

FERNANDA DE OLIVEIRA LANA

**MORFOLOGIA, IDENTIFICAÇÃO MOLECULAR E MOVIMENTAÇÃO DO TUBARÃO
LOMBO-PRETO, *Carcharhinus falciformis* (Bribon, 1939), NO ATLÂNTICO OESTE
TROPICAL**

RECIFE,

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UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

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TROPICAL

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Tese apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Doutora.

Prof. Dr. FÁBIO HISSA VIEIRA HAZIN
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Fernanda de Oliveira Lana

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Dedicatória

“Deus nos concede, a cada dia, uma página de vida nova no livro do tempo. Aquilo que colocarmos nela, corre por nossa conta.”

“Cada dia que amanhece assemelha-se a uma página em branco, na qual gravamos os nossos pensamentos, ações e atitudes. Na essência, cada dia é a preparação de nosso próprio amanhã.”

Chico Xavier

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Resumo

O tubarão lombo-preto, *Carcharhinus falciformis*, é uma espécie comum na zona pelágica, com distribuição em águas tropicais e equatoriais de todos os oceanos do mundo. O presente trabalho teve por objetivo estudar a ecologia do tubarão lombo-preto, incluindo estudar as características morfológicas, a identificação molecular e o deslocamento, via marcações eletrônicas, do tubarão lombo-preto no Oceano Atlântico Sudoeste e Equatorial, em particular com relação à sua distribuição no Arquipélago São Pedro e São Paulo (ASPSP), com o objetivo de esclarecer o seu padrão de movimentação diária e sazonal, além da estrutura local de sua população. As características morfológicas/ anatômicas que definem essa espécie foram também analisadas de forma comparativa com as outras espécies do gênero *Carcharhinus* que também ocorrem na área de estudo (ASPSP). Todas as marcações dos tubarões e coleta de material biológico foram realizadas no Oceano Atlântico Sudoeste e equatorial, nas imediações do ASPSP, na área localizada entre as latitudes 00°55'3,72"N e longitudes 029°20'11,22"W. Tubarões lombo-preto foram marcados com 3 (três) modelos distintos de transmissores via satélite, totalizando 10 (dez) indivíduos: 5 MiniPAT (*Pop-up Archival Tag*), 3 PSAT (*Pop-up Satellite Archival Transmitting Tag*), e 2 SPOT (*Smart Position or Temperature Transmitting Tag*), no período de 2010 a 2014, nas imediações do ASPSP. O condrocrânio de *Carcharhinus* (incluindo *Nasolamia*), apesar de ser conservador, mostrou diferenças que podem ser taxonomicamente significativas. Os componentes cranianos que apresentaram diferenças consistentes são: a forma do nó rostral e a margem posterior da fontanela anterior, dimensão rostral, presença ou ausência de fenestra rostral, entalhe epifisário, forame epifisário e aba nasal; direção das cápsulas nasais, formas do processo pré-orbital e fossa subetóide. De acordo com a técnica de Multiplex PCR e a comparação das características genéticas com outras espécies do gênero *Carcharhinus* que são registradas no ASPSP e apresentam características morfométricas próximas (por exemplo, *C. falciformis*, *C. galapagensis* e *C. obscurus*). Os resultados, baseados em 64 amostras, mostraram que quase todas as amostras eram tubarões lombo-preto. Os tubarões marcados mostraram preferência pelas águas quentes da camada mista em que permaneceram a maior parte do tempo, durante o dia. A faixa de temperatura preferencial se situou entre 26-28°C, com uma profundidade preferencial entre 1-10m. Apresentaram uma elevada residência para o ASPSP, assim os presentes resultados mostram claramente a importância da medida de ordenamento para a conservação de tubarões lombo-preto (ICCAT - Instrução Normativa Interministerial nº 8 – 06/11/2014), particularmente na proximidade de ilhas oceânicas, devido à forte associação desses tubarões com essas estruturas.

Palavras-chave: comportamento, deslocamento, anatomia, genética, Carcharhinidae.

Abstract

The silky shark, *Carcharhinus falciformis*, is a common species in the pelagic zone, with distribution in tropical and equatorial waters of all the oceans of the world. The objective of this study was to study the ecology of silky shark, including the study of the morphological characteristics, molecular identification and displacement, via electronic taggings of the silky shark in the Southwest and Equatorial Atlantic Ocean, particularly in relation to its distribution in the Saint Peter and Saint Paul Archipelago (SPSPA), in order to clarify their daily and seasonal movement patterns, as well as the local structure of their population. The morphological/anatomical characteristics that define this species was also analyzed in a comparative way with other species of the *Carcharhinus* genus that also occur in the study area (SPSPA). All tagged shark and collection of biological material were performed in the Southwest and Equatorial Atlantic Ocean, in the vicinity of the SPSPA, in the area between latitudes 00°55'3,72"N and longitudes 029°20'11,22"W. Silky sharks were tagged with three (3) different models of satellite transmitters, totaling ten (10) individuals: 5 MiniPAT (Pop-up archival tags), 3 PSAT (Pop-up Satellite Archival Transmitting Tag), and 2 SPOT (Smart Position or Temperature Transmitting Tag), in the period from 2010 to 2014, in the vicinity of SPSPA. The chondrocrania of *Carcharhinus* (including *Nasolamia*), despite being conservative, showed differences that can be taxonomically significant. The cranial components that presented specific consistent differences are: the shape of the rostral node and posterior margin of anterior fontanelle, rostral dimension, presence or absence of rostral fenestra, epiphysial notch, epiphysial foramen and nasal flap; direction of the nasal capsules, shapes of the preorbital process and subtemporal fossa. According to Multiplex PCR-technique and to compare its genetic characteristics with the other species of the *Carcharhinus* genus that are recorded in the (SPSPA) and have close morphometric characteristics (eg *C. falciformis*, *C. galapagensis* e *C. obscurus*). The results, based on 64 samples, showed that almost all samples were silky sharks. The tagged sharks showed a preference for warm waters of the mixed layer, where they remained most of the time, during the day. The preferred temperature range of the tagged animal was between 26-28°C, with a preferred depth between 1-10m. Exhibited an elevated residency to the SPSPA, so the present results clearly show the importance of the management measure for the conservation of silky sharks (ICCAT - Interministerial Normative Instruction nº 8 - 06/11/2014), particularly in the vicinity of oceanic islands, due to the strong association these sharks show to these structures.

Key words: Behavior, displacement, anatomy, genetics, *Carcharhinidae*.

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1- Introdução

Recentemente, o declínio populações de várias espécies de tubarões pelágicos tem gerado preocupações referentes à sua conservação (FOWLER et al., 2005; DULVY et al., 2008; AIRES-DA-SILVA E GALLUCCI, de 2008; CORTÉS et al., 2010; TOLOTTI et al., 2015). Essas espécies são capturadas tanto como alvo, como incidentalmente, como fauna acompanhante, por uma série de frotas, desde a artesanal costeira até embarcações industriais que operam em águas distantes (BONFIL, 1994; WORMET al., 2013). Uma questão inerente à pesca de elasmobrânquios, quando comparados com os seus homólogos teleósteos, é a sua baixa capacidade de recuperação resultante diretamente dos traços característicos de sua história de vida, como crescimento lento, maturação mais tardia e baixa fecundidade (CORTÉS, 2000). Em razão disso, esse grupo é geralmente muito mais vulnerável à sobrepesca do que os peixes teleósteos (MUSICK et al., 2002; COMPAGNO et al., 2005; TOLOTTI et al., 2015).

Estimativas globais indicam que cerca de 50% das capturas de elasmobrânquios ocorrem como fauna acompanhante (*bycatch*), grande parte das quais não são devidamente registradas nas estatísticas pesqueiras. Consequentemente, em muitos casos os dados de captura não são coletados, dificultando uma correta avaliação dos estoques e a decorrente implementação de medidas de manejo que possam assegurar a sustentabilidade da sua exploração.

Em 2008, último ano com estatística disponível, cerca de 750.000 toneladas de elasmobrânquios (FAO, 2009) foram desembarcadas em todo mundo. No Brasil, cerca de 20.000 t de tubarões e raias são capturadas anualmente, representando aproximadamente 4% da produção total da pesca extrativa marinha brasileira (ESTATPESCA, 2005). Desse total, aproximadamente 5.000 toneladas de tubarões são

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capturados pela pesca de espinhel que tem os atuns e o espadarte como espécies-alvo. Entre as espécies presentes na pesca com espinhel, o tubarão azul, *Prionace glauca*, e os tubarões do gênero *Carcharhinus* spp. se incluem entre os mais abundantes, totalizando 95% dos elasmobrânquios capturados (HAZIN et al., 1994).

Os tubarões do gênero *Carcharhinus* estão entre os elasmobrânquios mais capturados na pesca atuneira, em várias partes do mundo. No Brasil, essa espécie é frequentemente capturada tanto pela pesca artesanal como industrial, seja como espécie-alvo, seja como fauna acompanhante. A sua identificação específica, porém, é dificultada pela grande semelhança com os outros membros do gênero *Carcharhinus*, devido à similaridade na forma corpórea e coloração, além de suas distribuições comumente se sobreporem (OSHITANI et al., 2003).

As espécies do gênero *Carcharhinus* são morfologicamente muito próximas, ocasionando frequentemente erros de identificação dos exemplares capturados, fato muito comum para algumas dessas espécies, como no caso do *C. falciformis*, *C. galapagensis* e *C. obscurus*, todas três presentes no Arquipélago de São Pedro e São Paulo. Segundo MENDONÇA (2010), um dos maiores obstáculos para obtenção dos dados sobre capturas e comercialização de tubarões no nível de espécie é exatamente a dificuldade de identificação morfológica dos espécimes. A identificação e manutenção de estoques diferenciados, porém, são fundamentais para a atividade pesqueira, principalmente pela sua relação direta com a produtividade total e uso sustentável dos recursos.

No Brasil, mais de 10 espécies de elasmobrânquios compõem a fauna acompanhante da pesca com espinhel, respondendo por cerca de 20% do total capturado. Esse alto índice de captura incidental, associado à carência aguda de informações sobre a biologia das espécies, é preocupante, em razão das dificuldades

decorrentes para uma adequada avaliação dos seus estoques, conforme constatado nas duas últimas avaliações dos estoques de tubarões realizadas pelo Comitê Permanente de Pesquisa e Estatística (SCRS- Standing Committee on Research and Statistics) da ICCAT (Comissão Internacional para a Conservação do Atum Atlântico).

Na reunião da ICCAT, realizada no final de 2011, foi adotada uma medida de ordenamento para a conservação de tubarões lombo-preto capturados em associação com a pesca de atuns e afins com espinhel, a qual foi internalizada no Brasil por meio da Instrução Normativa Interministerial nº 8, de 06 de novembro de 2014. No referido ato normativo, se estabeleceu que todo tubarão lombo-preto capturado de forma incidental na pesca de atuns com espinhel deverá, obrigatoriamente, ser devolvido inteiro ao mar, vivo ou morto, no momento do recolhimento do aparelho de pesca, proibindo-se, ao mesmo tempo, a pesca direcionada, retenção a bordo, transbordo, desembarque, armazenamento, transporte e a comercialização dessa espécie em águas jurisdicionais brasileiras, em alto-mar ou em águas incluídas em acordos internacionais dos quais o Brasil seja signatário.

Recentemente, TOLOTTI et al. (2015) avaliaram os benefícios e desvantagens de medidas de proteção de determinadas espécies adotadas pelas Organizações Regionais de Ordenamento Pesqueiro (OROPs), responsáveis pela gestão da pesca de atuns e afins, a fim de avaliar a sua eficácia e o benefício geral a longo prazo para as populações protegidas. Segundo os referidos autores, embora haja aspectos positivos e negativos para a maioria das medidas de gestão adotadas, em geral, os aspectos positivos superam os negativos, garantindo benefícios importantes para os recursos pesqueiros e seus usuários em longo prazo. Várias dessas medidas de gestão dizem respeito à captura incidental de tubarões pelágicos, incluindo o tubarão lombo-preto (*C. falciformis*), galha-branca oceânico (*C. longimanus*), tubarão martelo (*Sphyrna spp.*) e

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tubarão raposa (*Alopias* spp.), os quais se encontram atualmente protegidos em pelo menos um oceano (Tabela I), sendo que o tubarão galha-branca oceânico é a única espécie abrangida por essas medidas em todos os oceanos (TOLOTTI et al., 2015).

Tabela I- Tubarões pelágicos atualmente sob medidas de proteção adotadas pelas diferentes OROPs de atuns e afins (Fonte: TOLOTTI et al., 2015).

		Espécies de Tubarão				
		Galha-branca oceânico	Lombo-preto	Bigeye	Raposa ssp.	Martelo ssp. ^a
ICCAT	Retenção	X	X	X		X
	Finning	X	X	X		X
	Comércio	X		X		X
	Desembarque		X	X		X
	REF	Rec. 10-07	Rec. 11-08	Rec. 09-07		Rec. 10-08
IOTC	Retenção	X			X	
	Finning	X			X	
	Comércio				X	
	Desembarque	X			X	
	REF	Res. 13-06			Res. 12-09	
WCPFC	Retenção	X	X			
	Finning	X	X			
	Comércio					
	Desembarque	X	X			
	REF	CMM 11-04	CMM 13-08			
IATTC	Retenção	X				
	Finning	X				
	Comércio	X				
	Desembarque	X				
	REF	Res. 11-10				

^aAs espécies de tubarão martelo *Sphyrna tiburo* é excluída dessa medida.

Vários estudos têm mostrado declínios populacionais substanciais para as espécies pelágicas, incluindo o tubarão lombo-preto, mortalidade muito provavelmente relacionada com o comércio global de barbatana de tubarão. Esta espécie está agora listada como "Criticamente em Perigo" (Near Threatened) mundialmente pela União Internacional para a Conservação da Natureza (IUCN) (BONFIL et al., 2009).

Diante deste contexto e da crescente pressão pesqueira sobre os elasmobrânquios, é importantíssimo que sejam aprofundados os conhecimentos disponíveis acerca dos

aspectos biológicos e populacionais dessas espécies, a exemplo do tubarão lombo-preto, espécie alvo deste estudo, de modo a subsidiar medidas de manejo que possam assegurar a sua conservação, em apoio às medidas de ordenamento adotadas pelas OROPs.

Com esse objetivo, o presente trabalho aporta informações sobre a morfologia, identificação molecular e comportamento do tubarão lombo-preto, elucidando os seus padrões de migração e sazonalidade no Oceano Atlântico Sudoeste e Equatorial, em particular em relação à sua distribuição no Arquipélago São Pedro e São Paulo (ASPSP). Buscar-se-á, ao mesmo tempo, avaliar a influência das variáveis ambientais e climáticas no seu comportamento, levando-se em consideração os fatores temporais e espaciais que influenciam o mesmo.

2- Revisão da Literatura

A ordem Carcharhiniformes contempla os tubarões mais abundantes em número de indivíduos e espécies, já que deste grupo fazem parte algumas famílias numerosas, sobretudo em regiões tropicais e subtropicais. No Brasil, a família de tubarões com maior diversidade é a Carcharhinidae, representada por 21 espécies (52,5% dos Carcharhiniformes brasileiros), sendo 8 os Carcharhinidae de ocorrência costeira, 8 de ocorrência oceânico-costeira e 5 de ocorrência pelágica. O gênero *Carcharhinus*, ao qual o tubarão lombo-preto (*Carcharhinus falciformis*) pertence, por sua vez, possui 15 espécies no Brasil (GADIG, 1998), com a maioria delas apresentando uma ampla distribuição na costa brasileira (LESSA et al., 1999; SILVÉRIO et al., 2010).

Os primeiros trabalhos a descreverem a distribuição, abundância, estrutura de tamanho, dieta, comportamento e reprodução da espécie *Carcharhinus falciformis* foram realizados há mais de 50 anos, na porção oeste do Atlântico Norte

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(BAUGHMAN e SPRINGER, 1950; MURPHY e SHOMURA, 1953) e no Pacífico Leste (STRASBURG, 1958). De acordo com STRASBURG (1958), o tubarão lombo-preto só foi relatado no Pacífico em 1953, quando ocorreu uma tentativa de identificação da espécie (MURPHY e SHOMURA, 1953), com a sua presença tendo sido confirmada, porém, apenas em 1956 (IVERSEN e YOSHIDA, 1956). Ao longo dos anos, foram sendo registradas ocorrências da espécie em vários estudos, principalmente para o Atlântico Norte, como BIGELOW e SCHROEDER, (1948); BANE (1966); BONFIL (1987), BRANSTETTER, (1987), BONFIL e ANDA (1993) e, a partir da década de 90, para o Oceano Índico (SANTANA et al., 1997; VARGHESE et al., 2015). Apenas ao final da década de 90, entretanto, é que dados provenientes do Atlântico Sul começaram a ser publicados (AMORIM et al., 1995; HAZIN et al., 1997, 2007; LESSA et al., 1999, 2001, 2002; NETO, 2011; LANA, 2012). Mais recentemente, diversos autores acrescentaram informações importantes acerca da distribuição, abundância relativa, biologia reprodutiva, hábito alimentar e idade e crescimento da espécie nos Oceanos Atlântico, Pacífico e Índico (OSHITANI et al., 2003; SHOUU-JENG et al., 2008; WATSON et al., 2009; FILMALTER, 2010; CABRERA-CHÁVEZ-COSTA et al., 2010; e NETO, 2011; GARCÍA-CORTÉS et al., 2011; LANA, 2012; VARGHESE et al., 2015).

A espécie analisada

O tubarão lombo-preto, *Carcharhinus falciformis* (Figura I), possui a seguinte classificação taxonômica:

Reino: Animalia

Filo: Chordata

Classe: Chondrichthyes

Sub Classe: Elasmobranchii

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Ordem: Carcharhiniformes

Família: Carcharhinidae

Gênero: *Carcharhinus*

Espécie: *Carcharhinus falciformes*



Figura I - O tubarão lombo-preto (*Carcharhinus falciformis*) (Fonte: <http://www.discoverlife.org>).

De acordo com JONES et al. (2015), Müller & Henle (1839) deram o crédito da descrição do *Carcharias (Prionodon) falciformis* (= *Carcharhinus falciformis*) para Bibron, observando ao final da descrição "A partir da comunicação de M. Bibron". Atualmente, porém, como no caso de Valenciennes, a autoridade para *C. falciformis* é dada a Müller & Henle (ESCHMEYER, 2014), e não a Bibron. Müller & Henle, porém, afirmaram especificamente que a descrição lhes foi comunicada por Bibron, não havendo a indicação de nenhum espécime que tenha sido examinado por eles mesmos. Bibron foi responsável pela descrição de muitos dos peixes e répteis coletados a partir de Cuba, por Ramón de la Sagra (ADLER, 1989). A origem do único espécime listado por Müller & Henle (1839) é Cuba, tendo sido coletado por de la Sagra. É razoável supor, portanto, que Bibron foi responsável pelo nome e descrição dessa espécie, devendo a sua autoria ser creditada a ele e não a Müller & Henle.

Entre os tubarões do gênero *Carcharhinus* existe uma natural similaridade morfológica que dificulta a identificação precisa das espécies, a qual é agravada sobremaneira quando associada à prática usual de retirada da cabeça, vísceras e nadadeiras ainda no mar, quando os espécimes são capturados, uma vez que estes caracteres são fundamentais para uma correta identificação morfológica (DOMINGUES e AMORIM, 2010). A dificuldade de identificação torna-se particularmente acentuada quando os animais são congelados a bordo.

O tubarão lombo-preto possui um corpo esguio e bastante alongado, com focinho moderadamente longo e nadadeira peitoral com algumas manchas negras nas pontas, particularmente em sua porção ventral. A segunda nadadeira dorsal é pequena, com margem livre comprida e origem coincidindo com a origem da nadadeira anal. Possui uma coloração cinza escura ou marrom acinzentada, tendendo a um profundo bronze metálico (Figura I). A espécie atinge um comprimento máximo de 3,30 a 3,50m (COMPAGNO, 1984; COMPAGNO & NIEM, 1998; VARGHESE et al., 2015), podendo ser distinguido dos outros grandes tubarões do mesmo gênero pela primeira barbatana dorsal relativamente pequena, com curvatura da sua margem posterior e nadadeiras peitorais em forma de foice (do latim *falciformis* significa forma de foice) BONFIL, 2008).

São considerados um dos tubarões mais abundantes na zona pelágica, podendo ser encontrados ao redor do mundo em águas equatoriais e tropicais (Figura II). É uma espécie pelágica que ocorre comumente próxima a recifes e bancos de areia, ao longo da borda da plataforma continental, bem como em ilhas oceânicas, em profundidades em geral de até 50m (BIGELOW & SCHROEDER, 1948; BASS et al., 1973; COMPAGNO, 1984). Comuns no Oceano Atlântico sudoeste e equatorial são bastante capturados principalmente por embarcações que operam com espinhel pelágico para

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atuns e espadarte, constituindo uma parte importante da fauna acompanhante dessa arte de pesca (HAZIN et al., 1991; MOURATO, 2007; BONFIL, 2008; FILMALTER et al., 2011, 2012, 2015; NETO, 2011; LANA, 2012; VARGHESE et al., 2015). Ocorre em praticamente toda a costa brasileira e na costa nordeste do Brasil, sendo particularmente abundante nas proximidades do ASPSP.

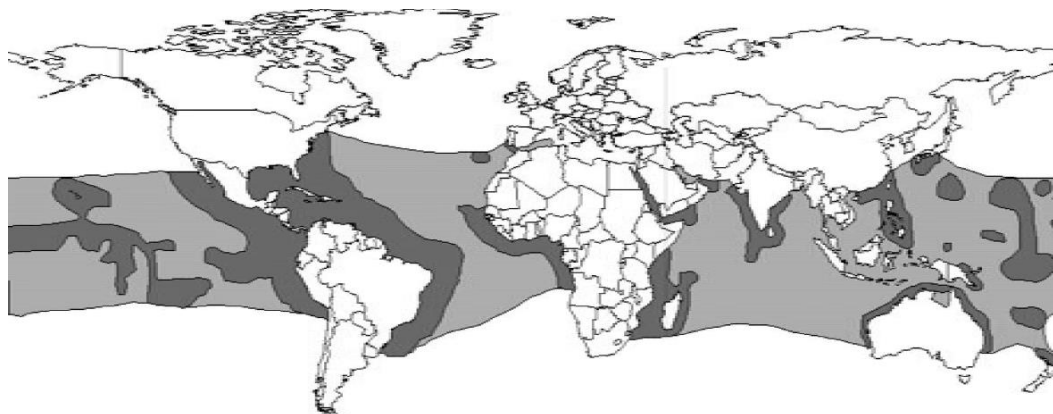


Figura II- Distribuição mundial do tubarão lombo-preto (*Carcharhinus falciformis*). O sombreado cinza mostra áreas de distribuição bem estabelecidas, enquanto o sombreado cinza claro mostra a distribuição incerta (presença esperada ou possível, ou registros que precisam de confirmação). (Fonte: BONFIL, 2008).

No Brasil, mais especificamente na região de Caiçara do Norte- RN, o tubarão lombo-preto parece utilizar as águas da porção terminal da plataforma interna (30-50 m) e plataforma continental externa como berçário, onde os jovens permanecem por, no máximo, um ano antes de assumirem uma distribuição mais oceânica (YOKOTA, 2005).

Como a maioria dos elasmobrânquios, o tubarão lombo-preto é considerado uma espécie K-estrategista, apresentando, portanto, uma susceptibilidade elevada à sobrepesca (HOLDEN, 1974a; 1977). Segundo HAZIN et al. (2007), apesar de o tubarão lombo preto ser uma espécie comum na costa brasileira, informações sobre sua biologia são ainda escassas.

Alguns estudos têm relatado aspectos da biologia reprodutiva de *C. falciformis*, descrevendo padrões de abundância, principalmente temporais e espaciais, tamanho de

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primeira maturação, fecundidade e período de gestação. No entanto, existem algumas inconsistências no que diz respeito ao período de estimativa de nascimento, gestação e tamanho na maturidade (LANA et al., 2012; GALVÁN-TIRADO et al. 2015).

Assim como os outros representantes da família Carcharhinidae, os tubarões lombo-preto são vivíparos placentários, com 2 a 16 filhotes, totalmente funcionais, por gestação, que dura aproximadamente 12 meses (COMPAGNO, 1984; BRANSTETTER, 1987; BONFIL, 2008; LANA, 2012; GALVÁN-TIRADO et al. 2015; VARGHESE et al., 2015). Observações sugerem que todo o ciclo reprodutivo se estende por 2 anos, com uma gravidez de 1 ano, seguido por um ano de "repouso" (BRANSTETTER, 1987; CORRENTE-CÁRDENAS, 2001; BONFIL et al., 2008).

O tamanho de nascimento estimado para a espécie varia normalmente entre 70 e 87 cm de comprimento total (CT) (COMPAGNO, 1984), com uma amplitude de 57,1 a 99,2 cm (BONFIL et al., 1993; OSHITANI et al., 2003; JOUNG et al., 2008; HALL et al., 2012; LANA, et al., 2012; VARGHESE et al., 2015). Em estudos sobre idade, crescimento e reprodução realizados no Oceano Pacífico, OSHITANI et al. (2003) documentaram um tamanho de nascimento para a espécie entre 65 e 81 cm do comprimento total (CT), muito próximo, portanto, ao que JOUNG et al. (2008) encontraram (63,5 a 75,5 cm CT) para o mesmo oceano. Já no Oceano Índico, BARTRON (2006) relatou 68 cm. Para o Oceano Atlântico, BONFIL et al. (1993, 2008), no entanto, relataram um tamanho de nascimento um pouco maior (65 a 80 cm CT) para a espécie no oeste do Atlântico Norte, enquanto LANA (2012), por sua vez, indicou um tamanho de nascimento em torno dos 80,0 cm, no Atlântico Sul.

A sazonalidade na reprodução não é evidente no Oceano Índico (BASS et al., 1973; HALL et al., 2012; VARGHESE et al., 2015), no Oceano Pacífico (STRASBURG, 1958; STEVENS, 1984 a; YOUNG et al., 2008; HOYOS-PADILLA et

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al., 2012) e nem no Atlântico Sul (LANA, 2012), ao passo que alguns estudos relataram atividade reprodutiva sazonal nesta espécie no noroeste do Golfo do México/ Oceano Atlântico (BRANSTETTER, 1987); Maldivas/ Oceano Índico (ANDERSON & AHMED, 1993); oeste do Atlântico Norte (BONFIL et al, 1993, 2008); e sul do Pacífico mexicano/ Oceano Pacífico (GALVÁN-TIRADO et al, 2015).

Estudo detalhado por HALL et al. (2012) sobre a idade e crescimento do tubarão lombo-preto revelou que o esta espécie no Oceano Índico oriental (maturação de 15 anos para fêmeas e 13 anos para os machos) cresce mais lentamente do que o relatado para as populações do Oceano Pacífico tropical (5-6 anos para machos e 6-7 anos para fêmeas) (OSHITANI et al., 2003) e Golfo do México (machos 6-7 anos e fêmeas 7-9 anos) (BRANSTETTER, 1987).

Essas diferenças nas taxas de crescimento, no tamanho máximo das mesmas espécies e na sazonalidade reprodutiva em diferentes áreas geográficas são geralmente atribuídas às diferenças nas latitudes (o efeito latitudinal) e nos parâmetros físicos, em especial à temperatura da água das regiões que habitam (BLACKBURN et al., 1999; HALL et al., 2012). No Atlântico Sul, LANA, et al. (2012) constataram a ação desse efeito latitudinal, quando os resultados encontrados foram comparados com os estudos realizados no Atlântico Norte/ Golfo do México (BRANSTETTER, 1987; BONFIL et al, 1993), já que, de acordo com LANA, et al. (2012), na proximidade do equador a presença de fêmeas grávidas ao longo de quase todo o ano sugere que os tubarões lombo-preto não apresentam um ciclo de gestação com uma sazonalidade muito definida nessa região, como já relatado por outros autores (STRASBURG, 1958; BANE, 1966; BASS et al., 1973). Esse padrão, porém, difere de BRANSTETTER (1987) e BONFIL et al. (1993), que sugeriram a ocorrência de sazonalidade reprodutiva para a espécie no Golfo do México, com o parto ocorrendo no início do verão depois de

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uma gestação de 12 meses. A amplitude sazonal relativamente menor da temperatura da superfície do mar próximo ao equador, como em LANA (2012), quando comparado com o Golfo do México, acima de 20°N de latitude, pode, porém, explicar essa diferença.

Estudos de idade e crescimento conduzidos no Oceano Atlântico Sul (SANTANDER-NETO, 2011) indicaram que machos e fêmeas maturam com 10,8 e 12,5 anos, respectivamente. BRANSTETTER (1987) relatou a maturação dos machos com 6-7 anos de idade, e das fêmeas com 7-9 anos de idade, para o noroeste do Golfo do México/ Atlântico Norte, enquanto BONFIL et al. (1993) no Caribe mexicano reportou idades de maturação de 10 anos para os machos e 12 anos para as fêmeas. Segundo JOUNG et al. (2008), a longevidade estimada para a espécie é de 28,6 anos para os machos e 35,8 anos para as fêmeas (VARGHESE et al., 2015).

Outro estudo conduzido sobre a reprodução da espécie no Atlântico Sudoeste e equatorial reportou um tamanho de primeira maturação para fêmeas em torno de 205-210 cm, e para os machos entre 180- 205 cm de comprimento total (LANA, 2012), comprimentos, portanto, inferiores aos observados por HAZIN et al. (2007) (210 a 230 cm para machos, e 230 cm para fêmeas), no mesmo oceano, e por BRANSTETTER (1987), no Golfo do México (210 cm para machos, e 225 cm para fêmeas). Segundo BONFIL (2008), o tamanho de maturação relatado para o Atlântico Norte para as fêmeas varia entre 232 e 246 cm CT, ao passo que para os machos essa amplitude vai de 215 a 225 cm CT (VARGHESE et al., 2015; GALVÁN-TIRADO et al, 2015). No oceano Pacífico, GALVÁN-TIRADO et al. (2015) encontraram um tamanho de maturação de 215,6 cm para fêmeas e de 207,6 cm para os machos.

Os tubarões lombo-preto são predadores generalistas que possuem um comportamento oportunista, com eventual formação de grupos de alimentação

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(SPRINGER, 1979). Análises de conteúdo estomacal indicam que são animais primariamente piscívoros, embora a sua alimentação também inclua moluscos e crustáceos (COMPAGNO, 1984; BRANSTETTER, 1987; GALVÁN-MAGAÑA et al., 1989; BONFIL et al., 1993; CABRERA-CHAVEZ-COSTA et al., 2010; VARGHESE et al., 2014, 2015; DUFFY et al., 2015).

Informações a respeito da migração e distribuição do lombo-preto no Oceano Atlântico são relativamente escassas. A maioria dos dados existentes é proveniente do Atlântico Noroeste, onde mais de 820 lombos-pretos foram marcados desde 1963 pelo *Cooperative Shark Tagging Program*, sob a responsabilidade do *National Marine Fisheries Service* (KOHLETER et al., 1998), do governo estadunidense (BONFIL, 2008). Os dados de recaptura indicam que esta espécie pode migrar longas distâncias em um espaço de tempo relativamente curto, ocasionalmente atravessando grandes extensões oceânicas, razão pela qual é frequentemente capturada por um grande número de países e frotas pesqueiras.

Segundo FILMALTER et al. (2013, 2015), o tubarão lombo-preto é a principal espécie de elasmobrânquio capturada como fauna acompanhante em conjunto com a pesca de cerco realizada em dispositivos de concentração de peixes (FADs) a deriva, resultando em mortalidades elevadas para a espécie.

Os tubarões lombo-preto dominam as capturas de elasmobrânquios nos FADs, constituindo aproximadamente 90% dos tubarões capturados nesta pescaria em todos os oceanos (GILMAN, 2011). No Oceano Índico, especificamente, a espécie representa pelo menos 75% da captura de tubarões, em número (AMANDE et al., 2010). Dois estudos relativamente recentes (DAGORN et al., 2007b; e FILMALTER et al., 2011), forneceram informações importantes sobre o comportamento dos tubarões lombo-preto associados com FADs. Como o uso de FADs continua aumentando (DAGORN et al.,

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2013), há uma grande necessidade de se entender como essas práticas influenciam as populações de tubarões lombo-preto.

Em um estudo ainda mais recente, realizado no Oceano Índico ocidental, entre 2010 e 2012, por FILMALTER et al. (2015), em 9 FADs à deriva equipados com receptores acústicos ligados a um satélite, 38 tubarões lombo-preto (CT 69-116 cm) foram monitorados com transmissores acústicos por um período total de 154 dias. Além desses 38 tubarões, 13 indivíduos também foram marcados com PSAT (Pop-up Satellite Archival Tag).

O uso de marcas eletrônicas, como as PSAT, tem permitido a obtenção de informações valiosas sobre a profundidade de distribuição, preferências de temperatura e movimentos migratórios de várias espécies de tubarões (SIMS et al., 2003; BONFIL et al., 2005; WENG et al., 2005; STOKESBURY et al., 2005; TOLOTTI et al., 2015), inclusive sobre o tubarão lombo-preto (FILMALTER et al., 2010, 2011, 2013, 2015), embora os resultados já publicados sobre essa espécie sejam ainda preliminares (DAGORN et al. 2007b, 2000; BURGESS et al., 2005; BERNAL et al., 2009).

Estudos conduzidos com marcas eletrônicas sobre os movimentos do tubarão lombo-preto (LANA, 2012¹) no Oceano Atlântico Sul indicam um padrão que coincide com os resultados encontrados por TOLLOTTI et al. (2011, 2015), com o tubarão galha branca, com os espécimes marcados tendo apresentado uma clara preferência por águas mais rasas no período diurno, a despeito de um comportamento nitidamente oscilatório. As causas desse padrão de movimento, observado não somente para o tubarão lombo-preto, mas para diversas espécies de tubarões, ainda não são bem definidas, podendo

¹ Cabe notar que, mesmo antes de terem sido publicados, os dados gerados nesse esforço de pesquisa já contribuíram para a adoção pela ICCAT da recomendação para conservação do tubarão lombo-preto.

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incluir causas tróficas, navegação por geomagnetismo ou celestial, termorregulação e conservação de energia.

3- Objetivos

3.1- Objetivo Geral

O presente trabalho pretende estudar as características morfológicas, a identificação molecular e o deslocamento, via marcações eletrônicas, do tubarão lombo-preto no Oceano Atlântico Sudoeste e Equatorial, em particular com relação à sua distribuição no Arquipélago São Pedro e São Paulo (ASPSP), com o objetivo de esclarecer o seu padrão de movimentação diária e sazonal, além da estrutura local de sua população. As características morfológicas/ anatômicas que definem essa espécie serão também analisadas de forma comparativa com as outras espécies do gênero *Carcharhinus* que também ocorrem na área de estudo (ASPSP).

3.2- Objetivos Específicos

1. Identificar as propriedades morfológicas que caracterizam o *Carcharhinus falciformis*, por meio da diferenciação por crânio, diferenciando-se os indivíduos juvenis dos adultos.
2. Identificar, por meio de análise molecular (PCR- Multiplex), o tubarão lombo-preto e comparar as suas características genéticas com as outras espécies do gênero *Carcharhinus* que possuem registro na área de estudo e que apresentam características morfométricas próximas (ex: *C. galapagensis* e *C. obscurus*).
3. Determinar os padrões de movimentação espacial e temporal, em pequena e média escala, dos tubarões lombo-preto no ASPSP, por meio de marcas MiniPAT, PSAT (*Pop-up Satellite Archival Transmitting Tag*) e SPOT (*Smart Position or Temperature Transmitting Tag*).

4- Material e Métodos

Todas as marcações dos tubarões e coleta de material biológico foram realizadas no Oceano Atlântico Sudoeste e equatorial, nas imediações do Arquipélago de São

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Pedro e São Paulo (ASPSP), na área localizada entre as latitudes 00°55'3,72"N e longitudes 029°20'11,22"W.

Descrição da área de estudo

A área de estudo compreende as águas do Oceano Atlântico Sudoeste e equatorial, cuja topografia oceânica é constituída predominantemente por planícies abissais, embora seja frequente a presença de vários bancos submarinos rasos, pertencentes às Cadeias Norte-Brasileira e de Fernando de Noronha, além de ilhas oceânicas, tais como o Atol das Rocas, o Arquipélago de Fernando de Noronha e o Arquipélago de São Pedro e São Paulo (ASPSP). Tanto os bancos submersos como as ilhas oceânicas se encontram sujeitas a importantes interações entre as correntes oceânicas locais e o relevo submarino, que podem promover transportes verticais de nutrientes (TRAVASSOS et al. 1999), incluindo a possível ocorrência de ressurgências localizadas. Segundo HAZIN (1993), no Atlântico equatorial, o forte gradiente térmico constitui um obstáculo adicional a movimentos verticais, dificultando a reposição de sais nutrientes na camada eufótica. Por essa razão, as áreas caracterizadas pela presença de tais acidentes topográficos representam os principais locais de captura de espécies pelágicas comercialmente explotadas na costa nordeste do Brasil, incluindo o tubarão lombo-preto.

O ASPSP constitui um ponto estratégico para o Brasil, em consequência da sua posição geográfica para a projeção do país no mar. É formado por um conjunto de ilhotas rochosas, sendo 6 ilhas maiores, 4 menores, e várias pontas de pedra, constituindo um “paredão” rochoso que se ergue de uma profundidade de 4.000 m (MIGUENS, 1995). Situado no Oceano Atlântico equatorial, no hemisfério Norte (00°55'3,72"N e 029°20'11,22"W), entre a América do Sul e a África, é o ponto do

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Brasil mais próximo do continente africano, distando 1.800 km de Dakar, no Senegal, 1.100 km do litoral do Rio Grande do Norte e 522 km do Arquipélago Fernando de Noronha (PE) (Figura III). O ASPSP é o resultado de uma falha transformante da Dorsal Meso-Atlântica, ocupando uma área total emersa de aproximadamente 17.500 m² e uma elevação máxima de 18 m acima do nível do mar (VIANA et al., 2008; VASKE-Jr, T. et al., 2010).

Divulgação/Secirm



Fonte: Secirm



http://www.scielo.br/scielo.php?pid=S0370-44672009000300011&script=sci_arttext

Figura III- Localização do ASPSP. Foto: PROARQUIPÉLAGO/ SECIRM- Marinha do Brasil.

4.1- Trabalho I

Morfologia

Entre os tubarões do gênero *Carcharhinus* existe uma natural similaridade morfológica que dificulta a identificação precisa das espécies. Dos espécimes coletados foram examinados anatomicamente os condrocânios. Uma vez coletados, os condrocânios foram colocados em um recipiente com água aquecida (sem ferver), à qual se adicionou carbonato de potássio calcinado, de forma a facilitar o desprendimento do tecido muscular. As arcadas foram retiradas antes de se aquecer a água, tomando-se o cuidado de não danificar o crânio, quando da sua remoção. O trabalho de morfologia foi realizado em parceria com o Laboratório de Taxonomia de Elasmobrânquios, do Departamento de Biologia Animal e Vegetal, do Instituto de Biologia da Universidade do Estado do Rio de Janeiro (UERJ).

4.2- Trabalho II

Identificação molecular

Dos indivíduos amostrados na área de estudo, foram coletadas amostras do tecido muscular da região dorsal próxima à cabeça e também da base da primeira nadadeira dorsal. Dos indivíduos que foram marcados por telemetria, no entanto, foram retiradas amostras do tecido muscular da base da primeira nadadeira dorsal, de forma a não lesionar o animal e a reduzir o estresse ao mínimo possível.

Os materiais coletados foram analisados por meio de uma cooperação já existente com o Laboratório de Genética de Organismos Aquáticos e Aquicultura (LAGOAA), pertencente ao Núcleo Integrado de Biotecnologia (NIB) da Universidade de Mogi das Cruzes (UMC/SP), e com o Instituto de Biociências de Botucatu, da Universidade Estadual Paulista “Júlio Mesquita Filho” (UNESP). Nessas instituições já

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foram desenvolvidos dois métodos práticos, rápidos e de baixo custo para identificação molecular de algumas espécies do gênero *Carcharhinus*, incluindo o *C. falciformis*, por meio das técnicas PCR- Multiplex, detalhadas por SHIVJI et al. (2002), as quais utilizam dois “primers” universais e nove “primers” espécie-específico, gerando padrões de bandas específicas com 100% de acurácia.

4.3- Trabalho III

Marcação e Monitoramento

No presente estudo foram marcados 10 (dez) tubarões lombo-preto, com 3 (três) modelos distintos de marcas eletrônicas: 5 MiniPAT (*Pop-up Archival Tag*), 3 PSAT (*Pop-up Satellite Archival Transmitting Tag*), e 2 SPOT (*Smart Position or Temperature Transmitting Tag*), no período de 2010 a 2014.

Os dados acerca da utilização do habitat e deslocamento dos animais coletados com as marcas eletrônicas foram comparados com informações ambientais obtidas por sensoriamento remoto. A marca PSAT permite o registro de dados relativos à temperatura da água, profundidade e posição geográfica estimada a partir da incidência de luz solar, os quais são transmitidos através do sistema ARGOS de satélites, tão logo a marca se solta do animal, no período pré-programado e emerge a superfície. Já a SPOT é capaz de armazenar apenas a localização e temperatura, enviando essas informações sempre que o animal marcado alcança a superfície. A marcação do tubarão lombo-preto ocorreu a bordo, por meio da captura, embarque e imobilização do animal, sempre que as suas dimensões o permitiram. As marcas foram aplicadas na 1ª nadadeira dorsal, com o auxílio de uma furadeira autônoma, realizando-se, para este fim, um pequeno furo distando 5 cm da margem anterior. O local escolhido para a fixação das marcas corresponde a uma zona da nadadeira onde a cartilagem é mais vigorosa e não

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há vasos sanguíneos. A PAT tag foi fixada por um monofilamento de náilon (0,80 mm) munido de um destorcedor, de forma a reduzir a interferência na natação do tubarão ao mínimo. Todo o procedimento de marcação foi bastante rápido, não ultrapassando 5 minutos.

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6- Artigo científico

6. 1 - Artigo científico I

Artigo científico a ser encaminhado a Marine Biology Research

Description of the chondrocranium of the silky shark *Carcharhinus falciformis* (Bribon, 1839) in the western equatorial Atlantic Ocean with comments on the cranial terminology in *Carcharhinus* (Chondrichthyes, Carcharhiniformes, Carcharhinidae)

Todas as normas de redação e citação, deste capítulo, atendem as estabelecidas pela referida revista (em anexo).

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Description of the chondrocranium of the silky shark *Carcharhinus falciformis* (Bribon, 1839) in the western equatorial Atlantic Ocean with comments on the cranial terminology in *Carcharhinus* (Chondrichthyes, Carcharhiniformes, Carcharhinidae)

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Abstract

The description of the neurocranium of the silk shark *C. falciformis* (Bribon, 1839) caught in Brazilian coast is herein presented. The cranial topographic terminology was revised with emphasis on the orbital region. Result was compared with the crania of other species of *Carcharhinus*. *C. falciformis* chondrocranium is characterized by a rounded rostral node, size of the median rostral cartilage, presence of rostral fenestra, epiphysial notch and nasal flap; posterior margin of the anterior fontanelle V-shaped; angular nasal capsule; triangular postorbital process; subethmoid fossa V-shaped. Epiphysial notch presented individual differences as well as the number of foramina for the passage of the trochlear nerve, which can vary even in the same individual. Sexual dimorphism wasn't found. Hypercalcification present. *C. falciformis* presents a well developed postorbital process, longer than other species, followed by the length and the width across the optic capsules. Cranial new terms have been proposed. In *Carcharhinus* specific differences was compared with crania of other species based in both literature and personal observations. The cranial components that presented specific consistent differences are: the shape of the rostral node and posterior margin of anterior fontanelle, rostral dimension, presence or absence of rostral fenestra, epiphysial notch, epiphysial foramen and nasal flap; direction of the nasal capsules, shapes of the preorbital process and subethmoid fossa. The rostral node showed great morphological plasticity among species. Possibly the rostral node is primitively massive and closed. In most derived forms the modification sequence of this character provides a breakdown of rostral fenestrae, vanishing with the opening of the transverse bar at the apex of the rostral cartilage. The taxonomic position of *Nasolamia*, formerly belonging to *Carcharhinus* and separated from this genus by having the distance between the nasal capsules greatly reduced, is called into question, since it shares almost all cranial proportions with *Carcharhinus*.

Keywords: cranial morphology, cranial topographic terminology, neurocranium, Carcharhinidae.

Introduction

The genus *Carcharhinus* has about 31 species known worldwide. They play an extremely important role in the trophic chain of tropical and subtropical marine ecosystems, where they are abundant (Garrick 1982, 1985; Gadig 1999). Despite their ecological importance and commercial value, there are few studies on this genus in Brazil. Along the Brazilian coast 15 species have been recorded corresponding to about 20% of the shark fauna and about 71% of the sharks of the Carcharhinidae family (Hazin et al, 1997, Gadig 1994, 1999), four of them being pelagic, four strictly coastal and seven oceanic-coastal, with most of them showing a wide distribution (Lessa et al. 1999; Silvério et al. 2010; Lana 2012).- Some Carcharhinids are known to travel long distances, occasionally crossing oceans, and are considered an important fishery resource, shared by many regions and nations. (Grace 2001; Lana 2012).

Carcharhinidae genera can be difficult to identify due to the similar body shape and color, and overlapping distribution, in special *Carcharhinus* and *Rhizoprionodon* species (Grace 2001).

The first attempt to subdivide *Carcharhinus* species in groups were done by Nichols & Breder (1927) for the presence or absence of an interdorsal (middorsal skin) ridge. Too much attention was given to this aspect. Nichols & Breder (1927) affirm that the interdorsal ridge, easy to find in fresh animals, sometimes disappears in preserved specimens.

Bigelow & Schroeder (1948), in their identification key, also separated the species in those with a low dermal ridge at the midline of the back between the first and second dorsal fins (*C. floridanus* [= *C. falciformis*], *C. falciformis*, *C. longimanus*, *C. obscurus*, *C. springeri*[= *C. perezii*] , *C. milberti* [= *C. plumbeus*]); and those without interdorsal ridge: again *C. longimanus*, *C. leucas*, *C. nicaraguensis* (= *C. leucas*), *C. acronotus*, *C. remotus* (= *C. acronotus*), *C. limbatus* and *C. maculipinnis* (= *C. brevipinna*).

Springer (1950) presented a taxonomic study of *Carcharhinus* and genera allied to it (*Scoliodon*, *Prionace*, *Aprionodon*, “*Pterolamia*”, “*Eulamia*”, “*Hypoprion*”, *Negaprion*, *Isogomphodon*). Within *Carcharhinus* he grouped *C. maculipinnis* (= *C. brevipinna*), *C. aethalorus* (= *C. limbatus*), *C. acronotus*, *C. porosus*, *C. velox* (= *Nasolamia*), *C. leucas*, *C. nicaraguensis* (= *C. leucas*), *C. azureus* (= *C. leucas*) and *C. gangeticus* (= *Glyphis gangeticus*). *Pterolamnia* with only *P. longimanus* (= *C.*

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longimanus) and the ridge-backed *Eulamia* (Gill 1862) with *E. altimus* (= *C. altimus*), *E. plumbeus* (= *C. plumbeus*), *E. milberti* (= *C. plumbeus*), *E. falciformis* (= *C. falciformis*), *E. springeri* (= *C. perezii*), *E. floridanus* (= *C. falciformis*), *E. obscurus* (= *C. obscurus*). He also discussed the possibility of including in *Eulamia* the following species: *Carcharias lamiella* (= *Carcharhinus brachyurus*), *Carcharias galapagensis* (= *Carcharhinus galapagensis*), *Carcharias dussumieri* (= *Carcharhinus dussumieri*), and *Carcharias japonicus* (= *Carcharhinus japonicus*).

Garrick (1982) observed the presence of an interdorsal ridge in nine species, being absent in other 11 species, but this feature can be both present or not in some of those species and in some cases the absence can be an artifact of preservation. He separated in species-group some *Carcharhinus* based mainly on tooth components and some external features: *sealei-dussumieri*, *leucas-amboiensis*, *limbatus-amblyrhynchoides*, *amblyrhynchos-wheeleri* and *obscurus-galapagensis*. Posteriorly, in the same work, the author presented a possible relationship between *falciformis-sorrah* group sharing the presence of a noticeably narrow and well defined middorsal ridge and also between the length of the second dorsal rear tip and height that is at the extreme for all other *Carcharhinus*. Both species differs from each other by the upper teeth shape, oblique lower teeth (*C. sorrah*) and lobe of the anterior nasal flap (absent in *Carcharhinus falciformis*).

Compagno (1988) also divided *Carcharhinus* in species-group as follow: *C. porosus* group (*C. porosus*, *C. bornensis* (= *C. sealei*), *C. dussumieri*, *C. sealei*, *C. macloiti*, *C. sorrah*, *C. hemiodon*, *C. fitzroyensis*), transitional group (*C. acronotus*, *C. melanopterus*, *C. cautus*, *C. falciformis*, *C. brachyurus*, *C. signatus*), *C. limbatus* group, (*C. limbatus*, *C. amblyrhynchoides*, *C. isodon*, *C. brevipinna*, *C. leiodon*), *C. leucas* group (*C. leucas* and *C. amboiensis*), *C. obscurus* group (*C. obscurus*, *C. galapagensis*, *C. albimarginatus*, *C. plumbeus*, *C. altimus*, *C. longimanus*). These species-groups were defined by their size (small-medium-large), shape of the snout (pointed, rounded, short, elongated or subangular), dimension of the anterior nasal flap (rudimentary or extended), dimension of the gill apertures (narrow or broad), morphology and dimension of the teeth (narrow/large, erect/oblique, strongly incised or not), number of vertebral countings, width of the basal plate at the orbital notches (being equal, less or greater across orbits), and dimension and morphology of the preorbital processes (large, triangular, narrow, reduced).

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The allozyme data proposed by Naylor (1992) suggested that *C. obscurus*, *C. galapagensis*, *C. longimanus*, *C. falciformis*, *C. plumbeus*, *C. altimus* and *C. perezii* all form a monophyletic group which also includes the blue shark *Prionace glauca* and all *Carcharhinus* members of the large ridge-backed group.

Recently Dosay-Akbulut (2008) took the ribosomal ITS1–2 regions sequence to differentiate the genus *Carcharhinus* from the blue shark (genus *Prionace*). As Naylor (1992), she concluded that the blue shark is a member of *Carcharhinus* instead of *Prionace*, or that there is a misclassification, since *Prionace* should not be considered a separate genus. In her work she also put *Carcharhinus* in groups by affinities. Eleven species of *Carcharhinus* were analyzed and compared with *Rhizoprionodon terranova*, *Prionace glauca*, *Negaprion brevirostris* and *Galeocerdo cuvier*. By affinities the cladogram presented united *C. porosus* + *C. isodon*, *C. falciformis* + *C. altimus* with *Prionace glauca*, *C. acronotus* + *C. limbatus*, and, outside, *Negaprion brevirostris* being sister group of *Carcharhinus* + *Prionace* and *C. brevipinna* placed outside from all other *Carcharhinus*.

The chondrocranium is the primitive cartilaginous skeletal structure of the fetal skull that grows to envelop the rapidly growing embryonic brain. Studies of chondrocranium have been developed to identify systematic taxonomic patterns of a species, genus or family (Compagno 1990). The chondrocranium of cartilaginous fishes of both recent and fossil species has proved to be a useful tool in phylogenetic and taxonomic studies (e.g. Gegenbaur 1872; Parker 1878; White 1937; Iselstöger 1937; Holmgren 1940, 1941; El-Toubi & Hamdy 1959, 1967, 1968; Hamdy 1960, 1964; Gohar & Mazhar 1964; Springer 1964; Stehmann 1970; Hulley 1972; Nakaya 1975; Capapé & Roux 1980; Maisey 1982, 1985, 2004; Compagno 1988; McEachran & Miyake 1990; Nishida 1990; Waller & Baranes 1991; Shirai 1992; Gomes & Siqueira 1995; Carvalho & Maisey 1996; Carvalho et al. 2008; Lane 2010; Carvalho et al. 2010; Vaz & Carvalho 2013; Maisey & Springer 2013).

Ishiyama (1958) and Qingwen et al. (1986) presented an identification key based only on neurocrania characters respectively for Japanese Rajidae and Chinese sharks.

Several species of *Carcharhinus* have had their cranium studied. Gegenbaur (1872) described *C. melanopterus*; Parker (1887), *Carcharhinus* sp.; Holmgren (1941), *C. mossambicus* (= ?); Gohar & Mazhar (1964), *Aprionodon brevipinna* (= *C. brevipinna*); Nakaya (1975), *C. dussumieri*, *Pterolamiopsis longimanus* (= *C.*

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longimanus); Qingwen et al. (1986), *Carcharhinus* sp; and Maisey (2004), *Prionodon* (= *Carcharhinus*).

Compagno (1979, 1988) compared the neurocrania topography (only the dorsal and ventral views) of *C. obscurus*, *C. macroti*, *C. amblyrhynchoides*, *C. falciformis*, *C. albimarginatus*, *C. galapagensis*, *C. isodon*, *C. sealei*, *C. porosus*, *C. borneensis*, *C. hemiodon*, *C. sorrah*, *C. signatus*, *C. amboinensis*, *C. melanopterus*, *C. limbatus*, *C. leucas* and *C. longimanus*.

According to that author, within this genus, neurocrania structures regarding species specificity are the rostral cartilages (stout to moderately slender), the medial rostral cartilage length from 1.7 to 3.6 in nasobasal length; and nasal capsules moderately broad, depressed or fairly high, with their widths about 0.7-1.5 times their lengths.

The silky shark (*C. falciformis*) has a slender and rather elongated body with moderately long snout and pectoral fin with some black spots on the tips, particularly in its ventral portion. The second dorsal fin is small, with free long edge and origin coinciding with the origin of the anal fin. It has a dark gray color or grayish brown, tending to a deep metallic bronze. According to D'Aubrey (1964), Bass et al. (1973, 1975), Garrick (1982), Compagno (1984), Grace (2001) and Varghese et al. (2015) *C. falciformis* is a large shark up to 3.30 to 3.50 m long. Identified by a moderately long and blunt pointed snout with internarial width from 1.2 to 1.6 times in preoral length. Eyes circular and moderately large, length 1.2 to 2.7% times in the total length. Upper labial furrows short and inconspicuous. Origin of first dorsal fin behind pectoral fin free rear tips. Origin of the second dorsal fin over or slightly behind the anal fin origin. A narrow interdorsal ridge always present. Upper teeth with fairly narrow, strongly serrated, erect to moderately oblique cusps, well-delimited from crown feet. Teeth with heavy serrations or small cusplets; lower teeth with erect, narrow, smooth-edged cusps and transverse roots. Dental formulae usually: 15 -2- 12/ 15 -1-15, varying from 14 or 16 -1 or 3- 14 or 16/14 or 17 -1 or 3- 14 or 17.

Dark grey or grey brown above, sometimes nearly blackish, white below; tips of fins other than first dorsal dusky but not black-tipped, an inconspicuous white band on flank.

The *C. falciformis* is a highly migratory apex predator distributed worldwide tropical and equatorial waters being oceanic epipelagic and offshore shark. According to

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Ebert & Stehmann (2013), it occurs in the Eastern Atlantic: Madeira Islands, in Atlantic Spain, and from Senegal to northern Angola, and in the Western Atlantic: from Massachusetts (U.S.A.) to southern Brazil. For synonyms see Bigelow & Schroeder (1948: 333) and Garrick (1982: 159). This species occurs from tropical to temperate areas, near reefs, sandbanks and oceanic islands (Compagno 1984). In the equatorial and southwestern Atlantic Ocean, the species has been often caught by longline vessels targeting tunas and swordfish, which has been the main cause of their fishing mortality. Occurs in the entire coast of Brazil, including Saint Peter and Saint Paul's Archipelago and it's particularly abundant close to seamounts (Gadig 2001; Gomes et al. 2010; Neto et al. 2011; Lana 2012). The species lives commonly near the edge of continental and insular shelves also occurring in the open sea and occasionally inshore, in shallow water. *C. falciformis* is often found over deep water reefs and near insular slopes, preferring temperatures of 23°C (Compagno et al. 2005; Ebert & Stehmann 2013).

In Brazil, the silky shark is known as “lombo-preto” or “cação-baía”. It may be confounded with *C. signatus*, differing from that species, however, by the tooth shape, a shorter snout and color. It has a gestation time of about 12 months, with 7 to 25 newborns ranging from 700 to 870 mm in total length (Compagno 1984; Gadig 1998, 1999, 2001; Gadig et al. 2000; Gomes et al. 2010; Lana 2012).

This species was firstly described from Cuba by Bibron in 1839 (Jones et al. 2015). According to Jones et al. (2015), Müller & Henle (1839) gave the credit of the description of the *Carcharias (Prionodon) falciformis* (= *Carcharhinus falciformis*) to Bibron, which can be noted at the end of the description "From the communication M. Bibron". However the authorship authority to *C. falciformis* is given to Müller & Henle (Eschmeyer 2014), not to Bibron. Müller & Henle stated specifically that the description was communicated to them by Bibron, with no indication of any specimen that has been examined by themselves. Bibron was responsible for the description of many of the fishes and reptiles collected from Cuba by Ramón de la Sagra (ADLER 1989). It's reasonable to assume, therefore, that Bibron was responsible for the name and description of this species, with the authorship be credited to him and not to Müller & Henle.

Besides studying shark taxonomy and phylogeny based on genetic data, most of the studies are based on the anatomy, with few anatomical studies being, however, available for *Carcharhinus*. Furthermore, the studies in which *Carcharhinus*

chondrocrania were described (cited above) show many inconsistencies, especially regarding the foramina of the orbital region.

This work, therefore, presents the description of the chondrocranium of the silky shark, *Carcharhinus falciformis*, in order to review the terminology of the components as well as the nomenclature of its foramina, and notes on individual modifications. The chondrocrania of other *Carcharhinus* were also analyzed in order to find consistent characters to be used in taxonomy.

Material and Methods

The specimens examined were caught in the western equatorial Atlantic Ocean, in the vicinity of the Saint Peter Saint Paul Archipelago (00°55.1'N latitude and 29°20.7' W longitude), by longline and hand-line. Other specimens were collected in southeastern Brazil, between São Paulo and Rio de Janeiro States. Compared species are listed below.

The crania were heated in water (without boiling) with calcinated potassium carbonate in order to easily take out the flesh.

The specimens were deposited at the anatomical cartilaginous fish collection, Departamento de Zoologia at Universidade do Estado do Rio de Janeiro "AC.UERJ".

The cranial terminology was based in Holmgren (1940, 1941), Devillers (1958), Hamdy (1960, 1964), Hulley (1972), Compagno & Garrick (1983) and Compagno (1979, 1985, 1988). Cranial measurements followed Compagno (1988) and are given as percentages of nasobasal length (Table I and VI) and will be compared along the text.

The list of species, with their respective collection number, sex, total length "TL" and procedures followed is presented below.

Material:

- *Carcharhinus falciformis* – "AC.UERJ" 058, sex: no data, "TL": no data, Atafona, São João da Barra, Rio de Janeiro; "AC.UERJ" 1452, male, 117 cm "TL", Saint Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1453, female, 107 cm "TL": no data, Saint Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1454, female, 1445 mm "TL", Saint Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1455, male, 1242 cm "TL", Saint Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1456, female, 1316 mm "TL", Saint

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Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1457, male, 1384 mm "TL", Saint Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1458, male, 1131 mm "TL", Saint Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1459 female, 1283mm "TL", Saint Peter and Saint Paul Archipelago, Rio Grande do Norte; "AC.UERJ" 1469, sex: no data, "TL": no data, off São Paulo State;

Compared material:

- ***Carcharhinus plumbeus*** – "AC.UERJ" 012, sex: no data, "TL": no data, Barra de Guaratiba, Rio de Janeiro; "AC.UERJ" 526, male, "TL": no data, Barra de Guaratiba, Rio de Janeiro;
- ***Carcharhinus longimanus*** "AC.UERJ" 1470 (4 specimens), sex: no data, "TL": no data, off Rio de Janeiro and São Paulo states;
- ***Galeocerdo cuvier*** "AC.UERJ" 1169, sex: no data, 1780 mm "TL", Ilha Rasa, Rio de Janeiro;
- ***Rioraja agassizi*** – "AC.UERJ" 299, male, 390 mm "TL", off Ilha Grande (Rio de Janeiro) and Paranaguá (Paraná);
- ***Atlantoraja cyclophora*** – "AC.UERJ" 634, female, 545 mm "TL", off Itajaí and São Francisco do Sul, Santa Catarina;
- ***Atlantoraja platana*** – "AC.UERJ" 1034, female, 753 mm "TL", off Santos, São Paulo;
- ***Atlantoraja castelnaui*** "AC.UERJ" 956, female, 850 mm "TL", Ilha Vitória, Ubatuba, São Paulo;
- ***Rhizoprionodon porosus*** – "AC.UERJ" 001, sex: no data, "TL": no data, Barra de Guaratiba, Rio de Janeiro; "AC.UERJ" 034, male, 1025 mm "TL", Barra de Guaratiba, Rio de Janeiro;
- ***Rhizoprionodon lalandii*** – "AC.UERJ" 041, male, "TL": no data, off Caraguatatuba and Ilha Bela, São Paulo.

Results

Description

The chondrocranium is typically Carcharhinidae (*sensu* Compagno, 1979, 1998) *i.e.* with medial rostral cartilage usually cylindrical and lateral rostral cartilages originating at the lateral edges of the anterior fontanelle; a rostral node not expanded

laterally; spherical, oval or subcylindrical nasal capsules; internarial septum usually high and compressed; nasal apertures oval to circular; nasal fontanelles usually absent. Ectethmoid condyles present and well marked. Subethmoid fossa present. Either medial and lateral ectethmoid foramina or only medial foramina present on the ectethmoid condyles. Anterior fontanelle subcircular, anteroposteriorly elongated or slightly expanded laterally. Cranial roof broadly arched to flattened in lateral view. Parietal fossa single and deep. Basal plate usually with a pair of internal carotid foramen and with stapedial foramina or fenestrae. Presence of a suborbital shelf. Preorbital process platelike or striplike. Postorbital process simple or rodlike. Sphenopterotic ridges weak, vestigial or absent. Occipital condyles well marked.

Excluding the pre- and postorbital processes, the cranium greatest width is verified in dorsal view at the nasal capsules becoming narrower at the orbital region in front of the anterior semicircular canal. For crania proportional dimensions see Table I.

Table I. Cranial proportional dimensions in % of nasobasal length in *C. falciformis*.

MEASUREMENTS (mm/%)	AC. UERJ 1452	AC. UERJ 1453	AC. UERJ 1454	AC. UERJ 1455	AC. UERJ 1456	AC. UERJ 1457	AC. UERJ 1458	AC. UERJ 1459
Total length	117	107	144.5	124	131.6	138.4	113.1	128.3
	109	113	147	128	129	138	117	135
Rostral length	2.4	2.2	2.4	2.2	2.4	2.4	2.5	2.4
Width across bases of lateral rostral cartilages	4.0	4.0	3.8	3.6	3.9	3.8	3.9	3.6
Width across nasal capsules	1.5	1.4	1.7	1.4	1.5	1.5	1.5	1.5
Width of nasal capsule	2.9	2.6	2.9	2.7	2.9	2.8	2.9	2.8
Length of nasal capsules	3.0	2.7	4.0	3.3	2.7	2.9	3.0	4.2
Width of nasal apertures	6.1	5.1	5.7	5.8	5.4	5.8	5.6	5.6
Width between nasal apertures	2.7	2.5	2.7	2.7	2.7	2.6	2.7	2.8
Distance from rostral base to edge of anterior fontanelle	2.9	2.6	3.3	3.5	3.4	3.7	4.0	3.6
Width of anterior fontanelle	3.4	3.3	3.3	3.3	3.2	3.4	3.5	3.2
Width of basal plate at orbital notches	3.6	3.5	3.6	3.4	3.4	3.5	3.5	3.5
Orbit length	2.5	2.4	2.4	2.2	2.2	2.3	2.3	2.3
Preorbital process length	4.2	3.8	3.9	3.2	3.7	4.5	3.8	3.8
Postorbital process length	6.4	5.7	7.0	4.4	8.1	3.9	7.8	4.4
Otic capsule length	2.5	2.6	2.5	2.4	2.3	2.4	2.5	2.5
Great width across suborbital shelves	2.9	2.7	1.6	1.5	1.5	1.6	1.6	1.6
Width across otic capsules	1.6	1.5	1.8	1.8	1.9	1.7	1.9	1.8
Width across preorbital process	1.3	1.2	1.2	1.2	1.2	1.3	1.3	1.2
Width across postorbital process	1.3	1.1	1.2	1.2	-	1.1	1.3	1.1
Width of stapedia fenestra	0.6	0.6	0.7	0.7	0.7	0.7	0.8	0.8
Endolymphatic foramen width	4.2	4.0	5.3	3.9	5.9	4.9	4.3	5.6
Width endolymphatic foramen/perilymphatic foramen	2.0	2.1	2.3	2.2	2.4	2.6	2.2	3.3
Height of the nasal capsule (Preprocess pre-orbital)	0.4	0.4	0.5	0.5	0.4	0.5	0.5	0.5
Height of the nasal capsule (Post-process pre-orbital)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Width optic foramen	1.8	1.7	2.3	2.2	2.1	2.0	2.2	2.0
Width of interorbital region before the anterior semicircular canal	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3

Carcharhinid sharks present a uniform pattern, reflected also in the chondrocranium. *Carcharhinus* chondrocrania are very similar within species with few differences that seem to be consistent (Table II).

Table II. Comparison of the length of stapedial fenestra (A) and the distance between them (B) in *C. falciformis*. Measurements in millimeters.

Specimen number	A	B	%
AC.UERJ 1452	13	31	2.38
AC.UERJ 1453	16	33	2.06
AC.UERJ 1454	14	41	2.15
AC.UERJ 1455	18	37	2.05
AC.UERJ 1456	12	38	2.23
AC.UERJ 1457	19	37	1.90
AC.UERJ 1458	15	35	2.30
AC.UERJ 1459	17	39	2.20

Rostrum (Figures 1-5 and Table I, V)

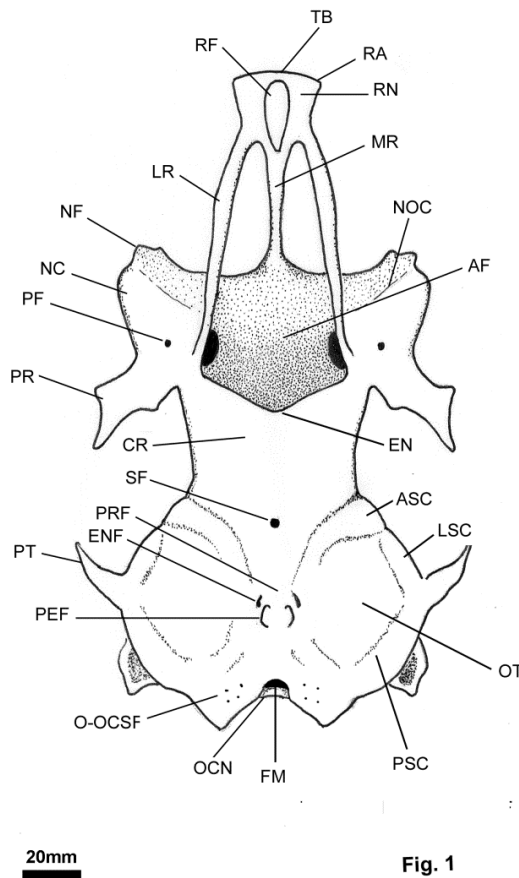


Fig. 1

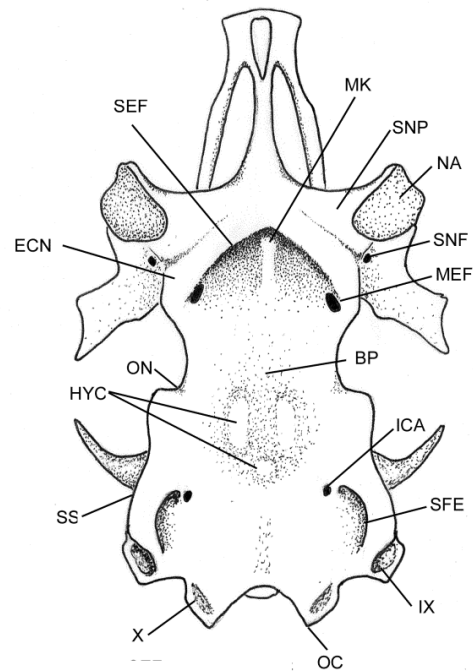


Fig. 2

Figure 1. Chondrocranium, dorsal view. (*C. falciformis* #AC.UERJ 1456, female, 1316 mm TL).

Figure 2. Chondrocranium, ventral view. (*C. falciformis* #AC.UERJ 1456, female, 1316 mm TL).

The limits of the rostrum are from its tip until the posterior margin of the anterior fontanelle (AF) (Figure 1). The rostrum represents 2.2 to 2.5 % in the nasobasal length (1.7 – 3.6 in *Carcharhinus*, 1.7 – 1.9 in *Nasolamia*).

The tripodal rostrum presents the median arched rostral cartilage (MR) which starts ventrally between the nasal capsules (Figures 2, 3, 4, 5). The lateral rostral cartilages (LR) arise from dorsal roof of nasal capsules (NC) near the level of a preorbital foramen, the ophthalmicus profundus nerve foramen (PF) (Figures 1, 3, 4, 5). In lateral view, the lateral rostral cartilage is arched at the level of nasal capsules (Figure 3). The three rostral cartilages come together in the most anterior point of the rostrum forming a platelike rostral node (RN) (Figures 1, 3).

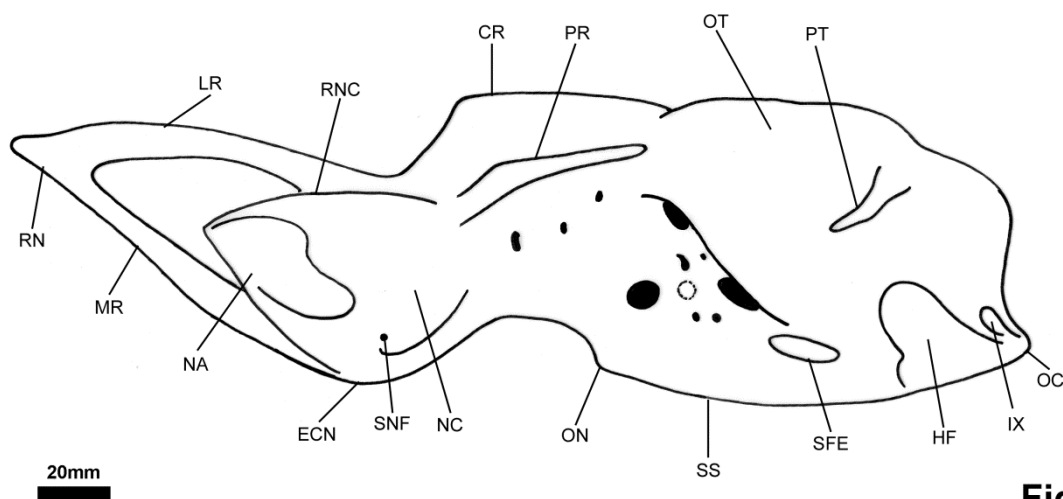


Fig.3

Figure 3. Chondrocranium, lateral view. (*C. falciformis* #AC.UERJ 1456, female, 1316 mm TL).

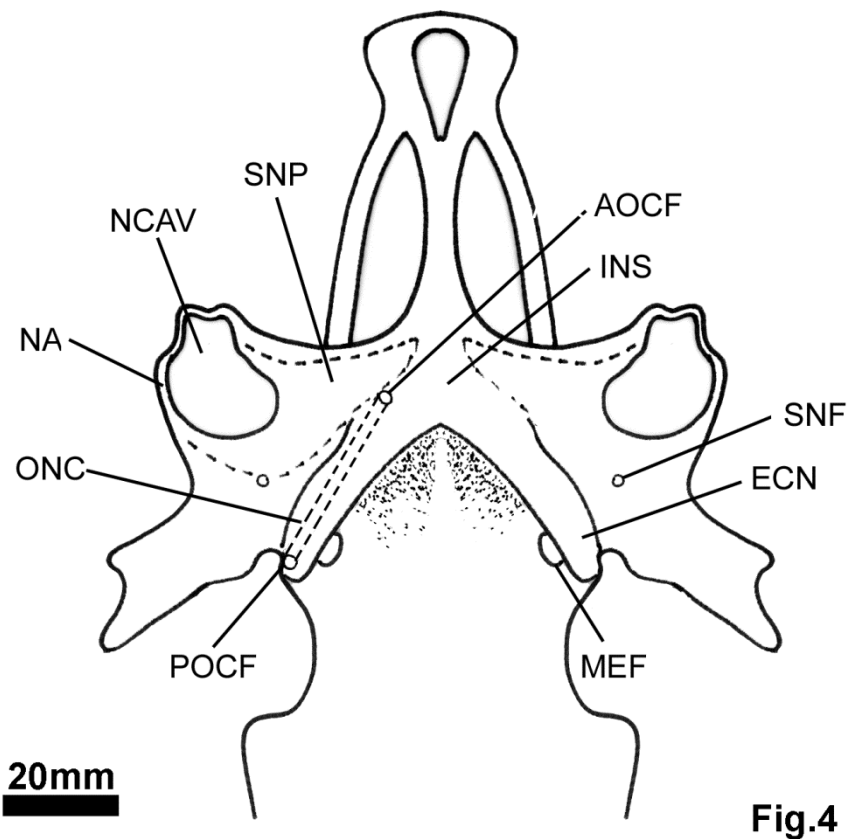


Fig.4

Figure 4. Rostrum ventral view. (*C. falciformis* #AC.UERJ 1456, female, 1316 mm TL. Detail of the nasal capsule region showing the anterior orbitonasal canal foramen (AOCF), the orbitonasal canal (ONC), the posterior orbitonasal canal foramen (POCF) and the subnasal plate (SNP).

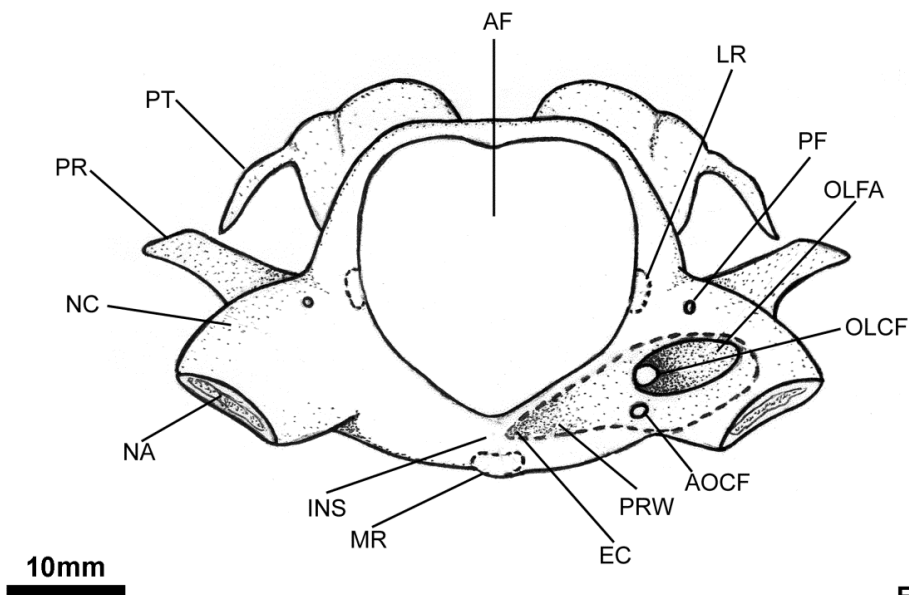


Fig.5

Figure 5. Chondrocranium anterior view. (*C. falciformis* #AC.UERJ 1469, no data. Detail of the nasal capsule region showing the internal structures.

The rostral node presents an inverted tear-shaped rostral fenestra (RF) distally expanded which is limited anteriorly by the transverse bar (TB) (Figures 1, 2). Posteriorly the rostral fenestra is straight, surpassing the point on which the three rostral cartilages are fused. A small rostral appendix (RA) can be present or not (Figures 1, 2).

Posteriorly, the anterior fontanelle (AF) is vertically oval, anteroposteriorly elongated, its width 3.2 - 3.5% in nasobasal length (2.6 - 4.2 in *Carcharhinus*; 4.0 - 4.3 in *Nasolamia*). At the end of the anterior fontanelle (beginning of the cranial roof) the border is medially undulated forming a tenuous epiphisial notch (EN) for the pineal body (Figure 1). Distance from rostral base to edge of anterior fontanelle 2.6 - 4.0 in nasoral length (2.5 - 4.1 in *Carcharhinus*, 2.7 - 4.0 in *Nasolamia*).

Nasal capsules (Figures 1-6; Tables I and V)

Behind and below the lateral rostral cartilages the elongated (quite tubular) nasal capsules (NC) present a transversally oval nasal aperture (NA) not so expanded, confined to their anteroventral surface (Figures 2, 3, 4, 5, 6). Nasal capsules width across nasal capsules 1.4 - 1.7 times in nasobasal length (1.1 - 1.8 in *Carcharhinus*, 1.8 - 2.0 in *Nasolamia*). Distance between nasal apertures 2.5 - 2.8 times the nasal aperture width (1.2 - 2.8 in *Carcharhinus*, 0.67 - 0.75 in *Nasolamia*). Width across the bases of the lateral rostral cartilages 1.4 - 1.7 (1.0 - 3.7 in *Carcharhinus*, 2.5 - 3.8 in *Nasolamia*) (Table V).

Dorsally, the roof of the nasal capsule (RNC, Figure 3) is formed by a thin curved wall and near the base of the preorbital process lies the small *ophthalmicus profundus* nerve foramen (PF) (Figures 1, 5). Ventrally they are limited by the narrow and V-shaped subethmoid fossa (SEF) (Figure 2). At the lateral extremity of the anterior margin there is a nasal flap (NF, Figure 1). The floor of the nasal capsule forms the subnasal plate (SNP, Figures 2, 4, 5). Inside the subnasal plate, between the nasal apertures there is the internasal septum (INS, Figures 4, 5). Near the nasal aperture, the subnasal plate is perforated by the subnasal foramen (SNF, Figures 2, 3, 4).

A low crest appears between the anterior margin of the nasal capsule and the *ophthalmicus profundus* nerve foramen, the nasal dorsal crest (NDC, new term) (Figure 1).

Posteriorly at a ventral view, the nasal capsules are limited medially from the basal plate by the long ectethmoid condyles (ECN), which converge to the direction of the median rostral cartilage (Figures 2, 3, 4).

The preorbital wall (PRW) forms the posterior wall of the nasal capsule as well as the anterior limit of the orbit (Figures 5, 6).

Inside the nasal aperture (taking off the subnasal plate) on the anterior preorbital wall, there is a large olfactory aperture for the olfactory canal (OLFA, new term) which bears the olfactory canal foramen (OLCF, new term) connecting the cerebral and nasal cavities (Figure 5).

Below the olfactory canal foramen, also on the preorbital wall lies the anterior pening of the orbitonasal canal (the anterior orbitonasal canal foramen, AOCF, new term, Figures 4, 5). The posterior orbitonasal canal foramen (POCF, new term) opens on the posterior preorbital wall on the orbital region (Figure 4,). The orbitonasal canal (ONC) runs inside the ectethmoid condyle (ECN) (Figure 4).

Near the internasal septum (INS, Figure 5) the preorbital wall (PRW) forms a shallow pit, the ectethmoid chamber (EC) or fossa (Figure 5).

Cranial roof (Figures 1, 3, 5 and Table I)

The cranial roof (CR Figures 1, 3) includes the coverage of the dorsal cerebral cavity, starting after the anterior fontanelle (AF), ending before the otic region (Figure 1, 5).

Posterior to the mild epiphysial notch (EN), in the central portion of the cranial roof there is a small opening, herein termed supracranial foramen (SF, Figure 1).

Basal plate (Figure 2 and Table I)

The floor of the skull or basal plate (BP) begins anteriorly at the ectethmoid condyles, extending posteriorly to the level of the fenestrae for stapedial (orbital) artery (SFE) under the auditory capsules, narrowing slightly under the hyomandibular facet (HF), ending in the region occiput (Figures 2, 3).

Internally and lateral to the ectethmoid condyle there is a medial ectethmoid foramen (MEF, Figures 2, 4) on the posterodorsal surfaces of the ectethmoid condyles. Between the ectethmoid condyles, in the subethmoid fossa there is a low medial keel (MK) (Figure 2). Width of basal plate at orbital notches 3.4 - 3.6 in nasobasal length (2.4 – 4.5 in *Carcharhinus*, 22,9 – 25, in *Nasolamia*)

The suborbital shelf (SS, Figures 2, 3) is somewhat indented immediately forming a weak orbital notch. Posterior to the weak orbital notch (ON) (Figures 2, 3) (for the articulation of the palaotoquadrate orbital process). Posterior to the orbital notch on each side there is the internal carotid foramen (ICA, Figure 2).

A large aperture, the stapedial fenestrae (SFE) (for the passage of the stapedial artery into the orbit) lies externally besides the internal carotid foramen (Figures 2, 3).

The posterior region of the basal plate between the orbital notch (ON) and the level of the internal carotid foramina (ICA), there are three masses of calcified tesserae, called

hypercalcification (HYC). A pair vertically elongated and an unpaired rounded one (parachordal centers of calcification) (Figure 2).

The width of basal plate across the orbital notch is almost equal to the great width across the orbit (1.04 - 1.16) (Table III).

Table III. Comparison of the width of the basal plate across the orbital notches (**A**) and the great width across the orbits (**B**) in *C. falciformis*. Measurements in millimeters.

Specimen number	A	B	%
AC.UERJ 1452	30	35	1.16
AC.UERJ 1453	33	38	1.15
AC.UERJ 1454	41	43	1.04
AC.UERJ 1455	38	43	1.10
AC.UERJ 1456	38	44	1.15
AC.UERJ 1457	40	46	1.15
AC.UERJ 1458	32	37	1.15
AC.UERJ 1459	39	43	1.10

Orbital region (Figures 3, 6, Tabs. I)

The orbital region is limited anteriorly by the preorbital wall (PRW) and posteriorly by the otic capsules (OT) (Figures 1, 3, 6). Dorsally this region is limited by the cranial roof (CR) that lacks the supraorbital crest and by the suborbital shelf (SS) (Figure 2, 3). Great width across the suborbital shelves 1.5 - 1.9 in the nasobasal length (1.2 - 1.9 in *Carcharhinus*, 1.7 - 1.9 in *Nasolamia*). The orbital wall (OW) is penetrated by foramina for nerves and blood vessels (artery and veins) and presents an optic pedicel (OP) (Figure 6).

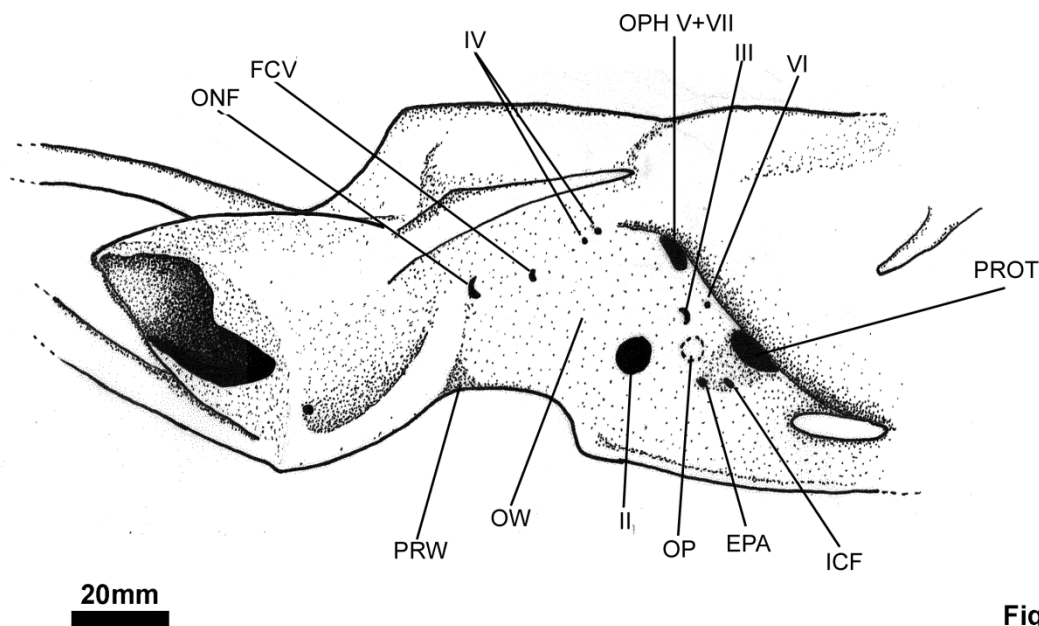


Fig.6

Figure 6. Orbital region structures. Lateral view. (*C. falciformis* #AC.UERJ 1469, no data. Detail of the nasal capsule region showing the anterior orbitonasal canal foramen (AOCF), the ectethmoid chamber or fossa (EC), the lateral rostral cartilages (LR), the olfactory aperture for the olfactory canal (OLFA), the medial rostral cartilage (MR) and the preorbital wall (PRW).

Located almost in the central position of the orbital wall there is the large rounded optic foramen (II). Backwards at the same level, lies the prootic foramen (PROT) for the passage of the rami of the *trigeminus* (the *ophthalmicus profundus* V, the *maxillaris* V, the *mandibularis* V, the *facialis* [= *bucalis* VII] or V+VII) and the *hyomandibularis ramus* of the *facialis*. Upwards, a separated large foramen is the superficial ophthalmic ramus of the *trigeminus* and the *facialis* (OPH V+VII) (Figure 6).

Between the foramen for the OPH V+VII and the prootic foramen, there is the small separated foramen for the abducens nerve (VI). The optic pedicel (OP) is located just posterior and in the same level of the optic foramen. Immediately dorsal to the base of the optic pedicel and just below the OPH V+VII lies the oculomotor foramen (III) (Figure 6).

The foramen for the trochlear nerve (IV) is positioned at the dorsal edge of the orbital wall, in front of the OPH V+VII foramen, dorsal and anterior to the II (Figure 6). Individually, the IV varies in both sides, in number and position. Mostly, two foramina were found. In the specimen AC.UERJ 1457, four foramina were found in the left side and two in the right.

The orbitonasal canal (ONC) penetrates the preorbital wall at the anterior orbitonasal canal foramen (AOCF) to end in the posterior orbitonasal canal foramen (POCF) (Figures 4, 6),

immediately posterior to the nasal capsule. From this foramen passes the anterior facial vein. Posterior to the orbitonasal foramen lies the foramen for the anterior cerebral vein (FCV) (Figure 6).

The interorbital canal foramen (ICF) is located downwards between the base of the optic peduncle and the prootic foramen. It serves for the passage of the pituitary vein in the *sella turcica* draining the cerebral cavity into the orbital sinus (Figure 6).

At the same level of the interorbital canal foramen and just in front of it lays the efferent pseudobranchial artery foramen (EPA), also laying in line below the abducens foramen (VI) (Figure 6).

Otic capsule (Figures 1, 3 and Tabs.

The rounded otic capsule (OT, Figures 1, 3) starts after the orbital wall, ending in front of the occiput. Three semicircular canals surround the otic capsule: the anterior semicircular canal (ASC), the lateral semicircular canal (LSC) and the posterior semicircular canal (PSC) (Figure 1). The otic capsule length 2.3 - 2.6 in the nasobasal length (1.6 - 2.5 in *Carcharhinus*, 2.3 - 2.7 in *Nasolamia*). Width across the otic capsules 1.5 - 1.9 (1.6 - 1.8 in *Carcharhinus*, 1.7 - 1.9 in *Nasolamia*).

Under the lateral semicircular canal there is a cylindrical postorbital process (PT, Figures 1, 3, 5).

Immediately after the supracranial foramen there is a deep parietal fossa (PRF), between the otic capsules. Anterolaterally to the otic capsules there is a pair of small circular endolymphatic foramina (ENF). The posterior pair are relatively larger and almost oval, named perilymphatic foramen (PEF) (Figure 1).

Occiput (Figures 1-3)

The largest aperture of the region is the foramen magnum (FM), positioned upwardly to the occipital centrum (OCN). Laterally from each occipital condyle there is the foramen for the vagus nerve (X). Lateral to the vagus nerve foramen, positioned posteriorly to the hyomandibula facet (HF) there is a glossopharyngeal foramen (IX). Two developed occipital condyles (OC) arise laterally from the foramen magnum.

In some specimens, the posterior wall of the occipital/otic regions presents perforations near the condyles (fused vertebral foramina), the otico-occipital spinal foramina (O-OCSF, Figure 1).

Terminology

Rostrum: Gegenbaur (1872), Wells (1917), Beer (1926), Lazier (1947), Hulley (1972) and Maisey (1980).

1) Rostral fenestrae (RF): (Compagno & Garrick 1983).

2) Anterior fontanelle (**AF**): (Gegenbaur 1872; Parker 1878; Wells 1917; Hulley 1972; Compagno & Garrick 1983; and Compagno 1988).

Synonymies:

- prefrontal fontanella (Holmgren 1941).
- precerebral fossa (Shirai 1992).
- precerebral fontanelle (Lane 2010).

OBS: For Hamdy (1960) it is important to name crania fontanelles according to their morphological relations with cranial cavity. The author presents four types of cranial fontanelle in elasmobranchs: the anterior supracranial, precerebral, posterior supracranial and supracranial. The last two are found in batoids. The author divides the fontanelle into two regions according to their position in relation to the cranium. The one located on the top of the cranium roof is called supracranial (anterior or posterior depending on their position on the cranium), while the anterior one is called precerebral, by its position outside (anterior) the braincase. Following this line of argument, the cranium of *Carcharhinus* (as well as in Carcharhiniformes) exemplified by *Triaenodon obesus* (Hamdy 1960, Figures 1 – 3) presents a precerebral fontanelle and an anterior supracranial fontanelle. While agreeing with these arguments, to simplify, we prefer to use the term “anterior fontanelle”.

Hulley (1972) discussing this subject affirms that the anterior fontanelle is an amalgamation of the precerebral fontanelle and the anterior supracranial fontanelle. The posterior fontanelle should therefore be correctly termed the posterior supracranial fontanelle (El-Toubi & Hamdy 1959; Hamdy 1960).

3) Epiphisial notch (**EN**) (Compagno 1988).

Synonymies:

- median notch (Wells 1917).
- epiphisial foramen (Lazier 1947).
- anterior supracranial fontanelle (Hamdy 1960).

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Nasal Capsules (NC): (Wells 1917; Compagno & Garrick 1983; Shirai 1992; Hulley 1972).

Synonymies:

- nasal sac (Parker 1878).
- olfactory capsule (Lazier 1947).

OBS: The nasal capsules in fishes develop in continuity with the front end of the embrionic trabecula (Goodrich 1958).

1) *Ophthalmicus profundus* nerve foramen (**PF**) - (Wells 1917; Compagno & Garrick 1983).

Synonymies:

- foramen for profundus nerve (Holmgren 1941).
- foramen for deep ophthalmic nerve (Lazier 1947).
- ethmoidal nerve foramen (Hulley 1972).
- profundus foramen (Compagno 1973)

2) Nasal cavity

Synonymies:

- *cavum nasi* (Goodrich 1958).

3) Nasal apertures (**NA**) (Compagno & Garrick 1983).

Synonymies:

- external naris (Wells 1917).
- fenestra narina (Goodrich 1958).

4) Subnasal plate (**SP**) (Compagno 1988).

5) Internasal septum (**INS**) (Compagno 1988).

Synonymy:

- internasal keel (Lane 2010).

6) Subnasal foramen (**SF**) (Compagno 1988).

7) Preorbital wall (**PRW**) (Compagno 1988).

8) Olfactory aperture for the olfactory canal (**OLFA**, new name).

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9) Olfactory canal (**OLF**) (Compagno 1988).

10) Ectethmoid chamber (**EC**) (Compagno 1988).

11) Anterior orbitonasal canal foramen (**AOCF**, new name).

Synonymies:

- ecthmoidal chamber (Compagno 1988).
- ecthmoidal fossa (Compagno 1988).

OBS: For the anterior orbitonasal canal foramen Compagno (1988) named it as ecthmoidal chamber or fossa. No chamber (nor fossa) was found herein. Compagno's definition as "anterior opening of the orbitonasal canal" represents a foramen rather than a fossa or chamber, being the name proposed by him not correct.

12) Posterior orbitonasal canal foramen (**POCF**, new name).

Synonymies:

- orbito-nasal canal foramen (Hulley 1972).
- orbitonasal foramen (Lazier 1947; Waller & Baranes 1991; Compagno 1988).

13) Orbitonasal canal (**ONC**) (El-Toubi & Hamdy 1967; Gohar & Mazhar 1964; Compagno 1973, 1988; Waller & Baranes 1991).

Synonymy:

- oro-nasal canal (Hulley 1972).

14) Lateral rostral cartilage (**LR**) (Compagno 1988).

15) Median rostral cartilage (**MR**) (Compagno 1988).

Synonymies:

- prenasal cartilage (Parker 1878).
- basitrabecular cartilage (Parker 1878).

Cranial Roof (CR): (Compagno 1988).

Synonymies:

- *tegmen cranii* (Parker 1878),

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- deck (Parker 1878).
- brain case (Wells 1917).
- dorsal surface (Hulley 1972).

1) Supracranial foramen (**SF**).

Synonymies:

- epiphysial foramen (Wells 1917; Devillers 1958).
- epiphysial fontanelle (Goodrich 1958).
- frontal fenestrae (Compagno 1973, 1988; Compagno & Garrick 1983).
- frontal fontanelle (Compagno 1973, 1988).

OBS: For the position of the supracranial foramen in *Squalus*, Devillers (1958) termed it as epiphysial foramen while Hamdy (1960) used it for *Squatina*. The epiphysial foramen according to Compagno (1988) occurs just behind the anterior fontanelle as seen in *Carcharhinus macroti*. Compagno (1973, 1988) instead of supracranial foramen, termed frontal fenestrae or even frontal fontanelle. Fenestra and fontanelle are wide apertures which do not happen in *Carcharhinus*, indeed represented by a small foramen. Compagno & Garrick (1983) and Compagno (1988) termed frontal fenestra although this term also seems inappropriate. Foramen is a small aperture, a better choice for this structure.

Basal Plate: (Gegenbaur 1872 [=Basalplatte]; Wells 1917; Beer 1926; Compagno & Garrick 1983).

Synonymies:

- basis cranii (Parker 1878).
- ventral surface (Hulley 1972).

1) Ectethmoid condyle (**ECN**) (Compagno & Garrick 1983).

OBS: Although the ectethmoid condyles are described as being a component of the nasal capsules, the medial ectethmoid foramen opens in the basal plate, being described in that region.

According to Compagno (1973, 1988) there are two types of ectethmoid foramen in accordance with its position through the ectethmoid condyle. The first one, the medial ectethmoid foramen, is found in *Carcharhinus* and other Carcharhinini (*sensu* Compagno 1988). It opens on the inner surface of each ectethmoid condyle. In the second type, the lateral ectethmoid foramen, opens on the outer surface of each condyle. This type is found in Sphyrnids and *Rhizoprionodon*.

The position of the medial ectethmoid foramen varies. In *Rhizoprionodon*, for example, this foramen lies anteriorly on the ectethmoid condyle, while in *Carcharhinus* it is located posteriorly (Compagno 1983, 1988; Gomes & Siqueira 1995).

Probably a more appropriate terminology could provide a reference to the position of these foramina (internal or external) on the ectethmoid condyle. Instead of a medial ectethmoid foramen it should be named internal ectethmoid foramen, being anterior or posterior as the case. The same serves to the lateral ectethmoid foramen that could be termed external ectethmoid foramen.

2) Subectethmoid fossa (**SEF**)

Synonymies:

- subethmoidal depression (Hulley 1972).

3) Medial ectethmoid foramen (**EF**) (Compagno 1988).

Synonymies:

- foramen for orbitonasal vein (Holmgren 1941).
- ectethmoid foramen – (Compagno 1983).

4) Orbital notch (**ON**) (Compagno, 1973, 1988).

Synonymies:

- notch for orbital process of palatoquadrate (Compagno & Garrick 1983)

5) Stapedial fenestra (**SFE**) (Compagno 1988).

Synonymies:

- foramen for orbital artery (*carotis externa*) (Holmgren 1940, 1941).
- fenestra for stapedial (orbital) artery (Compagno & Garrick 1983).

6) Medial keel in subethmoid fossa (**MK**) (Compagno & Garrick 1983; Compagno 1988).

7) Foramen for internal carotid artery (**ICA**) - (Beer 1926; Lane 2010).

Synonymies:

- internal carotid artery (Wells 1917).
- canal for carotid artery (Wells 1917).
- foramen of arteria carotis interna (Holmgren 1941).

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8) Suborbital shelf (**SS**): (Compagno & Garrick 1983; Compagno 1988; Carvalho & Maisey 1996; Lane 2010).

Synonymies:

- subocular shelf (Beer 1926).
- subotic shelf (Shirai, 1992).

Obs: for Carvalho & Maisey (1996) “The subotic shelf is the posterior continuation of the suborbital shelf, lying ventral to the orbital fissure and pierced by the orbital artery foramen (= Holmgren’s [1940, 1941] “*carotis externa*”). According to these authors, the subotic shelf originates from a separate chondrification center enclosing the orbital artery and joining with the parachordal chondrification forming a continuous shelf as pointed by Holmgren (1940) and Beer (1937) as cited by Carvalho & Maisey (1996).

Orbital Region

1) Orbital wall (**OW**) (Compagno 1988).

Synonymies:

- orbit (Wells 1917; Hamdy 1964).
- interorbital wall (Shirai 1992).

Carcharhinus falciformis followed Holmgren (1941) (his third arrangement), Miyake (1988) (his fourth type), and Shirai (1992) (*i.e.* a separate foramina for OPH V+VII). This arrangement was found by Holmgren (1941) in *Carcharhinus*, *Galeocerdo*, *Galeus*, *Mustelus canis* and *Orectolobus* while Gohar & Mazhar (1964) and Hamdy (1964) presented a different disposition in *C. brevipinna* (probably a wrong interpretation).

2) Superficial ophthalmic ramus of the trigeminus and the facialis (**OPH V+VII**).

Synonymies:

- orbitocerebral foramen for the superficial ophthalmic nerve (Compagno 1988).
- orbitocerebral foramen for the supraorbital trunk (Compagno 1988).

3) Foramen for the abducens nerve (**IV**) (Compagno 1988).

4) Optic pedicel (**OP**) (Wells 1917; Compagno 1988).

Synonymies:

- optic stalk (Hulley 1972).
- optic peduncle (Compagno 1973)

- eye stalk (Silva & Carvalho 2011)

5) Oculomotor foramen (**III**): (Wells 1917; Compagno 1988).

Synonymies:

- foramen for oculomotor nerve (Beer 1926).
- oculomotor nerve foramen (Hulley 1972).

6) Foramen for the trochlear (**IV**) (Beer 1926; Compagno 1988).

Synonymies:

- trochlear foramen (Lazier 1947).
- pathetic nerve foramen (Hulley 1972).
- trochlear nerve foramen (Hulley 1972).

OBS: the position of VI given by Heintz (1962) is erroneous and has obviously been misunderstood with the anterior cerebral vein.

According to Hulley (1972) this has been incorrectly termed the abducent foramen by Heintz (1962) although it has been labeled as IV in the figure (Figure 2b)

El-Toubi & Hamdy (1967) describing the cranium of *Rhizoprionodon acutus* (as *Mustelus manazo*) and Gomes & Siqueira (1995) describing and comparing *Rhizoprionodon lalandii* and *R. porosus*, made erroneous descriptions for the foramina III, IV and VII being successively corrected in the present study as foramen for anterior cerebral vein (not III), oculomotor (not IV) and trochlear (not *facialis*)

In modern elasmobranchs the trochlear foramen also can be located dorsally above the optic foramen as seen in *Galeus* (Gegenbaur 1872), *Gogolia filewoodi* (Compagno 1973), *Paragaleus pectoralis* (Compagno 1988), *Heterodontus* and also †*Synechodus* (Maisey 1985). In Rajidae (Stehmann 1970; Hulley 1972; Leible 1988; Carvalho et al. 2005; Moreira et al. 2011) the position of the trochlear can be posterior (*Cruriraja*, *Rajella*, *Malacoraja*) or dorsal to the optic foramen (*Bathyraja*, *Dipturus*, *Raja*, *Rostroraja*, *Amblyraja* or both (*Rioraja* and *Atlantoraja*, personal observation, see material and methods).

7) Posterior orbitonasal foramen (**POCF**, new name).

Synonymies:

- opening into nasal capsule (Wells 1917).
- orbitonasal canal (Lane 2010).

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8) Foramen for the anterior cerebral vein (**FCV**) (Hulley 1972 = anterior cerebral vein foramen).

Synonymy:

- anterior cerebral vein (Lane 2010).

9) Interorbital canal foramen (**ICF**) (Beer 1926; Compagno 1973, 1988).

Synonymies:

- transbasal canal (Wells 1917; Shirai 1992)
- interorbital canal (Lazier 1947).
- pituitary vein foramen– (El-Toubi & Hamdy 1959).
- interorbital vein foramen (Hulley 1972).

10) Efferent pseudobranchial artery foramen (**EPA**) (Beer 1926; Hamdy1960; Gohar & Mazhar 1964).

Synonymy:

- afferent pseudobranchial artery foramen (Hulley 1972).

11) Abducens foramen (**VI**) (Compagno 1988).

Synonymy:

- abducent foramen (Lazier 1947).

12) Foramen for the internal carotid artery (**ICF**).

Synonymy:

- efferent spiracular foramen (Compagno 1988).

13) Preorbital process (**PR**) (Wells 1917).

Synonymies:

- preorbital extension (Gegenbaur 1872 = Praeorbitalfortstaz).
- praeorbital cartilage (Parker 1878).
- antorbital process (Wells 1917, Maisey1980).
- preorbital cartilage (Holmgren 1940,1941).
- crista praeorbitalis (Heintz 1962).
- praeorbital process (Hulley 1972).
- antorbital (ectethmoid) process (Carvalho & Maisey 1996).

OBS:1- Wells (1917) in her text presented this process abbreviated as “p.a.” but in Plate 1, Figure 1. abbreviation is “a.p.”. Also, for the same structure she used two names (preorbital and antorbital process).

2- Carvalho & Maisey (1996) analyzing this structure considered the existence of confusion in the literature concerning the terms “antorbital process”, “ethmoid process” and “preorbital process”. According to them, the posterior nasal wall (orbitonasal lamina) of many chondrichthyans (both recent and fossil) contains lateral projections of varying developmental stages that can be recognized as one of these three cited structures. For example, when the projection is in dorsal position (as in *Carcharhinus*) it is called preorbital process. When it is ventral to nasal capsules, orbit and orbitonasal canal: ectethmoid or antorbital process (see discussion in Carvalho & Maisey 1996:17-22). The antorbital cartilage in Batoids is located on outer side of nasal capsules, separated from the neurocranium as seen in Garman (1913) and Nishida (1990). For this matter, Carvalho & Maisey (1996) consider this structure a further modification of the ectethmoid process.

14) Postorbital process (**PT**) (Wells 1917; Lazier 1947; Maisey 1980; Hulley 1972; Lane 2010).

Synonymy:

- postorbital extension (Gegenbaur 1872 = Postorbitalfortsatz).

Otic Capsule – (Wells 1917; Lane 2010).

Synonymies:

- labyrinth-Region (Gegenbaur 1872).

- auditory sac (Parker 1878).

- ear balls (Parker 1878).

- auditory capsule (Beer 1926; Hulley 1972).

1) Parietal fossa (**PRF**) (Gegenbaur 1872 = *fossa parietalis*; Lazier 1947; Compagno & Garrick 1983; Lane 2010).

Synonymies:

- Perietallöcher (Gegenbaur 1872).

- fossa endolymphaticus (Wells 1917).

- endolymphatic fossa (El-Toubi & Hamdy 1959).

- parietal depression (Hulley 1972).

2) Endolymphatic foramen (**ENF**) (Lazier 1947; Hulley 1972; Compagno 1988; Lane 2010).

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Synonymy:

- foramen for the endolymphatic duct (Wells 1917).

3) Perilymphatic foramen (**PEF**) (Hulley 1972).

Synonymies:

- opening for the perilymphatic duct (Wells 1917).

- fenestra of parietal fossa (Lazier 1947).

4) Anterior semicircular canal (**ASC**) (Parker 1878; Lane 2010)

Synonymies:

- superior semicircular canal (Wells 1917).

- proeminence of anterior semicircular canal (Lazie, 1947).

5) Lateral semicircular canal (**LSC**)

Synonymies:

- horizontal semicircular canal (Parker 1878; Wells 1917).

- prominence for horizontal semicircular canal (Lazier 1947).

- external semicircular canal (Lane 2010).

6) Posterior semicircular canal (**PSC**) (Parker 1878; Wells 1917; Lane 2010).

Occiput

Synonymies:

- occipital-region (Gegenbaur 1872).

- super occipitalis (Parker 1878).

OBS: Only Parker (1878) presented a subregion of the occipital region. The super occipitalis is the wall located immediately above the foramen magnum.

1) Foramen magnum (**FM**) (Gegenbaur 1872; Wells 1917; Lazier 1947; Hulley 1972; Compagno & Garrick 1983; Compagno 1988; Lane 2010).

Synonymies:

- foramen occipitale (Gegenbaur 1872).

2) Occipital centrum (**OCN**) (Compagno 1988; Compagno & Garrick 1983).

3) Foramen for the vagus nerve (**X**): Vagusloch (Gegenbaur 1872; Wells 1917)

Synonymy:

- passage for the vagus (Parker 1878).

4) Glossopharyngeal foramen (**IX**) (Wells 1917; Beer 1926; Lazier 1947).

Synonymies:

- passage for the glosso-pharyngeal (Parker 1878).
- foramen for the ninth-nerve (Wells 1917).
- foramen for the glossopharyngeal nerve (Wells 1917).
- glossopharyngeal nerve foramen (Hulley 1972).

5) Otico-Occipital spinal foramina (**O-OCSF**).

Synonymies:

- spino-occipital foramina (Goodrich 1958).
- occipito-spinal nerve (Goodrich 1958).

OBS: Goodrich (1958) presents two types of spinal foramina fused to neurocranium in *Hexanchus*, the anterior ones (the spino-occipital foramina) and the posterior, near the occipital condyles (occipito-spinal nerves foramina). This author affirms that while the side walls of the brain case is on formation, it shows signs of segmentation in the occipital region. This segmentation forms the occipital arch, which fuses above the auditory capsule surrounding the foramen magnum.

According to Bemis & Forey (2001), the occipital region is formed by a variable number of vertebral segments that become incorporated into the definitive skull. The number of vertebral segments varies according to the actinopterygians taxa (object of their studies). The vertebral elements fusing to the skull in sharks were described by El-Toubi (1949) for *Squalus acanthias* as reported by Bemis & Forey (2001).

Also those foramina was verified in *Etmopterus* by Holmgren (1940) named foramen “y” and “z”.

6) Occipital condyles (**OCN**) (Gegenbaur 1872 = condylus occipitalis Parker 1878; Wells 1917; Hulley 1972).

Synonymy:

- occipital cotylus (Lane 2010).

IV. Individual character

Individual character was found in the epiphysial notch (**EN**). Even though being weak (Figure 7) it can be almost indistinguishable in some specimens (Figure 8). Also the number of the trochlear nerve presented individual number in the same or different specimens (from 1 - 4).

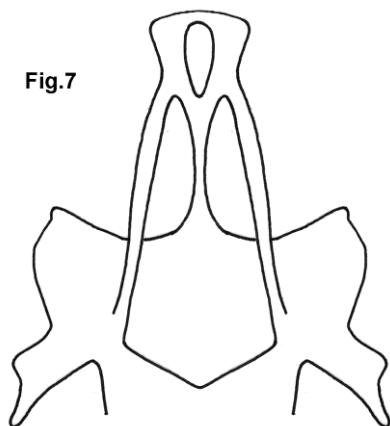


Figure 7. Individual character observed in the epiphysial notch (*C. falciformis* AC.UERJ 1457, male, showing the presence of a mild epiphysial notch (EN).

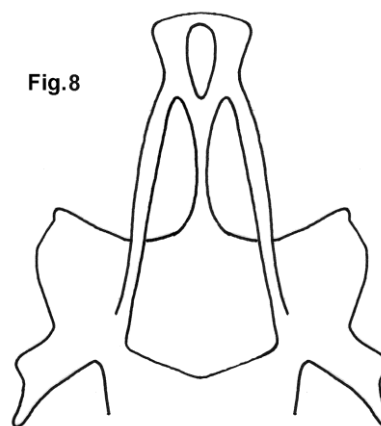


Figure 8. Individual character observed in the epiphysial notch (*C. falciformis* AC.UERJ 1459, female, 1283 mm TL. Showing an almost indistinguishable epiphysial notch).

V. Chondrocrania characters used in *Carcharhinus* taxonomy

According to the literature as well as to present observations, some structures seem to present an individual character rather than a specific one (possible ontogenetic rather than sexual) and should be looked with attention.

The morphology of the rostral node, the development of the rostral fenestrae, the preorbital process and the subethmoidal fossa, the presence or absence of the epiphysial notch (and its variations) as well as the presence or absence of the supracranial foramen, and epiphysial foramen and the position of the nasal capsules, as well as the presence or not of a nasal flap showed possible diagnostic characters (Table IV).

Table IV. Crania characters among *Carcharhinus* species useful in taxonomy species based in Holmgren (1940), Gohar & Mazhar (1964), Compagno & Garrick (1983), Compagno (1988) and present work.

	RN	rostral dimension	RF	AF	EN	EF	NC direction	PR shape	NF	SEF
albi	indented	median	+	Vshaped	-	-	angular	triangular	+	Vshaped
ambl	convex	median	+	Vshaped	-	-	angular	triangular	+	Ushaped
ambo	indented	short	-	Vshaped	+	-	angular	triangular	-	Ushaped
born	rounded	median	+	rounded	-	-	angular	triangular	+	Ushaped
falc	rounded	median	+	Vshaped	+	-	angular	triangular	+	Vshaped
gala	straight	median		Vshaped	-	-	angular	triangular	+	Ushaped
hemi	indented	short	-	straight	+	-	angular	triangular	-	Ushaped
isod	indented	median	-	Vshaped	-	-	angular	triangular	+	Ushaped
leuc	indented	short	-	Vshaped	-	-	straight	pin shaped	-	Ushaped
limb	straight	long	+	Vshaped	+	-	angular	triangular	+	Ushaped
long	indented	short	-	Vshaped	+	-	angular	rectangular	-	Ushaped
macl	rounded	long	+	rounded	-	+	angular	rectangular	+	Vshaped
mela	indented	short	-	Vshaped	-	-	angular	triangular	-	Ushaped
obsc	rounded	median	-	Vshaped	+	-	angular	rectangular	+	Ushaped
poro	rounded	median	+	straight	+	-	straight	triangular	+	Vshaped
seal	rounded	median	+	rounded	-	-	angular	triangular	-	Vshaped
sign	rounded	long	+	Vshaped	+	-	angular	triangular	+	Ushaped
sorr	straight	median	+	rounded	-	-	angular	triangular	+	Vshaped
moss	indented	short	+	?	?	?	straight	triangular	-	Ushaped

albi = *C. albimarginatus*, **ambl** = *C. amblyrhynchoides*, **ambo** = *C. amboiensis*, **born** = *C. bornensis*, **falc** = *C. falciformis*, **gala** = *C. galapagensis*, **hemi** = *C. hemiodon*, **isod** = *C. isodon*, **leuc** = *C. leucas*, **limb** = *C. limbatus*, **long** = *C. longimanus*, **macl** = *C. macloiti*, **mela** = *C. melanopterus*, **moss** = “*C. mossambicus*”, **obsc** = *C. obscurus*, **poro** = *C. porosus*, **seal** = *C. sealei*, **sign** = *C. signatus*, **sorr** = *C. sorrah*. **AF** = anterior fontanelle (posterior margin), **EF** = epiphisial foramen, **EN** = epiphisial notch, **NC** = nasal capsule, **NF** = nasal flap, **RF** = rostral fenestra, **RN** = rostral node, **SEF** – subethmoid fossa. (+) = present. (-) = absent.

The rostral node (RN) can be indented (in *C. amblyrhynchoides*, *C. hemiodon*, *C. amboiensis*, *C. melanopterus*, *C. leucas*, *C. longimanus*, Figure 9) or not (the other species, Figure 10) The indentation can be mild as in *C. amblyrhynchoides* in which the rostral fenestrae is still present (Figure 11) or highly indented without rostral fenestrae (as in *C. albimarginatus* (Figure 12).

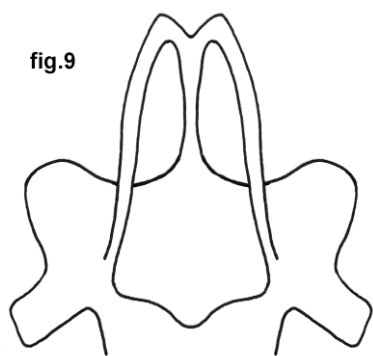


Figure 9. Rostral node in *C. longimanus* (modified from Compagno 1988).

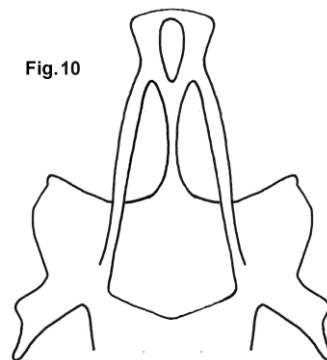


Figure 10. Rostral node in *C. falciformis* (modified from Compagno 1988) (*C. falciformis* #AC.UERJ 1458, male, 1131 TL).

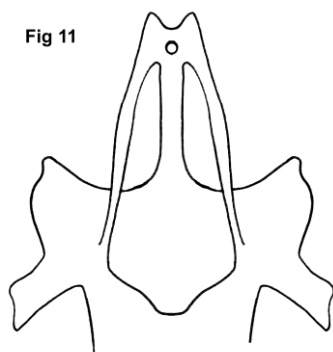


Figure 11 Rostral node in *C. amblyrhynchoides* (modified from Compagno 1988).

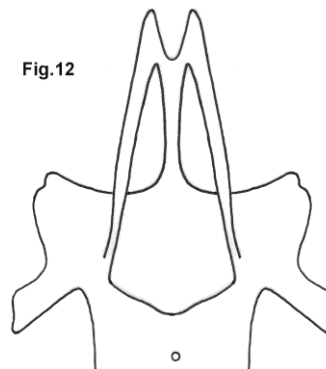


Figure 12. Rostral node in *C. albimarginatus* (modified from Compagno 1988).

The rostral node in *C. macroti* is massive not indented provided with hypercalcification (Figure 13)

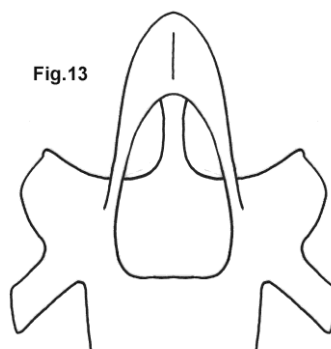


Figure 13. Rostral node in *C. macroti* (modified from Compagno 1988).

The rostral fenestra can be well developed (as in *C. obscurus*, *C. falciformis* (Figure 10) *C. galapagensis*, *C. sorrah*, *C. limbatus*, *C. plumbeus*) or reduced (*C. isodon*, *C. sealei* (Figure 14), *C. porosus* *C. borneensis*, *C. signatus*).

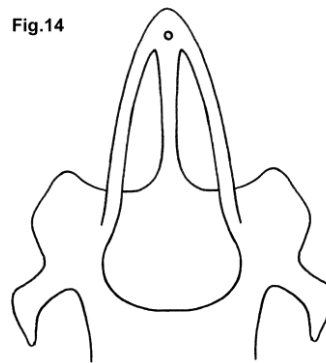


Figure 14. Rostral fenestra in *C. porosus*, *C. borneensis*, *C. signatus* (modified from Compagno 1988).

The preorbital process in most species is flat with a subtriangular extremity (Figure 1). In *C. longimanus* (Figure 9), *C. obscurus* and *C. plumbeus* (AC.UERJ 526) the subtriangular extremity can be absent. In *C. leucas* the preorbital process is reduced being similar to the postorbital process (Figure 15).

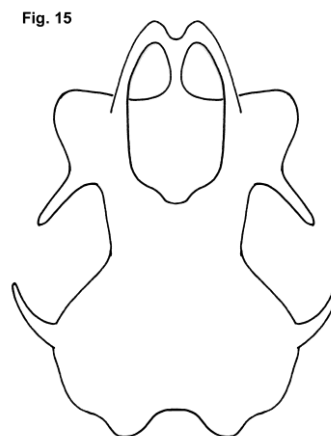


Figure 15. Preorbital process in *C. leucas* (modified from Compagno 1988).

The subethmoidal fossa can be V-shaped (ex. *C. macroti*, *C. falciformis*, *C. albimarginatus*, *C. sealei*, *C. signatus*, *C. plumbeus*, *C. sorrah* (Figure 16) or U-shaped (the other species, ex. Figure 17).

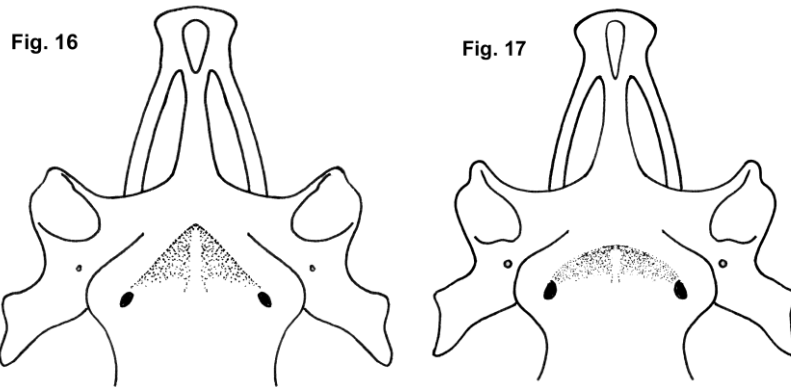


Figure 16. Subethmoidal fossa in *C. macroti*, *C. falciformis*, *C. albimarginatus*, *C. sealei*, *C. signatus*, *C. plumbeus*, *C. sorrah*.

Figure 17. Subethmoidal fossa in others species. The epiphysial notch can be well evident in *C. porosus*, *C. hemiodon* and *C. signatus* (Figure 18).

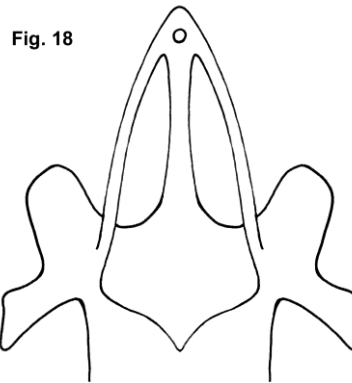


Figure 18. Epiphysial notch in *C. porosus*, *C. hemiodon* and *C. signatus* (modified from Compagno 1988). (*C. falciformis* #AC.UERJ 1452).

The taxonomic position of *Nasolamia*, however, formerly belonging to *Carcharhinus* and separated from this genus by having the distance between the nasal capsules greatly reduced, is called into question since it shares almost all cranial proportions with *Carcharhinus*. As seen in Table V, almost all percentages fits *Nasolamia* in *Carcharhinus*. Some characters as the position of nasal capsules, presence or not of a rostral fenestra (individual variation), V-shaped subethmoidal fossa, stapedial fenestra, pre- and pos orbital processes are similar to *Carcharhinus* chondrocrania.

Table V. Comparision between chondrocrania measurements (in % of nasobasal length, except in [*]) between *Nasolamia* and *Carcharhinus* (based in Compagno 1988).

Measurements	<i>Nasolamia</i>	<i>Carcharhinus</i>
Rostral length	1.7-1.9	1.7-3.6
Width across bases of lateral rostral cartilages	2.5-3.8	1.0-3.7
Nasal capsule width X their length [*]	0.7	0.7-1.5
Width across nasal capsule	1.8-2.0	1.1-1.8
Width between nasal apertures	0.67 – 0.75	1.2-2.8
Subethmoid fossa as wide as long [*]	0.67	0.5-1.0
Postorbital process length	5.0-7.0	3.3-6.7
Great width across suborbital shelves	1.7 -1.9	1.2-1.9

The posterior margin of the anterior fontanelle may be present an advance or entry toward the cranial roof (being a mild epiphysial notch) as seen in *C. falciformis* (Figure 1), *C. obscurus*, *C. amblyrhynchoides*, *C. albimarginatus*, *C. galapagensis*, *C. isodon*, *C. amboiensis*, *C. melanopterus*, *C. limbatus*, *C. leucas*, *C. longimanus*.

In *C. plumbeus*, *C. borneensis* and *C. sorrah* and *C. macloiti* and *C. sealei* (Figure 14) the epiphysial notch is absent. In *C. macloiti* (Figure 13) instead, there is a small epiphysial foramen (absent in all other *Carcharhinus*).

The position of the nasal capsule can be quite acute in most species (ex. *C. signatus*, Figure 18), and almost at a right angle in *C. porosus* and *C. leucas* (Figure 15).

A small nasal flap was found in *C. obscurus*, *C. falciformis* (Figure 1), *C. albimarginatus*, *C. galapagensis*, *C. plumbeus* and *C. limbatus*. In *C. macloiti*, *C. amblyrhynchoides*, *C. isodon*, *C. sealei*, *C. porosus*, *C. borneensis*, *C. hemiodon*, *C. sorrah*, *C. signatus*, *C. amboiensis*, *C. melanopterus*, *C. longimanus* and *C. leucas* (Figure 15) the nasal flap is absent.

The supracranial foramen was found in *C. falciformis* in all specimens of the present study (Figure 1) (but absent in Compagno's 1988 Figure 19B), *Carcharhinus longimanus* (AC.UERJ 1470, absent in Compagno's 1988, Figure 19B) and *Carcharhinus obscurus*. This foramen was also found in *C. albimarginatus* and absent in other species.

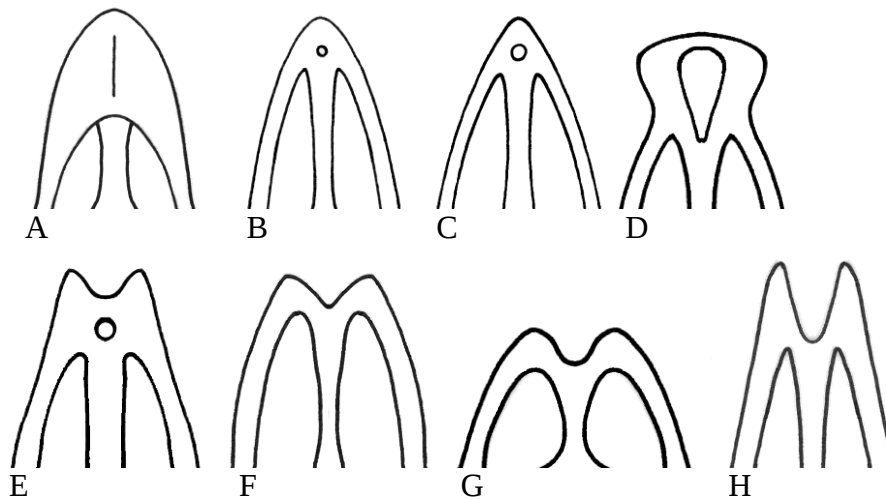


Figure 19. Morphological plasticity of the rostral node among species: A. *Carcharhinus macroti*; B. *C. sealei*; C. *C. porosus*; D. *C. falciformis*; E. *C. amblyrhynchoides*; F. *C. longimanus*; G. *C. leucas*; H. *C. albimarginatus* (modified from Compagno 1988).

Discussion

Compagno (1988) also defined *Carcharhinus* crania by the following characters: the nasal capsule's anterior margin varying from straight to strongly arched anterolaterally; width across nasal capsules 1.1 – 1.8 times in nasobasal length; nasal apertures oval and confined to anteroventrolateral surfaces of nasal capsules; distance between nasal apertures 1.2 – 2.8 times the nasal aperture widths; ectethmoid condyles low to high but not crestlike; orbitonasal foramina, visible in dorsal view of cranium or not; subethmoid fossa narrow to fairly broad, with its length 0.5 – 1.0 times in width; anterior fontanelle without an anteriorly directed dorsal flare, with or without a small epiphysial foramen; basal plate varying from very broad and somewhat wider than the distance across the orbital walls above it (*C. leucas*, *C. amboinensis*) to narrow and down to 0.67-0.75 of this distance (*C. sorrah*, *C. signatus*, *C. macroti*), but in most species the basal plate width is about equal to the distance across the orbital walls. Width across basal plate 2.4-4.5 in nasobasal length; stapedial fenestrae usually very large and longitudinally elongated, with their lengths greater than width of suborbital shelves lateral to them; stapedial fenestrae length 2.0-5.5 times in distance between them; internal carotid foramina close to the anterior ends of the stapedial fenestrae and separated from them by a space from less than one to three times the width of the stapedial fenestrae; basal plate moderately to strongly arched in lateral view, with or

without a weak keel just posterior to the subethmoid fossa; preorbital processes usually relatively large, rectangular to triangular and with narrow bases

The cranium of *C. falciformis* is typical Carcharhininae and Carcharhinini *sensu* (Compagno 1988). Most of the cranial proportions of this species, as described by Compagno (1988), suits in that found in the genus. *C. falciformis* differs from all other *Carcharhinus*, however, by the presence of a well-developed postorbital process, longer than other species, followed by the length of and the width across the optic capsules (Table I).

Carcharhinid sharks present a uniform pattern, reflected also in the chondrocranium. *Carcharhinus* chondrocrania are very similar within species with few differences that seem to be consistent (Table II). These variations may be useful in taxonomy, taking into account ontogenetic and individual differences. In literature, very few specimens of the same species were observed and ontogenetic and sexual dimorphism within them were overlooked. At least Compagno (1979) affirms that large adults of *C. obscurus* present slightly shorter rostrum than the immature ones.

The rostral node showed great morphological plasticity among species. *Galeocerdo* has been considered the most plesiomorphic taxon within carcharhinid (Compagno 1979, 1988) or *Galeocerdo* + *Rhizoprionodon*, being plesiomorphic sister-group of *Sphyrna* + *Prionace* + *Carcharhinus* (Naylor 1992). The rostral node of *Galeocerdo* is massive and closed (without rostral fenestra). The plesiomorphic rostral node in *Carcharhinus* was found in *C. macroti* (Figure 19A), without rostral fenestra. Continuing, the rostral fenestra is small, undeveloped (ex. *C. sealei*, Figure 19B and *C. porosus* Figure 19C) and followed by an opened one (ex. *C. falciformis*, Figure 19D). In most derived rostra, the modification sequence of this character provides a breakdown of rostral fenestrae, vanishing with the opening of the transverse bar at the apex of the rostral cartilage (ex. *C. amblyrhynchoides*, *C. longimanus*, *C. leucas* and *C. albimarginatus*, Figures 19E to 19H respectively).

More specimens of the same species must be analyzed to verify the consistency of a character. In *Rhizoprionodon porosus*, for example, the rostral fenestra proved to be an individual character, being present or not present, as showed by Gomes & Siqueira (1995).

Other character seen, the hypercalcification, is usually found in adults as observed by Benzer (1944) in *Squalus acanthias*, Gomes & Siqueira (1995) in

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Rhizoprionodon porosus and Maisey & Springer (2013) in *Lamna*. In imatures, the hypercalcification was also found in *Lamna ditropis*, by Compagno (1988). The hypercalcification in *Carcharhinus* was formely verified by Compagno (1979) in *C. macroti* (rostral node) and it was found in young *C. falciformis* herein (ex. Figure 2).

Waller & Baranes (1991) compared the chondrocrania of *Mustelus mosis* and *Iago omanensis* and found that the former had thickened neurocranium. The authors attributed this fact to the durophagic diet of *Mustelus mosis*. This same statement was followed by Gomes & Siqueira (1995) for *Rhizoprionodon porosus*, which food items included fishes, molluscs and crustaceans. *R. lalandii* that showed a more ichthyophagous diet. *R. porosus* also showed ontogenetic teeth dimorphism being serrated in adults and smooth in juveniles. Besides, *R. lalandii* doesn't have cranium hypercalcification.

In *Carcharhinus*, as seen in Garrick (1982) and Naylor & Marcus (1994), teeth morphology can be separated into three groups (based on the upper teeth): narrow teeth (*C. brevipinna*, *C. limbatus*, *C. amblyrhynchoides*, *C. wheeleri*, *C. amblyrhynchoides*, *C. melanopterus*, *C. acronotus*), intermediate teeth (*C. porosus*, *C. bornensis*, *C. sealei*, *C. dussumieri*, *C. cautus*, *C. fitzroyensis*, *C. sorrah*, *C. albimarginatus*, *C. falciformis*, *C. perezi*, *C. brachyurus*, *C. macroti*), and wide teeth (*C. longimanus*, *C. leucas*, *C. obscurus*, *C. galapagensis*, *C. plumbeus*, *C. macroti*).

Unlike what was observed by Baranes & Waller (1991) and Gomes & Siqueira (1995), *Carcharhinus* teeth, being either thin or wide, apparently do not present any effect in the diet since the species are generally piscivorous (Compagno 1984).

The chondrocrania hypercalcification, at least in known species, proved not to be an ontogenetic modification or even related to the species size. *Carcharhinus macroti* and *C. falciformis*, for instance, which are considered small (up to 89 cm in total length) and medium sized (about 3.30 cm in total length), respectively, show high concentrations of calcium in certain regions. The hypercalcification was not found in species of wide teeth, and large size (ex. *C. leucas*, reaching about 3.40 cm in total length and *C. obscurus*, up to 400 cm total length).

The chondrocrania topographic terminology may be different according to the authors as seen in synonymies. The most controversial region is the orbital, with their foramina proved to be the most complex leading to various erroneous interpretations in which some foramina presents distinct names.

Compagno (1988) was the first in separating *Carcharhinus* in groups of species including chondrocrania characters. *C. falciformis* fits in his transitional group in which the width across orbital notch is equal to its length across orbit and distally angular and broad, characters confirmed in the present study.

The taxonomic position of *Nasolamia*, however, formerly belonging to *Carcharhinus* and separated from this genus by having the distance between the nasal capsules greatly reduced, is called into question since it shares almost all cranial proportions with *Carcharhinus*. As was showed in Table V, almost all percentages fits *Nasolamia* in *Carcharhinus*.

Compagno & Garrick (1983) separates *N. velox* from *Carcharhinus* by an oval nasal capsule, with its axes nearly parallel to cranial axis. An oval nasal capsule is similar to *C. sealei*, while the position of the nasal capsule of *C. signatus* and *C. macroti* presents its positions almost similar to that of *Nasolamia*.

Compagno (1988) presents *Nasolamia* as a “satellite genus of *Carcharhinus*” and could be included in his “*Carcharhinus* transitional group of species” (as does *C. falciformis*), also very similar to *C. acronotus*. *Nasolamia* differs from *Carcharhinus* by the straight distance across nostrils with an elongated cranium. That author however affirms that *Nasolamia* could merely be an aberrant derivative of an *acronotus*-like ancestor. From our point of view, however, the cranial characters which differ *Nasolamia* from *Carcharhinus* could be included in the characters used in separating these species.

Conclusions

The chondrocrania of *Carcharhinus* (including *Nasolamia*), despite being conservative, showed differences that can be taxonomically significant.

The cranial components that presented specific consistent differences are: the shape of the rostral node and posterior margin of anterior fontanele, rostral dimension, presence or absence of rostral fenestra, epiphisial notch, epiphisial foramen and nasal flap; direction of the nasal capsules, shapes of the preorbital process and subetmoid fossa

In reviewing in detail the chondrocranium terminology of *C. falciformis* the orbit presented the most modified region.

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Hypercalcification is not an ontogenetic modification.

A rigorous analysis in *Nasolamia* should be done to elucidate its taxonomic status.

Abbreviations

AF -anterior fontanelle
 AOCF – anterior orbitonasal canal foramen
 ASC - anterior semicircular canal
 BP – basal plate
 EC – ectethmoid chamber or fossa
 ECN – ectethmoid condyle
 EN – epiphysial notch
 ENF - endolymphatic foramen
 EF – epiphysial foramen
 EPA – afferent pseudobranquial artery foramen
 FCN – foramen for the anterior cerebral vein
 FCV – foramen for the anterior cerebral vein
 FM – foramen magnum
 HF – hyomandibular facet
 ICA – foramen for internal carotid artery
 ICF – interorbital canal foramen
 INS – internasal septum
 LSC – lateral semicircular canal
 LR - lateral rostral cartilage
 MEF – medial ectethmoid foramen
 MK – median keel
 MR – medial rostral cartilage
 NA – nasal aperture
 NC - nasal capsule
 NDC - nasal dorsal crest
 NF - nasal flap
 NP – notch for orbital process of palatoquadrate
 OC – occipital condyle
 OCN – occipital centrum
 OLFA – olfactory aperture for the olfactory canal
 OLCF – olfactory canal foramen
 O-OCSF – otico-occipital spinal foramina
 ON - orbital notch
 ONC – orbitonasal canal
 ONF – orbitonasal foramen
 OP – otic pedicel
 OPH V+VII – foramen for the superficial ophthalmic ramus of the *trigeminus* and the *facialis*
 OT - otic capsule

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OW – orbital wall
 PEF – perilymphatic foramen
 PF - ophthalmicus profundus nerve foramen
 POC – posterior part of orbitonasal canal
 POCHF – posterior orbitonasal canal foramen
 PR -preorbital process
 PRF - parietal fossa
 PROT – prootic foramen
 PRW – preorbital wall
 PSC – posterior semicircular canal
 PT – postorbital process
 RA - rostral appendix
 RF – rostral fenestra
 RN - rostral node
 RNC – roof of nasal capsule
 SEF – subethmoid fossa
 SF - supracranial foramen
 SFE – fenestra for stapedial (orbital) artery
 SNF – subnasal foramen
 SNP –subnasal plate
 SS – suborbital shelf
 TB - transverse bar
 II – foramen for the optic nerve (optic foramen)
 III – foramen for the oculomotor nerve (oculomotor foramen)
 IV - foramen for the trochlear nerve
 VI – foramen for the abducens nerve
 IX – Foramen for the glossopharyngeal nerve
 X – foramen for the vagus nerve

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Figures

- Figure 1. *C. falciformis*. AC.UERJ 1456, female, 1316 mm TL. Dorsal view;
- Figure 2. *C. falciformis*. AC.UERJ 1456, female, 1316 mm TL. Ventral view;
- Figure 3. *C. falciformis*. AC.UERJ 1456, female, 1316 mm TL. Lateral view;
- Figure 4. *C. falciformis*. AC.UERJ 1456, female, 1316 mm TL. Detail of the nasal capsule region showing the anterior orbitonasal canal foramen (AOCF), the orbitonasal canal (ONC), the posterior orbitonasal canal foramen (POCF) and the subnasal plate (SNP);
- Figure 5. *C. falciformis*. AC.UERJ 1469, (no data). Detail of the nasal capsule region showing the internal structures;
- Figure 6. *C. falciformis*. AC.UERJ 1469, (no data). Detail of the nasal capsule region showing the anterior orbitonasal canal foramen (AOCF), the ectethmoid chamber or fossa (EC), the lateral rostral cartilages (LR), the olfactory aperture for the olfactory canal (OLFA), the medial rostral cartilage (MR) and the preorbital wall (PRW). Front view;
- Figure 7: *C. falciformis* AC.UERJ 1457, male, showing the presence of a mild epiphysial notch (EN);
- Figure 8: *C. falciformis* AC.UERJ 1459, female, 1283 mm TL. Showing an almost indistinguishable epiphysial notch;
- Figure 9. *C. longimanus* (modified from Compagno, 1988);
- Figure 10 *C. falciformis* (AC.UERJ, 1458, male, 1131 TL);
- Figure 11. *C. amblyrhynchoides* (modified from Compagno, 1988);
- Figure 12. *C. albimarginatus* (modified from Compagno, 1988);
- Figure 13. *C. macroti* (modified from Compagno, 1988);
- Figure 14. *C. sealei* (modified from Compagno, 1988);
- Figure 15. Preorbital process in *C. leucas* (modified from Compagno, 1988);
- Figure 16. Subethmoidal fossa in *C. macroti*, *C. falciformis*, *C. albimarginatus*, *C. sealei*, *C. signatus*, *C. plumbeus*, *C. sorrah*;
- Figure 17. Subethmoidal fossa in others species. The epiphysial notch can be well evident in *C. porosus*, *C. hemiodon* and *C. signatus* (Figure 18);
- Figure 18. Epiphysial notch in *C. porosus*, *C. hemiodon* and *C. signatus* (modified from Compagno 1988). (*C. falciformis* #AC.UERJ 1452);
- Figure 19. Morphological plasticity of the rostral node among species;
- Figure 19A. *C. macroti* (modified from Compagno, 1988);
- Figure 19B. *C. sealei* (modified from Compagno, 1988);
- Figure 19C. *C. porosus* (modified from Compagno, 1988);
- Figure 19D. *C. falciformis* (AC.UERJ, 1458, male, 1131 TL);
- Figure 19E. *C. amblyrhynchoides* (modified from Compagno, 1988);
- Figure 19F. *C. longimanus* (modified from Compagno, 1988);
- Figure 19G. *C. leucas* (modified from Compagno, 1988);
- Figure 19H. *C. albimarginatus* (modified from Compagno, 1988).

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6.2 - Artigo científico II

Nota científica a ser encaminhado a Journal of Genetics

Genetic Identification of the Silky Shark *Carcharhinus falciformis* (Bribon, 1839) in the South and Equatorial Atlantic Ocean

Todas as normas de redação e citação, deste capítulo, atendem as estabelecidas pela referida revista (em anexo).

LANA, F.O. Ecologia do Tubarão Lombo-preto *Carcharhinus falciformis* (Bribon, 1839) na margem Ocidental do Oceano Atlântico Equatorial.

GENETIC IDENTIFICATION OF THE SILKY SHARK *Carcharhinus falciformis* (BRIBON, 1839) IN THE SOUTH AND EQUATORIAL ATLANTIC OCEAN

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ABSTRACT

The silky shark, *Carcharhinus falciformis* is an oceanic-coastal pelagic shark species found worldwide in tropical and subtropical waters. It is one of the most frequently caught shark species by pelagic longline fisheries but, despite its commercial importance and ecological significance, the understanding of its biology and ecology is still unavailable for large geographical regions. The present study intended to identify, through molecular analysis, the silky shark, according to Multiplex PCR-technique and to compare its genetic characteristics with the other species of the genus *Carcharhinus* that are recorded in the Saint Paul and Saint Peter Archipelago (Brazil) and have close morphometric characteristics (eg *C. falciformis*, *C. galapagensis* e *C. obscurus*). The results, Based on 64 samples, the results showed that almost all samples were silky sharks (*C. falciformis*). Of these total, 57 were expected as silky sharks (89.1%), proving through the molecular identification method that 59 samples were silky sharks, totaling 92.2% of the samples. Only 1 sample that we expected to be *C. falciformis* was identified as *C. obscurus*. And among the total samples, 7 were expected to be *C. obscurus* (10.9%), and 5 samples were identified for the species, totaling 7.8% of the samples. There were 3 samples that we expected to be *C. obscurus* but were identified as *C. falciformis*, and any *C. galapagensis* was found in the field or identified by molecular markers.

Keywords: Genetic diversity, conservation genetics, fisheries management.

1. Introduction

The silky shark, *Carcharhinus falciformis*, is a pelagic species with equatorial and tropical distribution, occurring from tropical to temperate areas, near reefs, sandbanks and oceanic islands (COMPAGNO, 1984). In the equatorial and southwestern Atlantic Ocean, the species has been often caught by longline vessels targeting tunas and swordfish, which has been the main cause of their fishing mortality. In northeast Brazil, it's particularly abundant close to seamounts and in the vicinity of Saint Peter and Saint Paul Archipelago (SPSPA).

According to HAZIN et al. (2007), although the silky shark is a common species in the Brazilian coast, information on its biology is still virtually nonexistent. Information about the migration and distribution of the silky shark in the Atlantic Ocean is equally scarce. Most of the existing data are from the Northwest Atlantic, where more than 820 silky sharks were tagged since 1963 by the Cooperative Shark Tagging Program, of the U.S. National Marine Fisheries Service (KOHLE et al., 1998). The recapture data indicated that this species can migrate long distances in a relatively short period of time, being often caught by various countries and fishing fleets. Due to this fact, the management of their stocks can only be done efficiently by Regional Fisheries Management Organizations, such as, in the case of the Atlantic Ocean, the International Commission for the Conservation of Atlantic Tunas- ICCAT.

Morphological data were historically the first to be used in the identification of species simply because they were the first tools available to researchers who began systematizing knowledge about living beings. With the development of new methods of study, other methodologies became available for the study of biodiversity and approximately 30 years ago, with the advent of molecular techniques, the analysis of sequences of ribosomal DNA genes began to be used to investigate Evolutionary relationships at higher taxonomic levels (Woese and Fox, 1977; Ussami, 2015).

From this new time, the researches using mitochondrial DNA sequences dominated the Molecular Systematics, with an initial emphasis for the period of the late 70's and early 80's (Avice, 1994) and today's methodology constitutes one of the main of this type of research, with a growing number of scientific journals dedicated exclusively to this field (Ussami, 2015).

In taxonomic studies, some categories of data have always been associated with morphological data, without wishing to replace them, and examples of such integration are increasingly common. According to Ussami (2015) although molecular tools have provided a wide range of new opportunities to study issues in evolutionary biology, such as speciation processes and phylogenetic systematics, it has only recently been proposed that a short segment of 648 nucleotides from the 5' end of the mitochondrial cytochrome oxidase I gene (COI) would be sufficient in many metazoans to identify them at the species level (Hebert et al., 2003a, 2003b). The use of this methodology, called *DNA barcode*, gained relevance with the creation of the *Consortium for the Barcode of Life* (CBOL) in 2004, whose goal is the creation of a bar code database representing partial sequences of COI gene DNA. With the objective of facilitating the process of automation in the identification of the species and to provide a better understanding and interpretation of the global biodiversity.

For reasons of standardization and for its apparent better performance, CBOL adopted as standard sequence the cited fragment of the COI gene. However, it is considered that the association of methodologies and markers can and should always be used for a correct species identification.

The development of molecular tools applied to the identification of species has been of great importance, especially for the quantification of the exploitation of natural populations, evaluation and inspection of the trade of species under government protection, as well as can provide tools for the certification of processed products, adding value to its marketing. In the literature, fishermen are more likely to be identified in the literature, with some of them specifically targeting sharks (Blanco et al., 2008; Magnussen et al., 2007; Clarke et al., 2006; Shivji Et al., 2005; Ussami, 2015).

2. Material and Methods

Tissue samples of 64 silky sharks (*Carcharhinus falciformis*) collected in the area located between 00°55'3,72"N and 029°20'11,22"W, as well as in the vicinity of the Saint Peter Saint Paul Archipelago, by hand-line, have been examined. These samples were analysed in collaboration with the Genetics Laboratory of Aquatic Organisms and Aquaculture- LAGOAA from Mogi das Cruzes University- UMC, where they were tested for molecular identification and confirmation of the species.

DNA Extraction

For the extraction of total DNA, sub-sections of each of the samples (Table 1) were used. Extraction was performed using the QIAGEN kit following the procedures described by the manufacturer. This rapid purification kit was designed to have a high-performance for molecular analyzes, effectively reducing contaminants or enzyme inhibitors. After extraction, the samples were assessed for integrity and concentration of DNA by agarose gel 0.8% 1X TAE buffer (0.04M Tris-acetate, 0.001 M EDTA), stained with ethidium bromide (1 mg/ ml under UV light and scanned by spectrophotometer) (NanoDrop ® ND-1000).

Polymerase Chain Reaction (PCR)

Gens of ITS

For amplification of the gene region ITS, several PCR reactions were run in a thermocycler (Applied Biosystems Veriti™ Thermal Cycler) in order to standardize the reagents and determine the optimum temperature for annealing of the primers to the DNA (temperature gradient: 56, 58, 60, 62, 64 and 66°C). The conditions for the PCR reaction are provided in Table 1 and the program used for the amplifications in Table 2. To prepare the PCR reactions a negative control was used as an indicator of non-contamination of other samples, prepared with all reagents but no DNA.

Agarose gels (2% 90V) were used to verify the amplification, being subsequently scanned by Image Quant 300 (GE Healthcare Life Sciences), using UV light. The following primers, described by Panket et al. (2001), were used:

Fish 5.8SF (forward primer 5'-TTAGCGGTGGATCACTCGGCTCGT-3')

Fish 28SR (reverse primer 5'- TCCTCCGCTTAGTAATATGCTTAAATTCAGC – 3')

Silky Primer (5'-ACCGTGTGGGCCAGGGTC-3')

Table 1- Conditions of the PCR reactions used.

Reagents	Quantities (µl)
Water	13,0
Tampon	2,5
MgCl ₂	2,0
dNTP	4,0
Universal Primer 1	0,5
Universal Primer 2	0,5
Specific Primer	0,5
Polymerase TAQ	0,5
DNA	1,5
Total	25

Table 2- Program used for PCR reactions in the thermocycler.

Stage	Temperature (°C)	Time (minutes)
1	94	15
2	94	1
3	64*	1
4	72	2
5	Stage 2 for 35 times	
6	72	5

*64°C- optimal annealing temperature was determined based on the gradient performed in the previous step.

Gene IOC

For amplification of the gene region IOC, the samples were subject to the following conditions: 4.5 l of water, 2.0 l MgCl₂, 1.25 l Buffer, 1.25 l of dNTP, 1.0 ul Primer F2 (Forward), 1.0 l Primer R2 (Reverse), 0.5 l of TAQ polymerase and 1.0 l DNA, totaling a PCR reaction with 12.5 l. The thermocycler program used for the amplification was: 94°C for 15 min. (Stage 1), 94°C for 1 min. (Stage 2), 52°C for 1 min. (Stage 3) 72°C for 2 min. (Stage 4), 35 times per second stage (Stage 5) and 72°C for 5 min. (Stage 6). Agarose gels 2% (90V) were used to verify the amplification, being subsequently scanned by ImageQuant 300 (GE Healthcare Life Sciences) using UV light. The following primers, described by Ward et al. (2005), were used:

Fish F2 (forward primer 5' TCGACTAATCATAAAGATATCGGCAC 3')

Fish R2 (reverse primer 5' TCCTCCGCTTAGTAATATGCTTAAATTTCAGC 3')

Purification of the PCR product

After evaluation in agarose gel, the PCR product derived from the gene for the IOC to be sequenced was purified. Samples were purified using ExoSAP. The procedure consisted of: 5 l of PCR product, 0.13 and 1.87 ExoSAP l l of ultrapure water. The program in the thermocycler was 1 hour at 37°C and 15 minutes at 80°C. Verification of the quality of the purification was performed by electrophoresis in agarose gel (2% 80V) stained with ethidium bromide.

Sequencing

The sequencing was performed using the chain termination method (SANGER et al. 1977) using the DYEnamic ET terminator premix kit (GE Healthcare). The reactions were performed at the Center for Human Genome Studies, University of São Paulo. The protocol for preparation of the sequencing reaction is shown in Table 3. The temperatures to carry out the reactions were: 95°C for 20 seconds, 55°C for 15 seconds, and 60°C for 1 minute, for a total of 30 cycles. The material was analyzed in an automated sequencing ABI PRISM 3730 (Applied Biosystems). Sequences were evaluated for quality and prepared for insertion into the database using the program Codon Code.

Table 3- Reagents used in the sequencing reactions.

Reagents	Quantities
Sample of purified DNA	1
DYEnamic ET Terminator	4
Prímer	1
Ultrapure water	

3. Results

General view

Based on 64 samples, the results showed that almost all samples were silky sharks (*C. falciformis*) (Table 4). Of these total, 57 were expected as silky sharks (89.1%), proving through the molecular identification method that 59 samples were silky sharks, totaling 92.2% of the samples. Only 1 sample that we expected to be *C. falciformis* was identified as *C. obscurus*. And among the total samples, 7 were

expected to be *C. obscurus* (10.9%), and 5 samples were identified for the species, totaling 7.8% of the samples. There were 3 samples that we expected to be *C. obscurus* but were identified as *C. falciformis* (Table 5). In the sample, any *C. galapagensis* was found in the field or identified by molecular markers.

Table 4- Samples of *C. falciformis* and *C. obscurus* species analysed for molecular confirmation.

	Sample	Nº Coleção	Expected Species	Species	Sex	TL (cm)
1	350.3	AC UERJ 1452	<i>C. falciformis</i>	<i>C. falciformis</i>	M	117,00
2	350.2	AC UERJ 1453	<i>C. falciformis</i>	<i>C. falciformis</i>	F	107,00
3	12	AC UERJ 1454	<i>C. obscurus</i>	<i>C. falciformis</i>	F	144,50
4	10	AC UERJ 1456	<i>C. obscurus</i>	<i>C. falciformis</i>	F	131,60
5	9	AC UERJ 1455	<i>C. falciformis</i>	<i>C. falciformis</i>	M	124,02
6	11	AC UERJ 1457	<i>C. falciformis</i>	<i>C. falciformis</i>	M	138,40
7	7	AC UERJ 1458	<i>C. falciformis</i>	<i>C. falciformis</i>	M	113,10
8	8	AC UERJ 1459	<i>C. obscurus</i>	<i>C. falciformis</i>	F	128,30
9	350.4	350.4	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
10	350.5	350.5	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
11	350.6	350.6	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
12			<i>C. falciformis</i>	<i>C. falciformis</i>	F	
13			<i>C. falciformis</i>	<i>C. falciformis</i>	F	
14			<i>C. falciformis</i>	<i>C. falciformis</i>	M	
15			<i>C. falciformis</i>	<i>C. falciformis</i>	M	
16			<i>C. falciformis</i>	<i>C. falciformis</i>	F	
17			<i>C. falciformis</i>	<i>C. falciformis</i>	M	
18			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
19			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
20			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
21			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
22			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
23			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
24			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
25			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
26			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
27			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
28			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
29			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
30			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
31			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
32			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
33			<i>C. falciformis</i>	<i>C. falciformis</i>	-	
34	M2		<i>C. falciformis</i>	<i>C. falciformis</i>	M	115,20
35	M3		<i>C. falciformis</i>	<i>C. falciformis</i>	M	144,60
36	M4		<i>C. falciformis</i>	<i>C. falciformis</i>	F	152,40

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37	M380.2	<i>C. falciformis</i>	<i>C. falciformis</i>	M	139,30
38	M380.4*	<i>C. falciformis</i>	<i>C. falciformis</i>		180,00
39	M380.5*	<i>C. falciformis</i>	<i>C. falciformis</i>		140,00
40	M380.6	<i>C. falciformis</i>	<i>C. falciformis</i>		130,00
41	M380.7	<i>C. falciformis</i>	<i>C. falciformis</i>		132,00
42	377.L4.N2	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
43	378.1.5	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
44	378.2.1	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
45	378.2.2	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
46	378.2.3	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
47	378.2.4	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
48	378.2.5	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
49	378.2.6	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
50	379.1.1	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
51	379.1.2	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
52	379.1.3	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
53	379.1.4	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
54	379.1.5	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
55	379.2.1	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
56	379.2.2	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
57	379.2.3	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
58	379.2.4	<i>C. falciformis</i>	<i>C. falciformis</i>	F	
59	379.4.1	<i>C. falciformis</i>	<i>C. falciformis</i>	M	
60	351.7	351.7	<i>C. falciformis</i>	<i>C. obscurus</i>	F 213,00
61	M1		<i>C. obscurus</i>	<i>C. obscurus</i>	F 156,90
62	M5		<i>C. obscurus</i>	<i>C. obscurus</i>	M 140,50
63	M380.1		<i>C. obscurus</i>	<i>C. obscurus</i>	M 201,40
64	M380.3		<i>C. obscurus</i>	<i>C. obscurus</i>	F 135,00

TL: Total Length.

Table 5- General results of species analysed for molecular confirmation.

Specie	Expected Species (%)	Confirmed Species (%)
<i>C. falciformis</i>	89.1	92.2
<i>C. obscurus</i>	10.9	7.8

By the amplification generated by PCR gene ITS, it was possible to identify the shark species in 23 of 30 samples, by the presence of specific bands marked with number of base pairs related to sharks *Carcharhinus falciformis*, confirming the expectation (Figure 1). The seven subjects who showed no band (sample numbers: 1, 8, 26, 27, 28, 29 and 30) were remade but it was not possible to actually amplify them in the ITS region of the gene. It was then decided, to sequence only those individuals. For

this to be accomplished, the DNA extracted from these samples passed again by PCR, using, however, alternatively the primers for the COI region and not for the ITS.

This COI gene was a major component of the standardized methodology known as "DNA barcoding", whose goal is to form international alliances to research the diversity of eukaryotic life (Marshall, 2005), through the creation of a database of sequences of partial DNA of COI gene, thus promoting the automation in the process of species identification (Matta, 2010). This region located in the COI gene has some advantageous characteristics for its use in the program DNA barcoding, and these are: (1) it is a rather short region which can be easily obtained for a large number of taxa with a small amount of primers; (2) it is a straight line; (3) it is informative to distinguish closely related species, as it has similar variability to other protein-coding genes (Matta, 2010).

Samples that had yet to be identified were then amplified in the region of the COI gene showing strong bands. The PCR product of these samples were further purified and sent for sequencing. Samples 1 (Figure 2), 26 (Figure 3), 27 (Figure 4), 28 (Figure 5), 29 (Figure 6) and 30 (Figure 7) were confirmed as the species *Carcharhinus falciformis*, when inserted into its respective nucleotide sequences in the database available at: <http://boldsystem.org>. However, the sample number 8 (Figure 8), which went through the same verification process in the database, showed 100% similarity with the sequences already available for the species *Carcharhinus obscurus*, confirming that this individual was not a silky shark, as shown in Table 4.

Genetics of the silky shark *Carcharhinus falciformis*

By the amplification generated by PCR gene ITS, it was possible to positively identify the shark species in 64 samples, 59 of which were *Carcharhinus falciformis* and 5 were *Carcharhinus obscurus*, by the presence of specific bands marked with a number of base pairs related to sharks *Carcharhinus falciformis*, confirming the expectation (Figure 1). The subjects who showed no band (sample numbers: 1, 8, 26, 27, 28, 29 and 30) were remade but it was not possible to actually amplify them in the ITS region of the gene. It was then decided to sequence only those individuals. For this to be accomplished, the DNA extracted from these samples were passed again by PCR, using, however, alternatively the primers for the COI region and not for the ITS.

This COI gene was a major component of the standardized methodology known as "DNA barcoding", which goal is to form international alliances to research the diversity of eukaryotic life (Marshall, 2005), through the creation of a database of sequences of partial DNA of COI gene, thus promoting the automation in the process of species identification (Matta, 2010). This region located in the COI gene has some advantageous characteristics for its use in the program DNA barcoding, and these are: (1) it is a rather short region which can be easily obtained for a large number of taxa with a small amount of primers; (2) it is a straight line; (3) it is informative to distinguish closely related species, as it has similar variability to other protein-coding genes (Matta, 2010).

Samples that had yet to be identified were then amplified in the region of the COI gene showing strong bands. The PCR product of these samples were further purified and sent for sequencing. Samples 1 (Figure 2), 26 (Figure 3), 27 (Figure 4), 28 (Figure 5), 29 (Figure 6) and 30 (Figure 7) were confirmed as the species *Carcharhinus falciformis*, when inserted into its respective nucleotide sequences in the database available at: <http://boldsystem>. However, the sample 8 (Figure 8), which went through the same verification process in the database, showed 100% similarity with the sequences already available for the species *Carcharhinus obscurus*, confirming that this individual was not a silky shark, similarly to the other 4 samples analyzed, as shown in Table 4.

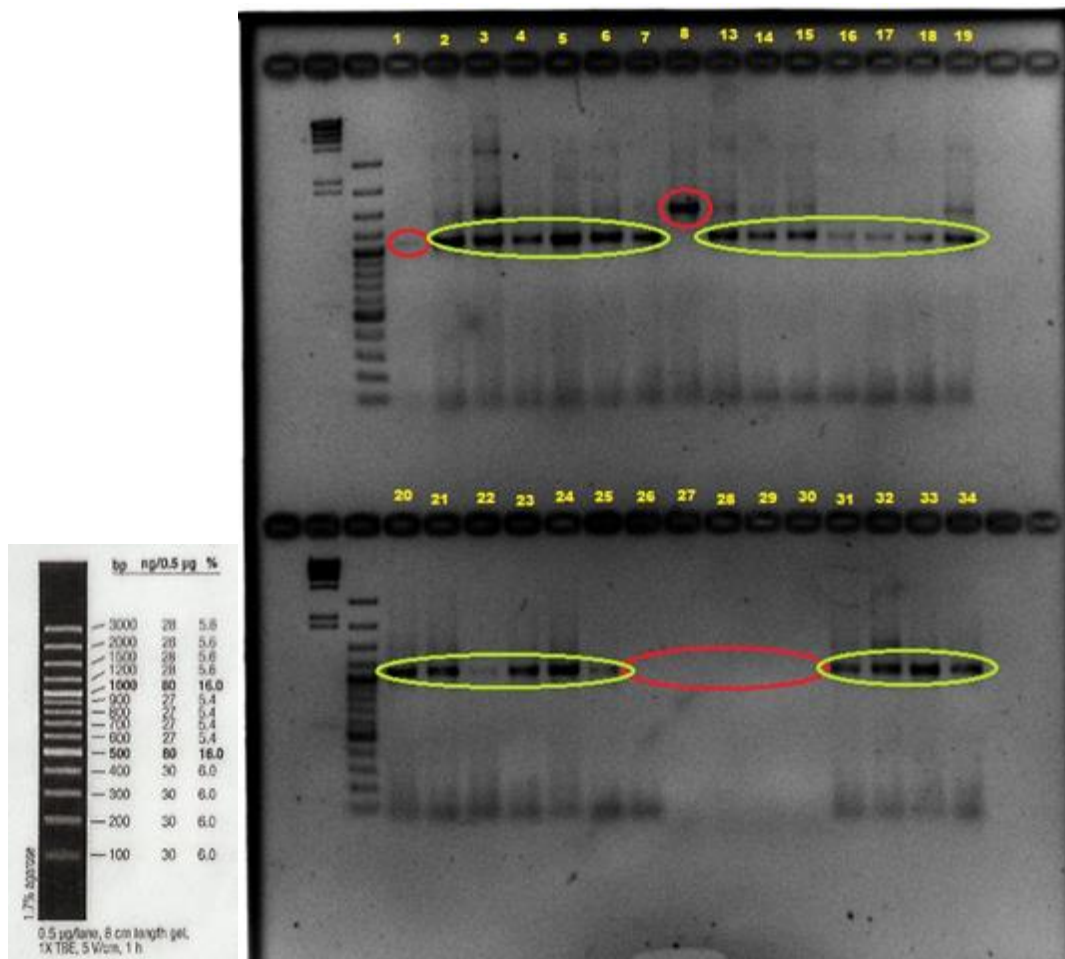


Figure 1- Agarose gel electrophoresis of products of PCR, amplifying the ITS gene. The specific bands of *Carcharhinus falciformis* are shown in green and were found in twenty-three samples. The samples in red were not amplified or were amplified only in the universal band (sample 8).

>CF 1

```
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AGTTCCTTTAATAATTGGTGCACCAGATATAGCCTTCCCACGAATAAATAAC
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AGTAGAAGCTGGAGCAGGTACTGGTTGAACAGTTTATCCTCCATTAGCTAGT
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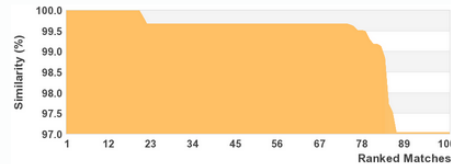
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TATTCTTGTAACCACTATTCTTCTCCTCCTATCACTTCCAGTTCTTGCAGCAG
GGATTACAATATTACTTACAGATCGTAACCTTAATACTACATTCTTTGATCC
TGCAGGTGGAGGAGACCCAATCCTTTATCAACATTTATTCTGATTCT

Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Chordata	100
Class	Elasmobranchii	100
Order	Carcharhiniformes	100
Family	Carcharhinidae	100
Genus	Carcharhinus	100

Similarity Scores of Top 100 Matches:



TOP 20 Matches:

Display option:

Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release

Figure 2- Output sequence and database check to confirm the species of shark sample number 1.

>CF 3

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TATAAAACCACCAGCCATTTCCMAATATCAAACACCATTATTTGTTTGATCT
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CAGGKGGAGGAGACCCAATCCTTTATCAASATTTATTCTGATTCT

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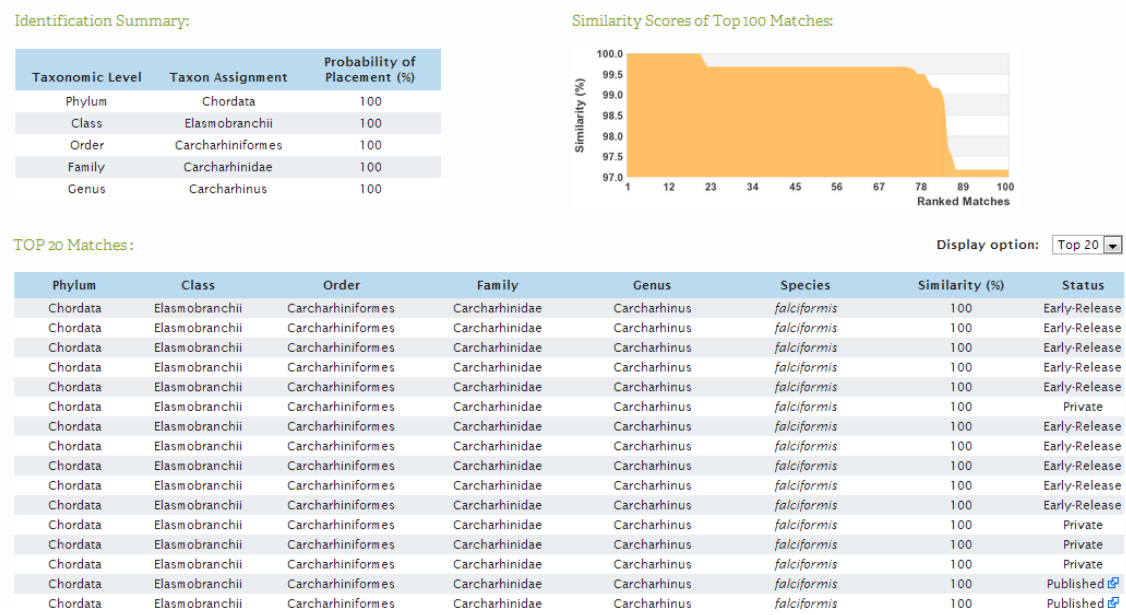


Figure 3- Output sequence and database check to confirm the species of shark sample number 26.

>CF 4

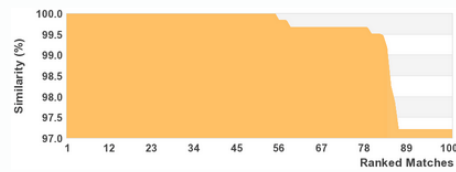
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 ACTAGTTCCTTTAATAATTGGTGCACCAGATATAGCCTTCCCACGAATAAAT
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 TGGAGTAGAAGCTGGAGCAGGTACTGGTTGAACAGTTTATCCTCCATTAGCT
 AGTAACCTAGCACATGCTGGACCATCTGTTGATTTAGCTATTTTCTCTCTTCA
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 CAGGGATTACAATATTACTTACAGATCGTAACCTTAATACTACATTCTTTGA
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Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Chordata	100
Class	Elasmobranchii	100
Order	Carcharhiniformes	100
Family	Carcharhinidae	100
Genus	Carcharhinus	100

Similarity Scores of Top 100 Matches:



TOP 20 Matches:

Display option:

Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release

Figure 4- Output sequence and database check to confirm the species of shark sample number 27.

>CF 5

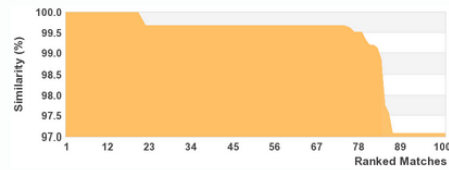
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 CCTGCAGGTGGAGGAGACCCAATCCTTTATCAACATTTATTCTGATTCT

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Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Chordata	100
Class	Elasmobranchii	100
Order	Carcharhiniformes	100
Family	Carcharhinidae	100
Genus	Carcharhinus	100

Similarity Scores of Top 100 Matches:



TOP 20 Matches:

Display option:

Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release

Figure 5- Output sequence and database check to confirm the species of shark sample number 28.

>CF 6

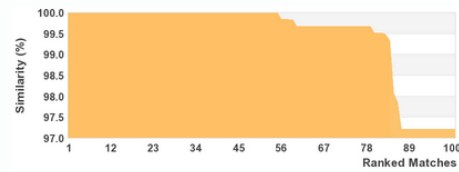
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```

LANA, F.O. Ecologia do Tubarão Lombo-preto *Carcharhinus falciformis* (Bribon, 1839) na margem Ocidental do Oceano Atlântico Equatorial.

Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Chordata	100
Class	Elasmobranchii	100
Order	Carcharhiniformes	100
Family	Carcharhinidae	100
Genus	Carcharhinus	100

Similarity Scores of Top 100 Matches:



TOP 20 Matches:

Display option:

Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published 🔗
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release

Figure 6- Output sequence and database check to confirm the species of shark sample number 29.

>CF 7

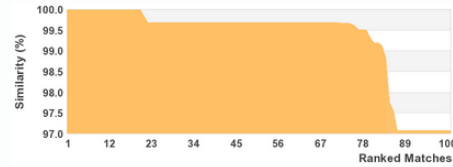
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LANA, F.O. Ecologia do Tubarão Lombo-preto *Carcharhinus falciformis* (Bribon, 1839) na margem Ocidental do Oceano Atlântico Equatorial.

Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Chordata	100
Class	Elasmobranchii	100
Order	Carcharhiniformes	100
Family	Carcharhinidae	100
Genus	Carcharhinus	100

Similarity Scores of Top 100 Matches:



TOP 20 Matches:

Display option:

Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Private
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Published P
Chordata	Elasmobranchii	Carcharhiniformes	Carcharhinidae	Carcharhinus	<i>falciformis</i>	100	Early-Release

Figure 7- Output sequence and database check to confirm the species of shark sample number 30.

>CF2

GGACAGCCCTAAGTCTCCTAATTCRAGCTGAACTTGGGCAACCTGGATCACT
 TTTAGGAGATGATCAGATTTTAAATGTAATCSTAACCGCCCACGCTTTTGTA
 TAATCTTTTTTCATGGTTATACCAATTATAATTGGTGGTTTCGGAAATTGATTA
 GTTCCTTTAATAATTGGAGCACCAGATATAGCCTTCCCACGAATAAATAACA
 TAAGTTTCTGACTTCTTCCACCATCATTCTTCTTCTCCTCGCCTCTGCTGGA
 GTAGAAGCTGGAGCAGGTACTGGTTGAACAGTTTATCCTCCATTAGCTAGC
 AATCTAGCACATGCTGGACCATCTGTTGATTAGCTATTTTTTCTCTTCACTT
 AGCCGGTGTTTCATCAATTTTAGCTTCAATTATTTTATCACAACCATTATTAA
 TATAAAACCACCAGCTATTTCCCAATATCAAACACCATTATTTGTTTGATCT
 ATTCTTGTAACCACTATTCTTCTTCTCCTCTCACTTCCAGTTCTTGCAGCAGG
 GATTACAATATTACTTACAGATCGTAACCTTAATACTACATTCTTTGACCCT
 GCAGGTGGAGGAGACCCAATCCTTTATCAACATTTATTCTGATTCT

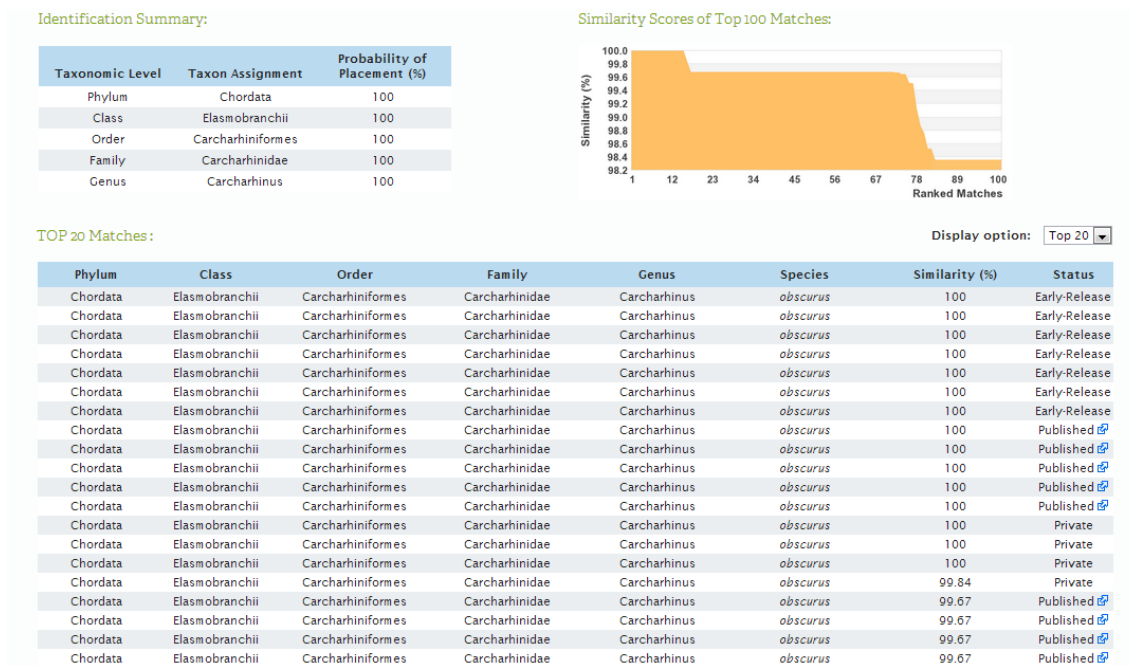


Figure 8- Output sequence and database check to confirm the species of shark sample number 8.

4. Discussion

In the last 20 years, genetic data have been incorporated into the management of marine species, mainly to delimit priority areas for conservation and management units (MUs) (Cardeñosa et al. 2014). Although, the identification of MUs is central to the short-term management, this is still a challenge in marine fish species, because many fishes have strong mobility potential and the marine environments have few geographic barriers, which favors the dispersion (Graves, 1998, Palsboll et al., 2007). Nevertheless, some factors, such as oceanic currents, sea floor topology, temperature, behavioral traits (e.g philopatry) and other features provide opportunities for isolation and differentiation of some species (Awise, 1994, Waples, 1998).

Sharks have been the focus of several studies on migration and/or site fidelity, using traditional methods such as capture-tagging-recapture and acoustic tracking (e. g. Howey-Jordan et al. 2013, Espinoza et al., 2014, Vaudo et al. 2014). Despite these methods can provide various insights about the behavioral traits and ecology of species, they can't detect whether the movement has resulted in reproduction, neither elucidate the genetic consequences of their dispersal (Ovenden, 2013, Waples, 1998). Furthermore, such methods are logistically difficult (Waples, 1998). On the other hand, genetics tools are important to respond various questions about genetic diversity,

population connectivity and historical processes that generated such patterns (Frankham et al. 2004).

Several authors have also suggested that oceanic currents may also affect the population structure of marine species (Rocha et al. 2003, 2005, White et al. 2010, Han et al. 2012, Mendonça et al. 2011, 2013). Silky sharks from the SPSPA presented connectivity with all localities except with USA (Domingues et al., in press). The connectivity between SPSPA and others location and the break with USA was concordant with the pattern of oceanic currents in the Atlantic Ocean. The main oceanic current passing through the ASPSP is the strong South Equatorial Current (SEC), localized between 20°S to 2-3°N, that flows westward toward the Brazilian shelf and bifurcates near 16°S with one branch heading northwards as the North Brazil Current (NBC) and the other, weaker southward branch, as the Brazil Current (BC), connecting ASPSP to SC and PA populations. On the other hand, the SEC reaches the NBC that continues flowing to northwestward to form the Guiana Current that reaches the Gulf of Mexico, connecting ASPSP and GM populations. This oceanic current pattern has explained the population structure of two coastal sharks along the Western Atlantic Ocean, *Rhizoprionodon porosus* (Mendonça et al. 2011) and *Rhizoprionodon lalandii* (Mendonça et al. 2013) and the bony fish *Macrodon ancylodon* (Santos et al. 2006).

For the 64 samples analyzed in this study, 59 confirmed that the silky shark is the most abundant species in the study area, representing 92.2% of the total samples. There were 3 samples that we expected to be *C. obscurus* but were identified as *C. falciformis*, of these specimens, all were identified through morphological characters before the molecular process, which confirms the similarity of both species in their visual, morphological and morphometric identification. The fact that it did not find *C. galapagensis* in this study demonstrates the need to increase the sample number and does not define the population status of the species in the study area.

The results presented in this work also demonstrate that the use of primers in PCR-Multiplex assays constitutes a fast and reliable method to discriminate with a 99% reliability efficiency species that present general distribution and have been intensively fished. Such methodology is extremely efficient in the correct identification of species, especially in those cases where the morphological characteristics that could serve as effective parameters of differentiation are very similar.

These data also reinforce and are in agreement with the results obtained in a study carried out by Pank et al. (2000), who identified with the application of the PCR-Multiplex technique two species of sharks of great representativity in the most morphologically similar catches, *C. obscurus* and *C. plumbeus*. This technique is characterized by being fast, efficient and inexpensive, differing from those methods that require steps of enzymatic restriction and sequencing that increase its application (Heist and Gold, 1998; Mendonça et al., 2009; Ussami, 2015).

Some disadvantages currently encountered in applying this methodology include limiting the current set of species-specific markers available, as well as the possibility that markers are not 100% accurate for species. Thus, in the present work other techniques of identification of species such as DNA barcoding, which uses universal *primers* to amplify part of the COI gene, were also used.

This methodology analyzes the variation of sequences within the species and, at a much higher level, between species. Sequences are submitted to comparisons in the Barcode of Life Data System (Ratnasingham and Hebert, 2007), where these are aligned and compared to the sequences deposited in the system, in an approach that complemented the process of identification of the species of sharks for which could not be identified by PCR-Multiplex.

The deficiency of scientific identification of the species leads to frequent situations of recognition and naming of the specimens by the popular name, which in many cases can relate more than one biological species. Thus, the excessive exploitation of stocks, coupled with certain biological characteristics of a large number of species of this group identified by a K-strategist life cycle, identifies them as fragile and susceptible to overexploitation, since they usually present slow growth, late sexual maturation, low fecundity and high longevity.

Considering this, more work related to genetic diversity and population structure of *Carcharhinus* found in SPSPA are needed to understand more about the ecology of the species. Some questions about its geographical distribution, on a global scale, and sex ratios, for instance, still remain.

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6.3 - Artigo científico III

Artigo científico a ser encaminhado a Journal of Fish Biology

Satellite Tagging And Standard Movement Space-Time Of
Silky Shark *Carcharhinus Falciformis* (Bribon, 1839) In
Southwest Atlantic Ocean And Equatorial

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MIGRATORY MOVEMENTS OF SILKY SHARK *Carcharhinus falciformis* (BRIBON, 1839) IN THE EQUATORIAL AND SOUTHWESTERN ATLANTIC OCEAN INFERRED FROM SATELLITE TAGGING

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ABSTRACT

The silky shark, *Carcharhinus falciformis*, is a highly migratory apex predator and pelagic species distributed in tropical and equatorial waters of all oceans. In northeastern Brazil, it is particularly abundant in the vicinity of the Saint Peter and Saint Paul Archipelago (SPSPA - Brazil). This work aimed at studying the movements of silky sharks in the equatorial and southwestern Atlantic Ocean, by three different types of satellite tags, particularly in the SPSPA. From 2010 to 2014, a total of 10 silky sharks were tagged: 3 with PSAT, 5 with MiniPAT tags and 2 with SPOT tags. From these tags, 6 reported data to the satellites as scheduled, 1 reported prematurely and 3 never reported. An inbuilt state-space model, the unscented Kalman filter- UKFsst, was then applied to the raw location estimates to correct anomalous positions, using SST data from Reynolds at 0.25° NOAA Optimum Interpolation. In general, the tagged sharks showed a preference for warm waters of the mixed layer, where they remained most of the time, during the day. The preferred temperature range of the tagged animal was between 26-28°C, with a preferred depth between 1-10m. Exhibited an elevated residency to the SPSPA, so the present results clearly show the importance of the management measure for the conservation of silky sharks (ICCAT - Interministerial Normative Instruction nº 8 - 06/11/2014), particularly in the vicinity of oceanic islands, due to the strong association these sharks show to these structures.

1. Introduction

The increased impact of pelagic longline fisheries, targeting tunas and swordfish, on less valuable species, particularly those which exhibit long reproductive cycles, such as the elasmobranchs, has been the cause of a growing concern worldwide. Global estimates indicate that almost half of the elasmobranchs caught correspond to bycatch. Besides the obvious impact of the fisheries on their populations, frequently, the lack of an accurate reporting of their catches hinders the assessment of their stocks and

the consequent adoption of management measures needed to ensure their conservation (STEVENSON, 2000; LOTZE et al., 2011).

In the case of the more abundant shark species, such as the blue shark, *Prionace glauca* (LINNAEUS, 1758), or the highly valued ones, such as the mako sharks, *Isurus oxyrinchus* (RAFINESQUE, 1810), catch and effort data are collected more regularly, allowing for a better monitoring of their stocks. The existing information on the catch and effort of these species in the Atlantic, for instance, in spite of some serious data deficiencies, has allowed the International Commission for the Conservation of Atlantic Tunas- ICCAT to assess the condition of their stocks in three different occasions already: in 2004, 2008 and 2012. In relation to the other species caught in conjunction with tuna fisheries, in light of the virtually total lack of data on their catches, ICCAT decided to run, in 2008, an Ecological Risk Assessment (ERA). According to the ERA results, the species subject to the highest level of risk were the bigeye thresher, *Alopias superciliosus* (R. T. LOWE, 1840), the shortfin mako (*I. oxyrinchus*), the longfin mako, *Isurus paucus* (GUITART-MANDAY, 1966), the oceanic whitetip, *Carcharhinus longimanus* (Poey, 1861), and the silky shark, *Carcharhinus falciformis* (BRIBON, 1839). Because of these results, landings of the bigeye thresher and of the oceanic whitetip were banned by the Commission, in 2009 and 2010, respectively, and those of the silky shark, in 2011, except for developing countries, for local consumption. In regard of the makos, in spite of their high risk, the much greater availability of catch and effort data, which consequently allows for a better monitoring of their stocks, has prevented the Commission from prohibiting its landings. In 2010, however, the taking of the species was conditioned to the supply of data, meaning that those contracting parties that do not provide information on their catches will be prohibited from taking them. Also in 2010, an historical year for shark protection in ICCAT, all species of oceanic hammerheads (*Sphyrna sp*) were also submitted to several restrictions of their catch.

Following the adoption by ICCAT, in 2011, of a management measure for the conservation of silky sharks, Brazil, by Interministerial Instruction Normative nº 8 (6 November, 2014), also made the release of all silky sharks caught incidentally in the tuna longline fishery mandatory, dead or alive, forbidding at the same time, the directed fishing, retention on board, transshipment, landing, storage, transportation and

marketing of this species in Brazilian territorial waters, on the high seas or in waters included in international agreements to which Brazil is a signatory.

Recently, TOLOTTI et al. (2015) evaluated the benefits and disadvantages of banning landings as a protective measure for certain species adopted by Regional Fishery Management Organizations (RFMOs), responsible for the management of tuna fisheries. Several of these management measures are now in force, concerning the incidental catch of pelagic sharks, including silky shark, oceanic white tip shark, oceanic hammerheads shark (*Sphyrna* spp.) and bigeye tresher shark (*Alopias* spp.).

The silky shark is a highly migratory apex predator distributed worldwide in tropical waters. It is an abundant pelagic shark species, usually found near the edge of continental and insular shelves, as well as in the open sea (COMPAGNO, 1984; LANA et al., 2012; VARGHESE et al., 2015). It has been assessed as near threatened by the IUCN Red List. Although it is not directly targeted, it is a lucrative by-catch because of their large fins (SIMPFENDORFER & BURGESS, 2009; LEA et al., 2015). According to FILMALTER et al. (2011), silky sharks are the primary elasmobranch bycatch species in tuna purse seine fisheries throughout the world and have been described as one of the three most common pelagic shark species (COMPAGNO, 1984), along with the blue shark and the oceanic white tip shark.

In light of the increasing fishing pressure on elasmobranch species, therefore, in spite of the conservation measures adopted by ICCAT and followed by other RFMOs, it is very important to advance the present knowledge on the various biological aspects of their populations, in order to support the adoption of management measures needed to ensure their conservation. The present paper, thus, aimed at providing new data on the silky shark movements in the southwestern equatorial Atlantic Ocean, in order to guide the adoption of measures needed to ensure their conservation.

2. Material and Methods

From 2010 to 2014, several scientific cruises were conducted to the Saint Peter and Saint Paul Archipelago (SPSPA) (Figure 1 and 2), near the Equator, in the western Atlantic Ocean (00°55'3,72"N and 029°20'11,22"W). During these cruises, 10 silky sharks, *Carcharhinus falciformis*, were caught with a small longline and tagged with electronic tags. The Archipelago is strategically located in the equatorial Atlantic,

between South America and Africa, lying 1,800 km from Dakar, Senegal, and 1,100 km from Rio Grande do Norte, Brazil (Figure 3) (VIANA et al., 2008; VASKE JR., T. et al., 2010).

2.1 Satellite Tag Details

Three different types of satellite tags were used in the present study, all of them manufactured by Wildlife Computers (Redmond, USA): PSAT (Mk10), MiniPAT (*Pop-up Tag*), and SPOT Tags (*Smart Position or Temperature Transmitting Tag*). The PSAT-tag allows for the tracking of temperature, depth and luminosity, which is used for geolocation estimation. The MiniPAT is the latest development in pop-up archival tags and includes several improvements over its predecessor, the Mk10-PAT tag. The SPOT-tag records temperature data and provides geolocation estimates each time the animal surfaces.

A total of 10 silky sharks were tagged: 3 with PSAT, 5 with MiniPAT tags and 2 with SPOT tags. From these tags, 6 reported data to the satellites as scheduled, 1 reported prematurely and 3 never reported (Table 1) (Figure 4, 28 and 29).

PSAT

The three PSAT tags were programmed to collect data on temperature, depth and geographical position every 10 seconds, summarizing them every three hours, with histograms of 14 bins (intervals). These tags were programmed to stay attached for 75 days. Unfortunately, one of the PSATs didn't transmit any signal.

MiniPATs

During the period of the study, five silky sharks were tagged with MiniPATs, and roughly 85% of the data were successfully transmitted and decoded. Due to a data recording and transmission error, however, it wasn't possible to separate the daytime and nighttime periods. Moreover, two of the MiniPATs failed to transmit. MiniPATs were programmed for release with 30, 45 and 60 days and collected data on temperature, depth and light-levels with a time series interval of 2.5 minutes, summarizing them every day and in a 14 bins (intervals) histograms. The internal archive data of depth, temperature and light level have been stored every 1 second.

SPOTs

Two silky sharks were tagged with SPOT tags in the Archipelago of Saint Peter and Saint Paul (ASPSP), between 29 and 30 May 2013. These tags were programmed to collect data on temperature and geographical position with a transmitting with fast repetition rate of 45,00 seconds and slow repetition rate of 90,00 seconds. SPOT tags record temperature data and provide geolocation estimates each time the animal surfaces. The transmission was programmed to start after 0 extra consecutive dry 1/4-sec wakeups, and they resume after being wet for 08 seconds, summarizing them every time the individual surfaces, with histograms of 12 bins (intervals).

2.2 Shark Capture and Handling

Sharks were captured, hauled and restrained on deck. Whenever the individual size jeopardized the animal handling and survival, the shark was restrained next to the vessel with a tail rope, which reduced its movement capacity. The tags were attached to the first dorsal fin by drilling a small hole at about 5 cm from its anterior margin with an autonomous portable drill. This region of the dorsal fin was chosen because it presents stiffer cartilage and absence of blood vessels, thus minimizing the risk of injury or bacterial infection. A monofilament (0.8 mm) coated by a plastic hose was inserted through the hole and crimped to the electronic tag, with a minimum interference on the swimming ability of the tagged individual. The monofilament was also equipped with a swivel to prevent the tag from spinning. The tagging procedure was quickly performed, and took less than 5 minutes in all specimens. In order to complete the tagging operation, a hose was placed inside the mouth of the shark to provide it with oxygen. A wet, opaque tissue was always placed on the eyes of the animal in order to minimize stress, during the whole time it was being manipulated.

2.3 Data Treatment and Statistical Analysis

Track processing

Light-levels recorded by the PSAT and MiniPAT tags were processed using the manufacturer's software (WC-GPE, global position estimator program suite) to determine geolocation estimates. An inbuilt state-space model, the unscented Kalman filter- UKFsst, was then applied to the raw location estimates to correct anomalous

positions, using SST data from Reynolds at 0.25° NOAA Optimum Interpolation. Thus, most probable tracks were obtained after removing improbable locations. For both SPOTs, Argos locations were calculated via the Doppler shift of consecutive transmissions via Argos satellites (CLS Argos). This system provides positions with variable accuracy from 150 m (LC 3) up to 10 km (LC B). Erroneous locations (e.g. on land, LC Z – Argos) were removed from the datasets (SOUSA et al 2016).

Table 1- Data on 10 silky sharks (*C. falciformis*) tagged in the Saint Peter and Saint Paul Archipelago (ASPSP).

	SERIAL NUMBER	PTT ID DEC	SEX	TL (CM)	TAG	PROGRAMMED (DAYS)	TAGGING				POP-UP	
							DATE	LAT	LONG	LOCAL	DATE	DURATION
1	11P0255	115348	F	125	MiniPAT	30	27/06/2012	00°55'3,72"N	029°20'11,22"W	SPSPA	28/07/2012	31
2	11P0261	115350	F	163	MiniPAT	45	23/06/2012	00°55'3,72"N	029°20'11,22"W	SPSPA	09/07/2012	16
3	11P0258	115349	F	135	MiniPAT	60	27/06/2012	00°55'3,72"N	029°20'11,22"W	SPSPA	01/08/2012	35
4	12P0118	128736	M*	180	MiniPAT	30	25/06/2013	00°55'3,72"N	029°20'11,22"W	SPSPA	NEVER REPORTED	
5	12P0119	128737	M	140	MiniPAT	45	25/06/2013	00°55'3,72"N	029°20'11,22"W	SPSPA	NEVER REPORTED	
6	11S1088	115352	M*	190	SPOT	-	29/05/2013	00°55'3,72"N	029°20'11,22"W	SPSPA	12/07/2013	44
7	11S1089	115353	M**	240	SPOT	-	30/05/2013	00°55'3,72"N	029°20'11,22"W	SPSPA	02/02/2014	248
8	10A0768	101017	M	157	PSAT	75	02/02/2012	00°55'3,72"N	029°20'11,22"W	SPSPA	15/04/2012	73
9	08A0848	90815	M	130	PSAT	75	02/10/2010	00°55'3,72"N	029°20'11,22"W	SPSPA	08/10/2010	6
10	09A0215	95273	M	100	PSAT	75	02/10/2010	00°55'3,72"N	029°20'11,22"W	SPSPA	NEVER REPORTED	

*Individuals in the range of the size at first maturity (180- 205° cm – Males; 205- 210 cm - Females).

**Individuals larger than the size at first maturity (180- 205° cm – Males).

Data from LANA et al., 2012.

Following the methods in Sousa et al., (2016) and to correct the retrieved shark positions, both Argos and light-level data were processed using a Kalman filter which corrects the locations with the respective established associated errors. Briefly, positions retained from Argos were corrected with the *crawl* package in R (JOHNSON, LONDON et al. 2008), whereas the light-level locations processed with the UKFsst were parameterised with standard deviation (SD) constants (K) (SIPPEL, HOLDSWORTH et al. 2011; SOUSA et al 2016).

Corrected tracks were then interpolated into regular time-steps for analysis ensuring at least one real location was maintained in the interpolation procedure (PSAT and SPOT tracks at every three days, and MiniPATs at daily time-steps). To avoid extra biases, consecutive locations exceeding 20 days were removed and respective tracks were split into sections (BLOCK, JONSEN et al. 2011). Final interpolated tracks were then plotted using ArcGIS (ESRI Inc., CA, USA) for spatial dynamics inspection. Spatial usage from all shark individuals was then inspected by means of a kernel density estimator (KDE) using ArcGIS, after reducing deployment area biases. Here, mean days per grid cel was calculated as the ratio between the total number of positions within each cell and the number of respective tags (WALLI, TEO et al., 2011), normalising the data set density by the tracking effort per cell.

Silky shark diving behaviour

Vertical profiles for four sharks were derived from the two PSAT and the three MiniPAT satellite relayed depth data, i.e. binned summaries of depth and temperature utilization at every three hours. This dataset was processed using the manufacturer's software and then analysed by means of a custom written function in R software. Binned data consisted in time-at-depth (TAD bins 1, 10, 20, 30, 50, 70, 90, 110, 150, 200, 300 and >300 m) and time-at-temperature (TAT bins 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29 and >29°C). Vertical profiles were then analysed in relation to the occupied temperature for each individual track. When possible, for the PSAT datasets, diel analysis was performed, splitting the dataset following the algorithm provided by the National Oceanic & Atmospheric Administration (NOAA) in the website <http://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html>. Data from bins encompassing either sunrise or sunset hours were excluded from the analysis, assuring no night hours are wrongly considered in daylight period or *vice versa*. Diel depth and temperature data

were then plotted as frequency histograms by summarising both the TAD and TAT matrices into a 24 hour window and by averaging the depth (or temperature) attained at every four (or six) hours. In addition, using information recorded on both minimum and maximum depth (from the retrieved PDT data) the vertical extent was summarised per individual.

3. Results

3.1. Tagging and monitoring

PSAT

- ID: 95273

Although the PSAT tag 95273 was programmed to stay attached for 75 days, it didn't transmit any signal, probably due to technical problems. The tagging occurred on October 2010, on a male silky shark with 100 cm in total length (TL).

- ID: 90815

The PSAT tag 90815 was deployed in a male silky shark, with 130 cm, total length (TL), on October 2010. Although it was programmed to stay attached for 75 days, it stored data for only 6 days, beginning its transmission eight days after deployment, with 92% of them successfully decoded.

During these 6 days the shark showed a remarkable preference for warm and shallow waters of the mixed layer (Figure 5), spending 72% of the time in water temperatures above 27°C and 39% in the first 10m. The silky shark remained in the vicinity of the Archipelago during all the time that the tag was attached to it (Figure 6). Therefore the distance from the tagging site to the point where the tag was released (pop-up) was 130 km (80,78 miles).

The tagged shark showed a higher frequency of incursions at depths greater than 10m during the night, remaining practically all the time above this depth during the day. The incursions in depths less than 50m were not very common, except for a dive at night to depths between 50 and 70 m. Exposure to water less than 25°C were also rare, with no record below 20°C.

- ID: 101017

The PSAT tag 101017, programmed to stay attached for 75 days, remained attached to the shark for 74 days, popping-off on April 15, 2012. The tagged specimen was a male of 157 cm total length (TL). About 78% of the transmitted data were successfully decoded.

During the period in which the tag was attached to the shark, the animal showed a preference for warmer waters of the mixed layer (Figure 7), remaining most of the time in temperatures above 24°C, but with a preference for the interval of 26-27°C. It spent 54.6% of the time in those temperatures during the day, and 45.4%, during the night. The minimum temperature reached by the tagged shark was 14- 16°C, during the day (0.02%), corresponding to maximum depths of more than 100m (Figure 8 and 9).

With regard to depth, the tagged animal showed a higher preference for waters between 20- 60 m, spending 54.1% of the time in the interval of 40- 60 m, during the day, and 35.5% during the night; and 37.3% between 20- 40 m, during the day, and 32.1%, during the night (Figure 8). The tagged shark stayed at lower depths during the night than during the day. During the day it spent almost all the time above 20m, being around 6% of this period in waters down to 1m of the water column. Dives into depths of more than 60m, in waters of less than 24°C, were very rare, but happened during both periods (day and night), being, however, more frequent during the day. Incursions between 60- 80m, represented 6.2% of the day-time, and only 3.0% of the night-time. The deepest incursion performed was about 130m. The minimum temperature incursion performed by the tagged shark reached 14- 16°C, during the day (0.02%), corresponding to maximum depths more than 100m (Figure 8 and 9).

The tagging of the silky shark occurred in the east side of the Archipelago, just like all other taggings, but during the whole time the tag was attached, the shark remained in the west side of the Archipelago (Figure 10).

MiniPATs

- ID: 115348

This MiniPAT tag was programmed to stay attached for 30 days and the release (pop-off) occurred on July 28, 2012, after 31 days attached to the shark, one day after the original programming. The specimen was a female of 125 cm total length (TL).

During the period in which the tag was attached to the shark, the animal showed a higher preference for warmer waters of the mixed layer (Figure 11), remaining most of the time in temperatures above 24°C, particularly in the interval of 26-28°C, in which the animal spent 76.8% of the time.

With regard to depth distribution, the animal showed a higher preference for shallow waters, above 20 m, spending 14.8% of the time between 10- 20 m, almost 29% in the interval of 1-10 m, and 20% at the surface (up to 1m of the water column) (Figure 11). The tagged silky shark displayed, however, various incursions into greater depths, remaining at layers from 50 to 70m for about 11% of its time. The shark performed dives into depths of more than 70m, spending 5.5% of the time in waters of less than 24°C, between 70-90m, with some deep dives, to temperatures between 10-12°C, where they stayed for 0.08% of the time. The deepest depth reached by this shark was about 450m, at a temperature of 9°C (Figures 12 and 13).

The tagged silky shark remained close to the Archipelago, but during the time the tag was attached, performed movements to the west side and towards the north of the island. (Figure 14).

- ID: 115350

The MiniPAT tag was programmed to stay attached for 45 days and the release (pop-off) occurred at the approximate date of July 9, 2012, at a depth of 56m, after remaining 16 days attached to the shark. The specimen was a female of 163 cm total length (TL).

The animal also showed a higher preference for warmer waters of the mixed layer (Figure 15), as in previous PSAT and the first MiniPAT (ID: 115348), remaining most of the time in temperatures above 24°C, with a preference for waters in the interval of 26-28°C, in which the animal spent almost 63% of the time. The shark displayed a few incursions below 24°C, being, however, less than 2% of the time in the range of 22-24°C.

The tagged silky shark showed a preference for waters between 30 m to 90m of the water column, spending 38% of the time between 50-70 m. Although it also visited waters above 20m, the time it spent in these shallower layers was about 7%, between 1-10 m, and 7% between 10-20 m (Figure 16). It also performed dives into depths of more than 70m, in waters of less than 24°C, spending 22% of the time between 70-90m. The

maximum depth reached by this shark was more than 300m, at a temperature between 10-12°C (Figure 16 and 17), representing 0.07% of the time that the shark spent on this temperature range.

After the tagging on the east side of the Archipelago, the silky shark moved south. It spent almost all the time, however, close to the island, moving northward later and concentrating its displacements in the north of the island (Figure 18).

- ID: 115349

The MiniPAT tag was programmed to stay attached for 60 days and the release (pop-off) occurred on August 1, 2012, at a depth of 88m, after remaining 35 days attached to the shark. The specimen was a female of 135 cm total length (TL).

During the period in which the tag was attached to the shark, the animal also showed a greater preference for warmer waters of the mixed layer (Figure 19), as it had been found in the previous results of the PSAT and MiniPATs (ID: 115348 and 115350). It remained most of the time in temperatures above 24°C, with a preference for the interval of 26-28°C, in which the animal spent almost 60% of the time, followed by the interval between 24-26°C, where it remained 33.4%.

The MiniPAT tag didn't record data for temperatures higher than 28°C, and the tagged shark displayed incursions below 24°C, where it stayed, however, less than 2% of the time. Analogously, the silky shark showed a preference for waters between 30 m and 90m of the water column, spending almost 33% of the time between 50-70 m. Although it also ventured into waters shallower than 30m, the percentage of time it spent from 10-20 m was equal to 9.7%, and to 8.1%, in depths between 20-30 m (Figure 20).

The shark performed dives into depths of more than 70m, in waters of less than 24°C, showing frequent incursions between 70-90 m, where it stayed for 21.7% of the time. It attained a maximum depth in the interval of 200-300 m, at a temperature between 12- 14°C. The maximum depth reached by this shark was about 220m, at a temperature around 14°C (Figure 20 and 21). Another incursion done by this shark reached 110m with 13°C of temperature. The shark stayed in the vicinity of the Archipelago while the tag was attached, performing displacements to the north of the island (Figure 22).

- ID: 128736

The MiniPAT tag was programmed to stay attached for 30 days and the tagging was performed on June 25, 2013. The specimen was a male of 180 cm total length (TL) and also received a plastic tag number #035 for further identification, in case it were captured again. Unfortunately, it didn't transmit any signal.

- ID: 128737

The MiniPAT tag was programmed to stay attached for 45 days and the tagging occurred on June 25, 2013. The specimen was a male of 140 cm total length (TL), and also received a plastic tag number #151 for further identification in case it were caught again. Unfortunately, it didn't transmit any signal either.

SPOTs

- ID: 115352

The SPOT tag (115352) had few data sent but had records in a few months between July and December of 2013, totalizing 44 days of data. The last signal occurred on July 12, 2013. The specimen was a male of 190 cm total length (TL), being in the range of the size at first maturity (180- 205 cm for Males; LANA et. al., 2012).

The tag didn't record temperatures above 25°C, probably due to technical problems. During the period in which the tag was attached to the shark, the animal showed a preference for warmer waters of the mixed layer, remaining most of the time in temperatures above 24°C, during day (48,7%) and night (46,7%), representing almost 50% in both periods of the total time recorded by the tag. The minimum temperature incursion performed by the tagged shark reached 16-18°C, during the day (5%) (Figure 23). Even with limited data, a close relationship between the tagged shark and the Archipelago is clear, since it didn't move away from the island in the period that the tag was transmitting (Figure 24).

- ID: 115353

The tag 115353 had about 52% of the data transmitted successfully decoded. The last transmission occurred on February 2, 2014, after 248 days, almost nine months,

attached to the silky shark. The specimen was a male of 240 cm total length (TL), being larger than the size at first maturity (180- 205 cm for Males; LANA et. al., 2012).

The tagged shark showed a preference for warmer waters of the mixed layer, remaining most of the time in temperatures about 27-28°C, representing 57.1% during the day and 60.2% at night. The minimum temperature incursion performed by the tagged shark reached 16-18°C, during the night (0.03%) (Figure 25).

During these almost nine months, the shark showed a movement around the Archipelago, being close to the island since the deployment of the tag, except for a few displacements to the 4000m isobath to the south of the Archipelago (Figure 26 A, B, C). The movement was concentrated in the eastern side of the Archipelago, near the site where the shark was tagged (Figure 27).

4. Discussion

The present study provides the first report of movement patterns and habitat use of silky sharks in the south Atlantic. Animal movements and its underlying motivations are important factors in the understanding of the ecology of a given population, particularly when it experiences threats that jeopardize its sustainability, such as overfishing, since an adequate understanding of their movements is crucial for the development of informed management strategies (HOWEY-JORDAN et al., 2013; TOLLOTTI et al. 2015; LEA, et al., 2015).

Elasmobranchs occupy the apical position in the food chain, and in general, also have slow growth, late sexual maturity and low fecundity, characteristics that make them particularly susceptible to overfishing (HOLDEN, 1974). Moreover, shark species are important bycatch components in many fisheries worldwide and, therefore, their declining catches usually do not lead to an economic failure of the activity, although the continuous fishing effort may lead to serious and irreversible damage of their stocks (MATTOS and HAZIN, 1997).

The findings of the present study support previous reports of *C. falciformis* to be a typically oceanic species, common in insular regions and known to be an epipelagic predator of warm tropical waters (COMPAGNO, 1984; FILMALTER et al, 2010; HOWEY-JORDAN et al., 2013). Here, the majority of the tracked animals remained

most of their time within temperatures between 26° and 28°C, with a minimum thermal layer above 22°C. Analysis of the depth profiles revealed an epipelagic zone occupancy, shallower than 200m, a behavior very similar to tagged conspecifics in the North Atlantic and Pacific Oceans, and also observed in other pelagic shark species, like the oceanic whitetip (FILMALTER et al, 2010, TOLLOTTI et al., 2011, 2015; HOWEY-JORDAN et al., 2013).

The thermal preference for silky sharks found in this study 26°-28°C, however, was higher than previously described (BONFIL, 2007), with the species preferring waters with temperatures around 23°C. Such a difference is likely related to the different geographic areas. Yet, the preference for the mixed layer (0-70 m) for all tracked individuals conforms to the known predominant permanence within the surface mixed layer of this species, rarely incurring in vertical depths exceeding 70 m or temperatures below 20°C. This daily surface occupancy of the silky shark contrasts to the diel vertical migration (DVM) exhibited by most large pelagic fish, that tend to be in deeper waters during the day and in shallower layers during the night (BRILL et al, 1999; HUSE and KORNELIUSSEN, 2000; MUSYL et al, 2003; SIMS et al. 2003; MUSYL et al., 2004; BONFIL et al., 2005; SIMS et al., 2005; WENG et al., 2005; STOKESBURY et al., 2005; PADE et al, 2009; TOLLOTTI et al, 2015). Notwithstanding, this shallow distribution during day hours has been already found in other species, such as the oceanic whitetip shark (*C. longimanus*), which also showed a preference for shallower waters during the day, in the South Atlantic Ocean (TOLLOTTI et al. 2011, 2015).

According to FILMALTER et al. (2015), the long residence time of silky sharks around FADs may suggest that they are able to find sufficient food in their vicinity to support their energetic requirements, which would certainly be lower than those of tunas. A similar hypothesis could also explain the association of the silky sharks tagged to the vicinity of St. Peter and St. Paul Archipelago, suggesting that the archipelago may be playing a role similar to a FAD. Oceanic islands are, in general, important fishing grounds for commercially exploited pelagic species that aggregate around them (HAZIN, 1993; TRAVASSOS et al., 1999; LANA et al., 2012), in a process not much different from a FAD. The high regularity of the time of the day silky sharks depart from the proximity of FADs, following sunset (FILMALTER et al., 2015), suggests that the onset of darkness is the major stimulus for sharks to initiate a switch in their

behavioral mode, which could also trigger the different behaviors during day and night times observed in the present study.

On the other hand, tracked silky sharks exhibited an elevated residency to the SPSPA. Geographically, the Archipelago is situated strategically between the two hemispheres and also between the American and African continents, exerting strong influence on the life cycle of several migratory species such as fish and crustaceans. The SPSPA is a well known feeding zone for yellowfin tuna (*Thunnus albacares*) and reproduction for several species, including the fourwing flyingfish (*Cypselurus cyanopterus*) (VIANA et al., 2008; VASKE-Jr et al., 2010), being this last considered the base of the food chain in this area.

The silky shark is probably attracted to the archipelago to feed on the teleost species aggregating there. The circadian changes in their vertical distribution, therefore, is likely related to similar changes of behavior of their prey species.

In this context, FILMALTER et al. (2015) provided important new insight into the behavior of the silky sharks tagged in the western Indian Ocean that can be compared to the results found in the present study on South Atlantic. The authors reported that silky sharks remained associated with the fish aggregating devices (FADs) where they were tagged for extended periods. Generally, individuals moved away from FADs after sunset and returned later at night, then remained closely associated to the FAD until the following evening. Their vertical behaviour also changed around sunset, with sharks using fairly constant depths (>25 m) during the day and switching to rapid vertical movements during the night, with descents over 250 m. The actively tracked individuals returned to a FAD from > 1.2 km away. The long residence times of silky sharks around FADs may suggest that they are able to find sufficient food in the vicinity of these devices to support their energetic requirements. This is also likely the case of silky sharks in the vicinity of SPSPA, suggesting that the archipelago ends up being similar to a FAD. Areas characterized by topographic features such as the Archipelago are commonly important fishing grounds for pelagic species in the northeastern coast of Brazil, including the silky shark (HAZIN, 1993; TRAVASSOS et al., 1999; LANA et al., 2012). FILMALTER et al. (2015) showed that the long residence times and close association of silky sharks to the FADs increase their vulnerability to incidental catch. Overall, this study revealed a high residency of tracked silky sharks in the vicinity of the SPSPA, with individuals remaining in that area for long periods of time (up to nine

months). Therefore, a similar concern also applies to the ASPSP and consequently to the potential impact of local fisheries on silky shark populations. As no long migration were detected in this study, further tracking of larger numbers of individuals is necessary to better understand the residency and/or philopatry of silky sharks in the equatorial Atlantic.

In 2011, ICCAT adopted a management measure for the conservation of silky sharks caught in association with fishing for tuna and related species, which was internalized in Brazil through the Interministerial Normative Instruction nº 8 (November 6, 2014). Both measures establish that any silky shark caught incidentally in the tuna longline fishery shall be returned to the sea, alive or dead, at the time of retrieval of the fishing gear, prohibiting, at the same time, any directed fishing, retention on board, transshipment, landing, storage, transport or marketing of this species. The present results clearly show the importance of such a measure, particularly in the vicinity of oceanic islands or FADs, due to the strong association these sharks show to these structures.

We acknowledge the limits imposed by the low number of tracked sharks and the associated temporal periods of satellite tracking, yet, we present here, for the first time to our knowledge, an assessment of this species in the region. Multidisciplinary studies integrating population genetic analyses and electronic tagging, as well as the development of improved monitoring instruments, are thus encouraged.

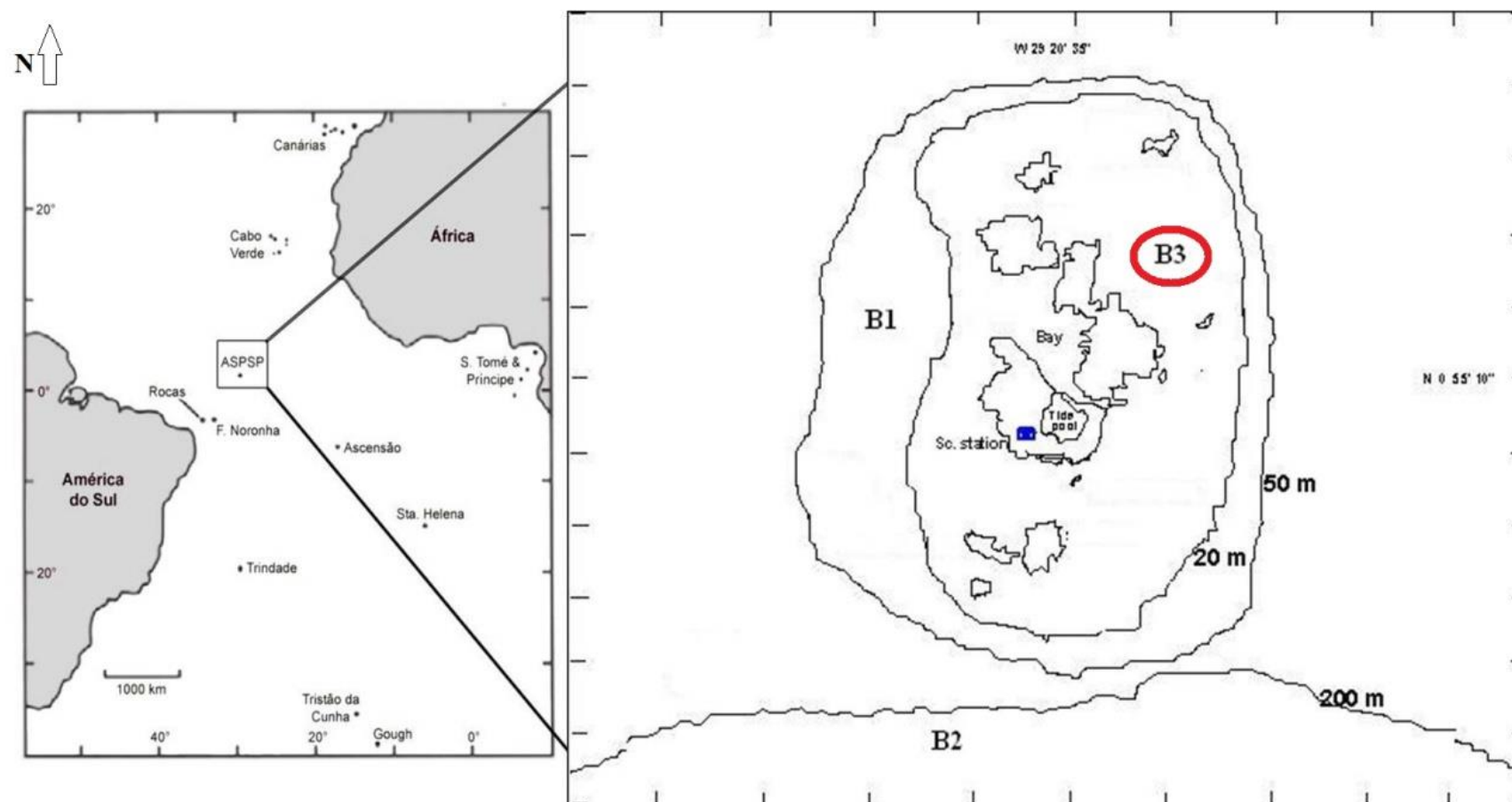


Figure 1 - Location of the Archipelago of Saint Peter and Saint Paul (left) and distribution of buoys B1, B2 and B3 to the west, south and east, respectively (right). The red circle indicates the float B3, around which the silky sharks were tagged with PSAT, MiniPAT and SPOT tags.

LANA, F. O. Morfologia, Identificação Molecular e Análise Comportamental Via Marcações Eletrônicas, do Tubarão lombo-preto, *Carcharhinus falciformis* (Muller & Henle, 1939), no Oceano Atlântico Sudoeste Equatorial.

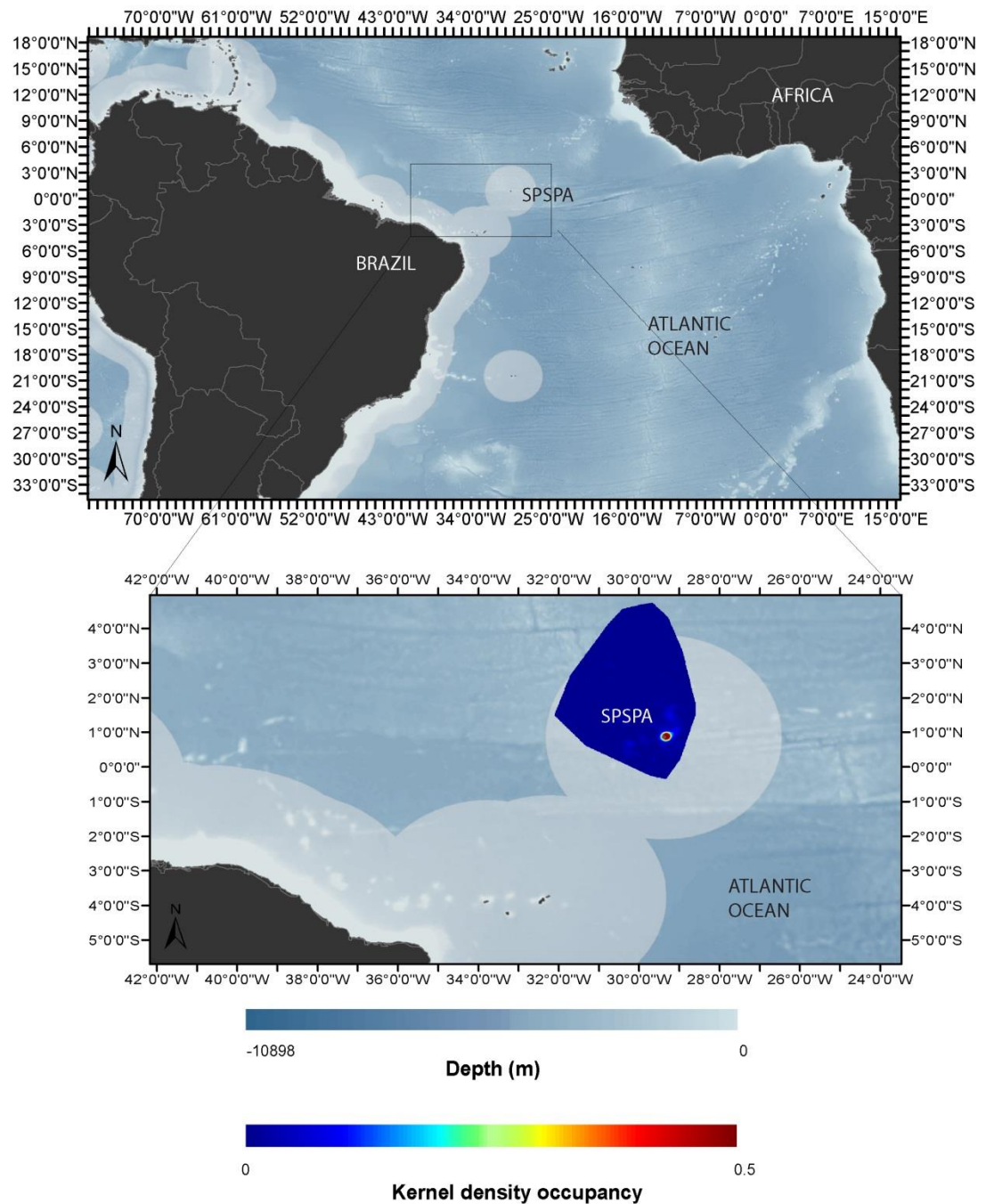


Figure 2- Location of the Archipelago of Saint Peter and Saint Paul – BRAZIL.

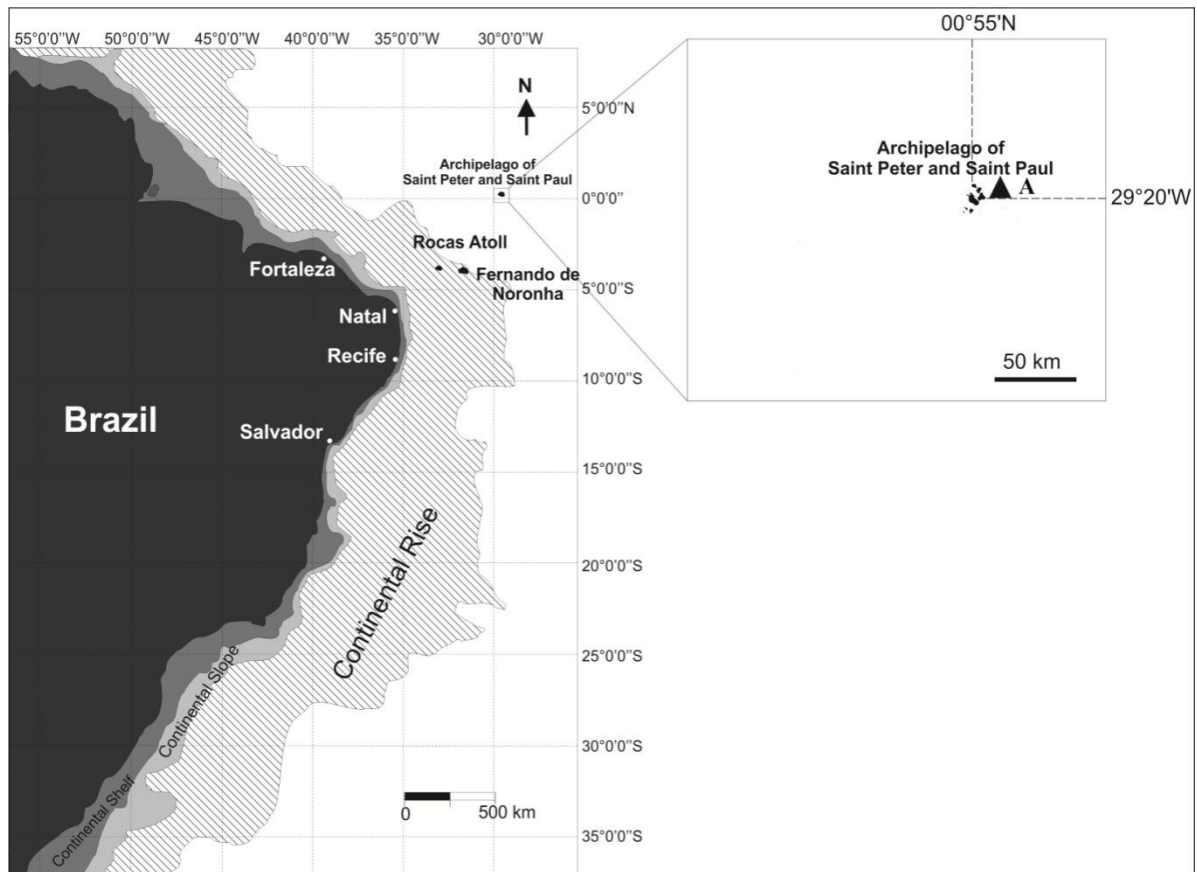


Figure 3- Local of tagging of the silky sharks, *Carcharhinus falciformis*, tagged with electronic tags: PSAT (Pop-up Satellite Archival Tags), MiniPAT *Pop-up Tag* and SPOT (*Smart Position or Temperature Transmitting Tag*) in the southwest and equatorial Atlantic Ocean.

Legend: ▲ Tagging of tags (Point A);

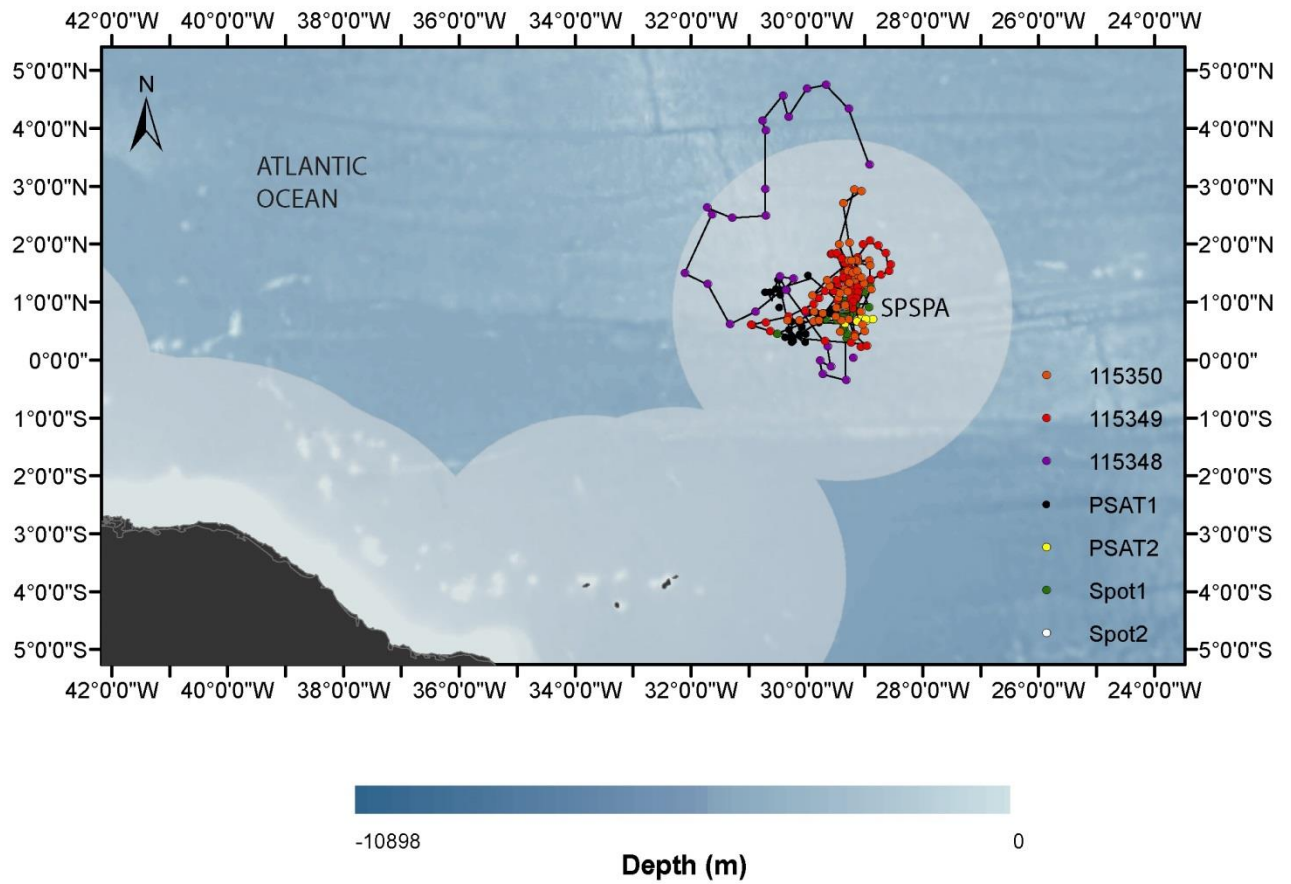


Figure 4- Interpolate tracks of the silky sharks, *Carcharhinus falciformis*, tagged with electronic tags: PSAT (Pop-up Satellite Archival Tags), MiniPAT and SPOT in the southwest and equatorial Atlantic Ocean.

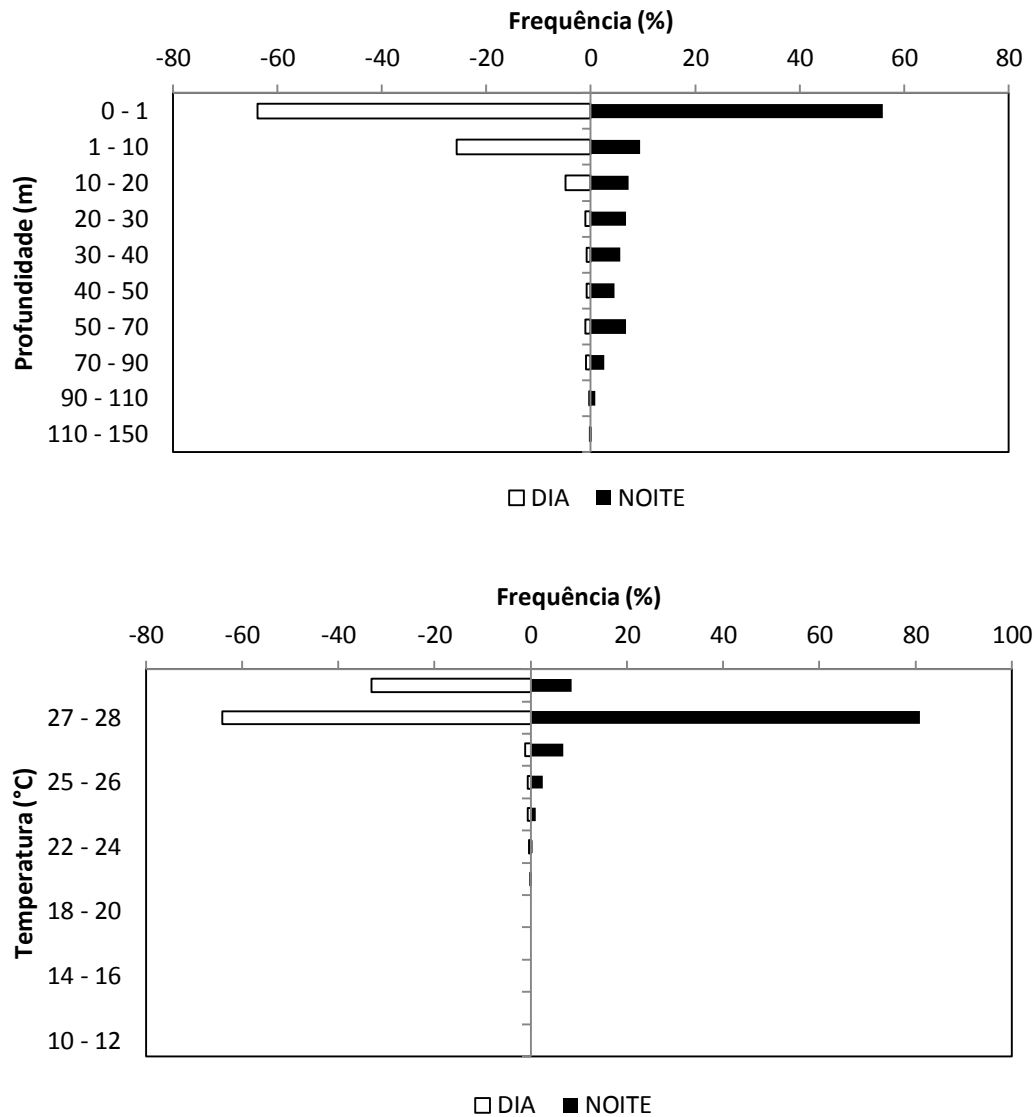


Figure 5- Depth and temperature distribution of a silky shark tagged with PSAT (90815) tag around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

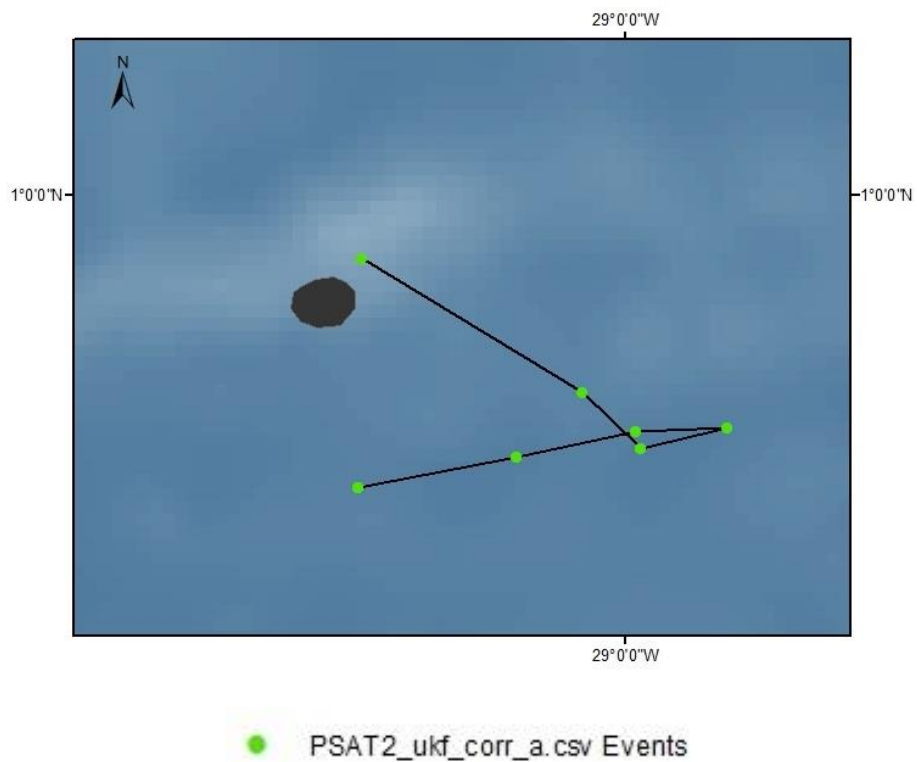


Figure 6- Geolocation of the silky shark tagged with PSAT (90815) around the St. Peter and St. Paul Archipelago.

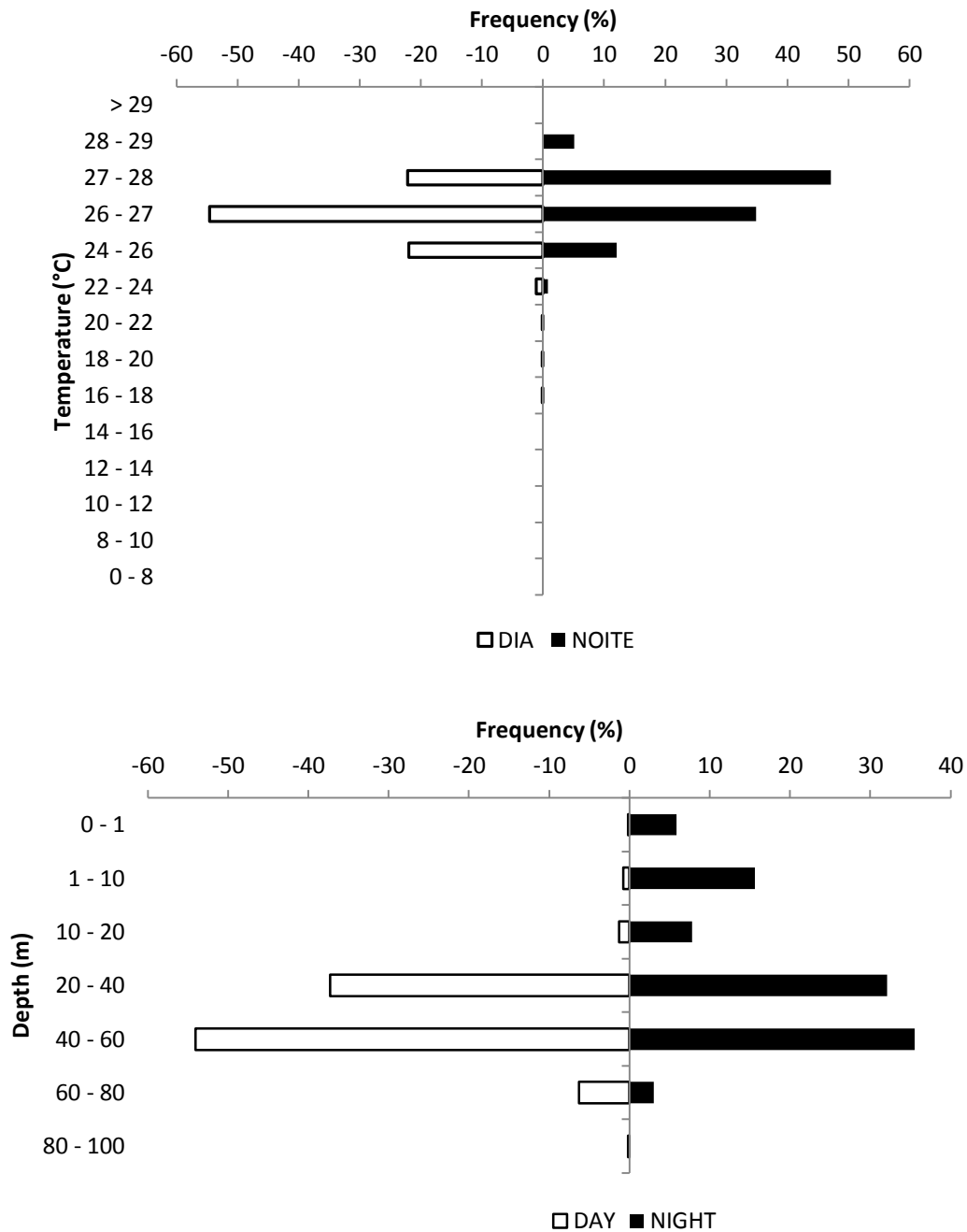


Figure 7- Depth and temperature distribution of a silky shark tagged with PSAT (101017) tag around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

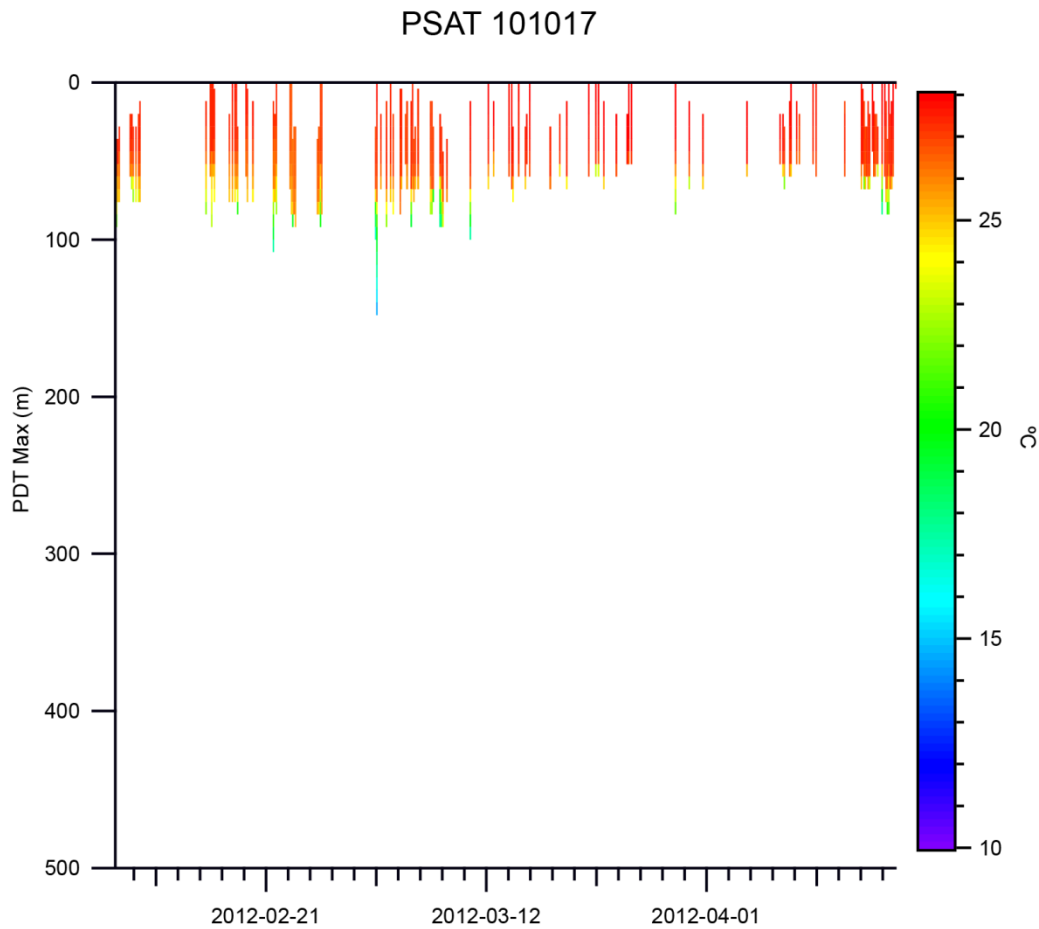


Figure 8- PDT Max(m) for the period of a silky shark tagged with PSAT (101017) tag around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

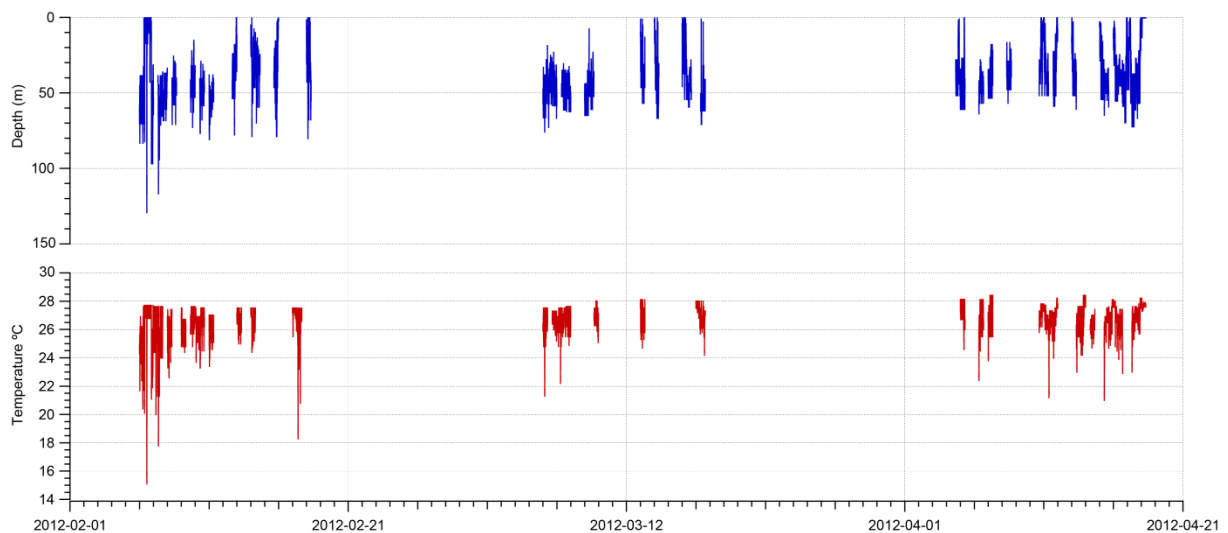


Figure 9- PDT Max(m) for the period of a silky shark tagged with PSAT (101017) tag around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

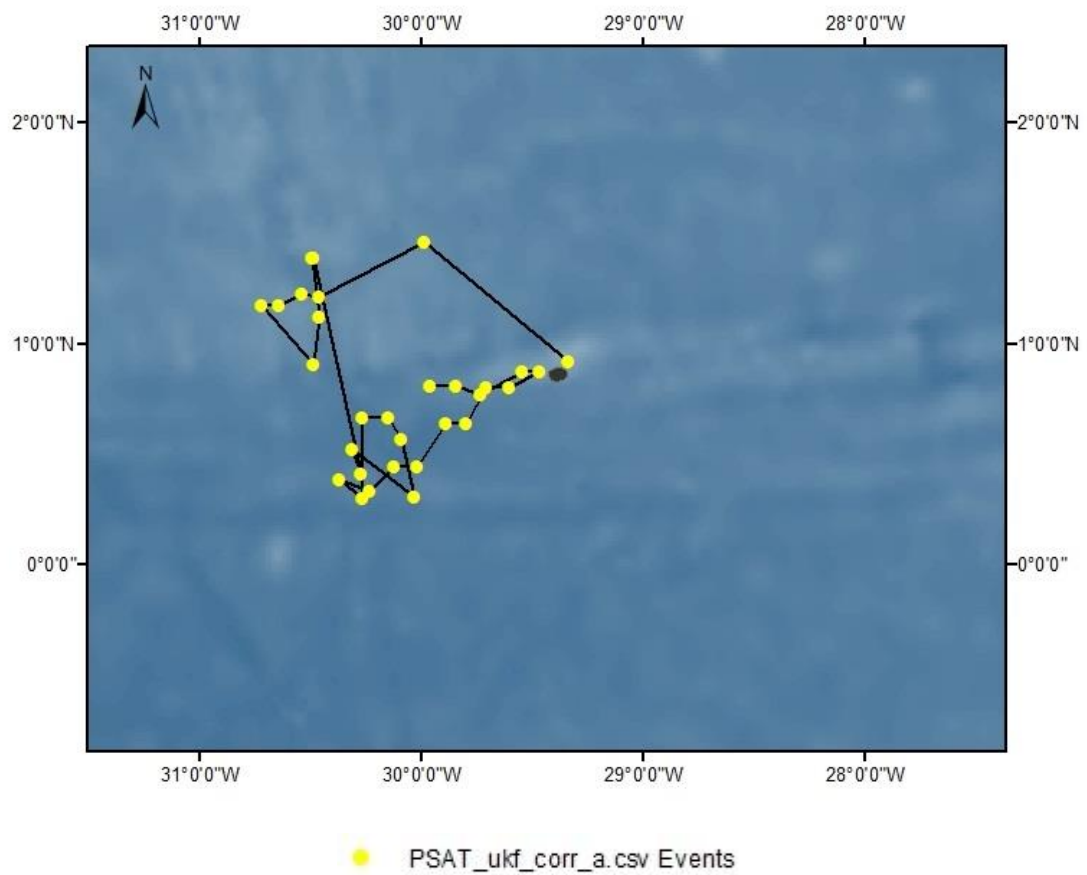


Figure 10- Geolocation of the silky shark tagged with PSAT (101017) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

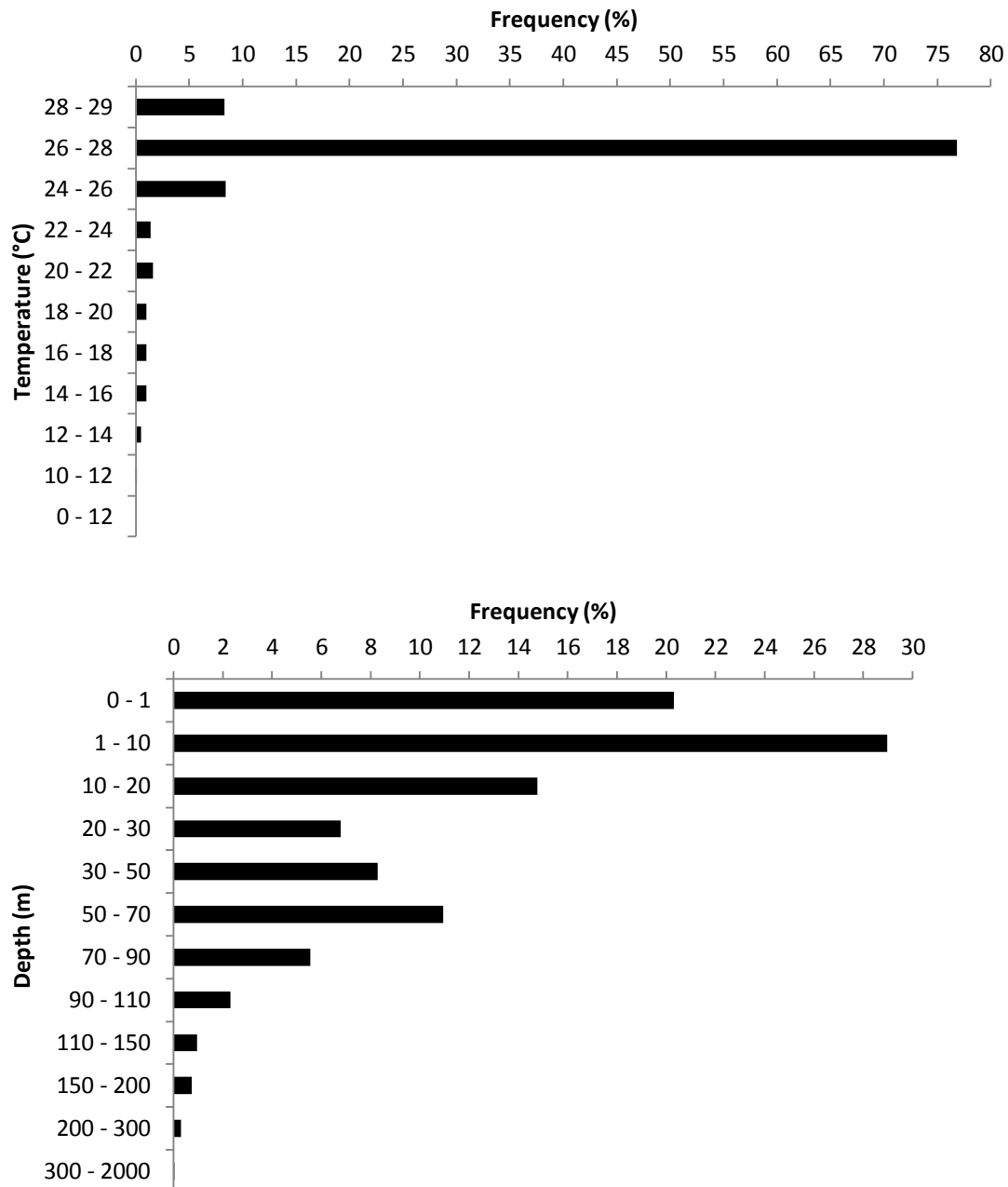


Figure 11- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115348) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

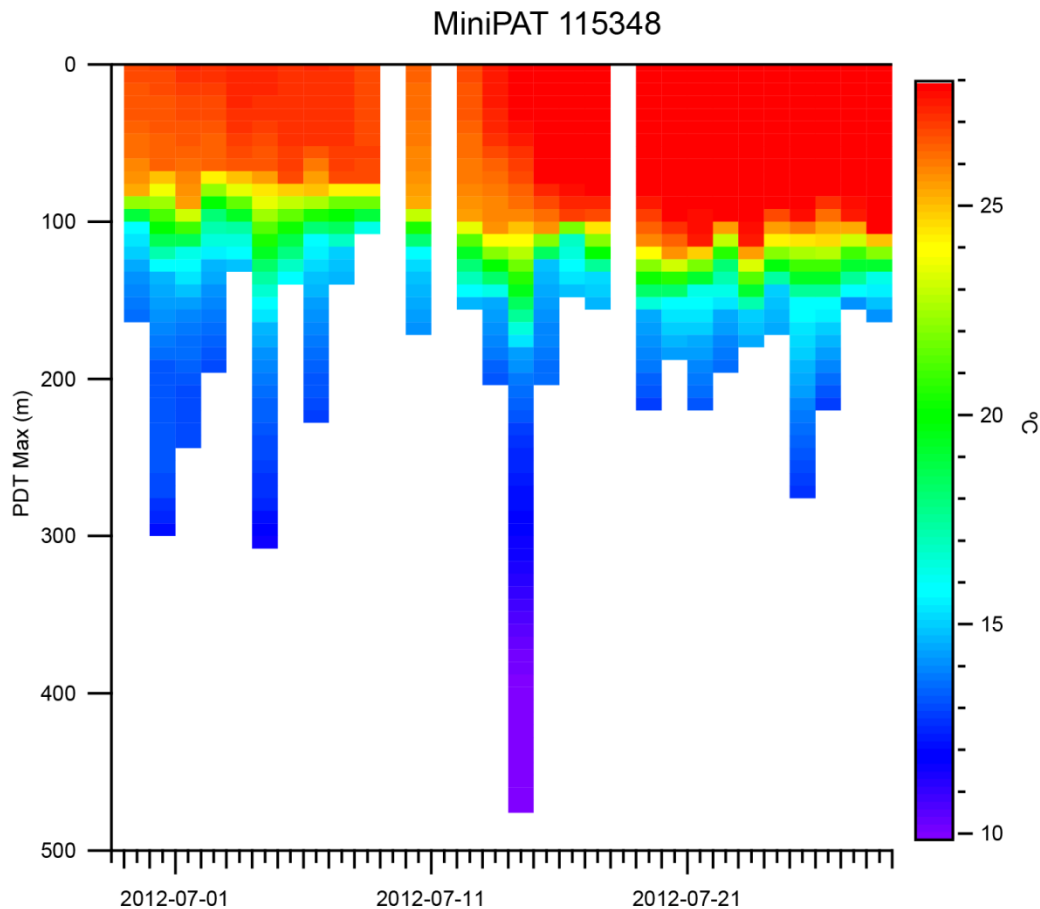


Figure 12- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115348) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

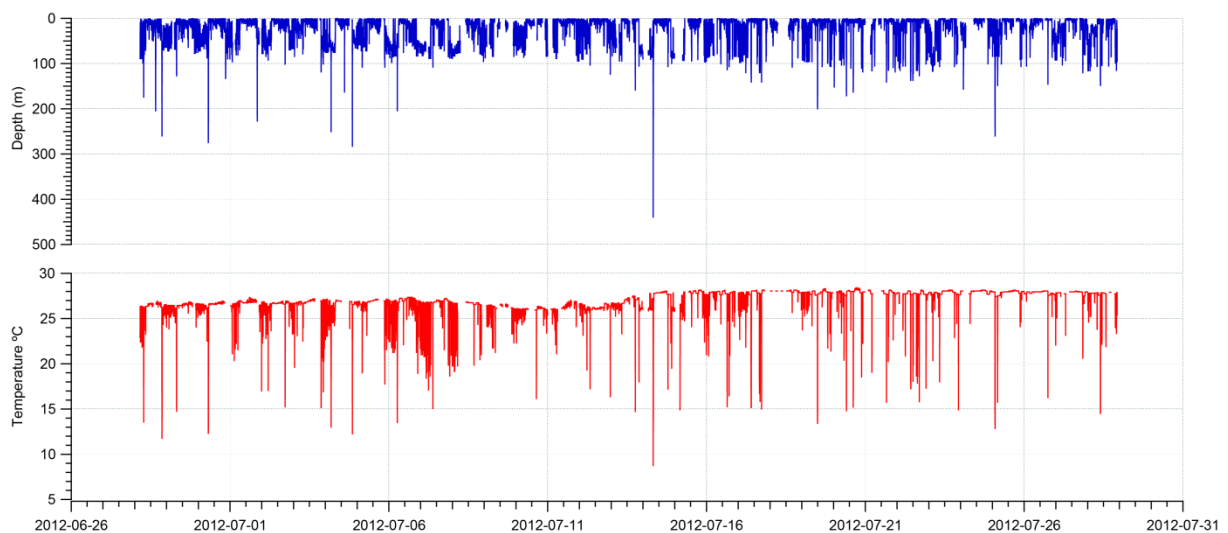


Figure 13- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115348) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

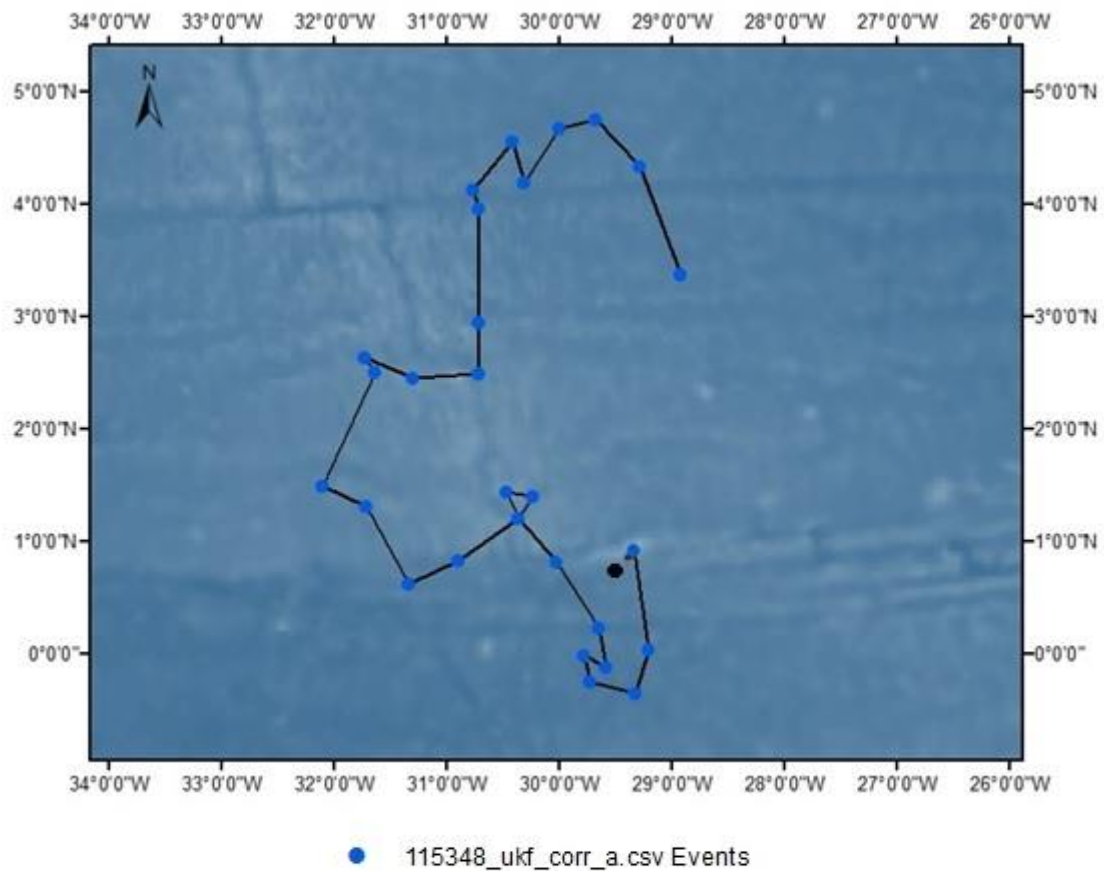


Figure 14- Geolocation of the silky shark tagged with MiniPAT (115348) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

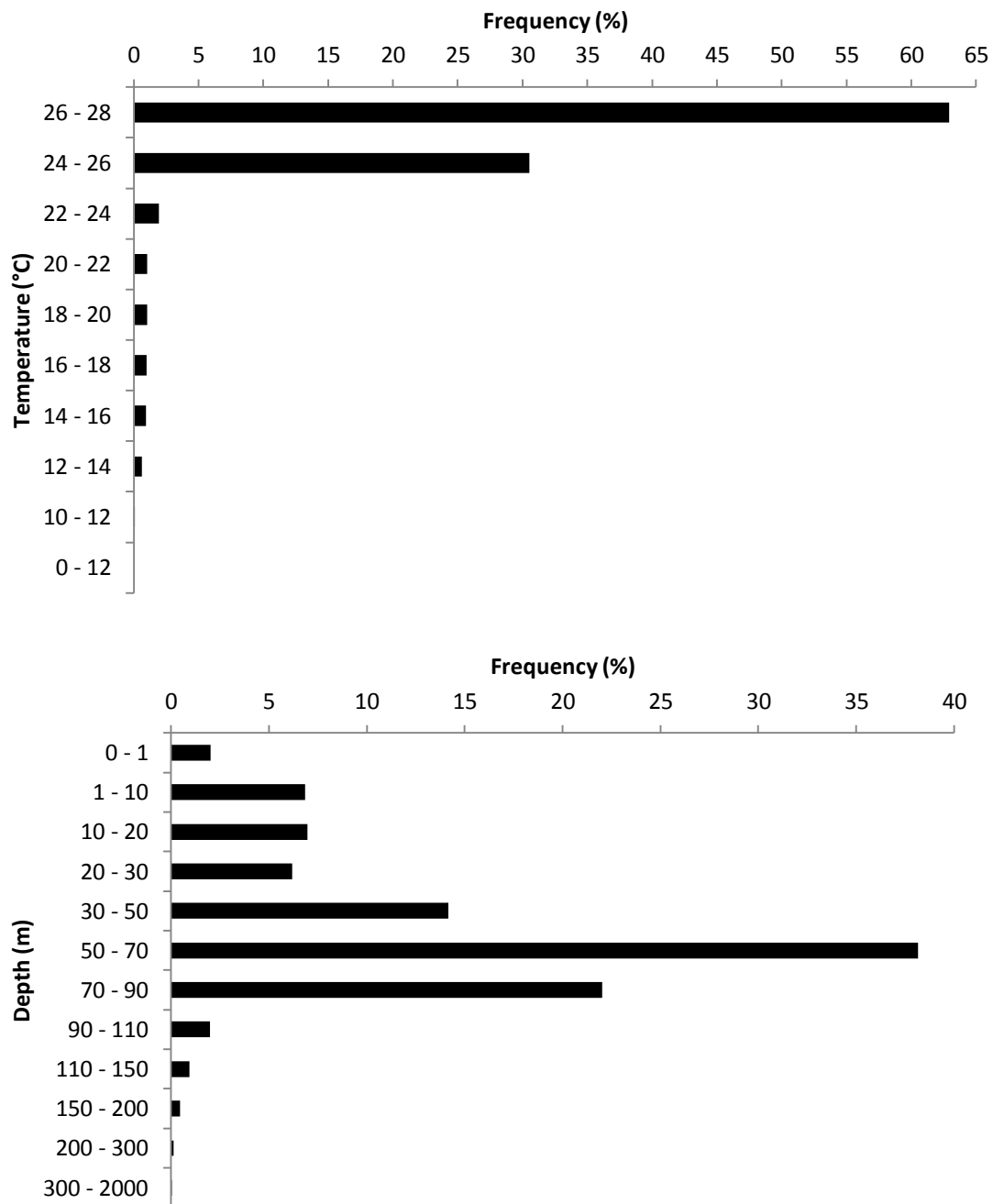


Figure 15- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115350) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

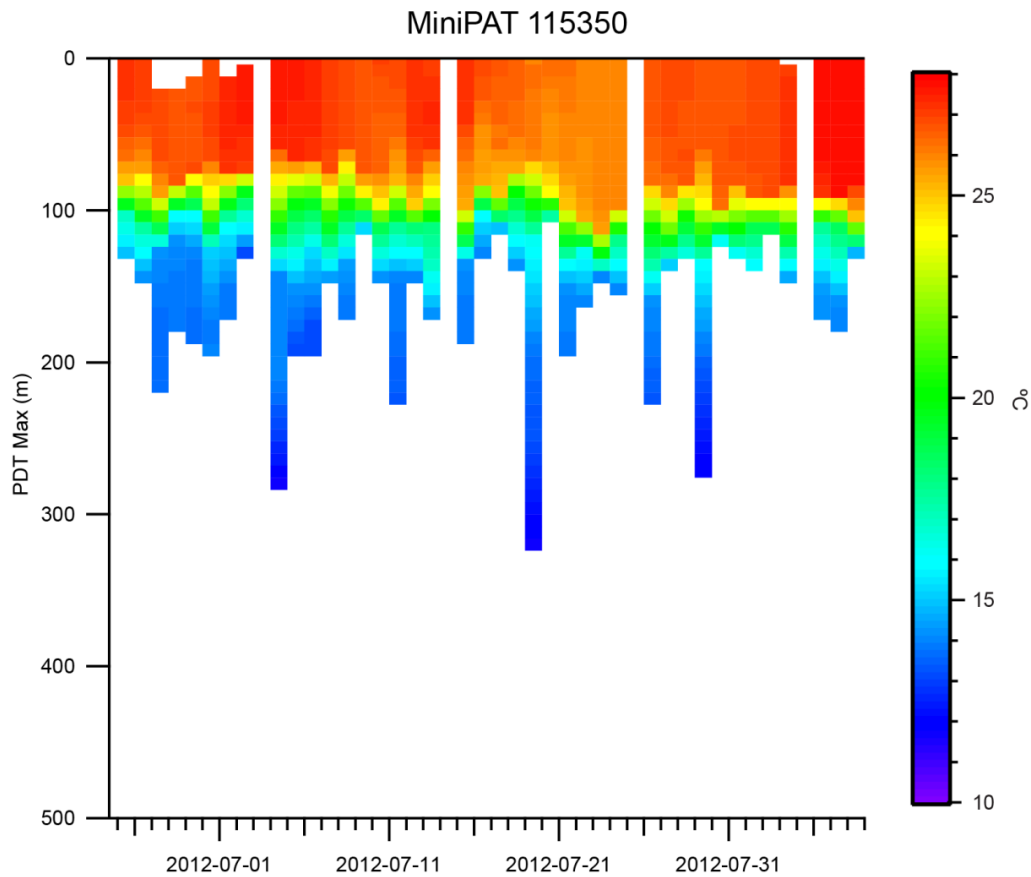


Figure 16- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115350) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

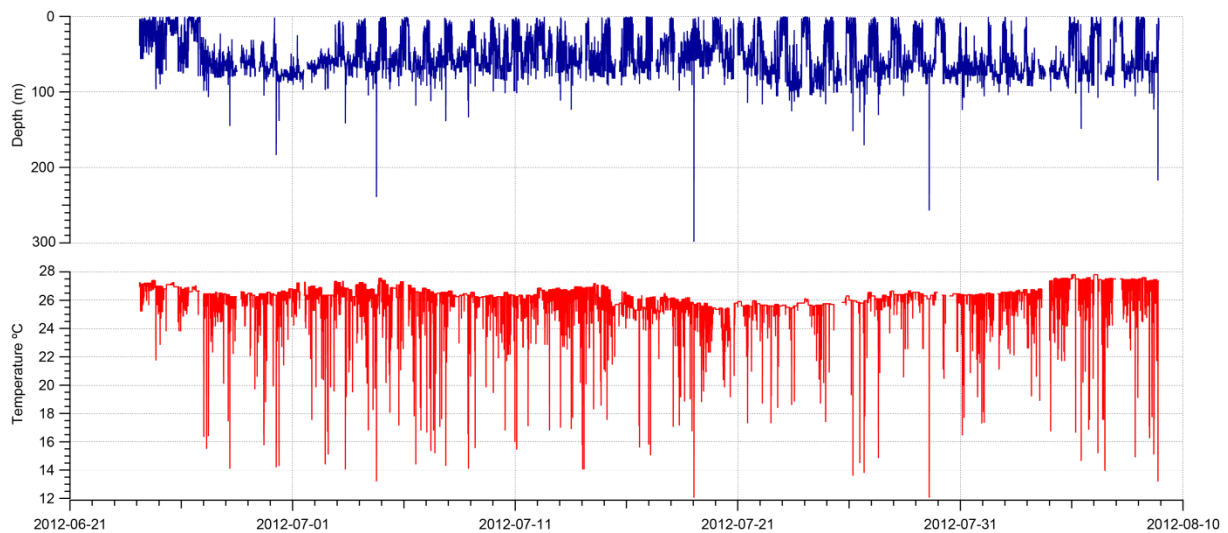


Figure 17- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115350) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

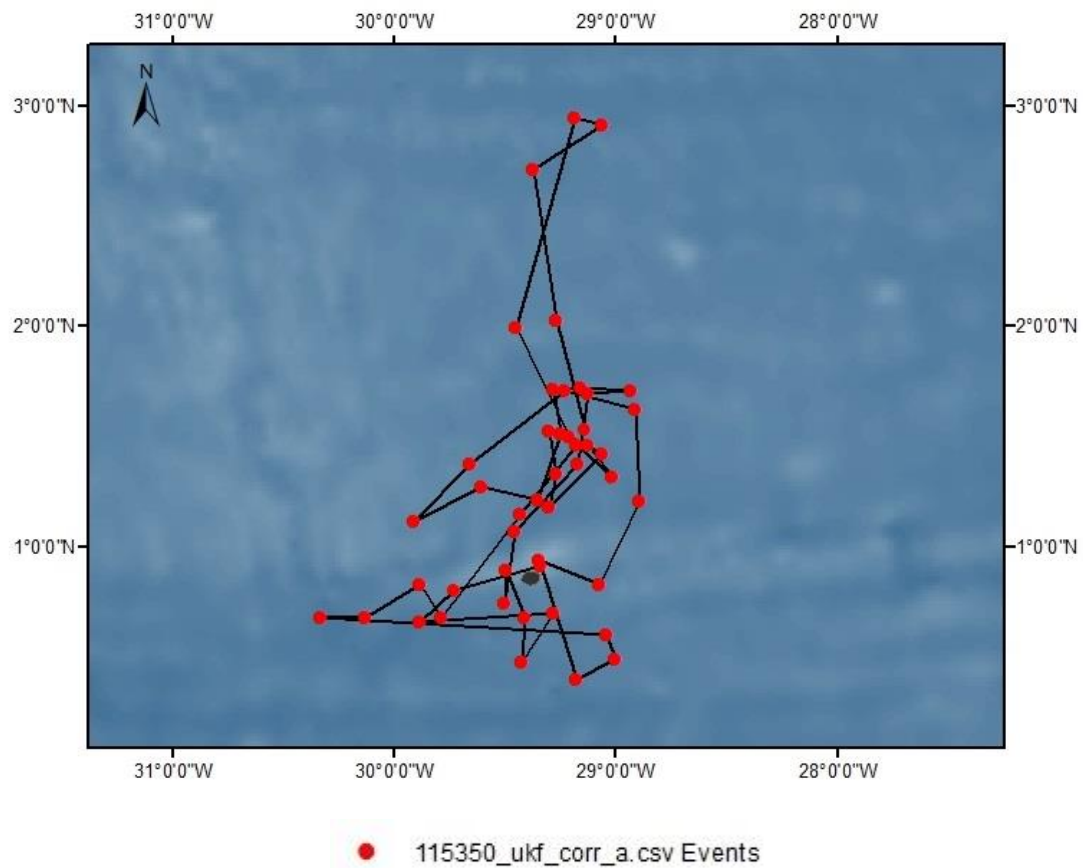


Figure 18- Geolocation of the silky shark tagged with MiniPAT (115350) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

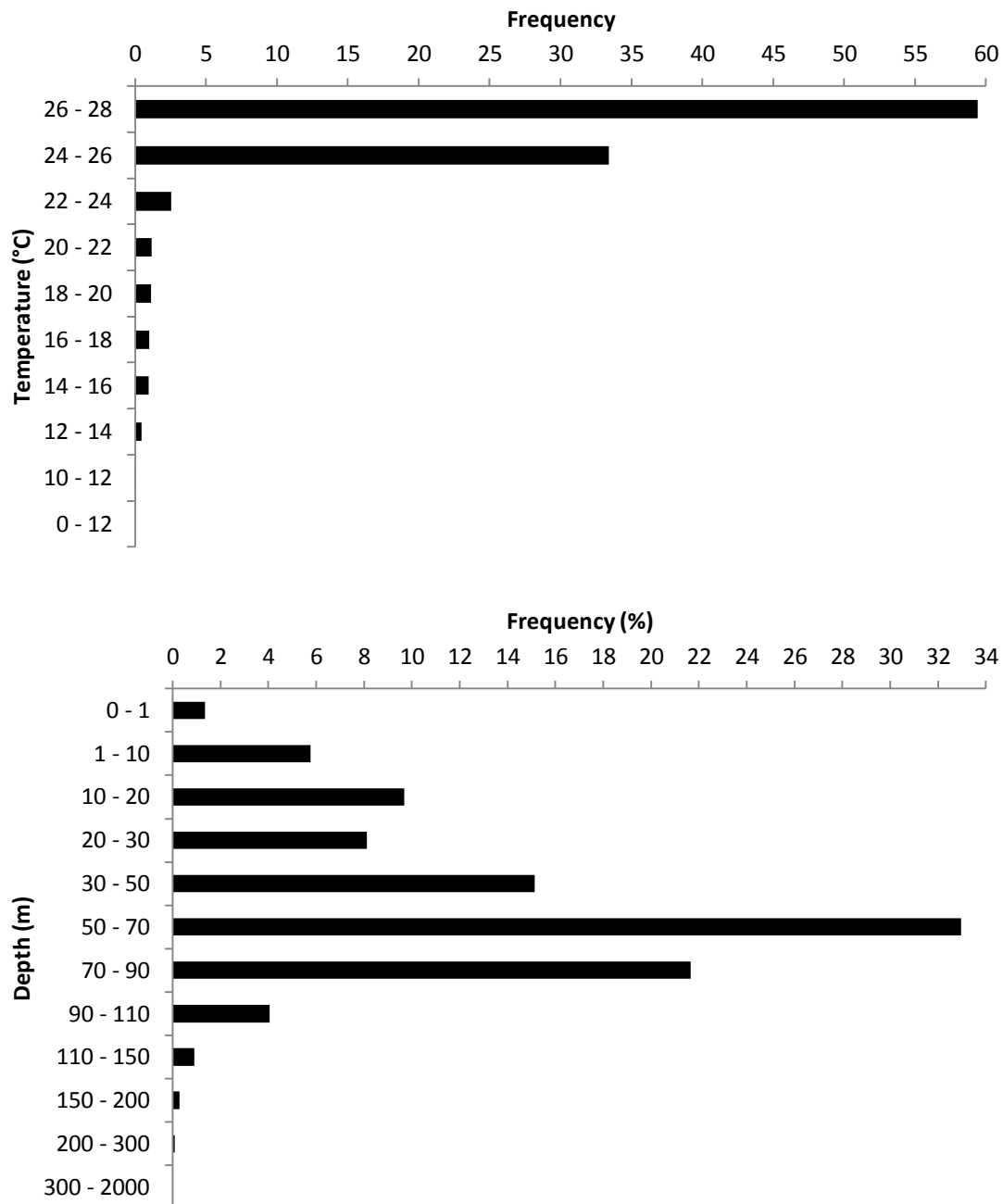


Figure 19- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115349) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

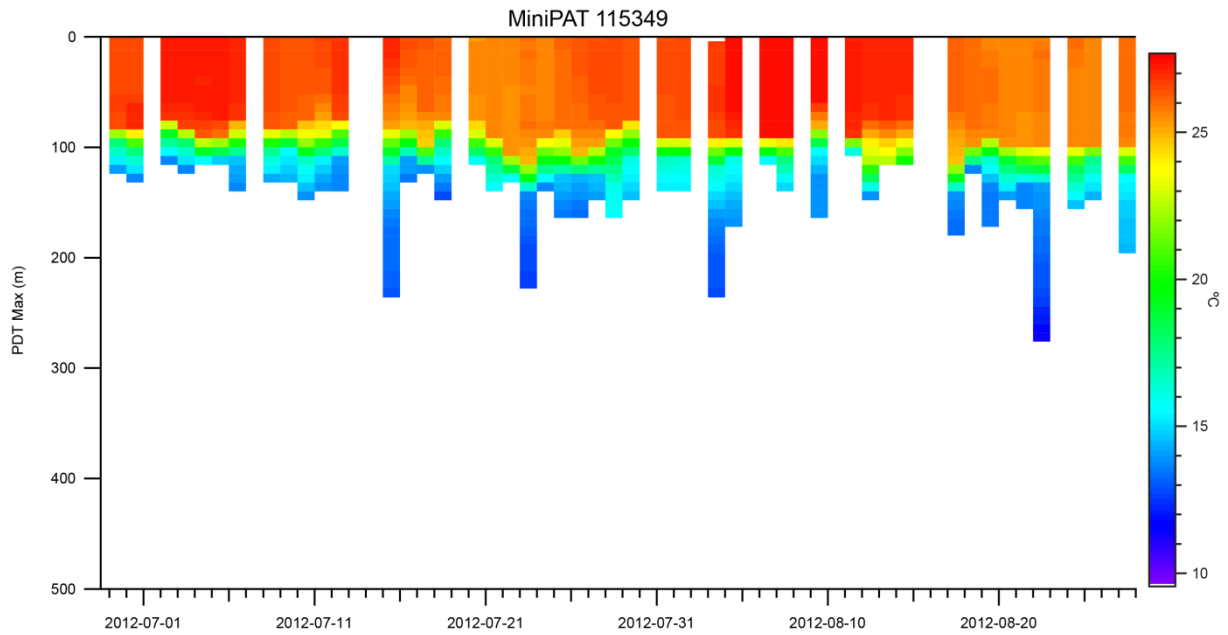


Figure 20- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115349) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

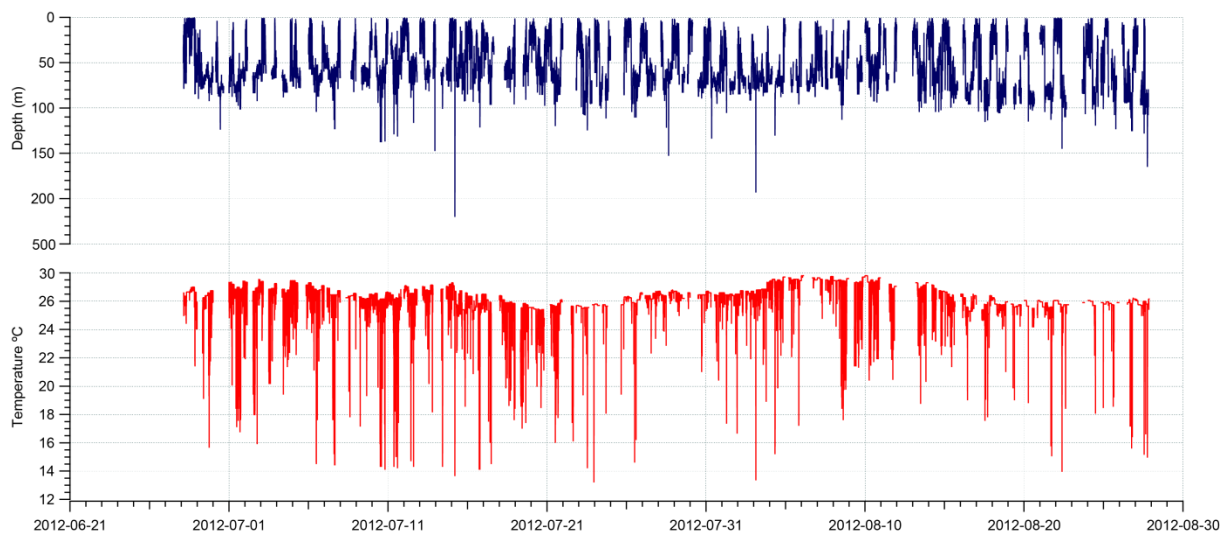


Figure 21- Depth and temperature distribution of a silky shark tagged with MiniPAT tag (ID 115349) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

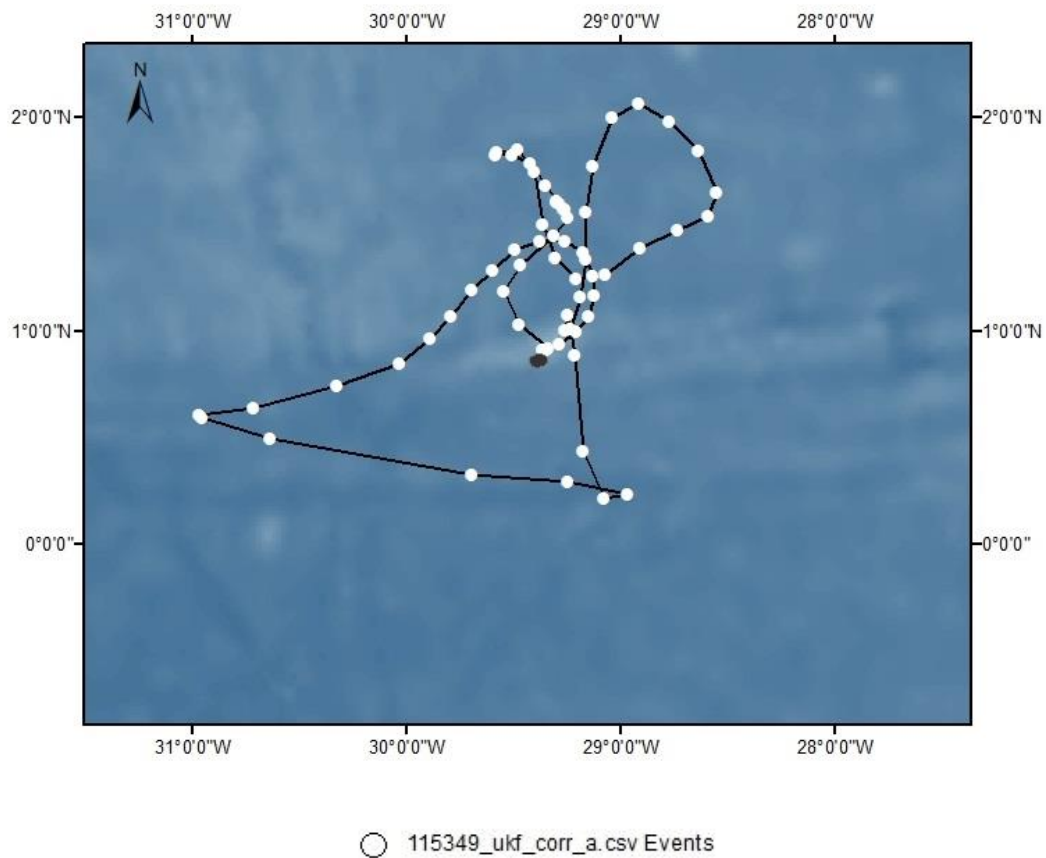


Figure 22- Geolocation of the silky shark tagged with MiniPAT (115349) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

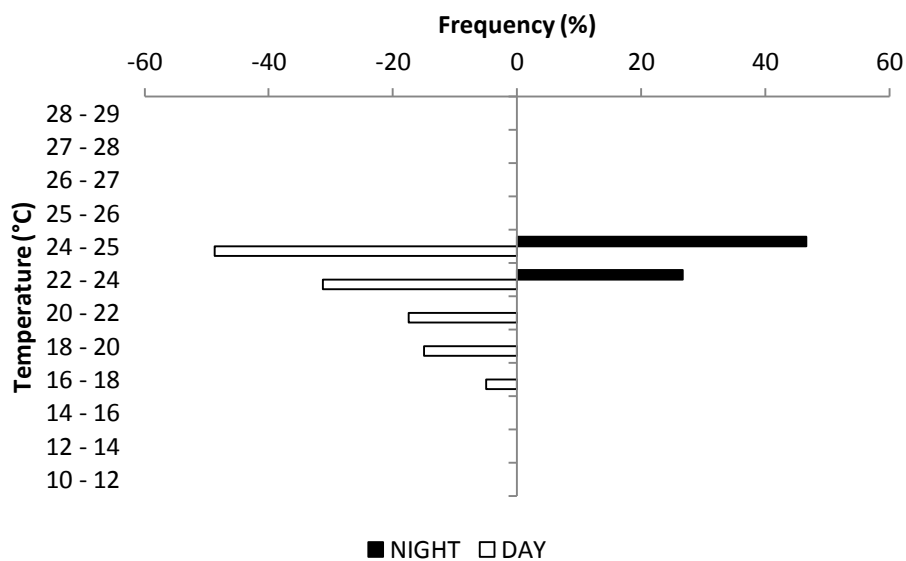


Figure 23- Temperature distribution of a silky shark tagged with SPOT (115352) tag around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

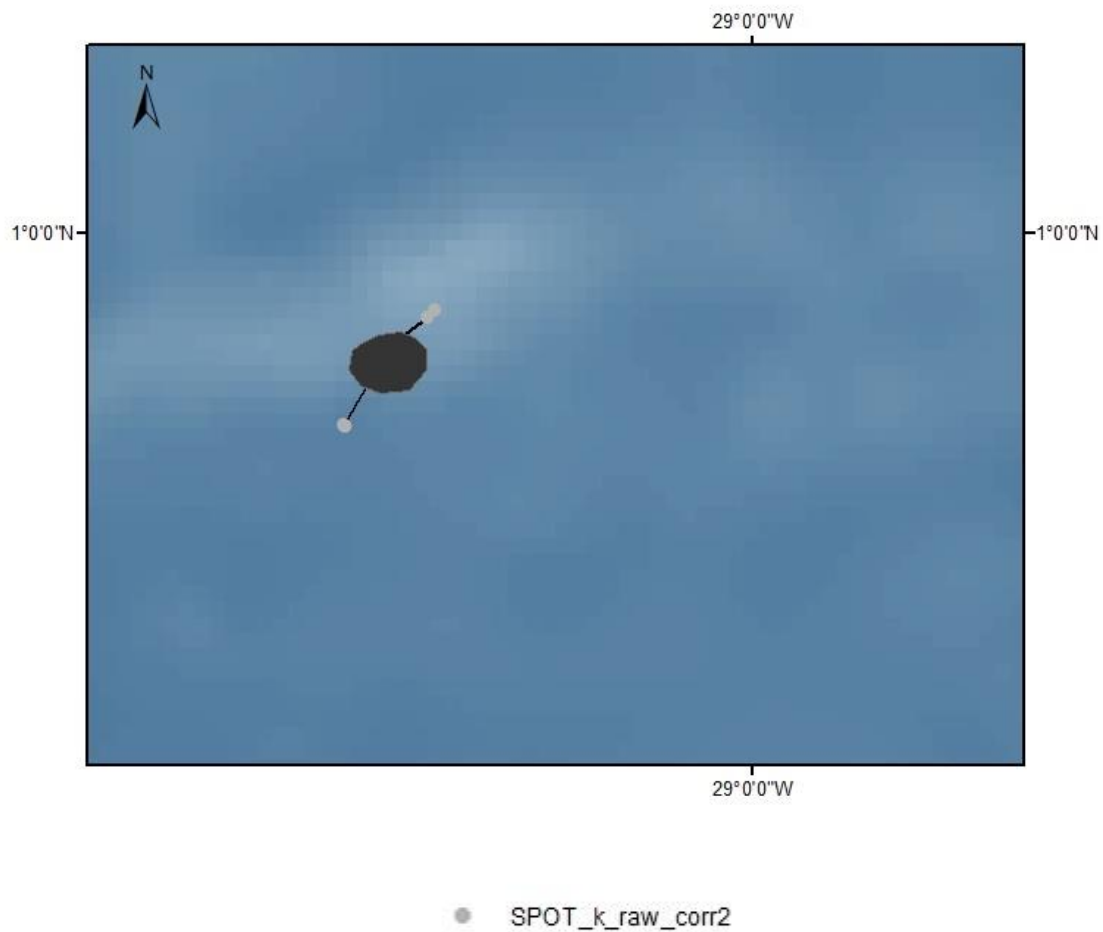


Figure 24- Geolocation of the silky shark tagged with SPOT (115352) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

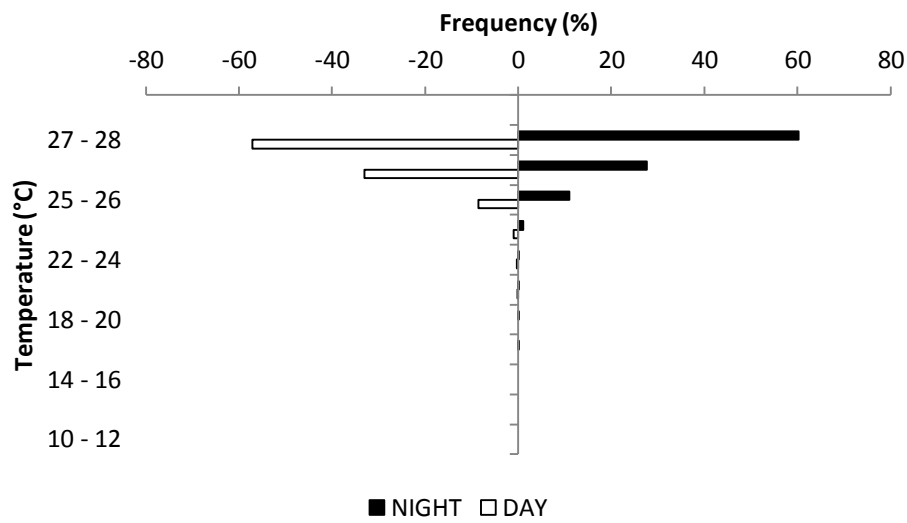
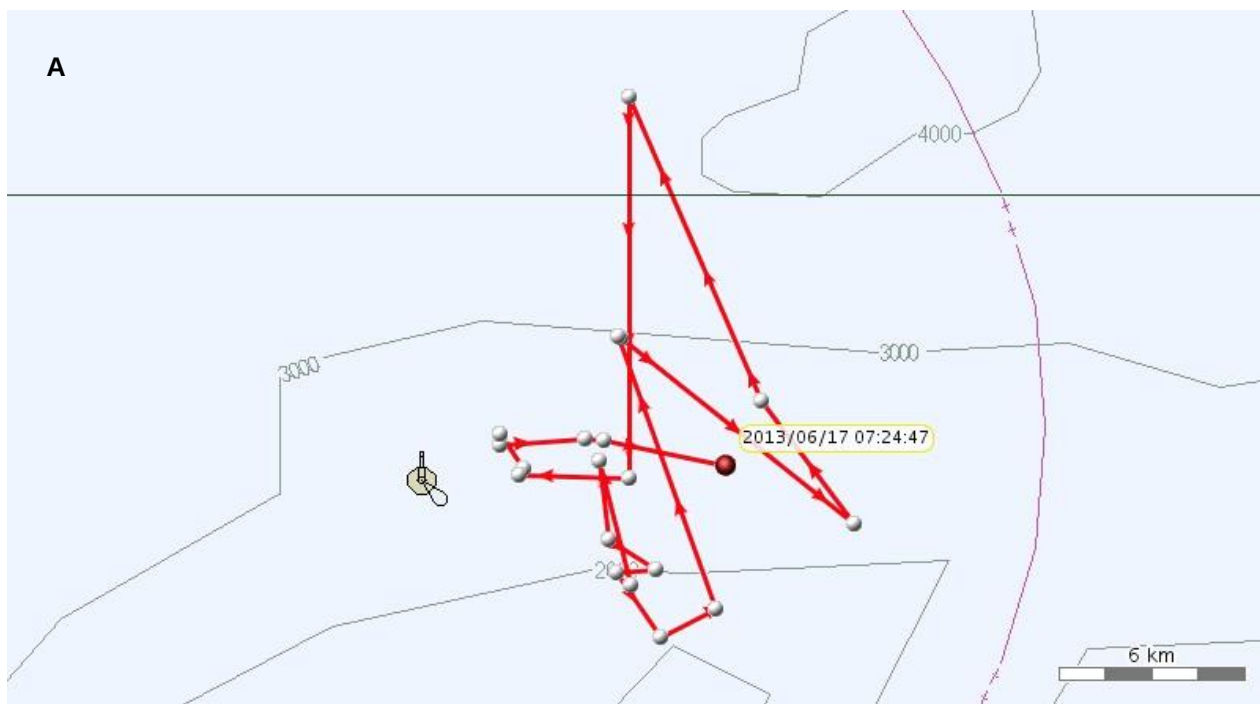


Figure 25- Temperature distribution of a silky shark tagged with SPOT (115353) tag around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.



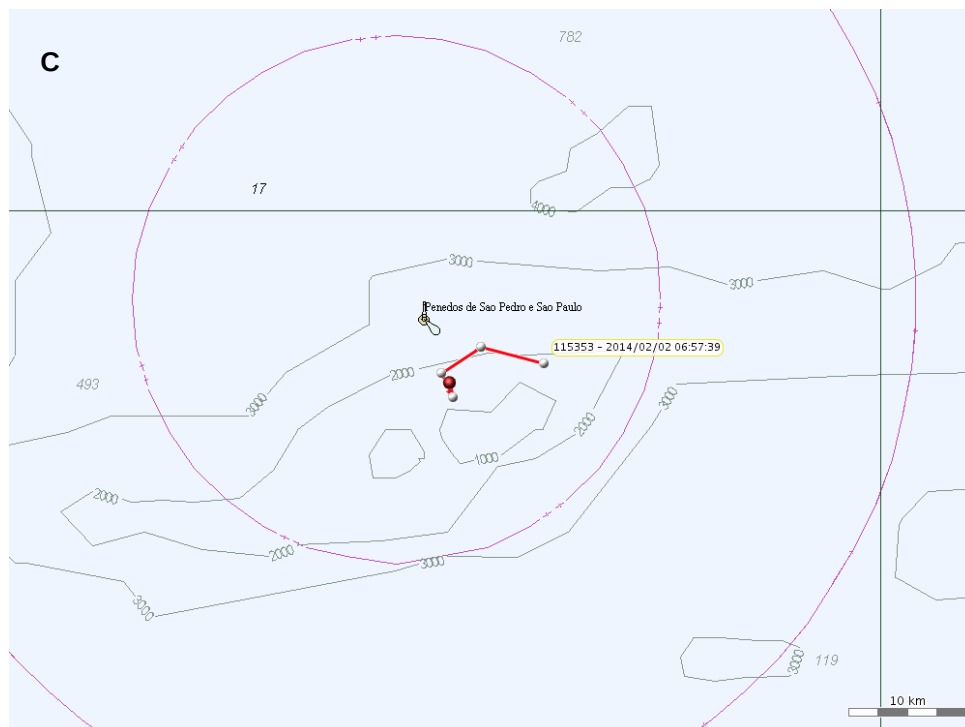
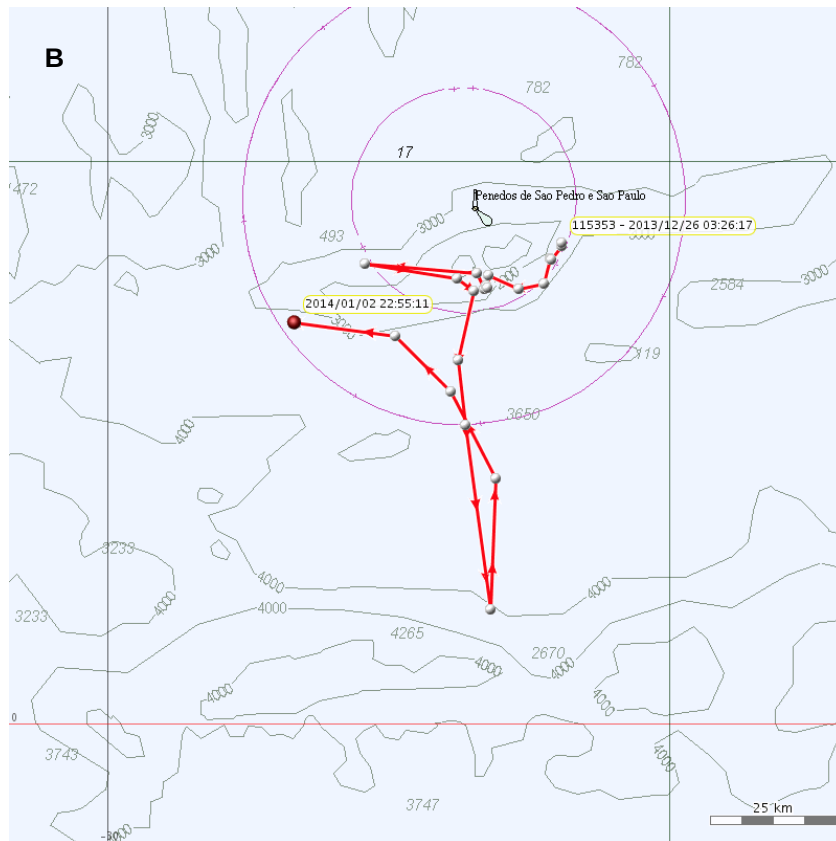


Figure 26- Movements of the silky shark tagged with SPOT (115353) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean. A. 17 June 2013, B. 02 Jan 2014, C. 02 Feb 2014 (Last signal)

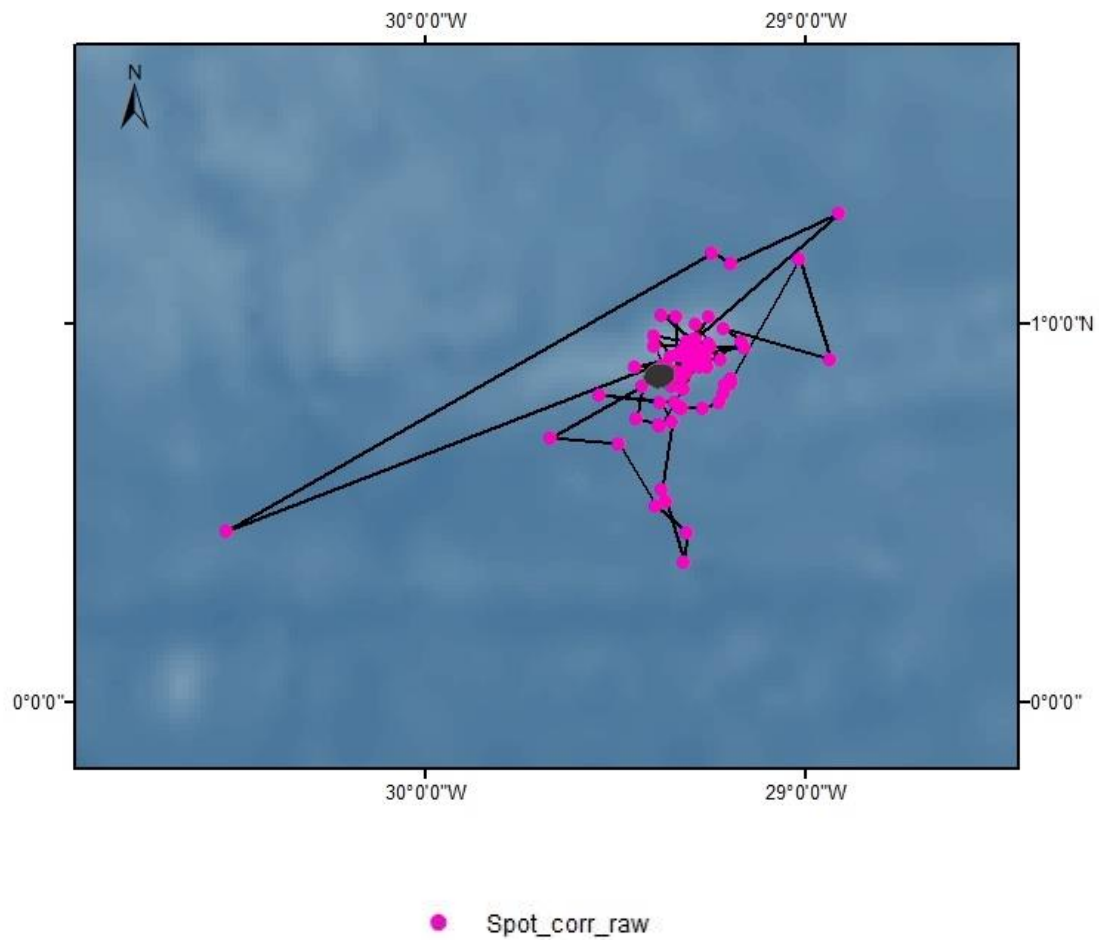


Figure 27- Geolocation of the silky shark tagged with SPOT (115353) around the Saint Peter and Saint Paul Archipelago (ASPSP) in the southwest and equatorial Atlantic Ocean.

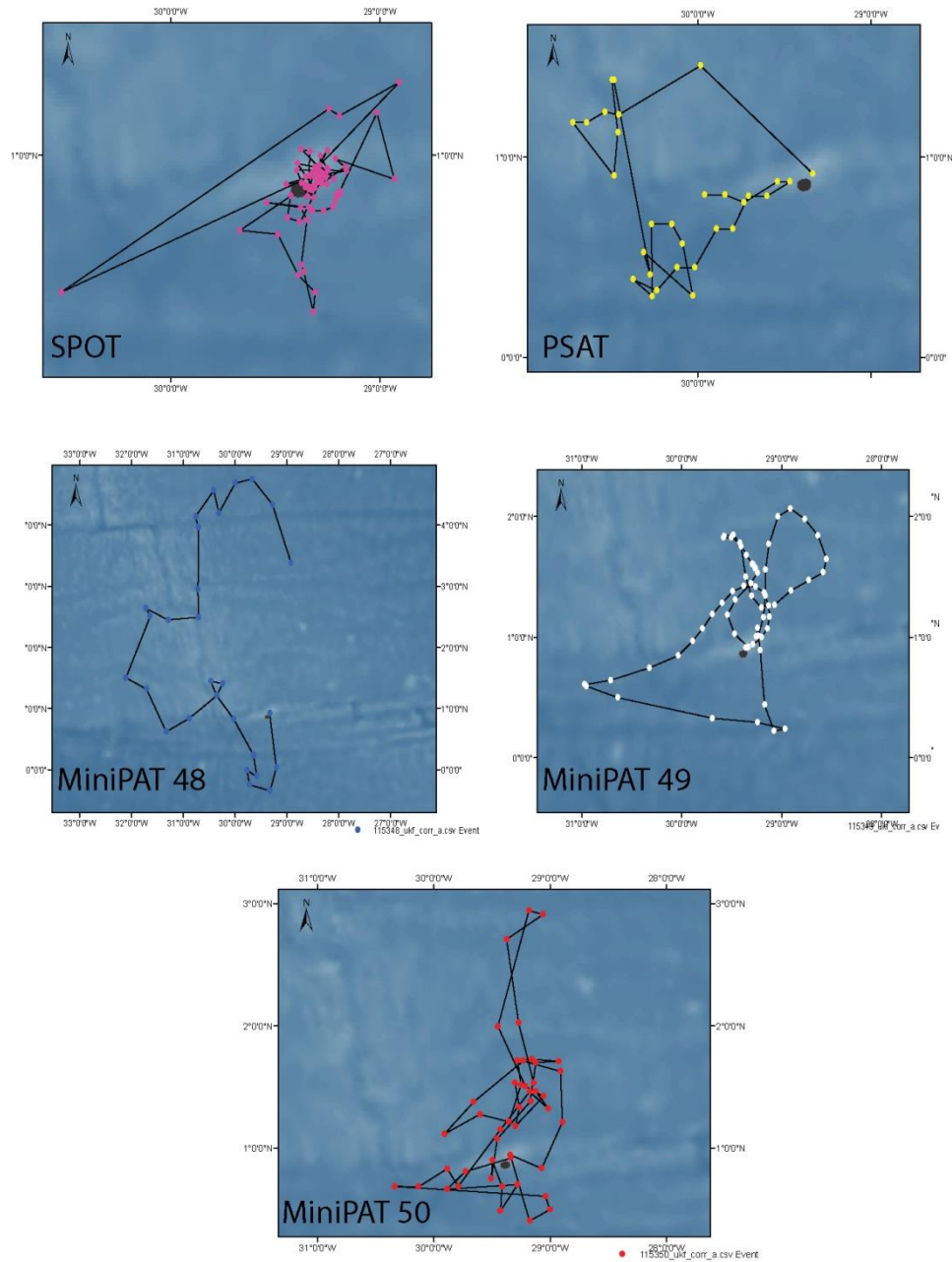


Figure 28- Tracks of the silky sharks, *Carcharhinus falciformis*, tagged with electronic tags: PSAT (Pop-up Satellite Archival Tags), MiniPAT and SPOT in the southwest and equatorial Atlantic Ocean.

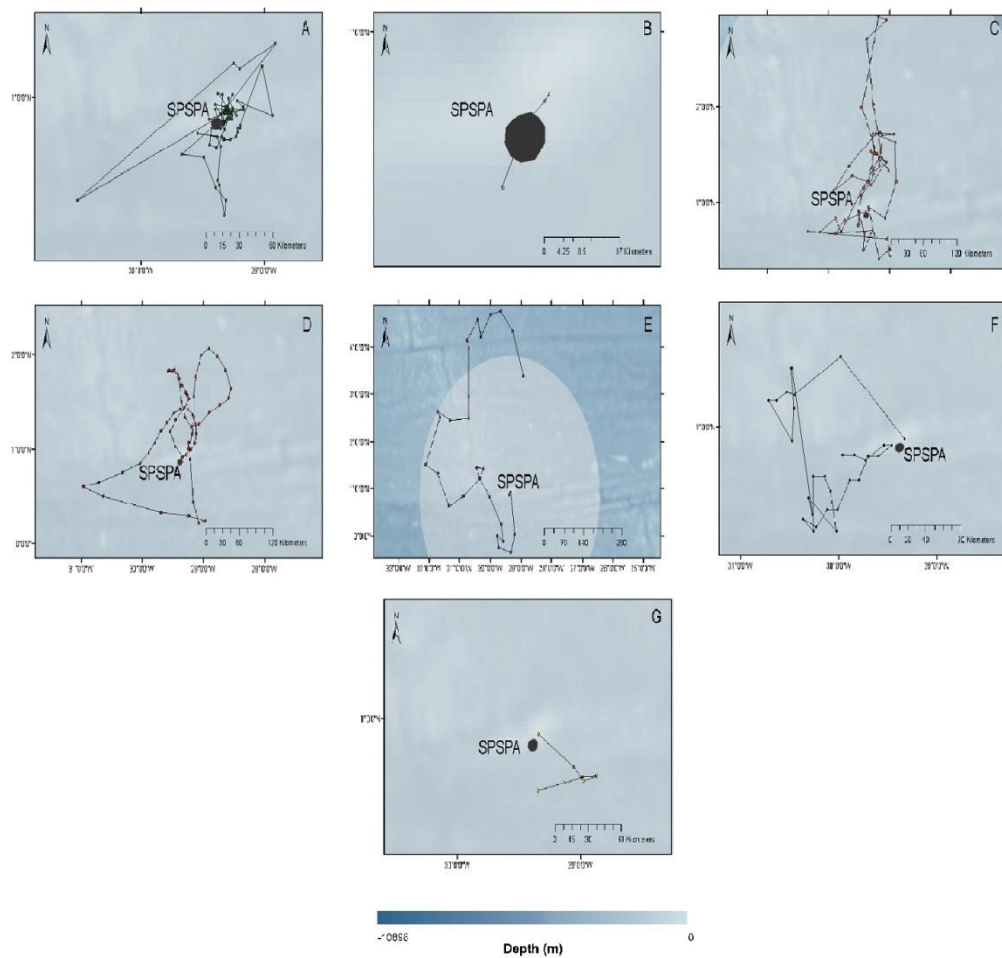


Figure 29- Tracks of the silky sharks, *Carcharhinus falciformis*, tagged with electronic tags: PSAT (Pop-up Satellite Archival Tags), MiniPAT and SPOT in the southwest and equatorial Atlantic Ocean:

- A- SPOT 1;
- B- SPOT 2;
- C- MiniPAT 115350;
- D- MiniPAT 115349;
- E- MiniPAT 115348;
- F- PSAT 1 (90815)
- G- PSAT 2 (101017)

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7- Final Considerations

The discoveries presented here and our lack of evidence of sex- or size-related patterns of space utilization in white sharks underscore the need for additional research. Multidisciplinary studies integrating population genetic analyses and electronic tagging, as well as the development of improved monitoring instruments, should be encouraged.

The present results on the morphology of the chondrocranium of the silky shark *Carcharhinus falciformis*:

- The chondrocrania of *Carcharhinus* (including *Nasolamia*), despite being conservative, showed differences that can be taxonomically significant.
- The cranial components that presented specific consistent differences are: the shape of the rostral node and posterior margin of anterior fontanelle, rostral dimension, presence or absence of rostral fenestra, epiphysial notch, epiphysial foramen and nasal flap; direction of the nasal capsules, shapes of the preorbital process and subetmoid fossa
- In reviewing in detail the chondrocranium terminology of *C. falciformis* the orbit presented the most modified region.
- Hypercalcification is not an ontogenetic modification.
- A rigorous analysis in *Nasolamia* should be done to elucidate its taxonomic status.

On the study of molecular identification it's observed that all samples were identified by morphological characters before the molecular process. Genetic tools are important to answer many questions about genetic diversity, population connectivity, and historical processes that have generated some pattern.

C. falciformis of the ASPSP showed connectivity with all localities, except with the USA (Domingues et al., In press). The connectivity between ASPSP and other locations and the break with the US corroborated the pattern of ocean currents in the Atlantic Ocean.

On this context can be concluded that:

- Confirms the similarity of both species in their visual, morphological and morphometric identification;
- The fact of not finding *C. galapagensis* demonstrates the need to increase the number of samples and does not define the population status of the species in the study area;
- Use of primers in PCR-Multiplex is a fast and reliable method to discriminate with a species of 99% reliability efficiency that have general distribution;

➤ Other techniques for identifying species such as DNA barcoding (uses universal primers to amplify part of the COI gene), It complemented the process of identification of the species of sharks for which it was not possible to identify by PCR-Multiplex;

➤ Need for more work related to the genetic diversity and structure of the *Carcharhinus* population found in the ASPSP. Geographic distribution (global scale) and sexual segregation are still missing;

On the telemetry study the results of all electronic tags have shown a clear behavior of shallow distribution, largely restricted to the mixed layer.

➤ It showed epipelagic distribution being closely connected to the surface, being closer to the surface during the day;

➤ The present results clearly show the importance of such a measure (Interministerial Normative Instruction nº 8 (November 6, 2014), particularly in the vicinity of oceanic islands or FADs, due to the strong association these sharks show to these structures;

➤ The results suggesting that the archipelago ends up being similar to a FAD, to the silky sharks in the vicinity of SPSPA. This study revealed a high residency of tracked silky sharks in the vicinity of the SPSPA, with individuals remaining in that area for long periods of time (up to nine months);

➤ The silky shark is probably attracted to the archipelago to feed on the teleost species aggregating there. The circadian changes in their vertical distribution, therefore, is likely related to similar changes of behavior of their prey species;

➤ As no long migration were detected in this study, further tracking of larger numbers of individuals is necessary to better understand the residency and/or philopatry of silky sharks in the equatorial Atlantic.

All this studies demonstrated that the deficiency in the scientific identification of species generates the recognition and naming of specimens by the popular name, which in many cases can relate more than one biological species. And the excessive exploitation of stocks coupled with certain biological characteristics of a large number of species in this group identifies them as fragile and susceptible to over-exploitation, confirming the need for more work related to ecology (morphological and genetic identification, as well as the genetic diversity and structure, movement and habitat use) of the *Carcharhinus* found in the ASPSP.

8- Normas Revistas

Os artigos elaborados ao longo dessa tese serão encaminhados aos seguintes periódicos, estando respectivamente disponibilizadas as normas das revistas nos links abaixo:

1- Marine Biology Research

<http://www.tandfonline.com/action/authorSubmission?show=instructions&journalCode=smar20>

2- Journal of Genetics

<http://www.springer.com/life+sciences/journal/12041>

3- Journal of Fish Biology

[http://onlinelibrary.wiley.com/journal/10.1111/\(ISSN\)1095-8649/homepage/ForAuthors.html](http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1095-8649/homepage/ForAuthors.html)