total length (TL), which corresponds to immature individuals according to previous studies. The minimum and maximum sizes observed were 45 cm and 302.9 cm TL, respectively. These observations were similar to previous studies, which indicated that southern Sinaloa, northern Nayarit and Oaxaca are breeding areas, with birth occurring between May and August, and a birth size usually between 45 and 47 cm TL. Since immature individuals were found in the coastal areas, this could make them more susceptible to artisanal fishery, especially during the birth months. On the other hand, adults that were caught offshore would apparently be less susceptible to be caught by fisheries when in these areas. As both a coastal and pelagic species, *S. lewini* depends on the coast for reproduction. However, in this study most of the sharks caught in the Mexican Pacific by the artisanal fishery were immature, which prevents them from reaching their first maturity size and from being recruited to the adult stock. Considering that most of the individuals occurring in the coastal area were immature, we encourage continued study of the distribution and habitat use of *S. lewini* in the Mexican Pacific. This is needed to provide more viable management solutions, such as the establishment of non-fishing zones to complement the existing temporal fisheries management measures, to achieve sustainable exploitation of this species.

INTRODUCCIÓN

Los tiburones son depredadores tope, por lo general altamente móviles y ampliamente distribuidos, muy vulnerables a la explotación al tener una baja resiliencia, debido a un crecimiento poblacional lento (Schindler et al. 2002). En México, la pesca de tiburones es una importante actividad desde el punto de vista económico, alimentario y social al generar empleos en su fase de captura, proceso, distribución y comercialización (CONAPESCA-INP 2004) siendo una de las pesquerías más importantes en el país; en el 2013 se capturaron 27 430 t, ubicándola en el noveno lugar de la producción pesquera en México; y por su valor en el octavo lugar ($ 425 364 000.00 M.N.). Los principales estados productores de tiburón se encuentran en el Pacífico mexicano, y son en orden descendente Sinaloa, Baja California (BC), Baja California Sur (BCS), Nayarit, Oaxaca, Sonora y Chiapas, que aportaron 19 633 t del total en el año 2013, que equivale a casi 72% de la producción nacional (CONAPESCA 2013).

El tiburón martillo común, *Sphyrna lewini* (Griffith y Smith 1834), es una especie migratoria pelágico-costera ampliamente distribuida en aguas tropicales y subtropicales a lo largo de las plataformas continentales e insulares; se encuentra tanto en áreas costeras como en zonas oceánicas hasta los 275 m de profundidad (Compagno et al. 1995). Típicamente, las hembras adultas expulsan a sus crías en la zona costera en donde los organismos inmaduros permanecen aproximadamente cinco años antes de iniciar sus migraciones hacia hábitats pelágicos (Holland et al. 1993, Duncan y Holland 2006). En el Pacífico mexicano, las hembras migran hacia aguas abiertas más temprano y a menor tamaño (115 cm) que los machos (160 cm) con el fin de alcanzar su longitud reproductiva lo antes posible (Klimley 1987). A diferencia de los organismos inmaduros, los adultos pasan la mayor parte de su vida en altamar donde generalmente se encuentran cerca de bajos oceánicos, volcanes submarinos o islas volcánicas (Klimley 1987, Klimley et al. 1988, Ketchum et al. 2014). Después de alcanzar su longitud de primera madurez, las hembras maduras emigran cada año hacia las zonas costeras para alumbrar (Clarke 1971, Bass et al. 1975, Klimley 1987, Stevens y Lyle 1989). La diferencia de estos rasgos ecológicos resulta en una segregación espacial de *S. lewini* por tamaño y sexo.

En el Pacífico mexicano, las crías nacen entre mayo y julio en áreas de crianza de poca profundidad (Torres-Huerta 1999, Ruiz Alvarado y Ixquiac-Cabrera 2000), como lo son las zonas costeras del Golfo de Tehuantepec (Soriano-Velásquez et al. 2002, Alejo Plata et al*.* 2006, 2007), del Pacífico central (Anislado-Tolentino 2000) y del sureste del Golfo de California (Mendizábal-Oriza *et al.* 2002, Torres-Huerta *et al.* 2008). Estos sitios han sido reportados como posibles áreas de crianza de *S. lewini* por la abundancia de capturas de organismos inmaduros. En el Pacífico mexicano, la longitud máxima registrada ha sido de 363 cm de LT (Torres-Huerta et al. 2008) y la madurez sexual se alcanza aproximadamente a 170 cm de LT en machos, y a 207 cm de LT en hembras (Anislado-Tolentino and Robinson-Mendoza 2001; Torres-Huerta et al. 2008), que alcanzan su madurez sexual a mayor tamaño que los machos (Klimley 1987).

La captura de *S. lewini* en el Pacífico mexicano es abundante, en pesquerías tanto artesanales como semi-industriales junto con otras especies de tiburón, como *Prionace glauca* (Linnaeus 1758), *Rhizoprionodon longurio* (Jordan y Gilbert 1882) y *Carcharhinus falciformis* (Bibron 1839), que contribuyen sustancialmente a sostener esta actividad (CONAPESCA-INP 2004). Es una de las especies de tiburón más importantes en las capturas artesanales de varias zonas del Pacífico mexicano, como en la zona centro (Jalisco, Colima, Michoacán y Guerrero) (Madrid et al. 1997, Anislado-Tolentino y Robinson-Mendoza 2001), la zona sur (Oaxaca y Chiapas) (Soriano-Velásquez et al. 2006, Bejarano-Álvarez et al. 2011) y en la entrada del Golfo de California (Pérez-Jiménez et al. 2005, Furlong-Estrada et al. 2014, 2015), en donde los organismos inmaduros dominan el desembarque total de esta especie.

Además de su importancia económica y pesquera, *S. lewini* también tiene un papel ecológico importante como depredador tope, ya que se considera como una de las especies de tiburones actuales evolutivamente más recientes (Lim et al. 2010, Klimley 2013, Gallagher et al. 2014). Sin embargo, a pesar de la importancia pesquera y ecológica de *S. lewini*, es poca la información disponible acerca de su estructura de longitudes a lo largo del Pacífico mexicano, y por tanto su manejo y conservación se dificultan al no conocerse adecuadamente estos aspectos biológicos básicos. Con base en las observaciones de distribución de tiburones por longitud y sexo, se pueden identificar áreas de importancia crítica para las especies y encontrar evidencias de segregación social. Mediante la utilización de datos de captura y frecuencia de longitudes, se puede analizar la captura con el fin de predecir la distribución de las longitudes, así como sus frecuencias de captura a lo largo del año, que pueden ser utilizadas para la evaluación de poblaciones (Maunder y Watters 2003).

El objetivo de este estudio fue analizar la estructura de longitudes de captura de las pesquerías artesanal y semi-industrial del tiburón martillo común *S. lewini* en diversos puntos del Pacífico mexicano que van desde Chiapas hasta el Golfo de California y Baja California Sur, a fin de determinar periodos y zonas de máxima susceptibilidad de captura de los diferentes estadios de madurez, y posibles zonas de crianza. Esta información complementará los datos biológicos y ecológicos de la especie, la cual puede ser utilizada para mejorar las medidas de manejo en el Pacífico mexicano.

MATERIALES Y MÉTODOS

Colecta de muestras

Todas las muestras fueron obtenidas de la captura comercial artesanal y semi-industrial de tiburón entre agosto de 2013 y abril de 2016 en aguas costeras y de mar abierto en diversos sitios del Pacífico mexicano: Las Lajitas, La Reforma, Mármol, El Verde Camacho, Mazatlán, Chametla, Las Cabras y Teacapán en Sinaloa; Cuautla, Boca de Camichín, Isla Isabel y Punta Santa Cruz en Nayarit; Bahía Navidad, Cuitzmala y Rebalsito en Jalisco; Marabasco y la Isla Clarión en Colima (a 200 km al suroeste de la isla cerca de bajos oceánicos). En el Golfo de Tehuantepec los muestreos incluyeron Salina Cruz en Oaxaca y El Paredón y Puerto Madero en Chiapas. El muestreo en cada campo pesquero se llevó a cabo de manera oportunista. Se incluyeron también organismos obtenidos en dos viajes de la pesca semi-industrial realizados en enero y abril de 2016, en una franja de aguas oceánicas de 200 millas náuticas de ancho ubicada al oeste de la península de BC entre Los Cabos al sur y Puerto San Carlos en Bahía Magdalena al norte (BCS) (Fig. 2.1).

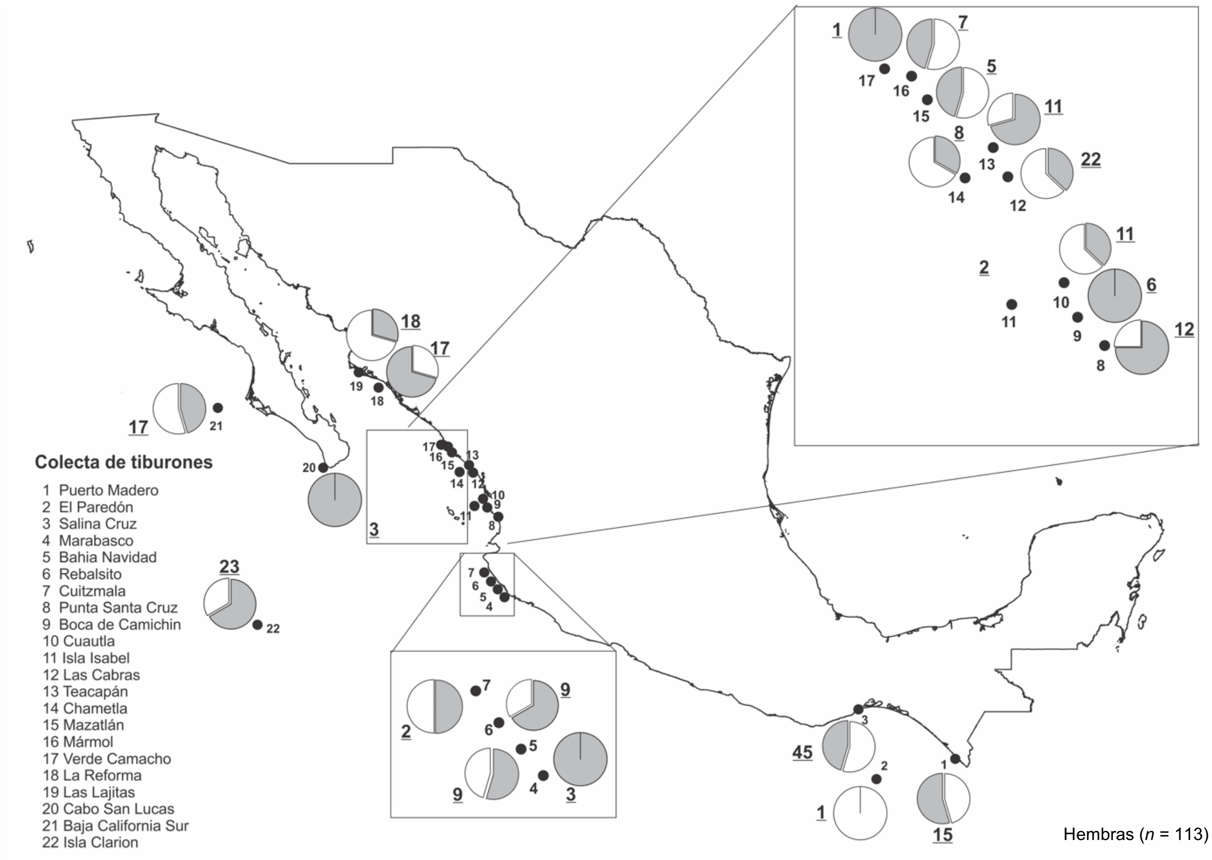

Fig. 2.1 Mapa del área de estudio y de los sitios de recolecta. Se señalan los números de organismos colectados en cada sitio y las proporciones de los sexos. *n* = 240

Los métodos de captura por la pesca artesanal fueron tanto redes de enmalle como palangres, mientras que en la flota semi-industrial se utilizó únicamente palangres. En cada muestreo se obtuvieron los datos de pesca (fecha, arte de pesca y ubicación). Cada tiburón recolectado se identificó *in situ* de acuerdo a Compagno et al. (1995), se registró su sexo y se tomaron las medidas de longitud total (LT) e interdosal (ID) en cm. En el caso de los organismos que no se pudieron conseguir enteros, la longitud total (LT) se estimó a partir de la longitud interdorsal (ID) medida en cada troncho. Para ello, se hizo una regresión linear simple de la ID con la LT de los organismos recolectados enteros descrita por el modelo LT = 3.4084 ID + 16.528 (r^2 = 0.977) (Fig. 2.2).

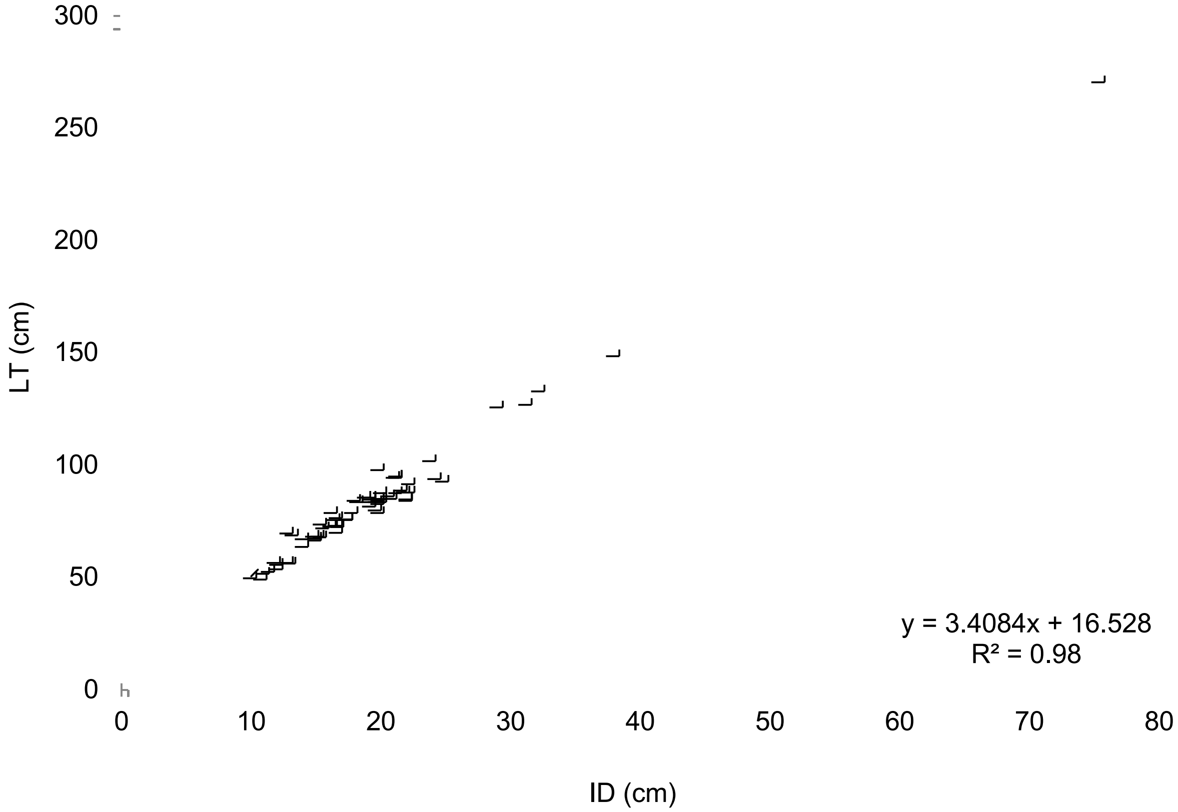

Fig. 2.2 Diagrama de dispersión de los datos de longitud total (LT) contra la longitud interdorsal (ID); n = 71

Análisis de datos

Mediante un análisis de χ^2 se analizó si la proporción de sexos fue diferente de la esperada de 1:1, utilizando para esto la corrección de Yates (Fowler et al. 1998). Considerando el pequeño tamaño de muestra, y a fin de obtener mayor información, la distribución de longitudes de esta especie se estimó mediante el método de Silverman (modelo matemático de Boostrap suavizado) (Silverman 1981, 1986), generando de manera aleatoria más datos de longitudes (1 000 muestras), y así poder confirmar la distribución multimodal de las longitudes de captura y ajustar los datos para obtener distribuciones más definidas. Posteriormente, se aplicó el método de Bhattacharya para la descomposición gaussiana de la distribución multimodal (Bhattacharya 1967, Matsumiya et al. 1991, Salgado-Ugarte et al. 1994, 2005), ajustar las curvas a la mejor distribución gaussiana, encontrar el número correcto de modas y determinar sus características (media, deviación estándar y tamaño). Para los análisis, se utilizaron los programas incluidos en Salgado-Ugarte et al. (1993, 1995a, 1995b), Salgado-Ugarte (2002) y Salgado-Ugarte y Mosqueda-Romo (2011).

Se construyeron histogramas de frecuencia de captura por mes, así como de longitudes de captura primero con todos los datos juntos y posteriormente por sexo y tipo de pesquería (semi-industrial o artesanal). En cada histograma se indicó la longitud de primera madurez sexual de acuerdo a Torres-Huerta et al. (2008), a fin de determinar el porcentaje de organismos inmaduros, así como de adultos capturados de cada sexo por cada pesquería.

RESULTADOS

Durante el periodo de estudio se registraron 240 organismos, de los cuales 197 fueron de la pesquería artesanal, y 43 de la semi-industrial, incluyendo 113 hembras (47.1%) y 127 machos (52.9%). Se capturaron más machos que hembras en ambas pesquerías, pero en ambos casos esta diferencia no fue significativa y se mantuvo la relación hembra: macho de 1:1 (Tabla 2.1).

Tabla 2.1 Organismos capturados por sexo y pesquería, y proporción macho: hembra. En todos los casos la prueba de X^2 fue no significativa (Valor crítico = 3.84)

Tipo de pesca	♀	♂	Total	Proporción ♂:♀	X^2
Artesanal	96	102	197	1.06: 1	0.126
Semi-industrial	17	26	43	1.53: 1	1.488
Total	113	128	240	1.13: 1	0.813

Los tiburones fueron capturados en su mayoría con palangre (57.7%) y en menor proporción con red de enmalle (41.9%) (Tabla 2.2). La mayoría de los ejemplares de *S. lewini* provinieron del suroeste del Golfo de California (sur de Sinaloa y norte de Nayarit; *n* = 64, 27%), Salina Cruz en Oaxaca (*n* = 39, 16.2%), y del suroeste de la Isla Clarión (*n* = 23, 9.5%) (Fig. 2.1).

Tabla 2.2 Organismos capturados por arte de pesca

Arte de pesca	Frecuencia	%
Red de enmalle	101	41.9
Palangre (o cimbra)	139	57.7
Total	240	100

Los meses con mayor cantidad de muestras recolectadas fueron septiembre (28.7%) y abril (17%), tanto para hembras como para machos (Fig. 2.3). Los meses entre mayo y julio corresponden al periodo de veda del tiburón en México por lo que casi no se recolectaron muestras de esta época (*n* = 2). La mayoría de los organismos inmaduros fueron recolectados entre agosto y diciembre (*n* = 123; 91%) mientras que la mayoría de los adultos fueron recolectados entre febrero y abril (*n* = 39; 81.2%).

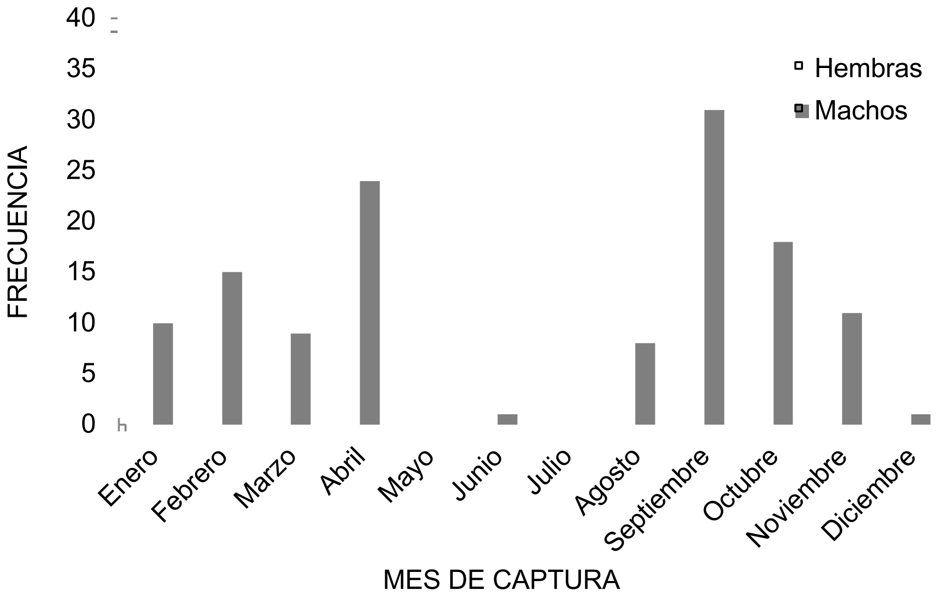

Fig. 2.3 Frecuencia mensual de captura de machos y hembras de *Sphyrna lewini* entre agosto 2013 y abril 2016. *n* = 240

Las longitudes mínimas (LT) correspondieron a un macho de 45 cm de LT capturado en Puerto Madero, Chiapas y a una hembra de 45 cm de LT capturada en La Reforma, Sinaloa. Las longitudes máximas fueron de 300.8 cm de LT para una hembra capturada en la Isla de la Piedra

(Mazatlán) y de 302 cm de LT para un macho capturado en la zona de altamar frente al suroeste de Baja California Sur (Fig. 2.4). La longitud promedio de las hembras fue de 100.5 cm de LT mientras que la de los machos fue 105.9 cm de LT. Durante el mes de abril, se capturaron dos hembras preñadas en Puerto Madero (267 cm y 291 cm de LT respectivamente), de las que se recuperaron cuatro embriones con LT promedio de 46.2 cm, y una hembra preñada de 266 cm de LT en Mazatlán con 15 embriones de 47 cm de LT promedio.

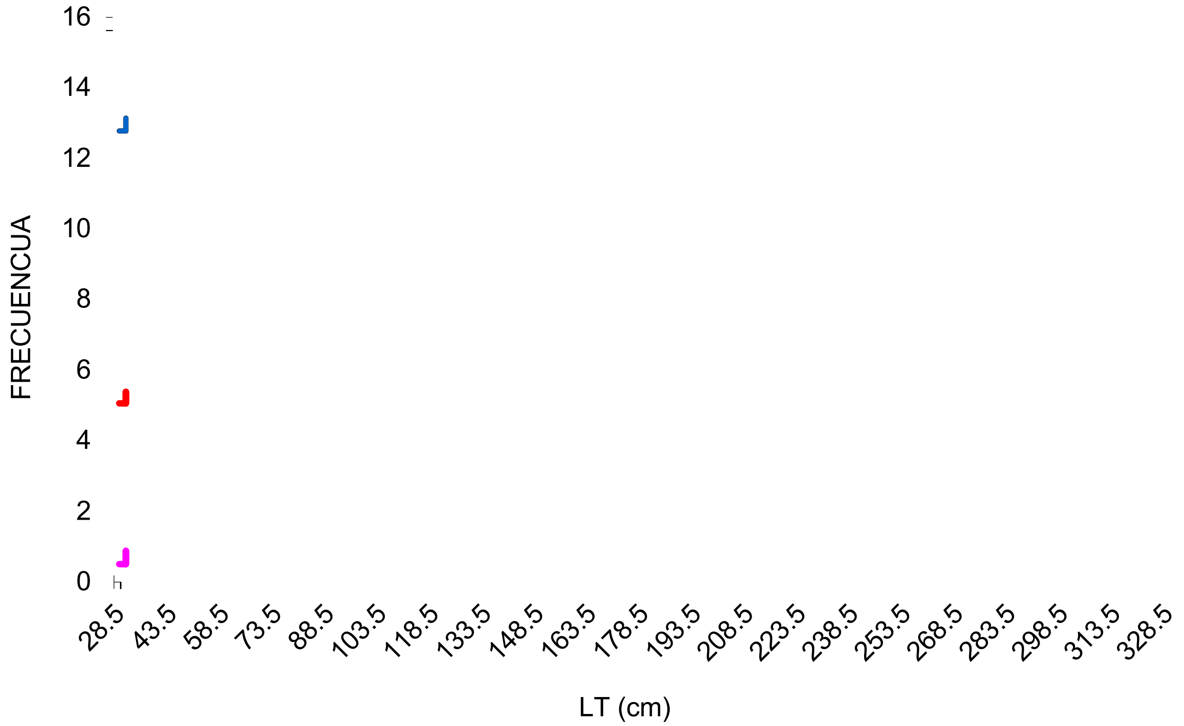

Fig. 2.4 Histograma de las frecuencias de longitudes (LT) de los tiburones recolectados entre agosto de 2013 y abril de 2016; se trazaron también las modas de las tres posibles cohortes. *n* = 240

Los organismos inmaduros fueron capturados en su totalidad en aguas costeras por pesquerías artesanales (*n* = 192), mientras que los organismos adultos se capturaron en su mayoría en aguas abiertas (> 50 km de distancia a la costa) por la pesquería semi-industrial (70%); 14 adultos fueron registrados en la zona costera (30%), en Puerto Madero (*n* = 10) y Mazatlán (*n* = 4) en abril 2016 específicamente. El análisis de las distribuciones de frecuencia de longitudes por sexo y tipo de pesquería mostraron multimodalidad.

La distribución de longitudes de hembras de la pesquería artesanal mostró una moda que contenía a la mayoría de los organismos (*n* = 81) con un promedio de LT de 67.3 cm (14.1 D.E.), una segunda moda de 12 organismos con LT promedio de 97.5 cm (3.9 D.E.), y una tercera moda con LT promedio de 270.7 cm (27.2 D.E.) y seis organismos (Fig. 2.5). 94% (*n* = 90) de las hembras capturadas por esta pesquería estuvieron por debajo de la longitud de primera madurez estimada en el Pacífico mexicano (*i.e.* 207 cm de LT; Torres-Huerta et al. 2008).

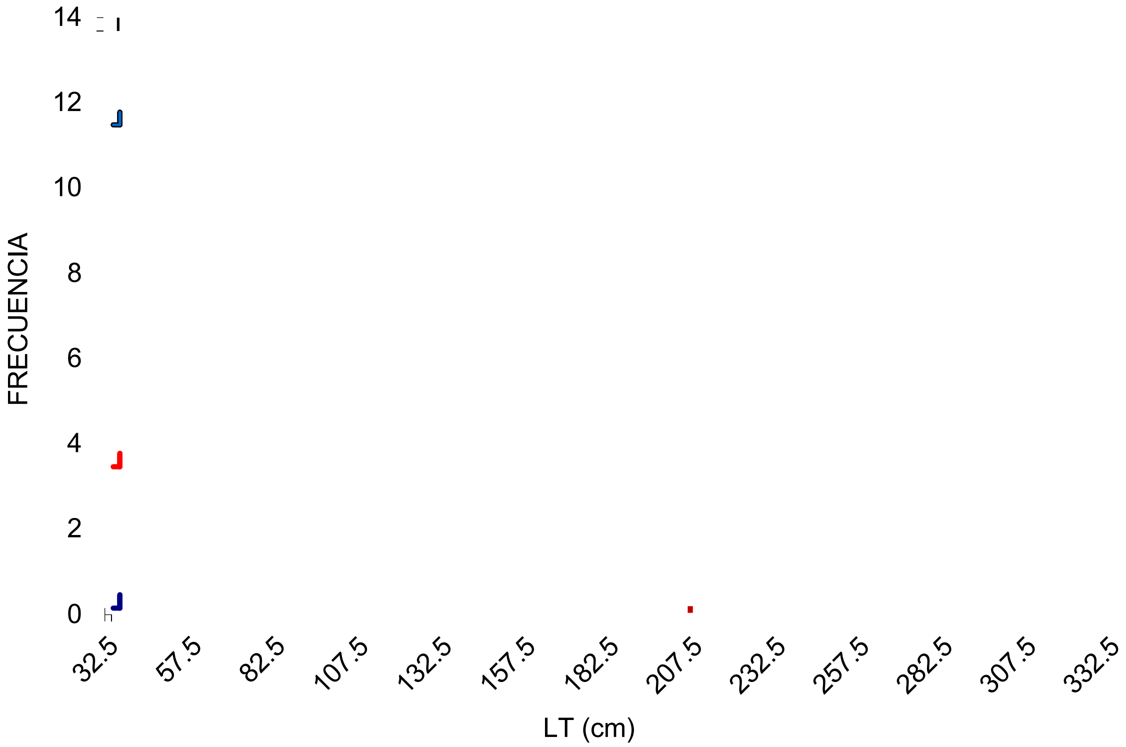

Fig. 2.5 Histograma de las frecuencias de longitudes de las hembras capturadas por la pesquería artesanal; se trazaron también las modas de las tres posibles cohortes. La línea azul claro representa la frecuencia esperada calculada con el método de máxima verosimilitud. La línea roja a la longitud total de 207 cm marca la longitud aproximada de primera madurez (Torres-Huerta et al. 2008). *n* = 96

En el caso de los machos (*n* = 102), las longitudes capturadas por la pesquería artesanal también mostraron tres modas, la primera con una LT promedio de 53.8 cm (3.4 D.E., *n* = 28), la segunda con una LT promedio de 77.3 cm (11.7 D.E., *n* = 73), y la tercera con una LT promedio de 212 cm (30 D.E., *n* = 7). Las dos primeras modas incluyeron 93% de los organismos, que también se encuentran por debajo de la longitud de primera madurez (*i.e.* 170 cm de LT) reportada por Anislado-Tolentino y Robinson-Mendoza (2001) y Torres-Huerta et al. (2008) (Fig. 2.6).

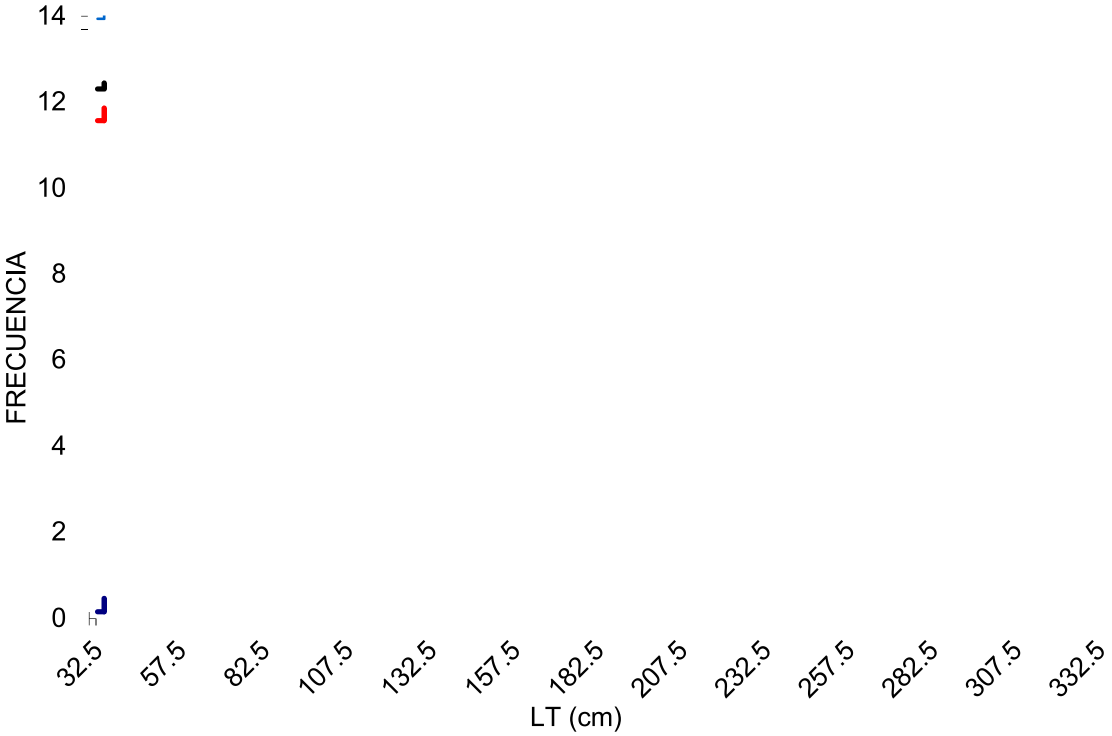

Fig. 2.6 Histograma de las frecuencias de longitudes de los machos capturados por la pesquería artesanal; se trazaron también las modas de las tres posibles cohortes. La línea azul claro representa la frecuencia esperada calculada con el método de máxima verosimilitud. La línea roja a la longitud total de 170 cm marca la longitud aproximada de primera madurez (Torres-Huerta et al. 2008). *n* = 102

La distribución de longitudes de los organismos provenientes de la pesquería semi-industrial registrados en este estudio mostró un comportamiento diferente al de las longitudes capturadas por la pesquería artesanal. La LT promedio de las hembras mostró dos modas, la primera a los 172 cm (D.E. 6.4, *n* = 9), y la segunda a los 199 cm (D.E. 11.5, *n* = 8). Si bien el intervalo de longitudes fue mayor al de los organismos capturados por la pesca artesanal, la mayoría de las hembras (*n* = 13; 76%) estuvieron por debajo de la longitud de primera madurez sexual (*i.e.* 207 cm de LT; Torres-Huerta et al. 2008) (Fig. 2.7).

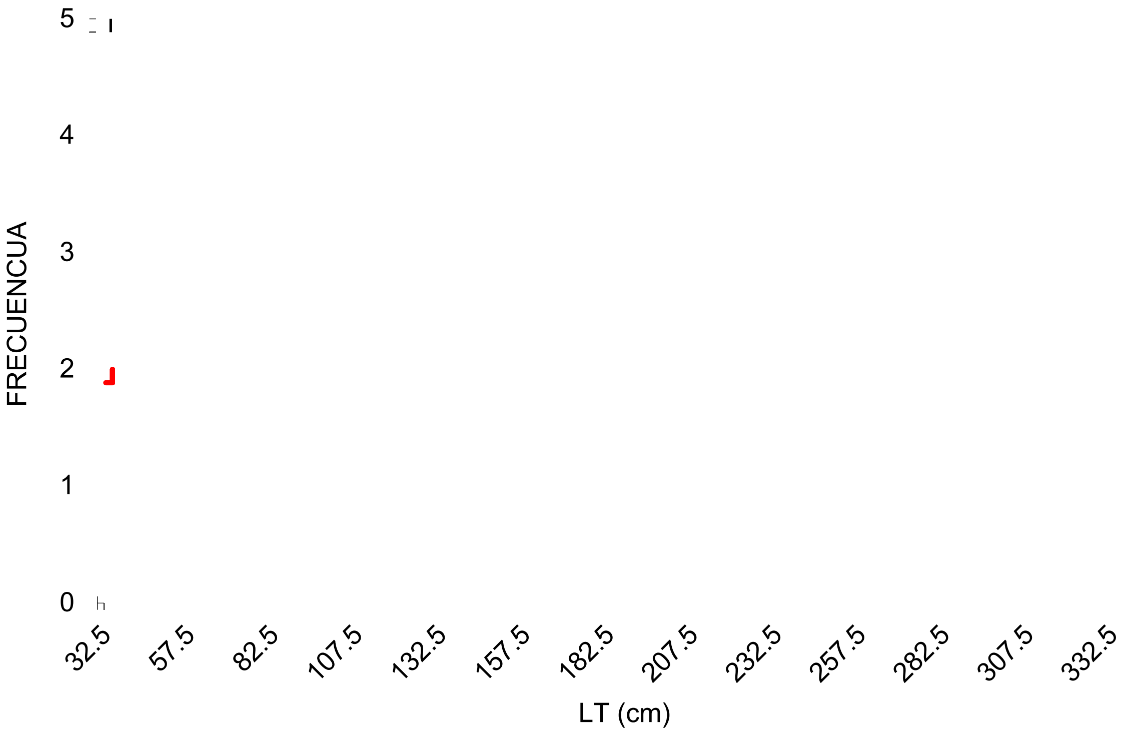

Fig. 2.7 Histograma de las frecuencias de longitudes de las hembras capturadas por la pesquería semi-industrial; se trazaron también las modas de las dos posibles cohortes. La línea azul claro representa la frecuencia esperada calculada con el método de máxima verosimilitud. La línea roja a la longitud total de 207 cm marca la longitud aproximada de primera madurez (Torres-Huerta et al. 2008). *n* = 17

Los machos presentaron tres modas con un intervalo de longitudes de 150 cm hasta 302.9 cm de LT. La primera moda fue a una LT promedio de 164.9 cm (D.E. 11.6, *n* = 19), la segunda moda tuvo una LT promedio de 177.3 cm (D.E. = 2.9, *n* = 12), y la tercera tuvo una LT promedio de 238.6 cm (D.E. = 40.4, *n* = 23). La mayoría de los organismos capturados eran maduros, pero 23% (*n* = 8) de los machos presentaron longitud menor a la de su primera madurez (*i.e.* 170 cm de LT; Anislado-Tolentino y Robinson-Mendoza 2001; Torres-Huerta et al. 2008) (Fig. 2.8).

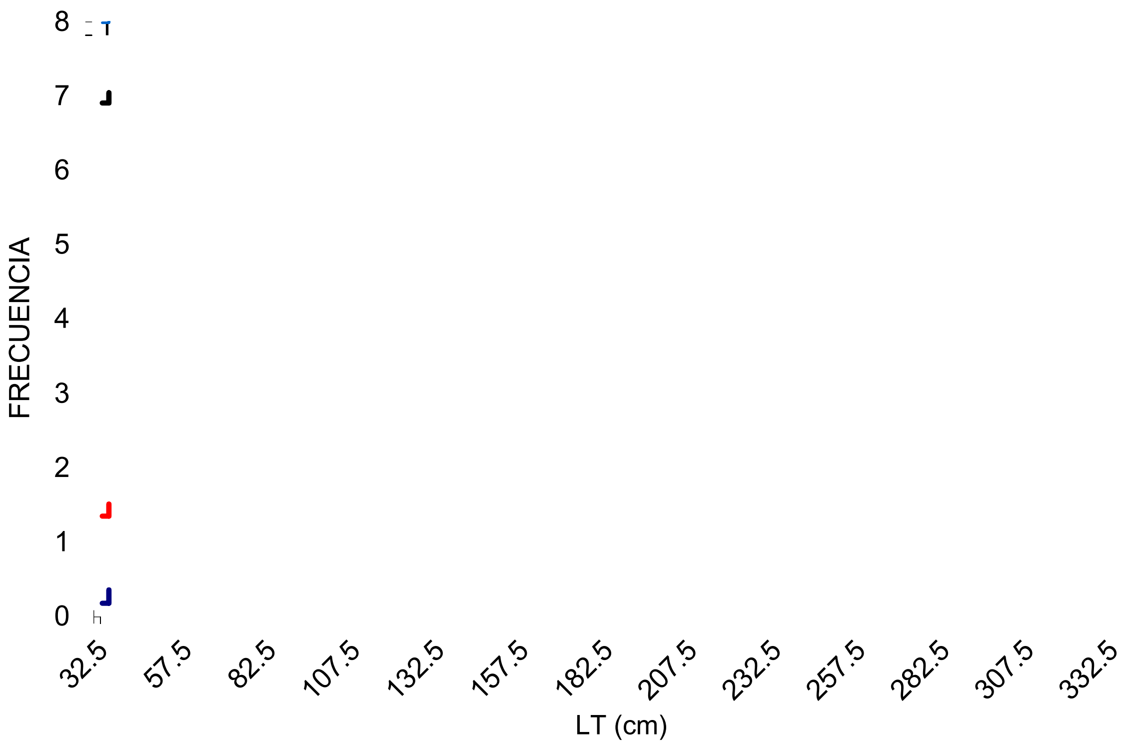

Fig. 2.8 Histograma de las frecuencias de longitudes de los machos capturados por la pesquería semi-industrial; se trazaron también las modas de las tres posibles cohortes. La línea azul claro representa la frecuencia esperada calculada con el método de máxima verosimilitud. La línea roja a la longitud total de 170 cm marca la longitud aproximada de primera madurez (Anislado-Tolentino y Robinson-Mendoza 2001; Torres-Huerta et al. 2008). *n* = 26

DISCUSIÓN

La presencia de *S. lewini* en campos pesqueros a lo largo del Pacífico mexicano, confirma su importancia comercial en el país, como se ha manifestado con anterioridad. Aunque existen varios estudios sobre *S. lewini* en diferentes puntos del Pacífico mexicano (Klimley et al. 1993, Anislado-Tolentino y Robinson-Mendoza 2001, Pérez-Jiménez et al. 2005, Bejarano-Alvarez et al. 2011, Gallegos-Camacho y Tovar-Ávila 2011, Furlong-Estrada et al. 2014, 2015 por destacar algunos), el presente trabajo es el primero que describe y compara las longitudes de las capturas de las flotas artesanal y semi-industrial a lo largo de la costa occidental de México, incluyendo datos de muestreos obtenidos desde Chiapas hasta el noroeste de México. Se documenta además por primera vez la presencia de la especie en la Isla Clarión y las aguas oceánicas de BCS.

Los resultados aquí presentados indican que actualmente la captura de esta especie pudiera no ser sustentable para el caso de esta pesquería debido a que son principalmente organismos de longitud inmadura, si se considera como explotación sustentable al uso o explotación de un recurso mediante un proceso de extracción, que permite o promueve su recuperación, de modo que garantiza su renovación y permanencia en el largo plazo (CONABIO 2016).

En el Pacífico mexicano, la pesquería artesanal de tiburón suele operar cercana a la costa, en una franja costera de 50 km de ancho, aunque hay excepciones (Pérez-Jiménez et al. 2005), generalmente utiliza el palangre, aunque en las operaciones hechas para la captura de escama con red de enmalle, también se captura esta especie. Por su parte, la pesquería semi-industrial captura tiburones exclusivamente con palangre y opera generalmente en zonas alejadas de la costa ubicadas entre 200 y 800 km de distancia a la costa. Esta diferencia espacial en las capturas, implica que las etapas de vida capturadas por cada pesquería sean diferentes, y por tanto el grado de impacto no sea igual.

Aunque los resultados indican que la captura de machos es ligeramente mayor al de hembras, estadísticamente estas diferencias no fueron significativas, y se necesitarían más datos para poder extrapolarlo a toda la población, por lo que no hay evidencias para sugerir un uso diferencial del hábitat de los sexos como había sido reportado en las costas de Oaxaca, donde los adultos tienen una distribución sesgada por sexo (1:3 hembra: macho) pero en los organismos inmaduros la proporción hembras: machos es similar (1:1) (Alejo-Plata et al. 2006, 2007). Los resultados de este estudio sugieren que ambos sexos se encuentran igualmente susceptibles a los artes de pesca, lo que coincide con lo reportado por Furlong-Estrada et al. (2014).

El método de *bootstrap* utilizado permitió aumentar el tamaño de muestra, con el fin de analizar los grupos modales que componen las capturas, este método se considera viable cuando el tamaño de muestra es pequeño (Salgado-Ugarte et al. 1994, 2005). La mayoría de los organismos analizados provinieron de la pesca con palangre, seguramente debido a que este sistema de captura está dirigido a este tipo de especies, aunque es necesario considerar que la red de enmalle representó más de 40% de los organismos capturados durante este estudio, sobre todo para organismos de longitudes inferiores a 100 cm de LT.

Al igual que en el presente trabajo, Furlong-Estrada et al. (2014) mencionan que *S. lewini* es una de las especies de tiburón más susceptibles a la captura tanto de redes como palangres y con mayor riesgo ecológico en la zona de entrada al Golfo de California. Ese mismo trabajo recomienda enfocar los esfuerzos de investigación, manejo y conservación en esta especie, debido a su importancia en la pesca. Además de que queda por primera vez en evidencia de que, si bien la pesca semi-industrial no captura una gran cantidad de organismos, podría tener un impacto en el stock reproductivo al capturar organismos pre-adultos, lo cual debe ser estudiado con más detalle a fin de evaluar cuál es el efecto de esta captura sobre la población.

La alta proporción de organismos inmaduros registrados en las capturas entre agosto y noviembre corrobora lo señalado en estudios anteriores respecto a que las crías de *S. lewini* nacen durante la época de lluvias, cuando se presentan las temperaturas más elevadas del año y se encuentra una alta disponibilidad de alimento en las áreas de crianza costeras. Por otra parte, las capturas en la zona costera durante los meses de invierno (diciembre-febrero) fueron menos abundantes, y los organismos presentaron longitudes entre 72 y 101 cm de LT, que corresponde a organismos inmaduros. Lo anterior sugiere que *S. lewini* migra hacia otras áreas después de la época reproductiva. Esta observación ya se había señalado en Sinaloa donde se propuso que una de las causas posibles es la necesidad de condiciones diferentes para la alimentación al alcanzar la longitud de madurez sexual (Saucedo-Barrón et al. 1982).

Para las capturas semi-industriales de altamar, es probable que la presencia de organismos adultos en la Isla Clarión (entre 151 y 193 cm de LT) en invierno esté relacionada con la presencia de bajos oceánicos al suroeste de la isla y a las corrientes frías de la zona que podrían favorecer la aparición de las presas (Klimley 2015). De la misma manera, la observación de organismos de longitudes entre 179 y 255 cm de LT en aguas abiertas de Cabo San Lucas solamente en invierno, podría estar relacionada con la intensificación de la corriente fría de California en esta época del año (Pantoja et al. 2012) y a la consecuente abundancia de presas, aunque estas aseveraciones aún no se pueden corroborar con los resultados de este trabajo por el número insuficiente de muestras y el tipo de muestreo llevado a cabo.

Debido a que *S. lewini* es una especie vivípara, con un periodo de gestación aproximado de 12 meses y reproducción anual (Liu et al. 1999, Alejo-Plata et al. 2007, White et al. 2008, Castro 2009), la aparición de hembras preñadas en la zona costera y la ausencia de organismos inmaduros en los muestreos de abril sugieren que las hembras se acercan a la costa antes de que empiece la temporada de lluvias para el alumbramiento durante los meses finales de primavera y los primeros meses de verano. El nacimiento de las crías entre mayo y agosto explicaría la abundancia de las capturas de neonatos al finalizar los meses de veda, es decir entre agosto y noviembre. La época de alumbramiento se había señalado ocurriendo entre mayo y agosto en el Pacífico centro mexicano (Anislado-Tolentino 2000), en junio en el Golfo de California (Torres-Huerta et al. 2008) y entre julio y agosto en Oaxaca (Alejo-Plata et al. 2007) lo que es consistente con las observaciones de captura de este estudio que incluye registros de captura en diferentes áreas del Pacífico mexicano.

La presencia de organismos inmaduros en la zona costera durante los meses de verano sugiere que *S. lewini* permanece en la costa durante las primeras etapas de su vida utilizando esta zona como área de crianza, lo cual ha sido documentado por otros autores para la especie (Duncan y Holland 2006, Zanella et al. 2009). La presencia de organismos inmaduros en el sur Sinaloa y Oaxaca, sugieren que estas zonas podrían ser áreas de crianza de la especie, ya que los organismos más pequeños se capturaron consistentemente en estas zonas en los sitios más someros (entre 3 y 45 m de profundidad) y más cercanos a la costa, entre 1 y 27 km de distancia, y es un área en la que diferentes estudios han reportado la presencia de organismos inmaduros (Anislado-Tolentino 2000, Mendizábal-Oriza et al. 2002, Soriano-Velásquez et al. 2002, Alejo-Plata et al. 2006, 2007, Torres-Huerta et al. 2008). De acuerdo a Heupel et al. (2007), un área de crianza de tiburones puede ser identificada porque: i) los tiburones se encuentran allí en mayor frecuencia que en otras áreas, ii) permanecen o regresan por periodos extensos, y iii) se utiliza por los tiburones repetidamente cada año. El uso de las áreas de crianza costeras por los tiburones inmaduros, como zona de protección contra los depredadores y de disponibilidad de alimento, los hace más susceptibles a la captura por las pesquerías artesanales, especialmente durante los meses de nacimiento, como ya se había mencionado en otros estudios para la especie (*e.g.* Klimley 1987, Torres 2004, Zanella et al. 2009).

Por otra parte, los resultados del presente estudio sugieren que los organismos adultos se encuentran en zonas más profundas y alejadas de la costa. Esto se atribuye a que en esas zonas pueden encontrar presas de mayor tamaño como calamares pelágicos (*e.g.* Jorgensen et al. 2009, Hoyos-Padilla et al. 2014, Klimley 2015), necesarias para suplir sus altas necesidades metabólicas (Lowe 2002, Bush 2003). Estos organismos de longitud grande se capturan principalmente por la pesquería de altura, siendo menos accesibles y menos susceptibles a las pesquerías artesanales. El histograma de frecuencia de longitudes muestra que la mayoría de los organismos recolectados fueron menores a 170 cm de LT, y por lo tanto no habían alcanzado su longitud de primera madurez (Anislado-Tolentino y Robinson-Mendoza 2001, Torres-Huerta et al. 2008). Por otro lado, la longitud máxima registrada en este estudio de 302.9 cm de LT coincide con las longitudes máximas reportadas anteriormente por otros autores; por ejemplo, en el Golfo de California y en el Pacífico central mexicano las longitudes máximas reportadas fueron de 307 cm y 363 cm de LT, respectivamente (Torres-Huerta et al. 2008), mientras que la longitud máxima reportada en Oaxaca fue de 281 cm de LT (Alejo Plata et al. 2006).

Respecto a la longitud de nacimiento, el tamaño mínimo observado en este trabajo (45 cm de LT) coincide con las de nacimiento reportadas en trabajos de reproducción de *S. lewini* en el Pacífico centro (46.5 cm de LT; Anislado-Tolentino 2000), en el Golfo de California (41-53 cm de

LT; Torres-Huerta et al. 2008) y en Oaxaca (45 cm de LT; Alejo-Plata et al. 2007). De igual manera, en el presente estudio se encontraron también embriones de 47 cm de LT no formados completamente.

Sphyrna lewini es una especie pelágico-costera cuya biomasa reproductora la constituyen los adultos en etapa reproductiva que se encuentran generalmente en mar abierto (Furlong-Estrada et al. 2015), pero que por otra parte, depende de la costa para la expulsión de crías, y como zona de crianza de organismos inmaduros, por lo que de una manera indirecta, el mantenimiento de su biomasa reproductora también depende de la zona costera. Los resultados de este estudio indican que la pesca artesanal captura en su gran mayoría organismos inmaduros de ambos sexos después de la veda, entre agosto y octubre, debido a la zona donde opera esta pesquería, que como ya se indicó son precisamente los hábitats que esta especie utiliza como áreas de crianza. Esta actividad pesquera limita las posibilidades de reclutamiento de las crías al stock adulto, al encontrarse principalmente en zonas de pesca artesanal costera. En este sentido, las medidas actuales de regulación pesquera (NOM-029-PESC 2006; DOF 2007) parecen no tener el resultado esperado en lo que se refiere a la protección y conservación de esta especie. Si bien es cierto que Furlong-Estrada et al. (2015) demostraron que los niveles de captura en el litoral de Nayarit y sur de Sinaloa se han mantenido estables para esta especie por al menos tres décadas, es necesario tener precaución con este resultado. Si se considera la teoría de selección de hábitat denso dependiente propuesta por MacCall (1990), es posible que esta zona sea el centro de la distribución y abundancia de un stock en esta zona del Pacífico. Estudios previos (Torres-Huerta et al. 2008, Alejo-Plata 2007) han señalado esta parte como un área sumamente importante para la crianza y reproducción de esta especie. De lo anterior se deriva que, si la teoría mencionada es cierta, es posible que no se vea una reducción inmediata, pero que cuando esta se dé, sea irreversible.

Por otro lado, la captura de hembras preñadas por parte de la pesquería artesanal durante el periodo antes del inicio de la veda, podría limitar las posibilidades de llevar a cabo su gestación completa, y ocasionar mortandad de organismos inmaduros, lo cual se observó durante el presente estudio. En estas condiciones, una manera de preservar el stock de *S. lewini* en el Pacífico mexicano sería estableciendo zonas de no pesca para esta especie cerca de la costa, ya que como se ha señalado en este trabajo, los organismos que ahí se encuentran son en su gran mayoría inmaduros que no han tendido ni un evento reproductivo al año, o bien hembras preñadas que se acercan a la costa para expulsar sus crías. El establecimiento de un tamaño mínimo de captura no parece ser una solución adecuada para esta especie, ya que por la anatomía de su cabeza, y la diversidad de artes de pesca empleadas en la zona, son susceptibles a ser capturados por redes de enmalle enfocadas a otras pesquerías, aun siendo inmaduros (Patrick et al. 2010). El problema con este planteamiento es que implicaría que los pescadores artesanales ya no pudieran capturar esta especie. Por lo anterior se recomienda continuar con estudios sobre el uso del hábitat de *S. lewini*, a fin de dar soluciones de manejo más viables, como podría ser el establecimiento de zonas de no pesca que complementen las limitaciones temporales ya existentes (DOF 2007). Las prácticas actuales dejan un escaso margen para que la población pueda regenerarse si se considera que tiene bajas tasas de crecimiento, madurez tardía y que es longeva como muchas otras especies de tiburones oceánicos. Los resultados de este trabajo indican que posiblemente la explotación de *S. lewini* no se da de manera sustentable en la actualidad. De ser el caso, el stock poblacional podría disminuir en el Pacífico mexicano, considerando además que esta especie se evaluó en 2007 como especie en peligro de extinción a nivel mundial en la Lista Roja de la IUCN (Baum et al. 2007).

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CAPÍTULO 3

PERIODICIDAD DE FORMACIÓN DE BANDAS DE CRECIMIENTO EN VÉRTEBRAS DE JUVENILES DEL TIBURÓN MARTILLO *Sphyrna lewini* EN EL PACÍFICO MEXICANO

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RESUMEN

La edad de 296 tiburones martillo (*Sphyrna lewini*), capturados por diferentes pesquerías en el Pacífico mexicano entre marzo 2007 y septiembre 2017, se estimó a partir del conteo del número de bandas de crecimiento en secciones sagitales de vértebras. Los análisis de incremento marginal (MIA) y de borde (CEA) se utilizaron para verificar la periodicidad de la formación de las bandas de crecimiento, mientras que los análisis de perfiles elementales obtenidos por LA-ICP-MS en las vértebras de 15 juveniles se usaron como método alternativo para verificar la edad de la especie. Las estimaciones de edad oscilaron entre 0 y 10+ años (42–158.7 cm LT). El índice de error promedio (IAPE 3.6%), el coeficiente de variación (CV 5.2%), los gráficos de sesgo y las pruebas de simetría de Bowker indicaron una estimación de edad precisa y poco sesgada. Tanto MIA como CEA indicaron que una única banda de crecimiento translúcida se forma en las vértebras de juveniles *S. lewini* durante el invierno (de noviembre a marzo) y una banda opaca durante el verano (de julio a septiembre), un período de crecimiento más rápido aparentemente correlacionado con las temperaturas superficiales del mar más altas. Los picos de concentración en los perfiles de P y Mn correspondían espacialmente con el patrón de bandas anual en la mayoría de las muestras, mostrando 1.19 y 0.88 pico por banda opaca, respectivamente, lo cual coincidía estrechamente con el patrón anual de deposición de bandas observado en este estudio. Aunque la periodicidad de la formación de bandas de crecimiento deba verificarse para todos los tamaños y edades de la población de la especie en la región, la demostración de la formación anual de las bandas de crecimiento en las vértebras de los juveniles debería llevar a una reestimación de los parámetros de crecimiento y productividad de la población para garantizar que su explotación se dé de manera sostenible.

ABSTRACT

The age of 296 juvenile scalloped hammerhead sharks (*Sphyrna lewini*), caught by several fisheries in the Mexican Pacific from March 2007 to September 2017, were estimated from growth band counts in thin-sectioned vertebrae. Marginal increment analysis (MIA) and centrum edge analysis (CEA) were used to verify the periodicity of formation of the growth bands, whereas elemental profiles obtained from LA-ICP-MS transect scans in vertebrae of 15 juveniles were used as an alternative approach to verify the age in the species for the first time. Age estimates ranged from 0 to 10+ years (42–158.7 cm L_T). The index of average percentage error (IAPE 3.6%), coefficient of variation (CV 5.2%), bias plots and Bowker's tests of symmetry showed precise and low-biased age estimation. Both MIA and CEA indicated that in the vertebrae of juvenile *S. lewini* a single translucent growth band was formed during winter (from November to March), and an opaque band during summer (from July to September), a period of faster growth apparently correlated with a higher sea surface temperature. Peaks in vertebral P and Mn content spatially corresponded with the annual banding pattern in most of the samples, displaying 1.19 and 0.88 peak per opaque band, respectively, which closely matched the annual deposition rate observed in this study. Although the periodicity of growth band formation needs to be verified for all sizes and ages representing the population of the species in the region, the demonstration of the annual formation of the growth bands in the vertebrae of juveniles should lead to a re-estimation of the growth parameters and productivity of the population to ensure that it is harvested at sustainable levels.

INTRODUCTION

The scalloped hammerhead shark *Sphyrna lewini* (Griffith & Smith 1834), is a large and highly migratory circumtropical species found in both coastal and oceanic waters (Compagno 1984). In the eastern Pacific Ocean, this species ranges from southern California southward to Ecuador (Castro 2011), where it is heavily harvested since it is frequently captured as target or by-catch in several fisheries. Young-of-the-year and juvenile individuals are captured with artisanal gillnets, bottom longlines and trawl nets in nearshore waters, whereas adults are mainly captured offshore with pelagic longlines (Coiraton et al. 2017; Kotas et al. 2014). Like most elasmobranchs, the species is susceptible to overfishing because of its slow growth, low fecundity, late age of maturity and the association of its reproductive cycle with coastal areas (Piercy et al. 2007; Torres-Huerta et al. 2008). Furthermore, its specialized traits and complex mating, feeding and predator behaviors (Irschick et al. 2005) have increased its vulnerability and hampered efforts at conservation (Gallagher et al. 2014). For these reasons, it has been categorized as Endangered by the IUCN (Baum et al. 2007).

Knowledge of the age structure and growth rate of a population is required for population models that estimate the productivity of a stock and demonstrate whether a species is harvested at sustainable levels (Cortés 2000). Studies of age and growth of *S. lewini* around the world (Branstetter 1987*a*; Chen et al. 1990; Drew et al. 2015; Harry et al. 2011; Kotas et al. 2011; Piercy et al. 2007) have included Mexico (Anislado-Tolentino et al. 2008; Anislado-Tolentino and Robinson-Mendoza 2001) but no consensus has been achieved regarding the growth pattern of the species. Some authors (Branstetter 1987a; Harry et al. 2011; Kotas et al. 2011; Piercy et al. 2007) have suggested that a single pair of growth bands are formed in the vertebrae whereas others (Anislado-Tolentino et al. 2008; Anislado-Tolentino and Robinson-Mendoza 2001; Chen et al. 1990) have suggested that two pairs of growth bands are formed each year. As yet, no complete age validation and verification have been achieved. It is unclear whether such differences result from the methods used, the widely variable sample sizes per month in each study or the existence of differing patterns of growth for the different stocks and age groups. Since population assessment and management are greatly affected by growth estimates (Cailliet et al. 1986; Campana 2001), erroneous age estimates of *S. lewini* can lead to inaccurate estimation of its resilience to fishing pressure (Beamish and McFarlane 1983; Campana 2001).

Age validation requires proof that growth bands are predictably deposited in the vertebrae. The process of evaluating growth band deposition in sharks can be categorized into the terms ‘verification’ and ‘validation’ (Cailliet 1990; Cailliet and Goldman 2004). Verification is defined as “confirming an age estimate by comparison with other indeterminate methods,” and validation as "proving the accuracy of age estimates by comparison with a determinate method" (Cailliet 1990; Cailliet and Goldman 2004). Because absolute age is only validated when the periodicity of growth band formation has been validated for all available age classes (Beamish and McFarlane, 1983; Cailliet, 1990; Campana, 2001), this process has often proven difficult when recapture of wild specimens is required (Cailliet and Goldman 2004), so it is the periodicity of the growth band formation for which validation is typically attempted. For this, centrum-edge analysis (CEA) and marginal-increment analysis (MIA) are the methods most frequently employed for elasmobranchs. However, these have often been hampered by small sample sizes across the species size ranges during incomplete annual periods and by the difficulty in objectively identifying growth bands in the vertebrae of some species (Cailliet et al. 1986). Interpretation is also hindered by lack of discrimination between annual and biannual cycles (Okamura et al. 2013). Hence, new simple and flexible approaches that directly account for periodicity have been developed to improve the accuracy and robustness of MIA (Okamura et al. 2013) and CEA (Okamura and Semba 2009).

As an alternative approach, microchemical analysis of vertebrae has also been used to validate the periodicity of the growth band formation in vertebrae of elasmobranchs because changes in the concentration of elements such as calcium (Ca) and phosphorus (P) can be related with growth band deposition or seasons (Jones and Geen 1977; Cailliet et al. 1986; Cailliet and Radtke 1987; Hale et al. 2006; Mohan et al. 2018). Variations in other element concentrations that are related to environmental variables such manganese (Mn) with temperature (Smith et al. 2013) and strontium (Sr) with salinity (Scharer et al. 2012; Tillett et al. 2011) can show a marked seasonality and also prove useful for validating ages (Raoult et al. 2016). This approach can complement the more traditional techniques (Cailliet et al. 1986) and is ideal for endangered species for which samples are few (Goldman et al. 2012; Scharer et al. 2012), or for species with few or no visible growth bands (Raoult et al. 2016).

The objective of this study was to provide new information to clarify the periodicity of the growth band formation in the vertebrae of *S. lewini* in the southeastern Gulf of California, Mexico, using MIA and CEA methods, together with the approaches developed by Okamura and Semba (2009) and Okamura et al. (2013) to improve the accuracy of the age estimations and verify the timing and frequency of the growth band formation. Age estimates based upon visual growth band counts were also compared for the first time for this species with the analysis of time-resolved elemental profiles (Ca, P and Mn) in its vertebrae as a complementary approach to verify the periodicity of the formation of growth bands.

MATERIAL AND METHODS

Sample collection

Vertebrae were opportunistically obtained from specimens captured between March 2007 and September 2017 in small-scale and industrial fisheries, landed in several locations of the southeastern Gulf of California in the states of Sinaloa (Las Lajitas, La Reforma, Marmól, Mazatlán, Chametla, Teacapán) and Nayarit (Cuautla, Isla Isabel, Boca de Camichín, San Blas, Punta Santa Cruz, Chacala and Bahía Banderas), Mexico (Fig. 3.1). Additional samples were obtained from the shrimp trawl fishery which operated off southern Sinaloa and northern Nayarit (Fig. 3.1). Fishing used surface and bottom gillnets, surface longlines and bottom trawls. Fisheries targeting sharks are prohibited during May to July in the Mexican Pacific to protect the main reproductive season, and the few specimens obtained during this period were incidentally caught by the shrimp trawl and Pacific sierra gillnet fisheries. Sex, total length (L_T) and interdorsal length (L_{ID}, distance between the two dorsal fin insertions) were recorded for each specimen. A set of 10 post-cephalic vertebrae was removed, stored on ice and later frozen until preparation for ageing and elemental analyses. L_T was measured to the nearest cm, without depressing the tail to be in line with the body axis. Estimation of L_T of commercially processed specimens (*i.e.* trunks) used L_{ID} measurement: $L_T = 3.4084\ L_{ID} + 16.528$ ($n = 71$, $R^2 = 0.97$; Coiraton et al. 2017) and $L_T = 3.402\ L_{ID} + 16.327$ ($n = 100$, $R^2 = 0.90$; Gallegos-Camacho & Tovar-Ávila 2011). Because of the difficulties in obtaining adequate sample sizes of adults (8 females and 16 males), the present study focused on immature specimens, of L_T below the estimated size at maturity in the region ($L_{T\ \mathrm{females}} < 207$ cm and $L_{T\ \mathrm{males}} < 170$ cm; Anislado-Tolentino and Robinson-Mendoza 2001; Torres-Huerta et al. 2008).

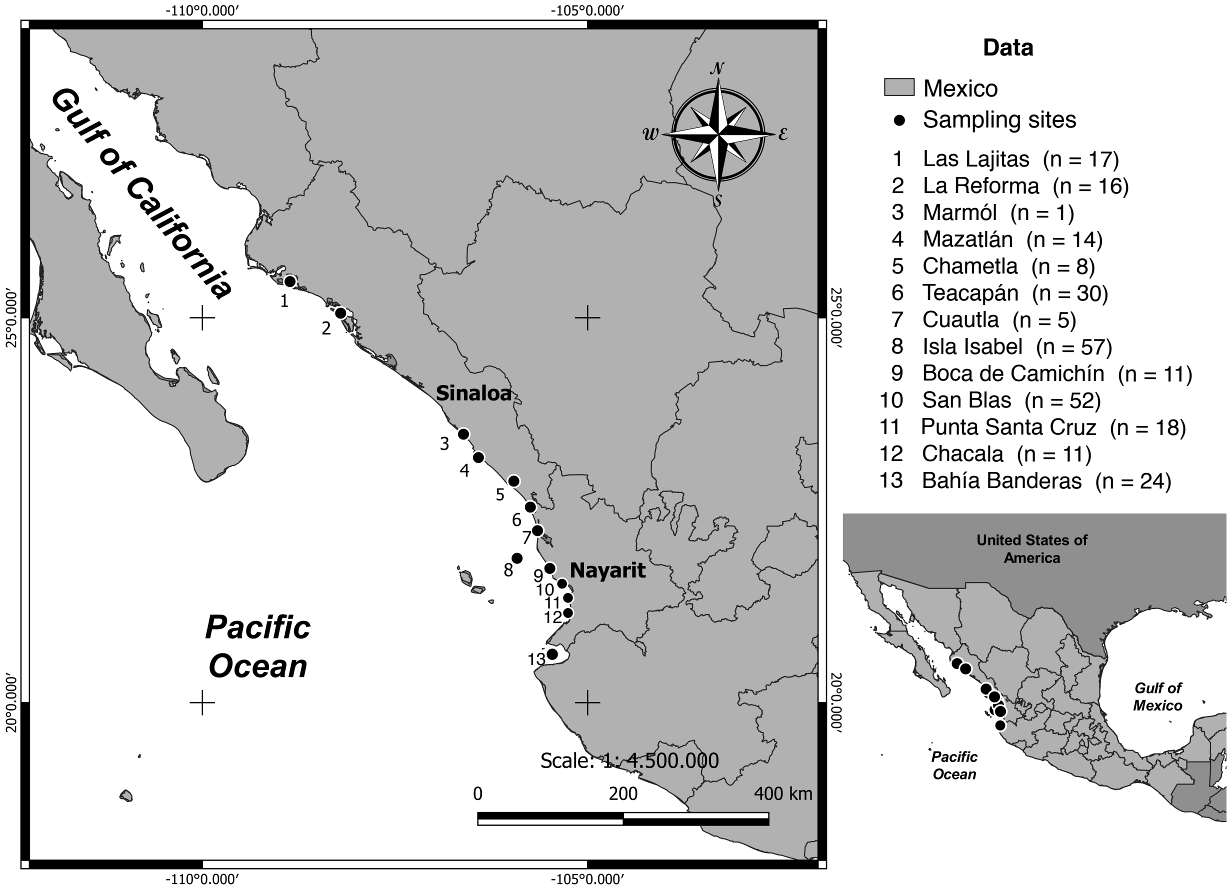

Fig. 3.1 Study area showing the *Sphyrna lewini* sampling sites. Additional samples (*n* = 32) were obtained from the shrimp trawl fishery operating off southern Sinaloa and northern Nayarit

Preparation of vertebrae for ageing

Following standard protocols recommended by Cailliet and Goldman (2004), vertebrae were defrosted and the neural arch and extraneous tissue removed, individual centra were soaked in 30% H_2O_2 to remove remaining tissue, thoroughly rinsed, air dried, mounted on wooden holders and later cut into 0.4 mm sagittal sections with a Buehler low-speed Isomet saw (www.buehler.co.uk).

Age estimation

The terminology for vertebrae recommended by Cailliet & Goldman (2004) is used throughout the text (Fig. 3.2). Vertebra sections were examined under transmitted light using a binocular dissecting microscope (Zeiss Stemi 508; www.zeiss.com) equipped with a digital camera (Zeiss AxioCam ERc5s) and software (Zen 2.3 Blue Edition; Zeiss). The birthmark was identified as the first translucent band closest to the focus of the vertebra and a change in angle along the *corpus calcareum* interface; this represented age 0. Each subsequent translucent growth band that extended across both sides of the *corpus calcareum* was counted (Goldman 2005). Each vertebra section was read by two readers, with the two readings being at different times. Each count was made with no knowledge of the previous count, or of the sex or size of the shark. When counts differed between the two readings, these sections were re-examined by each reader and a consensus was decided. If disagreement persisted, then those samples were excluded from the analyses. Vertebra centrum radius (R_V) was measured as a straight line from the centrum focus to the outer margin of the *corpus calcareum* to the finest scale possible (Goldman et al. 2012). Linear regression was used to determine the relationship between R_V and L_T. To confirm correct identification of the birthmark, length at the formation of the supposed birthmark was back-calculated by the Fraser–Lee method (Francis 1990):

$$L_t = [(R_T)(R_V)^{-1}](L_C - a) + a$$

where L_t is the back-calculated length corresponding to age t, R_t the distance between the focus and each translucent band at age t, R_V the centrum radius, L_C the length at the time of capture and a is the intercept of the regression between R_V and L_C. Whenever back-calculated length at birth differed from the reported lengths at birth of *S. lewini* in the area of study, the sections were re-examined for the birthmark position by both readers. Those samples where disagreement on the birthmark position persisted were excluded from analysis because of the risk of incorrectly assigning age 0. It is well established that in the southeastern Gulf of California, pregnant females of *S. lewini* give birth during the summer months, between May and July (Torres-Huerta et al. 2008), with the formation of the vertebral birthmark deposited at that moment being representative of the transition from an *in-utero* to a *post-partum* life history (Cailliet and Goldman 2004). Considering that the first translucent band is possibly formed during the following winter months (Piercy et al. 2007; Kotas et al. 2011), the first vertebral growth band pair of *S. lewini* would therefore represent less than one year (8–11 months of age) once such translucent band is fully formed. However, for the purposes of this study, it was assumed that the first growth band pair represented a complete year of growth to simplify the age estimates and comparisons with previous studies. It was also considered that such assumption would not affect the precision, bias and verifications process of the periodicity of the growth band formation because the analyses performed (*i.e.* MIA and CEA) are independent of the estimated age and the duration of the first growth band formation. It would be however necessary to determine in the future if considering the first growth band as a full year or estimating the fraction that it would represent could affect the estimation of the population growth parameters.

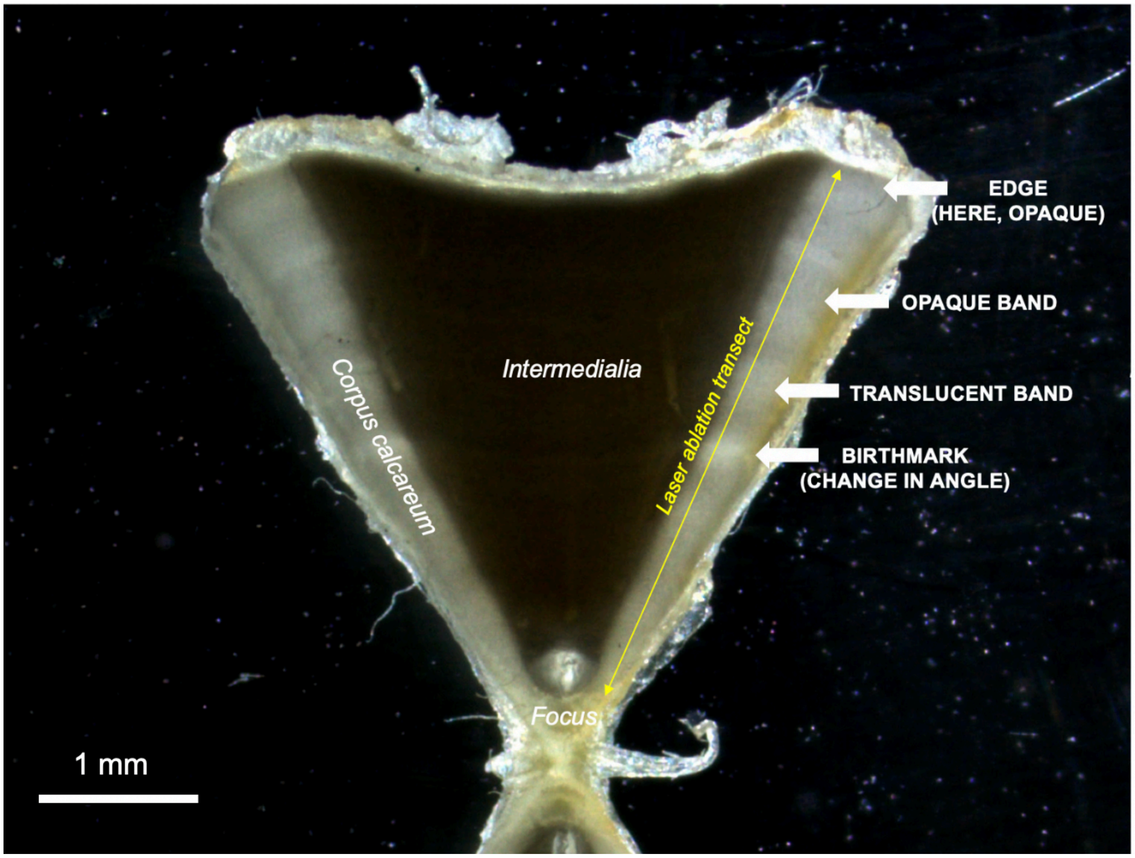

Fig. 3.2 Sagittal section of a vertebra centrum from a 4 year-old *Sphyrna lewini*, illustrating the terminology used throughout the text

Ageing precision and bias

Precision, defined as the reproducibility of repeated measurements on a given structure, was estimated through the traditional index of average percentage error (IAPE; Beamish and Fournier 1981):

$$IAPE = \frac{1}{n}\sum_{j=1}^{n}\left[\frac{1}{r}\sum_{i=1}^{r}\frac{|x_{ij} - x_j|}{x_j}\right]$$

where n is the number of sharks aged, r is the number of readings, x_{ij} is the i^{th} age estimation of the j^{th} shark at the i^{th} reading, and x_j is the mean age calculated for the j^{th} shark.

The coefficient of variation (CV) was also used as a precision measure because it has been described as statistically more rigorous and flexible (Chang 1982):

$$CVj = 100\% * \left(\frac{\sqrt{\frac{\sum_{i=1}^{R} (x_{ij} - x_j)^2}{R-1}}}{x_j} \right)$$

where CV$_j$ is the age precision estimate for the j^{th} fish. This can be averaged across sharks to produce a mean CV. An age-bias plot compared the bias of growth band counts between the two readers (Campana et al. 1995), and Bowker's test of symmetry (1948) determined whether differences between readers were systematic or due to random error (Hoenig *et al.,*1995):

$$\chi^2 = \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} \frac{(n_{ij} - n_{ji})^2}{n_{ij} + n_{ji}}$$

where *n* is the observed frequency in the i^{th} row and j^{th} column, and n_{ij} is the observed frequency in the j^{th} row and i^{th} column.

Validation of the periodicity of growth band formation

MIA, as a first validation method, determined the periodicity of the translucent growth band formation. Marginal increment ratios (R_{MI}) were derived (Branstetter and Musick 1994; Natanson et al. 1995):

$$R_{MI} = \frac{(R_V - R_n)}{(R_n - R_{n-1})}$$

where R_V is the vertebra centrum radius, R_n is the radius of the last complete translucent band and R_{n-1} is the radius of the penultimate complete translucent band. All measurements were made along the *corpus calcareum* using digital photographs and software (Zen 2.3 Blue Edition). Individuals of age 0 (young-of-the-year) were excluded from MIA because they have no fully formed winter translucent increments. Monthly averaged R_{MI} values were plotted to detect trends in the growth band formation. A one-way Kruskal-Wallis test assessed differences in R_{MI} by month and compared the results with previous studies. Following Okamura et al. (2013), a circular-linear regression model with random effects was used to adjust three models of growth periodicity (acyclic, annual and biannual cycle) to the R_{MI} data. The Akaike information criterion (AIC; Akaike 1973) determined which growth cycle best fitted the data.

CEA, as the second validation method, compared the monthly frequencies of translucent and opaque centrum edges. Again, young-of-the-year that only displayed birthmarks were excluded because translucent birthmark periodicity differs from normal growth band pairs and does not reflect growth seasonality. Following Okamura & Semba (2009), the data were adjusted to different periodicity models (acyclic, annual or biannual), which were compared with AIC to determine which best fit the data. Models were fitted to R_{MI} and CEA data using R software (R Core Team; www.r-project.org), ADMB platform (Fournier et al. 2012; http://admb-project.org/) and the program code available in Appendix SIII (Supporting Information) in Okamura et al. (2013) and online (http://cse.fra.affrc.go.jp/okamura/program/agevalid/index.html; Okamura and Semba 2009).

R_{MI} and CEA data were related to the monthly averaged sea surface temperature (SST) registered across the study area by the Coastwatch West Coast Regional Node of the National Oceanic and Atmospheric Administration (https://coastwatch.pfeg.noaa.gov/) for the years and months in which R_{MI} and CEA data were available. Pearson's correlation analysis determined whether vertebral growth was influenced by SST.

Elemental profile analysis

Elemental profiles were analyzed in a subset of vertebral sections as an attempt to verify the age estimates obtained by the traditional approaches. Vertebral sections were hand polished with a series of progressively finer grades of lapping paper until the growth bands were clearly visible, sonicated in Milli-Q water to remove surface contaminants, triple rinsed, dried for 24 h and randomly affixed to acid-washed petrographic slides (subsequently referred to as 'master slides'). One vertebra section from each specimen was used for analyses as it was shown that elemental signatures of *S. lewini* did not differ among vertebrae from a same shark (Schroeder 2011; Smith et al. 2016). All cleaning and drying procedures were performed under a Class-100 laminar-flow clean hood using trace-metal-grade reagents, non-metallic instruments and HNO_3 acid-washed glass slides.

A Photon-Machines Analyte.193 excimer UV laser ablation system (LA), connected to an Agilent Technologies 7500CX quadrupole inductively coupled plasma–mass spectrometer (ICP-MS), was employed to characterize the elemental profiles in the vertebrae of *S. lewini*, which were assayed along transects encompassing the area from the focus towards vertebral edge completely within the *corpus calcareum* (Fig. 3.2). Transects were pre-ablated prior to data acquisition in order to remove possible external contamination. Pre-ablation transect scan speed was 108 μm s^{-1}, with a repetition rate of 2 Hz and a spot size of 108 μm. For data acquisition, ablation transect scan speed was 10 μm s^{-1}, with a repetition rate of 10 Hz and a spot size of 83 μm.

Data were acquired by the ICP-MS which employed Agilent Technologies ChemStation software operating in time-resolved analysis mode to collect raw data of the ion count rates in counts per second (cps) for ^{31}P, ^{43}Ca and ^{55}Mn along the vertebral transects. As there is no matrix-matched standard available for shark vertebrae, NIST-612 silicate glass served as external calibration reference material (values given in Pearce *et al.* 1997) and was ablated with two replicates before and after every fifth vertebral section was sampled. MACS-3 microanalytical carbonate standard material (Koenig and Wilson 2007) was ablated in brackets before and after each master slide to estimate experiment-wide levels of precision. Raw cps data for ^{31}P and ^{55}Mn were standardized to the number of ^{43}Ca ions (cps) obtained simultaneously in the structure by deriving element-to-Ca cps data ratios (element:Ca) to adjust for variability in instrument sensitivity and the amount of ablated material and to provide accurate data on the relative distribution of the target elements along the transect accounting for potential differences in ^{43}Ca data within the vertebrae, which have been reported to occur between opaque and translucent growth bands for some species (*e.g.* Scharer et al. 2012; Raoult et al*.* 2016; Mohan et al. 2018). Background data corresponding to gas blanks were collected for 90 s before and after each transect was ablated. Parsing, processing and quality control of the raw cps data generated by the methods described above were performed using the free download Fathom Toolbox for Matlab$^{\text{TM}}$ (Jones, 2017).

Data for ^{43}Ca (cps) and the ^{31}P:^{43}Ca and ^{55}Mn:^{43}Ca ratios (no unit) were plotted *versus* vertebral transect distance (µm) for each sample. A 11-point running average window size was applied to filter/smooth the data, reduce the noise, and aid in identifying the underlying pattern of the elemental profiles (Sinclair et al. 1998). The degree to which smoothed peaks above the background noise in elemental profiles corresponded to opaque bands was evaluated by overlaying the position of opaque bands on plots obtained by measuring the distance (µm) from the focus to the mid-point of each opaque band of the vertebra. Linear regression analyses investigated how well the opaque growth band counts (independent variable) predicted peaks (dependent variables) in the ^{31}P:Ca, ^{55}Mn:Ca and ^{43}Ca transect profiles.

All data were assessed for normality and homogeneity of variances by Shapiro-Wilk's and Levene's tests before the statistical analyses. When required, data were $\log_{10}$-transformed to conform to the assumptions of parametric analyses.

RESULTS

Age estimation

Overall, the vertebrae from 296 young-of-the-year and juvenile specimens of *S. lewini* from the southeastern Gulf of California were analyzed: 135 females (47.3–193 cm L_T,) and 161 males (42–158.7 cm L_T) (Fig. 3.3a). Growth bands were relatively easy to discern in all vertebral sections except for those in sharks <2 years old. Excluding the pre-birth and birthmarks, the number of translucent growth bands counted along the *corpus calcareum* (and thus the estimated ages) ranged from 0 to 10+ years for females and 0 to 7+ years for males (Fig. 3.3b). R_V and L_T were strongly correlated, with a linear relationship ($L_T = 17.35R_V + 14.52$, $R^2 = 0.92$). Back-calculated length at the formation of the birthmark, L_T, ranged from 46.6 to 65.7 cm (mean ± S.D., 53.8± 3.3 cm).

Ageing precision

The IAPE and CV between the final counts of the two readers (4.3% and 6.1% respectively) indicated a high precision of growth band counts. Most of the counts agreed exactly between readers (84.4%) and 98.4% within one band pair. In addition, the age-bias plot (Fig. 3.4) and Bowker's test of symmetry showed no systematic bias, and low disagreement between readers ($\chi^2 = 8.12$, d.f. 8, $P = 0.5$).

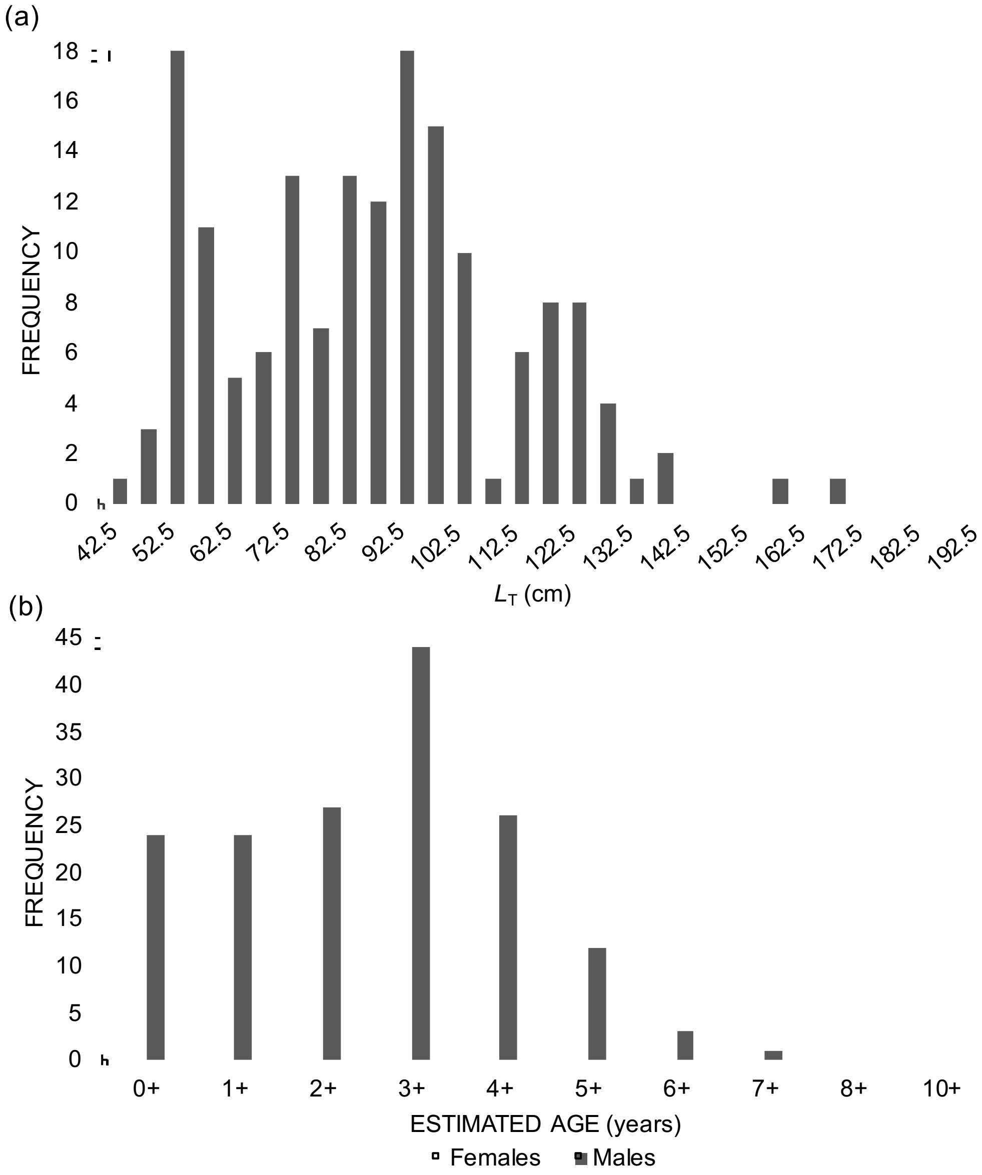

Fig. 3.3 (a) Total length (L_T; cm) and (b) estimated age (translucent growth band counts) frequency distribution of the male (*n* =161) and female (*n* = 135) juvenile specimens of *Sphyrna lewini* landed between March 2007 and September 2017 in several locations of the southeastern Gulf of California (*n* = 296)

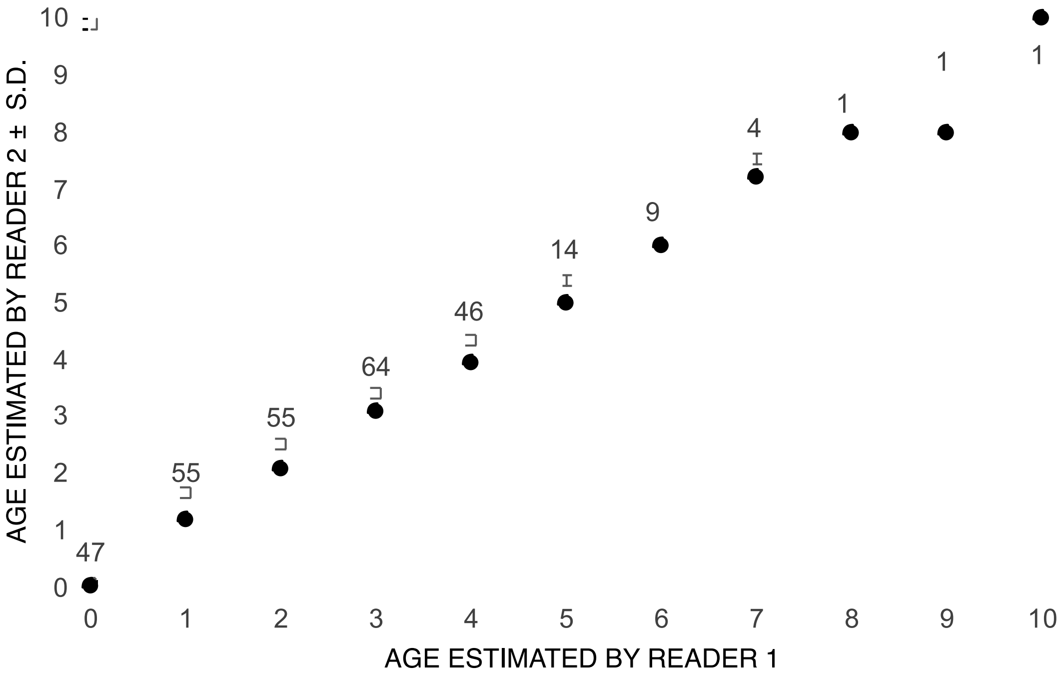

Fig. 3.4 Age-bias plot for pair-wise comparisons of juvenile *Sphyrna lewini* vertebral translucent growth band counts of age reader 2 (± SD) versus age reader 1 relative to the line of 1:1 equality (--). Numbers above the graph line denote sample size for the month

Validation of the periodicity of growth band formation

In total, 235 specimens of *S. lewini* were suitable for MIA and CEA. Despite low sample sizes in May (n = 2), June (n = 1), July (n = 2) and December (n = 6), the R_{MI} data differed significantly among months (Kruskal–Wallis χ^2= 60.02, $P < 0.001$); a trend to increase in June and to decline in October suggested that a single translucent growth band was formed between November and March (Fig. 3.5a). AIC values obtained with the Okamura *et al.* (2013) method (87.07 for acyclic model, 57.37 for annual and 95.85 for biannual cycle models) supported an annual cycle of growth band formation. The trend was positively correlated with the annual variation pattern of the averaged SST (Pearson's correlation: r = 0.74; $P < 0.01$) (Fig. 3.5a), indicating that vertebral growth of *S. lewini* is probably strongly related to SST.

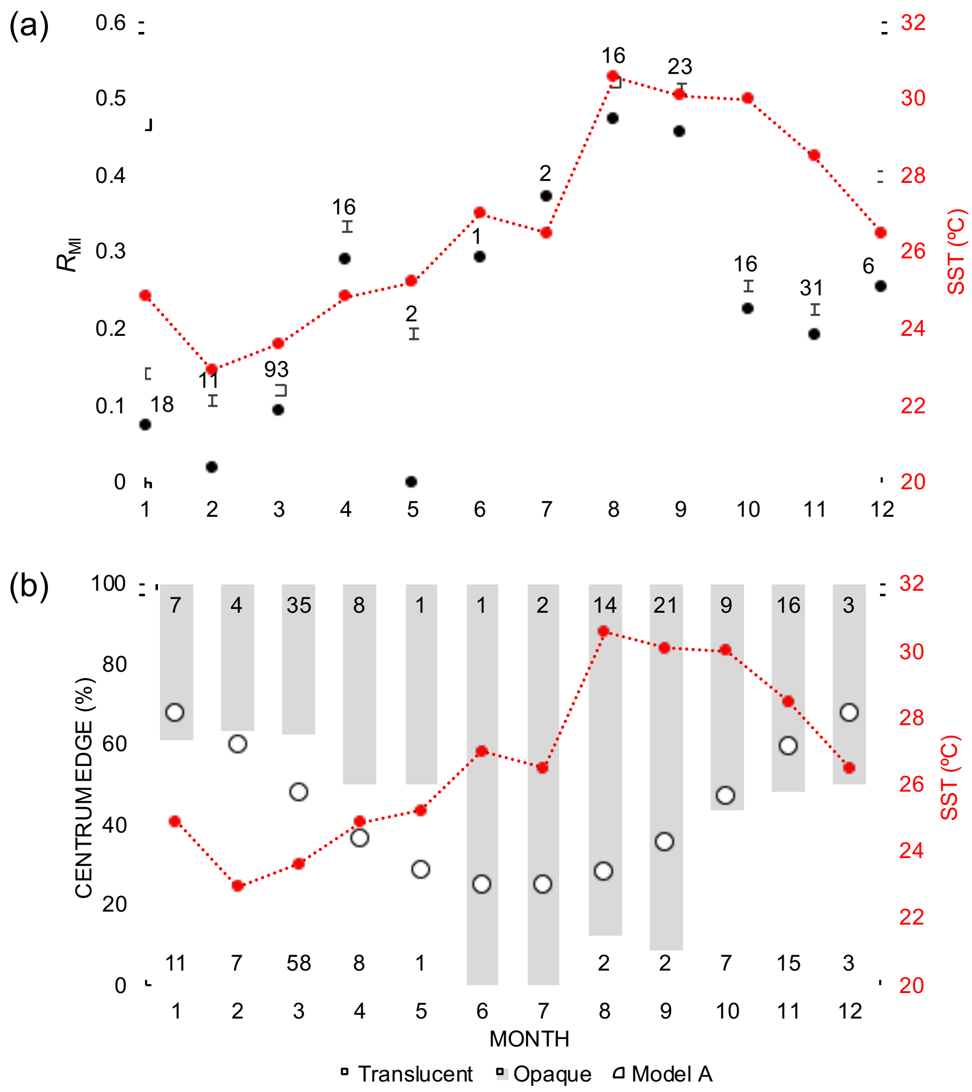

Fig. 3.5 (a) Monthly averaged marginal increment ratios (R_{MI}) ± S.D. and (b) monthly percentages of opaque and translucent centrum edges in vertebrae of juvenile *Sphyrna lewini* (*n* = 235), compared with the monthly averaged sea surface temperatures (SST) between March 2007 and September 2017 in the southeastern Gulf of California. Open circles: translucent edge percentage values predicted by model A from Okamura & Semba (2009). Numbers above each bar (a) and within columns (b) give sample sizes

Of the 235 vertebrae considered for CEA, 48.5% had a translucent centrum edge, with the highest frequency occurring in January and the lowest in June and July (Fig. 3.5b). The variation of the monthly proportions of translucent edges was seasonal and closely followed the trend exhibited by the monthly averaged R_{MI} (Figs. 3.5 a,b), supporting the hypothesis that a single translucent growth band was formed during the winter months. AIC values obtained with the

Okamura & Semba (2009) method (327.6 for acyclic model, 317.1 for annual and 331.4 for biannual models) similarly supported an annual cycle of the growth band formation in the vertebrae of juveniles of *S. lewini* (Fig. 3.5b). This trend also followed the annual variation pattern of the averaged SST registered in the study area with the highest proportion of translucent edges occurring during the coldest months (22.9–24.5°C) and the lowest proportion when the temperatures were the highest (27–30°C) (Pearson's correlation: $r = 0.67$; $P = 0.01$) (Fig. 3.5b).

Elemental profile analysis

Vertebrae samples from 15 juvenile specimens of *S. lewini* aged from 1 to 5 years (66.4–90.8 cm L_T) collected in La Reforma ($n = 5$), Chametla ($n = 5$) and Teacapán ($n = 5$) were made available for the LA-ICP-MS analyses performed as an attempt at age verification (Table 3.1).

The spatial variation of ^{55}Mn:Ca along the vertebral transects was significantly correlated with the visible pattern of opaque bands in the samples ($R^2 = 0.48$) (Figs. 3.6a and 3.7). The corresponding linear relationship between the number of smoothed peaks identified in the ^{55}Mn:Ca profile (dependent variable) and the number of opaque growth bands (independent variable) was $y = 0.88x + 0.34$ ($P < 0.01$) (Fig. 3.6a). ^{55}Mn:Ca data accurately predicted the number of opaque growth bands in 66.6% ($n = 10$) of the vertebrae sampled. For the remaining 5 samples, the number of peaks in the ^{55}Mn:Ca profiles differed from the number of opaque growth bands by -2 to +2 counts (Fig. 3.6a).

Although the spatial variation of ^{31}P:Ca along the vertebral transects was also significantly correlated with the visible pattern of opaque bands in the samples ($R^2 = 0.54$) (Figs. 3.6b and 3.7), ^{31}P:Ca data could accurately predict the number of opaque growth bands in only 46.6% of the samples ($n = 7$). For the remaining 8 samples, the number of peaks in the ^{31}P:Ca profiles differed from the number of opaque growth bands by -1 to +4 counts. The corresponding linear relationship was $y = 1.19x + 0.19$ ($P < 0.01$) (Fig.3.6b).

The ^{43}Ca profiles did not exhibit a consistent pattern among individuals. ^{43}Ca values either showed a progressive increase from the focus to the edge ($n = 6$), a progressive decline from the focus to the edge ($n = 3$; Fig. 3.7), or a constant pattern along the transects profiles ($n = 6$). The predictions of the ^{43}Ca data were highly variable ($R^2 = 0.1$, $P > 0.5$) and thus are not presented here (but see Table 3.1).

Table 3.1 Number of peaks counted in the smoothed profiles of the ^{43}Ca data (cps) and ^{31}P:Ca and ^{55}Mn:Ca (no unit), obtained from LA-ICP-MS transect scans encompassing the area from the focus to the edge of the vertebrae from 15 juvenile specimens of *Sphyrna lewini*, compared with the number of opaque growth bands

Sampling site and date	Sample	Size (L_T; cm)	Sex	Opaque bands	^{43}Ca	^{31}P:Ca	^{55}Mn:Ca
LA REFORMA Apr 2015	RE-1	84	M	3	2	5	5
	RE-2	86.8	M	3	2	7	1
	RE-3	90.8	M	3	3	4	5
	RE-4	84.4	H	3	4	4	3
	RE-5	71.2	M	2	2	2	2
CHAMETLA Nov 2014	CH-1	72.8	M	2	1	1	2
	CH-2	83	H	2	2	3	2
	CH-3	87	M	4	5	6	3
	CH-4	83	H	2	3	3	2
	CH-5	83	M	5	0	5	5
TEACAPÁN Feb 2014	TC-1	78.2	M	4	3	4	4
	TC-2	84	M	4	4	4	3
	TC-3	69	H	2	1	2	2
	TC-4*	66.4	H	2	1	2	2
	TC-5	66.6	H	1	1	1	1

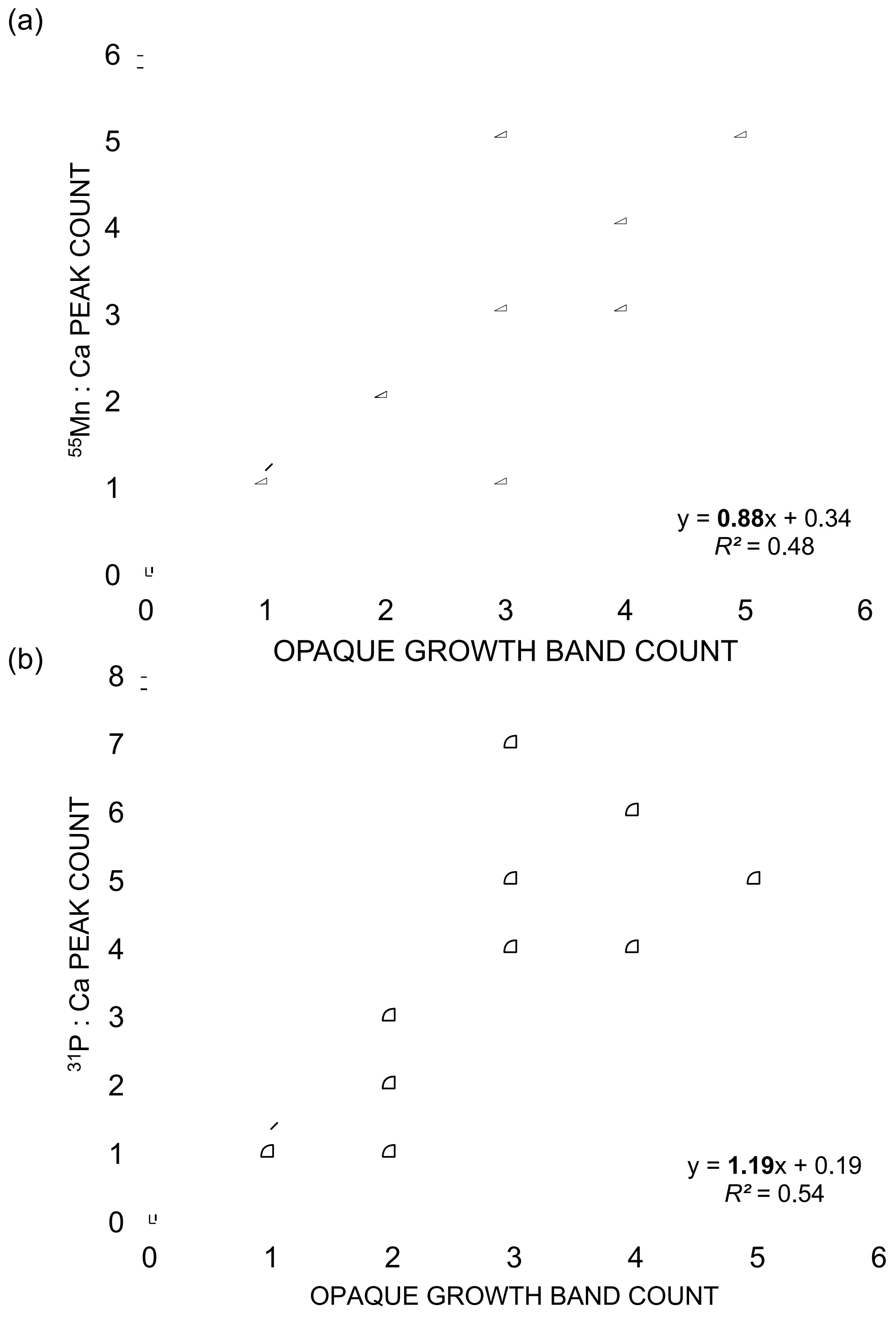

Fig. 3.6 Number of smoothed peaks in the (a) ^{55}Mn:Ca and (b) ^{31}P:Ca ratio profiles versus number of opaque growth bands measured in the vertebrae from 15 juvenile specimens of *Sphyrna lewini*. Dashed lines represent 1:1 equality

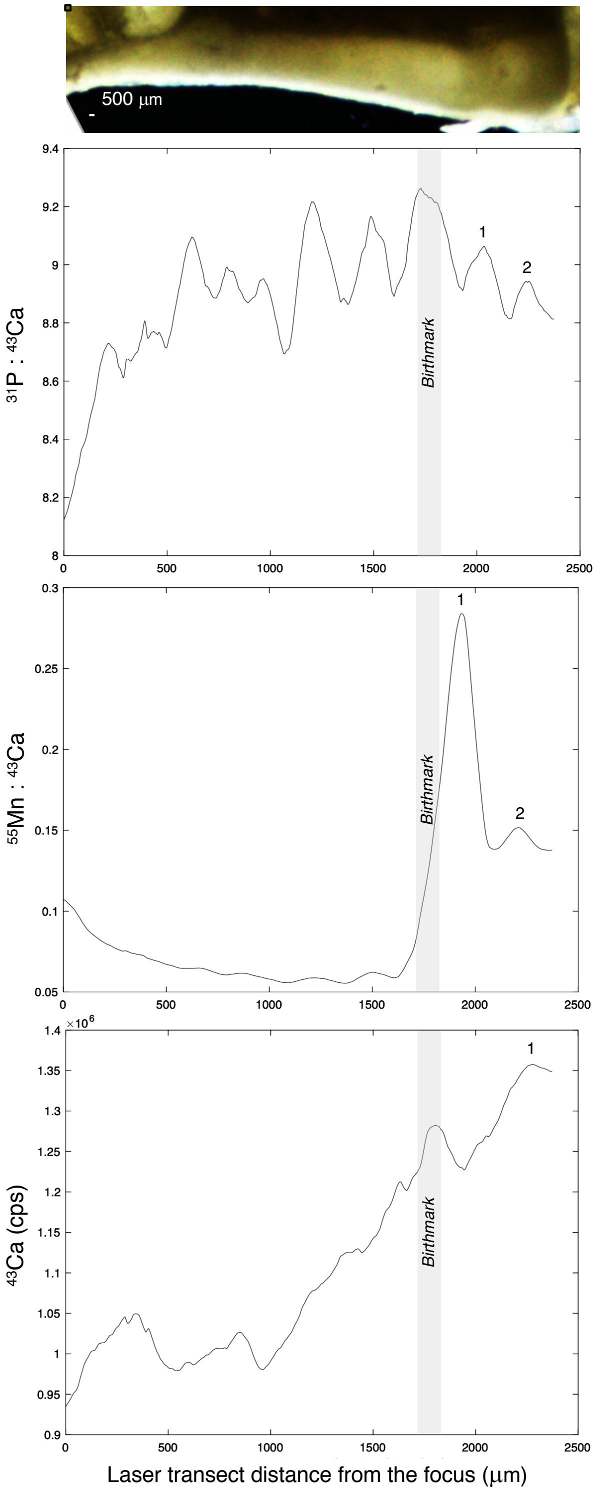

Fig. 3.7 (a) Representative photograph of the *corpus calcareum* of a vertebral section from a juvenile *Sphyrna lewini* aged 1 year (sample: TC-4), together with the corresponding smoothed profiles of the (b) ^{31}P:Ca, (c) ^{55}Mn:Ca and (d) ^{43}Ca data encompassing the area from the focus to the vertebral edge. Numbers show peaks identified

DISCUSSION

Age estimation

There is no *a priori* value of precision that can be designated as a target for ageing studies, since precision is highly influenced not only by the species biology but also by the age reader (Campana 2001). However, a review (Campana 2001) of 131 ageing papers stated that all studies that have estimated the age of sharks based on vertebral growth band counts reported CV precision values >10%. On that basis, Campana (2001) recommended that ageing studies be carried out with a CV of < 7.6%, corresponding to an IAPE of 5.5%, serving as a reference point. Accordingly, the CV value obtained in this study (6.1%) was consistent with a precise age estimation, especially since CV values as low as 6.8% (Drew et al. 2015) and as high as 17.9% (Harry et al. 2011) have been reported in studies of *S. lewini*. In addition, the IAPE value obtained here (4.3%) was also consistent with those obtained from previous ageing studies of *S. lewini* (3.2%, Piercy et al. 2007; 3.7%, Anislado-Tolentino et al. 2008; 5.6%, Kotas et al. 2011), a further indication of a precise age estimation with low bias.

The validation of the first increment is mandatory in ageing studies because without a correctly defined starting point age estimations would be consistently biased, especially when the goal of a study is to validate the periodicity of the growth band formation rather than the absolute age, as chemical tagging studies do (Campana 2001). According to the length-at-birth of *S. lewini* reported in the southeastern Gulf of California (41–53 cm L_T; Anislado-Tolentino 2000; Torres-Huerta et al. 2008, Coiraton et al. 2017), the back-calculated length-at-birth obtained in the present study (53.8 ± 3.3 cm, mean ± S.D.) indicated that the birthmark was correctly discerned by readers in all vertebral sections.

Validation of the periodicity of the growth band formation

Age estimates derived from visual counts of concentric growth zones in calcified structures such as vertebrae rely on validating the temporal periodicity of formation of these zones over the lifespan of the species (Beamish and McFarlane 1983; Cailliet et al. 1986; Campana 2001). Although validation of the periodicity of growth band formation in vertebrae of *S. lewini* was attempted in previous studies (Branstetter 1987a; Anislado-Tolentino and Robinson-Mendoza 2001; Anislado-Tolentino et al*.* 2008; Harry et al. 2011; Drew et al. 2015), few have provided conclusive results; the use of a single method of validation (either MIA or CEA) precluded an objective comparison of results, samples and ages were lacking for some months, and there was a mixture of individuals of different sizes and ages (in some cases even young-of-the-year). It is important to separate the life stages in *S. lewini* ageing studies to avoid sex and life-stage bias, since these can be subject to different growth patterns (Campana 2001).

In this study, only immature specimens were considered because of the difficulties in obtaining adequate sample sizes of all life stages forming the population. The MIA and CEA methods were combined as a validation process, and results indicated that in the Mexican Pacific a single translucent growth band was formed in the vertebrae of the juveniles during the winter months (November to March), a period of slower growth. A single opaque growth band was formed during the summer months, between June and September, a period of faster growth. A similar pattern of growth has been reported for *S. lewini* in the northwestern Atlantic Ocean and Gulf of Mexico (Piercy et al. 2007; Branstetter 1987a), southern Brazil (Kotas et al. 2011), eastern Australia (Harry et al. 2011) and Indonesia (Drew et al. 2015).

Although MIA was inconclusive for elucidating the periodicity of growth band formation in the vertebrae of *S. lewini* in previous studies (Drew et al. 2015), here it allowed visual discernment of a trend in the monthly R_{MI} data in spite of the low sample size in some months. This observation was statistically supported by a Kruskal-Wallis test and the model developed by Okamura et al. (2013), which provided strong evidence for an annual growth cycle for R_{MI} data. Burnham & Anderson (2002) suggested that a difference of >2 in AIC values should be required to identify with certainty the best model in terms of the Kullback–Leibler divergence. Here, the annual cycle model had an AIC difference of > 10, confirming that this model was the best fitted to the R_{MI} data. Moreover, CEA also graphically revealed a contrast of growth between the winter (higher proportions of translucent edges) and summer (higher proportions of opaque edges) in spite of the lack of data between May and July, and the predictions of the annual cycle model developed by Okamura and Semba (2009) clearly showed this trend. This model is applicable to data sets that lack data for some months (Okamura and Semba 2009), as in the present study.

Evidence of latitudinal variations in growth have been found for various species of elasmobranchs (Driggers et al. 2004; Licandeo and Cerna 2007), including other hammerhead sharks such as *S. tiburo* (Lombardi-Carlson et al. 2003). Those variations could be occurring also for *S. lewini* among geographical regions (*e.g.* Taiwan; Chen et al. 1990). Although this hypothesis must be tested, the assumption of the existence of different patterns of growth within the Mexican Pacific (*i.e.* Anislado-Tolentino and Robinson-Mendoza 2001; Anislado-Tolentino et al. 2008) seems unlikely for *S. lewini*. The observed differences among studies may be due in part to differences in the vertebra preparation and growth band interpretation. The annual formation of growth bands in vertebrae of two other *Sphyrna* species, *S. mokarran* (Passerotti et al. 2010,) and *S. tiburo* (Parsons 1993; Carlson and Parsons 1997) have been directly validated by bomb radiocarbon and chemical tagging in vertebrae, which gives support to the findings of the present study and those of the earlier ones that also supported an annual growth cycle for *S. lewini* in other regions of the world. Considerable progress has been made in age validation efforts since the results of Chen et al. (1990), Anislado-Tolentino and Robinson-Mendoza (2001) and Anislado-Tolentino et al. (2008) were published, and the particularly robust and statistically rigorous methods developed by Okamura and Semba (2009) and Okamura et al. (2013) were not available at the time to facilitate the verification process of the periodicity of growth band formation. Here, the use of such approaches addressed this uncertainty for *S. lewini* in the Mexican Pacific, even though only juveniles could be assessed. It is still necessary to verify the timing and frequency of the growth band formation in the vertebrae of adult specimens across the area, because growth can change once individuals mature (see Wells et al. 2013; Kinney et al. 2016). Although the periodicity of growth band formation needs to be validated for all sizes and ages representing the population of the species in the region, the annual formation of the growth bands in the vertebrae of juveniles makes it necessary to re-estimate growth parameters and productivity to ensure that the population is harvested at sustainable levels.

Banding patterns in vertebrae of elasmobranchs may be related to growth in size rather than age (Natanson and Cailliet 1990; Natanson et al. 2008; Baremore et al. 2009). The fact that some species do not show an annual banding pattern (*e.g.* basking shark *Cetorhinus maximus*; Natanson et al. 2008) or exhibit ontogenetic changes in their growth cycle over the life history (*e.g.* shortfin mako *Isurus oxyrinchus*; Wells et al. 2013; Kinney et al. 2016) reinforces the need to confirm the temporal meaning of these growth increments for each species (Natanson 1993). Seasonal changes in temperature, light and food availability, and/or migrations, may cause the deposition of periodic growth bands in the vertebrae of elasmobranchs (Pratt and Casey 1983; Branstetter 1987b), although causes can vary depending on the species. *S. lewini* is a large and migratory species that cannot be maintained in captivity to conduct controlled experiments and explore those cues. However, it appeared that seasonal variations in temperature influenced in part its vertebral growth rate and banding pattern in the Mexican Pacific. The highest temperatures that occurred during the summer months were related to the highest growth increments and opaque band formation at the edge of these shark vertebrae. Similar results were also observed for *I. oxyrinchus* (Ribot-Carballal et al. 2005), and recent experimental studies have shown that increased temperatures had a positive effect on the mean growth rates of the round stingray *Urobatis halleri* (Smith et al. 2013). Although the apparent relationship that relates vertebral growth and seawater temperature allowed to verify the annual banding pattern in vertebrae of juveniles of *S. lewini* from the Mexican Pacific, the forecasted end-of- century increases in temperature might also have detrimental effects on these sharks (Pistevos et al. 2015). Recent experiments showed that more elevated temperatures increased food intake and energetic demand which, when combined with elevated CO2, could also affect the hunting behavior of sharks (Pistevos et al. 2015). Even though investigating the effects of global warming on *S. lewini* was not the purpose of this study, these are of concern and need to be investigated in the near-future.

Elemental profile analysis

Analyses of elemental profiles (^{43}Ca, ^{31}P and ^{55}Mn) in vertebrae of juvenile specimens of *S. lewini* were used in this study as an attempt to verify for the first time the periodicity of the growth band formation and age estimates derived from visual counts of the opaque growth bands by matching peaks in elemental concentrations with the seasonal banding pattern. The use of strontium (^{88}Sr) was not explored for *S. lewini* because little is known about the age-related movement patterns of this species (Harry et al. 2011; Ketchum et al. 2014) and seawater chemistry was not available to compare with the variations of Sr in its vertebrae.

No significant relationship was found between peaks in ^{43}Ca profiles and the opaque growth bands; however, the peaks in ^{31}P:Ca and ^{55}Mn:Ca spatially corresponded with the annual banding pattern in most of the samples. ^{31}P:Ca profiles displayed 1.19 peak per opaque band while ^{55}Mn:Ca displayed 0.88 peak per opaque band; this closely matched the annual deposition rate in juveniles obtained from the verification analyses of this and previous studies (Branstetter 1987a; Piercy et al. 2007; Harry et al. 2011; Kotas et al. 2011). However, these interpretations remain tentative because age estimates explained only half of the variability of the elemental patterns, as shown by the low (but significant) correlation coefficients. When ^{31}P:Ca and ^{55}Mn:Ca data could not accurately predict the number of opaque growth bands, the predictions did not vary in a predictable manner and could be both less than or greater than opaque band counts; this indicates that the incorporation of these elements in the vertebrae of *S. lewini* might be mediated by factors other than growth, which perhaps do not show any temporal cycle.

Several authors have explored the elemental variations (*e.g.* ^{43}Ca, ^{31}P, ^{55}Mn and ^{88}Sr) in vertebrae of elasmobranchs as they can relate to growth band deposition (Jones and Geen 1977; Cailliet and Radtke 1987; Hale et al. 2006; Scharer et al. 2012; Raoult et al. 2016; Mohan et al. 2018). Ca was not useful for verifying ages in most of the species (*Pristis pectinata*, *Carcharodon carcharias*, *S. zygaena*, *Heterodontus portusjacksoni*, *Carcharhinus obscurus*, *C. brevipinna, Prionace glauca*, *Alopias vulpinus* and *Isurus oxyrinchus*; Scharer et al. 2012; Raoult et al. 2016; Mohan et al. 2018), although the variations of Ca were strongly related with opaque growth bands in the vertebrae of the round stingray *Urobatis halleri* and spiny dogfish *Squalus acanthias* (Jones and Geen 1977; Hale et al. 2006). Raoult et al. (2016) stated that a stronger correlation might be found if more individuals of these species were analyzed; however, the results of the present study, as in previous studies (Scharer et al. 2012; Raoult et al. 2016; Mohan et al. 2018), showed that banding pattern in vertebrae of elasmobranchs may be more closely related to element absorption than to calcification or growth. One explanation for the discrepancy observed among the elemental patterns in vertebrae of these various elasmobranch species is that they exhibit contrasting patterns of migration that have possibly impeded interpretation and comparison of the data obtained. The different species occupy different habitats (*i.e.* coastal, oceanic or both) and vary widely in their elemental patterns, with some elements being more accurate than others for predicting the age estimates of a species.

Variations of ^{31}P also exhibited a yearly period in the vertebrae of *S. acanthias* (Jones and Geen 1977), which they concluded was an independent way to estimate age. Hale et al. (2006) and Scharer et al. (2012) found no apparent relationship of peaks in P in the vertebrae of *P. pectinata* and *U. halleri* with opaque growth bands. In coastal waters, concentrations of P typically exhibit winter maxima and summer minima, thus showing a yearly period of change; Ca is by contrast relatively abundant and remains practically constant year-round (Jones and Geen 1977). This might explain why Ca has not been useful for verifying the ages of most species.

In studies of *P. glauca*, *A. vulpinus* and *I. oxyrinchus* (Mohan et al. 2018), as in the present study, ^{55}Mn was the most consistent in accurately predicting the ages of the species. Incorporation of ^{55}Mn in vertebrae is enhanced by a rise in temperature (Smith et al. 2013), as are the growth rates of elasmobranchs (Pistevos et al. 2015). These findings may explain why variations in ^{55}Mn could be correlated with the number of growth bands, since the species differ in their movement patterns (Sepulveda et al. 2004; Nakano & Stevens, 2008; Cartamil et al. 2010; Wells et al. 2013, 2017). As a coastal and oceanic species, *S. lewini* has complex patterns of habitat use (Klimley 1987; Duncan and Holland 2006; Hoyos-Padilla et al. 2014), and despite extensive studies little is known about the movements of juveniles (Harry et al. 2011; Ketchum et al. 2014). The use of Mn might help to verify the age estimates of juvenile *S. lewini* when combined with other approaches, especially since the vertebral growth rate and banding pattern of these sharks is related to seasonal variation in the SST of the eastern Gulf of California.

All previous studies that have assessed vertebral microchemistry of sharks using LA-ICP-MS analyses used ^{43}Ca as an internal standard element to standardize the concentration data of the elements of interest because the elemental composition of the hydroxyapatite matrix that constitutes the vertebrae of sharks is dominated by Ca (Urist 1961; Clement 1992) and this element was assumed to be homogenously distributed within the vertebrae for most species, being this often quantified in advance as a mean species or family-specific percentage mass of Ca value (%Ca) of the vertebrae (*e.g.* Izzo et al. 2016; Lewis et al. 2016; McMillan et al. 2018; Mohan et al. 2018). The fact that in this study Ca did not exhibit a constant pattern along the vertebral transects of most samples therefore raises a question about the suitability of this element as an internal standard to derive the element:Ca ratios, especially since a precondition for the successful use of an element as internal standard for LA-ICP-MS analyses is that it is homogeneously distributed within the samples (Limbeck et al. 2015). Nevertheless, Ca appears to be the only major element occurring naturally in the vertebrae of sharks that could be optimally used as an internal standard to account for fluctuations in the laser ablation yield, ensure reliability of the concentration measurements of the elements of interest, and allow comparisons among individuals and species. In addition, the fact that the ^{55}Mn and ^{31}P data were standardized to ^{43}Ca in spite of the apparent spatial heterogeneity of this element within the vertebrae of *S. lewini* probably did not affect the results of this study because ^{43}Ca was measured simultaneously along the transects (McMillan et al. 2017) rather than being estimated in advance as a mean species-specific %Ca value using solution based ICP-MS (*i.e.* whole vertebrae) or stoichiometrically calculated, which would have not indicated whether the element was actually homogenously distributed within the structure (Limbeck et al. 2015).

In conclusion, analyses of ^{31}P and ^{55}Mn profiles in vertebrae of sharks might help in verifying the banding pattern of species that exhibit seasonal movements among habitats or that are subjected to environmental changes during growth, as in *S. lewini*. It seems necessary, however, to emphasize the importance of standardizing the elemental data to the actual number of ^{43}Ca ions measured simultaneously in the structure in order to obtain accurate element:Ca values that account for potential differences in ^{43}Ca along the vertebral transects. Since banding patterns in vertebrae of elasmobranchs are also caused by changes in environmental conditions, it is also possible that this method would provide better results when seawater chemistry is available to compare with the patterns observed in the vertebrae and when older specimens can be assessed to fully appreciate the possible cyclical variations of the elements. Reliable interpretation of variations in vertebral elemental patterns to estimate ages of a species would require, however, substantially stronger statistical relationships with the number of opaque growth bands.

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CAPÍTULO 4

NUEVAS PERSPECTIVAS SOBRE LOS PATRONES MIGRATORIOS DEL TIBURÓN MARTILLO *Sphyrna lewini* EN EL PACÍFICO MEXICANO

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CAPÍTULO 4 – PATRONES MIGRATORIOS Y USO DE HÁBITAT

RESUMEN

El tiburón martillo *Sphyrna lewini* es una especie migratoria con patrones de organización espacial complejos relacionados con el uso tanto de hábitats costeros como oceánicos. La información existente acerca de sus movimientos y uso de hábitat es escasa, pero estudios recientes mostraron que las firmas microquímicas depositadas en sus vértebras podrían servir como valiosos marcadores espaciales de uso de hábitat. En este sentido, el objetivo de este estudio fue inferir los patrones de movimiento de *S. lewini* en el Pacífico mexicano por medio del análisis de su microquímica vertebral. Las vértebras se obtuvieron de 48 tiburones capturados en 2016 y las firmas elementales se midieron usando un sistema de ablación láser acoplado inductivamente con un espectrómetro plasma de masas (LA-ICP-MS). La variabilidad espacial de la microquímica del borde vertebral de *S. lewini* se evaluó de manera preliminar para verificar si esta técnica permite distinguir con precisión diferentes tiburones de acuerdo a su uso de hábitat reciente, antes de hacer inferencias referentes al ambiente. Posteriormente se evaluaron patrones de movimientos mediante el análisis de las variaciones de los radios Sr:Ca, Ba:Ca, Sr:Ba y Pb:Ca a lo largo de transectos vertebrales que abarcaron toda la historia de vida de cada espécimen. Los análisis de perfiles elementales sugirieron que *S. lewini* tiene patrones de movimiento probablemente más plásticos de lo que se suponía anteriormente, con una afiliación a la costa más fuerte de lo esperado y un uso relativamente limitado de los hábitats oceánicos. Más específicamente, las hembras utilizaron ambientes muy variables a lo largo de su vida mientras que los machos alternaron entre dos estrategias de migración, costera o pelágica, ya que algunos permanecieron cerca de la costa durante toda su vida mientras que otros regresaron a la costa después de haber migrado hacia hábitats oceánicos. Contingentes migratorios o migración parcial podrían ser una estrategia de *S. lewini* para diferenciar su vulnerabilidad a factores estresantes entre las regiones y su persistencia en el Pacífico mexicano a pesar de ser muy explotado podría en realidad estar más relacionado con su plasticidad de movimiento que con sus características de historia de vida.

ABSTRACT

The scalloped hammerhead shark *Sphyrna lewini* is a migratory species that exhibits complex patterns of spatial organization associated with the use of both coastal and oceanic habitats. Information about its movements and habitat use is fragmentary and recent study showed that elemental signatures deposited in its vertebrae served as reliable site-specific markers. The age-related movements of *S. lewini* were assessed in the Mexican Pacific based on its vertebral microchemistry. Vertebrae were obtained from 48 sharks captured in 2016. Elemental signatures were quantified using laser ablation inductively coupled plasma mass spectrometry. Spatial variation of the vertebral edge elemental signatures was first assessed to verify that microchemistry could distinguish among sharks based on recent habitat use before inferences on environmental histories could be made. Age-related movements were then assessed by quantifying changes in the Sr:Ca, Ba:Ca, Sr:Ba and Pb:Ca ratios along vertebral transects encompassing complete life histories. Analysis of elemental profiles suggested that *S. lewini* exhibits movements that are likely more plastic than previously assumed, with a probable stronger affiliation with coastal habitats than expected. Females used highly variable habitats over lifetime and males alternated between two migratory patterns, coastal or pelagic, as they either remained nearshore for their entire life or migrated offshore to later return to coastal habitats. Migratory contingents or partial migration might be a strategy of *S. lewini* to reduce its vulnerability to stressors among regions and its persistence in the Mexican Pacific despite being heavily fished might be linked to its behavioral plasticity of movement rather than its life history characteristics.

INTRODUCTION

Migration is vital for marine species adaptability as it determines the colonization rate of new habitats and the resilience of a population to harvest by the exchange of individuals among geographically separate groups (Cowen et al. 2000). Identifying patterns of habitat use and migration is therefore crucial for their successful conservation and fisheries management (Bonfil 1997; Thorrold et al. 2001; Fogarty and Botsford 2007) as this knowledge allows the impacts of environmental and human disturbances on the species to be understood (Heithaus et al. 2002), although this can be difficult due to the mobility of large and wide-ranging species such as sharks that often have complex life histories and are difficult to observe in the open ocean (Castro 2011a).

Migrations of sharks can be assessed forward or backward in time through a variety of techniques which provide different scales of resolution (Chapman et al. 2015). Physical tagging is widely applied and typically involves telemetry and mark–recapture studies (Speed et al. 2010). Such individually-based techniques, particularly telemetry, allow fine (*e.g.* Hoyos-Padilla et al. 2014) and large (*e.g.* Ketchum et al. 2014) scale movements to be mapped on ecologically relevant time scales, although they are restricted to the number of tagged individuals. Genetic markers and DNA profile analyses, on the other hand, can be used to infer stock structure (*e.g.* Nance et al. 2011), philopatry (*e.g.* Tillett et al. 2012) and population mixing over evolutionary time scales (Dudgeon et al. 2012), while the stable isotope analyses provide a tool for examining individual movements on time scales that range from weeks to months depending on the tissue analyzed (Hussey et al. 2012).

Although not yet widely used for sharks, analyses of vertebral microchemistry using laser ablation inductively-coupled mass spectrometry (LA-ICP-MS) have recently proven a powerful tool for studying the stock structure, natal origin and age-related movements of coastal and oceanic species over lifetime periods (Tillett et al. 2011; Scharer et al. 2012; Izzo et al. 2016; Lewis et al. 2016; McMillan et al. 2016, 2018; Smith et al. 2016; Mohan et al. 2018), thereby complementing the other techniques. This approach is similar to studying life history of teleosts based on otolith microchemistry (see Elsdon et al. 2008) and relies on fine-scale and discrete changes in the elemental composition of the vertebrae that reflect changes in the chemistry of the surrounding water. Chemical elements from the water are incorporated within the continuously growing vertebrae where they are retained even after the individuals disperse, serving as discrete site-specific markers. Where groups have spatially distinct environmental signatures, then connectivity of individuals among those groups can be distinguished without information about water chemistry (Thorrold et al. 1998). In addition, elemental profiles in vertebrae can be used to (1) reconstruct time-resolved records of the individual environmental histories when related to growth bands

(Tillett et al. 2011; Scharer et al. 2012), if their periodicity of formation (*i.e.* annual or biannual) was verified, and (2) help to reconstruct periods of residency and movements of a species between habitats (Elsdon et al. 2008; Tillett et al. 2011).

More specifically, the life history transitions of sharks across environments of different salinities can be assessed by screening the changes in strontium (Sr) and barium (Ba) along vertebral transects (Tillett et al. 2011; Scharer et al. 2012) because: (1) Sr is relatively abundant in fully marine environments where it is uniform and low salinity regions are typically enriched in Ba (McCulloch et al. 2005; Crook et al. 2006; Allen et al. 2009), (2) these elements are not physiologically regulated (Walther and Thorrold 2006; Pistevos et al. 2019) and (3), their incorporation in the vertebrae, primarily derived from branchial uptake, is representative of the ambient concentrations (Smith et al. 2013), positively influenced by temperature (Pistevos et al. 2019), but not affected by somatic growth nor vertebral precipitation rate (Smith et al. 2013). Accordingly, the Sr:Ca and Ba:Ca ratios can serve as indicators in the vertebrae for differentiating less saline/nearshore from more saline/oceanic residence of the sharks (Tillett et al. 2011), although the combined use of Sr and Ba as unique ratio (Sr:Ba) can be a better indicator of salinity changes as it combines the differences observed for both Sr:Ca and Ba:Ca and also yields larger magnitude of differences between the environments (Allen et al. 2009). Other environmental tracers such as lead (Pb) have also proven useful in otoliths as indicators of teleost environmental histories (Ranaldi and Gagnon 2008, 2010; Selleslagh et al. 2016) because dissolved Pb concentrations are primarily derived from anthropogenic sources, which typically results in a pattern of Pb being elevated in nearshore/coastal habitats and comparatively depleted offshore. Although the utility of this element has not been explored for sharks, laboratory experiments showed that incorporation of Pb in the otoliths was representative of the ambient concentrations and reflected the environmental exposure history to contamination of a fish (Geffen et al. 1998; Selleslagh et al. 2016).

The scalloped hammerhead shark, *Sphyrna lewini* (Griffith & Smith 1834), is a globally endangered species (Baum et al. 2007) that inhabits both coastal and oceanic areas of the circumtropical seas (Compagno 1984). *S. lewini* exhibits complex patterns of spatial organization with neonates, juveniles, adult females and adult males often occupying different habitats (Klimley 1987). Despite extensive studies, information about its movements and habitat use is fragmentary (Castro 2011a), especially following departure of the juveniles from the estuarine and coastal habitats (Duncan and Holland 2006; Hoyos-Padilla et al. 2014). Juvenile females of *S. lewini* usually migrate offshore at a smaller size (105-123 cm of total length) than do males (150-165 cm of total length), possibly to exploit energy-rich pelagic preys and grow more rapidly to reproductive size (Klimley 1987). Adult females later return to nearshore habitats for parturition (possibly annually, Torres-Huerta et al. 2008) and depart shortly afterwards (Clarke 1971; Klimley 1987; Stevens and Lyle 1989). Although males do not have such requirement, Harry et al. (2011) suggested that they may exhibit two migratory patterns: coastal or pelagic. Pelagic strategists migrate from the coastal habitats to pelagic habitats, like females (Harry et al. 2011), thus providing an opportunity for gene flow across oceanic expanses (Daly-Engel et al. 2012). Coastal strategists, instead, may remain nearshore for their entire life, thus being able to mate opportunistically with females entering coastal waters to give birth (Harry et al. 2011), which has been postulated to occur directly after parturition (Chen 1988). Studies into the spatial organization and habitat use of *S. lewini* are necessary to further investigate this behavior (Harry et al. 2011).

The objective of this study was to explore the use of vertebral microchemistry to assess the age-related movements of *S. lewini* in the Mexican Pacific and more specifically provide more insights into the (1) migratory patterns of males (coastal or pelagic) and females, and (2) ontogenetic habitat shifts. Strontium (^{88}Sr) and barium (^{137}Ba) were used as salinity change indicators of the individual environmental histories to obtain more information on the patterns of spatial organization and habitat use of the species. The variations of lead (^{208}Pb) in the vertebrae were also assessed as a tentative alternate indicator of habitat use that may further inform the observations based on Sr and Ba. Because elemental signatures deposited at the vertebral edge provide a known spatial and temporal reference that corresponds to the time immediately prior to capture and reflects recent habitat use (Smith et al. 2016), spatial variation of the vertebral edge elemental signatures was examined as a preliminary analysis to verify that vertebral microchemistry could reliably distinguish among sharks from the distinct sites of capture or habitat (coastal versus pelagic) before inferences on the age-related movements of the species could be made. Only adults (*i.e.* $L_{\mathrm{T\ females}} > 207$ cm and $L_{\mathrm{T\ males}} > 170$ cm; Anislado-Tolentino and Robinson-Mendoza 2001; Torres-Huerta et al. 2008) and pelagic juveniles (*i.e.* immature specimens captured offshore that have left the coastal habitats) were considered for this study to encompass the most complete range of movements as possible of the species and address the different objectives. Knowledge obtained by Smith et al. (2016) about the factors that influence the elemental incorporation in the vertebrae of *S. lewini* was used as a baseline before further inferences on the life history of the species could be made.

MATERIAL AND METHODS

Study area

The age-related movements of *S. lewini* were assessed through the vertebral microchemistry analysis of individuals occupying four geographically distinct areas of the Mexican Pacific: two offshore sites (Rocas Alijos, west of Baja California Sur, and Shimada Bank, southwest off Clarion Island, the most remote island of the Revillagigedo Archipelago) and two inshore sites (Mazatlán and Puerto Madero, in the Gulf of Tehuantepec) (Fig. 4.1).

The offshore western area off the Baja California Peninsula is characterized by intense year-round coastal upwelling events induced by westward wind-forcing that strongly influences the water conditions on the inner part of the continental shelf and produce cross-shelf gradients in temperature, nutrients (Petersen et al. 1986), and trace element distribution patterns (Bruland 1980). The Revillagigedo Archipelago consists of four volcanic oceanic islands (San Benedicto, Socorro, Roca Partida and Clarion) located southwest off the tip of Baja California Peninsula, where the connection between the California Current and the North Equatorial Current occurs (Bennett and Schaefer 1960). The offshore region around Clarion Island is characterized by strong hydrothermal activity influence and high densities of trace element-rich nodules (Fe, Co, Mn Cu and Ni; (Rosales Hoz and Carranza Edwards 1993). The coastal area off Mazatlán, located in the southeastern Gulf of California, is highly urbanized and strongly influenced by anthropogenic trace metal and pollutant inputs (Pb, Hg and Cd) that principally originate from the wastewater discharges and harbor, industry, agriculture and tourism activities (Soto-Jiménez and Páez-Osuna 2001; Jara-Marini et al. 2008; Raygoza-Viera et al. 2014). The coastal area off Puerto Madero in the Gulf of Tehuantepec exhibits highly contrasting oceanographic dynamics compared to the rest of the Mexican Pacific as it is influenced by intense northern winds that induce considerable upwelling events and a quasi-permanent westward coastal current (Flores-Vidal et al. 2011).

Vertebrae collection

Vertebrae were opportunistically obtained from adult and juvenile specimens of *S. lewini* captured offshore between February and April 2016 by the industrial fishery operating within two offshore areas of the Mexican Pacific: (1) off Rocas Alijos, 370 km west off Baja California Sur, between Puerto San Carlos and Cabo San Lucas and (2), Shimada Bank, 185 km southwest off Clarion Island (see rectangular striped areas; Fig. 4.1). In addition, small-scale artisanal fishers provided samples from inshore areas of adult specimens, including late-term pregnant females, captured nearshore in April 2016 approximately (1) 55 km off Mazatlán and (2) 55 km off Puerto Madero (Fig. 4.1). All fishers used surface longlines. Sex and total length (L_T) were recorded for each specimen after which a set of post-cephalic vertebrae was removed and stored frozen before preparation for ageing and elemental analyses.

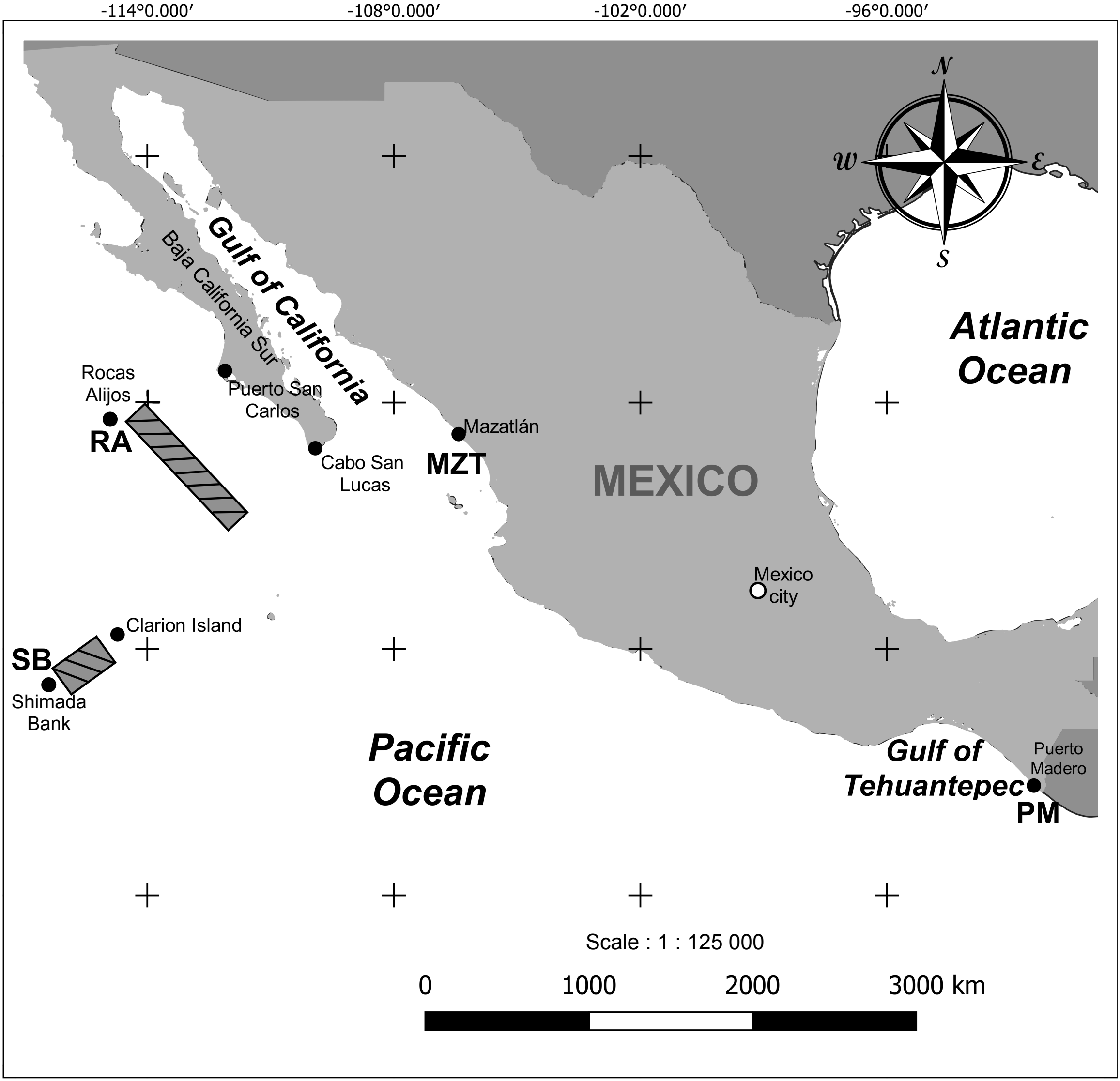

Fig. 4.1 Map of the study area showing the sampling sites of *Sphyrna lewini* in the Mexican Pacific. Rectangular striped areas correspond to the offshore operating areas of the industrial fishery. RA: Rocas Alijos; MZT: Mazatlán; SB: Shimada Bank; PM: Puerto Madero

Preparation of vertebrae for ageing and LA-ICP-MS analyses

Vertebrae were defrosted and the neural arch and extraneous tissue were removed. Individual centra of the vertebrae were exposed, thoroughly rinsed, air dried, mounted on wooden holders and later cut into 0.4 mm thick sagittal sections with a Buehler low-speed Isomet saw. Vertebral sections were then hand polished with a series of progressively finer grades of lapping paper (220, 800, and 3 µm grit) until the growth bands were clearly visible, sonicated in Milli-Q water for 5 minutes to remove surface contaminants, triple rinsed and dried for 24 hours. One vertebra section from each specimen was used for analyses as it was shown that elemental signatures of *S. lewini* did not differ in individual sharks (Schroeder 2011; Smith et al. 2016). Up to 10 vertebral sections were affixed with double-sided tape to acid-washed petrographic slides (subsequently referred to as 'master slides'). Samples arrangements on master slides were randomized to prevent systematic bias. All cleaning and drying procedures were performed under a Class-100 laminar flow clean hood using trace-metal grade reagents, non-metallic instruments and HNO_3 acid-washed glass slides.

Ageing

Vertebral sections were examined under transmitted light using a binocular dissecting microscope (Zeiss Stemi 508) equipped with digital camera (Zeiss AxioCam ERc5s) and software (Zen 2.3 Blue Edition). The birthmark (representing age 0) was identified as the first translucent band closest to the focus of the vertebra and a change in angle along the *corpus calcareum* interface (Fig. 4.2). Individual ages were estimated by counting each subsequent translucent growth band, that extended across both sides of the *corpus calcareum* (Fig. 4.2). Each vertebral section was read by two readers, with the two readings being performed at different times. All details regarding ageing procedures used in this study are documented in (Coiraton et al. 2019). Annual growth band deposition was recently verified for *S. lewini* in the Mexican Pacific (Coiraton et al. 2019).

Fig. 4.2 Sagittal section of a vertebra centrum from an 11-year-old female *Sphyrna lewini*, illustrating the terminology for vertebrae used throughout the text and the regions of the vertebrae sampled. Arrows show the translucent growth bands counted for estimating individual ages

LA-ICP-MS analyses

A Photon-Machines Analyte.193 excimer UV laser ablation system (LA), connected to an Agilent Technologies 7500CX quadrupole inductively coupled plasma–mass spectrometer (ICP-MS), was employed to quantify the elemental composition of the vertebrae. Raw data of the ion counts per second (cps) were collected for the following 23 elements using this instrumentation: ^{7}Li, Na^{23}, ^{24}Mg, ^{43}Ca, ^{45}Sc, ^{51}V, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{64}Zn, ^{72}Ge, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{114}Cd, ^{118}Sn,

^{137}Ba, ^{197}Au, ^{208}Pb, ^{232}Th and ^{238}U with ^{43}Ca being quantified for use as internal standard. These were screened in the vertebrae of *S. lewini* because this combination of masses minimizes potential interferences that can arise from isobaric spectral overlap, sample matrix effects, and the presence of molecular ions (Jones et al. 2013), and was successfully used in previous studies for inferring the life history of sharks (Tillett et al. 2011; Izzo et al. 2016; Lewis et al. 2016; McMillan et al. 2016, 2018; Smith et al. 2016; Mohan et al. 2018; Pistevos et al. 2019). Even though it is the ^{138}Ba isotope that is usually assayed in studies of calcified structures, in this study it is ^{137}Ba that was screened in the vertebrae of *S. lewini* for the sake of comparison with results reported in analogous study (Tillett et al. 2011). As the less abundant isotope (11.2%; Zolfonouna et al. 2016), ^{137}Ba is slightly more challenging to assay reliably than its counterpart, ^{138}Ba (71.7%; Zolfonouna et al. 2016), however this was not considered as a detrimental flaw to the present study because the use of a less abundant isotope would only increase the magnitude of change of the Sr:Ba ratio, though not the general pattern.

A sequence of n = 3 replicate 83 µm diameter circular spot scans of 60 s duration with a repetition rate of 5 Hz targeting vertebral edge (Fig. 4.2) was ablated for each sample to quantify the elemental signature deposited at the site of capture (*i.e.* discrete data reflecting recent habitat use) and test the hypothesis that vertebral microchemistry could reliably distinguish among sharks from the distinct sites of capture and habitats (coastal versus pelagic).

The utility of vertebral microchemistry to assess the age-related movements of *S. lewini* was explored by screening the changes in ^{88}Sr,^{137}Ba and ^{208}Pb along vertebral transects encompassing complete life histories (*i.e.* time-series data), running from the birthmark to the edge within the *corpus calcareum* of each individual vertebral section (Fig. 4.2). Transects were pre-ablated prior to data acquisition in order to remove possible external contamination. Pre-ablation transect scan speed was 108 µm.s^{-1}, with a repetition rate of 2 Hz and a spot size of 108 µm. For data acquisition, ablation transect scan speed was 10 µm.s^{-1}, with a repetition rate of 10 Hz and a spot size of 83 µm.

Data for the 23 target elements were acquired by the ICP-MS which employed Agilent Technologies ChemStation software operating in time-resolved analysis mode to collect raw cps data. NIST-612 silicate glass served as external calibration reference material (Pearce et al. 1997) and was ablated with two replicates before and after every fifth vertebral section was sampled. MACS-3 microanalytical carbonate standard material (Koenig and Wilson 2007) was ablated before and after each master slide to estimate experiment-wide levels of precision. Background data corresponding to gas blanks were collected for 60s before and after each spot or transect was ablated. Prior to data acquisition, the ICP-MS instrument was fine-tuned while ablating NIST-

612 using 108 µm wide transect scans running at 5 Hz and 86% power in order to maximize element counts and minimize noise.

All laboratory facilities and instrumentation used for elemental analyses were located on the campus of the College of Marine Science, University of South Florida, St. Petersburg, FL, USA.

Data analyses

Assessing spatial variation of elemental signatures

Raw spot data (cps) of vertebral edge were parsed as discrete, uniquely named variables corresponding to individual spot ablation samples. The quality of the signal representing each ablation sample was visually assessed within the software, and those portions of the signal displaying peaks likely associated with surface contaminants or other forms of instabilities were excluded from further processing. The following operations were then applied to the data associated with each spot sample: (1) background levels were removed by subtraction; (2) mass-specific spikes detected by the Grubbs test ($\alpha = 0.05$) were replaced with mean values when present; (3) mass-specific drift in the sensitivity (ions. s^{-1}.ppm^{-1}) of the ICP-MS detector was corrected via linear interpolation when R^2 values of acquisition time versus yield from spot scans of NIST-612 were ≥ 0.55; and (4) raw spot data (cps) were converted to single, mean (*i.e.* averaged across replicates) elemental concentration values (ppm) using NIST-612 data for external calibration and standardized to the ^{43}Ca data obtained simultaneously in the structure by deriving element:Ca ratios (µmole·mole^{-1}) to adjust for variability in instrument sensitivity and the amount of ablated material. Limits of detection (LOD) were estimated for samples based on 3·SD of the ion count rates (cps) of the corresponding gas blanks and converted to concentrations (ppm). Elements with ≥ 10% of the measures of concentration below LOD were omitted from subsequent analyses. Outliers among replicate spot scans were identified using a multivariate measure of outlyingness (Breiman and Cutler 2003) based on elemental concentrations (ppm). Replicates with outlyingness values > 10 were excluded before reducing the data to mean ppm for each vertebral section.

Spatial variability in the microchemistry of vertebral edge of *S. lewini* was examined among the four sites of capture (*i.e.* RA, MZT, SB and PM) and habitat (*i.e.* coastal versus pelagic) using permutational analysis of variance (PERMANOVA; (Anderson et al. 2017). This a distribution-free variant of MANOVA that accounts for unbalanced designs with heterogenous multivariate dispersion and appropriate for analyzing hard-part microchemistry data, which even after transformation often fail to meet the underlying assumptions of any one distributional model (*e.g.* (Lara et al. 2008; Mercier et al. 2011; Jones et al. 2013), and that accounts for unbalanced designs with heterogenous multivariate dispersion (Anderson et al. 2017). Where significant differences were found, *post hoc* pair-wise comparisons were used to determine which pairs of sites differed. A canonical analysis of principal coordinates (CAP; (Anderson and Willis 2003) was employed to visualize the spatial differences detected using PERMANOVA, test the ability of the model to accurately classify individuals to their known site of capture or habitat based on elemental data of vertebral edge and determine which elements were driving most of the differences among sites or habitats. Leave-one-out cross-validation (LOO-CV) was used to assess the overall classification accuracy of the CAP model and build a confusion matrix summarizing the occurrence of site-specific misclassifications. The proportional chance criterion (PCC) was used to test the significance of the observed overall classification success rate of the CAP models compared with that expected by chance (White and Ruttenberg 2007). Elemental concentration data (ppm) of vertebral edge were analyzed as multi-elemental signatures (*i.e.* discrete data) of the element:Ca ratios.

A Euclidean distance-based dissimilarity matrix constructed from the mean element:Ca ratios (μmole·mole^{-1}) served as multiple, quantitative explanatory variables in PERMANOVA and CAP design, with sites of capture or habitat serving as the categorical response variable.

Assessing the age-related movements

Raw transect data for ^{88}Sr,^{137}Ba and ^{208}Pb (used as Sr:Ca, Ba:Ca, Sr:Ba and Pb:Ca raw cps data ratios) were plotted versus vertebral transect distance from the birthmark (µm) for each sample. Data were used as raw cps for the sake of comparison with analogous study involving euryhaline bull (*Carcharhinus leucas*) and fully coastal pig-eye (*Carcharhinus amboinensis*) sharks (Tillett et al. 2011). An 11-point running average window size was applied to filter/smooth the data, reduce the noise, and aid in identifying the underlying pattern of the elemental profiles (Sinclair et al. 1998). Prior analyses, the utility of Sr and Ba as a metric of salinity change in the vertebrae of *S. lewini* was verified by determining whether Sr:Ca and Ba:Ca were effectively inversely related relative to each other (as generally assumed) within each individual vertebra with respect to the nearshore-offshore salinity gradient. The overall magnitude of change of Sr:Ca, Ba:Ca and Sr:Ba was then compared in order to find the most sensitive measure of environmental change and select the best ratio to use as indicator of environmental histories among habitats in subsequent analyses. When a marked shift was observed in the elemental profile of a sample, the total length of that individual at the time of the shift was back-calculated using the Fraser–Lee method (Francis 1990):

$$L_t = [(R_t)(R_V)^{-1}](L_C - a) + a$$

where L_t is the back-calculated total length corresponding to age t (*i.e.* time of the habitat shift), R_t corresponds to the transect distance to the shift observed in the selected ratios *(i.e.* Sr:Ca, Ba:Ca, Sr:Ba and Pb:Ca) at age t, R_V is the vertebrae centrum radius, L_C the length at the time of capture and a is intercept of an established linear regression between R_V and L_C (Francis 1990), which is $L_C = 17.349\ R_V + 14.516$ for *S. lewini* in the study area (Coiraton et al. 2019). Age-specific habitat shifts were identified by overlaying the position of annual translucent growth bands on plots.

All elemental data processing and multivariate statistical analyses were performed using the free download Fathom Toolbox for Matlab^{TM} (Jones 2017). For more details on the data processing and multivariate statistical analyses, please refer to the readme files and corresponding Matlab codes available on GitHub.

RESULTS

The vertebrae from 31 adults and 17 pelagic juveniles of *Sphyrna lewini* captured in the Mexican Pacific were used for this study. Females (*n* = 22) ranged from 159 to 301 cm of L_T (mean ± SD = 217.1 ± 43.3 cm) and were aged 9 to 20 years. Males (*n* = 26) were also aged 9 to 20 years and ranged from 151 to 303 cm of L_T (193.8 ± 37.1 cm) (Table 4.1). Based on the estimated size at first maturity in the region, the proportion of juvenile (54.5%) and adult (45.5%) stages of females analyzed was similar whereas males mainly consisted of adults (80.7%) (Table 1). Samples analyzed in Puerto Madero (*n* = 10) consisted of adult males (*n* = 4) and late-term pregnant females (*n* = 6), likely entering coastal waters for parturition (Table 1). Sample size was low in Mazatlán (*n* = 3) and consisted of 2 female and 1 male adult specimens (Table 4.1).

Table 4.1 Sampling sites, site codes, sample size (*n*) per habitat and sex, total length range (L_T; cm) and age (years) of the 31 adults and 17 pelagic juveniles of *Sphyrna lewini* captured in the Mexican Pacific between February and April 2016

Sampling site	Month	Site code	Habitat	Sex	$n_{\text{juveniles}}$	L_T	Age	n_{adults}	L_T	Age
Rocas Alijos	April	RA (*n* = 16)	Pelagic	Females	6	177 - 205	9 - 12	2	224 - 245	14
				Males	2	163 - 166	10	6	173 - 303	11 - 20
Shimada Bank	February & March	SB (*n* = 19)	Pelagic	Females	6	159 - 203	10 - 12	1	215	13
				Males	3	151	9 - 10	9	171 - 221	11 -16
Mazatlán	April	MZT (*n* = 3)	Coastal	Females	0	_	_	1	301	20
				Males	0	_	_	2	215 - 266	14 - 18
Puerto Madero	April	PM (*n* = 10)	Coastal	Females	0	_	_	6	240 - 277	16 - 20
				Males	0	_	_	4	189 -220	15 - 18

Assessing spatial variation of elemental signatures

The mean percentage of the elemental concentration data below the limits of detection (LOD) was ≤10% for all elements (*i.e.* ^{7}Li, ^{23}Na, ^{24}Mg, ^{51}V, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{64}Zn, ^{85}Rb, ^{88}Sr, ^{118}Sn, ^{137}Ba, ^{208}Pb, ^{238}U), except ^{45}Sc (34.9%), ^{53}Cr (45.5%), ^{72}Ge (65.1%), ^{89}Y (81.4%), ^{114}Cd (13.9%), ^{197}Au (88.4%) and ^{232}Th (65.1%) which were not regularly detected at all sites.

The multi-elemental signatures of vertebral edge differed significantly among the four sites of capture (PERMANOVA: $F = 2.97$, $P = 0.001$, $i = 1000$) and *post hoc* pair-wise comparisons indicated that significant differences existed between all pairs of sites ($0.006 < P < 0.04$). The canonical analysis of principal coordinates employed to visualize the spatial variation of the edge signatures (CAP$_{\text{4SITES}}$: $m = 7$, $G_{\text{prop}} = 83.9\%$, $Trc_{\text{stat}} = 1.65$, $P = 0.001$; Fig. 4.3) accurately classified 79.1% of the sharks to their known sites of capture, which was significantly better than the 28.7% accuracy rate expected by chance (PCC: $P = 0.001$), with ^{55}Mn, ^{208}Pb, ^{7}Li, ^{137}Ba, ^{88}Sr and ^{238}U driving most of the differences among sites (Fig. 4.3). The highest classification accuracy (81.8%) was obtained for the individuals from Rocas Alijos while the lowest (66.7%) was obtained for the individuals from Mazatlán (Fig. 4.4).

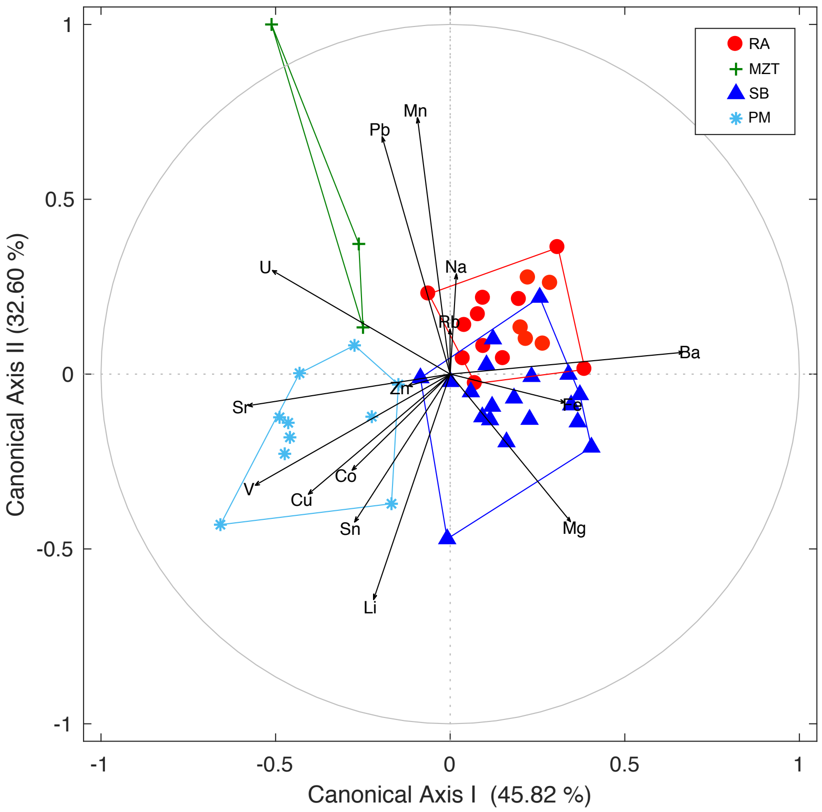

Fig. 4.3 Canonical analysis of principal coordinates (CAP_{4SITES}) ordination diagram and overlaid vector plot comparing multi-elemental signatures of vertebral edge of *Sphyrna lewini* (*n* = 48) among the four sites of capture from the Mexican Pacific, with 78.42% of the total variation among sites represented by canonical axes I and II

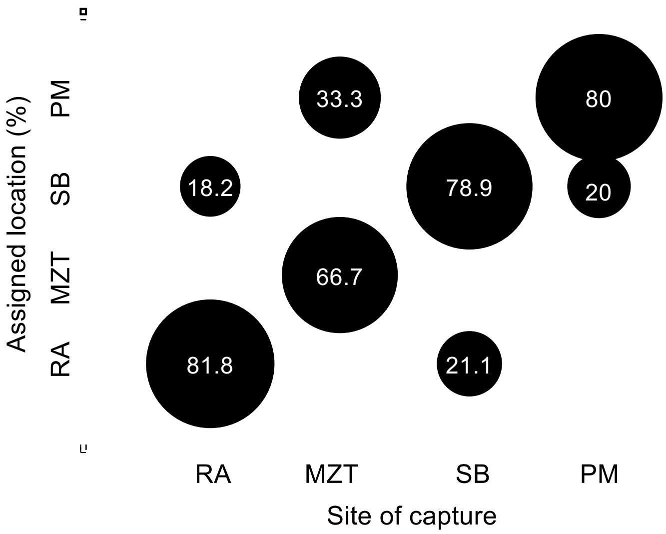

Fig. 4.4 Allocation of *Sphyrna lewini* individuals to their known sites of capture based on the results of the canonical principal coordinates analysis ($\text{CAP}_{\text{4SITES}}$) model fitted on multi-elemental data of vertebral edge (*n* = 48). Bubble size is scaled relative to reclassification success rates of the confusion matrix obtained using the LOO-CV procedure, which are shown (%) within the bubbles

When pooling the coastal and offshore pelagic samples within two separate groups and performing the same procedure of analysis, overall classification accuracy of the CAP model reached 95.4% ($\text{CAP}_{\text{COASTAL/PELAGIC}}$: $m = 8$, $G_{\text{prop}} = 87.9\%$, $Trc_{\text{stat}} = 0.75$, $P = 0.001$) suggesting that recent use of coastal or pelagic habitat could be distinguished based on vertebral microchemistry (Fig. 4.5). Although Sr and Ba were driving most of the differences among habitats, this did not happen in the expected way as Ba was characteristic of the pelagic samples and Sr discriminated the coastal ones (Fig. 4.5). Despite this observation, all individuals from offshore areas captured off Rocas Alijos and Shimada Bank (100%) were correctly assigned to the pelagic habitat, whereas 15.4% of the individuals captured in Mazatlán and Puerto Madero were misclassified to the pelagic group suggesting that those individuals were recently moving into pelagic habitats.

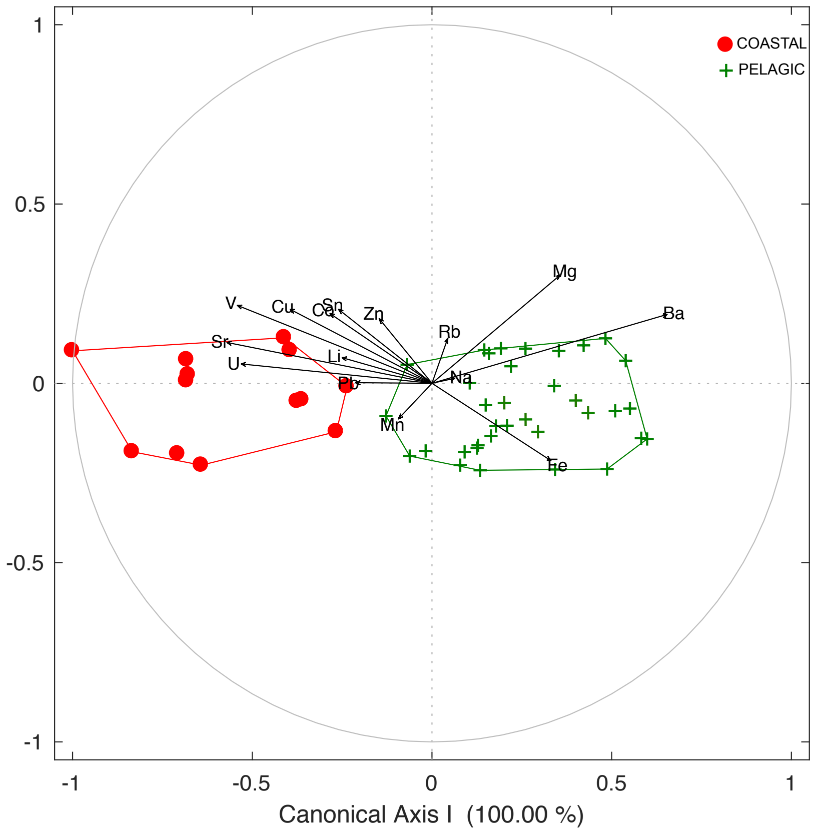

Fig. 4.5 Canonical analysis of principal coordinates ($\mathrm{CAP}_{\mathrm{COASTAL/PELAGIC}}$) ordination diagram and overlaid vector plot comparing multi-elemental signatures of vertebral edge of *Sphyrna lewini* (*n* = 48) between coastal and offshore pelagic habitats in the Mexican Pacific, with 100% of the total variation between habitats represented by canonical axis I

Assessing the age-related movements

Regarding the utility of the Sr:Ca, Ba:Ca and Sr:Ba ratios as indicators of salinity changes, all individuals captured nearshore (*i.e.* MZT and PM; *n* = 13) exhibited a Sr:Ca decline prior to capture (*i.e.* vertebral edge) and a Ba:Ca increase at the same time, whereas most of the individuals captured offshore (*i.e.* RA and SB; *n* = 19, 54.2%) exhibited the opposite pattern, thus confirming that Sr:Ca and Ba:Ca are inversely related relative to each other based on the nearshore-offshore gradient of salinity change (Fig. 4.6). Even though the Sr:Ca and Ba:Ca patterns of the remaining individuals (*n* = 16, 45.7%) were also inversely related to each other, a Sr:Ca decline associated with a Ba:Ca increase were observed prior to capture. Regardless of the among-individuals consistency of the Sr:Ca and Ba:Ca patterns with respect to the nearshore-offshore gradient of salinity, the Sr:Ba ratio overall provided a better tool for examining individual environmental histories rather than the joint comparison of the Sr:Ca and Ba:Ca ratios because it combined the differences observed for both and also yielded better magnitude of change within individuals (*i.e.* 4.57 versus 1.33 (Sr:Ca) and 0.29 (Ba:Ca)), which allowed to highlight the differences observed among habitats.

Regarding variations of Pb, most individuals (*n* = 31; 65%) exhibited comparatively higher Pb:Ca values following birth than later in life (see example in Fig. 4.7*a*), likely indicative of an early-life nearshore residence in contaminated areas. Similarly, all individuals captured off Mazatlán exhibited a sharp increase of the Pb:Ca ratio prior to capture (see example in Fig. 4.7*b*). Because Pb was also found to be characteristic of the samples captured off Mazatlán based on microchemistry of vertebral edge (Fig. 4.3), the Pb:Ca ratio was used in subsequent analyses as indicator of contaminated habitat use to aid in interpreting the observations based on the Sr:Ba profiles.

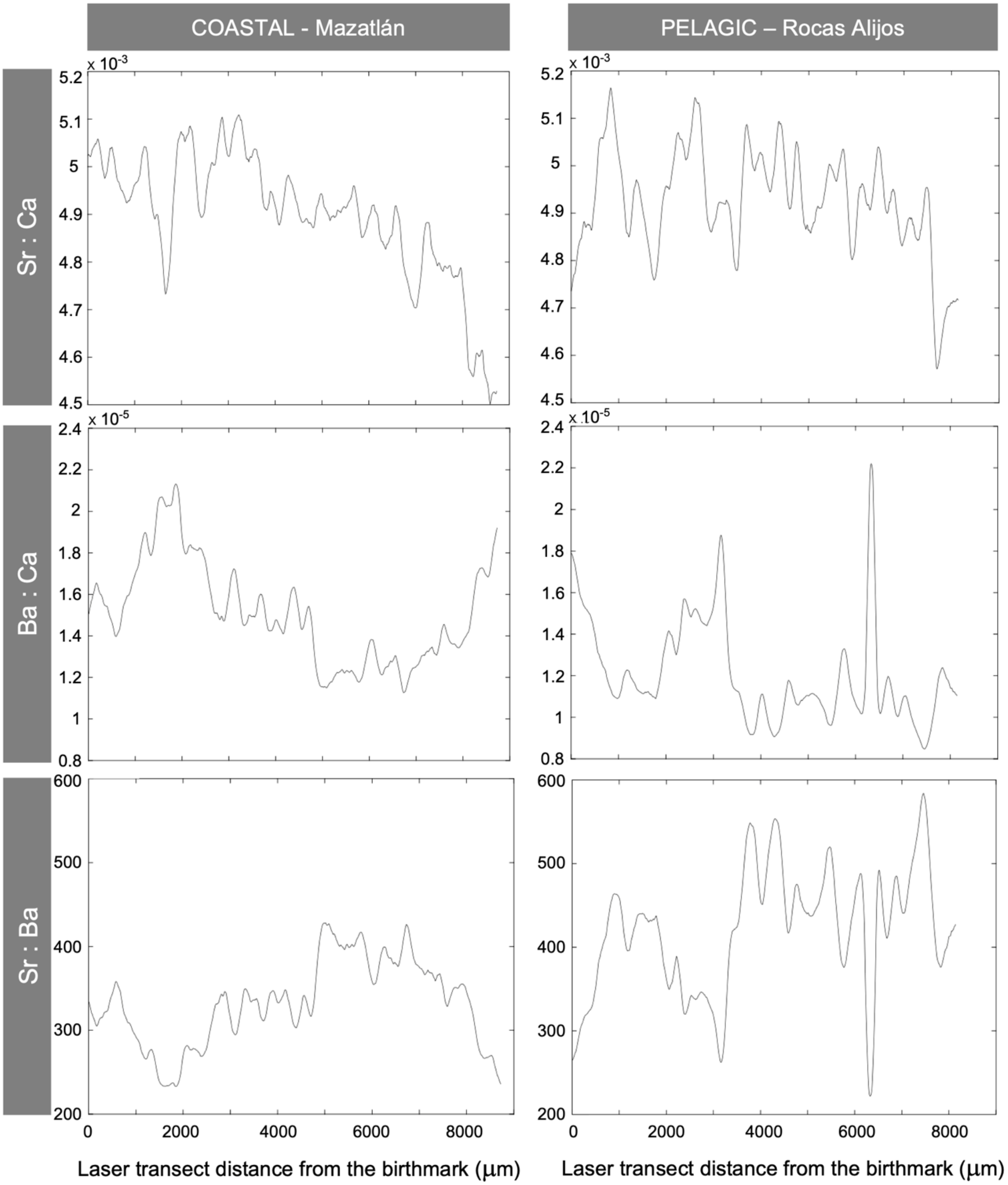

Fig. 4.6 Individual transect profiles of the Sr:Ca, Ba:Ca and Sr:Ba ratios quantified in the vertebrae of two specimens of *Sphyrna lewini* captured off Mazatlán and Rocas Alijos

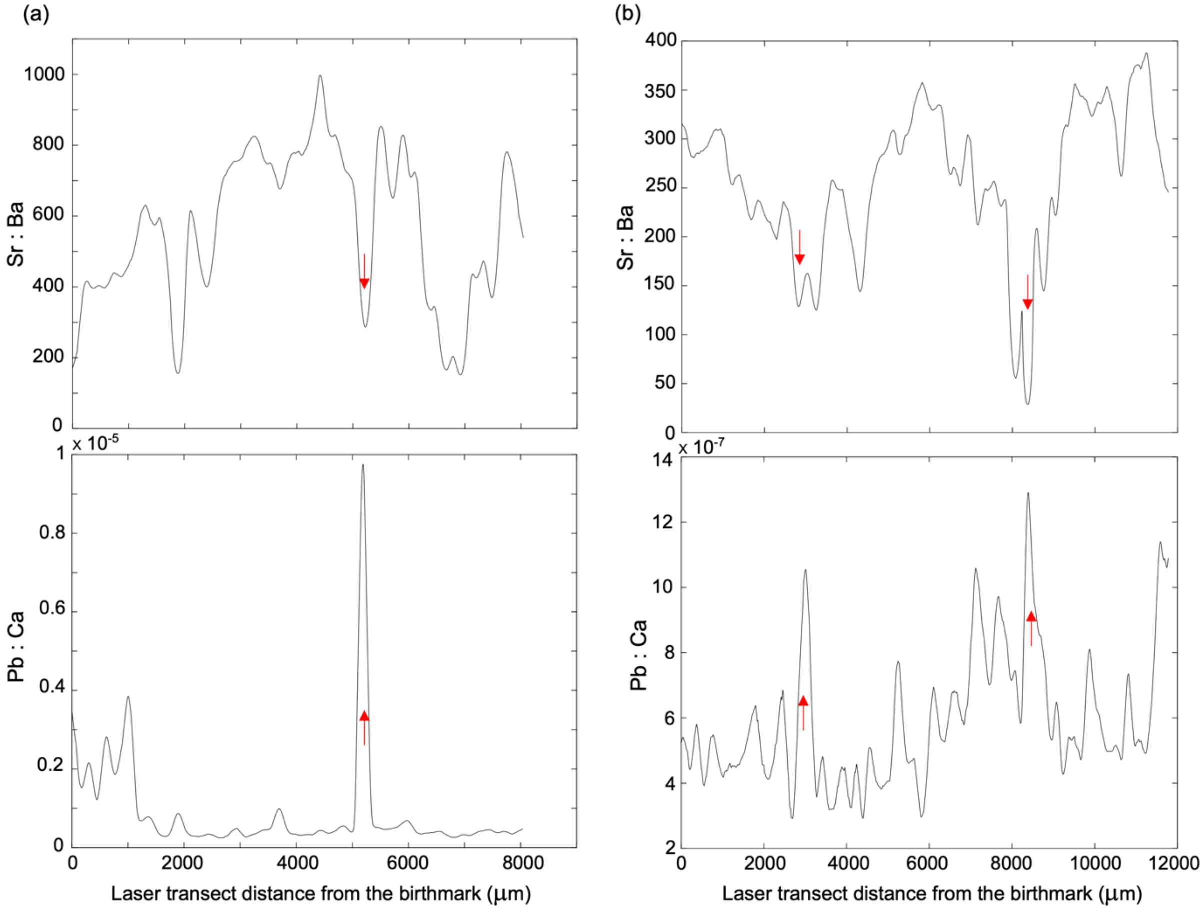

Fig. 4.7 Individual transect profiles of the Sr:Ba and Pb:Ca ratios quantified in the vertebrae of (a) an adult female *Sphyrna lewini* (301 cm L_T) captured off Mazatlán and (b) an adult male (194 cm L_T) captured off Puerto Madero. Arrows indicate possible exposure to contaminated nearshore habitats

S. lewini showed complex patterns of variation in Sr:Ba among individuals, with overall values varying from 0 to 1200. The Sr:Ba ratio quantified at the vertebral birthmark (age 0) was highly variable among individuals (from 75 to 750), suggesting that those sharks were born in environments of contrasting salinities. A marked decline in Sr:Ba to values ranging between 150 and 400 was also observed at the vertebral edge (*i.e.* time of capture) in all individuals captured off Mazatlán (*n* = 3) and Puerto Madero (*n* = 10), confirming that Sr:Ba typically drops when sharks enter coastal habitats (see Fig. 4.8 and 4.10). In addition, and irrespective of sex, the individuals captured off Puerto Madero appeared to have inhabited more oceanic environments when compared to individuals captured in the other sites, with the highest individual Sr:Ba values ranging between 700 and 1200.

The reported pattern of offshore ontogenetic migration of females (subsequently referred to as ‘Pattern 1’; Fig. 4.8) was detected in only few individuals (*n* = 7; 30.4%); Sr:Ba remained relatively low and constant (0 - 200) until females reached a size of approximately 133.3 ± 17.7 cm of $L_{T,}$ after which the ratio rapidly increased to the highest values for the individual (400 - 600), suggesting a habitat shift towards a more oceanic environment (marked by spots in Fig. 4.8). When related to the annual growth bands, this habitat shift occurred between 3 to 5 years of age (see Fig. 4.10a). In addition, evidence of mature females possibly returning to coastal habitats for parturition were found in two of these females that shown a marked decline in the Sr:Ba ratio from values of 500 to < 200 at approximately 207.3 and 228.5 cm of L_T, respectively (marked by a star in Fig. 4.10a), and a sharp increase of the Pb:Ca ratio at the same time for one of them (Fig. 4.7a). When related to the annual growth bands, this shift in Sr:Ba corresponded to 10 and 11 years of age, respectively (see Fig. 4.10a). The remaining females (*n* = 15; 69.6%) did not exhibit a discernible age-related pattern in their Sr:Ba profiles; there was no evidence of ontogenetic habitat shift based on these data and Sr:Ba was highly variable over their lifetimes (subsequently referred to as ‘Pattern 2’; Fig. 4.8).

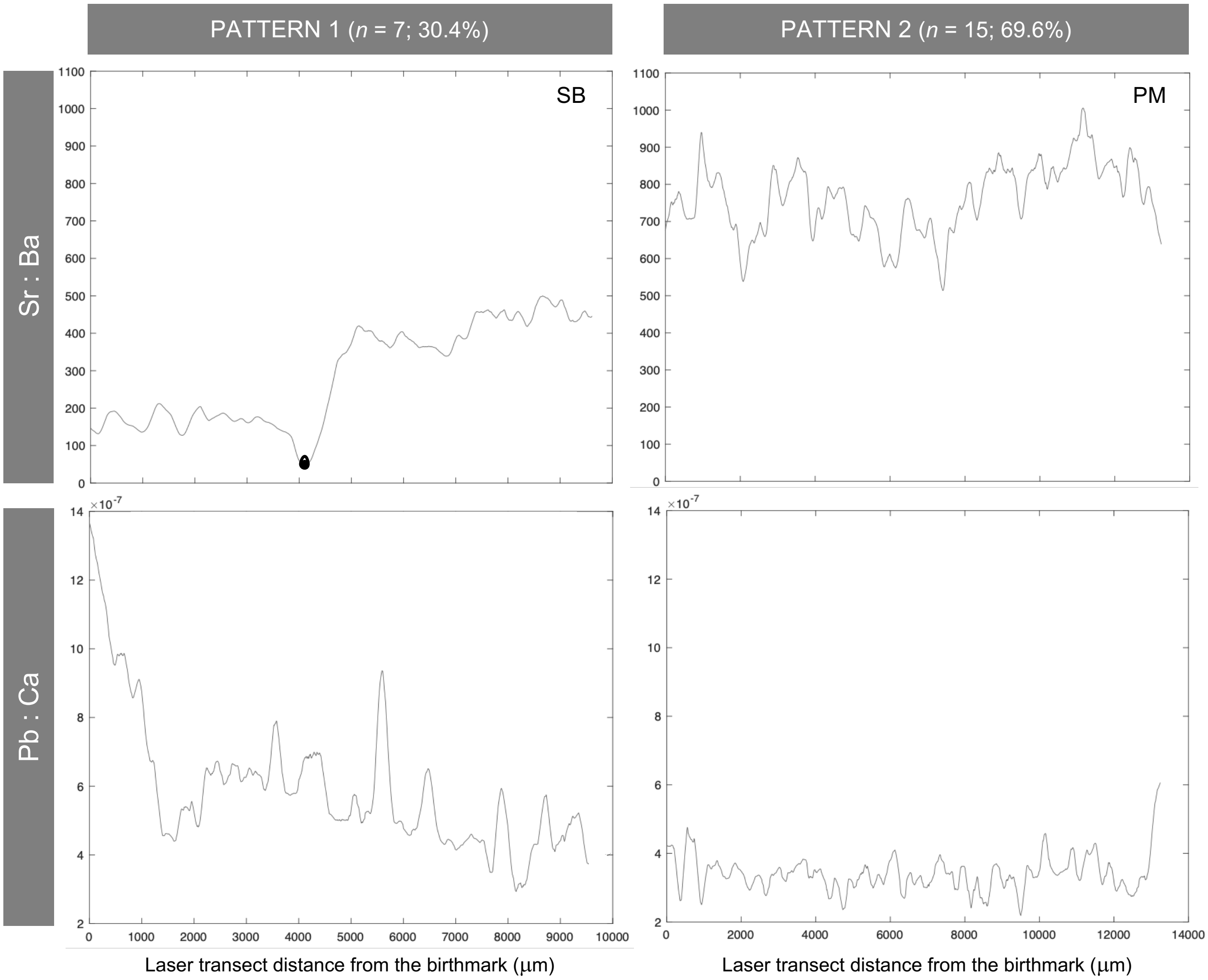

Fig. 4.8 Examples of individual transect profiles of the Sr:Ba and Pb:Ca ratios quantified in the vertebrae of two females of *Sphyrna lewini* exhibiting the reported pattern of offshore ontogenetic migration (*i.e.* ‘Pattern 1’) or no discernible pattern (*i.e.* ‘Pattern 2’). Dot shows possible habitat shift towards more oceanic habitat

The migratory patterns of males were more evident to discern than females, and two apparently distinct patterns were detected. Some males (*n* = 6; 23.1%) distinctively exhibited the reported pattern of ontogenetic migration; Sr:Ba remained relatively constant until individuals reached a size of approximately 148.4 ± 22.2 cm of L_T before Sr:Ba progressively increased to the highest individual values (500 - 1200), suggesting a habitat shift towards a more oceanic environment between 4 to 7 years of age (*i.e.* Pattern 1; Fig. 4.9). The analyses also suggested that some of those males (*n* = 4) later returned to coastal habitats, as shown by a marked decline in the Sr:Ba ratio to values between 100 and 300 prior to capture (marked by a star in Fig. 4.10b), which was further evidenced by a sharp increase of the Pb:Ca ratio at the same time (see example in Fig. 4.7b). The remaining individuals (*n* = 20; 76.9%) exhibited a relatively low but constant Sr:Ba ratio (between 200 and 500) across vertebral transects, eventually showing cyclical variations with fairly small magnitudes of change (100 to 200), suggesting that those males remained in less saline/more coastal habitats over lifetime (*i.e.* 'Pattern 2'; Fig. 4.9).

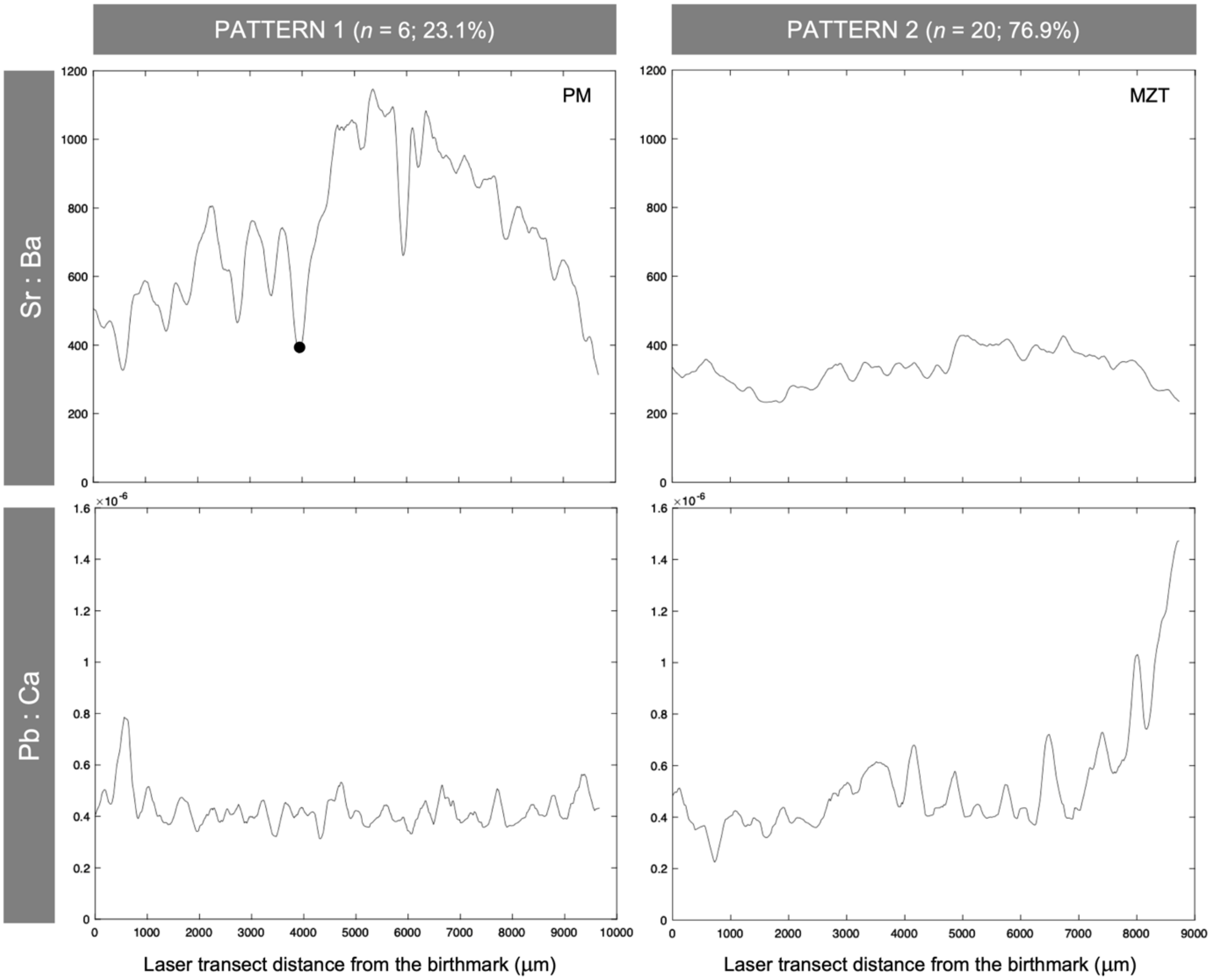

Fig. 4.9 Individual transect profiles of the Sr:Ba and Pb:Ca ratios quantified in the vertebrae of two males of *Sphyrna lewini* exhibiting the reported pattern of offshore ontogenetic migration (*i.e.* 'Pattern 1'), or a constant nearshore pattern (*i.e.* 'Pattern 2'). Dot shows possible habitat shift towards more oceanic habitats

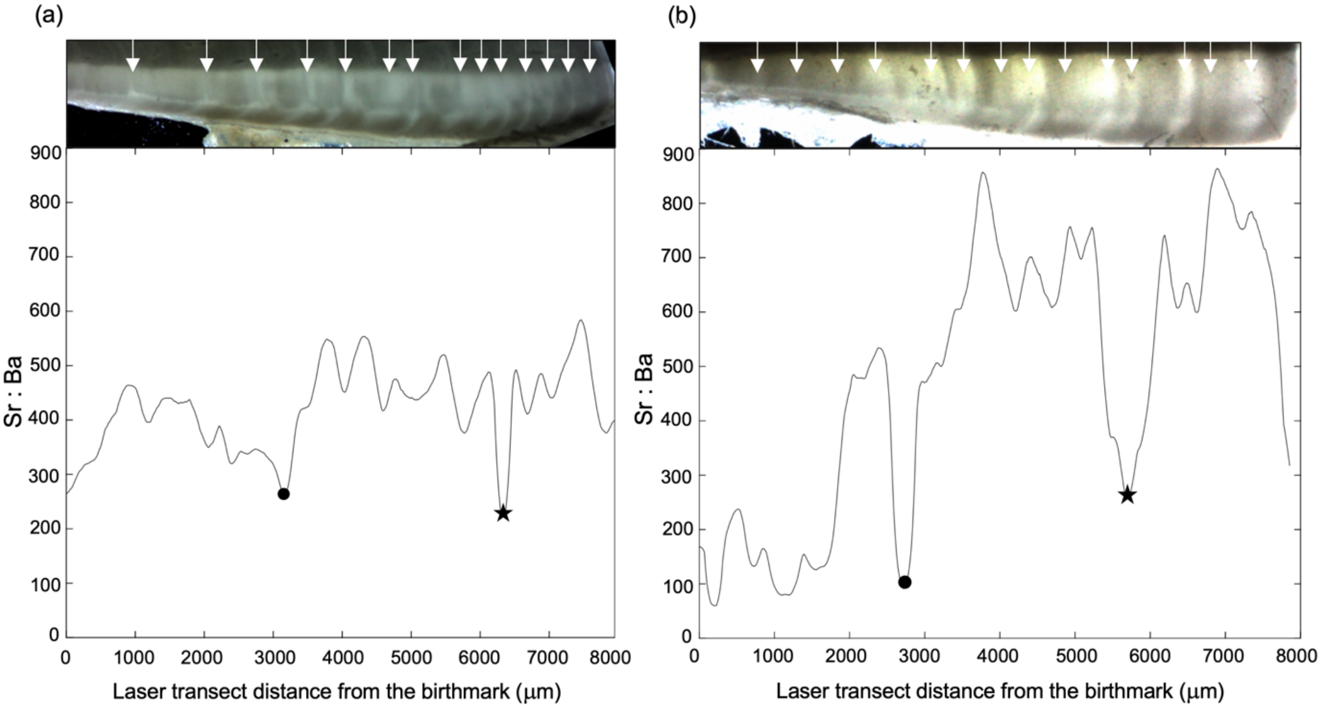

Fig. 4.10 Individual transect profiles of the Sr:Ba ratio quantified in the vertebrae of (a) a 14-year-old adult female *Sphyrna lewini* (245 cm L_T) captured off Rocas Alijos and (b), a 12-year-old adult male (215 cm L_T) captured off Mazatlán. Arrows show the translucent annual growth bands counted for estimating individual ages. Dots indicate possible habitat shift towards more oceanic habitats. Stars indicate possible return to nearshore habitats

DISCUSSION

Assessing spatial variation of elemental signatures

Elemental signatures are powerful discriminators of groups when individuals have different environmental histories, but of negligible value when differences among those individuals cannot be detected in spite of a potential geographic separation (Campana 2005). Marine environments are relatively homogenous in terms of the water chemistry gradients, however the analyses of the vertebral microchemistry of *S. lewini* performed in this study allowed to successfully distinguish (79.1%-95.4%) among individuals that had recently occupied geographically distinct (separated by 400 to > 1000 km) marine environments. Nevertheless, the comparison at such geographical scales was opportunistic (*i.e.* fishery-dependent) and the sample size was small. Restricting analyses to samples collected within the same year and season was essential to avoid bias induced by possible site-specific temporal variations in the water chemistry and maximize the overall classification accuracy (Smith et al. 2016). Given that vertebral microchemistry of *S. lewini* could accurately distinguish among neonates from distinct coastal nursery areas of the eastern Pacific, including Mexico, across fine (10s km) and broad spatial scales (> 1000 km) in a previous study (Smith et al. 2016), the results of the present study provided evidence that elemental signatures of the species can serve as broad-scale effective spatial markers whether individuals have been occupying nearshore or more oceanic environments, and irrespective of their ontogenetic stage.

Nevertheless, and irrespective of the classification accuracy among habitats, the fact that in this study elemental signatures at vertebral edge were found to be mostly driven by Ba in individuals captured offshore (*i.e*. Rocas Alijos and Shimada Bank) raised an important question regarding the time required for changes in the surrounding water chemistry to be incorporated into the vertebrae of *S. lewini* because Ba is typically enriched in freshwater and low salinity regions (McCulloch et al. 2005; Crook and Gillanders 2006). Experimental evidences showed that changes in vertebral chemistry in response to external changes in the surrounding water can be incorporated in the vertebrae of the bull shark *C. leucas* after at least 3 weeks of residency in a particular area (Werry et al. 2011), however these rates of uptake were estimated based on only 6 captive specimens and might primarily reflect the sharp environmental gradients that specimens were subject to (riverine-estuarine and marine treatments) in the study. Even if the lack of analogous study for *S. lewini* clearly limits the power of such inferences, yet it is possible that some of the individuals captured offshore might have recently migrated from less saline/nearshore habitats. This might also explain why both of the CAP models (*i.e.* $\mathrm{CAP}_{\mathrm{4SITES}}$ and $\mathrm{CAP}_{\mathrm{COASTAL/PELAGIC}}$) indicated that Ba was driving most of the differences in samples being captured offshore, especially since almost half of these were estimated to be juveniles (n = 17, 48.5%) based on the sex-specific sizes at first maturity estimated for the species in the region (Anislado-Tolentino and Robinson-Mendoza 2001; Torres-Huerta et al. 2008).

In addition, subsequent analyses of the elemental changes along vertebral transects to assess the age-related movements of *S. lewini* allowed to verify the utility of the Sr:Ba ratio as indicator of salinity change because this ratio was found to drop prior to capture in the sharks being captured nearshore. Even though most of half of individuals captured offshore (n = 19, 54.2%) exhibited the opposite pattern (*i.e.* an increase of the Sr:Ba ratio prior to capture) being this likely indicative of sharks recently migrating into more offshore habitats, the remaining individuals (n = 16, 45.7%) did exhibit a Sr:Ba decline. The latter is consistent with the hypothesis proposed above that some of the sharks captured offshore in this study might have recently migrated from more coastal habitats, thus precluding elemental signatures characteristic of the offshore habitat to be incorporated in the vertebrae.

Ultimately, even though laboratory experiments reported that incorporation of Pb in the otoliths of teleosts reflected the exposure of a fish to contamination derived from anthropogenic sources (Geffen et al. 1998; Selleslagh et al. 2016), this assumption has not been explicitly tested for sharks. This was not considered as a detrimental flaw in this study because the fact that (1) nearshore individuals of *S. lewini* exhibited particularly higher Pb:Ca values at vertebral edge when compared to earlier in their life history and that (2), Pb was found by the CAP models to be characteristic of the individuals captured off Mazatlán (an area strongly influenced by anthropogenic sources of trace metal and pollutant inputs (Soto-Jiménez and Páez-Osuna 2001; Jara-Marini et al. 2008; Raygoza-Viera et al. 2014) indicated that Pb could be used as indicator of the individual environmental histories of *S. lewini* and support the observations based on Sr and Ba.

Assessing the age-related movements

In this study, the Sr:Ba ratio appeared a useful indicator of environmental histories for studying the migratory patterns of *S. lewini* in the Mexican Pacific. Declines in Sr:Ba profiles to values <300 at vertebral edge in individuals captured nearshore were consistent with movements of the sharks into nearshore and estuarine habitats as observed in euryhaline bull shark *C. leucas* (Tillett et al. 2011). The resulting individual Sr:Ba profiles allowed to detect apparently distinct migratory patterns of the species in terms of the movements between nearshore and more oceanic environments within each site of capture and sex, which were further informed by the analyses of the Pb:Ca profiles when sharks apparently entered nearshore contaminated areas. In some sharks, variations in Sr:Ba correlated well with the previously described ontogenetic and sex-specific changes in habitat use of *S. lewini* in the Mexican Pacific based on size distribution (Coiraton et al. 2017), telemetric (Hoyos-Padilla et al. 2014) and stomach content (Klimley 1987) data analyses, whereas the remaining ones exhibited apparently distinct migratory patterns.

Before going further with such inferences, it might be stated that the among-individual differences observed in the Sr:Ba patterns might be driven by other factors than salinity such as variations in temperature, dietary preferences or individual-specific physiology (as shown for some teleosts; e.g.(Walther et al. 2010; Sturrock et al. 2014), and hence be not fully reflecting the movements of the sharks across the nearshore-offshore gradient of salinity change. However, experimental evidences shown that: (1) Sr and Ba are not physiologically regulated and their incorporation in the vertebrae being primarily derived from branchial uptake, representative of the environmental concentrations, but not affected by somatic growth nor vertebral precipitation rate (Smith et al. 2013; Pistevos et al. 2019) and (2), that temperature positively affected the incorporation of both Sr and Ba in the vertebrae of sharks (Pistevos et al. 2019) and hence would not affect the overall Sr:Ba ratio variations if the sharks were to move into warmer (or cooler) waters, regardless of the nearshore-offshore gradient of salinity. Caution should be however taken regarding this latter point because conflicting evidences on the effect of temperature were found with round stingray *Urobatis halleri,* as temperature negatively affected the incorporation of Ba, though not that of Sr in its vertebrae (Smith et al. 2013). Nevertheless, analyses of the Pb:Ca ratio were used in this study as a complementary indicator of the individual environmental histories of *S. lewini* to further evidence movements of the sharks into nearshore habitats based on the Sr:Ba data and the results were consistent, which allowed to provide more insights on the species life history.

Regarding inferences on its early-life history, Sr:Ba was often distinctively lower around birth within individual profiles in accordance with neonate and juvenile stages occupying less saline nearshore habitats. Nevertheless, Sr:Ba was also found to differ widely among individuals (from 75 to 750), suggesting that parturition may occur in variable environments and/or that the use of coastal nursery areas may be less defined for this species than previously assumed. In the Mexican Pacific females *S. lewini* typically give birth between May and July (Torres-Huerta et al. 2008), and rainfall and river discharge that primarily influence the nearshore salinity (and hence the Sr and Ba values; McCulloch et al. 2005; Crook et al. 2006; Allen et al. 2009) are restricted to a relatively narrow season during these summer months in the region (Amezcua et al. 2019). Accordingly, the assumption that variations observed in vertebrae in the Sr:Ba ratio among individuals following birth might be driven by other factors than rainfall, such as neonate dietary preferences or individual-specific physiology seems unlikely because Sr and Ba were found to be not physiologically regulated and their incorporation in the vertebrae being primarily derived from branchial uptake (Walther and Thorrold 2006; Smith et al. 2013; Pistevos et al. 2019). In a previous study, Duncan and Holland (2006) showed that confined coastal nurseries of *S. lewini* could be more important in providing protection from predators than in providing a plentiful source of food for the juveniles, thus often causing mortality from starvation. It is therefore possible that, in the Mexican Pacific, females might be more plastic in selecting the nurseries for parturition, perhaps to aid in differing the neonate vulnerability to predators and a potential lack of foraging success among regions. More information is needed to elucidate this important aspect of the spatial ecology of *S. lewini.*

Regarding the migratory patterns of females, analyses of the Sr.Ba and Pb:Ca profiles showed that some females (*n* = 7; 30.4%) likely remained nearshore for the first 3 to 5 years of life and later presumably migrated offshore at approximately 133 cm of L_T, thus confirming that ontogenetic migration of juvenile females occurs around 105 -123 cm of L_T (Klimley 1987; Hoyos-Padilla et al. 2014). Analyses of the Sr.Ba and Pb:Ca profiles also indicated that mature females (L_T > 207 cm; Anislado-Tolentino and Robinson-Mendoza 2001; Torres-Huerta et al. 2008) eventually returned to coastal habitats for parturition between 207 to 277 cm of L_T, corresponding to 11 to 20 years of age. The lack of any discernible age-related pattern and the high variability in Sr:Ba for many other females (*n* = 15; 69.6%) suggested the use of vastly different habitats over lifetimes.

Although the reproductive cycle of *S. lewini* is thought to be annual in the Mexican Pacific (Torres-Huerta et al. 2008; Bejarano-Álvarez et al. 2011), the frequency of return of females to the coast for parturition could not be documented in this study based on vertebral microchemistry. The latter was found for the bull shark *C. leucas* for which periodic returns of females into estuarine habitats for parturition were shown by cyclic declines of the Sr:Ba ratio to values around 300 (Tillett et al. 2011). Considering that this same approach concurrently failed in distinguishing movements between oceanic and coastal habitats of the pig-eye shark *C. amboinensis* (a fully marine species characterized by a narrow range of movements) since only subtle changes in Sr:Ba were detected (Tillett et al. 2011), it is possible that periodic returns of females of *S. lewini* could not be detected in the present study because these either (1) moved into less saline habitats and departed shortly afterward (< 3 weeks; Werry et al. 2011), thus precluding elemental signatures of the recently used habitat to be incorporated into the vertebrae, or (2) gave birth in variable environments, being this possibly less nearshore than previously assumed. Although these conclusions are based on a data set that does not include all potential sources of the population of *S. lewini* in the region, the fact that in this study some females exhibited highly contrasted patterns in their Sr:Ba profiles that corresponded well with the previously described ontogenetic habitat use changes (Klimley 1987; Hoyos-Padilla et al. 2014) whereas other females from the same sites of capture did not exhibit a discernible pattern supports the assumption of a less defined use of coastal nurseries by the species rather than a downfall of the technique.

As a placental viviparous species, *S. lewini* exhibits a high degree of maternal investment in its offspring. During their first weeks of development, the embryos derive their nourishment from the yolk stored in the fetal yolk sac after which a placenta is formed allowing them to be directly nourished from the mother's blood stream (Castro 2011b). Considering that in the Mexican Pacific the gestation of *S. lewini* lasts about 10-11 months (Torres-Huerta et al. 2008; Bejarano-Álvarez et al. 2011), maternal supply of nutrients to the embryos might possibly influence their vertebral microchemistry while *in-utero*, at least a few weeks after fertilization. This requires further investigations as the pre-natal elemental signatures in vertebrae of any specimen of the species might thus inform about the movements of females during the gestation.

Regarding the migratory patterns of males, comparisons of the age-related Sr:Ba and Pb:Ca profiles showed that males are probably divided between two distinct strategies of movements. Some males (*n* = 6; 23.1%) likely remained nearshore for the first 4 to 7 years of life and presumably later migrated offshore at approximately 150 cm of $L_{T,}$ in accordance with sex, size, reproductive stage and stomach content data that showed that males may remain in coastal habitats until they reach a size of approximately 160 cm of L_T before migrating offshore (Klimley 1987). Importantly, this observation also confirmed that, in the Mexican Pacific, females migrate offshore at a smaller size (*i.e.* 105 to 133 cm of L_T) than do males (*i.e.* 148 to 160 cm of L_T) as evidenced by telemetric and stomach content studies (Klimley 1987; Hoyos-Padilla et al. 2014). This strategy has been attributed to increase their foraging success by feeding on energy-rich pelagic preys in order to attain the larger body size required for embryonic development and later match their reproductive lifetime to that of males (Klimley 1987).

The Sr:Ba and Pb:Ca analyses also indicated that some of those males apparently returned to coastal habitats after having inhabited more offshore habitats. For *S. lewini*, the necessary conditions of the female reproductive strategy are both coastal and pelagic habitats as they depend on coastal habitats for parturition and offspring development. Males do not have such requirement and the occurrence of reproductive males in coastal habitats after having recently inhabited more oceanic habitats suggests that males may enter coastal habitats for reproductive purposes (Clarke 1971; Castro 2011a). This behavior was documented in only few occasions for *S. lewini* in other regions of the species range (Clarke 1971; Castro 2011a; Harry et al. 2011), however the reasons why males are presumably following females are unknown. The occurrence of reproductive males in coastal waters may be coincidental or in response to exuded pheromones in anticipation of mating after parturition (Castro 2011a) after foraging offshore on energy-rich pelagic preys to increase their body size (Klimley 1987).

While the reported pattern of offshore ontogenetic migration of males was effectively detected in some individuals (*n* = 6; 23.1%), it is worth noting that most (*n* = 20; 76.9%) likely remained nearshore for their entire life. This was evidenced by a low but constant Sr:Ba ratio across vertebral transects and higher Pb:Ca values in these individuals when compared to the individuals captured offshore. Given these observations, the fact that previous studies documented the occurrence of adult males offshore as well as nearshore (Clarke 1971; Castro

2011a; Harry et al. 2011), and that the results of the present study indicated that males may either (1) constantly remain nearshore or (2), migrate offshore to later return nearshore, possibly for reproductive purposes, supports the assumption that males of *S. lewini* can exhibit two strategies of movements, coastal or pelagic (Harry et al. 2011). Alternating among these two strategies might have important impacts on the resilience of the population to the fishing pressure because reproductive individuals might be more susceptible to fisheries while remaining nearshore. A coastal strategy might also influence the genetic architecture and adaptability of the species as the gene flow of *S. lewini* has been thus far thought to be largely male-mediated (Daly-Engel et al. 2012).

CONCLUSIONS

Vertebral microchemistry has some limitations in detecting movements in widely distributed marine species (Allen et al. 2009; Tillett et al. 2011), however this approach allowed to further document important aspects of the life history of *S. lewini* in the Mexican Pacific, in spite of the low sample size available and the lack of analogous studies in sharks against which to compare the results of this study (*i.e.* Tillett et al. 2011). The results indicated that *S. lewini* exhibits migratory patterns that are likely more plastic than previously assumed, with a probable stronger affiliation with coastal habitats than expected and a relatively limited use of highly oceanic habitats, in particular because several males appeared to have returned to the coast after having inhabited more offshore habitats while others even remained nearshore for their entire life.

Six of the nine hammerhead shark species (Sphrynidae) used to occur in the Mexican Pacific: bonnethead *S. tiburo,* great hammerhead *S. mokarran,* scalloped bonnethead *S. corona,* scoophead *S. media,* smooth hammerhead *S. zygaena* and *S. lewini* (Compagno 1984). Though *S. lewini* and *S. zygaena* are the most frequent hammerheads documented in the fishery surveys of that area, *S. media*, *S. mokarran* and *S. tiburo* have considerably declined during the last decades, and even became extirpated from the Gulf of California (Bizzarro et al. 2009; Smith et al. 2009; Pérez-Jiménez 2014). Considering that *S. tiburo,* a coastal species with restricted-movements exhibiting one of the highest population growth rates among sharks (Castro 2011a; Cortés et al. 2016), has practically disappeared from the Mexican Pacific (Pérez-Jiménez 2014), it is quite surprising that *S. lewini* is still a frequent component of the small-scale and industrial fishery landings in spite of being heavily fished (Bizzarro et al. 2009; Smith et al. 2009; Pérez-Jiménez 2014). The persistence of *S. lewini* in the Mexican Pacific might be linked to its behavioral plasticity of movement and habitat use rather than its life history characteristics (*i.e.* slow growth, late age at maturity). Migratory contingents or partial migration might be a strategy of the species to reduce its vulnerability to stressors or unfavorable environmental conditions among regions (*e.g.* overexploitation or habitat loss) and vary its ability among sub-populations to recover from population depletions (Kerr et al. 2010; Chapman et al. 2011; Parsons et al. 2011). Nevertheless, the effects of these variable and complex movement patterns on the resilience of this globally endangered species require further investigations to precisely identify the migratory routes of *S. lewini* across spatially distinct habitats (coastal versus offshore) and ensure that the populations of scalloped hammerhead sharks in the Mexican Pacific are properly protected and managed.

DATA AVAILABILITY

The Matlab codes used to undertake the analyses performed in this study and prepare the figures presented in the article are freely available on GitHub (https://github.com/clairecoiraton/HammerheadMigratoryPatterns_MatlabCode.git)

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CAPÍTULO 5

USO DE MICROQUÍMICA VERTEBRAL PRENATAL DEL TIBURÓN MARTILLO *Sphyrna lewini* PARA INFERENCIAS DE PATRONES MIGRATORIOS DE HEMBRAS PREÑADAS

Trabajo publicado en: Coiraton C, Amezcua F (2020) *In utero* elemental tags in vertebrae of the scalloped hammerhead shark *Sphyrna lewini* reveal migration patterns of pregnant females. Scientific Reports (ACCEPTADO)

RESUMEN

Estudios recientes sobre la microquímica vertebral del tiburón martillo *Sphyrna lewini* permitieron la inferencia de sus patrones migratorios en el Pacífico mexicano, sin embargo conclusiones sobre los movimientos de las hembras preñadas se vieron obstaculizadas por el pequeño tamaño de muestra. Teniendo en cuenta que *S. lewini* es una especie vivípara placentaria, el suministro materno de nutrientes a los embriones podría influir en su microquímica vertebral mientras ellos estén *in utero* y entonces proporcionar marcadores intrínsecos de las historias ambientales de las hembras preñadas durante su gestación. El objetivo de este estudio fue de probar esta hipótesis para luego intentar determinar los patrones migratorios de las hembras preñadas por medio del análisis de los perfiles elementales cuantificados en las vértebras de neonatos costeros que son más frecuentemente capturados. Las vértebras se obtuvieron de tiburones capturados a lo largo de la costa del Pacífico mexicano en 2016. Las firmas elementales se midieron por medio de un sistema de ablación láser acoplado inductivamente con un espectrómetro plasma de masas (LA-ICP-MS). Las firmas microquímicas de las vértebras fueron consistentes entre cada hembra y sus respectivos embriones, demostrando la viabilidad de utilizar las firmas elementales depositadas en las vértebras *in utero* como indicadores de historia ambiental de las madres durante su gestación. El análisis subsecuente de los perfiles Sr:Ba y Pb:Ca cuantificados *in utero* en vértebras de neonatos demostraron que las hembras preñadas (1) se dispersaron progresivamente hacia el altamar antes de migrar a los hábitats de crianza justo antes del parto, o bien (2) permanecieron cerca de la costa durante toda la gestación. Debido a que *S. lewini* se encuentra en peligro de extinción, las medidas de manejo actuales podrían ser insuficientes para el manejo sostenible y conservación de esta población ya que las hembras preñadas son muy susceptibles a ser capturadas al permanecer cerca de la costa o cuando se acercan a esta poco antes del parto, lo cual podría limitar las posibilidades de llevar a cabo su gestación completa y reducir el potencial reproductivo de la población al centralizarse brevemente en las áreas costeras.

ABSTRACT

Vertebral microchemistry recently allowed to infer the migration patterns of the scalloped hammerhead shark *Sphyrna lewini* in the Mexican Pacific, however conclusions regarding the movements of reproductive females were hindered by the small sample size. Considering that *S. lewini* is a placental viviparous species, maternal supply of nutrients to the embryos might influence their vertebral microchemistry while *in utero* and provide intrinsic markers of the pregnant female environmental histories. This hypothesis was tested before attempting to infer the migration patterns of pregnant females through the analyses of the *in utero* elemental profiles quantified in the vertebrae of coastal young-of-the-year ('YOY'). Vertebrae were obtained from sharks captured along the Mexican Pacific coast in 2016. Vertebral microchemistry was quantified using laser ablation inductively-coupled plasma mass spectrometry. Elemental signatures at vertebral edge were consistent between each pregnant female and her embryos demonstrating the viability of employing *in utero* elemental signatures as a maternal tag of the gestation-related environmental histories. Analyses of the YOY *in utero* Sr:Ba and Pb:Ca profiles suggested that pregnant females either (1) progressively migrated offshore before quickly returning to coastal habitats before term or (2) remained nearshore during complete gestation. Considering the endangered status of *S. lewini,* current management measures may be insufficient for the sustainable management of the population as pregnant females may be particularly susceptible to fisheries when remaining nearshore or entering coastal habitats prior to pupping.

INTRODUCTION

The scalloped hammerhead shark, *Sphyrna lewini* (Griffith and Smith 1834), is a circumtropical migratory species that uses both oceanic and coastal habitats for its dispersal and reproductive strategy(Compagno 1984). In the Mexican Pacific, neonate and juvenile stages of *S. lewini* are typically found in shallow estuarine and coastal habitats (Anislado-Tolentino 2000; Alejo-Plata et al. 2007; Coiraton et al. 2017) for their first 3 to 7 years of life (Klimley 1987; Hoyos-Padilla et al. 2014; Coiraton et al. 2020) before migrating offshore to maximize their foraging opportunities and grow more rapidly to reproductive size (Klimley 1987). Adults, on the other hand, seasonally form schools near seamounts and oceanic islands (Klimley 1983, 1985; Klimley and Nelson 1984) and females later return to coastal habitats for parturition (possibly annually; Torres-Huerta et al. 2008; Bejarano-Álvarez et al. 2011). Although males do not have such requirement, recent studies indicated that some individuals may also later return to coastal habitats or even remain nearshore for their entire life (Harry et al. 2011; Coiraton et al. 2020), thus being able to mate opportunistically with females entering coastal waters to give birth (Clarke 1971; Castro 2011b; Harry et al. 2011). Though these findings provided more insights into the life history of males, the present state of knowledge on the migratory patterns of females remains fragmentary, particularly during the gestation.

Microchemical signatures derived from elements deposited in the vertebrae of sharks during growth can reflect changes in the surrounding water chemistry (Tillett et al. 2011; Scharer et al. 2012; Smith et al. 2013; Pistevos et al. 2019), environmental conditions (Smith et al. 2013; Mohan et al. 2018; Pistevos et al. 2019) and diet (Estrada et al. 2006; Carlisle et al. 2014), and thus serve as discrete site-specific markers (Tillett et al. 2011; Izzo et al. 2016; Lewis et al. 2016; McMillan et al. 2016, 2018; Smith et al. 2016) or time-resolved records of the individual environmental histories when related to growth bands (Coiraton et al.; Tillett et al. 2011; Scharer et al. 2012; Raoult et al. 2016; Mohan et al. 2018). More specifically, it was recently showed that vertebral microchemistry of the scalloped hammerhead shark *S. lewini* could accurately distinguish among individuals from different locations of the Mexican Pacific, whether these had been occupying coastal or more oceanic habitats, and address important questions concerning the recent habitat use and natal origin (Smith et al. 2016; Coiraton et al. 2020) of the species in the region. Concentrations of strontium ^{88}Sr, barium ^{137}Ba and lead ^{208}Pb were assessed along vertebral transects encompassing complete life histories, which allowed to document several aspects on the migratory patterns of *S. lewini* because these elements were found to reflect the nearshore-offshore movements of the sharks across the apparent salinity gradient (Allen et al. 2009; Tillett et al. 2011; Scharer et al. 2012; Coiraton et al. 2020) and periods of nearshore residency into contaminated habitats (Ranaldi and Gagnon 2008, 2010; Selleslagh et al. 2016; Coiraton et al. 2020). Robust conclusions regarding the migration patterns of females, and particularly mature individuals, were however hindered by the small sample size and the lack of discernible pattern in the age-related elemental profiles for some individuals (Coiraton et al. 2020).

As a placental viviparous species, *S. lewini* exhibits high degree of maternal investment in its offspring. During their first weeks of development, embryos derive their nourishment from their yolk-sacs after which these transform to become a highly vascularized placenta that directly provides nutrients to the embryos through the mother’s blood stream (Wourms 1977). Given the long gestation period of *S. lewini* (*i.e.* 10-11 months; Torres-Huerta et al. 2008; Bejarano-Álvarez et al. 2011), maternal supply of nutrients to embryos has the potential to influence the vertebral microchemistry of embryos while *in utero* (Coiraton et al. 2020), at least after placental formation (Castro 2011a). Accordingly, validating the relationships between maternal vertebral microchemistry and elemental composition of embryos may provide novel proxies for inferring the migration patterns of pregnant females and facilitate further investigations on the species population connectivity through the examination of *in utero* elemental signatures or transect profiles quantified in the vertebrae of coastal juvenile stages (Coiraton et al. 2020), that are a more frequent component of the small-scale fisheries operating along the Mexican Pacific coast when compared to pregnant females (Pérez-Jiménez et al. 2005; Alejo-Plata et al. 2007; Coiraton et al. 2017).

The objective of this study was to establish the viability of employing the *in utero* elemental signatures deposited in vertebrae of *S. lewini* as intrinsic markers of the environmental histories of pregnant females during their gestation in the Mexican Pacific. More specifically, this study sought to test the hypotheses that: (1) elemental signatures in vertebrae and (2) elemental profiles encompassing complete *in utero* development were consistent among embryos within each pregnant female’s litter and (3) vertebral microchemistry of embryos reflected that of pregnant females during the gestation in order to (4) attempt to assess the gestation-related movements of pregnant females using *in utero* maternal tags in the vertebrae of coastal juveniles. Only young-of-the-year specimens (age 0) were considered to avoid potential site-specific temporal variations in the water chemistry that might result in different elemental signatures for individuals of different ages originating from the same nurseries (Smith et al. 2016).

MATERIAL AND METHODS

Sample collection

Vertebrae samples from pregnant females of *S. lewini* and their embryos were obtained from specimens captured in April 2016 by the artisanal shark fishery operating off Puerto Madero (Fig. 5.1; Table 5.1). Vertebrae samples from young-of-the-year specimens were obtained between August and October 2016 from three artisanal fishery landings along the Mexican Pacific coast, in La Reforma, Teacapán and Salina Cruz (Fig. 5.1; Table 5.2). These sampling locations were selected to address the questions of this study because they were reported as important nursery areas for *S. lewini* in the Mexican Pacific (Anislado-Tolentino 2000; Mendizábal-Oriza et al. 2002; Alejo-Plata et al. 2006, 2007; Torres-Huerta et al. 2008; Coiraton et al. 2017). Fishers used surface and bottom gillnets and surface longlines. All sample collection was opportunistic and carried out in accordance with relevant national guidelines and regulations. Sex and total length (L_T) were recorded for each specimen and a set of post-cephalic vertebrae was removed and stored frozen until preparation for analyses.

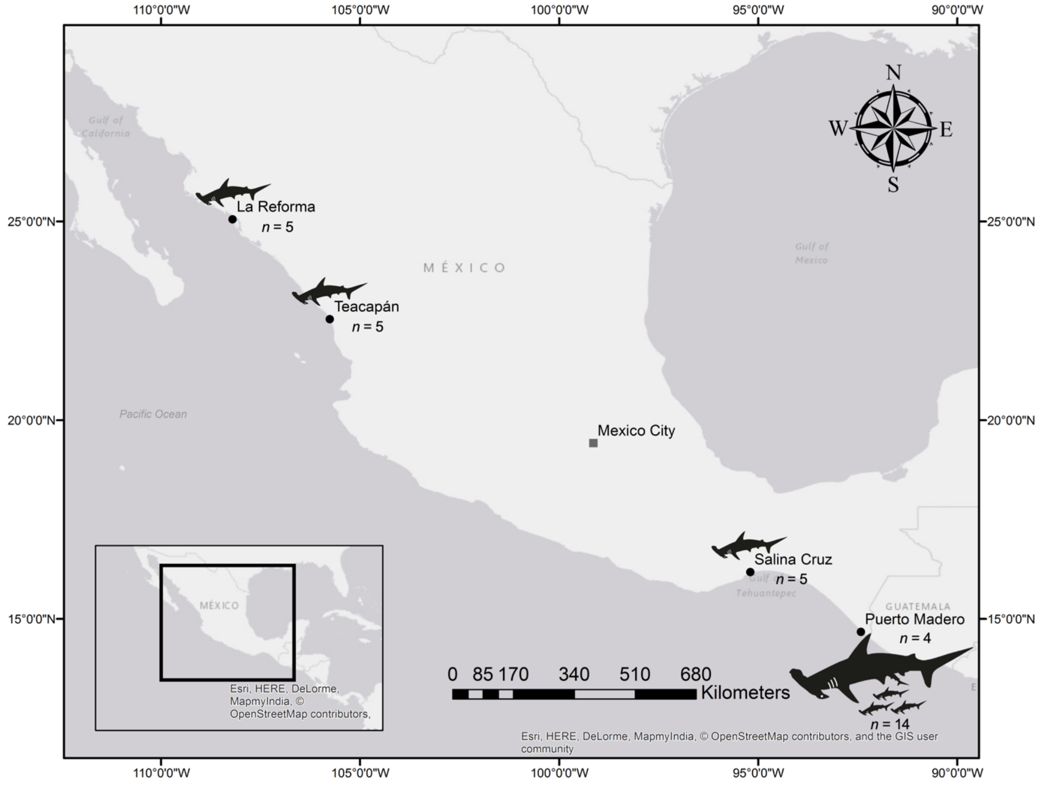

Figure 5.1. Map of the region of study showing the sampling sites of pregnant females (*n* = 4), embryos (*n* = 14) and young-of-the-year (*n* = 15) specimens of *Sphyrna lewini* in the Mexican Pacific (hammerhead illustrations used with permission ©*Flora pixelia*)

Table 5.1. Codes, total length (L_T; cm) range and mean (± standard deviation) of the 4 pregnant females of *Sphyrna lewini* and their near-term embryos (*n* = 14) captured off Puerto Madero in April 2016

Female's litter code	$L_{T\ \text{females}}$	*n* embryos	$L_{T\ \text{embryos}}$
1	291	4	41 - 43 (42.1 ± 0.9 cm)
2	277	3	41 - 47 (44.5 ± 3.1 cm)
3	253	3	47
4	240	4	43 - 49 (46.6 ± 2.6 cm)

Table 5.2. Sampling details, total length (L_T; cm) range and mean (± standard deviation) of the 15 young-of-the-year specimens of *Sphyrna lewini* collected in 2016 in three coastal nurseries of the Mexican Pacific

Sampling site	Date of capture	*n*	L_T
La Reforma	Sept 2016	5	55 - 63. (59.4 ± 3.4 cm)
Teacapán	Aug 2016	5	52.8 – 56.8 (54.2 ± 1.7 cm)
Salina Cruz	Oct 2016	5	49.2 – 59.6 (55.3 ± 4.5 cm)

Vertebrae preparation for LA-ICP-MS analyses

Vertebrae were defrosted and the neural arch and extraneous tissue were removed. Individual centra were exposed, thoroughly rinsed, air dried, mounted on wooden holders with polyester resin and later cut into 0.4 mm thick sagittal sections with a Buehler low-speed Isomet saw. Vertebral sections were hand-polished with a series of progressively finer grades of lapping paper (220, 800, and 3 µm grit), ultrasonically cleaned for 5 minutes in Milli-Q water to remove surface contaminants, triple rinsed, dried for 24 hours and randomly affixed with double-sided tape to acid-washed petrographic slides (subsequently referred to as 'master slides'). One vertebra section from each specimen was used for analyses as it was shown that elemental signatures of *S. lewini* did not differ in individual sharks (Schroeder 2011; Smith et al. 2016). All cleaning and drying procedures were performed under a Class-100 laminar flow clean hood using trace-metal grade reagents, non-metallic instruments and HNO_3 acid-washed glass slides to minimize contamination.

LA-ICP-MS analyses

The elemental composition of the vertebrae of *S. lewini* was quantified using a Photon-Machines Analyte 193 excimer UV laser ablation system (LA), connected to an Agilent Technologies 7500CX quadrupole inductively coupled plasma–mass spectrometer (ICP-MS). Raw data of the ion counts per second (cps) were collected for the following 21 elements: ^{7}Li, ^{24}Mg, ^{43}Ca, ^{45}Sc, ^{51}V, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{72}Ge, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{114}Cd, ^{118}Sn, ^{137}Ba, ^{197}Au, ^{208}Pb, ^{232}Th and ^{238}U with ^{43}Ca being quantified for use as internal standard. These were screened in the vertebrae of *S. lewini* because this combination of masses minimizes potential interferences that can arise from isobaric spectral overlap, sample matrix effects, and the presence of molecular ions (Jones et al. 2013) and was successfully used in previous studies for inferring the life history of sharks (Tillett et al. 2011; Izzo et al. 2016; Lewis et al. 2016; McMillan et al. 2016, 2018; Smith et al. 2016; Mohan et al. 2018). Even though it is the ^{138}Ba isotope that is usually assayed in studies of calcified structures, ^{137}Ba was screened in the vertebrae of *S. lewini* in this study for the sake of comparison with results reported in analogous study (Tillett et al. 2011). As the less abundant isotope (11.2%; Zolfonouna et al. 2016), ^{137}Ba is slightly more challenging to assay reliably than its counterpart, ^{138}Ba (71.7%; Zolfonouna et al. 2016), however this was not considered as a detrimental flaw to the present study because the use of a less abundant isotope would only increase the magnitude of change of the Sr:Ba ratio, though not the general pattern.

The consistency in vertebral microchemistry among embryos within each pregnant female's litter was tested before determining whether the elemental signatures deposited in the vertebrae of embryos reflected maternal vertebral microchemistry (Hypothesis 1; Table 5.3). Spots targeting the vertebral focus and edge of each embryo were ablated in order to quantify the elemental signatures deposited at the beginning of the gestation and at the time immediately prior to capture, respectively, and test this hypothesis (Fig. 5.2).

The temporal consistency in elemental profiles among embryos within each pregnant female's litter was also examined to verify that results obtained separately for the focus and the edge in the vertebrae of embryos were consistent for the time period between the beginning of the gestation and the time immediately prior to capture (*i.e. in utero* embryonic development) (Hypothesis 2; Table 5.3). Transects encompassing the area from the focus to the edge in the vertebrae from each embryo were ablated to characterize the individual elemental profiles and test this hypothesis (Fig. 5.2).

The hypothesis that vertebral microchemistry of embryos reflected that of pregnant females during the gestation period was tested by comparing the elemental signatures deposited at the vertebral edge of each pregnant female with her respective embryos (Fig. 5.2), as this region of the vertebrae provided a known spatial and temporal reference (*i.e.* time immediately prior to capture) that made the comparisons possible (Hypothesis 3; Table 5.3). Spots targeting the vertebral edge of each pregnant female were ablated so the resulting elemental signatures could be compared with the elemental signatures of vertebral edge previously quantified in their embryos.

Ultimately, the potential to infer the gestation-related movements of pregnant females using the *in utero* vertebral microchemistry of their offspring as a proxy was evaluated by measuring changes in ^{88}Sr, ^{137}Ba and ^{208}Pb along transects encompassing complete *in utero* embryonic development from the focus to the birthmark (Fig. 2) of the young-of-the-year vertebrae (Hypothesis 4; Table 3). Strontium (^{88}Sr) and barium (^{137}Ba) were used as salinity change indicators of the pregnant female environmental histories because: (1) adult females of *S. lewini* primarily inhabit fully marine offshore habitats (Klimley 1983, 1985; Klimley and Nelson 1984) and (2), nursery areas of the species (*i.e.* estuaries and coastal bays; Clarke 1971; Anislado-Tolentino 2000; Duncan and Holland 2006; Brown et al. 2016; Coiraton et al. 2017; Marie et al. 2017) are typically characterized by significant freshwater inputs during the birthing season (*i.e.* May-August; Alejo-Plata et al. 2007; Torres-Huerta et al. 2008; Coiraton et al. 2017) in the Mexican Pacific (Amezcua et al. 2019), which was expected to influence the nearshore-offshore gradient of salinity and hence the Sr and Ba values (McCulloch et al. 2005; Crook et al. 2006; Allen et al. 2009) during this period. The *in utero* variations of lead (^{208}Pb) in the vertebrae were assessed as an alternate indicator of contaminated habitat use to aid in interpreting the observations based on ^{88}Sr and ^{137}Ba.

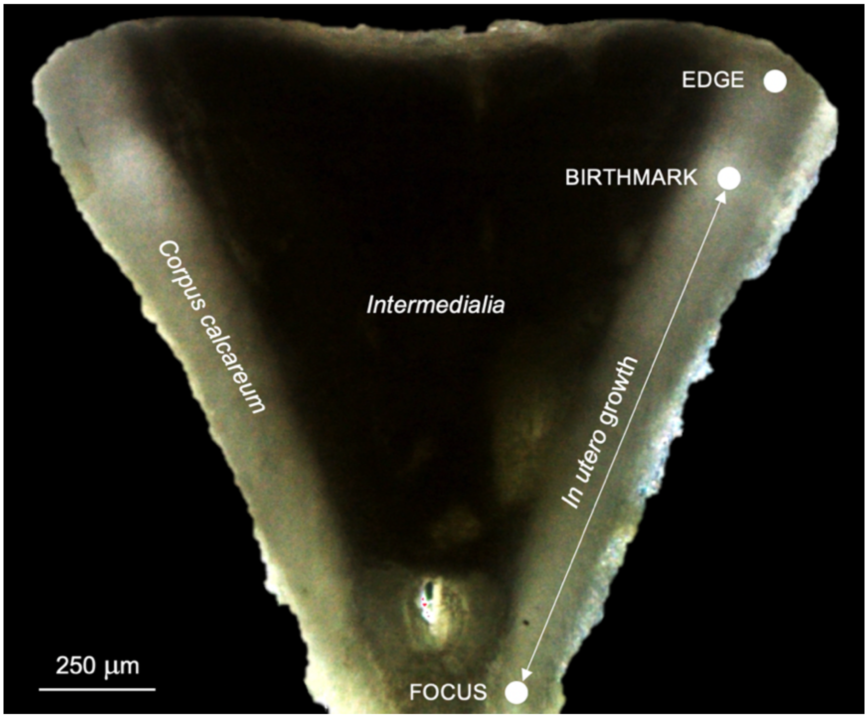

Figure 5.2. Sagittal section of a vertebra centrum from a young-of-the-year specimen of *Sphyrna lewini*, illustrating the terminology used for LA-ICP-MS analyses

Table 5.3. Summary of the methodology employed to address the different hypotheses of this study. See figure 5.2 for the target areas of the vertebral sections (*i.e* focus, birthmark or edge) from the embryo, pregnant female and young-of-the-year ('YOY') specimens

Hypothesis	Vertebrae	Ablation samples
1. Elemental signatures in vertebrae of embryos from a same litter are similar	EMBRYOS	SPOTS (Focus and Edge)
2. Elemental profiles in vertebrae of embryos from a same litter are similar	EMBRYOS	TRANSECTS (Focus - Edge)
3. Elemental signatures in vertebrae of a mother match with those of her embryos	EMBRYOS vs MOTHERS	SPOTS (Edge)
4. *In utero* elemental profiles in the offspring's vertebrae reflect migration patterns of pregnant females	YOY	TRANSECTS (Focus - Birthmark

Spots were ablated with $n = 3$ replicates; circular spot size was 83 μm with a laser repetition rate of 5 Hz and a 60 s duration. Transects were pre-ablated to remove possible external contamination. Pre-ablation scan speed was 108 $\mu m.s^{-1}$, with a repetition rate of 2 Hz and a 108 μm spot size. For data acquisition, ablation scan speed was 10 $\mu m.s^{-1}$, with a repetition rate of 10 Hz and an 83 μm spot size. All ablation samples were entirely positioned within and along the *corpus calcareum* of the vertebral sections (Fig. 5.2).

Elemental data were acquired using an ICP-MS which employed Agilent Technologies ChemStation software operating in time-resolved analysis mode to collect raw data (cps) for the 21 target elements. NIST-612 silicate glass served as external calibration reference material (Pearce et al. 1997) and was ablated with two replicates before and after every fifth vertebral section was sampled. MACS-3 microanalytical carbonate standard material (Koenig and Wilson 2007) was ablated in brackets before and after each master slide to estimate experiment-wide levels of precision. Background data corresponding to gas blanks were collected for 60 s before and after each spot or transect scan was performed. Prior to data acquisition, the ICP-MS instrument was fine-tuned while ablating NIST-612 using 108 µm wide transect scans running at 5 Hz and 86% power in order to maximize element counts and minimize noise. All laboratory facilities and instrumentation used for elemental analyses were located on the campus of the College of Marine Science, University of South Florida, St. Petersburg, FL, USA.

Data analyses

Elemental signatures in vertebrae: embryos versus pregnant females

Raw spot data (cps) were visually assessed within the software, and those portions of the signals displaying peaks likely associated with surface contaminants or other forms of instabilities were excluded from further processing. The following operations were then applied to the data associated with each spot sample: (1) background levels were removed by subtraction; (2) mass-specific spikes detected by the Grubbs test ($\alpha = 0.05$) were replaced with mean values; and (3) mass-specific drift in the sensitivity of the ICP-MS detector was corrected via linear interpolation. Raw spot data (cps) were then converted to single, mean (*i.e.* averaged across replicates) elemental concentration values (ppm) using NIST-612 data for external calibration and standardized to the ^{43}Ca data obtained simultaneously in the structure by deriving element:Ca ratios ($\mu mole \cdot mole^{-1}$) to adjust for variability in instrument sensitivity and the amount of ablated material. Limits of detection (LOD) were estimated for samples based on 3·SD of the ion count rates (cps) of the corresponding gas blanks and converted to concentrations (ppm). Elements with

≥ 10% of the measures of concentration below LOD were omitted from subsequent analyses. Outliers among replicate spot scans were identified using a multivariate measure of outlyingness (Breiman and Cutler 2003) based on elemental concentrations (ppm). Replicates with outlyingness values > 10 were excluded before reducing the data to mean ppm concentration value for each sample. Raw transect data (cps) were made compatible for direct comparisons by interpolating cell array of each vertebral transect so they all had the same number of data points as the longest one and generating mean integrated transect data of the retained elements (*i.e.* consistently recorded above LOD).

The null hypotheses that (1) no difference existed in the elemental signatures deposited at the vertebral focus and edge of embryos within each pregnant female's litter, (2) no difference existed among the elemental profiles of embryos within each litter, and that (3) no difference in the elemental signatures of edge existed between a pregnant female and her embryos were tested using permutational analysis of variance (PERMANOVA; Anderson et al. 2017). Canonical analyses of principal coordinates (CAP; Anderson and Willis 2003) were subsequently employed to visualize the within-group similarities detected using PERMANOVA and test the ability of the models to accurately distinguish among pregnant females and their corresponding embryos based on vertebral microchemistry. Leave-one-out cross-validation (LOO-CV) was used to assess the overall classification accuracy of each CAP model and build a confusion matrix summarizing the occurrence of group-specific misclassifications. Proportional chance criterion (PCC) was used to test the significance of the observed overall classification success rates of the CAP models compared with that expected by chance; White and Ruttenberg 2007).

Spot (*i.e.* focus and edge) and transect data were analyzed as multi-elemental signatures of the mean (*i.e.* averaged across replicates) element:Ca ratios and mean integrated transect data of the retained elements, respectively. A Euclidean distance-based dissimilarity matrix constructed from the mean element:Ca ratios or elemental profiles served as multiple, quantitative explanatory variables in PERMANOVA and CAP design, with individual pregnant females and/or litters serving as the categorical response variables.

Inferring the gestation-related movements of pregnant females

Transect data for ^{88}Sr,^{137}Ba and ^{208}Pb (used as Sr:Ba and Pb:Ca raw cps ratios) were plotted versus vertebral transect distance from the focus (μm) of each young-of-the-year to evaluate the viability to employ the *in utero* elemental profiles of *S. lewini* to infer the gestation-related movements of pregnant females. Data were used as raw cps for the sake of comparison with analogous study involving *S. lewini* in the Mexican Pacific (Coiraton et al. 2020). An 11-point running average window size was used to filter/smooth the data, reduce the noise, and aid in identifying the underlying pattern of the Sr:Ba and Pb:Ca profiles (Sinclair et al. 1998). The same procedure was subsequently applied to the embryo transect data to compare the patterns observed in their elemental profiles with those generated from the young-of-the-year while *in utero*. Evidences of pregnant females possibly entering estuarine or nearshore habitats for parturition were expected to be shown by a sharp decline of the Sr:Ba ratio (between 150 and 400) in the *in utero* profiles prior to birth combined with an increase of the Pb:Ca ratio at the same time (Coiraton et al. 2020). Although previous study recently confirmed that Sr:Ca and Ba:Ca were inversely related relative to each other in the vertebrae of *S. lewini* relative to the nearshore-offshore environmental gradient in the region of study (Coiraton et al. 2020), this assumption was also verified in the present study before inferences on the movements of pregnant females could be made based on their offsprings' *in utero* Sr:Ba profiles.

When a marked shift was observed in the *in utero* profiles of a sample, the total length of the individual at that time *t* of the embryonic development was estimated using the back-calculation Fraser–Lee method (Francis 1990):

$$L_t = [(R_t)(R_V)^{-1}](L_C - \mathrm{a}) + \mathrm{a}$$

where L_t is the back-calculated total length corresponding to age *t* (*i.e.* time of the pregnant female habitat shift), R_t corresponds to the vertebral transect distance from the focus to the elemental shift identified *in utero* at age *t*, R_V is the vertebrae centrum radius, L_C the total length at the time of capture of the individual (*i.e.* young-of-the-year or embryo) and *a* is intercept of an established linear relationship between R_V and L_C (Francis 1990), which is $L_C = 17.349\ R_V + 14.516$ for juvenile specimens of *S. lewini* in the Mexican Pacific (Coiraton et al. 2019).

The Fraser–Lee equation was selected as a back-calculation method in order to correct for bias induced when the R_V-L_C linear regression does not pass through the origin (such as is the case for *S. lewini*) by using the intercept *a* estimated from the R_V-L_C relationship as a correcting factor in the equation (Quist and Isermann 2017). Estimating the *in utero* back-calculated length at the time of the pregnant female habitat shift was considered necessary in this study to emphasize the importance of protecting these individuals when entering nearshore habitats a few cm before the embryos have reached a full-term total length. Although back-calculation may not adequately describe faster embryonic growth during the early stages of the gestation (Branstetter 1987a), this was not considered as an issue in the present study because the *in utero* total lengths of the individuals at the time of the pregnant female habitat shift were estimated for the period corresponding to the late stages of the gestation and the parameters used in the equation were derived from juveniles, which allowed to avoid bias induced by mature individuals exhibiting different growth rates (Ricker 1979).

All elemental data processing and multivariate statistical analyses were performed using the free download Fathom Toolbox for Matlab^{TM} (Jones 2017). For more details on the data processing and multivariate statistical analyses, please refer to the readme files and corresponding Matlab codes available on GitHub.

RESULTS

Elemental signatures in vertebrae: embryos versus pregnant females

The vertebrae from 4 pregnant females of *S. lewini* captured off Puerto Madero and 3 to 4 embryos from their respective litters were available for this study (Hypotheses 1-3; Table 5.3). Pregnant females ranged from 240 to 291 cm of L_T (mean ± SD = 265 ± 23 cm L_T; Table 5.1). Embryos ranged from 41 to 49 cm of L_T (n = 14; 44.8 ± 2.8 cm L_T; Table 5.1). Based on the reported length-at-birth of *S. lewini* in the Mexican Pacific (*i.e.* 41-53 cm L_T; Anislado-Tolentino 2000; Alejo-Plata et al. 2007; Torres-Huerta et al. 2008; Coiraton et al. 2017), these embryos were about to be born.

The mean percentage of the elemental concentration data below the limits of detection (LOD) was ≤10% for all elements (*i.e.* ^{7}Li, ^{24}Mg, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{114}Cd, ^{118}Sn, ^{137}Ba, ^{208}Pb), except for ^{45}Sc (42.9%), ^{51}V (57.1%), ^{53}Cr (64.3%), ^{72}Ge (71.4%), ^{89}Y (64.3%), ^{114}Cd (57.1%), ^{197}Au (71.4%), ^{232}Th (63.3%) and ^{238}U (78.6%) which were not regularly detected in the vertebrae of embryos and pregnant females. For more details on the mean (± standard deviation) element:Ca ratios (μmole·mole^{-1}) quantified at the vertebral focus and edge of embryos and pregnant females, please refer to Table 5.S1 from the Supplementary Information.

The multi-elemental signatures deposited at the vertebral focus of embryos were consistent within each pregnant female's litter (PERMANOVA: $0.68 < P < 1$; Hypothesis 1; Table 5.3), 85.7% of the embryos were correctly assigned within each corresponding litter by the $\text{CAP}_{\text{FOCUS}}$ model classifier ($\text{CAP}_{\text{FOCUS}}$: $m = 8$, G_{prop} = 100%, $Trc_{\text{stat}} = 2.66$, $P = 0.001$; Fig. 5.3a) and this was significantly better than the 25.5% accuracy rate expected by chance (PCC: $P = 0.001$).

The multi-elemental profiles were also consistent among embryos within each pregnant female's litter (PERMANOVA: $0.36 < P < 1$, Hypothesis 2; Table 5.3); 60% of the embryos were correctly assigned within each corresponding litter by the $\text{CAP}_{\text{TRANSECT}}$ model classifier ($\text{CAP}_{\text{TRANSECT}}$: $m = 11$, G_{prop} = 86.9%, $Trc_{\text{stat}} = 2.7$, $P = 0.04$; Fig. 5.3b) and this was significantly better than the 40% accuracy rate expected by chance (PCC: $P = 0.04$).

The multi-elemental signatures deposited at the vertebral edge were consistent between each pregnant female and her embryos (PERMANOVA: *P* = 1; Hypothesis 3; Table 5.3); 88.9% of the samples were correctly assigned within each corresponding group (combining embryos and pregnant females) by the CAP_{EDGE} model classifier (CAP_{EDGE}: $m = 12$, $G_{\text{prop}} = 100\%$, $Trc_{\text{stat}} = 2.85$, $P = 0.001$; Fig. 5.4), which was significantly better than the 26.4% accuracy rate expected by chance (PCC: *P* = 0.001). For details on the elements driving most of the differences among elemental signatures deposited at the vertebral focus and edge of embryos and pregnant females, please refer to Figures 5.S1 and 5.S2 from the Supplementary Information.

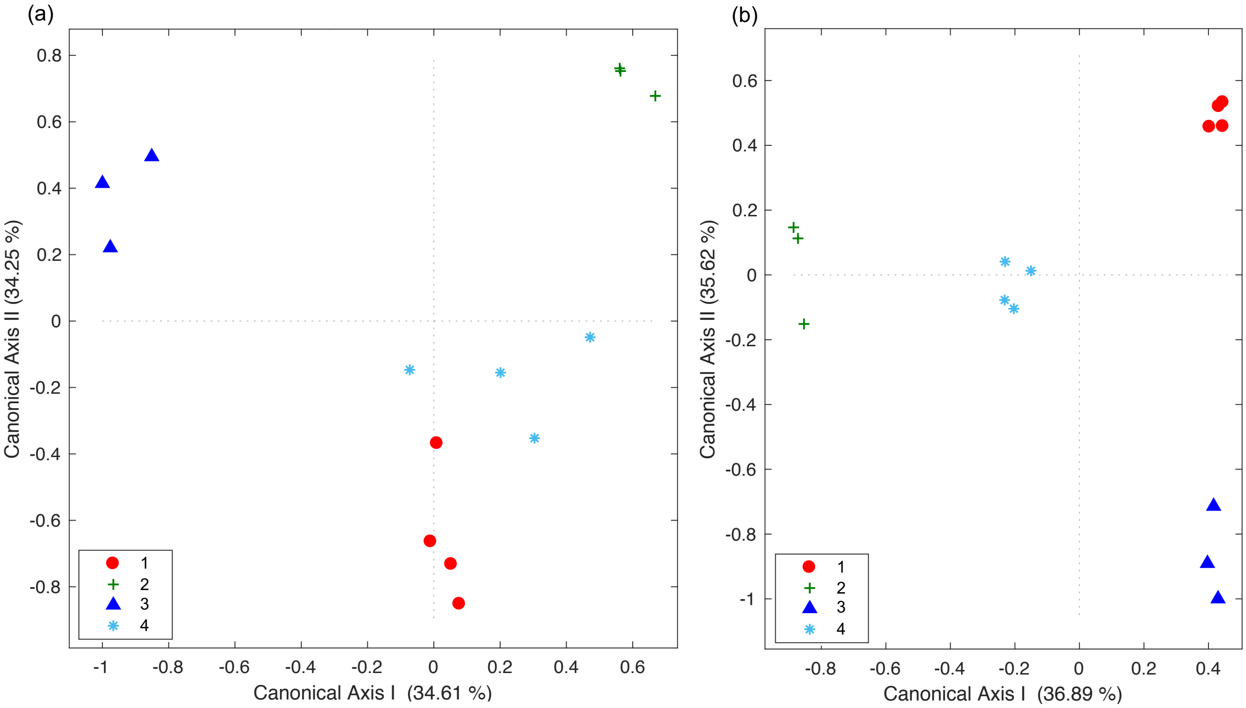

Figure 5.3. Canonical analyses of principal coordinates ordination diagrams illustrating the spatial variation of (a) multi-elemental signatures deposited at the vertebral focus ($\text{CAP}_{\text{FOCUS}}$) and (b) multi-elemental profiles ($\text{CAP}_{\text{TRANSECT}}$) quantified in the embryos of *Sphyrna lewini* (*n* = 14). Numbers refer to the female's litter codes (see Table 5.1)

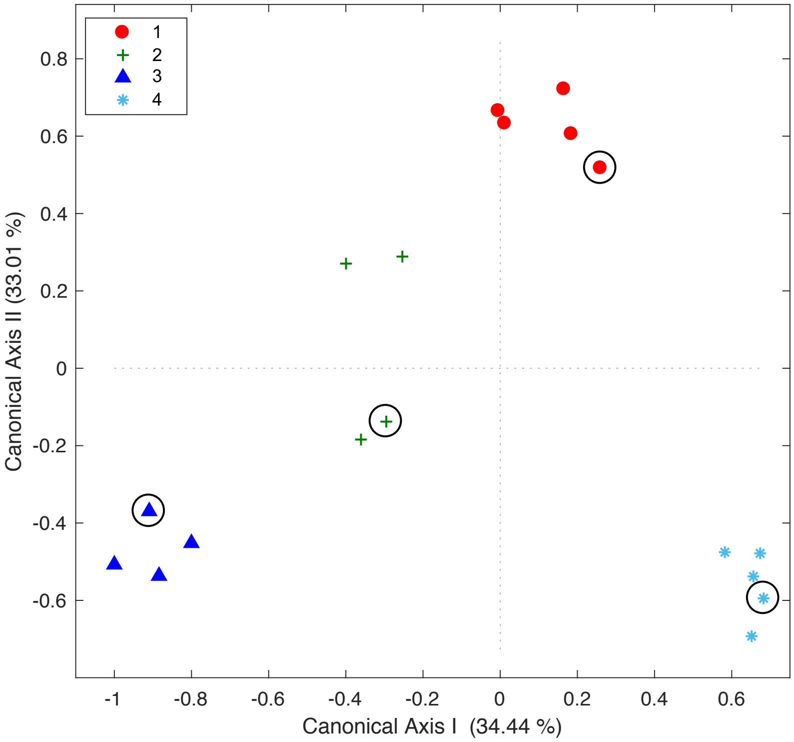

Figure 5.4. Canonical analysis of principal coordinates (CAP_{EDGE}) ordination diagram illustrating the spatial variation of elemental signatures deposited at the vertebral edge of the pregnant females of *Sphyrna lewini* (*n* = 4) and their embryos (*n* = 14). Numbers refer to the female's litter codes (see Table 5.1). Symbols with black circles correspond to the litter's female

Inferring the gestation-related movements of pregnant females

The vertebrae from 15 young-of-the-year specimens of *S. lewini* ranging from 49.2 to 59.6 cm of L_T (mean ± SD = 55.7 ± 4.1 cm L_T; Table 5.2) were available for this study. Elemental profiles quantified in their vertebrae were compared with those previously quantified in the vertebrae of the 14 near-term embryos (Table 5.1).

All individuals (*i.e.* embryos and young-of-the-year) exhibited the same pattern of variation in their *in utero* Sr:Ca and Ba:Ca profiles. The Sr:Ca ratio progressively increased throughout gestation and eventually slightly declined prior to parturition whereas the Ba:Ca ratio drastically declined at the beginning of the gestation (*i.e.* focus), remained particularly low and constant during its intermediate stage and then increased prior to birth (*i.e.* vertebral edge or birthmark; see example in Fig. 5.5). Although Sr:Ca and Ba:Ca were effectively inversely related relative to each other, Sr:Ba alone provided a better tool for examining the individual environmental histories rather than the joint comparison of the Sr:Ca and Ba:Ca ratios because it allowed to combine and highlight the differences observed for both (Fig. 5.5). Regarding variations of Pb along the *in utero* profiles, all samples exhibited a sharp increase of the Pb:Ca ratio prior to birth when compared to earlier in the gestation (Fig. 5.5). Accordingly, Sr:Ba was used as primary environmental indicator combined with Pb:Ca in order to support the observations based on Sr and Ba.

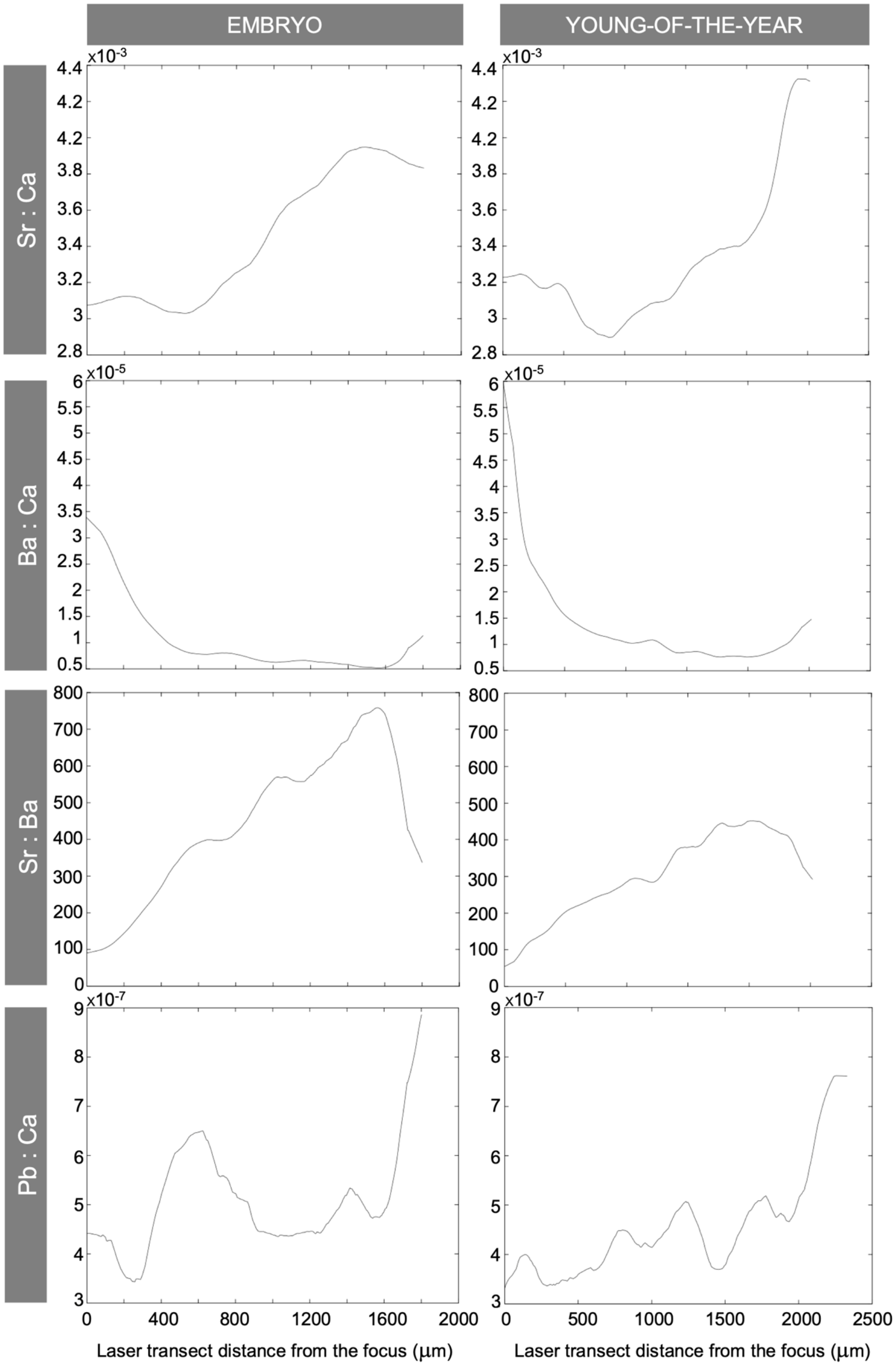

Figure 5.5. Individual *in utero* profiles of the Sr:Ca, Ba:Ca, Sr:Ba and Pb:Ca ratios quantified in the vertebrae of an embryo (left) and young-of-the-year (right) specimen of *Sphyrna lewini*

Embryos and young-of-the-year of *S. lewini* displayed large variations in Sr:Ba while *in utero*, with overall values that could vary from < 100 to 1200 within individuals, suggesting that pregnant females remained in environments of contrasting salinities throughout gestation (Fig. 5.5). The Sr:Ba ratio also differed widely among individuals, with the maximum individual values ranging between 300 and 1200 and the samples from Salina Cruz displaying the highest (1050 - 1200). Overall, all embryos and young-of-the-year exhibited relatively low Sr:Ba values at the vertebral focus (0 - 200) while the Sr:Ba values quantified at the vertebral birthmark of the young-of-the-year were more variable (100 - 600) among individuals (Fig. 5.5 and 5.6).

A single pattern was found in the *in utero* Sr:Ba profiles of embryos (subsequently referred to as 'Pattern 1'); Sr:Ba gradually increased until the highest individual value was reached (between 600 and 1300) and then rapidly declined to values between 300 and <100 shortly prior to the female capture, which corresponded to a sharp increase of the Pb:Ca ratio at the same time (Fig. 5.5). Back-calculated length of embryos at the time of the decline in Sr:Ba ranged between 37.6 cm and 45.9 cm of L_T (41.1 ± 2.1 cm L_T), which corresponded to a difference of size of approximately 4.2 ± 1.9 cm of L_T with that of the embryos at the pregnant female capture.

The same pattern of variation was observed in the *in utero* Sr:Ba profiles of most young-of-the-year (*n* = 11; 73.3%) (*i.e.* 'Pattern 1'; Fig. 5.6); Sr:Ba gradually increased until the highest individual value was reached (600-1200), and then rapidly declined to values between 350 and <100 shortly prior to birth, which also corresponded to an increase of the Pb:Ca ratio at the same time (Fig. 5.6). The *in utero* back-calculated length at the time of the Sr:Ba decline ranged between 36.3 and 52.3 cm of L_T (43.9 ± 5.4 cm L_T) for these samples, which was not significantly different from that estimated for the embryos (*i.e.* 41.1 ± 2.1 cm; ANOVA: $F = 2.57$, $P > 0.05$). The remaining young-of-the-year specimens (*n* = 4; 26.6%) exhibited low but relatively constant values of the Sr:Ba ratio (0-200) while *in utero* (subsequently referred to as 'Pattern 2'; Fig. 5.6), suggesting that these specimens' mothers possibly remained nearshore throughout gestation. These specimens were also found to exhibit significantly higher values of the Pb:Ca ratio at the beginning of the gestation when compared to later in the embryonic development (*i.e.* focus; $1.25 \times 10^{-6} \pm 1.03 \times 10^{-6}$ versus $0.46 \times 10^{-6} \pm 0.77 \times 10^{-6}$ in specimens exhibiting 'Pattern 1'; Mann–Whitney test: $P < 0.001$), after which the ratio remained relatively constant (Fig. 5.6).

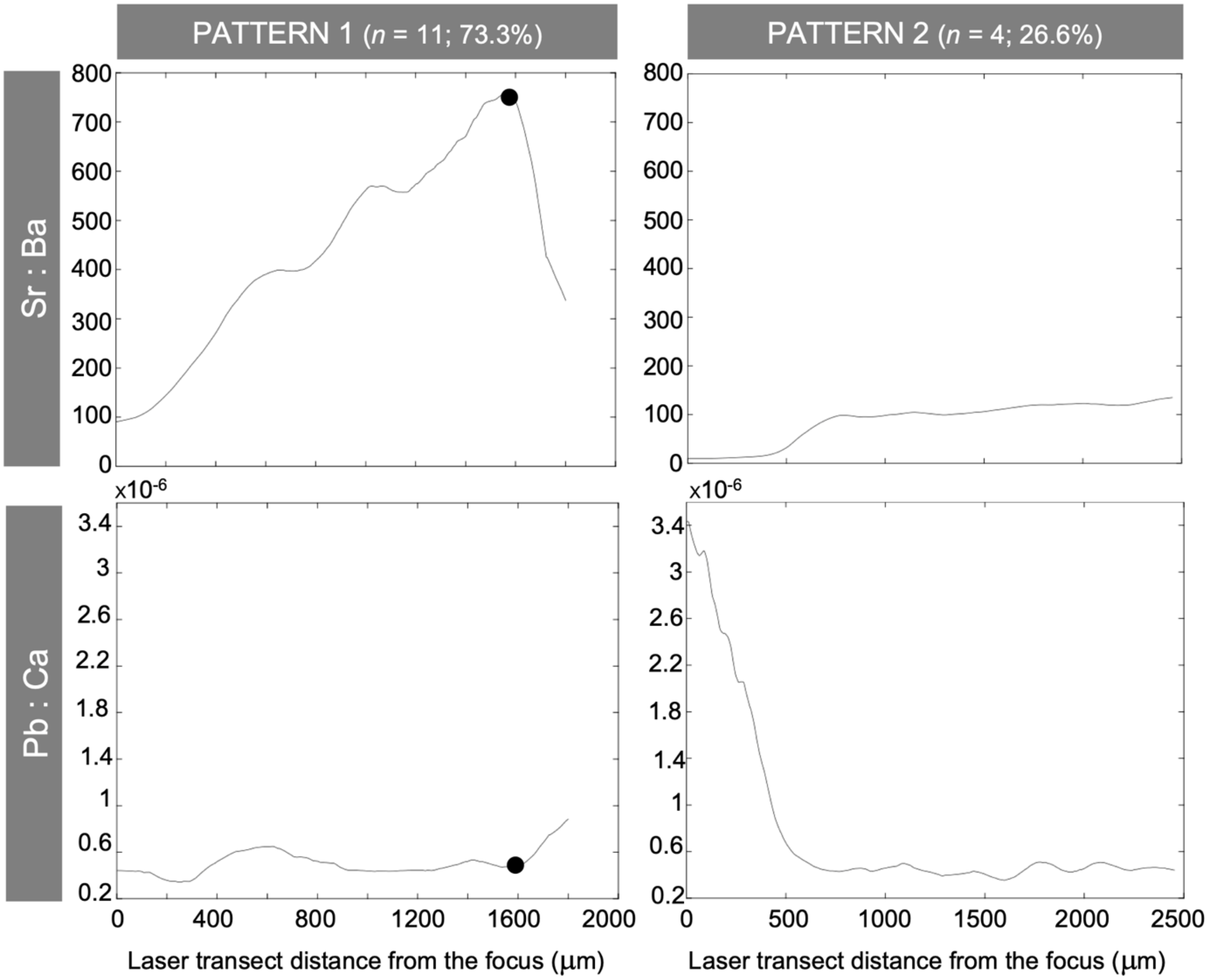

Figure 5.6. Examples of the *in utero* Sr:Ba and Pb:Ca ratio profiles quantified in the vertebrae from two young-of-the-year specimens of *Sphyrna lewini,* illustrating the two patterns identified (*i.e.* 'Pattern 1' and 'Pattern 2'). Dots indicate possible habitat shift of pregnant females towards nearshore habitats

DISCUSSION

Elemental signatures in vertebrae: embryos versus pregnant females

Vertebral microchemistry of pregnant females of *S. lewini* and some of their near-term embryos was analyzed in this study to address important questions concerning the gestation-related migratory patterns of the species in the Mexican Pacific. Previous studies recently demonstrated that elemental signatures in the vertebrae of *S. lewini* can serve as broad-scale effective spatial markers in the Mexican Pacific whether individuals have occupied coastal or oceanic environments and irrespective of the ontogenetic stage (Smith et al. 2016; Coiraton et al. 2020) , and the results of this study indicated that elemental signatures of the species can also serve as maternal tags, when these are quantified in the area of the vertebrae that was deposited during the *in utero* embryonic development. Although only few samples could be collected, vertebral microchemistry of *S. lewini* allowed to successfully distinguish among embryos from the distinct litters (60-88.9%), whether based on discrete elemental signatures deposited at the vertebral focus and edge (Hypothesis 1; Table 5.3) or elemental profiles that encompassed complete *in utero* development (Hypothesis 2; Table 5.3), demonstrating the temporal consistency in the elemental deposition among embryos of the species within a same litter.

Vertebral microchemistry of embryos was also found to reflect that of pregnant females during the gestation (Hypothesis 3; Table 5.3), at least at the edge (*i.e*. region of the vertebrae deposited immediately prior to capture), as this region of the vertebrae provided the only known spatial and temporal reference from which the comparisons could be made. Considering to compare elemental signatures deposited at another time such as the beginning of the gestation (*i.e.* focus) was not possible because there is no temporal reference in the vertebrae of pregnant females allowing to calibrate the period of the gestation in terms of months (*i.e.* 10-11 months (Torres-Huerta et al. 2008; Bejarano-Álvarez et al. 2011). Nevertheless, the fact that elemental signatures were temporally consistent among embryos within each litter and that vertebral microchemistry allowed to successfully classified (88.9%) pregnant females with their respective embryos based on their unique elemental signatures of vertebral edge indicated that *in utero* vertebral microchemistry of *S. lewini* can serve as maternal tag from a few weeks after fertilization, when the formation of a placenta allows the embryos to be directly nourished from the mother's blood stream, to the end of the gestation.

It was recently stated that pregnant female's transfer of substantial amounts of organic contaminants (*i.e.* polychlorinated biphenyls and chlorinated pesticides) to their embryos through the process of fetal nutrition (Lyons and Adams 2015) might potentially alter the maternal site-specific markers deposited in the vertebrae of embryos while *in utero* (Smith et al. 2016). Although it is unknown whether maternal contaminant offloading can effectively alter the vertebral microchemistry of embryos of *S. lewini*, the lipophilic nature of organochlorines does not support this assumption as such contaminants primarily accumulate within the hepatic tissue (Borga et al. 2004; Torres et al. 2016). Furthermore, the fact that in this study vertebral microchemistry of pregnant females was not significantly different from that of their embryos indicated that elemental signatures in vertebrae of embryos are likely not altered through the mechanisms of contaminant transfer, thus confirming that *in utero* elemental signatures of *S. lewini* can serve as intrinsic markers of the environmental histories of pregnant females during the gestation.

Inferring the gestation-related movements of pregnant females

Conventional tagging studies indicated that adult females of *S. lewini* typically aggregate offshore and later return to coastal habitats for parturition (Klimley 1983, 1985; Klimley and Nelson 1984). Although the patterns of migration and habitat use of pregnant individuals have remained unknown despite extensive study, yet it could be assumed that the female reliance on coastal habitats for parturition combined with an annual reproductive cycle (Torres-Huerta et al. 2008; Bejarano-Álvarez et al. 2011) had restricted their dispersal to highly oceanic habitats during the gestation. The novel approach developed in this study allowed to elucidate some of these aspects through the analysis of the female offspring's *in utero* vertebral microchemistry, using Sr:Ba and Pb:Ca as indicators of environmental histories (Hypothesis 4; Table 5.3). Even though the lack of water chemistry data and telemetric data against which to compare the results of this study limits the power of such inferences, analyses of the *in utero* Sr:Ba and Pb:Ca profiles allowed to detect two apparently distinct migratory patterns of the pregnant females of *S. lewini* in terms of the movements between nearshore and more oceanic environments.

More specifically, the results indicated that most pregnant females (73.3%) apparently progressively migrated offshore from the initial stages of the gestation (*i.e.* a few weeks after fertilization) before quickly heading back to the coastal nurseries prior to parturition ('Pattern 1'), when embryos had reached a size between approximately 41.1 and 43.9 cm of L_T. Similarities in such habitat shift among pregnant females at this time of the gestation were shown by marked decline of the Sr:Ba ratio to values between 300 and <100 prior to birth in the *in utero* Sr:Ba profiles of young-of-the-year and embryos, in accordance with previously observed changes in the vertebral microchemistry of adult specimens of *S. lewini* when entering nearshore habitats (150-400) (Coiraton et al. 2020), assuming that variations in Sr and Ba effectively reflect the gradient of salinity changes of the environment (Allen et al. 2009; Scharer et al. 2012).

These observations were further informed by the analyses of the *in utero* Pb:Ca profiles that showed a sharp increase of the ratio prior to birth in both embryos and young-of-the-year, possibly indicative of pregnant females entering nearshore contaminated habitats for parturition, even though the assumption that incorporation of Pb in the vertebrae effectively reflects the exposure of a shark to contamination derived from anthropogenic sources has not been explicitly tested. Nevertheless, the fact that (1) embryos of pregnant females of *S. lewini* captured nearshore were found in this study to exhibit particularly higher Pb:Ca values at the vertebral edge when compared to earlier in the gestation, and that (2) ^{208}Pb in the vertebrae of *S. lewini* was found in previous study to be characteristic of the specimens captured in nearshore areas (Coiraton et al. 2020) strongly influenced by anthropogenic sources of trace metal and pollutant inputs (Soto-Jiménez and Páez-Osuna 2001; Jara-Marini et al. 2008; Raygoza-Viera et al. 2014) (when compared to individuals captured offshore; Coiraton et al. 2020) indicated that Pb could be used as a complementary indicator of environmental history of *S. lewini* to support the conclusions based on Sr and Ba.

Taking this into account, the results of this study also suggested that some pregnant females (23.3%) apparently remained nearshore during complete gestation, as shown by low but constant values of the Sr:Ba ratio (< 200) along their offspring's *in utero* profiles, associated with relatively high but constant values of the Pb:Ca ratio ('Pattern 2'). This possible behavior has not been documented before for *S. lewini* and the reasons why some females presumably remained nearshore during the gestation are unknown, especially considering that adult stages of *S. lewini* are thought to migrate offshore as a strategy to increase their foraging success by feeding on energy-rich pelagic preys (Klimley 1987). Nevertheless, the fact that only a small proportion (26.6%) of the individuals were found to exhibit this pattern precluded robust conclusion regarding this point, especially since sample size available for this study was in fact relatively low.

On the other hand, it might be stated that the among-individual differences observed in the Sr:Ba patterns and shifts might be driven by other factors than salinity such as variations in temperature, dietary preferences or individual-specific physiology (as shown for some teleosts (Walther et al. 2010; Sturrock et al. 2014)), and hence be not fully reflecting the movements of females across the nearshore-offshore gradient of salinity change. However, experimental evidences showed that: (1) Sr and Ba are not physiologically regulated and their incorporation in the vertebrae being primarily derived from branchial uptake, representative of the environmental concentrations, but not affected by somatic growth nor vertebral precipitation rate (Smith et al. 2013; Pistevos et al. 2019) and (2), that temperature positively affected the incorporation of both Sr and Ba in the vertebrae of sharks (Pistevos et al. 2019), and hence would not affect the overall Sr:Ba ratio variations if females were to move into warmer (or cooler) waters during the gestation, regardless of the nearshore-offshore gradient of salinity. Caution should be however taken regarding the latter because conflicting evidences on the effect of temperature were found with round stingray *Urobatis halleri,* as temperature negatively affected the incorporation of Ba, though not that of Sr, in its vertebrae (Smith et al. 2013). Nevertheless, analyses of the Pb:Ca ratio were used in this study as a complementary indicator of environmental history to further evidence the movements of pregnant females into the nearshore habitats, and the results were consistent with the observations based on Sr:Ba.

It was recently suggested that in the Mexican Pacific females of *S. lewini* may probably give birth in variable environments and/or that the use of coastal nurseries may be less defined for this species than previously assumed (Coiraton et al. 2020), and the fact that in this study Sr:Ba values quantified at the young-of-the-year vertebral birthmark was found to differ widely among individuals (<100 - 600) also supports this assumption. Behavioral plasticity towards nursery habitat selection for parturition might effectively be a strategy of the females to enhance their offspring survival by differing its vulnerability to predators and a potential lack of foraging success among regions (Coiraton et al. 2020).

It is also important to note that even if the results of this study suggested that females remained nearshore at the beginning of the gestation because the Sr:Ba values quantified at the vertebral focus were found to be particularly low (50 - 200) in all individuals (while Pb:Ca was comparatively higher), conclusions regarding this point remain tentative because elemental data for this area of the vertebrae might primarily reflect vitellogenesis (*i.e.* yolk deposition) rather than the actual initial stage of the gestation, as placentation generally occurs within a few weeks after fertilization (Castro 2011a). Yolk-based nourishing of embryos during the first weeks of gestation may possibly lead to a lag in the time required for the embryo vertebral microchemistry to reflect variations in the chemistry of the surrounding water to which the pregnant female was exposed during these weeks. Accordingly, the *in utero* elemental profiles of *S. lewini* may be informative on the migration patterns of pregnant females only once embryos were directly fed through the mother's blood stream, a few weeks after fertilization (Castro 2011a).

Nevertheless, yet it appeared that pregnant females of *S. lewini* likely exhibited two distinct migratory patterns in the Mexican Pacific, as they either (1) progressively migrated offshore before quickly returning to coastal nurseries before term, or (2) possibly remained nearshore during complete embryonic development, supporting the assumption that overall females might exhibit relatively short dispersal to the oceanic habitats during the gestation. Alternating between two migratory patterns during the gestation may be a strategy of females to reduce their vulnerability to stressors or unfavorable environmental conditions among regions (Kerr et al. 2010; Chapman et al. 2011; Parsons et al. 2011) and optimize chances to complete a full-term gestation.

Considering the life history and global endangered status of *S. lewini* (Baum et al. 2007), current management measures in the Mexican Pacific such as the regulation norm of the commercial shark fishery (NOM-029-PESC-2006; DOF 2007) and its seasonal prohibition between May and July (NOM-009-SAG/PESC-2015; DOF 2016) may be insufficient for the sustainable management of the population. Pregnant females may be particularly susceptible to fisheries when remaining nearshore or entering coastal nurseries slightly before term, therefore limiting their possibilities to complete gestation to the term (Coiraton et al. 2017) and reducing the reproductive potential of the population as it is briefly centralized in coastal areas (Smith et al. 2016). Additional information on the movements of reproductive females provided by fine-scale telemetric studies is required to determine how such strategies of migration could potentially impact the reproductive potential of the population.

DATA AVAILABILITY

The Matlab codes used to undertake the analyses performed in this study and prepare the figures and tables presented is freely available on GitHub (https://github.com/clairecoiraton/HammerheadInUteroMicrochemistry_MatlabCode.git).

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SUPPLEMENTARY INFORMATION

Table S5.1. Mean (± standard deviation) element:Ca ratios (µmole·mole^{-1}) quantified at the vertebral focus and edge of embryos and pregnant females of *Sphyrna lewini* captured in April 2016 off Puerto Madero in the Mexican Pacific.

ELEMENT	FOCUS		EDGE (EMBRYOS)		EDGE (MOTHERS)	
	MEAN	± SD	MEAN	± SD	MEAN	± SD
Li^{7}	1.05	0.18	1.35	0.34	1.32	0.17
Na^{23}	6241.42	1033.46	7963.09	2591.18	7327.39	2032.91
Mg^{24}	4746.61	480.22	6202.11	1065.93	6877.59	328.29
Mn^{55}	30.56	7.38	19.35	6.94	20.87	3.67
Fe^{57}	185.14	10.56	164.74	30.18	180.28	5.04
Co^{59}	0.25	0.07	0.20	0.15	0.23	0.04
Cu^{63}	48.62	61.52	44.63	72.85	18.91	16.27
Rb^{85}	1.26	0.74	1.04	1.25	1.74	1.39
Sr^{88}	971.25	71.42	1463.65	318.60	1285.39	80.50
Sn^{118}	0.94	1.60	0.47	0.65	0.28	0.07
Ba^{137}	14.44	5.25	1.97	1.53	1.60	0.97
Pb^{208}	0.34	0.30	0.24	0.05	0.28	0.06

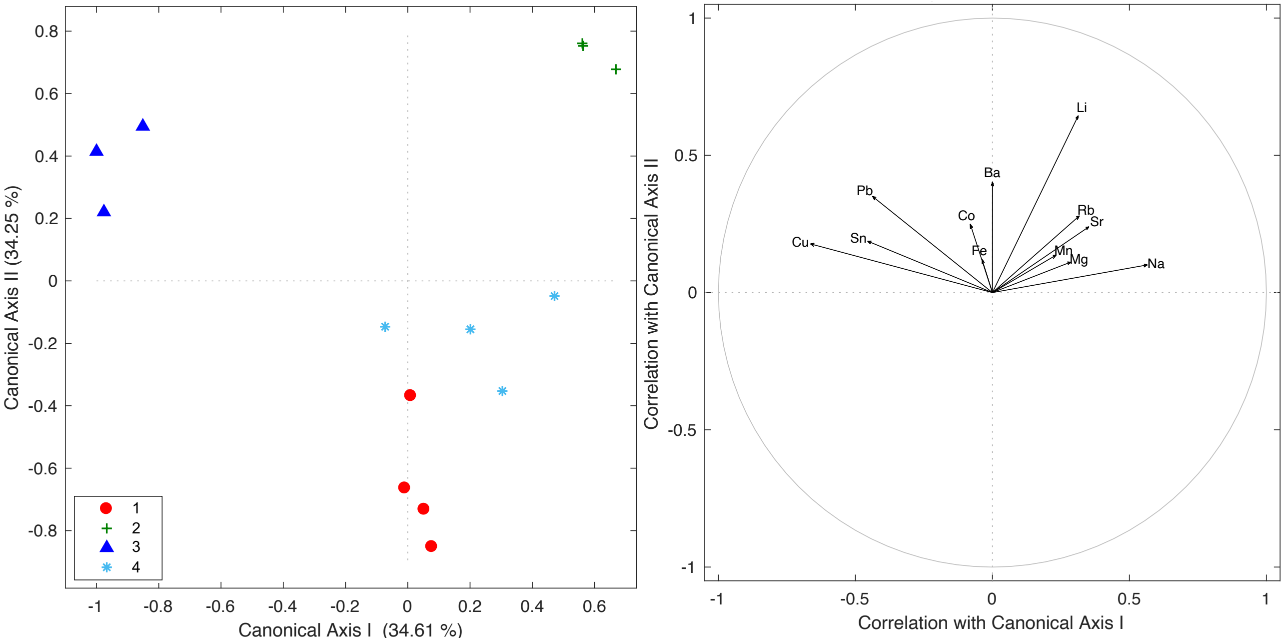

Figure S5.1. Canonical analysis of principal coordinates ($\text{CAP}_{\text{FOCUS}}$) ordination diagram and vector plot illustrating the spatial variation of multi-elemental signatures deposited at the vertebral focus quantified in the embryos of *Sphyrna lewini* (*n* = 14). Numbers refer to the female's litter code (see Table 5.1).

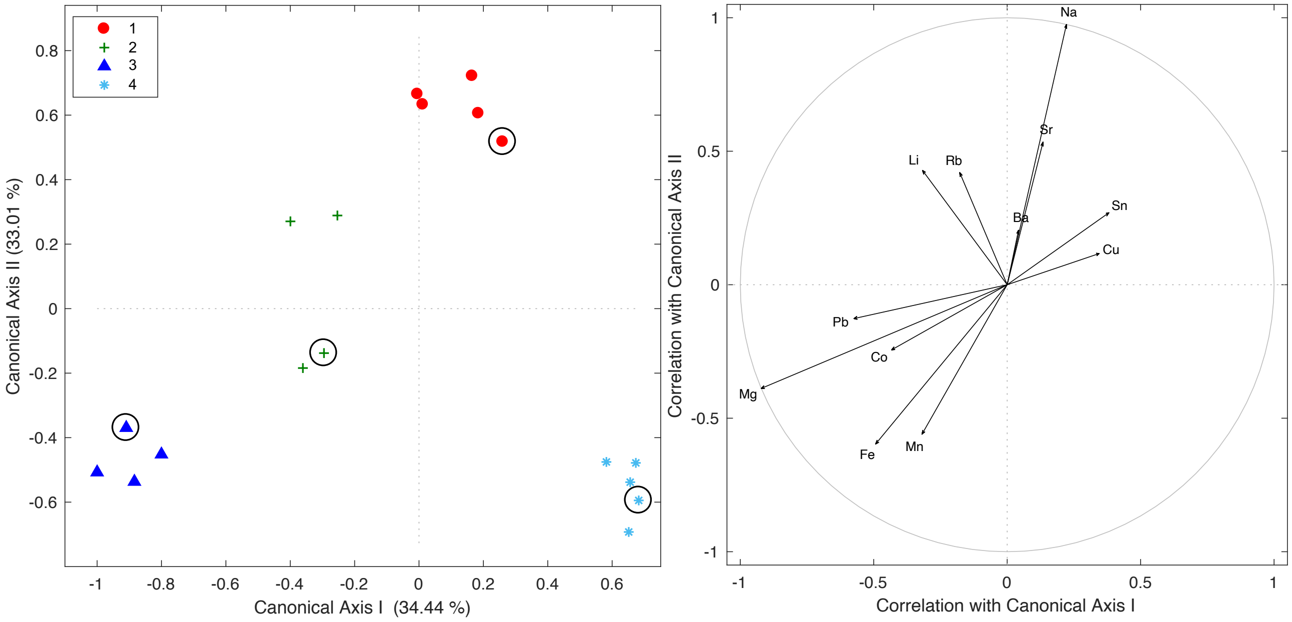

Figure S5.2. Canonical analysis of principal coordinates (CAP$_{\text{EDGE}}$) ordination diagram and vector plot illustrating the spatial variation of elemental signatures deposited at the vertebral edge of the pregnant females of *Sphyrna lewini* (*n* = 4) and their embryos (*n* = 14). Numbers refer to the female's litter codes (see Table 5.1). Symbols with black circles correspond to the litter's female.

CAPÍTULO 6

CONCLUSIÓN

Contar con un conocimiento detallado y actualizado de la distribución, estructura de tallas, parámetros de crecimiento, patrones de movimientos y uso de hábitat del tiburón martillo *Sphyrna lewini,* es esencial a fin de definir zonas de protección o refugio para esta especie y mejorar las medidas de conservación actualmente implementadas (FAO 1998), especialmente considerando su importancia comercial en México (Ruiz y Madrid 1997; Anislado-Tolentino y Robinson-Mendoza 2001; CONAPESCA-INP 2004). Lo anterior es particularmente importante sobretodo si se considera la elevada disminución en la abundancia de esta especie en varias regiones de su área de distribución durante las ultimas décadas debido al incremento de la presión pesquera (Myers et al. 2007; Ferretti et al. 2008; Baum y Blanchard 2010), aunado a que esta especie tiene particularidades en las características de su historia de vida (*e.g.* Piercy et al. 2007; Torres-Huerta et al. 2008), junto con adaptaciones morfológicas (Gallagher et al. 2014a) y fisiológicas (*e.g.* Morgan and Burgess 2007; Gulak et al. 2015) además de comportamiento (*e.g.* Klimley y Nelson 1981; Klimley 1985), que limitan drásticamente las posibilidades de recuperarse de una captura excesiva (Gallagher et al. 2014b).

En el presente trabajo se lograron documentar aspectos novedosos y relevantes de la historia de vida de *S. lewini* en el Pacifico mexicano, gracias al análisis de capturas provenientes tanto de la pesca de pequeña escala, como industrial, lo cual permitió tener acceso a organismos de diferentes tallas, y hábitats. Específicamente, en el presente estudio se llevaron a cabo análisis de tallas capturadas por las diferentes pesquerías, estimaciones de edad con base al conteo de bandas de crecimiento en vértebras y validación subsecuente de su ciclo de crecimiento, así como la caracterización de sus patrones migratorios y cambios ontogenéticos y sexuales de uso de hábitat en sus zonas de distribución por medio de análisis microquímicos de sus vértebras. Es necesario considerar que estos resultados se obtuvieron a pesar de que esta es una especie relativamente difícil de estudiar, debido a su capacidad migratoria en largas distancias (*e.g.* Bessudo et al. 2011; Hoyos-Padilla et al. 2014; Ketchum et al. 2014; Spaet et al. 2017; Wells et al. 2018) y que a la vez pueda encontrarse tanto en estuarios y bahías costeras (*e.g.* Clarke 1971; Duncan y Holland 2006; Brown et al. 2016; Marie et al. 2017) como en aguas pelágicas de plataformas continentales e islas oceánicas (*e.g.* Klimley 1983, 1985; Klimley y Nelson 1984; Ketchum et al. 2014b).

Los resultados aquí presentados mostraron que las medidas de manejo actuales podrían ser insuficientes para el manejo sostenible de la población de *S. lewini* en el Pacífico mexicano, debido a que en esta región: (1) se capturan en mayoría organismos inmaduros, especialmente al finalizar los meses de veda y el periodo de nacimiento entre agosto y noviembre, lo que impide a una gran cantidad de organismos alcanzar su talla de primera madurez y reclutarse al stock adulto (Capítulo 2); (2) el ciclo de crecimiento de la población es más lento (*i.e.* anual) de lo que se había reportado anteriormente (*i.e.* bianual), al menos para los organismos juveniles (Capítulo 3); (3) los organismos adultos, y en particular los machos, ocupan hábitats costeros durante más tiempo de lo inicialmente contemplado, siendo por lo tanto más susceptibles a ser capturados por las pesquerías de pequeña escala operando en la zona (Capítulo 4); y (4) las hembras preñadas son altamente susceptibles a ser capturadas por la pesca de pequeña escala ya que algunas permanecen cerca de la costa durante toda su gestación, o entran en las áreas de crianza antes de que los embriones estén completamente formados. Esto no solo podría afectar al stock de organismos neonatos, sino que además podría tener efectos en el potencial reproductivo de la población al centralizarse brevemente en áreas costeras, más accesibles por las pesquerías (Capítulo 5).

Por otra parte, algunos resultados de este trabajo indicaron también que el uso de áreas de crianza costeras por *S. lewini* para el nacimiento de sus crías podría ser menos definido para la especie, principalmente debido a que las firmas microquímicas de origen natal, cuantificadas tanto en vértebras de adultos pelágicos como de neonatos costeros y embriones, reflejaron hábitats variables para el parto, y posiblemente también más alejados de la costa de lo que se suponía antes. Estudios previos del uso de hábitat de *S. lewini* reportaron que en algunos casos las áreas de crianza en realidad sirven más como zona de protección de las crías contra los depredadores que para brindar una alta disponibilidad de alimento, pudiendo generar tasas de mortalidad importante por inanición, especialmente durante el primer año de vida (Duncan y Holland 2006). Por estas razones, es posible que la probable plasticidad de comportamiento observada en el presente estudio por parte de las hembras hacia la selección de áreas de crianza para el parto sea una estrategia de la especie para mejorar la supervivencia de sus crías al diferenciar su vulnerabilidad a los depredadores y una posible falta de alimento entre las diferentes regiones del Pacífico mexicano, aunque más información se requiere para poder elucidar este aspecto importante de la ecología espacial de *S. lewini*.

Un objetivo importante de esta tesis desarrollado en el marco del estudio de los cambios ontogenéticos de uso de hábitat de *S. lewini* (Capítulo 4), era la construcción de un atlas de sus áreas de crianza en el Pacífico mexicano generado para cada cohorte (*i.e.* misma clase de edad y año de captura) a partir de las firmas microquímicas natales de los organismos colectados dentro de cada posible área de crianza identificada para la especie en la región, a fin de poder evaluar el grado de intercambio de organismos a lo largo de la costa y conectividad de hábitat. Esta valiosa información podría ayudar en determinar como se distribuyen espacialmente los hábitats de crianza de *S. lewini* en la región (*i.e*. áreas discretas especificas o franja costera continua), sin embargo, el insuficiente número de muestras obtenido de cada cohorte por sitio (*n* < 5) no permitió llevar a cabo este objetivo. Generar un atlas especifico a cada cohorte es primordial para evitar sesgos derivados de posibles variaciones inter-anuales de la química del agua de mar dentro de cada sitio de captura (Smith et al. 2016) las cuales podrían resultar en distintas firmas microquímicas natales para organismos, que si bien nacieron en las mismas áreas de crianza, pertenecían en diferentes cohortes (Begg et al. 1998; Campana et al. 2000). Debido a que las normas de manejo actualmente implementadas en México toman en cuenta una distribución espacial discontinua de las áreas de crianza de *S. lewini* (NOM-029-PESC-2006; DOF 2007) pero que por otro lado estudios genéticos indicaron que estas áreas pudieran estar distribuidas de manera continua a lo largo de la franja costera (Duncan et al. 2006), se recomienda continuar con muestreos de *S. lewini* para contar con una colección de muestras más amplia y lograr en construir este altas de áreas de crianza. Esto permitiría evaluar la mezcla poblacional y conectividad de hábitats de *S. lewini* a lo largo de la costa lo cual, en combinación con datos de telemetría de escala-fina, ayudaría a definir zonas de refugio más amplias para proteger el proceso de reproducción y nacimiento de la especie en el marco de la norma reglamentaria para la pesca responsable de tiburones (NOM-029-PESC-2006; DOF 2007) ya que en la actualidad estas se consideran insuficientes para la conservación de la especie en el Pacífico mexicano.

Finalmente, si bien este trabajo de tesis contribuyó en aumentar sustancialmente la información disponible acerca de la historia de vida de *S. lewini* en el Pacífico mexicano, aún hace falta determinar y validar el ciclo de crecimiento de bandas de crecimiento en vértebras de organismos adultos (*i.e.* anual o bianual) debido a que este parámetro puede cambiar cuando los organismos maduran (*e.g.* Wells et al. 2013; Kinney et al. 2016) y desafortunadamente el insuficiente número de muestras por meses de captura no permitió llevar a cabo este objetivo en el marco de esta tesis. Ahora si bien es cierto que la periodicidad de la formación de bandas de crecimiento debe validarse para todos los tamaños y edades que representan la población de *S. lewini* en el Pacífico mexicano, la determinación de un ciclo anual de formación de bandas de crecimiento en las vértebras de los juveniles requiere de todas formas una reestimación de los parámetros de crecimiento (*i.e.* tasas de crecimiento, talla y edad máxima) e índices de productividad de la población, ya que a la fecha estos se han estimado con base a la estimación de un ciclo de crecimiento bianual (*i.e.* dos pares de bandas de crecimiento al año) para *S. lewini* en la región, sin que este parámetro se haya en realidad validado como tal (Anislado-Tolentino y Robinson-Mendoza 2001; Anislado-Tolentino et al. 2008).

En conclusión, las medidas de manejo implementadas actualmente en el Pacífico mexicano para la pesca comercial responsable de tiburones y rayas (NOM-029-PESC-2006; DOF 2007) y su prohibición estacional entre mayo y julio (NOM-009-SAG/PESC-2015; DOF 2016), se consideran insuficientes para la conservación de la población de *S. lewini,* la cual se encuentra con una tendencia a la baja en sus poblaciones a nivel mundial (Baum et al. 2007). Por lo anterior, es recomendable dar soluciones de manejo más viables, como podría ser el establecimiento de más zonas de refugio costeras para esta especie, y las otras especies de tiburones, que complementen las limitaciones temporales ya existentes (DOF 2007), ya que como se ha señalado en este trabajo, los organismos que ahí se encuentran son en su gran mayoría inmaduros que no han tendido eventos reproductivos, machos adultos o hembras preñadas que se acercan a la costa para expulsar sus crías. El establecimiento de un tamaño mínimo de captura no parece ser una solución adecuada para esta especie ya que por la anatomía de su cabeza y la diversidad de artes de pesca que se emplean en esta región, los tiburones de esta especie son susceptibles a ser capturados por redes de enmalle enfocadas a otras pesquerías que operan a lo largo del año, como la pesquería de sierra (*Scomberomorus sierra*). Las prácticas actuales de pesca de *S. lewini* en el Pacífico mexicano dejan un escaso margen para que la población se pueda mantener y regenerar teniendo en cuenta sus características de historia de vida en esta región, y es posible que aun si no se aprecia una reducción inmediata en sus capturas y abundancia, es posible que cuando esta se dé, sea un proceso irreversible, como se reportó para otras tres especies de este género, a saber *S. media, S. mokarran* y *S. tiburo*, que disminuyeron considerablemente durante las últimas décadas hasta incluso desaparecer del Golfo de California (Bizzarro et al. 2009; Smith et al. 2009; Pérez-Jiménez 2014).

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