GOVERNO DO ESTADO DE SÃO PAULO

SECRETARIA DE AGRICULTURA E ABASTECIMENTO
AGÊNCIA PAULISTA DE TECNOLOGIA DOS AGRONEGÓCIOS
INSTITUTO DE PESCA
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA E PESCA

IDENTIFICAÇÃO MOLECULAR DE LARVAS E OVOS DE PEIXES-DE-BICO DAS FAMÍLIAS ISTIOPHORIDAE E XIPHIIDAE (PERCIFORMES) DA COSTA SUDESTE DO BRASIL ATRAVÉS DO DNA BARCODE

Tiago Rodrigues

Orientador: Prof. Dra. Katharina Eichbaum Esteves
Co-orientador: Prof. Dr. Alberto Ferreira de Amorim

Dissertação apresentada ao Programa de Pós-graduação em Aquicultura e Pesca do Instituto de Pesca - APTA - SAA, como parte dos requisitos para obtenção do título de Mestre em Aquicultura e Pesca.

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CERTIFICADO DE APROVAÇÃO

"IDENTIFICAÇÃO MOLECULAR DE LARVAS E OVOS DE PEIXES-DE-BICO DAS FAMÍLIAS ISTIOPHORIDAE E XIPHIIDAE (PERCIFORMES) DA COSTA SUDESTE DO BRASIL ATRAVÉS DO DNA BARCODE"

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Aprovado como parte das exigências para obtenção do título de MESTRE EM AQUICULTURA E PESCA, Área de Concentração em Pesca, pela Comissão Examinadora:

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SUMÁRIO

RESUMO

O presente estudo teve como objetivo identificar e relatar a ocorrência de larvas dos peixes-de-bico no Sudoeste do Atlântico e das demais larvas de peixe na massa de água superficial da região costeira de Vitória (ES). Durante o verão dos últimos dois anos (2012/2013 e 2013/2014) foram realizadas 64 arrastos de superfície utilizando uma rede de ictioplâncton na costa de Vitória (ES), Rio de Janeiro (RJ) e Ilhabela (SP) e 391 larvas de peixes foram coletadas, sendo 27 deles em Vitória e capturando 151 larvas nessa região. A identificação das larvas de Istiophoridae até o nível taxonômico de família foi feita através de características morfológicas, e no nível de espécie foi realizada através da biologia molecular utilizando a técnica de DNA *barcode*, mesmo método utilizado nas demais larvas de peixe coletadas. O fragmento *barcode* do gene mitocondrial citocromo c oxidase subunidade I (COI) foi amplificado e sequenciado para a identificação das espécies. Durante a temporada 2012/2013, oito larvas de agulhão-vela foram identificados na costa de Vitória (ES). Na temporada 2013/2014 uma larva de agulhão-branco e dois ovos de espadarte também foram identificados na amostragem proveniente de Vitória, além de outros dois exemplares de agulhão-vela e um agulhão-branco, coletados na costa de Ilhabela em janeiro de 2014. Identificou-se nos 27 arrastos de Vitória um total de 13 espécies e 10 Famílias, com ampla dominância da espécie *Dactylopterus volitans* (Dactylopteridae) no início de 2013 e peixes da Família Carangidae e Exocoetidae na temporada 2013/2014. As larvas de *D. volitans* foram fotografadas sob microscópio eletrônico de varredura com o apoio do Departamento de Cirurgia da Faculdade de Medicina Veterinária e Zootecnia (USP-SP). Durante os dois períodos de amostragem também foram coletadas larvas de *Thunnus atlanticus* (Scombridae), espécie de elevado interesse comercial. Para avaliar a importância dos parâmetros ambientais sobre a abundância de larvas de peixe a Análise de Correspondência Canônica (CCA) foi realizada, análise que explicou 77,2% da variação dos dados (valor de inércia). O presente estudo identificou larvas de Istiophoridae e Xiphiidae pelo DNA *barcode* pela primeira vez na costa brasileira e deve ter continuidade, para que inferências sobre a área e período de desova e desenvolvimento de fases iniciais de vida destes peixes possam ser feitas com mais precisão. Além disso, esses dados podem contribuir para a gestão e conservação destas espécies no Sudeste do Atlântico.

Palavras-chave: : barcode, agulhão, sailfish, larva de peixe, ictioplâncton, Dactylopteridae.

ABSTRACT

This study had the aim to identify and relat the occurrence of billfish larvae and eggs in the Southeast Atlantic and the other fish larvae caugh on the coastal region of Vitoria (ES). During the summer in the last two years (2012/2013 and 2013/2014) 64 surface trawls were done using an ichthyoplankton net on the coast of Vitoria (ES), Rio de Janeiro (RJ) and Ilhabela (SP) and 391 fish larvae were collected, 27 trawls made in Vitoria with 151 fish larvae collected. The identification of the Istiophoridae larvae to the family taxonomic level was done by morphological characteristics, and the species of Istiophoridae and other fish larvae by molecular biology using the DNA barcode technique. The fragment barcode of the mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified and sequenced for identifying the species. During the 2012/2013 season, eight sailfish larvae were identified off the coast of Vitoria (ES), Southeast Brazil. In the 2013/2014 season, one white marlin larvae and two swordfish eggs were also identified in the sample that came from Vitoria, along with another two examples of sailfish and one white marlin that were collected on the coast of Ilhabela in January 2014. From the 27 collection stations on the coast of Vitoria, 13 species and 10 Families were identified, widely dominated by the *Dactylopterus volitans* (Dactylopteridae) species in the beginning of 2013 and fish from the Caragnidae and Exocoetidae families in the 2013/2014 season. The *D. volitans* larvae were photographed under a electronic scanning microscope with support from the Department of Surgery at the College of Veterinary Medicine and Zootechny (*Departamento de Cirurgia da Faculdade de Medicina Veterinária e Zootecnia* - USP-SP). During the two sampling periods, *Thunnus atlanticus* (Scombridae) larvae were also collected, a species of heightened commercial interest. To evaluate the importance of the environmental parameters on the abundance of fish larvae, the Canonical Correspondence Analysis (CCA) was performed, which explained 77.2% of data variation (inertia value). The occurrence of billfish larvae should be studied in detail so that inferences about the area and the spawning and initial life development phases of these fish can be made with more precision. This data can also contribute to the management and conservation of these species in the Southeast Atlantic.

Keywords: barcode, marlin, sailfish, fish larvae, ichthyoplankton, Dactylopteridae.

INTRODUÇÃO GERAL

Os peixes-de-bico são grandes peixes pelágicos que ocupam um importante nicho ecológico no seu ambiente como predadores de topo (HOESE e MOORE, 1998). As espécies presentes no Atlântico Sul são o agulhão-negro (*Makaira nigricans*), agulhão-vela (*Istiophorus platypterus*), agulhão-branco (*Kajikia albida*), agulhão-estilete (*Tetrapturus pfluegeri*) e marlim-polegar (*Tetrapturus georgii*), todos pertencentes à família Istiophoridae; além do espadarte (*Xiphias gladius*), único representante da família Xiphiidae. Acreditava-se que o marlim-polegar era o único ausente da costa brasileira, no entanto, sua presença foi assinalada no litoral do nordeste em 2008, em janeiro de 2009 na pesca esportiva do Iate Clube do Rio de Janeiro e nos desembarques da frota atuneira de São Paulo que atuou no sudeste-sul do Brasil. As demais espécies estão presentes nas capturas da pesca esportiva e comercial feita por embarcações nacionais e estrangeiras na costa brasileira (AMORIM et al*.,* 2011).

Peixes-de-bico capturados pela pesca

A zona costeira brasileira vem sofrendo diversos tipos de pressões ambientais, tais como o crescimento populacional que acelera a ocupação de áreas litorâneas e utilização de seus recursos, liberação de esgotos domésticos, vazamentos de óleos de navios e plataformas, contaminação proveniente da descarga da água de lastro e superexploração dos recursos pesqueiros (CASTRO e MIRANDA, 1998). Destaca-se portanto a necessidade cada vez maior de elaborar planos bem estruturados de gestão ambiental da costa brasileira, especialmente das atividades de grande significado econômico, como a indústria petrolífera e a pesqueira.

Os recursos pesqueiros são fundamentalmente importantes na manutenção do ecossistema marinho e têm grande valor como alimento (MENDONÇA et al., 2009). Assim deve-se buscar um manejo sustentável dos alvos de captura, pois além da importância econômica, são de grande relevância ambiental constituindo parte da biota marinha.

Segundo dados de *The State of World Fisheries and Aquaculture* da FAO (2010), agravantes significativos devem ser considerados no estudo de espécies alvo da pesca extrativa marinha, tais como: o elevado número de colônias de pesca artesanal não monitoradas; os descartes excessivos de pesca; o *bycatch* ou captura acidental; a captura de espécies chave de grande valor ecológico e ainda a captura de exemplares juvenis e neonatos. Estima-se que os descartes do produto pesqueiro cheguem a sete milhões de toneladas anuais (FAO, 2010). Entre as espécies capturadas acidentalmente, especialmente pelos espinhéis segundo Amorim e Arfelli (2003) estão os peixes-de-bico como o agulhão vela (*Istiophorus platypterus*), o agulhão branco (*Tetrapturus albidus*) e o agulhão negro (*Makaira nigricans*). Adicionalmente, essas espécies são alvos potenciais da modalidade de pesca esportiva e portanto podem ser monitoradas (ARFELLI et al., 1994; ICCAT, 2011).

Tratando-se de peixes de alto potencial migratório e de ampla distribuição, os agulhões são capturados acidentalmente por vários países em diversas regiões do Oceano Atlântico, ficando expostos a uma significativa pressão pesqueira, resultando no declínio dos estoques (OLIVEIRA et al., 2007).

A captura dos peixes-de-bico representou cerca de 8% do total da frota espinheleira japonesa no Atlântico na década de 1960; entretanto até o final da década de 1980 esse percentual caiu para apenas 2% (UOZUMI e NAKANO, 1994). O baixo valor econômico da carne desses peixes, segundo Uozumi e Nakano (1994) associado à captura acidental resultou em descartes pesqueiros de intensidade desconhecida, dificultando estratégias de manejo sustentável dessa atividade.

A captura do agulhão negro no Atlântico pelas frotas japonesas apresentou os maiores valores de CPUE (captura por unidade de esforço) na década de 1960, assim como a captura do agulhão vela, ambas ocorrendo do nordeste ao sudeste da costa brasileira (UEYANAGI et al., 1970). Uozumi e Nakano (1994) observaram o declínio dessas capturas até a década de 1980, possivelmente devido ao desenvolvimento dos espinhéis de fundo somado à

mudança da área de pesca, concentrando-se no Atlântico oriental, região onde os peixes-de-bico possuem menor distribuição.

O agulhão branco é capturado pelos espinhéis na costa brasileira durante o ano todo, afirmaram Antero-Silva et al. (1994). Com base em dados das frotas espinheleiras japonesas, Ueyanagi et al. (1970) e Mather et al. (1972) afirmaram que a maior concentração do agulhão branco no Atlântico se dá entre os meses de Setembro a Janeiro do nordeste ao sudeste da costa do Brasil. Entretanto, a captura dessa espécie pela frota japonesa apresentou forte declínio nas décadas de 1970 e 1980, observando-se uma menor ocorrência no sudeste do Brasil (UOZUMI e NAKANO, 1994; AMORIM e ARFELLI, 2003;). A captura dessa e das demais espécies de peixes-de-bico do Atlântico também é comum durante os meses de Outubro a Fevereiro, compreendendo a primavera e o verão no hemisfério Sul, quando ocorrem os torneios de pesca esportiva de peixes-de-bico nos iate clubes das cidades de Vitória (ES), Rio de Janeiro (RJ), Cabo Frio (RJ) e Ilhabela (SP) (PAIVA e PIRES-JUNIOR, 1983; ARFELLI et al., 1994; AMORIM e ARFELLI, 2003).

Atualmente a comercialização dos agulhões branco e negro é proibida em todo o território nacional e para exportação, segundo a Instrução Normativa número 12 de 2005, editada pela Secretaria Especial de Aquicultura e Pesca (SEAP).

Importância dos peixes-de-bico e desova

Embora os peixes-de-bico ocupem importante papel na biota marinha do Atlântico, diversos fatores dificultam o manejo sustentável dessas espécies, tais como o baixo valor econômico, que resulta em descartes pesqueiros quando esses peixes são capturados acidentalmente, somado ao declínio dos estoques e sua ampla distribuição (sendo explotados por diversas nações) (UOZUMI e NAKANO, 1994; OLIVEIRA et al., 2007). Segundo Graves e Horodysky (2010), a maioria dos estoques já está superexplotada. Por outro lado, são peixes de interesse da pesca esportiva e de apelo conservacionista, ainda com poucas informações biológicas sobre as espécies e os estoques atuais (ARFELLI et al., 1994; ICCAT, 2005; MAYER e ANDRADE, 2009). Segundo Ditton e Stoll (2003), os peixes-de-bico dão suporte à pescaria

esportiva, que gera 200 milhões de dólares ao ano apenas nos Estados Unidos. Por essas razões, torna-se imprescindível realizar estudos de biologia pesqueira e reprodutiva das espécies de Istiophoridae e Xiphiidae, subsidiando dados para medidas de manejo espécie específicas desses peixes no Atlântico Sul (ICCAT, 2005; ICCAT, 2011).

Historicamente, tem-se noticiado a ocorrência de fêmeas em diferentes estágios de desenvolvimento gonadal e a presença de larvas de Istiophoridae e Xiphiidae no Atlântico, incluindo um detalhamento para o litoral sudeste e sul do Brasil (YABE, et al., 1959; UEYANAGI et al., 1970). Larvas de Istiophoridae foram registradas e descritas em diferentes áreas do Atlântico por Mather et al., (1972) e Shomura e Williams (1975). A desova e o desenvolvimento das larvas estão diretamente ligados à presença de massas de água produtivas, como as provenientes de ressurgência, afetando a disponibilidade de alimento planctônico para as larvas e o sucesso no recrutamento (MATSUURA et al., 1992)

A maior área de desova do agulhão-branco no Atlântico, segundo Ueyanagi et al. (1970) ocorre no sudeste-sul da costa brasileira, entre 20º e 30ºS. Locais de desova foram assinalados em águas oceânicas em frente à Flórida e ao norte de Porto Rico e do Caribe de abril a junho (BAGLIN, 1979; AROCHA et al. 2005). No mês de junho, em frente ao Cabo Hatteras (Maryland) e ao sul de Nova Jersey foram observadas fêmeas em repouso, comportamento típico pós-desova. No Atlântico Equatorial (5º N a 5º S), eles se reproduzem frente ao litoral nordeste do Brasil (AROCHA E ORTIZ, 2006). No sudeste e sul do Brasil, durante sua migração na costa brasileira, é provável que a fêmea desove parceladamente do Espírito Santo a Santa Catarina (18º S a 27º S), de novembro a março segundo Arfelli et al. (1986). Em fevereiro de 1997 foi observada grande concentração de ovos e algas em frente ao Cabo de Santa Marta (25º 55’ a 26º 20’ S e 45º 10’ a 45º 50’ W), onde outras espécies também se reproduziam, como o agulhão vela, espadarte, dourado e outros (AMORIM et al., 2011). Essa massa é levada para áreas distantes da costa brasileira provavelmente pelas correntes marítimas. Ainda não foi encontrado um juvenil de agulhão branco, mesmo através dos estudos de conteúdo estomacal realizados no sudeste e sul do Brasil no período de 1972 a 1980 (AMORIM et al., 2011). As coleções de larvas de agulhão-branco são

bastante escassas, pois seu crescimento é bastante rápido, dificultando obtenção de exemplares. Historicamente, foram coletadas larvas de agulhão-branco em áreas oceânicas do Atlântico frente à costa brasileira: uma a 22º S e 32º W e outra a 8º S e 35º W (Ueyanagi et al., 1970).

Para o agulhão-negro, embora as áreas de desova ainda sejam pouco conhecidas, foram encontradas fêmeas desovando na costa da Geórgia e estreitos da Flórida, nos Estados Unidos, em Porto Rico, Bahamas, Jamaica e Bermuda (CALDWELL, 1962; ERDMAN, 1968; SERAFY et al., 2003; LUTHY, 2004; LUCKHURST et al.,2006). Na costa brasileira foram observadas fêmeas prontas para desova na região de Abrolhos no período de março a abril (UEYANAGI et al., 1970; AMORIM et al., 1998). No litoral de Cabo Frio, Rio de Janeiro, as fêmeas se encontram em fase de maturação nos meses de janeiro e fevereiro (AMORIM et al., 1998).

Estudos com a descrição de séries completas de desenvolvimento larval de agulhão-vela foram feitas por Voss (1953) e Yabe (1953) no Atlântico e Pacífico, respectivamente. Descrições larvais foram também realizadas por Gehringer (1956 e 1970). Durante o verão no Atlântico, o agulhão-vela se reproduz em áreas tropicais e subtropicais nos dois hemisférios. No Atlântico Norte foram encontradas fêmeas ovadas, ovos e larvas de agulhão-vela no Estreito da Flórida e áreas adjacentes, de abril a outubro (DE SYLVA e BREDER, 1997; POST et al., 1997; LUTHY, 2004; RICHARDSON et al., 2009). Foram observadas fêmeas ovadas de junho a dezembro no mar do Caribe, Venezuela, Guianas e Suriname (AROCHA e MARCANO, 2006). No Atlântico Equatorial (5º a 13º N), há registro de desova de fevereiro a setembro, já no Atlântico oriental, a espécie desova frente à costa do Senegal, de julho a agosto (LIMOUUNZY e CAYRE, 1981; HAZIN et al., 1994). No Brasil, as desovas acontecem desde o Espírito Santo à Santa Catarina, entre novembro e fevereiro, com pico em janeiro (ARFELLI e AMORIM, 1981). Um cardume de agulhão-vela juvenil, com exemplares de cerca de 15 centímetros foi observado por pescadores esportivos na última semana de dezembro de 2010, no Parcel dos Reis, no litoral sul de São Paulo (24º 20’ S e 46º 36’ W). Em dezembro de 2009, examinando os estômagos dos peixes capturados pelos atuneiros de Santos, foram encontradas duas formas juvenis com 16 e 20 centímetros, em um estômago de dourado *Coryphaena hippurus* (AMORIM et

al. 2011). Entre 1972 a 1980 também foram encontrados dois exemplares juvenis (20 a 22 cm) no conteúdo estomacal de dourado e agulhão-vela por Zavala-Camin (1982). Segundo arquivo pessoal de Amorim (não publicado) outro juvenil com 70 centímetros de comprimento foi capturado incidentalmente pelo corrico de pesca esportiva, em maio de 1999 e um agulhão vela de 1,12 metro e 4,8 quilos foi desembarcado em Cabo Frio, em 30 de maio de 2004. Provavelmente os exemplos de juvenis mencionados nasceram em uma temporada entre novembro e janeiro. Portanto, levanta-se a hipótese de que uma das importantes áreas de desova e crescimento de agulhão-vela, no Atlântico Sul Ocidental, é o litoral sudeste-sul do Brasil (AMORIM et al. 2011).

A época de desova coincide com a realização dos torneios de pesca esportiva de peixes-de-bico, que operam anualmente entre os meses de outubro a fevereiro, partindo das cidades de Vitória, Cabo Frio, Rio de Janeiro e Ilhabela (Arfelli et al., 1986).

A pesca esportiva é praticada nessas áreas em águas produtivas, decorrentes da ressurgência, fenômeno que ocorre quando a água superficial é deslocada de uma área para a outra proporcionando uma elevação da água de fundo, rica em oxigênio e nutrientes, provocada pela ação de ventos ou correntes (GARRISON, 2010). Inicia-se nessa região as depressões do talude, marcando os limites da plataforma continental. Os pescadores costumam atuar em pontos onde a temperatura da água é aferida, buscando-se a faixa dos 24º Celsius. Ueyanagi et al. (1970) encontraram larvas de peixes-de-bico em termoclinas de 24ºC a 26ºC no sudeste brasileiro, evidência que corrobora para a indubitável necessidade de se realizar uma amostragem na região visando o comprovar a ocorrência de larvas dessas espécies (MATHER et al., 1972).

Os peixes-de-bico são animais epipelágicos em todos os estágios de vida, possibilitando dessa forma, estudos de época e área de desova a partir da análise das larvas, amostradas por arrastos planctônicos de superfície, técnica de rápida execução inerente ao substrato (DE SYLVA et al., 2000). Tidwell et al. (2007) afirmam que provavelmente a área do conhecimento menos conhecida dos peixes-de-bico é a dos primeiros estágios de vida. A informação básica sobre os primeiros estágios do desenvolvimento, bem como a identificação dos locais de berçário, preferências de hábitat e hábitos

alimentares ainda é escassa para esse grupo de peixes (TIDWELL et al., 2007).

Estudo das larvas

O conhecimento da distribuição do zooplâncton e ictioplâncton (larvas e ovos de peixe) no Brasil ganhou força nos últimos 30 anos (Katsuragawa et al., 2006). Matsuura (1971; 1972) foi um dos pioneiros estudando o clico de vida da sardinha *Sardinella brasiliensis*. Ambientes mais costeiros, com maior disponibilidade de alimento, padrões de circulação que facilitam a retenção dos estágios planctônicos e com baixa abundância de predadores são favoráveis ao desenvolvimento do ictioplâncton, dessa forma estudos de material planctônico oceânico são realizados em menor escala, dada a dificuldade de obtenção do material biológico e sua escassez em áreas abertas (FRANK e LEGGETT, 1983).

As larvas de peixe são frágeis e extremamente suceptíveis às variações dos parâmetros físico-químicos da água e do ambiente, dessa forma um impacto ambiental de ação antrópica pode ser catastrófico para essas populações, o que pode afetar diretamente o recrutamento do estoque de algum recurso pesqueiro (KATSURAGAWA et al., 2011). O conhecimento sobre o recrutamento de espécies de interesse comercial é essencial na busca da sustentabilidade pesqueira, portanto o detalhamento da biologia dos estágios iniciais de vida dos peixes e de outros recursos é imprescindível para a conservação dessas espécies.

O estudo das larvas de Istiophoridae deve preencher importantes lacunas do conhecimento biológico dos peixes-de-bico, uma vez que pode fornecer dados sobre a ocorrência de uma ou mais espécies e as áreas e épocas de desova numa determinada região, conhecimento que servirá de base para o desenvolvimento de uma atividade pesqueira sustentável desse grupo, que segundo a *International Commission for the Conservation of Atlantic Tunas* - ICCAT (2006) tem como meta internacional a redução da mortalidade pela pesca acidental.

Estudos da biologia reprodutiva dos peixes-de-bico do Atlântico em comparação aos demais pelágicos de grande porte ainda são relativamente escassos (DE SYLVA e BREDER, 1997). Exemplifica-se com os trabalhos de

De Sylva (1963), Erdman (1968), Jolley (1977), Baglin (1979), Harvey (1990), Souza et al. (1994), De Sylva e Breder (1997) e Richardson et al. (2009); todos do Atlântico Norte.

Recentemente, foram realizados trabalhos com larvas de Istiophoridae vivas, estudos que compreendem suas características biológicas tais como: crescimento, dieta, mortalidade e padrões de movimentação (POST et al., 1997; SERAFY et al., 2006; SERAFY et al., 2008; SPONAUGLE et al., 2010).

Primeiramente, as pesquisas de biologia e ecologia básica das larvas de peixes-de-bico encontraram severas dificuldades na identificação das espécies em razão da semelhança e do pouco conhecimento de aspectos morfológicos e morfométricos que as distinguem nos primeiros estágios de vida (RICHARDS, 1974). Todavia, técnicas de genética e biologia molecular têm ajudado a solucionar esses problemas, destacando-se os trabalhos de McDowell e Graves (2002), Hyde et al. (2005) e Luthy et al. (2005), modelos recentes dessa ferramenta aplicada à taxonomia dos peixes-de-bico no Atlântico Norte.

Identificação molecular

Visando elucidar grandes emblemas taxonômicos, o DNA tem sido amplamente utilizado pela genética através das técnicas de biologia molecular para auxiliar na identificação de espécies (BARTLETT e DAVIDSON, 1991). Trata-se de uma molécula relativamente estável, presente em todas as células de um organismo, que pode fornecer uma gama de informações, sejam espécie específicas, populacionais ou filogenéticas (HOLSINGER e MASON-GAMER, 1996).

O estudo genético de espécies, além da dinâmica de populações e da padronização de estoques pesqueiros de grandes pelágicos tem sido empregado em diversos grupos, incluindo elasmobrânquios, atuns e peixes-de-bico (BARTLETT e DAVIDSON, 1991; CHOW, 1994; DANIEL e GRAVES, 1994; WARD, 1995 ; ROCHA-OLIVARES, 1998 ; WAPLES, 1998 ; GREIG et al., 1999; APPLEYARD et al., 2001; MCDOWELL e GRAVES, 2002; RODRIGUES-FILHO et al., 2009 ; MENDONÇA, 2010 ; DOMINGUES, 2011;).

Chow (1994) investigou o polimorfismo no comprimento dos fragmentos de restrição – RFLP em dez espécies de peixes-de-bico. Desde então a técnica

do PCR-RFLP tem sido utilizada na identificação de adultos e larvas de Istiophoridae e Xiphiidae (INNES et al., 1998; LUTHY et al., 2005; MCDOWELL e GRAVES, 2002). McDowell e Graves (2002) elaboraram uma chave molecular de identificação taxonômica dos peixes-de-bico através da análise de marcadores moleculares de regiões independentes do DNA mitocondrial e nuclear, utilizando o material genético de mais de 500 amostras de Istiophoridae. Primeiramente buscou-se amplificações consistentes de sequências de tamanhos similares entre todos os taxa, definindo posteriormente quais enzimas de restrição exibiram sequências discriminantes entre as espécies, porém mantendo baixa variação intraespecífica (MCDOWELL e GRAVES, 2002).

Posteriormente, após o desenvolvimento da técnica de PCR multiplex, que utiliza vários primers para espécies diferentes na mesma reação, outros estudos optaram por esse novo método. Shivji et al. (2002) utilizaram a técnica de PCR multiplex na identificação de amostras de tubarões e Shivji, M. e Magnussen, J. desenvolveram um protocolo para aplicação dessa técnica na identificação dos peixes-de-bico (não publicado). Baseado em Shivji et al. (2002) e no protocolo de Shivji e Magnussen, Hyde et al. (2005) desenvolveram mais um método de PCR multiplex para identificar ovos e larvas de peixes-de-bico, incluindo primers para dourado (*Coryphaena equiselis* e *Coryphaena hippurus*) e cavala wahoo (*Acanthocybium solandri*). Estudos mais recentes de amostras do Golfo do México também se basearam em Shivji e Magnussen e utilizaram o multiplex para identificar larvas de Istiophoridae, demonstrando a viabilidade da técnica (ROOKER et al., 2012; SIMMS et al., 2010).

A utilização do gene citocromo oxidase subunidade I (COI) veio após anos de utilização de diferentes sequências do DNA para identificação que não podiam ser comparadas. Segundo Herbert et al. (2003) a sequência de um único gene, desde que evolutivamente conservada poderia ser utilizada para identificar e distinguir ao menos a maioria das espécies animais. Herbert et al., (2003) ainda propuseram a utilização do gene mitochondrial COI como um sistema global de bioidentificação, mais tarde denominado de DNA Barcoding.

A sequência de nucleotídeos contida entre as posições 58 e 705 da extremidade 5'do gene COI é o fragmento *barcode* (WAUGH, 2007). O sistema

global de bioidentificação iniciado por Herbert et al. (2003) alavancou estudos do fragmento *barcode* em diversos grupos taxonômicos, tais como invertebrados (BARRETT e HERBERT, 2005; COSTA et al., 2007), peixes (WARD et al., 2005), aves (YOO et al., 2006; WARD et al., 2009) e fungos (BEGEROW et al., 2010). Para fungos e plantas o fragmento *barcode* não se mostrou adequado na discriminação da maioria das espécies, uma vez que nesses organismos a taxa mutacional do gene COI é mais baixa (PALMER e HERBON, 1988; CHO et al., 1998).

A utilização do DNA Barcoding é vantajosa segundo Herbert et al. (2003) por se tratar de uma região relativamente curta, obtida para um elevado número de taxa com poucos primers, além da sequência ser facilmente alinhada e capaz de distinguir mesmo espécies próximas. Além disso, a técnica é viável para a identificação de pequenos fragmentos de organismos, espécies crípticas e espécimes imaturos, tais como as larvas de peixes coletadas no presente estudo (HERBERT et al., 2003).

OBJETIVOS

Objetivo geral

Identificar e comprovar a ocorrência das espécies de larvas de peixes-de-bico (Istiophoridae e Xiphiidae) na massa de água superficial do Atlântico Sul Ocidental, na região costeira entre Vitória-ES e Ilhbela-SP através do DNA *barcode* e caracterizar possíveis hábitats de berçário desses peixes.

Objetivos específicos

Testar a hipótese de que a área de estudo é um sítio de desova e de desenvolvimento larval desses peixes.

Testar a identificação de ovos fertilizados através do DNA *barcode* e caracterizá-los morfologicamente.

Identificar as demais larvas de peixes coletadas também através do sequenciamento do fragmento do DNA *barcode*.

Identificar se os parâmetros ambientais exercem alguma influência na abundância do ictioplâncton e quais são esses parâmetros através da Análise de Correspondência Canônica – CCA.

ORGANIZAÇÃO DA DISSERTAÇÃO

Os métodos utilizados e resultados que foram obtidos na presente dissertação foram apresentados e discutidos sob a forma de dois artigos científicos, ambos a serem submetidos à publicação nas revistas indicadas. Cada artigo corresponde a um capítulo, conforme listados abaixo. As Considerações Finais referem-se aos dois capítulos abordados e concluem a dissertação.

Capítulo 1. Molecular identification of Istiophoridae larvae and Xiphiidae eggs on the Southeastern coast of Brazil using Barcode DNA

O primeiro artigo trata do tema principal da dissertação, visando testar a hipótese de que a área costeira entre Vitória e Ilhabela, situada no Sudoeste do Atlântico faz parte dos sítios de desova e berçário das larvas dos peixes-de-bico do Atlântico. Também é abordada a aplicação da técnica do DNA *barcode* como importante ferramenta de identificação molecular. Artigo a ser submetido à revista ***Fisheries Research***.

Capítulo 2. Molecular identification (Barcode DNA) and taxonomic composition of fish larvae caught off Espirito Santo State, Southeastern coast of Brazil

Consiste na identificação molecular por sequenciamento do fragmento *barcode* do DNA das demais larvas de peixe (ictioplâncton) capturadas durante as amostragens feitas em Vitória durante o anos de 2013 e 2014. A Análise de Correspondência Canônica – CCA foi realizada buscando possíveis influências dos parâmetros ambientais na abundância das larvas. Artigo a ser submetido à revista ***Fisheries Research***.

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CAPÍTULO 1.

MOLECULAR IDENTIFICATION OF ISTIOPHORIDAE LARVAE AND XIPHIIDAE EGGS ON THE SOUTHEASTERN COAST OF BRAZIL USING BARCODE DNA

Molecular identification of Istiophoridae larvae and Xiphiidae eggs on the Southeastern coast of Brazil using Barcode DNA

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Abstract

This study had the aim to identify and relat the occurrence of billfish larvae and eggs in the Southeast Atlantic. During the summer in the last two years (2012/2013 and 2013/2014) 64 surface trawls were done using an ichthyoplankton net on the coast of Vitória-ES, Rio de Janeiro-RJ and Ilhabela-SP and 391 fish larvae were collected. The identification of the Istiophoridae larvae to the family taxonomic level was done by morphological characteristics, and the species by molecular biology using the DNA barcode technique. The fragment barcode of the mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified and sequenced for identifying the species. During the 2012/2013 season, eight sailfish larvae were identified off the coast of Vitória (ES), Southeast Brazil. In the 2013/2014 season, one white marlin larvae and two swordfish eggs were also identified in the sample that came from Vitoria, along with another two examples of sailfish and one white marlin that were collected on the coast of Ilhabela in January 2014. The occurrence of billfish larvae should be studied in detail so that inferences about the area and the spawning and initial life development phases of these fish can be made with more precision. This data can also contribute to the management and conservation of these species in the Southeast Atlantic.

Key words: *barcode, billfish, swordfish, marlin, sailfish.*

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1. Introduction

The billfish species present in the Southwestern Atlantic are the blue marlin (*Makaira nigricans* – Lacépède, 1852), sailfish (*Istiophorus platypterus* – Shaw, 1792), white marlin (*Kajikia albida* – Poey, 1850), longbill spearfish (*Tetrapturus pfluegeri* – Robins and De Sylva, 1963), roundscale spearfish (*Tetrapturus georgii* – Lowe, 1841) and swordfish (*Xiphias gladius* - Linneaeus 1752) (AMORIM et al., 2011). These fish are captured by longlines on commercial fishing and by trolling on sports fishing, especially during the spawning period on the coast of Brazil, from November to March (Arfelli and Amorim, 1981; Amorim and Arfelli, 1984, 1987; Arfelli et al., 1986; Amorim et al., 2011).

The occurrence of females in the final stages of gonadal development as well as the presence of billfish larvae were reported in the Atlantic (Voss, 1953; Yabe, 1953; Ueyanagi et al., 1970; Mather et al., 1972; Richards, 1974; Shomura and Williams, 1975; Arfelli and Amorim, 1981; Amorim and Arfelli, 1984, 1987; Arfelli et al., 1986; Post et al., 1997; Luthy et al., 2005; Tidwell et al., 2007; Amorim et al., 2011).

During summer in the Atlantic, sailfish reproduction occurs in tropical and subtropical areas in both hemispheres. In Brazil, the spawning takes place since Espirito Santo to Santa Catarina States, between November and February, with a peak in January (Arfelli and Amorim, 1981).

According to Ueyanagi et al. (1970), maturing of the white marlin gonads in the western part of the Southern Atlantic occurred between 20ºS and 30ºS, in the months from November to March, and the presence of larvae was observed between 10ºS and 25ºS. Arfelli et al. (1986) observed females in the final stages of maturing and post-spawning in the same period, in the area that goes from the coast of the state of Espirito Santo to Santa Catarina (18ºS to 27ºS). In the Gulf of Mexico and in the Caribbean, spawning was observed in ocean waters from April to June (Baglin, 1979; Arocha et al., 2005; Arocha and Ortiz, 2006). However, in the western part of the Equatorial Atlantic (5ºN to 13ºN), there are records of females spawning between February and September, while off the coast of Senegal in the Southeast Atlantic, spawning was observed between July and August (Limouzy and Cayre, 1981; De Sylva and Breder, 1997; Post et al., 1997; Luthy, 2004; Arocha and Marcano, 2006).

On the brazilian coast, blue marlin females ready to spawn were observed in the costal region of Bahia State (Abrolhos - Northeastern Brazil) during March to April (Ueyanagi et al, 1970; Amorim et al., 1998.), while on the coast of Cabo Frio, Rio de Janeiro, the females are maturing in January and February (Amorim et al., 1998).

Tidwell et al (2007) state that information about the first stages of development, nursery areas, billfish larvae habitat preferences and eating habits is still scarce, especially for the Southern Atlantic. Studies on Istiophoridae and Xiphiidae larvae are most concentred in the Northern Atlantic, such as those by De Sylva (1963), Erdman (1968), Jolley (1977), Baglin (1979), Harvey (1990), Souza et al. (1994), De Sylva and Breder (1997) and Richardson et al. (2009).

The morphological identification of the billfish larvae is met with severe difficulties due to the lack of distinctive characteristics between the species. However, the application of genetic techniques and molecular biology have been essential tools in larvae taxonomy, shown by McDowell and Graves (2002); Luthy et al. (2005), and Hyde et al. (2005). According to Herbert et al. (2003) the sequence of a single gene as long as evolutionarily conserved could be used to identify and distinguish at least the most animal species. Herbert et al., (2003) also proposed the use of mitochondrial COI gene as a global system of bioidentification, later called DNA barcoding.

The aim of this study was to identify and report the occurrence of billfish larvae and eggs in the superficial water mass in the Southwestern Atlantic, between the coastal region of Vitoria (ES), Rio de Janeiro (RJ) and Ilhabela (SP) using DNA barcode and characterizing possible nursery habitats of these fish.

2. Material and Methods

2.1 Study area and larvae collection

The area of study is located in the southeastern coast of Brazil, encompassing the continental shelf off the coast of Vitoria (ES), Rio de Janeiro (RJ) and Ilhabela (SP) (Figure 1). This deals with a traditional oceanic area for

billfish sport fishing: coastal region of South of Bahia, Espirito Santo, Rio de Janeiro and Sao Paulo States.

With the support of the sport fishing vessels from the Espirito Santo Yacht Club (ICES), the Rio de Janeiro Yacht Club (ICRJ) and the Ilhabela Yacht Club (YCI), it was possible to embark during the oceanic fishing seasons from 2012/2013 and 2013/2014 to collect the biological material. The collections were made from October/2012 to January/2013 in the first season and from November/2013 to February/2014 in the second season. The trips for collection were made on fishing tournament or training days. The collection stations were limited by the area in which the fishing vessels worked.

The larvae were collected with surface trawls using a 1.0m diameter and 2.90m long conical ichthyoplankton net made of 500µm mesh in the body and 600µm in the cup. The average speed of the trawls was 2 knots. There were made a total of 64 surface trawls, 14 of which were during the 2012/2013 season and 50 during the 2013/2014 season.

Each sampled station was numbered and the following data was taken: geographic position, date and time, air temperature, weather conditions, water temperature and depth. When possible, the wind speed was also checked using an anemometer.

The samples with remaining sea water were first preserved in a solution with 95% alcohol for material conservation (Luthy et al., 2005) and initially, all of the fish larvae were separated from the accompanying planktonic macrofauna and photographed. The probable Istiophoridae larvae were identified (Fahay, 2007), based on the four backwards-facing spines with a pronounced snouth. After being photographed the total length was measured, then they were separated for later DNA extraction and specific identification. The other fish larvae and planktonic macro fauna were stored for future identification. Some eggs were separated from the samples for testing since they were morphologically similar to those described by Hyde et al. (2005) as possible billfish.

2.2 Molecular identification

2.2.1 DNA extraction and amplification of the gene COI by PCR

The molecular tests were performed in the Integrated Center for Biotechnology - NIB at the University of Mogi das Cruzes - UMC, under the supervision of Professor Dr. Alexandre Wagner S. Hilsdorf.

The total DNA extraction of the Istiophoridae larvae and some of the eggs tested was done with a DNeasy Blood & Tissue – *Qiagen* kit according to the protocol offered by the manufacturer. Larvae smaller than 4.0mm were completely macerated for DNA extraction, just as for the eggs. Larvae larger than 4.0mm were partially used. In general, the tail was cut and then fragmented, avoiding the intestinal region, which is a possible area for contamination.

A fragment of approximately 650 pairs of bases from the mitochondrial gene COI (cytochrome c oxidase subunit I) was amplified using a pair of primers developed by Ward et al. (2005) FishF2 (5'TCGACTAATCATAAAG ATATCGGCAC3') and FishR2 (5'ACTTCAGGGTGACCGAAGAATCAGAA3'), both universal for fish. The primers FishF1 (5'TCAACCAACCACAAAGACATTGGCAC3') and Fish R1 (5'TAGACTTCTGGGTGGCCAAAGAATCA3') were also tested, but they amplified a smaller number of samples.

The Polymerase Chain Reaction - PCR to amplify the fragment of gene COI was done from a mix containing 18.7 µl of ultra pure water; 1.5µl of $MgCl_2$ (50mM); 1.0µl of KCl (50mM); 1.0µl of dNTP mix (2mM); 0.5µl of each primer (10µM); 0.3µl of *Taq* DNA Polymerase (Fermentas Life Sciences) and 1.5µl of the DNA template. The PCR was done in a Peltier Thermal Cicler PTC 200 (MJ Research).

A negative control was done without the DNA template to indicate the absence of contamination. The program used was: 94ºC for 2min; 35 cycles of 94ºC – 30s, 58ºC – 1min (annealing), 72ºC – 1min and 72ºC – 10min; finalizing with cooling at 4ºC.

The result of the amplification was observed in 1.5% agarose gel that was colored with GelRed and viewed under UV light in the photodocumenter ImageQuant 300 (GE Healthcare Life Sciences).

The purification was done using ExoSap (ExoSap-it, GE Healthcare) in a mix containing 10µl of the product of the PCR and 4µl of ExoSap according to the program of 60min at 37ºC and 15min at 80ºC and the samples were prepared for sequencing.

2.2.2 Sequencing and data analysis

The samples were sent to the section of DNA Sequencing on the Center for Human Genome Studies - USP (SP), where purified PCR products were subjected to sequencing reaction with BigDye Terminator v.3.1 @ Cycle Sequencing kit (GE Healthcare Life Sciences) according to the protocols provided by the manufacturer and sequenced bidirectionally in a sequencer ABI PRISM 3730 DNA Analyzer (Applied Biosystems). After receiving the forward and reverse sequences, the software *BioEdit Sequence Alignment Editor* (Hall, 1999) and *CodonCode Aligner* (CodonCode Corp., Dedham, MA, USA) were used to align them and obtain a consensus sequence.

The consensus sequence of each specimen was compared with the barcode sequences that already existed in the data banks of the Barcode of Life Data Systems – BOLD; Fish Barcode of Life (FISH-BOL) and with the *Nucleotidae BLAST* (GenBak - NCBI) tool for the analysis of similarity and identification of the species.

3. Results

During 2012/2013 and 2013/2014 seasons, 64 trawls were done. Figure 2 shows the geographic distribution of the collection stations in the area of the study.

The presence of ichthyoplankton was observed in 59.4% of the samples. A total of 391 fish larvae were captured at 38 stations. Of the 391 fish larvae captured, only 12 (3,07%) were morphologically identified as probable billfish:

Istiophoridae (Fahay, 2007). The first five Istiophoridae larvae captured during the 2012/2013 season in Vitoria were identified as sailfish (*Istiophorus platypterus*) by the PCR multiplex in partnership with Dr. Mahmood Shivji – Nova Southern University, Florida (method not published) and their occurrence was previously described by Schmidt (2013, not published). The reason for this previous identification was that at that time (early 2013) the project had no budget approved for carrying out the molecular assays in Brazil. The *I. platypterus* larvae cited were collected in January 2013, three of which (4.4mm; 3.8mm and 3.9mm) were at the same station (sea surface temperature - SST 25.4ºC) at 08:40, the fourth (5.6mm) at 13:55, and the fifth (4.8mm) at 14:20 (SST 25.5ºC for both), as shown in Table 1.

The other Istiophoridae larvae were stored for identification by mitochondrial gene COI barcode fragment sequencing. After sequencing, three more *I. platypterus* larvae were identified (3.3mm, 4.1mm and 3.2mm) coming from Vitória (January 2013) and one sample of *Kajikia albida* (3.1mm) collected in November 2013 also in Vitória; beyond this, there were three larvae collected in Ilhabela: one *K. albida* (6.7mm) and one *I. platypterus* (6.8mm) collected in December 2013 and another *I. platypterus* (4.4mm) collected in January 2014 at a point that is only 38.5 meters deep with a water temperature of 25.9ºC (Table 1).

In addiction to the described larvae, four fertilized eggs were tested that presented the following morphological characteristics: around 1.5mm diameter, opaque white coloring in 95% ethanol with melanophores (pigmentation) uniformly distributed over the entire egg and one pigmented embryo in development circling the sphere, without oil globules. Of the four eggs tested, two did not amplify even in tests with other primers (FishF1 and FishR1) and variations in the PCR program, while the other two amplified and were identified after sequencing as *Xiphias gladius.* Both were collected in Vitória, the first in November and the second in December of 2013.

The total number of larvae and eggs, geographic position, date, time and environmental parameters of the collection stations where the larvae and eggs were collected are also described in Table 1. The average temperature of the

points where the larvae were found was 25.7ºC.

A greater specific abundance was observed in January 2013 off the coast of Vitória where eight specimens of sailfish were collected at three collection points on the same sampling day.

Figure 3 illustrates the morphology of two *Istiophorus platypterus* larvae in different stages of development collected in this study, along with one *Kajikia albida* larvae and two eggs identified as *Xiphias gladius*.

Of the 12 Istiophoridae larvae identified, only three presented a total length greater than 5.0mm. The relationship between the total length of each specimen and the month of collection is illustrated in Figure 4.

4. Discussion

Identification by gene COI barcode fragment was considered satisfactory in this study since the sequenced fragments distinguished up to the taxonomic level of species of Istiophoridae and Xiphiidae in the data banks that were consulted. Identification by COI can be highly precise when the most appropriate database for reference is used (Herbert et al., 2003; Dawnay et al., 2007). The database from the Barcode of Life Data Systems (BOLD) contains the sequences of more than 144 thousand animal species. The Fish Barcode of Life (FISH-BOL) archives more than 100 thousand sequences of around 10 thousand fish species.

Despite the sampling efforts, the occurrence of the larvae was extremely rare in other ichthyoplankton studies on the coast of Brazil. Mafalda Jr. et al. (2004) collected ichthyoplankton on the coast of Bahia in December 1993 and March 1994 and they captured 826 larvae identified in 33 families, none of which were Istiophoridae or Xiphiidae. A study of ichthyoplankton diversity in the Arvoredo Marine Reserve (SC) was done by Rutkowski et al. (2011) with collections in the winter and summer of the years 1997/1998, 2007/2008 and 2008/2009, having collected 467 larvae identified in 19 families and again, there were no examples of Istiophoridae or Xiphiidae. Studying the distribution of ichthyoplankton in the Baia de Todos os Santos and Camamu (Northeastern

Brazil), Katsuragawa et al. (2011) made trawls during the winter and summer of 2003, winter 2004 and summer 2005, identifying 11 families and once again, no billfish were found. Finally, Bonecker et al. (2012) collected ichthyoplankton on the coast of Espirito Santo and north of Rio de Janeiro between February and April and between August and September 2009. In this study, the samples were collected at five depths: surface, 250m, 800m, 1200m and 2300m and a total of 10,978 fish larvae were collected. 75 families and 169 taxa were identified, and even so, only two specimen of Istiophoridae were collected, both in the superficial water mass, which could not be identified to a smaller taxonomic level (Bonecker et al., 2012). The present study even with limited sampling effort succeeded collecting 12 billfish larvae and two eggs, more than any others studies from Brazilian coast previously described.

The nursery area of the billfish larvae in the Gulf of Mexico is found to be widely studied and the points of high density of these fish were already covered in other studies. Tidwell et al. (2007) did a sampling with 287 collection stations from May to September 2005 and 2006 and captured 2,587 billfish larvae to analyze the stomach content. The total length of the larvae varied from 2.2 to 31.0mm and the main food item was composed of copepods from the Corycaeidae Family. The average temperature where the larvae were found by Tidwell et al. (2007) was 27.5ºC; 1.8ºC higher than the average temperature of the present study.

During the months of June and July from 2006 to 2008 Rooker et al. (2012) also found high densities in the Gulf of Mexico with ichthyoplankton trawls and captured 3,152 billfish larvae, 264 of which were swordfish (*Xiphias gladius*). The authors observed that the sailfish larvae presented a very wide horizontal distribution, different from that of the white marlin, blue marlin and swordfish, which seem to be less tolerant of environmental variations. Although Tidwell et al. (2007) had captured larvae in waters with elevated temperatures, Rooker et al. (2012) observed a greater abundance in lower temperatures, however with greater salinity. According to Rooker et al. (2012), areas with a mixture of continental water masses, presenting low salinity and higher temperatures, characterize the environments with a rare presence of billfish larvae. Despite this, in our study, one sailfish larvae was captured in a coastal

region just 8 nautical miles off the coast, at a point that is only 38m deep with a water temperature that was considered elevated for the region at 25.9ºC.

From July to November 2003, Serafy et al. (2008) captured 19 live Istiophoridae larvae on the coast of Miami, Florida for observation of the vertical movement. The temperature varied from 28.8 to 30.4ºC and the total length was from 3.3 to 17.7 mm. In August 2005, Serafy et al. (2006) tested the use of the Continuous Access Neuston Observation Net – CANON, a special kind of trawl net designed for capturing live billfish larvae, collecting a total of 104 larvae that were 3.5 to 12mm long, also on the coast of Miami.

Istiophoridae and Xiphiidae larvae and eggs were also collected by Hyde et al. (2005) on the coast of Kona, Hawaii on a scientific cruiser in May 2003 and another in July 2004, identified by PCR multiplex on board. During the first cruise, 76 larvae were captured and during the second, 215. The larvae captured by Hyde et al. (2005) were only shortbill spearfish (*Tetrapturus angustirostris*) and blue marlin (*Makaira nigricans*). There were 54 swordfish and 2 blue marlin eggs identified in the first sampling; in the second 57 swordfish and 8 blue marlin were identified. The morphological description of the swordfish eggs by Hyde et al. (2005) was similar to the characteristics observed in the eggs collected in this study, which were then submitted to sequencing to prove the hypothesis of being from the *Xiphias gladius* species.

Further studies should be done in the area, amplifying the sampling months in order to capture larvae in more advanced stages of development as well as test the hypothesis of blue marlin *Makaira nigricans*, longbill spearfish *Tetrapturus pfluegeri* and roundscale spearfish *Tetrapturus georgii* spawning.

5. Conclusion

The results of this study confirm the presence of *Istiophorus platypterus* and *Kajikia albida* (Istiophoridae) larvae as well as *Xiphias gladius* (Xiphiidae) eggs in the superficial water mass of the Southwestern Atlantic, between the coastal region of Vitoria (ES) and Ilhabela (SP) in the summer. These data suggest new evidence of billfish spawning and larvae nurseries in the southeast coast of Brazil. The larvae appear to exhibit an elevated dispersion in the area

of study, since no hotspot with a great specific abundance was found. The gene COI barcode fragment distinguished the billfish to the level of the species, being an important tool for specific identification that was applicable to these fish.

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Artwork and tables

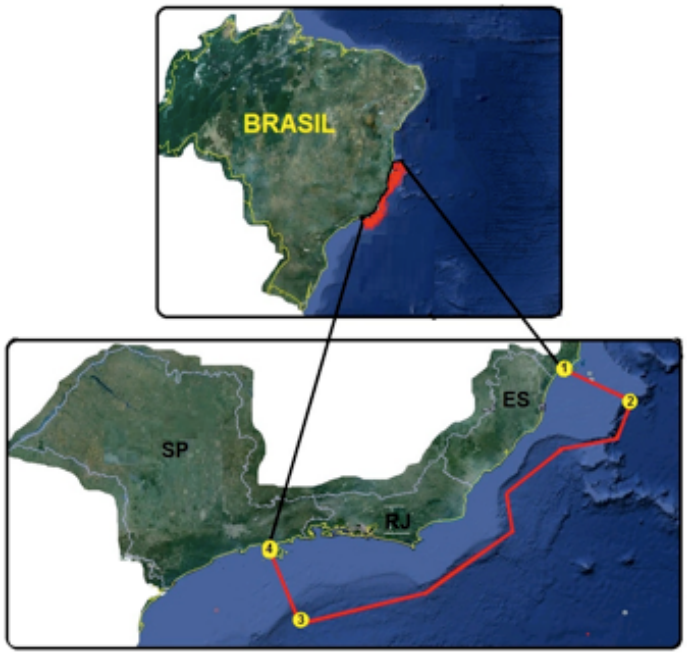

Fig. 1. Representation of the area of study. The limits are the points: (1)-18°01'S/39°30'W; (2)-18°16'S/37°31'W; (3)- 25°22'S/44°23'W;(4)- 23°50'S/45°34'W. Ilustration by Roberta Schmidt. Google Earth.

Fig. 2. Geographic distribution of the collection stations. Each point represents a trawl, collected off the coast of Ilhabela and Rio de Janeiro (Map A) and on the coast of Vitoria (Map B).

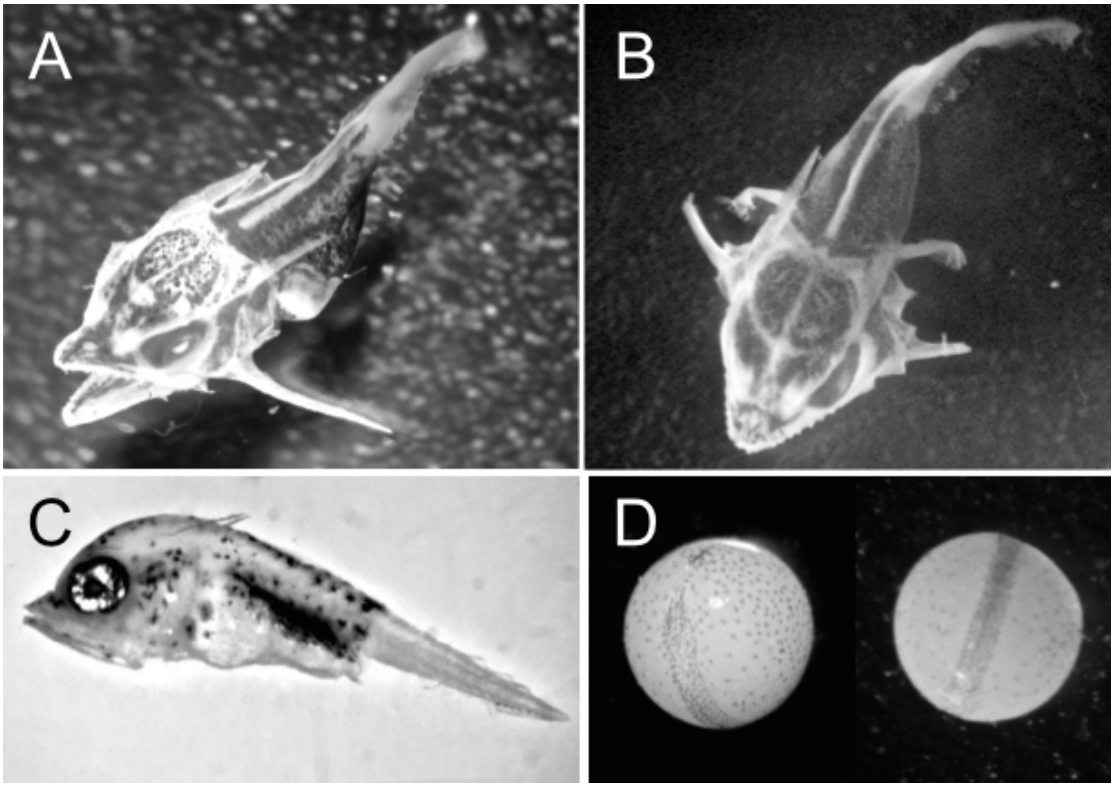

Fig. 3. Sailfish larvae (A: 6.8mm and C: 3.2mm), white marlin larvae (B: 6.7mm) and swordfish eggs (D: 1.5mm).

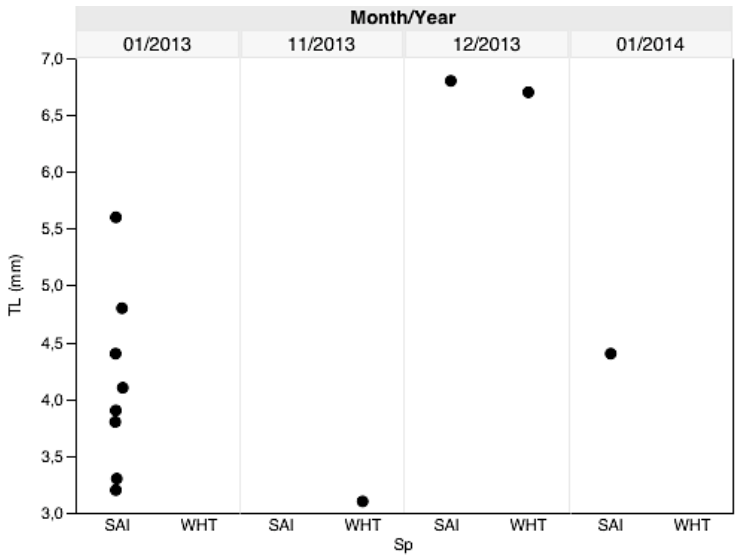

Fig. 4. Relationship between the total length (TL) of the 12 Istiophoridae larvae and the month of collection. SAI – sailfish (*Istiophorus platypterus*), WHT – White marlin (*Kajikia albida*).

Table 1. Description of the collection stations with the presence of Istiophoridae larvae and Xiphiidae (*) eggs.

Date	Time	Lat (S)	Long (W)	Species (N)	SST ºC	Depth m	Wind m/s
25/01/13	08:40	20 30.470	39 53.323	*Istiophorus platypterus* (5)	25,4	100	4,0
25/01/13	13:55	20 22. 173	39 53.092	*Istiophorus platypterus* (2)	25,5	53	10,0
25/01/13	14:20	20 22.121	39 53.356	*Istiophorus platypterus* (1)	25,5	53	6,0
20/11/13	11:30	20 44.059	39 56.453	*Kajikia albida* (1)	25,1	165	2,1
20/11/13	15:26	20 38.659	39 55.743	*Xiphias gladius* (1)*	24,9	67	1,5
07/12/13	16:00	20 24.069	39 52.793	*Xiphias gladius* (1)*	26,2	62	1,9
16/12/13	09:00	24 15.000	44 26.000	*Kajikia albida* (1) *Istiophorus platypterus* (1)	24,6	141	/
07/01/14	07:20	23 52.000	45 05.000	*Istiophorus platypterus* (1)	25,9	38,5	/

CAPÍTULO 2.

MOLECULAR IDENTIFICATION (BARCODE DNA) AND TAXONOMIC COMPOSITION OF FISH LARVAE CAUGHT OFF ESPIRITO SANTO STATE, SOUTHEASTERN COAST OF BRAZIL

Molecular identification (Barcode DNA) and taxonomic composition of fish larvae caught off Espirito Santo State, Southeastern coast of Brazil

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Abstract

Fishes initial stages of life are directly associated to fish recruitment, especially for the species captured by commercial fishing. This study aimed to identify and report the occurrence of species fish larvae in the superficial water mass in the Southwest Atlantic off the coast of Espirito Santo State, Southeastern Brazil by barcode DNA. During the summer in the last two years (2013 and 2013/2014) 27 surface trawls were done using an Ichthyoplankton net and 151 fish larvae were collected. The identification of the larvae up to the species level was made through molecular biology using the DNA Barcoding technique. The barcode fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified and sequenced. A total of 13 species and 10 Families were identified, widely dominated by the *Dactylopterus volitans* (Dactylopteridae) species in the beginning of 2013 and fish from the Caragnidae and Exocoetidae families in the 2013/2014 season. The *D. volitans* larvae were photographed under a electronic scanning microscope with support from the Department of Surgery at the College of Veterinary Medicine and Zootechny (*Departamento de Cirurgia da Faculdade de Medicina Veterinária e Zootecnia* - USP-SP). During the two sampling periods, *Thunnus atlanticus* (Scombridae) larvae were also collected, a species of heightened commercial interest. To evaluate the importance of the environmental parameters on the abundance of fish larvae, the Canonical Correspondence Analysis (CCA) was performed, which explained 77.2% of data variation (inertia value). The abundances and specific composition of the larvae were different on the two seasons. Therefore, it is important to perform new similar studies to record other species that are developed in the area.

Key words: *barcode, fish larvae, flying gurnard, Ichthyoplankton, dactylopteridae.*

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1. Introduction

The Ichthyoplankton, composed by eggs and fish larvae, represents the initial stage of life of fish, which, in this phase, are extremely fragile and sensitive to environmental factors and water quality (Katsuragawa et al., 2011). In this way, variations in abiotic factors and environmental impacts can be the determinants in the survival rate and in the fish recruitment, including the species that are target on comercial fishing (Sassa et al., 2004).

The life cycle of fishes and successful recruitment for the fishing stocks greatly depends on the initial phase of life, therefore, studies on fish larvae in fishing areas could represent a great importance to fisheries biology (Moser et al., 1984).

Several studies on the distribution of Ichthyoplankton have been done in Brazil in recent years (Katsuragawa et al., 1993; Ekau et al., 1999; Nonaka et al., 2000; Barletta-Bergan et al., 2002; Mafalda Jr. et al., 2004; Castro et al., 2005; Campos et al., 2010; Goçalo et al., 2011). Matsuura (1971) was one of the first to study the development of eggs and larvae in the life cycle of the sardine, *Sardinella brasiliensis,* in the region of Ilha Grande (RJ).

Looking for clarifying large taxonomic emblems, DNA has been widely used by genetics with molecular biology techniques to help identify the species (Bartlett and Davidson, 1991). The genetic study of the species, in addition to the dynamics of the populations, has been applied to large pelagic fish stocks in various groups, including elasmobranchs, tuna and billfishes (Chow, 1994; Daniel and Graves, 1994 Ward, 1995; Rocha-Olivares, 1998; Waples, 1998; Greig et al., 1999; Appleyard et al., 2001; Mcdowell and Graves, 2002; Hyde et al., 2005; Rodrigues-Filho et al., 2009).

The use of cytochrome c oxidase subunit I gene (COI) came after years of using different DNA sequences for identifying that which could not be compared. According to Herbert et al. (2003), the sequence of a single gene, as long as it is evolutionarily preserved, could be used to identify and distinguish at least the majority of the animal species. Herbert et al., (2003) proposed the use of gene mitochonrial COI as a global system for bioidentification, which was later called the barcode DNA.

The aim of this study was to identify and report the occurrence of fish larvae species in the superficial water mass in the Southwest Atlantic off the coast of Espirito Santo State by DNA barcode, as well as investigate possible relationships of the most represented taxa with the environmental parameters.

2. Materials and Methods

2.1 Study area, larval fish collection and electronic scanning microscopy

The study area is located on the southeast coast of Brazil, over the continental shelf off Vitoria (Espirito Santo), which is also an area for the traditional practice of oceanic sport fishing.

With the support of sport fishing vessels from the Espirito Santo Yacht Club (ICES), it was possible to embark during the ocean fishing seasons from 2012/2013 and 2013/2014 to collect the biological material. The sampling were made in January/2013 in the first season and from November/2013 to January/2014 in the second season. The collection stations were determined by the activity area of the fishing vessel, being limited in some cases by the sea conditions.

The larvae collection was done by surface trawls using a 1.0m diameter and 2.90m long conical ichthyoplankton net with 500µm mesh in the body and 600µm in the cup. The average speed of the trawls was 2 knots.

Each collection station was numbered and the following data was taken: geographic position, date and time, air temperature, weather conditions, water temperature and local depth. The wind speed was also checked using an anemometer. During the 2013/2014 season, a multiparameter meter, HANNA HI9828, was used to measure the pH, sea surface temperature (SST) and salinity.

The samples with remaining sea water were set in a solution with 95% alcohol for material preservation and the fish larvae were separated from the accompanying planktonic macrofauna and photographed. The planktonic macro fauna were stored for future identification and fish larvae were then separated for later DNA extraction and specific identification.

With the support of the Surgery Department at the of Veterinary Medicine and Zootechny College - USP (SP), an electronic microscopic scan was performing using some of the larvae from the Dactylopteridae Family, the most abundant specimens in this study. Some precautions were necessary for the electronic microscopic scan: setting the larvae with 3% Glutaraldehyde and 1% Osmium tetroxide; dehydration was done in 50% to 100% alcohol in each weighing; the sample was glued with carbon glue (so there is interaction with the electron) and inserted into a device with gold, palladium or silver.

2.2 Molecular identification

2.2.1 DNA extraction and amplification of the gene COI by PCR

The molecular tests were performed in the Integrated Center for Biotechnology - NIB at the University of Mogi das Cruzes - UMC, under the supervision of Professor Dr. Alexandre Wagner S. Hilsdorf.

The total DNA extraction of the Istiophoridae larvae and some of the eggs tested was done with a DNeasy Blood & Tissue – *Qiagen* kit according to the protocol offered by the manufacturer. Larvae smaller than 4.0mm were completely macerated for DNA extraction, just as for the eggs. Larvae larger than 4.0mm were partially used. In general, the tail was cut and then fragmented, avoiding the intestinal region, which is a possible area for contamination.

A fragment of approximately 650 pairs of bases from the mitochondrial gene COI (cytochrome c oxidase subunit I) was amplified using a pair of primers developed by Ward et al. (2005) FishF2 (5'TCGACTAATCATAAAG ATATCGGCAC3') and FishR2 (5'ACTTCAGGGTGACCGAAGAATCAGAA3'), both universal for fish. The primers FishF1 (5'TCAACCAACCACAAAGACATTGGCAC3') and Fish R1 (5'TAGACTTCTGGGTGGCCAAAGAATCA3') were also tested, but they amplified a smaller number of samples.

The Polymerase Chain Reaction - PCR to amplify the fragment of gene COI was done from a mix containing 18.7 µl of ultra pure water; 1.5µl of $MgCl_2$

(50mM); 1.0µl of KCl (50mM); 1.0µl of dNTP mix (2mM); 0.5µl of each primer (10µM); 0.3µl of *Taq* DNA Polymerase (Fermentas Life Sciences) and 1.5µl of the DNA template. The PCR was done in a Peltier Thermal Cicler PTC 200 (MJ Research).

A negative control was done without the DNA template to indicate the absence of contamination. The program used was: 94ºC for 2min; 35 cycles of 94ºC – 30s, 58ºC – 1min (annealing), 72ºC – 1min and 72ºC – 10min; finalizing with cooling at 4ºC.

The result of the amplification was observed in 1.5% agarose gel that was colored with GelRed and viewed under UV light in the photodocumenter ImageQuant 300 (GE Healthcare Life Sciences).

The purification was done using ExoSap (ExoSap-it, GE Healthcare) in a mix containing 10µl of the product of the PCR and 4µl of ExoSap according to the program of 60min at 37ºC and 15min at 80ºC and the samples were prepared for sequencing.

2.2.2 Sequencing

The samples were sent to the section of DNA Sequencing on the Center for Human Genome Studies - USP (SP), where purified PCR products were subjected to sequencing reaction with BigDye Terminator v.3.1 @ Cycle Sequencing kit (GE Healthcare Life Sciences) according to the protocols provided by the manufacturer and sequenced bidirectionally in a sequencer ABI PRISM 3730 DNA Analyzer (Applied Biosystems). After receiving the forward and reverse sequences, the software *BioEdit Sequence Alignment Editor* (Hall, 1999) and *CodonCode Aligner* (CodonCode Corp., Dedham, MA, USA) were used to align them and obtain a consensus sequence.

The consensus sequence of each specimen was compared with the barcode sequences that already existed in the data banks of the Barcode of Life Data Systems – BOLD; Fish Barcode of Life (FISH-BOL) and with the *Nucleotidae BLAST* (GenBak - NCBI) tool for the analysis of similarity and identification of the species.

2.3 Data analysis

An exploratory analysis of the data was done to identify the distribution of values and the normality, aiming at pointing to errors in measurement and readings that could compromise the multivariate analysis.

Data were put into two matrices: the abundance matrix, containing the abundance of fish larvae captured in the stations and the environmental matrix, which consists of the the diverse environmental and physical-chemical parameters of the water that were gathered at each collection station.

The environmental matrix was used in the Agglomerative Hierarchal Cluster Grouping Analysis (AHC), with the stations where larvae were captured, aiming at grouping the most similar collection stations and later compare them to the larval abundance and diversity. The parameters used in the analysis were: time of the trawl, local depth (m), water temperature (ºC), latitude, longitude, wind (m/s), pH and salinity (ppm). Dissimilarity and the Euclidean Distance criteria was used with the Ward Method for the AHC.

The multivariate analysis Canonical Correspondence Analysis (CCA) was done to test if there was an influence from the environmental matrix on the abundance matrix, looking to show which measured parameters seem to be connected to the occurrence of fish larvae. The parameters used were: local depth (m), water temperature (ºC), latitude, wind (m/s), pH and salinity (ppm). The exploratory analysis of the data was done on the *JMP Version 10.0* (SAS Institute) software and the multivariate analysis (AHC and CCA) on the *XLStat* (Addinsoft) software.

3. Results

During the two seasons, 27 surface drags were done, five of them in January/2013 and 22 during November 2013 to January 2014. Figure 1 shows the geographic distribution of the collection stations in the area of study with an indication of where there was a presence or absence of fish larvae.

The presence of larvae was observed in 55.5% of the samples. A total of 151 fish larvae were captured at 15 stations. 125 larvae were collected in

January 2013 and only 26 were captured from November 2013 to January 2014, even with a greater sampling effort.

The geographic position, date, time and environmental parameters of the collection stations where the larvae were collected and their abundance are described in Table 1. The average salinity of the points where the larvae were found was 31.5 and the average temperature was 25.8ºC.

Five larvae of *Istiophorus platypterus* (Istiophoridae) captured in January 2013 were first identified by PCR multiplex in conjunction with Dr. Mahmood Shivji – Nova Southern University, Florida (method not published) and their occurrence was previously described by Schmidt et al. (not published). The reason for this previous identification was that at that time (early 2013) the project had no budget approved for carrying out the molecular assays in Brazil.

The other fish larvae were identified by mitochondrial gene COI barcode fragment sequencing according to the described methods.

Of the 151 fish larvae collected, 134 specimens (88.7%) were identified to the species level. The other 17 (11.3%) were identified to the Family (Exocoetidae) level since they were not found in the database fragments of the gene COI that aligns these specimen with high similarity.

The taxonomic composition was formed by 13 species, distributed in 10 Families: *Aluterus monoceros* (Monacanthidae)*, Balistes capriscus* (Balistidae)*, Caranx crysos* (Carangidae)*, Dactylopterus volitans* (Dactylopteridae)*, Decapterus punctatus* (Carangidae)*, Harengula clupeola* (Clupeidae)*, Hemiramphus brasiliensis* (Hemiramphidae)*, Istiophorus platypterus* (Istiophoridae)*, Kajikia albida* (Istiophoridae)*, Prognichthys occidentalis* (Exocoetidae)*, Selar crumenophthalmus* (Carangidae)*, Sphoeroides spengleri* (Tetraodontidae) *and Thunnus atlanticus* (Scombridae)*.* The list of species captured in the collection stations with their respective abundances are in Table 2.

Station 2V was the one which presented the greatest diversity of species (10) and at station 3V, the greatest abundance of larvae (36) was observed, both in January 2013. The greatest specific abundance was of *Dactylopterus*

volitans with 82 examples, representing 54.3% of all of the larvae collected in this study. Figure 2 shows the photographs from the electronic microscopic scan of these larvae, where it is possible to see the four bony spines in the head, two superior and two inferior, both turned backwards. The presence of *Caranx crysos* was also highlighted, with 27 specimens.

The dendogram resulting from the Agglomerative Hierarchal Cluster (AHC) was illustrated in Figure 3, where it was possible to see the grouping of the stations into three large groups. It was observed that the first grouping (1) was from the stations in January 2013, then two other groups were formed (2 and 3) containing the other stations.

The Canonical Correspondence Analysis was done using the abundance matrix for *Dactylopterus* volitans, *Caranx* crysos, *Thunnus* atlanticus, the Exocoetidae Family and Total N of the fish larvae. The inertia value was 77.2, indicating that the CCA explained 77.2% of data variation, of which 70.7% were expressed by the axis one and 23.6% by the axis two of the ordination diagram (Figure 4).

The variables most related to the axis one were salinity, date and local time (highest score and regression coefficients values). Wind was the variable that presented the least relationship with the axis one. The sea surface temperature (SST) was most related to the axis two, as was latitude. For *Dactylopterus volitans* a greater relationship was observed with greater wind values, salinity and lower latitudes, pH values and sea surface temperature. *Caranx crysos* species showed a greater relationship with higher salinity and sea surface temperature. The presence of *Thunnus atlanticus* exhibited a positive relationship with latitude, salinity and sea surface temperature, and the Exocoetidae Family specimens, beyond presenting a similar relationship to that of the *T. Atlanticus* (however less expressively), exhibited a relationship with greater pH values. The total N (abundance) has not exhibited expressive tendencies for any variable, being in the center of the vectors on the ordination diagram, probably because this group was unspecific.

4. Discussion

The occurrence of fish larvae has also been reported in other Ichthyoplankton studies on the coast of Brazil. A diversity study of Ichthyoplankton in the Arvoredo Marine Reserve (SC) was done by Rutkowski et al. (2011) with collections in the winter and summer of the years 1997/1998, 2007/2008 and 2008/2009, having collected 467 larvae identified in 19 families and 21 species. The area of study for Rutkowski et al. (2011) was situated further to the south of Brazil and only two families and one species that were found in this study were also identified by them: Clupeidae (*Harengula clupeola* species) and Carangidae, which represent the greater part of the specimens captured in the summer. Rutkowski et al. (2011) also did a Canonical Correspondence Analysis and the relationship to the most abundant family (Eugralidae) were similar to those shown by *Dactylopterus volitans* in this study.

Mafalda Jr. et al. (2004) collected Ichthyoplankton on the coast of Bahia in December 1993 and March 1994 and have captured 826 larvae, identified in 33 families and 20 species. The taxonomic groups with the greatest representation were Engraulidae, Carangidae, Clupeidae and Gerreidae (Mafalda Jr. et al., 2004) and within the 33 families that were captured, those that were also found in this study were the Tetraodontidae, Hemiramphidae, Scombridae, Clupeidae, Carangidae, Exocoetidae and the Balistidae.

Studying the distribution of Ichthyoplankton in the Baia de Todos os Santos and Camamu (BA), Katsuragawa et al. (2011) made trawls during the winter and summer of 2003, winter of 2004 and summer of 2005; and identified 11 families, among which are Clupeidae, Hemiramphidae, Carangidae and the Tetraodontidae, which were also present off the coast of Vitoria according to our results.

In the study made by Bonecker et al. (2012) Ichthyoplankton on the coast of Espirito Santo and north of Rio de Janeiro was collected between February and April and between August and September 2009. In their study, the samples were collected at five depths: surface, 250m, 800m, 1200m and 2300m and a total of 10,978 fish larvae were captured. There were 75 families

and 169 taxa identified (Bonecker et al., 2012). Even with this elevated sampling effort and the diversity of the taxonomic composition, only the Clupeidae (n=87), Carangidae (*Caranx crysos* n=1), Exocoetidae (n=1), Scombridae (*Thunnus atlanticus* n=1), Balistidae (*Balistes capriscus* n=1), Tetraodontidae (n=2) and Istiophoridae (n=2) were similar to our study. Again, like in the previously cited studies, no Dactylopteridae larvae were found.

The first large group formed by the Grouping Analysis is composed by trawls done in January 2013, characterized by the constant water temperature between 25.4 and 25.5 ºC and lower latitudes. Even at different day times, these stations were those that presented the greatest abundance and diversity of larvae. Apparently, the water temperature and latitude were the determining parameters for grouping these stations. The second group contained the first and last trawls from the 2013/2014 season, which were then grouped again in smaller groups, the first of which showing similar temperatures (around 25ºC) and higher salinity (over 31 ppm) and the last ones with higher temperatures (over 27ºC) and lower salinity values (below 30). The third group contained the stations with the least abundance of larvae: 15V, 16V and 17V, with only one larvae collected at each point and was characterized by the lower pH values, with an average of 6.88 instead of the average of 7.21 at the other points.

Despite the low value of abundance of species, the Canonical Correspondence Analysis explained 77% of the variation in the data and some relationships were observed. The use of relationships should mainly serve as an investigative tools for future studies, however, it is essential to highlight that this analysis showed numerical relationships between objects (abundance of the species) and variables (physical-chemical, environmental, etc. parameters), being able to diverge from the ecological relationships and habitat preferences of each specie.

The presence of fish larvae on the summers of 2013 and 2014 showed an elevated difference, with 125 larvae captured in 5 trawls in January 2013 and only 26 captured in another 22 trawls between November 2013 and January 2014. The results suggest a highly heterogeneous spawning standard in the

period studied, possibly related to oceanographic and climactic factors. During the 2013/2014 season, the fishermen in the area have argued that the capture of the fish in oceanic sports fishing was atypical, with a significant delay comparing to the previous year and some species that were frequently captured were rare, like the dolphinfish *Coryphaena hippurus*.

Performing new samples and studies in the area in the same period is important for elucidate the occurrence of some larvae that were not present in the samples from the two seasons, taking the *Dactylopterus volitans* specie as an example, which was found in large quantities in the first samples and was not captured again in the 2013/2014 season, despited the great increase in sampling efforts.

5. Conclusion

The results of our study report the presence of *Dactylopterus volitans* (Dactylopteridae) larvae as well as *Caranx crysos* (Carangidae), Exocoetidae Family and *Thunnus atlanticus* (Scombridae) in the superficial water mass of the Southwestern Atlantic, on the coastal region of Espirito Santo State (Southeastern Brazil) during the summer. These data suggests the evidence of larvae nurseries for these species on the southeast coast of Brazil, especially of *D. volitans*. The barcode fragment of COI gene distinguished the larger part of the specimens until the level of the species, and was an essential tool for taxonomic identification on the present study.

Acknowledgments

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Artwork and tables

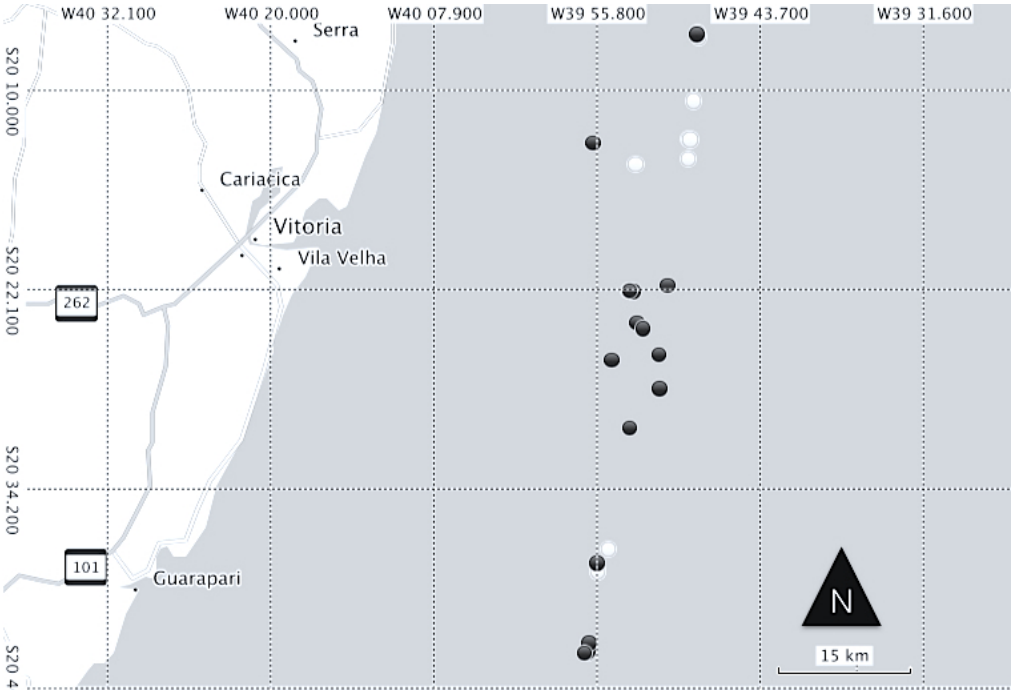

Fig. 1. Geographic distribution of the collection stations off the coast of Vitoria, Espirto Santo State. Each point represents a trawl. Black points: presence of fish larvae; white points: absence of fish larvae.

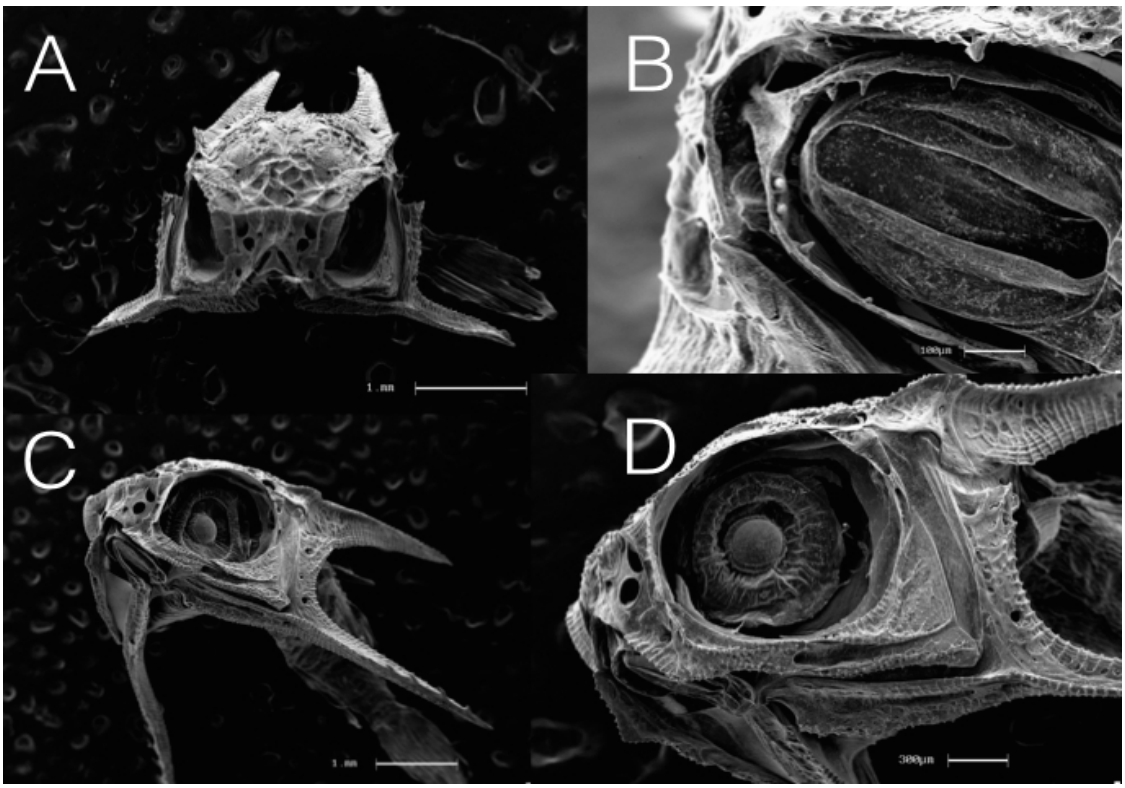

Fig. 2. Electronic Microscopic Scan of *Dactylopterus volitans*. A: front view; B: under view of the mouth; C: lateral inferior view; D: lateral view of the head.

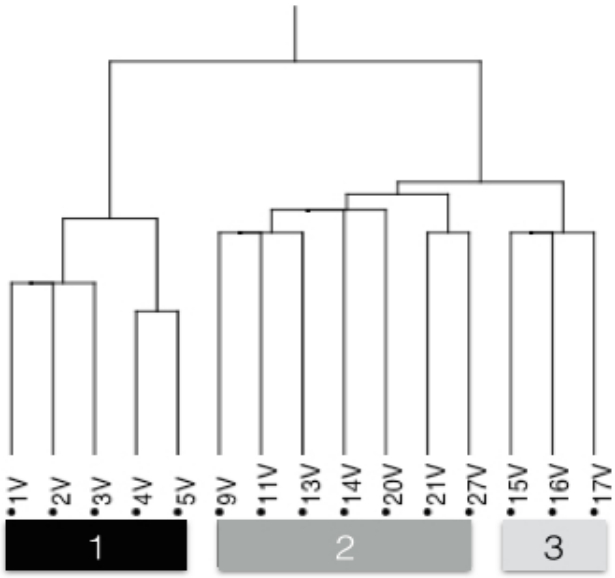

Fig. 3. Agglomerative Hierarchal Cluster (AHC) of the larvae collection stations. Formation of three main groups of agglomerates.

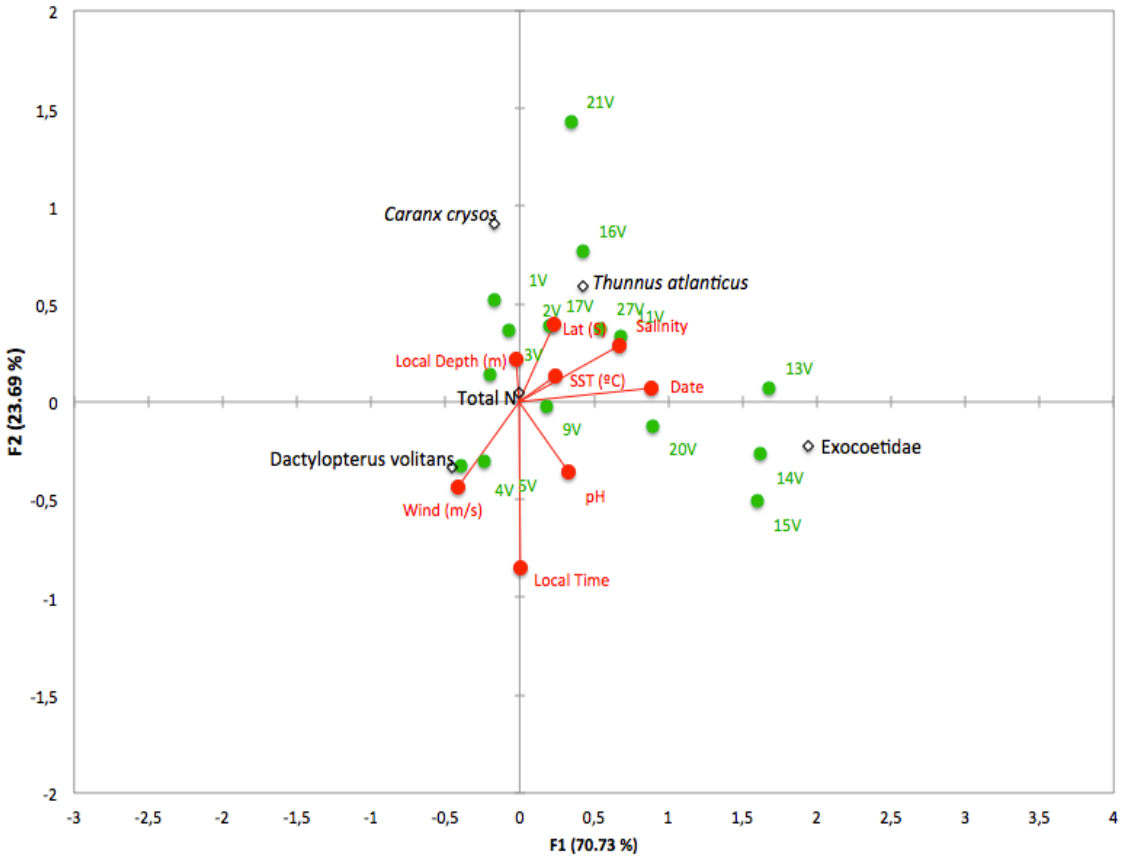

Fig. 4. Canonical Correspondence Analysis - CCA Ordination Diagram. The vectors represent the environmental variables, the points are relative to the collection stations and the diamonds represent the species.

Table 1. Description of the stations where fish larvae was caught and the total abundance (total N) of the larvae in each station. Stations were numbered from 1V to 27V.

Station	Date	Local Time	Lat (S)	Long (W)	Local Depth (m)	Wind (m/s)	SST (ºC)	Salinity	Total N
1V	25/01/13	07:30	20 26.342	39 54.684	63	3,8	25,40	31,50	9
2V	25/01/13	08:40	20 30.470	39 53.323	100	4,0	25,40	31,00	24
3V	25/01/13	10:45	20 28.073	39 51.113	1100	8,0	25,42	31,50	36
4V	25/01/13	13:55	20 22.173	39 53.092	53	10,0	25,51	30,00	35
5V	25/01/13	14:20	20 22.121	39 53.356	53	4,0	25,55	29,00	21
9V	16/11/13	13:40	20 44.105	39 56.676	102	5,5	25,08	32,1	2
11V	20/11/13	11:30	20 44.059	39 56.453	165	2,1	25,19	31,7	1
13V	23/11/13	10:19	20 24.438	39 52.364	59	4,5	25,46	36,58	3
14V	23/11/13	12:35	20 25.993	39 51.148	455	5,2	25,55	34,30	10
15V	23/11/13	14:50	20 21.815	39 50.542	86	6,1	26,08	34,04	1
16V	04/12/13	10:00	20 44.010	39 56.419	116	0,7	25,01	28,99	1
17V	04/12/13	15:10	20 43.529	39 56.329	130	5,5	26,35	29,58	1
20V	07/12/13	16:00	20 24.069	39 52.793	62	1,9	26,27	29,81	3
21V	29/01/14	07:19	20 13.136	39 56.069	48	3,5	27,02	29,32	2
27V	29/01/14	16:24	20 06.560	39 48.342	72	0,4	28,28	29,49	2

Table 2. Composition, total and relative abundance species and Families captured in the collection stations off Espirito Santo State, Southeastern Brazil.

Species	Family	Total N	%	Station
Caranx crysos	Carangidae	7	4,64	1V
Dactylopterus volitans	Dactylopteridae	2	1,32	
Balistes capriscus	Balistidae	1	0,66	2V
Caranx crysos	Carangidae	4	2,65	
Dactylopterus volitans	Dactylopteridae	7	4,64	
Decapterus punctatus	Carangidae	1	0,66	
Hemiramphus brasiliensis	Hemiramphidae	1	0,66	
Istiophorus platypterus	Istiophoridae	5	3,31	
Prognichthys occidentalis	Exocoetidae	1	0,66	
Selar crumenophthalmus	Carangidae	1	0,66	
Sphoeroides spengleri	Tetraodontidae	1	0,66	
Thunnus atlanticus	Scombridae	2	1,32	
Caranx crysos	Carangidae	11	7,28	3V
Dactylopterus volitans	Dactylopteridae	23	15,23	
Prognichthys occidentalis	Exocoetidae	2	1,32	
Caranx crysos	Carangidae	1	0,66	4V
Dactylopterus volitans	Dactylopteridae	31	20,53	
Istiophorus platypterus	Istiophoridae	2	1,32	
Sphoeroides spengleri	Tetraodontidae	1	0,66	
Dactylopterus volitans	Dactylopteridae	19	12,58	5V
Istiophorus platypterus	Istiophoridae	1	0,66	
unidentified larvae of Exocoetidae	Exocoetidae	1	0,66	
Harengula clupeola	Clupeidae	1	0,66	9V
Sphoeroides spengleri	Tetraodontidae	1	0,66	
Kajikia albida	Istiophoridae	1	0,66	11V
unidentified larvae of Exocoetidae	Exocoetidae	3	1,99	13V
unidentified larvae of Exocoetidae	Exocoetidae	10	6,62	14V
unidentified larvae of Exocoetidae	Exocoetidae	1	0,66	15V
unidentified larvae of Exocoetidae	Exocoetidae	1	0,66	16V
Caranx crysos	Carangidae	1	0,66	17V
Caranx crysos	Carangidae	1	0,66	20V
Thunnus atlanticus	Scombridae	1	0,66	
unidentified larvae of Exocoetidae	Exocoetidae	1	0,66	
Aluterus monoceros	Monacanthidae	1	0,66	21V
Caranx crysos	Carangidae	1	0,66	
Caranx crysos	Carangidae	1	0,66	27V
Prognichthys occidentalis	Exocoetidae	1	0,66	

CONSIDERAÇÕES FINAIS

A ocorrência de larvas de *Istiophorus* platypterus e *Kajikia albida* (Istiophoridae), além de ovos de *Xiphias gladius* (Xiphiidae) foi confirmada na massa de água superficial do Atlântico Sul Ocidental na região costeira entre Vitória-ES e Ilhbela-SP durante o verão. Essa ocorrência corrobora a hipótese que os peixes-de-bico desovam e as larvas eclodem dos ovos e se desenvolvem na costa sudeste do Brasil.

Apesar da elevada dificuldade para obtenção das larvas, acredita-se que a baixa densidade encontrada ocorreu devido às limitações de amostragem. Os arrastos foram realizados apenas na superfície, entretanto não é conhecido o padrão de migração vertical dessas espécies nesse estágio de vida. Assim, infere-se a possibilidade delas estarem presentes também em outras profundidades.

As larvas aparentam exibir elevada dispersão pela área de estudo, uma vez que nenhum hotspot com elevada densidade foi encontrado. A ampla distribuição atribui ainda mais dificuldades de amostragem desses espécimes, o que foi observado no presente estudo e na literatura.

Sugere-se que ao menos uma das razões da migração dos peixes-de-bico para o Atlântico Sul seja para reprodução e desova. Desta forma, os dados do presente estudo são registros que devem ser futuramente estudados a fundo, podendo fornecer subsídios para medidas de conservação dessas espécies, alvos potenciais da pesca recreacional oceânica.

A hipótese de que as larvas e ovos sejam trazidos por correntes oceânicas ao acaso não é descartada, mas possui lacunas e poucas evidências. Além da presença de larvas e ovos, fêmeas com gônadas em maturação e maduras já foram observadas nas últimas décadas, sustentando a hipótese de desova na área.

A presença de larvas de *Dactylopterus volitans* (Dactylopteridae), *Caranx crysos* (Carangidae), Família Exocoetidae e *Thunnus atlanticus*

(Scombridae) na massa de água superficial na região costeira do Espírito Santo também foi reportada. Salienta-se a importância de novas amostragens na área afim de observar se existe sazonalidade e a distribuição espaço-temporal das larvas dessas espécies.

O fragmento barcode do gene COI distinguiu os peixes-de-bico e a maior parte das larvas de peixes analisadas a nível de espécie, sendo uma importante ferramenta na identificação aplicável a peixes, inclusive pequenos fragmentos e ovos.