

**UNIVERSIDADE FEDERAL DO RIO GRANDE
PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA**

**ECOLOGIA TRÓFICA, USO DE HABITAT E
CONTAMINAÇÃO POR MERCÚRIO EM GAROUPA-
VERDADEIRA (*Epinephelus marginatus*) EM UMA
REGIÃO LITORÂNEA E OUTRA NERÍTICA NA COSTA
DO RIO GRANDE DO SUL**

MARIO VINICIUS CONDINI

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Orientador: Prof. Dr. Alexandre Miranda Garcia

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RESUMO

A garoupa-verdadeira é um peixe de grande porte (até 2,5 m e 400 Kg) que é explorado pela pesca comercial e esportiva ao longo da sua distribuição no Atlântico Oeste, Oceano Índico e Mediterrâneo, sendo atualmente considerado em perigo de extinção (IUCN). Devido ao tamanho avantajado e hábito carnívoro, a espécie pode exercer forte influência sobre a cadeia alimentar dos ecossistemas no qual está inserida. Apesar da sua importância econômica e ecológica, existem vários aspectos ainda desconhecidos sobre seu ciclo de vida e comportamento, especialmente no extremo sul do Brasil da sua distribuição no Atlântico Oeste, aonde a espécie ocorre em fundos rochosos na região litorânea e nerítica como os Molhes da Barra de Rio Grande (MB) e o Parcel do Carpinteiro (PC), respectivamente. A presente tese teve como principais objetivos principais investigar a ecologia trófica, padrões de movimento, uso de hábitat e contaminação de mercúrio da garoupa-verdadeira (*Epinephelus marginatus*) entre as regiões litorânea e nerítica no extremo sul do Brasil. Entre 2008 e 2011 foram coletadas 348 garoupas-verdadeiras (n=133 do MB e n=215 do PC) junto aos pescadores artesanais e frota pesqueira industrial. A partir desses indivíduos foi possível investigar a ecologia trófica da garoupa-verdadeira através de análises de conteúdo estomacal e isótopos estáveis e determinar que a espécie é um predador generalista com elevada posição trófica comparada às demais espécies de peixes nas duas áreas de estudo. As técnicas de análise empregadas mostraram diferenças na composição dos itens ingeridos e efetivamente assimilados pela espécie, que foram principalmente peixes e camarões. Através da análise de microquímica de otólitos (^{138}Ba : ^{43}Ca) foi possível determinar, pela primeira vez, que alguns indivíduos

da garoupa-verdadeira podem permanecer períodos prolongados (2-3 anos) em ambientes estuarinos, mostrando que a espécie apresenta maior plasticidade no uso de habitat do que registrado até então na literatura. Foram analisadas amostras de músculos da garoupa-verdadeira a fim de determinar o grau de contaminação por mercúrio da espécie em ambas as áreas de estudo. Os indivíduos de grande porte (> 650 mm CT) apresentaram elevados níveis de contaminação de mercúrio, os quais podem ser prejudiciais para a saúde da espécie e também mostrou limites acima dos aceitáveis para o consumo humano. Quando foram considerados apenas os indivíduos de classes de tamanhos similares, foi possível observar que os indivíduos que habitam o habitat litorâneo apresentaram maiores concentrações de mercúrio, provavelmente devido à proximidade com as fontes de poluição associados com as atividades humanas na região estuarina e sua bacia de drenagem. As informações científicas obtidas sobre a bioecologia da garoupa-verdadeira, inéditas para o extremo sul do Brasil e alguns casos para as demais áreas aonde a espécie ocorre, podem auxiliar os gestores e as autoridades competentes na criação de planos de conservação dessa espécie que atualmente está ameaçada de extinção.

Palavras chaves: Atlântico sudoeste; conservação; espécie ameaçada; isótopos estáveis; mercúrio total; microquímica de otólito.

ABSTRACT

Dusky grouper is a large-bodied marine species (up 1.4 m and 60 kg) exploited by commercial fishing and in spearfishing along its distribution in Atlantic Ocean, Indian Ocean and Mediterranean Sea, and is currently included in the IUCN red list. Due to its large body and carnivorous feeding habit, this species plays an important functional role in food webs where they are inserted. Despite its economic and ecological importance, there are several aspects unknown on the dusky grouper's life cycle and behavior, mainly on southern Atlantic, where this species inhabits rocky bottoms in littoral and neritic zones, locally known as *Molhes da Barra de Rio Grande* (MB) and *Parcel do Carpinteiro* (PC), respectively. The main goals of the current thesis were to investigate the trophic ecology, habitat use and mercury contamination of the dusky grouper (*Epinephelus marginatus*) between littoral and neritic areas of extreme Southern Brazil. Between 2008 and 2011, 348 dusky grouper (n= 133 MB and n= 215 PC) were sampled in collaboration with local fisherman. Based on these individuals we investigated the trophic ecology using stomach content and stable isotope analyzes, which revealed that this species is a generalist predator with a high trophic position among the fish species in both study areas. The methods showed differences between the composition of ingested food items and those effectively assimilated by the species, which were mainly fishes and shrimps. Microchemical analysis of otoliths ($^{138}\text{Ba}:^{43}\text{Ca}$) indicated for the first time that individuals of *E. marginatus* can remain for prolonged time periods in brackish waters (2-3 years), revealing that the species has higher plasticity in habitat use that previously shown in the literature. We also analyzed mercury in dusky grouper muscles and found that larger individuals (>650

mm) showed highest mercury contamination levels that are potentially harmful for this endangered fish species and above the acceptable limits for human consumption. When considering similar body sizes, individuals inhabiting littoral rocky habitats adjacent to Patos Lagoon showed higher concentrations of mercury, probably due to proximity to pollution sources associated with human activities in the estuary and its drainage basin. These results are useful to future management plans to ensure the sustainability of the dusky grouper fisheries in southern Brazil and hopefully contribute to reduce its current extinction risk status worldwide.

Key words: Southwestern Atlantic; conservation; endangered species; stable isotope; total mercury; otolith microchemistry

1 Introdução geral

1.1 Garoupas e conservação

Através de estudos de morfologia e genética, Smith & Craig (2007) concluíram que a família Serranidae (*sensu* Nelson, 2006) era um grupo polifilético, onde as garoupas e afins (Subfamília Epinephelinae) estariam separadas das demais espécies das Subfamílias Anthiinae e Serraninae. Assim, esses autores propuseram que a Subfamília Epinephelinae fosse elevada a família Epinephelidae, e as outras duas Subfamílias continuassem como família Serranidae, sendo agora um grupo monofilético. Essas famílias redefinidas incluem 163 (Epinephelidae) e 245 (Serranidae) espécies.

As garoupas, agora pertencentes à família Epinephelidae, englobam diversas espécies de peixes de pequeno a grande porte e com ampla distribuição geográfica desde os mares tropicais aos subtropicais rasos ao longo dos Oceanos Atlântico, Índico e Pacífico, sendo diversas espécies alvo da pesca artesanal, industrial e esportiva (Coleman *et al.* 2000; Craig *et al.* 2011). Grande parte das garoupas são peixes predadores de emboscada, com hábito territorialista, estando frequentemente associada a fundos coralíneos e rochosos, habitando tocas e fendas entre rochas e recifes de corais, sendo comuns em águas costeiras tropicais e subtropicais, especialmente em recifes, parcéis e costões rochosos (Heemstra & Randall 1993; Craig *et al.* 2011). Devido a essas características, diversas espécies exercem forte influência sobre a cadeia alimentar dos ecossistemas nos quais estão inseridas, atuando como predadores de topo (Heemstra & Randall 1993; Sluka *et al.* 2001). Sendo assim,

alterações em suas populações podem ter consequências comerciais e ecológicas significativas (Bruslé *et al.* 2005).

Diversas espécies de garoupas exibem um comportamento K-estrategista, visto que elas apresentam baixas taxas de crescimento, são espécies longevas e maturação tardia (Manooch & Mason 1987; Heemstra & Randall 1993; Craig *et al.* 2011). Essas características biológicas, juntamente com uma complexa estratégia de vida (hermafroditismo e agregações reprodutivas) e a exposição a uma elevada pressão pesqueira, acabam tornando esse grupo de peixes vulneráveis à sobre-exploração (Hunstman *et al.* 1999; Sadovy *et al.* 2013). Apesar de ser uma família com grande interesse econômico, ainda há muitas espécies com poucas informações sobre sua biologia, ecologia, comportamento e dados sobre sua exploração pesqueira, dificultando enormemente o planejamento de ações para sua conservação. De acordo com a União Internacional de Conservação da Natureza (IUCN - *International Union for Conservation of Nature*), das 163 espécies da família Epinephelidae, cerca de um terço das espécies (50) estão classificadas como Dados Deficientes (*Data Deficient* - DD). Essa classificação é caracterizada pela falta de informações que permitam enquadrar uma espécie dentro de algum critério de ameaça ou de não ameaça. Portanto, a categoria DD é muito perigosa para a conservação, pois a espécie pode estar em perigo eminente e nenhuma providência está sendo tomada para protegê-la. Outras 71 espécies encontram-se no critério de Menor Preocupação (*Least Concern* – LC), ou seja, espécies livres de ameaças. Outras 22 espécies estão classificadas como Quase Ameaçadas (*Near Threatened* – NT). Por fim, 20 espécies estão dentro dos critérios de ameaças, estando 12 espécies como Vulnerável (*Vulnerable* – VU), 5 como Em Perigo

(*Endangered* – EN) e 3 como Criticamente em Perigo (*Critically Endangered* – CR) (IUCN 2016).

O maior exemplo de vulnerabilidade das espécies de Epinephelidae é o mero *Epinephelus itajara*, sendo essa a maior espécie da família, podendo atingir mais de 2 m de comprimento total e pesar até 400 kg (Bullock *et al.* 1992). O mero foi intensamente pescado tanto pela pesca comercial quanto esportiva, mostrando acentuado declínio em sua captura durante meados da década de 1970 (Bullock *et al.* 1992), até o quase desaparecimento total no final da década de 1990. A partir de então começaram haver diversos esforços visando sua preservação, até a proibição completa de sua pesca ao longo de toda sua distribuição. Medida essa que vem resultando num aumento de sua população (Sluka & Sullivan 1998), mas mesmo assim, o mero ainda se encontra no critério de Criticamente em Perigo (CR) segundo a IUCN (Craig 2011).

Devido ao elevado número de espécies de Epinephelidae ameaçados ou quase ameaçados, medidas de conservação têm sido criadas ao redor do mundo, desde proibição da pesca, tamanho mínimo de captura, período de defeso e criações de Áreas Marinhas Protegidas (AMP) visando à conservação das garoupas.

Os ecossistemas marinhos costeiros são áreas altamente produtivas, apresentando papel fundamental para ecologia de diferentes grupos de vertebrados e invertebrados, os quais podem servir como áreas de alimentação, crescimento e/ou reprodução, no entanto, esses ambientes estão sendo indiscriminadamente explorados e devastados pela atividade humana (Jackson 2008; Halpern *et al.* 2008).

Com intuito de manter esse ecossistema em harmonia, são propostas implementações de Áreas Marinhas Protegidas (AMP), sendo essas zonas marinhas e/ou costeiras que abrigam ecossistemas importantes e, por isso, são protegidas através de legislações específicas, no sentido de conservar a biodiversidade (Kelleher & Kenchington 1992) e assegurar a produtividade pesqueira dentro e fora de seus limites através de *spillover* e exportação larval (Roberts *et al.* 2001; Goñi *et al.* 2008). Nos últimos anos, houve um aumento significativo de AMP ao redor do mundo (Benedetti 2013), sendo que apenas no Mar Mediterrâneo existem ao menos 100 dessas áreas de proteção (Andrelo *et al.* 2013). No entanto, mesmo com o aumento significativa de AMP em alguns locais, as áreas marinhas protegidas estão muito abaixo do proposto pela Convenção de Diversidade Biológica (*Convention on Biological Diversity* – CBD) em 2010 (www.cbd.int/cop10).

As AMPs apresentam tamanha importância que hoje elas estão incluídas nas avaliações da IUCN, onde de acordo com suas normas é possível distinguir duas categorias de áreas protegidas: a) as áreas de proteção restrita, caracterizadas pela ausência de espécies exóticas, de atividades e de instalações humanas e b) as zonas de extração protegidas, que são zonas de pesca e de caça, onde uma exploração limitada e controlada dos recursos naturais é autorizada (Kelleher & Kenchington 1992). Em ambas as modalidades, as AMPs têm se mostrado bastante eficientes para diversas espécies de Epinephelidae, devido ao fato de algumas dessas espécies serem peixes territorialistas de pouca mobilidade, que mantem sua dispersão larval através de suas larvas vageis (Halpern 2003; Andrelo *et al.* 2013).

Dentre as espécies da família Epinephelidae, apenas 19 espécies de oito gêneros (*Alphestes*, *Cephalopholis*, *Dermatolepis*, *Epinephelus*, *Gonioplectrus*, *Hyporthodus*, *Mycteroperca*, *Paranthias*) ocorrem em ambientes costeiros do litoral brasileiro (Hostim-Silva *et al.* 2006; Craig *et al.* 2011), das quais sete espécies se encontram classificadas em algum dos critérios de ameaça determinado pela IUCN. Dentre os Epinephelidae, os gêneros *Mycteroperca* e *Epinephelus* apresentam maior riqueza de espécies na costa brasileira (6 e 5 espécies, respectivamente).

1.2 Comportamento, distribuição, biologia e pesca

A espécie de garoupa investigada na presente tese é a garoupa-verdadeira *Epinephelus marginatus* (Lowe, 1834) (Fig. 1). A garoupa-verdadeira é uma espécie territorialista de hábito sedentário e solitário, com alta fidelidade ao seu habitat, principalmente os indivíduos maiores de 20 cm, os quais permanecem a maior parte do tempo em suas tocas, saindo apenas para curtas expedições à procura de alimento (Pastor *et al.* 2009; Koeck *et al.* 2014; Afonso *et al.* 2016). Em contrapartida, os juvenis, menores que 20 cm, preferem áreas abertas, vagando à procura de alimento, sendo que nessa fase eles chegam a apresentar uma formação de pequenos grupos, com até três indivíduos (Gibran 2007). A garoupa-verdadeira é uma espécie de grande porte, podendo atingir 143 cm de comprimento total (Craig *et al.* 2011) e pesar até 60 kg (Figueiredo & Menezes 1980), sendo predominantemente carnívora, predando preferencialmente crustáceos, peixes e moluscos (Barreiro & Santos 1998; Reñones *et al.* 2002; Linde *et al.* 2004; Machado *et al.* 2008; Condini *et al.* 2015). Essa espécie tem

uma importante função nos fundos rochosos, no sentido de manutenção do equilíbrio ecológico desses ambientes, pois geralmente constitui um dos principais predadores na sua área de ocorrência (Parrish 1987; Reñones *et al.* 2002; Condini *et al.* 2015).



Figura 1 – Imagem da garoupa-verdadeira *Epinephelus marginatus* em seu ambiente natural (A, Foto: Áthila Bertoncini Andrade) e de um jovem (~30 cm) recém-coletado (B, Foto: Alexandre Miranda Garcia).

A garoupa-verdadeira apresenta um ciclo de vida bastante complexo, pois são espécies longevas, com idade máxima estimada em 61 anos (Reñones *et al.* 2007), de lentas taxas de crescimento, hermafrodita protogínica monândrica (Bruslé & Bruslé 1975; Marino *et al.* 2001), ou seja, os indivíduos iniciam sua vida como fêmeas e atingem sua primeira maturação ainda com o mesmo sexo, mas alguns indivíduos podem sofrer transição sexual, entre os 7 e 17 anos de idade (Reñones *et al.* 2010), tornando-se machos funcionais (Marino *et al.* 2003; Condini *et al.* 2014a). A transição sexual pode ocorrer devido à pressão populacional (Sadovy de Mitcheson & Liu 2008; Erisman *et al.* 2010). Portanto, todos os machos da população são provenientes de fêmeas sexualmente maduras. Outra característica importante da espécie está relacionada ao comportamento reprodutivo, pois assim como outras garoupas, a garoupa-verdadeira também apresenta agregações reprodutivas na forma de haréns, onde há várias fêmeas para cada macho (Zabala *et al.* 1997a,b).

A partir de todas essas peculiaridades da ecologia e ciclo de vida da espécie, juntamente com o aumento da pressão pesqueira sobre a espécie, a garoupa-verdadeira encontrasse na lista vermelha da IUCN, classificada como Em Perigo devido à redução da população (EN A2d) (Cornish & Harmelin-Vivien 2004).

A garoupa-verdadeira possui uma ampla distribuição, ocorrendo ao longo do Mar Mediterrâneo e em ambas as costas do Oceano Atlântico. Na costa leste do Oceano Atlântico distribui-se desde as Ilhas Britânicas até a África do Sul (Heemstra & Randall 1993), estendendo-se ao sudoeste do Oceano Índico (Fennessy 2006). Na costa oeste do Oceano Atlântico, a garoupa-verdadeira ocorre na costa brasileira do sul da

Bahia até ao sul da costa gaúcha, ocorrendo na costa uruguaia e estendendo-se até o Golfo Novo na Argentina (Figueiredo & Menezes 1980; Irigoyen *et al.* 2005; Condini *et al.* 2016a). A espécie está frequentemente associada a fundos rochosos habitando tocas e fendas entre rochas, sendo comum em águas costeiras, especialmente em recifes, parcéis e costões rochosos. Os adultos ocorrem, em maior densidade, em locais com até 50 m de profundidade, mas podem ser encontrados em profundidades de até 250 m (Bruslé 1985; Heemstra & Randall 1993), enquanto os juvenis podem ser comumente encontrados em poças de marés (Azevedo *et al.* 1995) e enseadas rasas na região estuarina (Condini *et al.* 2016b).

No Brasil, a garoupa-verdadeira é um dos peixes costeiros mais conhecidos da costa, sendo uma espécie emblemática, como demonstra sua escolha para ilustrar a cédula de 100 Reais (Escobar 2015). A garoupa-verdadeira é amplamente capturada pela pesca comercial e recreativa em toda sua distribuição na costa brasileira (Figueiredo & Menezes 1980). Ao longo das últimas décadas houve um declínio populacional, o que levou a espécie ao status de Vulnerável devido à redução da população (VU A2d) nas duas últimas listas de espécies ameaçadas da fauna brasileira (Instrução Normativa nº 5, de 21 de maio de 2004 e Portaria MMA nº 445, de 17 de dezembro de 2014). Há poucos estudos relacionados à conservação da espécie no Brasil, e a maioria dos estudos sobre a espécie se concentra na região sul, sendo estes restritos a estudos de biologia reprodutiva (Andrade *et al.* 2003; Seyboth *et al.* 2011; Condini *et al.* 2013, 2014a), estrutura de idade e crescimento (Seyboth *et al.* 2011; Condini *et al.* 2014b), além dos estudos de ecologia trófica (Machado *et al.* 2008; Begossi & Silvano 2008; Condini *et al.* 2011, 2015), pesca (Condini *et al.* 2007) e

comportamento (Gibran 2007; Condini *et al.* 2016b), havendo assim a necessidade de maiores informações sobre a espécie ao longo de toda a costa brasileira, para que se possa estruturar planos de manejo integrado de maneira eficiente.

Na costa do Rio Grande do Sul, a espécie ocorre de forma ocasional ao longo da faixa litorânea, provavelmente, devido ao fato de que os fundos arenosos que predominam nessa região não são habitats propícios para a espécie. No entanto, são comuns e abundantes em fundos rochosos (parcéis) localizados na região nerítica, tais como os Parcéis do Carpinteiro, Hermenegildo, Solidão e Torres, aonde são comumente pescadas (Cardoso & Haimovici 2011). Porém, as informações ainda são insuficientes para determinar o *status* da pesca e da vulnerabilidade da espécie na costa gaúcha. Apenas na última década, surgiram os primeiros trabalhos acerca da biologia e ecologia da espécie na região, primeiramente com grupos de indivíduos habitando a faixa litorânea (molhe oeste da Barra de Rio Grande) e mais recentemente na faixa nerítica (Parcel do Carpinteiro). Nessa região, já foram realizados estudos com a garoupa-verdadeira dos molhes da Barra acerca do perfil sócio econômico do pescador de garoupa (Condini *et al.* 2007), dieta e hábitos alimentares (Condini *et al.* 2011), bem como estrutura de idade e aspectos da biologia reprodutiva (Seyboth *et al.* 2011). Mais recentemente, essas pesquisas têm sido estendidas para a região nerítica (Parcel do Carpinteiro) aonde foram investigados aspectos da reprodução (Condini *et al.* 2014a), inversão sexual (Condini *et al.* 2013), estrutura de idade e crescimento (Condini *et al.* 2014b) e ecologia trófica (Condini *et al.* 2015).

Esses trabalhos permitiram conhecer aspectos fundamentais sobre a bioecologia da espécie, que eram desconhecidos para a região, tais como: as garoupas-verdadeiras da região litorânea apresentam indivíduos, em média, com menor tamanho e idade (Seyboth *et al.* 2011) quando comparado aos indivíduos da região nerítica (Condini *et al.* 2014b); as garoupas-verdadeiras que habitam a região litorânea não se reproduzem nesse ambiente (Seyboth *et al.* 2011), diferentemente do que ocorre na região nerítica (Condini *et al.* 2014a) Porém, questões importantes sobre o ciclo de vida, comportamento e o papel funcional dessa espécie no ecossistema precisam ser mais bem compreendidos para que seja possível subsidiar ações de conservação na costa gaúcha dessa espécie ameaçada. Por exemplo, a determinação de áreas reprodutivas de uma espécie é de fundamental importância para sua conservação, pois somente a partir do conhecimento de que áreas são responsáveis pela manutenção de cada um dos estoques, será possível planejar ações de conservação. Sendo assim, estudos sobre os fluxos de matéria e energia que sustentam a espécie na zona litorânea e nerítica, e seu papel trófico como predador, além de padrões de movimentos e de uso de hábitat são tópicos fundamentais para o planejamento de ações de conservação. Ainda, por se tratar de uma espécie de interesse econômico de grande porte, longa e que ocupa altos níveis tróficos nas cadeias as quais estão inseridas, informações sobre contaminações por poluentes, como metais pesados, são fundamentais para melhor entender a saúde da espécie e determinar se há risco de contaminação para o consumo humano.

1.3 Ecologia trófica

As informações sobre a ecologia trófica da garoupa-verdadeira, na maioria dos casos, estão restritas à descrição da dieta com base na análise de conteúdo estomacal (ACE) (Reñones *et al.* 2002; Linde *et al.* 2004; Machado *et al.* 2008; Condini *et al.* 2011). Estudos com dieta mostram que a garoupa-verdadeira apresenta um comportamento generalista, alimentando-se desde pequenos caranguejos e camarões a peixes e polvos de maior porte, apresentando ainda variação da dieta ao longo de sua ontogenia, predando preferencialmente caranguejo na sua fase juvenil, mudando para peixe e polvo na fase adulta (Reñones *et al.* 2002; Linde *et al.* 2004; Machado *et al.* 2008; Condini *et al.* 2011). Porém, poucos trabalhos utilizam técnicas mais inovadoras para avaliar os fluxos de matéria e energia na cadeia alimentar que sustenta essa espécie (Reñones *et al.* 2002). Uma dessas técnicas é a análise de isótopos estáveis (AIE), principalmente das razões isotópicas de carbono ($^{13}\text{C}/^{12}\text{C}$) e nitrogênio ($^{15}\text{N}/^{14}\text{N}$) (Fry 2006).

Isótopos são átomos de um mesmo elemento que possuem diferente número de nêutrons como, por exemplo, o carbono (^{12}C , ^{13}C) e o nitrogênio (^{14}N , ^{15}N), sendo esses os elementos mais comumente utilizados como marcadores naturais para inferir relações tróficas. As razões atômicas entre esses elementos ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$) podem ser utilizadas para identificar as fontes orgânicas desses materiais para os produtores e também para conhecer os caminhos subsequentes da matéria, na medida em que transitam entre os vários consumidores (Fry 2006). Ao contrário da ACE, a AIE indica o material que foi realmente assimilado pelo consumidor e, dependendo do tecido que

está sendo analisado, representa uma informação sobre o que foi consumido num intervalo de tempo maior (1-3 meses; Herzka & Holtz 2000). Vários autores sugerem que a AIE seja feita em combinação com a tradicional ACE, já que a combinação dos dois métodos fornece maior resolução e precisão nas estimativas (Winemiller *et al.* 2007; Davis *et al.* 2012). Assim, essa aplicação concomitante dos dois métodos permite investigar com maior profundidade e precisão os fluxos de energia e matéria que conectam os consumidores numa teia alimentar (Winemiller *et al.* 2007).

1.4 Uso de hábitat

Estudos sobre uso de hábitat e padrões de movimentos são essenciais para uma adequada avaliação da dinâmica populacional de peixes de grande porte e consequente adoção de medidas apropriadas de ordenamento e manejo da pesca (Andrello *et al.* 2014; Davoren *et al.* 2015). Com esse intuito, diversos estudos têm sido realizados para conhecer o uso de habitat, extensão de área de vida e de fidelidade ao local de ocorrência da garoupa-verdadeira, principalmente no Mar Mediterrâneo (Lembo *et al.* 1999, 2002; Spedicato *et al.* 2005; Pastor *et al.* 2009; Koeck *et al.* 2014; Afonso *et al.* 2016), sendo inexistente no Oceano Atlântico Sudoeste. Todos esses estudos têm sido realizados através de telemetria, não havendo estudos utilizando marcadores naturais para responder essas perguntas para a *E. marginatus*, como por exemplo, estudos através de química de otólitos. Estudos utilizando a análise química dos otólitos para inferir padrões de deslocamento e uso de

habitat têm sido amplamente utilizados em estudos com espécies de peixes (Hobson 1999; Elsdon *et al.* 2008; Sturrock *et al.* 2012).

Otólitos são estruturas basicamente constituídas por carbonato de cálcio, matéria orgânica e elementos traços (Campana 2001), situadas no ouvido interno dos peixes teleósteos que têm como principal função controlar o equilíbrio dos peixes (King 2007). Os otólitos são comumente utilizados para determinação da idade dos peixes (King 2007), porém, ao longo das últimas décadas os otólitos têm sido muito utilizados em estudos relacionados a migração, identificação de estoques, determinação de locais de nascimento e reconstruções de temperatura e salinidade do ambiente (Campana 1999). Isso é possível, devido ao fato de que à medida que o animal cresce, além de carbonato de cálcio e otolina, são depositados ao menos mais outros 40 elementos em pequenas concentrações em seus otólitos, que guardam uma relação com sua disponibilidade na água em que os peixes se desenvolvem (Fowler *et al.* 1995; Campana 1999). Além disso, após serem depositados nos otólitos, esses elementos não sofrem transformações ou modificações químicas ao longo do tempo, o que significa dizer que são quimicamente inertes (Campana & Neilson 1985). Os elementos apresentam concentrações que variam, dentro de uma mesma espécie, entre diferentes localidades, devido a variações ambientais tais como, temperatura e salinidade, associados a padrões migratórios e de alimentação, aos quais os indivíduos estão expostos dentro da sua distribuição (Newman *et al.* 2000; Ayvazian *et al.* 2004). Sendo assim, é possível determinar, através desses elementos químicos, informações sobre os ambientes e as condições químicas experimentadas pelo peixe que ficam registradas nos otólitos ao longo do desenvolvimento do indivíduo.

A análise é baseada na leitura das concentrações de elementos químicos no otólito de peixes teleósteos, sendo os mais difundidos o estrôncio (Sr) e bário (Ba). O Sr é um metal existente em maior concentração em ambiente marinho, e tem sido usado como ferramenta para estudos de migração de várias espécies (Vries *et al.* 2005). Sendo assim, peixes que habitam ambientes marinhos tendem a apresentar maiores concentrações de Sr, comparados a peixes que habitam ambientes estuarinos e ou límnicos. O Ba, ao contrário, é um indicador de ambientes de água doce, pois é mais abundante no ambiente límnicico do que marinho (Vries *et al.* 2005).

1.5 Contaminação por mercúrio

No ambiente aquático um dos contaminantes que oferecem maior risco a saúde humana e dos demais organismos devido sua alta toxicidade é o mercúrio (Hg) (WHO 1991). O mercúrio é um metal naturalmente presente no ambiente, porém em baixas concentrações (Bens *et al.* 1976). No entanto, atividades antropogênicas, como a mineração de ouro e prata, principalmente durante o período colonial até o século passado, intensificação da produção agrícola, farmacêutica e da indústria, são responsáveis pelo acúmulo desse metal no ambiente. O mercúrio apresenta alta capacidade de biomagnificação (Gray 2002), tornando-se assim extremamente perigoso à saúde humana e dos demais organismos (WHO 1991; Choy *et al.* 2002).

Peixes são considerados bons indicadores para a avaliação de poluição crônica, principalmente espécies territorialistas, como é o caso das garoupas, pois esses tendem a refletir as características do corpo d'água onde ocorre, tornando-se assim

reflexo da variabilidade ambiental local (Jahanbakht *et al.* 2002). As espécies predadoras de topo de cadeia estão sujeitas ao fenômeno da biomagnificação desses metais, ou seja, o acúmulo de contaminantes ao longo da cadeia trófica (Evers *et al.* 2009). O consumo de peixe tem sido positivamente correlacionado com o aumento de níveis de mercúrio em seres humanos (Choy *et al.* 2002), e o consumo de peixes contaminados tem sido a principal fonte de contaminação humana por mercúrio nos EUA (Tremain & Adams 2012). Como o mercúrio sofre biomagnificação, os peixes carnívoros acumulam maior quantidade de mercúrio comparado às espécies onívoras e herbívoras (Zhou & Wong 2000), sendo assim, as garoupas têm sido alvo de preocupação em estudos nos EUA e em águas internacionais (Tremain & Adams 2012), pois em muitos locais as garoupas têm apresentados valores acima do limite aceitável pela Organização Mundial da Saúde (WHO, 1991). Entretanto, esses estudos têm sido realizados exclusivamente considerando as 'garoupas' de modo geral (Sunderland 2007), havendo a necessidade de realizar estudos espécie-específicos visando aprimorar a aplicabilidade desses resultados em medidas de conservação da espécie e segurança alimentar para consumo humano.

2 Objetivos

A presente tese teve como objetivos principais investigar a ecologia trófica, padrões de movimento, uso de hábitat e contaminação por mercúrio da garoupa-verdadeira (*Epinephelus marginatus*) entre as regiões litorânea e nerítica no extremo sul do Brasil.

Objetivos Específicos:

2.1 Revisar o estado atual de conhecimento da bioecologia da garoupa-verdadeira, identificando lacunas de conhecimento e tópicos promissores em pesquisas futuras (Anexo I).

2.2 Determinar os elos tróficos dominantes que sustentam energeticamente à garoupa-verdadeira (*Epinephelus marginatus*) na região litorânea e nerítica da costa gaúcha, bem como sua posição trófica em ambos os ambientes (Anexo II).

2.3 Determinar o padrão de uso de habitat neríticos e litorâneos da garoupa-verdadeira (*Epinephelus marginatus*) na costa gaúcha (Anexo III).

2.4 Determinar a concentração de mercúrio total na garoupa-verdadeira (*Epinephelus marginatus*) nas regiões litorânea e nerítica da costa gaúcha e ao longo de seu incremento em tamanho (Anexo IV).

3 Material e Métodos

3.1 Áreas de Estudo

A presente tese apresenta duas áreas de estudo: os molhes da Barra de Rio Grande (MB) (32°09'S; 052°05'O) e o Parcel do Carpinteiro (PC) (32°16'S; 051°47'O), as quais são referidas como região litorânea e nerítica, respectivamente. Os molhes foram construídos no início do século XX e possuem atualmente cerca de 4,5 km de extensão. Além da sua importância econômica, os molhes constituem um importante habitat rochoso para uma diversa fauna bentônica (Capítoli 1996) e área de desova para algumas espécies de crustáceos (Alaniz *et al.* 2011). Por estar localizado na desembocadura do estuário da Lagoa dos Patos (ELP), sofre influência da descarga continental, apresentando variação temporal em parâmetros ambientais como a salinidade (PELD-ICTIO 2005-2009: Média: 26,9; DP: 7,5; Min-Máx: 9,1-35,1). O PC é um promontório submarino composto por rochas praias altamente cimentados por carbonato de cálcio recristalizado e superficialmente colonizado por briozoários, esponjas, crustáceos e poliquetas (Buchmann *et al.* 2001). O PC está situado entre as isóbatas dos 20 e 30 m e aproximadamente 40 km distante do litoral (Abreu & Calliari 2005), sendo pouco influenciado pela pluma do ELP, apresentando salinidade média pouco variável ao longo do ano (Osmar Moller; dados não publicados: 2002-2007; Média: 32,9; DP: 2,1; Min-Máx: 26,8-36,6).

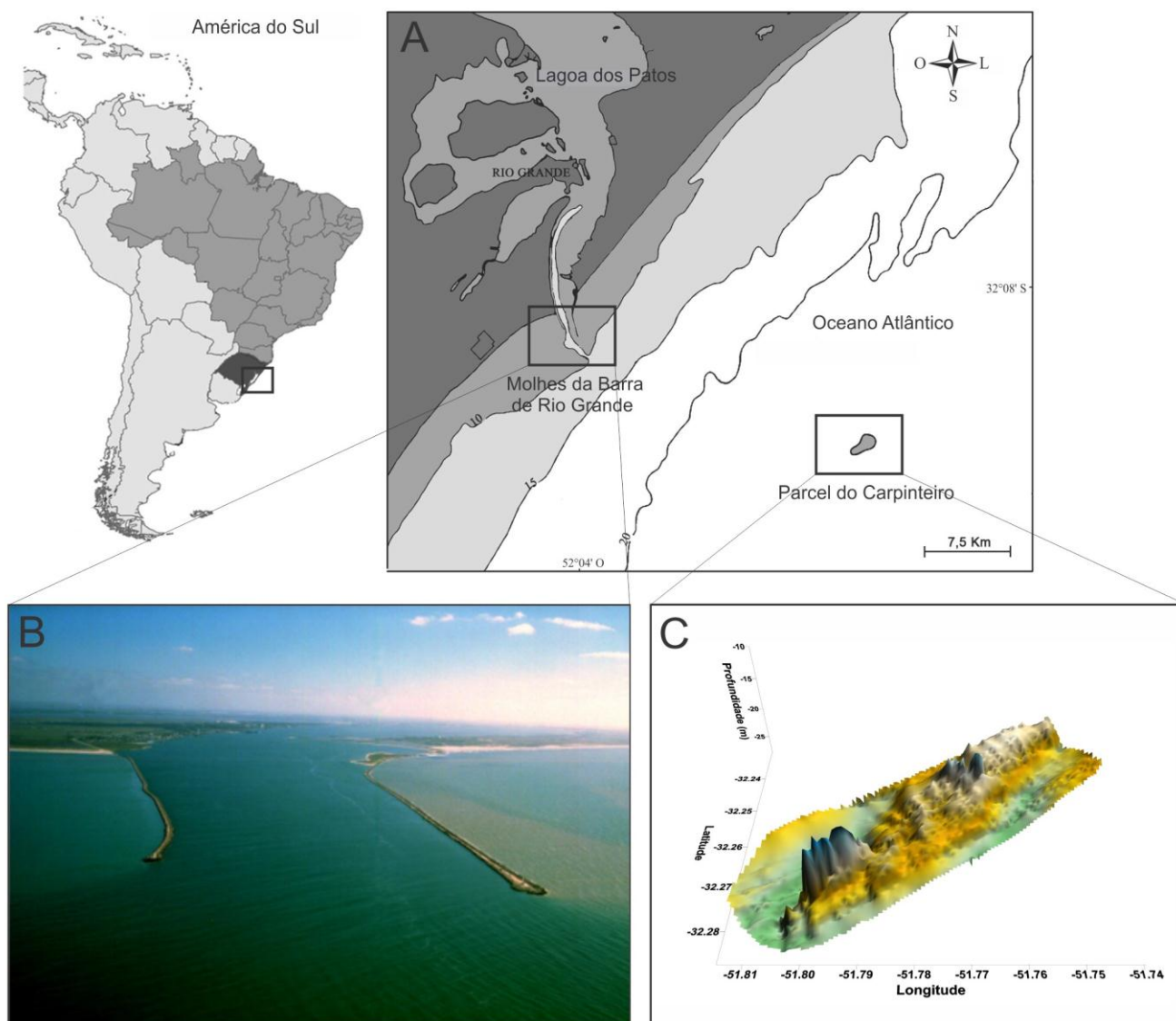


Figura 2 – Região estuarina da Lagoa dos Patos (10.360 km²) na região sul do Rio Grande do Sul (A), apresentando as áreas de estudo: molhes da barra de Rio Grande (B) na região litorânea e Parcel do Carpinteiro na região nerítica adjacente (C). Linhas ao longo da costa (A) indicando as isóbatas de 10, 15 e 20 m, respectivamente.

3.2 Amostragem

Os indivíduos da região litorânea foram obtidos entre janeiro 2008 e abril de 2009 junto aos pescadores artesanais de Rio Grande, enquanto que os indivíduos da região nerítica foram adquiridos junto à frota pesqueira de Rio Grande, entre o período de janeiro de 2010 e maio de 2011, com oito indivíduos coletados entre 2008 e 2009. As garoupas-verdadeiras foram congeladas para posterior processamento. Esses indivíduos (n=133 da região litorânea e n=215 da região nerítica) foram utilizados nas análises dos objetivos 2.2, 2.3 e 2.4. Adicionalmente para o objetivo 2.2, foram coletados, em dezembro de 2010, componentes representativos das teias tróficas na região litorânea e nerítica como: potenciais presas consumidas pela garoupa-verdadeira, outros predadores (peixe-espada, anchova, corvina, entre outros) e fontes alimentares basais (*e.g.* séston) para análise de isótopos estáveis. A escolha desses organismos foi baseada em trabalhos anteriores sobre a dieta da espécie na região litorânea (Condini *et al.* 2011) e em outras regiões da sua distribuição (Reñones *et al.* 2002; Linde *et al.* 2004; Machado *et al.* 2008).

3.3 Revisão científica

Para a realização da revisão científica, foram utilizados apenas artigos científicos publicados em revistas com corpo editorial e comitê revisor, obtidos através de banco de dados especializados, tais como: *Web of Science* (<http://apps.webofknowledge.com/>), *Science Direct* (<http://www.sciencedirect.com/>) e *Google Scholar* (<http://scholar.google.com/>). As pesquisas nas três plataformas

ocorreram a partir dos termos: nome popular da espécie (em inglês), nome científico atual e antigos nomes científicos, tais como: “*dusky grouper*”, “*Epinephelus marginatus*”, “*Epinephelus guaza*”, “*Mycteroperca marginata*”, “*Epinephelus marginatus*”, “*Serranus marginatus*”, “*Perca gigas*”, “*Epinephelus gigas*”, “*Serranus gigas*” e “*Serranus guaza*”. O período compreendeu até julho de 2016. Publicações consideradas como ‘literatura cinza’ (e.g.. relatórios técnicos, boletins, dissertações, monografias) não foram incluídas.

3.4 Ecologia trófica

A ecologia trófica foi estudada a partir da combinação dos métodos de análises de isótopos estáveis (AIE) e das análises de conteúdos estomacais (ACE). Para a AIE foram analisadas 72 garoupas-verdadeiras da região litorânea e 190 da região nerítica, além de 157 espécies de potenciais presas consumidas pela garoupa-verdadeira em ambas as áreas de estudo. Já para ACE foi analisado um total de 348 indivíduos de garoupa-verdadeira (133 do litoral e 215 do nerítico), os quais foram coletados ao longo de todas as estações do ano e em diferentes classes de tamanho.

3.4.1 Análises de Isótopos Estáveis (AIE)

Em laboratório, foram extraídos aproximadamente 5 g de tecido de cada indivíduo amostrado, nos peixes foram coletados tecidos da região antero-dorsal, nos crustáceos tecido da região abdominal, para os bivalves foi coletado o músculo adutor,

dos cefalópodes foram coletados tecido dos tentáculos, os poliquetas foram processados por inteiro assim como o zooplâncton, os quais foram identificados e separados em três grandes grupos. Após a coleta dos tecidos, as amostras foram cuidadosamente inspecionadas para remover tecidos ou estruturas (por exemplo, ossos ou escamas no tecido de peixe) que não fossem o tecido alvo de interesse (i.e., músculo). Em seguida, as amostras foram lavadas com água destilada, colocadas em placas de Petri esterilizadas, e levadas ao forno a temperatura constante de 60 °C, por no mínimo 48 h, ou até estarem inteiramente desidratadas. As amostras secas foram moídas a um pó fino com um auxílio de gral e pistilo e, posteriormente, foram armazenadas em frascos de vidro esterilizados com solução de HCl.

As amostras foram pesadas em balança analítica de precisão (entre 1 e 3 mg de tecido por amostra), e armazenadas em cápsulas de estanho ultra pura (*Costech Analytical*, Valencia, Califórnia). As cápsulas foram armazenadas em bandejas plásticas apropriadas e enviadas ao Laboratório de Química Analítica do Instituto de Ecologia da Universidade de Geórgia (EUA) para determinação das razões isotópicas de carbono ($^{13}\text{C}/^{12}\text{C}$) e nitrogênio ($^{15}\text{N}/^{14}\text{N}$).

Os resultados foram expressos em notação de delta (partes por mil de desvio a partir de um padrão correspondente): $\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, onde $R = ^{13}\text{C}/^{12}\text{C}$ ou $^{15}\text{N}/^{14}\text{N}$. O padrão de carbono foi *Pee Dee Belemnite* (PDB) e o padrão de nitrogênio foi o nitrogênio atmosférico. Com base no desvio padrão dos padrões internos, a precisão analítica foi de 0,08‰ e 0,07‰ para o $\delta^{13}\text{C}$ e para $\delta^{15}\text{N}$, respectivamente.

Amostras com elevados teores de lipídeos podem afetar os valores de $\delta^{13}\text{C}$ e a extração de lipídeos ou normalização matemática podem ser usadas para controlar este efeito (Jardine *et al.* 2003; Post *et al.* 2007). Amostras com menos de 5% de teor de lipídeos, o que corresponde a uma relação C:N inferior a 3,5, tem um efeito negligenciável sobre os valores de $\delta^{13}\text{C}$ e não precisam ser corrigidas (Post *et al.* 2007). No entanto, a extração de lipídeos pode causar efeitos indesejados nos valores de $\delta^{15}\text{N}$, principalmente quando a concentração de conteúdo lipídico é baixa (Ingram *et al.* 2007). Sendo assim, como a maioria das amostras (> 75%) apresentaram baixa concentração de conteúdo lipídico (C:N < 3,5) não foram feitas extrações de lipídeos. A maioria das amostras que apresentaram razão C:N > 3,5 foram presas que não estavam incluídas na dieta da garoupa.

3.4.2 Análise de conteúdo estomacal (ACE)

Em laboratório, cada exemplar de garoupa-verdadeira foi medido (comprimento total, CT, mm), pesado (peso total, g) e eviscerado para extração do estômago, o qual foi fixado em formaldeído 10% por um período de 7 dias e, posteriormente, conservado em álcool 70% até sua análise. Os itens encontrados em cada estômago foram identificados até o menor nível taxonômico possível. Os itens encontrados nos estômagos foram agrupados em cinco categorias: peixes, camarões, caranguejos (todos os anomuras e brachyuras, exceto os portunídeos), siri (todos os portunídeos) e moluscos. A distinção entre “siri” e “caranguejo” deu-se devido à diferença significativa entre os tamanho e peso das espécies desses dois grupos.

3.5 Análises químicas dos otólitos

Os otólitos foram emblocados e cortados em serra metalográfica de acordo com Condini *et al.* (2014b) e analisados em um sistema 'Laser Ablation Inductively Plasma Mass Spectrometer' (LA-ICPMS) composto por um laser modelo CETAC LSX 100 e um ICPMS modelo ELAN 6000, PerkinElmer – SCIEX, no Laboratório de Espectrometria de Massas da PUC-RJ. O princípio desta técnica baseia-se na remoção de partes da superfície dos otólitos através de ablação a laser e na medição das concentrações de alguns elementos presentes nesse material removido. Foram analisados estrôncio, bário, cálcio, magnésio e manganês para a medição de suas massas relativas e absolutas. O laser foi ajustado para prover uma espessura de ablação de aproximadamente 20 μm , que permitiu uma resolução temporal variável conforme a espessura dos anéis de crescimento presentes nos otólitos da garoupa-verdadeira. Foram realizados perfis de ablação desde o núcleo (assinatura referente a origem de sua vida) até a borda (assinatura referente ao ambiente onde foi capturado) de cada otólito, de forma que todo seu eixo principal de crescimento foi analisado.

3.6 Análises de concentração de mercúrio total

A mesma metodologia utilizada para o processamento das amostras de isótopos estáveis (supracitado) foi utilizada para processar as amostras de mercúrio no tecido da garoupa-verdadeira, visto que os tecidos utilizados aqui foram sub-amostras do material utilizado para a análise de isótopos estáveis.

As amostras para análise de mercúrio total (HgT) foram pesadas em balança analítica de precisão (aproximadamente 0,3 mg peso seco de tecido por amostra), sendo 68 amostras da região litorânea e 176 da nerítica. As análises da concentração de HgT no tecido muscular dos indivíduos foram realizadas com um analisador direto de mercúrio (*Direct Mercury Analyser* - DMA-80, Milestone Inc.) no Departamento de Ciências Biológicas e Instituto Aplicado a Ciência na Universidade do Norte do Texas, EUA, usando decomposição térmica, fusão de ouro e espectrometria de absorção atômica (USEPA 1998).

A calibração das curvas geradas utilizando três materiais de referencia do Conselho Nacional de Pesquisa do Canada: MESS-3 (sedimento marinho: valor certificado = 91 ± 9 ng de HgT\g de peso seco), TORT-2 (hepatopâncreas de lagosta: valor certificado = 270 ± 60 ng\g), e DOLT-4 (tecido de fígado de *dogfish*: valor certificado = 2580 ± 220 ng\g). Para garantir a qualidade das amostras, foram incluídas amostras “branco” e amostras duplicadas. A amostra “branco”, nada mais foi do que amostras vazias (barcos de amostras sem material), as quais foram analisadas a cada 20 amostras, com uma média Hg entre $0,0004 \pm 0,0005$ mg/kg (variação = 0 - 0,0015 mg/kg, n = 22). As amostras duplicadas também foram analisadas a cada 20 amostras, mas com uma diferença relativa média de $3,69 \pm 2,85\%$ (variação = 0,41 - 9,36%, n = 15). Enquanto que as amostras de referência (MESS-3, TORT-2 e DOLT-4) foram analisadas a cada 10 amostras: MESS-3 $101,35 \pm 3,54 \%$ (n=8), TORT-2 $110,01 \pm 13,63 \%$ (n = 8) e DOLT-4 $98,90 \pm 2,44 \%$ (n=8).

3.7 Análises estatísticas dos dados

3.7.1 Ecologia trófica

3.7.1.1 Isótopos estáveis: Foram utilizados *bi-plots* dos valores de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ das garoupas e demais fontes alimentares para a visualização do padrão de variação isotópica dentre e entre locais. Análise de variância de uma via (ANOVA), com comparações de pares usando o teste de Tukey, foi usada para comparar valores de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ da garoupa entre locais e classes de tamanho.

Para identificação e a quantificação da importância relativa das presas como fonte de energia para as garoupas-verdadeiras, foi utilizado o modelo de mistura isotópico Bayesiano, SIAR (*Stable Isotope Analysis in R* versão 3.0.1; Parnell *et al.* 2010). Esse modelo permite a inclusão dos valores das razões isotópicas ($\delta^{13}\text{C}$ e $\delta^{15}\text{N}$), concentração elementar (%C, %N) e fracionamento isotópico, juntamente com a incerteza (desvio padrão) desses valores (Parnell *et al.* 2010). Considerando a forte influência que esses fatores podem ter sobre os resultados do modelo (Bond & Diamond 2011), bem como a variabilidade conhecida no enriquecimento entre os grupos tróficos, tipos de tecido analisados e outros fatores (Caut *et al.* 2009) optamos por valores mais conservadores, assim os valores de fracionamento isotópico utilizados foram de $1,0 \pm 0,5\%$ para $\delta^{13}\text{C}$ e $3,0 \pm 0,5\%$ para $\delta^{15}\text{N}$, incluindo assim uma gama de estimativas de enriquecimento e variabilidade de diferentes categorias tróficas (Post 2002; Vanderklift & Ponsard 2003). O modelo de mistura foi calculado por classe de tamanho e por local, e para que o modelo fosse mais fiel a realidade da espécie, foram incluídas apenas presas em potencial determinadas por estudos prévios com a espécie

(Reñones *et al.* 2002; Linde *et al.* 2004; López & Orvay 2005; Machado *et al.* 2008; Condini *et al.* 2011).

3.7.1.2 Dieta: Os itens ou categorias alimentares encontrados nos estômagos das garoupas analisadas foram quantificados utilizando os seguintes parâmetros: (a) frequência de ocorrência (%F), que representa o número de estômagos não vazios (em porcentagem) que um determinado item ou categoria alimentar foi encontrado; (b) abundância numérica (%N), representa o número total (em porcentagem) de um determinado item ou categoria alimentar em relação ao número total de itens ou categorias alimentares encontrados em todos os estômagos não vazios; (c) abundância em massa (%W), representa o massa total (em porcentagem) de um determinado item ou categoria alimentar em relação ao número total de itens ou categorias alimentares encontrados em todos os estômagos não vazios (Hyslop 1980). Esses parâmetros foram utilizados para calcular o índice de importância relativa (%IRI), proposto por Pinkas *et al.* (1971): $\%IRI = \%F * [\%N + \%W]$.

Para avaliar a variabilidade da dieta da garoupa-verdadeira ao longo de seu incremento em tamanho, os indivíduos foram agrupados em quatro classes de tamanho: <351 mm CT; 351-500 mm CT; 501-650 mm CT; >650 mm CT. Foi calculado o %IRI de todas as categorias para as quatro classes de tamanho para ambas as áreas de estudo. Foi usada uma análise multivariada NMDS (*Non-Metric Multidimensional Scaling*), calculada pelo índice de Bray-Curtis, para comparar a composição e biomassa relativa dos itens da dieta da garoupa-verdadeira entre locais e classes de tamanho.

Para comparação entre o tamanho médio e biomassa das presas, entre local e classe de tamanho, foi utilizado uma análise de variância de uma via (ANOVA), com comparações de pares usando o teste de Tukey. Os testes de normalidade e homogeneidade de variância foram sempre avaliados quando necessário, utilizando testes de Kolmogorov-Smirnov e Cochran, (Zar 1994), respectivamente. Para essa análise, os dados foram log-transformados antes da análise para atender os pressupostos estatísticos.

3.7.2. Químicas dos otólitos

Análise de variância de uma via (ANOVA) foi usada para testar possíveis diferenças entre as concentrações dos elementos analisados entre núcleo e borda dos otólitos, entre a região litorânea e nerítica, bem como ao longo do incremento de tamanho da garoupa. Foram usadas comparações de pares usando o teste de Tukey quando necessário.

3.7.3. Concentração de mercúrio

Regressões lineares foram utilizadas para descrever as relações entre tamanho, peso e idade da garoupa-verdadeira por concentração de mercúrio total (mg/kg peso seco). Análise de variância de uma via (ANOVA) foi usada para testar possíveis diferenças nos níveis de concentração de mercúrio entre: tamanho, sexo, regiões (litorânea e nerítica) e ao longo do incremento de tamanho da garoupa-verdadeira. Foram usadas comparações de pares usando o teste de Tukey quando necessário.

A relação comprimento-peso da garoupa-verdadeira foi determinada pela equação $PT = a \times CT^b$ para ambos os locais, onde 'a' e 'b' são coeficientes de regressões.

4. Síntese dos Resultados

4.1 Capítulo 1 - Revisão sobre ecologia, biologia e comportamento da garoupa-*Epinephelus marginatus* (Lowe 1834). (ANEXO I - *A review of ecology, biology and behavior of dusky grouper Epinephelus marginatus* (Lowe 1834))

Nesse capítulo foi possível fazer uma síntese dos diversos estudos sobre a ecologia, biologia, comportamento, pesca e conservação da garoupa-verdadeira *Epinephelus marginatus*, espécie ameaçada de extinção a qual apresenta grande relevância ecológica e econômica. A revisão científica da literatura analisou apenas artigos científicos nos quais a garoupa-verdadeira *E. marginatus* era considerada a espécie alvo do estudo. Foi possível encontrar 169 trabalhos publicados entre 1969 e 2016, os quais foram distribuídos em 87 diferentes periódicos nacionais e internacionais. Ao longo da revisão científica foi possível identificar um conhecimento substancial sobre alguns temas, como: ecologia alimentar, biologia reprodutiva e estrutura etária ao longo de sua distribuição geográfica. No entanto, estudos relacionados a comportamento e padrões de uso do habitat estão restritos a região do Mar Mediterrâneo, sendo quase que inexistente nos Oceanos Atlântico e Índico. Mesmo com uma quantidade significativa de estudos sobre a espécie, ainda existem diversas questões em aberto ou com respostas insipientes, tais como: (i) os locais e as condições específicas em que as agregações reprodutivas ocorrem; (ii) padrões de

dispersão de ovos e larvas e seus padrões de conectividade com os estoques de adultos e colonização de fundos rochosos; (iii) requisitos de habitat ao longo do ciclo de vida da espécie (com base em modelos de habitats e nichos em várias escalas espaciais); (iv) esforço pesqueiro atual empregado ao longo de sua distribuição; (v) eficácia dos planos e medidas de conservação da espécie; (vi) níveis de contaminação por metais pesados. Também foi possível sugerir novos focos de estudo e áreas de investigação sobre a bioecologia da garoupa-verdadeira a fim de subsidiar da melhor forma possível os tomadores de decisão a criarem leis e normas que protejam essa espécie ameaçada.

4.2 Capítulo 2 - Ecologia trófica da garoupa-verdadeira *Epinephelus marginatus* (Actinopterygii, Epinephelidae) na região litoral e nerítica do sul do Brasil, elucidada por conteúdo estomacal e isótopos estáveis. (ANEXO II - *Trophic ecology of dusky grouper *Epinephelus marginatus* (Actinopterygii, Epinephelidae) in littoral and neritic habitats of southern Brazil as elucidated by stomach contents and stable isotope analyses*)

O presente capítulo investigou a ecologia alimentar da garoupa-verdadeira através da integração de duas metodologias, análises de conteúdo estomacal (ACE) e análises de isótopos estáveis (AIE). Foram analisados 348 indivíduos, 133 da região litorânea e 215 da região nerítica, das quais 78 e 113, respectivamente, apresentaram conteúdo alimentar em seus estômagos. A ACE revelou variação ontogenética da dieta das garoupas-verdadeiras em ambos os locais, sendo que na região litorânea houve

predomínio de pequenos caranguejos nas três classes de tamanho analisados (<351, 351-500 e 501-650), porém com decréscimo de importância relativa dessa presa ao longo do incremento em tamanho da garoupa-verdadeira, e aumento da importância de presas maiores como siris e peixes. Enquanto que as garoupas-verdadeiras da região nerítica também apresentaram predomínio de pequenos caranguejos nas três primeiras classes de tamanho analisados (<351, 351-500 e 501-650), porém a maior classe de tamanho analisada (>650) foi dominada por peixes.

Para a AIE, as razões isotópicas de carbono ($\delta^{13}\text{C}$) e nitrogênio ($\delta^{15}\text{N}$) foram analisadas em amostras de músculo de 262 garoupas (n= 72 litorânea e n= 190 nerítica) além de 157 amostras (n= 58 litorânea e n= 99 nerítica) de diversos taxa possivelmente consumidos pelas garoupas. Em contraste com os dados da dieta, as razões isotópicas de carbono e nitrogênio das garoupas-verdadeiras apresentaram pequenas diferenças entre locais e entre as classes de tamanho. Os modelos de mistura apresentaram padrões diferentes dos observados nos conteúdos estomacais. Para as garoupas da região litorânea, a técnica determinou que peixe foi o item mais assimilado pela garoupa-verdadeira ao longo das classes de tamanho analisadas (<351, 351-500 e 501-650). Enquanto que as presas camarão e peixe foram as presas mais assimiladas pela garoupa-verdadeira na região nerítica. Nessa região, camarão diminui e peixe aumenta de importância na dieta à medida que a garoupa aumentou de tamanho. Essa diferença entre os resultados encontrados pelas duas técnicas (ACE e AIE), provavelmente, está relacionada ao maior tempo de digestão do material refratário (e.g. carapaça de caranguejo) encontrado no estômago das garoupas, comparado com o tempo de digestão de peixes e camarões.

4.3 Capítulo 3 - Uso de habitat prolongado pela *Epinephelus marginatus* em latitudes subtropical revelado por microquímica de otólito. (ANEXO III - *Prolonged estuarine habitat use by dusky grouper Epinephelus marginatus at subtropical latitudes revealed by otolith microchemistry*)

O presente capítulo avaliou o padrão de uso de habitat da garoupa-verdadeira através da análise de microquímica de otólitos. Foram analisados 72 indivíduos, sendo 28 da região litorânea e 44 da nerítica, as quais variaram entre 2 e 12 anos e 2 e 40 anos, respectivamente. Quando analisados quimicamente ($^{138}\text{Ba}:$ ^{43}Ca e $^{87}\text{Sr}:$ ^{43}Ca) a região do núcleo e da borda dos otólitos das garoupas de ambos os locais, não foram encontradas diferenças significativas, sendo assim, as amostras foram analisadas como um todo. Através das análises da razão $^{138}\text{Ba}:$ ^{43}Ca , foi possível determinar, pela primeira vez para a espécie, a entrada e permanência de espécimes juvenis em região estuarina. Foi possível ainda determinar três padrões bem marcados de uso de habitat da garoupa, principalmente durante sua fase juvenil: a) a maioria dos indivíduos analisados (> 80%) mostrou o uso exclusivo de águas marinhas ao longo da sua história de vida; b) cerca de 10% dos espécimes analisados indicaram que a garoupa-verdadeira, em sua fase juvenil, apresenta incursões e residência temporária na região estuarina, porém num curto período de permanência, provavelmente, menor que um ano; c) por fim, 5% das garoupas, em sua fase juvenil, apresentaram entradas na região estuarina da Lagoa dos Patos, permanecendo longos períodos, de até dois anos, sendo esse registro inédito para a espécie ao longo de sua distribuição global.

4.4 Capítulo 4 - Concentração de mercúrio na garoupa-verdadeira *Epinephelus marginatus* em ambientes litoral e nerítico ao longo da costa sudoeste brasileira. (ANEXO IV - *Mercury concentrations in dusky grouper Epinephelus marginatus in littoral and neritic habitats along the Southern Brazilian coast*)

Esse capítulo incorporou um conjunto abrangente de parâmetros (i.e., tamanho do corpo, idade, estágio de maturação sexual, dieta e ecologia trófica) para investigar a concentração de mercúrio na garoupa-verdadeira, espécie longeva de grande porte e altamente consumida pelo homem. Foi ainda determinada a variação espacial das concentrações de mercúrio em indivíduos que habitam as zonas litorâneas e neríticas no extremo sul do Brasil. Um total de 244 garoupas-verdadeiras (n = 68 litoral, n = 176 nerítica) foi analisado para determinar as concentrações de mercúrio total. Indivíduos de mesma classe de tamanho da região litorânea apresentaram maiores concentrações de mercúrio total, quando comparado com os indivíduos que habitam a região nerítica. Isso ocorreu, provavelmente, devido à proximidade da região litorânea com as fontes de poluição associados com as atividades humanas no estuário da Lagoa dos Patos e sua bacia de drenagem. Também foi determinado que os indivíduos maiores e mais velhos (>650 mm e >8 anos de idade) apresentaram níveis de contaminação por mercúrio acima dos limites estipulados pela Organização Mundial da Saúde, apresentando assim risco a saúde dos peixes e a saúde humana, quando consumidos. Estudos para determinar as principais fontes de contaminação de mercúrio na área de estudo se fazem premente para melhor entender as rotas de contaminação de mercúrio nessa espécie ameaçada.

5. Conclusões dos capítulos

A partir da presente tese pode-se concluir que:

- Existe um elevado número de artigos científicos (169 ate 2016) sobre a ecologia, biologia, comportamento, pesca e conservação da garoupa-verdadeira *Epinephelus marginatus*, porém ainda existem lacunas importantes sobre o conhecimento da bioecologia da espécie (Anexo I).
- As principais lacunas no conhecimento da espécie estão relacionadas as suas agregações reprodutivas, dispersão de ovos e larvas e conectividade populacional, uso de habitat ao longo do ciclo de vida, esforço pesqueiro atual empregado ao longo de sua distribuição, eficácia dos planos e medidas de conservação da espécie e níveis de contaminação por metais pesados (Anexo I).
- A garoupa-verdadeira apresenta comportamento predatório generalista com uma ampla diversidade de presas (pequenos caranguejos, siri, peixes, camarões, moluscos) na sua dieta em ambas as áreas de estudo, porém a espécie assimila nos seus tecidos musculares predominantemente peixes e camarões (Anexo II).
- A garoupa-verdadeira alimenta-se de presas maiores à medida que cresce em tamanho, mudando de pequenos crustáceos para presas maiores como peixes (Anexo II).
- É um predador generalista de elevada posição trófica que parece integrar vias tróficas bentônicas e pelágicas nos ecossistemas em que habita (Anexo II).

- Não foram encontrados diferenças nas razões de $^{138}\text{Ba}:^{43}\text{Ca}$ e $^{87}\text{Sr}:^{43}\text{Ca}$ nas análises de borda dos otólitos da garoupa-verdadeira entre as regiões litorânea e nerítica (Anexo III).
- A análise da razão $^{138}\text{Ba}:^{43}\text{Ca}$ determinou três diferentes padrões de uso de habitat da garoupa-verdadeira no sul da costa do Rio Grande do Sul (Anexo III).
- Alguns indivíduos jovens da garoupa-verdadeira podem permanecer períodos prolongados (2-3 anos) em ambientes estuarinos, mostrando que a espécie apresenta maior plasticidade no uso de habitats, especialmente nos primeiros anos de vida, do que registrado até então na literatura (Anexo III).
- Os indivíduos de grande porte (> 650 mm CT) de garoupa-verdadeira apresentaram níveis de contaminação de mercúrio acima do aceitável pela Organização Mundial e Saúde (Anexo IV).
- As garoupas-verdadeiras da região litorânea apresentaram maior concentração de mercúrio total, quando comparada aos indivíduos de mesma classe de tamanho oriundos da região nerítica (Anexo IV).
- O consumo de peixe (principalmente de espécies estuarino-dependente) é o elo trófico mais provável por onde ocorre a contaminação por mercúrio, especialmente para as garoupas-verdadeiras da região nerítica (Anexo IV).

6. Considerações finais

Escolhida para ilustrar a nota de 100 reais, a garoupa-verdadeira é uma espécie emblemática da fauna brasileira, sendo um dos peixes mais conhecidos de nossa costa, sendo considerada ameaçada tanto no Brasil como no exterior. A revisão da literatura efetuada na presente tese (Anexo I) revela que vários aspectos da sua bioecologia vêm sendo investigados em toda a sua área de distribuição desde o final da década de 60. No Brasil, as pesquisas sobre essa espécie têm avançado especialmente nos últimos 10 anos. Porém, ainda existem diversas lacunas ou respostas insipientes sobre vários aspectos da bioecologia dessa espécie, especialmente acerca das suas agregações reprodutivas, dos padrões de dispersão de ovos e larvas, conectividade entre populações e uso de habitat ao longo da vida (Anexo I). Do ponto de vista da conservação, causa especial preocupação a ausência de monitoramento do esforço pesqueiro na captura da espécie ao longo de praticamente toda a sua distribuição. Além das preocupações com a pesca intensiva e predatória que ameaçam a espécie, a presente tese revelou que a espécie está sujeita a contaminação por mercúrio, especialmente os indivíduos adultos de maior porte, que são o alvo usual das pescarias (Anexo IV). A contaminação por metais pesados constitui risco não apenas a manutenção das populações de garoupas-verdadeiras, mas também as populações humanas que consomem essa espécie. O fato de a espécie ser um predador carnívoro que ocupa uma posição trófica elevada no ecossistema (Anexo II) potencializa a transferência de contaminantes como o mercúrio através da cadeia alimentar (fenômeno conhecido como biomagnificação), impondo riscos os consumidores no topo das cadeias alimentares, como o Homem. Além disso, o fato da espécie habitar

ambientes litorâneos junto a desembocadura de estuários, incluindo períodos de permanência prolongada no interior dos estuários por alguns indivíduos (Anexo III), favorece a exposição da espécie a pesca artesanal e esportiva (*e.g.*, nos Molhes da Barra de Rio Grande) e aumenta a proximidade com fontes de poluição associadas a atividades urbana e industrial. Nesse contexto, a presente tese chama a atenção sobre a necessidade premente de medidas que visem a conservação da garoupa-verdadeira no extremo sul do Brasil. Dentre as várias medidas possíveis, a presente tese sugere aos órgãos responsáveis: 1) que a região do Parcel do Carpinteiro torne-se uma área de proteção ambiental, visto que essa é uma importante região para o ciclo reprodutivo da espécie, 2) que a pesca da garoupa-verdadeira ocorra de forma ordenada, onde apenas pescadores artesanais, devidamente registrados, possam realiza-la, e de forma sustentável, como descrita na portaria 445 do MMA, 3) conscientizar a Sociedade sobre os perigos que o consumo de garoupa-verdadeira pode causar devido sua contaminação por mercúrio total.

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8. Anexo I

A review of ecology, biology and behavior of dusky grouper *Epinephelus marginatus* (Lowe 1834)

Mario V. Condini^{1*}, José A. García-Charton², Alexandre M. Garcia¹

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A review of ecology, biology and behavior of dusky grouper *Epinephelus marginatus*

(Lowe 1834)

Mario Vinicius Condini · José Antonio García-Charton · Alexandre Miranda Garcia

M. V. Condini (corresponding author) · A. M. Garcia

Laboratory of Ichthyology, Institute of Oceanography, Federal University of Rio

Grande, Av. Itália Km 8, Carreiros, Rio Grande, RS 96.201-900, Brazil

e-mail: mvcondini@gmail.com

J. A. García-Charton

Departamento de Ecología e Hidrología, Universidad de Murcia, Campus de Espinardo,

30100 Murcia, Spain

Abstract

We conducted a literature review on bio-ecological aspects and conservation status of the endangered dusky grouper *Epinephelus marginatus*. Dusky grouper is a large-bodied marine species usually associated with rocky bottoms and reefs. This species plays an important functional role in rocky bottoms in which they inhabit, usually occupying the highest trophic levels among the fish fauna. They have a complex life cycle characterized by high longevity, slow growth rates and a monandric protogynous hermaphroditic mode of reproduction. Aside its ecological importance, this species has high commercial value for professional fishermen and is highly prized in spearfishing. These biological features associated with increasing fishing pressure along its geographical distribution led *E. marginatus* to be included since 2004 in the IUCN Red List as Endangered (ENa2d). Despite a great number of research conducted on this species in the last five decades using several tools and techniques, key questions regarding its biology still remain unknown. In terms of conservation and management plans to safe guard this endangered species, we identified the crucial need to improve fishing landing statistics for this species and to increase the number of Marine Protected Areas along its distribution beyond the Mediterranean Sea. We also discussed the feasibility of still poorly explored conservation actions like population enhancement through the release of hatchery-reared or captured-from-the-wild post-larvae as complementary measures to help protect natural populations of dusky groupers.

Keywords Epinephelidae · Fisheries · Conservation · Endangered species · Top fish predator · Protogynous hermaphroditic fish

Introduction

Groupers¹ are large-bodied fishes with a broad global distribution and large commercial and ecological importance (Sadovy de Mitcheson et al. 2013). Groupers are targeted by artisanal, industrial, and sport fishing throughout their ranges (Heemstra and Randall 1993; Craig et al. 2011) and often have strong influences on food webs and ecosystems through their role as top predators (Heemstra and Randall 1993; Sluka et al. 2001). Alterations to grouper populations can thus have significant commercial and ecological consequences (Parrish 1987; Brulé et al. 2005). Several groupers have slow growth rates, late maturation and a complex mode of seasonal reproduction, including spawning aggregations and sexual reversal (Manooch and Mason 1987; Craig et al. 2011), which combined with high fishing pressure, led to vulnerability and/or over-exploitation of many grouper populations (Huntsman et al. 1999; Coleman et al. 2000; Sadovy de Mitcheson et al. 2013). Epinephelidae Family has several important species to world fishery (Sadovy de Mitcheson et al. 2013), but little

¹ Based on evidences provided by molecular and prior morphological studies, Smith and Craig (2007) recommended taxonomic changes to the Serranidae Family (*sensu* Nelson 2006) because it was a polyphyletic group. They proposed that groupers and seabasses (Subfamily: Epinephelinae) should be separate from Anthiinae and Serraninae Subfamilies. These two subfamilies were considered a new Serranidae Family, being now a monophyletic group, whereas the Subfamily Epinephelinae was elevated to Family Epinephelidae. These two reset families (Epinephelidae and Serranidae) include approximately 160 and 245 species, respectively.

is known about many of them. According to the International Union for Conservation of Nature (IUCN), almost a third of grouper species (50 out of 163 species) are classified as Data Deficient (i.e., there are not enough information to classify them into criteria of threat or no threat). Another 71 species are classified as Least Concern (i.e., they are not threatened) and 22 species as Near Threatened. The remaining 20 species are classified into some criteria of threaten; 12 species as Vulnerable, five as Endangered and three as Critically Endangered. Dusky grouper *Epinephelus marginatus* (Cornish and Harmelin-Vivien 2004) is one example of Epinephelidae currently included in the IUCN red list.

Epinephelus marginatus was originally described in the 18th century as *Perca gigas* Brünnich 1768 and, since then, has been attributed several scientific names: *Serranus gigas* Valenciennes 1828, *Serranus marginatus* Lowe 1834, *Epinephelus gigas* Jordan and Swain 1885, *Epinephelus guaza* (non Linnaeus): Jordan and Evermann 1896 and, currently, as *Epinephelus marginatus* (Lowe 1834) (Heemstra 1991).

Dusky grouper has a large-body reaching up 143 cm (total length, TL) (Craig et al. 2011) and weighs up to 60 kg (Figueiredo and Menezes 1980). It has an important functional role in the rocky bottoms in which they inhabit, especially as a main predator occupying the highest trophic levels in the food web (Parrish 1987; Reñones et al. 2002; Condini et al. 2015). They have a complex life cycle characterized by high longevity (may attain 60 years) (Reñones et al. 2007), slow growth rates (Reñones et al. 2007; Condini et al. 2014a) and a monandric protogynous hermaphroditic mode of reproduction (Bruslé and Bruslé 1975; Marino et al. 2001). Due to its reproduction

strategy, all specimens are born as females, but some individuals from 7 years old can undergo sexual transition and become functional males (Reñones et al. 2010). Sexual transition may occur due to population pressure (Sadovy de Mitcheson and Liu 2008; Erisman et al. 2010). Another significant feature about this species is a spawning aggregations behavior (Zabala et al. 1997a, b; Hereu et al. 2006), which makes it vulnerable to fishing activity.

Epinephelus marginatus has great economic importance and high commercial value for artisanal fishing (Heemstra and Randall 1993; Condini et al. 2007; Begossi and Silvano 2008; Lloret and Riera 2008; Begossi et al. 2012). Additionally, this species is widely caught by underwater sport fishing using spear guns along most of its distribution (Heemstra and Randall 1993; Machado et al. 2003; Coll et al. 2004; Lloret et al. 2008; Diogo and Pereira 2014). Due to above mentioned biological peculiarities associated with increasing fishing pressure along its geographical distribution, *E. marginatus* has been included since 2004 in the IUCN Red List as Endangered (ENa2d) (Cornish and Harmelin-Vivien 2004).

Several studies have been conducted along the last years on the biology and ecology of this endangered species. The main topics that have been addressed in these studies are behavior (Azevedo et al. 1995; Zabala et al. 1997a, b; Lembo et al. 2002; Machado et al. 2003; Gallego et al. 2013), reproduction and growth (Marino et al. 2001; Fennessy 2006; Reñones et al. 2007, 2010; Condini et al. 2014a, b), trophic ecology (Derbal and Kara 1996; Barreiros 1988; Reñones et al. 2002; Linde et al. 2004; López and Orvay 2005; Machado et al. 2008; Condini et al. 2011, 2015), parasite

communities (Oliver 1986; Balci et al. 2006; Marzouk et al. 2010; Roumbedakis et al. 2014), genetic structure (Maggio et al. 2005; Innocentiis et al. 2008; Schunter et al. 2011), fishing (Condini et al. 2007; Kouassi et al. 2010; Cardoso and Haimovici 2011; Begossi et al. 2012), and genetic identification in fish markets (Trotta et al. 2005; Asensio et al. 2008; Mottola et al. 2014). Studies on dusky grouper aquaculture aiming to repopulate fish stocks (Marino et al. 2003; Sarter et al. 2006; Cunha et al. 2009) or for commercial purposes are increasing in the last ten years (Sanches et al. 2006; Pierre et al. 2008). Such biological and ecological studies are crucial sources of information to guide conservation and sustainable fishery management of this endangered species (Tzanatos et al. 2008; Damalas et al. 2010). This review critically evaluates the available literature on *E. marginatus* focusing on biology, ecology, stock structure and fisheries, and suggests future research directions to fulfill current knowledge gaps.

Morphological characteristics

According to Heemstra and Randall (1993), *E. marginatus* shows an elongate body form, with head length contained 2.3 to 2.5 times in standard length (SL), depth body about 2.6 to 3.1 times in the SL, and the depth body less than head length. The preopercle is rounded and serrate in the edge, with subopercle and interopercle smooth. The midlateral part of lower jaw has 2 to 4 rows of subequal teeth, and 22 to 25 gill rakers; 7 to 10 in the upper arch and 14 to 16 at the lower arch. Caudal fin is rounded (in juveniles) or truncate with rounded corners (large adults). Dorsal fin

harbor 11 spines and 14 to 16 rays, being the third or fourth spine longest; pectoral-fin has 17 to 19 rays; pelvic fins are distinctly shorter than pectoral fins, not reaching anus (except in some fish less than 20 cm SL); anal fin shows 3 spines and 8 rays (some fishes can have 9 rays). These authors also described general characteristics like head and body dark reddish brown or greyish dorsally, usually yellowish gold ventrally; with irregular blotches in the body and head ranging from white, pale greenish, yellow or silvery grey. A remarkable characteristic of the species are a narrow white band in the distal edge of the caudal, anal and pectoral fins.

Geographical distribution and habitat

Dusky grouper *E. marginatus* (Lowe, 1834) has a wide distribution, occurring in the Mediterranean Sea (Oliver 1986; Kara and Derbal 1995a, b; Zabala et al. 1997a, b; Bouchereau et al. 1999; La Mesa et al. 2002; Lembo et al. 2002; Reñones et al. 2002, 2007, 2010; Bertucci et al. 2015), in Indian Ocean (Smale 1986; Fennessy 2006; Maggs et al. 2013a, b), and Atlantic Ocean. Along the western coast of the Atlantic, the dusky grouper has been recorded from Rio de Janeiro (Brazil) to Patagonia (Argentina) (Figueiredo and Menezes 1980; Bertoncini et al. 2003; Machado et al. 2003; Rico and Acha 2003; Irigoyen et al. 2005; Gibran 2007; Seyboth et al. 2011; Condini et al. 2011, 2013, 2014a, b, 2015, 2016a), whereas along the eastern Atlantic coast, this species occurs from the British Isles to South Africa (Azevedo et al. 1995; Barreiros and Santos 1998; Cunha et al. 2009; Kouassi et al. 2010; Mahé et al. 2012).

This species often inhabits rocky bottoms, such as reefs, banks and rocky shores. Overall, adults are more abundant in depths down to 50 m, and they can be found occasionally at depths down to 250 m (Bruslé 1985; Heemstra and Randall 1993; Harmelin and Harmelin-Vivien 1999). In contrast, juveniles are usually found in coastal tide pools (Azevedo et al. 1995; Machado et al. et al. 2003), highly structurally-complex shallow rocky habitats (La Mesa et al. 2002; Bussotti and Guidetti 2009), small bays in estuarine regions (Condini et al. 2016b).

Habitat use

A better knowledge of habitat use patterns among marine fish populations is extremely important to determine population dynamics and guide appropriate measures of conservation and fisheries management (Andrello et al. 2014; Davoren et al. 2015). Hence, many studies on dusky groupers have been conducted to evaluate their habitat use, home range and site fidelity.

Epinephelus marginatus is a sedentary species, displaying strong site fidelity with small home ranges (Chauvet and Francour 1990; Lembo et al. 1999, 2002; Spedicato et al. 2005, Pastor et al. 2009; Koeck et al. 2014; Afonso et al. 2016). Koeck and collaborators (2014) studied a dusky grouper population in a marine reserve at Cerbère-Banyuls (France) using telemetry and showed diurnal movement patterns throughout the year, with an active phase during the day (high detection frequencies) and a resting phase at night (low detections). A similar pattern was described by an acoustic-telemetry study by Hackradt (2012) in a marine reserve at Cabo de Palos -

Islas Hormigas (Spain), although with a strong inter-individual variability, since 15 out of 27 tagged individuals displayed a clearly diurnal pattern of activity, other 10 specimens did not differ among day and night, and only one fish showed greater probability of detection at night than day. Based on visual observation during scuba dive, Gibran (2007) reported that majority of dusky grouper were active during daytime, whereas few individuals showed activity during the night in rocky bottoms in southern Brazil. He also noticed that *E. marginatus* appeared “excited” during the twilight, and could be found outside their hiding places foraging for preys.

Several authors have claimed a relationship between dusky groupers’ body sizes and depth range. During early juvenile stages, the species preferentially inhabits shallow rocky habitats rich in shelter (Derbal and Kara 1995; Harmelin and Harmelin-Vivien 1999; La Mesa et al. 2002), the entrance of shallow caves (Bussotti & Guidetti 2009) and intertidal pool (Azevedo et al. 1995; Machado et al. 2003; Cottalorda et al. 2009), and shifting to deeper water as they grow older (Chauvet 1991; Kara and Derbal 1995a; Francour and Ganteaume 1999; La Mesa et al. 2002; Ruitton et al. 2010; Hackradt 2012; Bodilis et al. 2013; Condini et al. 2014a, b).

Other relationships with body size are related with behavior. Smaller individuals may form groups up to three individuals and actively swim in open waters over the soft bottom. In contrast, larger individuals have solitaires habits, are less active, usually found nearby their shelters among rocks or inside hiding places, from where they rarely come out (Kara and Derbal 1995a; Gibran 2007).

A strong seasonal variation in dusky groupers' densities has also been reported, with higher abundance during summer probably associated with spawning aggregations (see below). After peaking in activity during summer, they decrease their activity along the year, becoming more sedentary and, sometimes, may remain "invisible" for most of the winter (Zabala et al. 1997b).

Feeding and trophic ecology

Currently, studies on trophic ecology of dusky groupers are basically restricted to stomach contents analyses (Smale 1986; Azevedo et al. 1995; Derbal and Kara 1996; Barreiros and Santos 1998; Linde et al. 2004; López and Orvay 2005; López and Orvay 2005; Begossi and Silvano 2008; Machado et al. 2008; Condini et al. 2011), with only two studies using more advanced approaches as stable isotopes analyses (Reñones et al. 2002; Condini et al. 2015) (Table 1).

The high frequency of empty stomachs is usually higher in carnivorous fishes when compared to detritivorous and omnivorous fishes (Arrington et al. 2002), and this pattern is consistently reported in all feeding studies with dusky grouper along its geographical distribution (Table 1). Most studies showed ontogenetic diet shifts, transitioning from consumption of crustaceans at smaller sizes to fishes and cephalopods by larger body size individuals. The only exception to this general trend is reported by Machado and collaborators (2003) in Santa Catarina, Brazil. These authors that did not found relevant ontogenetic diet changes in the proportions of dominant

prey groups, with brachyurans remained the most representative food category along the species lifespan.

These studies on ontogenetic diet shifts also revealed a tendency of consuming preys with greater sizes or biomasses as dusky grouper increases in body size (Reñones et al. 2002; Linde et al. 2004; Machado et al. 2008; Condini et al. 2015). Linde and collaborators (2004) suggested that ontogenetic diet shifts in groupers are related with changes in foraging strategies, from an active predator searching for smaller preys (e.g., crabs) to an ambush predator targeting larger preys (e.g., fishes and cephalopods).

Overall, these feeding habits studies point out that dusky grouper is a carnivorous fish feeding essentially on crustaceans, fish, and mollusks. However, as expected, there are differences in the taxonomic composition of the diet due to differences in environmental conditions and prey availability among distant geographic areas (Reñones et al. 2002; Linde et al. 2004; Machado et al. 2008) or between adjacent sites (e.g., neritic vs. littoral; Condini et al. 2011, 2015). It is worth noting the importance of cephalopods in the diet of dusky grouper populations from the Mediterranean Sea, Southern Western Indian and eastern coast of the Atlantic Ocean, in contrast with its low representation or absence in population from the western coast of the Atlantic Ocean (Machado et al. 2008; Begossi and Silvano 2008; Condini et al. 2011, 2015). Other food items reported in very low abundance in the dusky grouper's stomach contents are plants fragments, polychaetes, small bivalves and gastropods (Azevedo et al. 1995; Derbal and Kara 1996; Linde et al. 2004; Machado et

al. 2008). It is still unclear if these items, especially plant fragments, are ingested accidentally when they are preying upon their preferential preys.

Despite providing a detailed view of diet composition of larger carnivorous like dusky groupers, stomach contents studies have some important limitations, such as the difficult to ascertain the actual assimilation by the consumer of refractory food items like preys with hard carapaces or shells (Jepsen and Winemiller 2002). An alternative approach overcoming these limitations is the analyses of stable isotope ratios (e.g., $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$) in food items and consumers' tissues, based on the assumption that consumers reflect isotopically their assimilated food (Fry 2006). This method reveals not only which food was in fact assimilated in the consumer's tissue, but also provides a time-integrated insight (days, weeks, months according with the isotopic turnover of the tissue) of the feeding habits. Moreover, it allows the evaluation of the relative importance of primary producers (e.g., seagrass beds, macroalgae, phytoplankton) in sustaining populations of carnivorous consumers (Garcia et al. 2007, Claudino et al. 2015). Nitrogen isotope ratios ($\delta^{15}\text{N}$), in particular, have been used to estimate consumer's trophic position in the food chain (Post 2002). The combination of stomach content and stable isotopes have become one of the most powerful approaches currently available to investigate feeding ecology in fishes (Layman et al. 2012).

Only two studies employed these combined approaches to study trophic ecology of dusky groupers. Reñones et al. (2002) observed intermediate $\delta^{15}\text{N}$ values in dusky groupers from Mediterranean (between 8.8 to 13.1‰) and a positive correlation

with body size. In contrast, Condini et al. (2015) did not find a significant relationship between $\delta^{15}\text{N}$ and body sizes in populations at Southern Western Atlantic. Moreover, they observed highest $\delta^{15}\text{N}$ values compared to other fish predators in their habitat suggesting dusky grouper occupy the upper most trophic-levels both at neritic and littoral rocky bottoms at Southwestern Atlantic. Carbon isotope ratios ($\delta^{13}\text{C}$) of dusky grouper in the Mediterranean provided evidence of strong linkages to the benthic food web, whereas at the Southwestern Atlantic suggest that this top marine predator integrates both pelagic and benthic food web pathways (Reñones et al. 2002, Condini et al. 2015).

It is also worth noting that stable isotopes may reveal that preys are not assimilated in consumer's tissues (e.g. muscle) in the same proportion they appear in the stomach content of dusky groupers (Condini et al. 2015). Stable isotope mixing models indicated that fishes are assimilated in greater proportion than crabs by dusky groupers, which usually is the dominant food item found in their stomach contents. This difference is likely due to relatively greater amount of refractory material in crabs (e.g., carapace) versus fishes. Thus, crabs may be more frequently encountered in stomach contents due to slower digestion rates, but may contribute less (per unit ingested biomass) to assimilated energy. Condini et al. (2015) suggested that stomach contents and stable isotope analyses are highly complementary for elucidating the trophic ecology of dusky groupers, and calls attention that relying solely in one technique could render inaccurate or misleading results.

Feeding behavior

The dusky grouper is a specialist and opportunistic predator (Gibran 2007; Condini et al. 2011), using different feeding strategies to capture its preys, such as “drift feeding”, “ambush hunting” and “roving or patrolling” and feed mainly at twilight hours (diurnal and crepuscular) (Gibran 2007).

The dusky grouper is a curious species, which favors symbiotic association beneficial to follower species (Bessa 2011). This characteristic is well documented on several studies showing that their opportunistic and learning behavior can turn into feeding tactics, such as following snake eel *Myrichthys ocellatus* (Gerhardinger et al. 2006), octopus *Octopus vulgaris* (Machado and Barreiros 2008) and other teleost fishes like the Brazilian damselfish *Stegastes fuscus* (Bessa 2011) and even the common dentex *Dentex dentex* in the Mediterranean (García-Charton J.A., pers. observ.).

Reproductive biology

As a monandric protogynous hermaphroditic species (Bouain and Siau 1983; Bruslé and Bruslé 1975; Marino et al. 2001), it is usually difficult to observe dusky grouper individuals undergoing sexual transition due to the low number of specimens engaged in this phase of the life cycle (Condini et al. 2014b). Additionally, this phase is probably significantly faster and more brief compared with other ones like development and maturation to adulthood (Sadovy and Shapiro 1987). Dusky grouper individuals undergoing sexual transition have been found in all studies carried out in the

Mediterranean Sea and Indian Ocean, whereas only one individual was collected in this phase in all reproductive studies conducted in the Atlantic Ocean (Table 2). As a protogynous hermaphrodite species, it is expected a higher proportion of females compared to the number of males in the population and all studies reporting sex (male:female) ratios corroborate this pattern (Bruslé and Bruslé 1975; Marino et al. 2001; Bertoncini et al. 2003; Fennessy 2006; Gerhardinger et al. 2006; Reñones et al. 2010; Özen and Balcı 2011; Condini et al. 2014b; Tsikliras and Stergiou 2014). Higher sex ratios have been reported for dusky groupers in the Atlantic Ocean (Table 2), except Seyboth et al. (2011) who did not collected males.

The reproductive cycle of dusky groupers has been established based on the histological analysis of the gonads, temporal analysis of the gonadosomatic index (GSI) and/or percentage frequency distribution of the gonadal development stages. Histological analyses of gonads indicated that the species spawns between late spring and late summer (Bruslé and Bruslé 1975; Marino et al. 2001; Bertoncini et al. 2003; Reñones et al. 2010; Condini et al. 2014b). Dusky groupers from South Hemisphere showed spawning peaks from November to January (Bertoncini et al. 2003; Fennessy 2006; Gerhardinger et al. 2006; Condini et al. 2014b), whereas in the North Hemisphere the peaks occurred between July and September (Bruslé and Bruslé 1975; Kara and Derbal 1999; Marino et al. 2001; Reñones et al. 2010; Özen and Balcı 2011) (Table 2). The body length at first maturation (L50) reported for *E. marginatus* ranged from 391 mm TL in Turkey to 622 mm TL in South Africa (Table 2). This parameter is crucial for conservation and management purposes and can change, for example,

among populations from different geographic areas, environmental conditions and levels of fishery pressure.

Another important reproductive parameter is fecundity, but only one study by Reñones et al. (2010) provided information on this aspect. These authors estimated the fecundity in a total of 39 females with sizes ranging from 386 to 915 mm TL and ages between 6 and 42 years, and demonstrated they are spawners with asynchronous oocyte development. The potential fecundity ranged from 65 thousand to 8 million oocytes in females, with a mean relative fecundity of 334×10^3 oocytes kg^{-1} . Females would spawn until 10 batches during the spawning season, with an average batch fecundity of 75×10^3 oocytes kg^{-1} .

Reproductive behavior

Dusky grouper has a complex life-history strategy characterized by a monandric protogynous hermaphrodite mode, where all individuals are born as females with potential to change their sex to males throughout their life span (Bruslé and Bruslé 1975; Heemstra and Randall 1993; Marino et al. 2001; Reñones et al. 2010). Sadovy and Shapiro (1987) reported that several monandric species have bimodal frequency distributions for body size and age, with females being both smaller and younger than males. Such bimodal frequency distributions can cause a considerable population imbalance, because larger individuals are the main target of fishermen and the first ones to be taken out of the fish stock (Gulland 1983). Consequently, there are higher withdrawn of males by fishery, thus forcing sexual transition to occur earlier. This

hypothesis seems to be corroborated when age structure of dusky groupers are compared among studies carried out in the last 30 years. Prior studies in Mediterranean Sea showed that sexual transition occurred in individuals 9 to 16 years old and with body sizes ranging from 68 to 90 cm TL (Chauvet 1988). In contrast, recent observations suggested that sexual transition can occur in much younger and smaller individuals (from 7 to 17 years old and from 52.1 to 76.9 cm TL, respectively) (Reñones et al. 2010).

One of the most relevant although least understood aspects of the complex life-history of dusky grouper is its aggregations during spawning behavior (Zabala et al. 1997a, b). The current knowledge about spawning aggregations in this species is limited to observations within Marine Protected Areas (MPA) located in the Mediterranean Sea, such as Medes Islands Marine Reserve (Zabala et al. 1997a, b) and Cerbère-Banyuls Marine Reserve (Mathieu-Tissot 1999). Other authors have been reported only indirect evidences on the occurrence of spawning aggregations sites in the Mediterranean Sea (Marino et al. 2001), southeastern Africa (Fennessy 2006) and southwestern Atlantic (Bertoncini et al. 2003, 2012; Gerhardinger et al. 2006; Condini et al. 2014b), which still need to be corroborated by direct observations.

Spawning of dusky groupers is reported to occur between late spring and late summer. The population density usually achieves the greatest peaks during spawning aggregations, when can increase by five to 12 times compared to densities during winter (Zabala et al. 1997b; Louisy and Culioli 1999). Another feature is the appearance of large individuals (> 80 cm TL) during aggregations. Some of the larger ones remain

throughout the year in these reproductive areas, but most appears at late spring and remain only until late summer. Usually, large males are the first ones to arrive. During the reproductive gathering, large males comprised only 10 to 17%, smaller females (40 to 60 cm TL) constitute 45 to 60% and large females (> 60 cm TL) represent 10 to 25%. There are no juveniles dusky groupers (<40 cm TL) in areas where adults are concentrated for reproduction, at least in summer (Louisy and Culioli 1999). After spawning, progressively the dominant males abandoned their territorial behavior until the next reproductive event in the incoming summer (Zabala et al. 1997b).

Identification of females and males individuals during underwater surveys is extremely hard. Zabala et al. (1997b) proposed gender identification during the reproductive period based on their color patterns (CP). They identified eight CPs, where the first three are probably shared by all size classes and sex throughout the year. The CP1 is a common pattern exhibited along the year and may be related with different behaviors, such as rest (CP1bis), aggression (CP2) and perplexity (CP3). The immature individuals always exhibit patterns CP1 or CP2, whereas patterns CP4 to CP8 are observed only during the reproductive season. CP4 is characterized by light colour and is mainly observed in small females (40 to 60 cm TL). CP5 is distinguished by a dark streaked and occurs in dusky groupers with body sizes (TL) between 40 to 95 cm, larger females (> 60 cm TL) and younger males (< 80 cm TL), being differenced for sex estimation such as, swollen abdomen (egg-swollen bellies). The dominant larger males (> 95 cm TL) usually exhibit the patterns CP6 (silver streaked), CP7 (basic male) and CP8 (light male) (see Fig. 2-3 in Zabala et al. 1997b).

Once in the reproductive area, there is a marked segregation between females and males. Males establish a territory in deeper water (from 15 and 35 m), generally below the thermocline, at temperatures of 17-20 °C. In contrast, females tend to remain in less deep waters (above or between 5-20 m most often) above the thermocline (Louisy and Culioli 1999). During a spawning event, a dominant male performs courtship activities within a small area of its territory, showing agonistic behavior against males entering in his territory. The spawning event has different phases: a) Approach, where males exhibit lateral display in the bottom sea, below of the thermocline; b) Ascent and false rise, males join females and they rise vertically along the water column in parallel, side by side, less than 20 cm apart. Just after reaching the upper level of the thermocline they abort the rise and come back to the bottom. The false rise can be repeated two or three times in a few minutes before the actual spawning; c) Actual spawning, males and females approach again and they move up the water column below the thermocline. Then, the rising process is followed by a short but frenetic swimming acceleration that ends up with the emission of gametes and the immediate separation of the pair. After gametes liberation each mate goes back to the bottom (see Zabala et al. 1997a, Fig. 4). A single male is able to repeat a successful spawning several times in a few minutes, and with different females. Courtship activities have been observed at sunrise and sunset (Zabala et al. 1997a; Pelaprat 1999).

The first observation of dusky grouper spawning at Medes Islands (Zabala et al. 1997a, b) suggested a close relationship with the lunar cycle, specifically the New Moon phase. However, information obtained from observation of spawning

aggregations in other regions did not confirm these earlier reports (Louisy and Culioli 1999). Monitoring of spawning aggregations of dusky groupers at Medes Islands for three consecutive years revealed that the date of gametes releasing in relation to the lunar cycle change every year (Louisy and Culioli 1999); for their part, Hereu et al. (2006) in the same MPA did not find such relationship with lunar phases, attributing this lack of synchronicity to the relatively long reproduction period of *E. marginatus* (2 to 4 wk) compared to tropical species (e.g. 7 d for *E. striatus*; Sala et al. 2001). Although it has been observed in other grouper species (Johannes 1978; Colin 1992; Shapiro et al. 1993; Sadovy et al. 1994; Samoilys and Squire 1994), the synchronization between lunar cycle and gametes releasing in dusky grouper populations still need validation. Regarding other environmental factors triggering spawning aggregations in this species, the seasonal variation in temperature undoubtedly plays a major role (Louisy and Culioli 1999; Hereu et al. 2006).

There is a clear knowledge gap regarding the planktonic life stage of Epinephelidae in general. Not surprisingly, almost nothing is known about the occurrence of eggs and larvae of *E. marginatus* along its entire geographic distribution. The first reported of less than 10 cm LT dusky groupers occurred in 1989 in the south of Corsica and in Cerbère-Banyuls, with further collections of individuals in subsequent years in different sites along the French coast (Bodilis et al. 2003). Marinaro and collaborators (2005) employed a bongo net and sampled dusky groupers' eggs at the marine reserve of Cerbère-Banyuls and were able to monitor their development until hatching. These eggs were perfectly spherical, had a diameter between 0.79 and 0.90 mm (average of 0.855 mm) and a single drop of oil. This oil drop measured 0.16 to 0.18

mm in diameter and was usually colorless, but in some cases exhibited a yellowish color. Crec'hriou et al. (2010), using both towed bongo nets and fixed plankton nets around the Cabrera National Park (Balearic Islands, Western Mediterranean Sea) during the period between 2nd and 14th of July 2004, documented that eggs of *Epinephelus* spp. (mostly *E. marginatus*) appeared to be continuously abundant through time, thus confirming continuous spawning during the entire sampling period. Muntoni et al. (2011) caught two post-larvae of the *E. marginatus* measuring 2.2 and 2.5 cm LT with light traps in September and October 2010 at the southern Gulf of Cagliari (Italy). Félix-Hackradt et al. (2013) also employed light traps and collected a few specimens smaller than 6 cm LT in shallow reefs surrounding Cabo de Palos–Islas Hormigas Marine Reserve (Spain).

Age and growth

Fennessy (2006) used tetracycline markings to reveal annual depositions in the otoliths and clearly demonstrated that a single annulus was deposited each year for dusky groupers at the southeastern Africa. Studies carried out elsewhere had not employed direct tetracycline markings as age validation, but rather, they relied on patterns of alternating translucent and opaque bands and marginal increment analyses (Reñones et al. 2007; Condini et al. 2014a). Although marginal increment analysis is not considered the most trustworthy technique for validation of growth increments in otoliths (Campana 2001), these studies corroborate the experimental approach with tetracycline conducted by Fennessy (2006). The annual deposition of growth

increments in otoliths has been found in other grouper species as well, such as *E. flavolimbatus* (Manickchand-Heileman and Phillip 2000), *Mycteroperca interstitialis* (Manickchand-Heileman and Phillip 2000), *M. rosacea* (Díaz-Urbe et al. 2001), *E. coioides* (Grandcourt et al. 2005), and *M. fusca* (Bustos et al. 2009), which suggests that is a common feature in large epinephelids.

Data currently available on size, age, length-weight relationships and growth parameters of *E. marginatus* are compiled in Table 3. These studies have relied both on counting of annuli on scales collected beneath the pectoral fins (Rafail et al. 1969; Bouain 1986; Chauvet 1988; Azevedo et al. 1995; Kara and Derbal 1995; Bouchereau et al. 1999; Ximenes-Carvalho et al. 2012) and readings of growth rings at sectioned *sagittae* otoliths (Fennessy 2006; Reñones et al. 2007; Seyboth et al. 2011; Conдини et al. 2014a). In a few cases, age estimates were also based on analysis of whole *sagittae* otolith (Chauvet 1988). The comparison among studies revealed some differences in body size and age structure (Table 3). The absence of older ages in some regions may be a consequence of (a) fishing, which can selectively remove older individuals (Ricker 1975), (b) insufficient sampling of the entire population (e.g., Algeria in Mediterranean Sea, Kara and Derbal 1995), or (c) simply because that region contains mostly immature individuals (e.g., littoral zone, Seyboth et al. 2011).

The parameters of the von Bertalanffy's growth equation (L_{∞} , K , and t_0) have been widely used to determine growth rates in many species, including *E. marginatus* (Table 3). A comparison of these studies revealed considerable differences among sites, mainly for the theoretical maximum length (L_{∞}) and growth coefficient (K). Such

differences could be partially explained by inherent differences between the reading structures (otoliths vs. scales). Otoliths showed low variation for both parameters (L_{∞} and K between 900 and 1249 mm and 0.069 and 0.129, respectively), when compared with age reading obtained from scales (785 to 1850 mm and 0.028 to 0.160, respectively) (Table 3). These differences may be due to the fact that otolith analysis tends to be slightly more precise than scales (Campana 2001). Alternatively, this may be simply because some of these studies (e.g., Kara and Derbal 1999) failed to sample large-sized individuals, which strongly underestimate the value of L_{∞} . The growth rate of *E. marginatus* increases rapidly during the first 5 years of life, when reaches in average approximately 45% of the theoretical maximum length. After this age, the growth rate tends to decline rapidly and nearly ceases after reaching 30 years old (Chauvet 1988; Kara and Derbal 1995b; Bouchereau et al. 1999; Fennessy 2006; Reñones et al. 2007; Condini et al. 2014a).

According to available studies on length-weight relationships, there are low variations among these parameters, with individuals of the same body size showing similar weight (Fig. 1 and Table 3). Exceptions are the studies from Kara and Derbal (1995) and Ximenes-Carvalho et al. (2012) that showed values below and above average, respectively, probably due the lack of the largest individuals in their dataset. However, as length-weight relationship may vary along the life cycle and according to the environmental conditions, seasonality, and individual physiological state, this parameter is not well recommended for comparing different populations (Froese 2006).

Parasitology and disease

Parasitological approaches have been used as biological tags or indicators to provide information for stock identification, movement patterns and population connectivity of fishing stocks (Williams et al. 1992; MacKenzie and Abaunza 1998). The principle of the method is that a fish can only become infected with a parasite when entering its endemic range (MacKenzie and Abaunza 1998, 2005). The first studies using this approach began in the middle of the last century, when Herrington et al. (1939) observed occurrence and distribution of the parasite *Sphyrion lumpi* in redfish (Sebastes, Scorpaenidae). These studies continue to be employed nowadays, for instance, by Vignon et al. (2008) ascertaining fine-scale spatial structure and movement of bluestripe snapper (*Lutjanus kasmira*) in Moorea (French Polynesia) or Weston et al. (2015) that distinguished a sardine (*Sardinops sagax*) stock in South African using this method.

MacKenzie and Abaunza (1998) pointed some advantages and limitations of the parasites as biological tags compared to artificial ones. The main advantages pointed out by these authors are its applicability in small, fragile species of fish and crustaceans, lower cost than artificial tags, and the absence of potential abnormal behavior of the host that are expected when using artificially tags. Among limitations of the use of parasites as biological tags are: (a) lack of adequate information on the complex ecology and biology of aquatic parasites that hinders their efficient use as tags; (b) the correct identification of many parasite species is still uncertain and subject

to disagreements among taxonomists; (c) it is usually desirable to know the age of host individuals, but in some species of fish and invertebrates the techniques of age determination have not been validated. Thus, different methods of tagging should be seen as complementing one another, and in any fish population study the interests of efficiency and accuracy are best served by using as many different methods as possible (Calò et al. 2013).

There are few investigations concerning the parasite fauna of dusky grouper along its occurrence (Oliver 1984; Scholz et al. 1993; Santos et al. 2000; Bartoli et al. 2003; Merella et al. 2005; Genc 2007; Marzouk et al. 2010; Roumbedakis et al. 2013) (Table 4) and no studies using parasites as biological tags to determined stock identification, movements patterns and population connectivity. The only exception within the Epinephelidae family is the recent study carried out by Espínola-Novelo et al. (2015) with *Mycteroperca bonaci* in the coast of Yucatán, México. The aim was investigate possible differences between parasite communities in two different environments, but they were not able to ascertain definitive patterns regarding the host ecology. Future studies could explore the potential of this approach to advance our current knowledge on population structure and connectivity of *E. marginatus* and other groupers of the Epinephelidae family.

Other studies on parasites of dusky grouper have called attention to symbiotic relationships with other fishes. For instance, Weitzmann and Mercader (2012) revealed that dusky groupers may have a symbiotic relation with a clingfish (*Lepadogaster candolii*) who removes parasites of its mouth. In aquaculture settings,

Souza and collaborators (2014) showed a symbiotic behavior of the barber goby *Elacatinus figaro* removing the parasite *Neobenedenia melleni* from dusky grouper individuals.

Groupers are subject to a number of diseases in aquaculture facilities (Harikrishnan et al. 2010). Regarding diseases of reared dusky grouper, the most commonly reported are infection by vibrios, *Pseudomonas* spp. and *Aeromonas* spp. (La Mesa et al. 2008). Frequent observations of mortalities of wild fish worldwide (including dusky groupers in the Mediterranean Sea) have been associated to viral encephalopathy and retinopathy, a disease caused by infectious agents of the *Betanodavirus* genus (Munday et al. 2002). This phenomenon has been observed in Sicily (Marino and Azzurro 2001), other Italian sites and Greece (Panzarin et al. 2012), northern Ionian Sea (Vendramin et al. 2013), Tunisia (Haddad-Boubaker et al. 2014), Balearic islands and Algeria (Kara et al. 2014), Corsica, Libya, Malta and Crete (GEM 2015). This disease seems to be originated from aquaculture facilities, thus showing the risk of viral exchange between wild and farmed fish populations (Vendramin et al. 2013; Haddad-Boubaker et al. 2014). Also, Rizgalla et al. (2016) recently reported upon a so-called 'dusky grouper dermatitis' in Libyan coastal waters, whose causes have yet to be established. There is concern that seawater warming due to climate change may increase the occurrence of infection diseases in the near future, which could cause pose another risk to the endangered dusky groupers (Vendramin et al. 2013).

Fisheries

Groupers have been caught by man for more than ten thousand years, as pointed out by well-preserved archaeological deposits on the Mediterranean coast. For instance, Desse and Desse-Berset (1999) found records of *E. marginatus* in deposits of the Neolithic in Cyprus, whereas Morales and Roselló (2004) found this species in Neolithic sites in the Nerja Cave (Málaga, Spain), and Mannino et al. (2012) registered the occurrence of dusky grouper in Mediterranean Island of Favignana (Italy) between the late Mesolithic and the early Neolithic. Groupers have been also recorded in archaeological sites around the world, such as in Northeastern Atlantic (Roselló and Morales 1990), Bahamas (Blick 2007), and the Pacific islands (Dalzell 1998).

Groupers have high economic value, and several species are broadly consumed worldwide (Heemstra and Randall 1993). According to the Food and Agricultural Organization of the United Nations (FAO), there was an increase in fishing global captures of groupers since 1950, when it was caught approximately 16.000 tons per year, until 2009 when captures achieved approximately 275.000 tons per year. Out of the total grouper production in 2008, more than 80% was reported from Asia. This increasing trend is probably substantially underestimated because of the unreported catches from many small-scale, multispecies fisheries (Sadovy de Mitcheson et al. 2013).

Dusky grouper is targeted by artisanal, industrial and sport fishing throughout their geographical ranges (Heemstra and Randall 1993; Craig et al. 2011). However, there are few information about dusky grouper fisheries and no fish landing data

available for this species in important areas of its geographic distribution, such as Mediterranean Sea and Western Atlantic. Dusky grouper is not a target species in the industrial fishery, but an accessory species with high economic value. In contrast, dusky groupers are the main target species in many artisanal fisheries around the world. The main fishing gears employed to catch dusky grouper is bottom longline (Galili et al. 2004; Gomez et al. 2006; Cardoso and Haimovici 2011; Martín et al. 2012; Lloret and Font 2013) both in artisanal and industrial fishing. The species may also be caught by hook-and-line (Galili et al. 2004; Condini et al. 2007) or eventually by gillnet (Galili et al. 2004; Martín et al. 2012). In the case of sport fishing dusky groupers are usually captured by spear gun (Coll et al. 2004; Lloret et al. 2008; Lloret and Font 2013).

A crucial prerequisite for efficient fishery management is the knowledge of the health of the fish stocks. Therefore, not only information about the biology of the target species is important, but also adequate fishery statistics data. Data on fishing landing and fishing effort have been used since 19th century when England scientists realized that they could not investigate fishing effects on fish stocks due to lack of adequate fishing statistics (Williams 1977). Information on the capture and fishing effort are also fundamental to determine public policies and management measures for guarantee the maintenance of fish stocks and conservation of the ecosystems involved. Therefore, the lack of fishing landings and fishing efforts currently employed to catch dusky grouper worldwide is a major obstacle to sustainable management of this endangered species.

FAO (1992) reported in 1990 catches of dusky grouper in the Atlantic Ocean and in the Mediterranean Sea of 1,261 and 3,308 tons, respectively. The available information on *E. marginatus* landing is currently fragmented and restricted to a few sites and occasions. For instance, there are only two records of dusky grouper catches in the Atlantic Ocean: firstly, in the Eastern Atlantic (Gulf Cádiz) by Silva et al. (2002) who registered landings of 7,200 kg (1996) and 11,260 kg (1997) by the artisanal fleet; and secondly, in Southern Western Atlantic (south Brazil) by Cardoso and Haimovici (2011) who described annual landing around 7,600 kg by the industrial fleet. In the Indian Ocean, Smale and Buxton (1985) estimated an extremely low total catch of 185 Kg in 1979 and 322 Kg in 1980 at Port Elizabeth (South Africa). Pradervand et al. (2007) reported catches of nearly 450 kg by sport fishers along the KwaZulu-Natal shoreline between 1977 and 2000. Unal et al. (2009) reported landing of only 56 kg by longline and gillnets fisheries at Gökova Bay (Turkey). Lloret and Riera (2008) reported dusky groupers landing along almost 20 years in Cape Creus (Spain) with low annual landings, with maximum values around 100 kg in 2002. Finally, Gallego and Reyes (2015) showed landings ranging from approximately 750 kg to 4,500 kg between 2000 and 2010, with a considerable increase in 2011, with peaks of nearly 9,000 kg in the southern coast of Spain. However, this increase in dusky grouper catches was associated with improvement and increase of fishing fleet and not with recovered of dusky grouper's stock. Aside government- or scientific-oriented fish landing reports, another way to reconstruct past fish stock levels is by estimating changes in fishing activity and fishing catches based on fishers' perceptions and other sources of information (Sáenz-Arroyo et al. 2005). For instance, Coll et al. (2014) documented

that Spanish Mediterranean fishers mention grouper species as being depleted in the area since mid-twentieth century.

Another aspect to highlight when assessing dusky groupers catches and subsequently marketing is the occurrence of fraudulent reports when other species are commercialized as dusky groupers. For example, Asensio et al. (2009) using PCR-based methodology detected fraudulent labeling of 58 out of 70 grouper fillets samples in different fish market in Madrid, which were in fact originally from other species, such as Nile perch *Lates niloticus* and wreck fish *Polyprion americanus*. Cutarelli et al. (2014) also detected by DNA barcoding the substitution of filets of dusky grouper by the pleuronectid *Reinhardtius hippoglossoides* in Italian markets.

Dusky groupers contamination by heavy metals and other pollutants are also cause of concerns, especially considering that this species is highly appreciated by human consumption around its geographical distribution. Since dusky grouper is a large-bodied and long-lived fish species, there is a great risk of metal contamination (e.g. mercury) via biomagnification (Evers et al. 2009; Tremain and Adams 2012). Mercury is a non-essential toxic element commonly found in aquatic ecosystems. It is released through a variety of natural and anthropogenic processes including geologic weathering, gold mining, and burning of coal (Pacyna et al. 2010). In aquatic systems, mercury can be transformed by microbial activity into organic methylmercury, which biomagnifies in the food web and results in high concentrations of methylmercury (MeHg) in piscivorous species (including humans) (Chen et al. 2008).

Despite the potential risk of mercury contamination in dusky groupers, there are few studies addressing this topic around the geographical distribution of the species. The first study about mercury contamination with dusky grouper was registered to Mediterranean Sea in Israel by Levitan et al. (1974). These authors analyzed total mercury and methyl-mercury in 18 individuals and 13 showed concentrations above the safety levels (World Health Organization - WHO) for human consumption. They also found higher contamination values in older specimens. The mercury source in this study was probably waste water from nearby industrial plants discharging into the streams or directly into the study site.

Two recently studies also revealed mercury contamination in dusky grouper populations. Banana et al. (2016) reported the dusky grouper showed the second higher Hg concentration above the safety levels (WHO) for human consumption among different fishes, oysters and cuttlefish species evaluated in Mediterranean Sea (west Zawya, Libya). Authors did not found relationships between mercury concentration and species biological parameters like length, weight or age. They also found high Hg concentration in sediment samples, which was associated with heavy pollution by chemical industries during more than 40 years. Condini et al. (*in press*) analyzed a suite of parameters (i.e., body size, age, diet and trophic position) to investigate mercury concentration in 224 dusky groupers in southern Brazil. They revealed that larger (> 650 mm TL) and older (> 8 years old) individuals showed mercury contamination levels that are potentially harmful for human consumption. When considering similar body sizes, individuals inhabiting littoral habitats had higher concentrations of mercury than

neritic habitats, probably due to proximity to pollution sources associated with human activities.

Aquaculture

In order to supply the worldwide demand that has not been fulfilled by decreasing fishing landings, grouper mariculture has been developing in several regions (Harikrishnan et al. 2010), especially in Asia where this species achieves high commercial value in fishing markets of Hong Kong, Singapore and Taiwan (Pierre et al. 2008). Ottolenghi et al. (2004) reported an increase annual aquaculture production of groupers from 1991 to 2000 (approximately from 2,000 tons to 9,300 tons, respectively). Most grouper are cultured in floating net cages either in the open sea or at the seaward end of estuaries (Pierre et al. 2008). However, since mariculture depends heavily on wild-caught seed to its farming, this activity seems unsustainable at current levels and could also aggravate ongoing overfishing of this species (Pierre et al. 2008; Sadovy de Mitcheson et al. 2013).

Despite the usefulness for restocking practices and the high economic potential, few aquaculture studies have been performed and rearing is still on an experimental scale (Glamuzina et al. 1999). The first attempts of artificial reproduction were made in 1995 by research groups in Italy and Croatia (Glamuzina et al. 1998; Marino et al. 1998). Studies about larval rearing were undertaken (Glamuzina et al. 1998; Spedicato et al. 1998; Russo et al. 2009; Kerber et al. 2012; Cunha et al. 2013), but larval rearing still is a bottleneck in the mariculture of this species (Pierre et al.

2008; Cunha et al. 2009). During its cultivation, mortality is mainly linked to low feeding rates and starvation, particularly before or during exogenous feeding onset (Glamuzina et al. 1998; Toledo et al. 2002; López and Orvay 2003; Russo et al. 2009; Kerber et al. 2012). Therefore, dusky grouper reproduction in captivity still poses many problems (Pierre et al. 2008), despite some effort to successfully rearing this species in different conditions (Boglione et al. 2009).

Conservation and Management

Humans have altered the oceans through many direct and indirect ways (Jackson et al. 2001; Myers and Worm 2003; Lotze et al. 2006) and to refrain ongoing degradation of marine ecosystems and recovered their balance, several management strategies have been proposed, such as the establishment of Marine Protected Areas (MPA) (Lubchenco et al. 2003). MPAs have been an effective conservation tool (Claudet et al. 2008; García-Charton et al. 2008; Wood et al 2008; Guidetti et al. 2014) in face of increasing degradation of marine ecosystems by overfishing (Pauly et al. 2002). On the other hand, Lester et al. (2009) pointed out that despite considerable evidences of the effectiveness of MPAs in rebuilding stocks, it has proven much more difficult to demonstrate total recovery of populations or the export of biomass from MPAs to adjacent habitats.

MPAs is usually build to prevent against overfishing and to promote protection of marine biodiversity and conservation of marine exploitable resources and ecosystem services (Kelleher and Kenchington 1992; Claudet et al. 2008), both within

and around areas protected from fishing (Alcala et al. 2005; Goñi et al. 2008; Christie et al. 2010). Another relevant feature is related with size of the organisms; specimens living within marine reserves tend to grow larger and live longer than individuals in adjacent unprotected areas (Halpern 2003; Lester et al. 2009), hence promoting population fecundity and larval survival rate (Francis et al. 2007). In the last decades, there has been a considerable increase of MPA designations around the world (Lubchenco and Grorud-Colvert 2015). The creation of MPAs in Mediterranean Sea was extremely beneficial for dusky grouper, since this region constitutes one of the most relevant sites in the geographical distribution of the species. Biological attributes of dusky groupers such as sedentarity and territorial behavior in adults (Chauvet and Francour 1990; Lembo et al. 1999, 2002; Spedicato et al. 2005; Pastor et al. 2009; Koeck et al. 2014) and a planktonic phase during its larvae development, where individuals can be passively dispersed by sea currents (passive dispersal) (Andrello et al. 2013), make this species a good candidate to respond positively to the protection provided by MPAs. In fact, a number of studies in Mediterranean and Atlantic MPAs emphasized that dusky groupers are beneficiated by protected areas (e.g. Harmelin et al. 1995; Reñones et al. 1999; Lenfant et al. 2003; García-Charton et al. 2004, 2008; Tuya et al. 2006; Afonso et al. 2011; Coll et al. 2012, 2013; García-Rubies et al. 2013; Harmelin 2013). The same is true for others Epinephelidae in MPAs worldwide (e.g. Chiappone et al. 2000; Russ and Alcala 2003, 2010; Anderson et al. 2014; Arai 2015). In a recent study performed in several MPAs in Mediterranean Sea, Hackradt et al. (2014) showed that average abundance, biomass and individual weight of dusky groupers were higher within the protected areas than immediately outside. Hackradt et al.

(2014) also reported grouper biomass gradients across MPA limits in most studied cases, thus indicating that spillover is likely to occur, although its spatial scale is probably small (hundreds of meters). Regarding the early life history stages, Crec'hriou et al. (2010) documented a gradient in eggs' abundance of *Epinephelus* spp. across the limits of Cabrera National Park as a clue to indicate that this MPA acts as a source of grouper propagules to neighboring unprotected areas.

Andrello et al. (2013) used a biophysical model to evaluate self-recruitment, larval supply and connectivity for Mediterranean MPAs. However, they could not determinate MPA productivity because crucial knowledge on larval biology is still scarce. Hence, precise evaluations of recruitment levels in Mediterranean MPAs and recruitment supply beyond MPA borders remain a challenge. On the other hand, Andrello et al. (2013) showed a potential high level of self-recruitment, but low connectivity among Mediterranean MPAs. These modeling results corroborate the empirical data of De Innocentis et al. (2001) on the genetic structure of *E. marginatus* in the west-central Mediterranean. Based on these results, the strategic plan for biodiversity adopted by the Convention on Biological Diversity (COP10) will be more challenging, with the creation of new MPAs that ensures connectivity within and among MPAs as a major measure to be taken in order to supply larvae all over the Mediterranean continental shelf. This conservation action may be even more crucial in a near future considering that climate change is likely to affect connectivity patterns of dusky grouper in the whole Mediterranean region (Andrello et al. 2014). Nevertheless, connectivity studies based on field data at local and regional scales are still needed to

confirm the outcomes of these large-scale modeling studies and to assist in the design of MPAs networks (Calò et al. 2013).

Conclusions and future research perspectives

Here, we have shown several studies about ecology, biology, behavior, fisheries and conservation of the dusky grouper *Epinephelus marginatus*, an endangered species with great ecological and economical relevance. We identified substantial knowledge available on feeding ecology, reproductive biology and age structure along its geographic distribution. In contrast, studies on habitat use and behavior are restricted to Mediterranean Sea and a few works in Atlantic and Indian Oceans. Important questions still remain unanswered regarding its life cycle, such as (i) the specific sites and conditions where reproductive aggregations occurs, (ii) dispersion patterns of eggs and larvae in the sea, and subsequent connectivity patterns with adult stocks and colonization of rocky bottoms, (iii) habitat requirements along life cycle of the species (based on habitat and niche models at several spatial scales), or (iv) the current fishing efforts employed worldwide to catch this endangered species, and management options to maintain viable populations.

A suite of tools and techniques have been used to investigate the biology and ecology of dusky groupers worldwide, such as stable isotope analysis (Reñones et al. 2001, Condini et al. 2015), otolith microchemistry (Condini et al. 2016b), parasite taxonomy (Marzouk et al. 2010; Roumbedakis et al. 2014) and genetics (Ramirez 2006; Schunter et al. 2011). Future studies on *E. marginatus* should strive to further

integrate these tools to better understand dusky grouper population connectivity and habitat use along its geographic distribution. In order to have more efficient public policies and management strategies to safe guard this species worldwide is crucial to increase fishing landing statistics, both in terms of geographic representation and data quality. Concomitantly, establishing MPAs in other areas beyond the Mediterranean Sea (while further advancing the MPA networks within this regional sea) would be highly beneficial to help conservation of this species along its geographic distribution. The feasibility of grouper population enhancement through the release of hatchery-reared or captured-from-the-wild post-larvae (once reaching a competent size) is still to be explored in future studies.

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Table 1 - Bibliographical review of the known parasites of wild dusky grouper *Epinephelus marginatus*; Study site, frequency of empty stomachs (Vacuity %), range of total lengths (TLs, mm), class sizes (ontogeny), number of the individuals analyzed of the stomach contents and stable isotope.

Reference	Study site	Vacuity (%)	TL (mm)	Ontogeny (TL, mm)	n Stomach Contents Analyzed	n Stable Isotope Analyzed
Smale (1986)	Southern Western Indian - South Africa	71.8	250-875	300-400; 401-500; 501-700	319	no
Azevedo et al. (1995)	Atlantic Ocean - Azores (Portugal)	33.8	-	40-70; 130-250; >360	71	no
Derbal and Kara (1996)	Mediterranean Sea - Algeria	48.5	160-980	no	68	no
Barreiros and Santos (1998)	Atlantic Ocean - Azores (Portugal)	38.6	600-1380	<900; >900	57	no
Reñones et al. (2002)	Western Mediterranean - Spain	56.1	144-1035	<300; 300-450; 450-600; >600	123	53
Linde et al. (2004)	Western Mediterranean - Spain	32.5	134-1056	<400; 401-500; 501-600; >600	203	no
López and Orvay (2005)	Western Mediterranean - Spain	16.6	0.325-20.88*	<3; 3-6; 6-9; 9-12; 12-15; 15-18; 18-21; >21*	139	no
Begossi and Silvano (2008)	Southeastern Western Atlantic - Brazil	34.4	-	<401; >400	61	no
Machado et al. (2008)	Southern Western Atlantic - Brazil	37.7	197-920	<400; 401-500; 501-600; >600	257	no
Condini et al. (2015)	Southern Western Atlantic - Brazil	48.3	150-1160	<351; 351-500; 501-650; >650	348	262

*Total size was described by weight (kg), such as to ontogeny classes.

Table 2 - Bibliographical review of the known parasites of wild dusky grouper *Epinephelus marginatus*; Study site, range of total lengths (TLs), number of specimens sampled (n) . Number of females, sexual transition and males collected, relative proportions of adults male and female (M:F), spawning season peaks and average female length at first maturity (L50).

Reference	Study site	LT range (mm)	n	Females (n)	Transitionals (n)	Males (n)	Sex ratio (M:F)	Spawning season	L50 (mm)
Brusle and Brusle (1975)	Mediterranean Sea - Tunisia	-	419	279	19	121	1:2.1	July-August	-
Kara and Derbal (1999)	Mediterranean Sea - Algeria	XX-795	-	-	-	-	-	July-August	570
Marino et al. (2001)	Southern Mediterranean - Italy	245-1050*	405	321	25	59	1:3.5	July-August	438*
Bertoncini et al. (2003)	Southern Western Atlantic - Brazil	197-1002	109	107	0	5	1:15.6	December	470
Fennessy (2006)	Southern Indian Western - South Africa	95-1100	395	352	1	42	1:5.5	December-January	622
Gerhardinger et al. (2006)	Southern Western Atlantic - Brazil	300-1000	193	190	0	3	1:46	November-December	460
Reñones et al. (2010)	Western Mediterranean - Spain	66-1056	399	346	10	43	1:7.4	July-August	491
Özen and Balcı (2011)	Eastern Mediterranean - Turkey	240-1110	104	77	8	19	1:3.5	July-September	391
Seyboth et al. (2011)	Southern Western Atlantic - Brazil	260-800	130	130	0	0	-	-	451
Condini et al. (2014)	Southern Western Atlantic - Brazil	278-1160	201	184	1	16	1:6.6	November-January	496

*Marino et al. (2001) used standard length (SL) in their study.

Table 3 - Bibliographical review of the known parasites of wild dusky grouper *Epinephelus marginatus*; Study site, aging structure, range of total lengths (TLs), age range, number of specimens sampled (n), and von Bertalanffy growth parameters of *E. marginatus* examined in this study and in previous investigations. Length-weight relationship, W = total weight ; $*W$ = gutted weight. Ageing structures used were scales (Sc), sectioned otolith (So), and whole otolith (Wo). Von Bertalanffy growth parameters included L_{∞} , the mean theoretical maximum TL; t_0 , the theoretical age at zero length; and K , the growth coefficient or the rate at which the curve approaches the asymptote.

Reference	Study site	Ageing structure	LT range (mm)	Age range (years)	n	L_{∞} (mm)	k (years ⁻¹)	t_0 (years)	Length-weight relationship ($W = a \cdot LT^b$)
Rafail et al. (1969)	Mediterranean Sea - Egypt	Sc	166-475	1-7	251	800	0.112	-1.08	$W = 0.0069LT^{3.222}$
Bouain (1986)	Mediterranean Sea - Tunisia	Sc	150-890	0-16	97	1850	0.028	-1.53	
Chauvet (1988)	Mediterranean Sea - Tunisia	Sc and Wo	53-1180	0-36	270	1144	0.093	-0.75	$*W = 0.01256LT^{3.073}$
Chalabi et al. (1992)	Mediterranean Sea - Algeria		250-900	1-8	60	1774	0.073	1.24	$W = 0.0000243LT^{2.971}$
Azevedo et al. (1995)	Atlantic Ocean - Azores (Portugal)	Sc	40-360	0-4	74				$W = 0.0139LT^{3.065}$
Kara and Derbal (1995)	Mediterranean Sea - Algeria	Sc	197-567	1-7	41	785	0.160	-0.73	$W = 0.0081LT^{3.140}$
Bouchereau et al. (1999)	Mediterranean Sea - France	Sc	200-1200	1-14	22	1359	0.080	-0.80	$W = 0.123LT^{2.60}$
Fennessy (2006)	Southern Western Indian - South Africa	So	95-1020	1-16	215	1249	0.090	-1.43	
Reñones et al. (2007)	Western Mediterranean - Spain	So	66 - 1056	0-61	358	955	0.087	-1.12	$W = 0.0098LT^{3.14}$
Seyboth et al. (2011)	Southern Western Atlantic - Brazil	So	260-800	2-12	108	1249	0.069	-1.49	
Ximenes-Carvalho et al. (2012)	Southeastern Western Atlantic - Brazil	Sc	115-705	1-14	135	1010	0.075	2.20	$W = 0.00008LT^{3.221}$
Condini et al. (2014)	Southern Western Atlantic - Brazil	So	150-1160	1-40	190	900	0.129	-1.45	$W = 0.0125LT^{3.094}$

Table 4 - Bibliographical review of the known parasites of wild dusky grouper *Epinephelus marginatus*; geographical location, identification of class and species of the parasite and position of the parasites in the body.

Reference	Study site	Class	Família	Parasite Species	Position
Oliver (1984)	Mediterranean Sea - France	Monogenea	Diplectanidae	<i>Cycloplectanum riouxi</i>	Gills
Oliver (1984)	Atlantic Ocean - Ireland	Monogenea	Diplectanidae	<i>Cycloplectanum riouxi</i>	Gills
Scholz et al. (1993)	Southern Mediterranean - Italy	Cestoda	Lacistorhynchidae	<i>Grillotia epinepheli</i>	Body cavity and Peritoneum
Santos et al. (2000)	Southeastern Western Atlantic - Brazil	Monogenea	Diplectanidae	<i>Pseudorhabdosynochus beverleyburtonae</i>	Gills
Bartoli et al. (2003)	Mediterranean Sea - France	Trematoda	Opecoelidae	<i>Podocotyle temensis</i>	Cephalic glands, testes, ovary, digestive and excretory systems
Merella et al. (2005)	Western Mediterranean Spain	Chromadorea	Philometridae	<i>Philometra jordanoi</i>	Gonads
Genc (2007)	Northeast Mediterranean Sea Turkey	Malacostraca	Gnathiidae	<i>Gnathia</i> sp.	Buccal cavity
Marzouk et al. (2010)	Mediterranean Sea - Egypt	Microsporea	Gluguidea	<i>Glugea anomala</i>	Gills
Roumbedakis et al. (2013)	Southeastern Western Atlantic - Brazil	Monogenea	Diplectanidae	<i>Pseudorhabdosynochus beverleyburtonae</i>	Gills
Roumbedakis et al. (2013)	Southeastern Western Atlantic - Brazil	Monogenea	Capsalidae	<i>Neobenedenia melleni</i>	Boby surface
Roumbedakis et al. (2013)	Southeastern Western Atlantic - Brazil	Trematoda	Opecoelidae	<i>Helicometrina nimia</i>	Stomach and intestine
Roumbedakis et al. (2013)	Southeastern Western Atlantic - Brazil	Chromadorea	Anisakidae	<i>Contracaecum</i> sp.	Mesentery
Roumbedakis et al. (2013)	Southeastern Western Atlantic - Brazil	Monogenea	Ancyrocephalidae	<i>Pseudempleurosoma</i> sp.	Stomach

9. Anexo II

Trophic ecology of dusky grouper *Epinephelus marginatus* (Actinopterygii, Epinephelidae) in littoral and neritic habitats of southern Brazil as elucidated by stomach contents and stable isotope analyses

Mario V. Condini^{1*}, David J. Hoeinghaus², Alexandre M. Garcia¹

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Trophic ecology of dusky grouper *Epinephelus marginatus* (Actinopterygii, Epinephelidae) in littoral and neritic habitats of southern Brazil as elucidated by stomach contents and stable isotope analyses

Mario V. Condini · David J. Hoeinghaus ·
Alexandre M. Garcia

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Abstract Groupers are large-bodied fishes with broad global distribution and commercial and ecological importance. Many grouper species are endangered, but information on trophic ecology of groupers outside the Mediterranean Sea is limited. We integrated stomach contents analyses (SCA) and stable isotope analyses (SIA) to test the hypotheses that diets of dusky grouper *Epinephelus marginatus* differ between littoral and neritic zones in the southwestern Atlantic, but exhibit similar ontogenetic shifts in prey size and consumption of fishes. SCA were dominated by fishes, crabs, and shrimp and differed significantly between habitats, but prey size and consumption of fishes increased with grouper body size at both sites. Grouper stable isotope ratios were similar between sites and among size classes, but integrating SCA and

SIA distinguished ingested versus assimilated dietary components, improved dietary resolution, and provided a more comprehensive assessment of grouper in the food webs. Dusky grouper integrate benthic and pelagic trophic pathways, as well as estuarine and marine food webs via consumption of migratory prey. Our findings have applications to other threatened grouper populations, for example, in managing fragmented populations or for reserve designs considering inclusion of artificial substrates as grouper habitat, and more broadly for approaches examining trophic ecology of generalist predators.

Keywords Food web · IUCN red list · Man-made habitats · Migration · Ontogenetic shifts · Subsidies

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M. V. Condini (✉) · A. M. Garcia
Laboratory of Ichthyology, Institute of Oceanography,
Federal University of Rio Grande, Av. Itália Km 8,
Carreiros, Rio Grande, RS 96.201-900, Brazil
e-mail: mvcondini@gmail.com

D. J. Hoeinghaus
Department of Biological Sciences and the Institute of
Applied Sciences, University of North Texas, 1155 Union
Circle #310559, Denton, TX 76203-5017, USA

Introduction

Groupers are large-bodied fishes of the families Serranidae and Epinephelidae with a broad global distribution and large commercial and ecological importance (Sadovy de Mitcheson et al., 2013). Groupers are targeted by artisanal, industrial, and sport fishing throughout their ranges (Heemstra & Randall, 1993; Craig et al., 2011) and often have strong influences on food webs and ecosystems through their roles as top predators (Heemstra & Randall, 1993; Sluka et al., 2001). Alterations to grouper populations can thus have significant commercial and ecological

consequences (Parrish, 1987; Brulé et al., 2005). Most grouper species exemplify a periodic life-history strategy (Winemiller, 2005) with slow growth rates, late maturation and a complex mode of seasonal reproduction including spawning aggregations and sexual reversal (Manooch & Mason, 1987), that combined with high fishing pressure leads to vulnerability and/or over-exploitation of many grouper populations (Huntsman et al., 1999; Coleman et al., 2000). One such example is the dusky grouper *Epinephelus marginatus* (Lowe, 1834), that like many other species of the family Epinephelidae, is included in the International Union for the Conservation of Nature (IUCN) red list as “endangered” (EN a2d), probably due to its complex life-history strategy and increasing fishing pressure (Cornish & Harmelin-Vivien, 2004).

The dusky grouper occurs along the southwestern Atlantic coastline with Argentina, Uruguay, and south-eastern Brazil (Figueiredo & Menezes, 1980; Irigoyen et al., 2005), throughout the British Isles and the Mediterranean Sea (Heemstra & Randall, 1993) and along the African coast to Mozambique in the extreme southeastern Atlantic (Fennessy, 2006). Dusky grouper typically inhabit rocky substrates up to 250 m depth but with a preference for shallower waters up to 50 m depth (Bruslé, 1985; Heemstra & Randall, 1993; Harmelin & Harmelin-Vivien, 1999). Similar to other grouper species (Beckley, 2000), dusky grouper juveniles are commonly found in the coastal zone, including tidal pools (Azevedo et al., 1995) and small bays in estuarine regions (MVC unpublished data). Rocky substrates are utilized as cover by this ambush predator and individuals may use different habitat structures and body positioning depending on grouper body size and what prey item is being sought (e.g., fishes vs. octopii; Barreiros & Santos, 1998). The dusky grouper is a monandric protogynous hermaphroditic species (Marino et al., 2001) and sexual transition can occur in individuals between 52 and 77 cm TL (Reñones et al., 2010). In extreme southern Brazil, individuals larger than 75 cm TL have usually undergone sexual transition (Condini et al., 2013, 2014).

Studies on the trophic ecology of dusky grouper are relevant for basic understanding of the ecology of the species, as well as insights into the trophic organization of the ecosystem and mechanisms affecting interactions among species (Herrán, 1988). Furthermore, such information is relevant for applications such as fisheries management and selection and monitoring of marine

protected areas (e.g., Reñones et al., 2002). That being said, there is very limited information on the trophic ecology of dusky grouper outside of the Mediterranean Sea (e.g. Machado et al., 2008; Begossi & Silvano, 2008; Condini et al., 2011). This is especially true for the southern Atlantic coast of Brazil, even though the dusky grouper is an important high-value species harvested by small-scale fisheries (Condini et al., 2007) and is broadly recognized for its cultural and conservation importance (e.g., gracing the Brazilian R\$100 banknote; Fig. S1). For groupers in general, most trophic ecology studies are restricted to stomach contents analyses (Grover, 1993; Brulé et al., 2005; Condini et al., 2011) and few studies utilize stable isotope analyses (Reñones et al., 2002; Nelson et al., 2012).

In this study, we integrate stomach contents and stable isotope analyses to describe the trophic ecology of dusky grouper at two locations along the southern Brazilian coast. Our objectives were to identify key prey species, test for ontogenetic shifts in diet and between males and females, and finally to compare trophic ecology of dusky grouper populations between natural and man-made habitats. We expect grouper diets to differ between sites, specifically with greater importance of estuarine-dependent prey for littoral grouper and marine-dependent prey for neritic grouper, but exhibit similar ontogenetic diet shifts of increasing prey size and greater dependence on fishes with increasing grouper body size at both sites. Carbon and nitrogen stable isotope values should complement the stomach contents data, specifically, we expect carbon stable isotope values of grouper to differ between sites due to differences in carbon sources supporting littoral and neritic food webs and grouper nitrogen stable isotope values to increase with increasing body size as larger individuals feed higher in the food web (i.e., consume greater biomass of fishes). This study contributes to the limited knowledge currently available for this endangered species in the southern Atlantic and has direct application to management and conservation activities for this region and elsewhere.

Materials and methods

Study area and field collections

This research was conducted at two locations on the southern Brazilian coast. The first location is in the

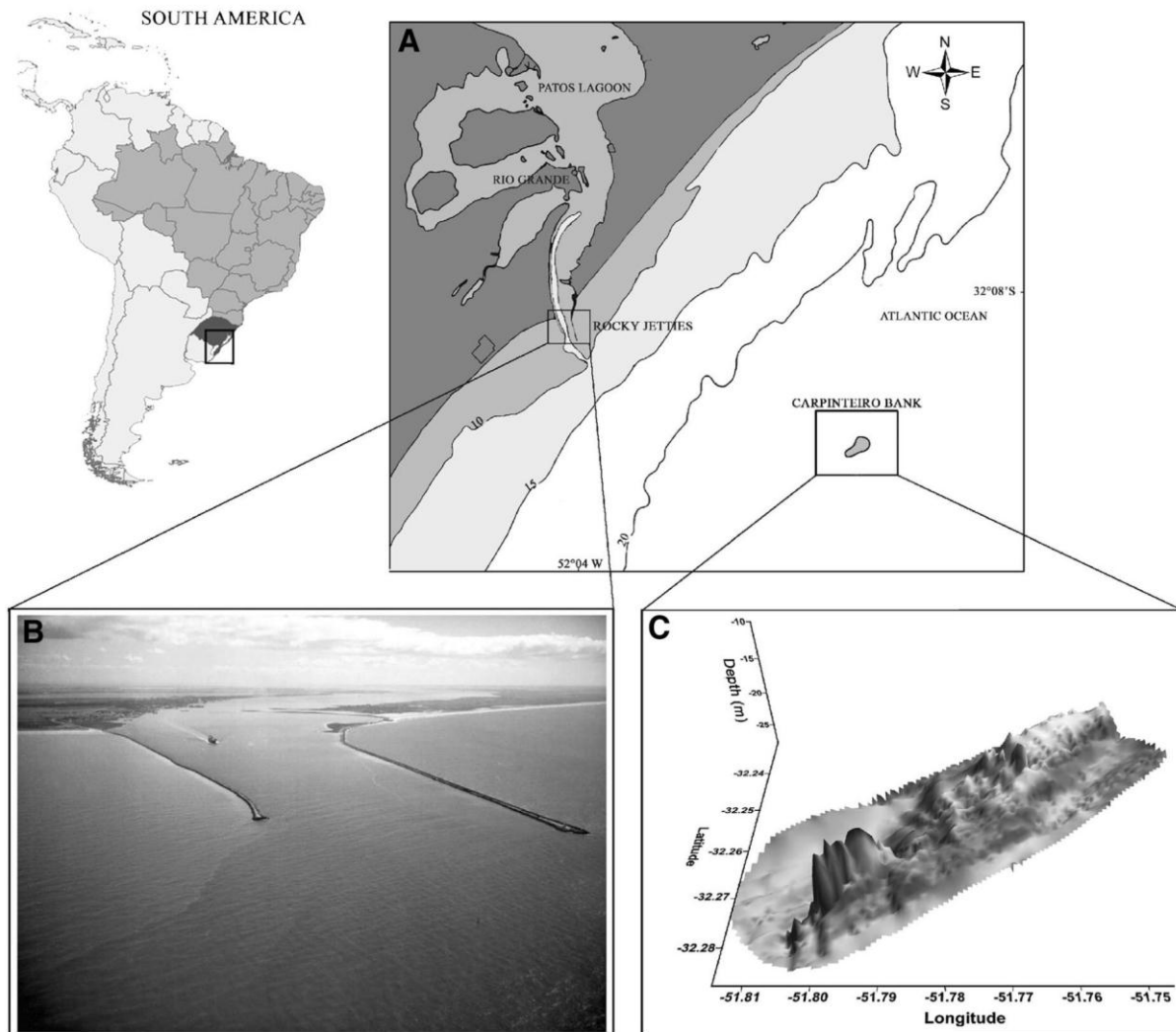


Fig. 1 Patos Lagoon (10,360 km²) and its estuarine zone in the state of Rio Grande do Sul, southern Brazil (A), showing the locations of the two sampling sites, the pair of rocky jetties at the

mouth of the lagoon (B), and Carpinteiro Bank in the adjacent marine area (C). The lines along the coast (A) denote 10, 15, and 20 m isobaths, respectively

littoral zone along a pair of rocky jetties (Molhes da Barra de Rio Grande) located at the mouth of Patos Lagoon Estuary (Fig. 1; 32°09'S, 52°05'W). The jetties were constructed at the beginning of the twentieth century to maintain the channel connecting Patos Lagoon with the southern Atlantic Ocean for navigation purposes. In addition to their logistical and strategic importance for the regional economy, the jetties constitute an important rocky habitat for a diverse benthic fauna (Capitoli, 1996), an area of reproduction for some crustacean species (Alaniz et al., 2011) and also serve as cover for some fish species (Condini et al., 2007). Due to its position at the

mouth of the estuary, this study location is characterized by varying hydrological conditions, primarily in regard to salinity (Möller et al., 2001). The second study location (Carpinteiro Bank) is a group of elevated submarine beach rocks in the neritic zone approximately 16 nautical miles from the coast and between 20 and 30 m depth (Fig. 1; 32°16'S, 51°47'W; Abreu & Calliari, 2005). The rocks are highly cemented by re-crystallized calcium carbonate and superficially colonized by bryozoans, sponges, crustaceans, and polychaetes (Buchmann et al., 2001). This study location experiences minor, if any, influence from the estuarine plume of Patos Lagoon and

average salinity is nearly constant year-round (Möller et al., unpublished dataset). Both locations represent rocky habitats within a coastal landscape that is otherwise dominated by homogeneous sandy substrates.

Field collections were conducted at both locations to obtain dusky grouper for stomach contents and stable isotope analyses, as well as potential diet items of dusky grouper and other components of the food web for stable isotope analyses. Dusky grouper were collected from the littoral zone between January 2008 and April 2009 in collaboration with regional artisanal fishermen using baited lines. Dusky grouper from the neritic zone were collected with the fishing fleet primarily between January 2010 and May 2011, with eight additional individuals collected in 2008 and 2009. An additional expedition to each study location was conducted in December 2010 to collect potential diet items of dusky grouper and other key components of the food web for stable isotope analyses. Stable carbon and nitrogen isotope values of the large-bodied consumers in this study have slow tissue turnover rates and thus reflect long-term feeding trends rather than episodic events, and as such we are confident in our use of samples from multiple expeditions. We also used conservative values for variability in our mixing models (see below) which should further reduce potential bias due to temporal variability in isotope values of diet items. In the littoral zone, fishes and crustaceans were collected using beach seines and minnow traps, mollusks and anemones were collected by hand, a beam trawl grab was used to collect polychaetes, and zooplankton were collected using a 30-cm-diameter plankton net with 500 μm mesh. Fishes and mollusks were captured from the neritic zone using benthic trawls, crustaceans, and polychaetes were captured using a van Veen grab, and zooplankton were collected using a 50-cm plankton net with 500 μm mesh. Samples from the littoral zone were transported on ice and frozen in the lab for subsequent processing, whereas samples from the neritic zone were frozen on the vessel during transport (Jardine et al., 2003; Garcia et al., 2007; Hoeinghaus et al., 2011).

Laboratory methodology and sample preparation

In the laboratory, each individual dusky grouper was measured for total length (TL, mm), weighed (g), and

eviscerated. The entire stomach was fixed in 10% formaldehyde for a period of 7 days then stored in 70% alcohol until analyzed. See Condini et al. (2011) for detail on methodology employed for stomach contents analyses. Briefly, each stomach was weighed before and after removing the contents. Numerical abundance, weight (0.01 g) and total length (TL, mm) were recorded for all diet items recovered, with prey TL measured only for items that were whole and not overly digested. Items encountered in the stomach contents were identified to the lowest taxonomic level and assigned to one of the following five broad categories: fishes, shrimps, non-blue crabs (all of the anomurans and brachyurans, but excluding portunids), blue crabs (all of the portunids), and mollusks. The distinction between non-blue crabs and blue crabs from stomach contents is facilitated by significant differences in size and weight of individuals between the two groups.

All samples for stable isotope analyses were processed in the laboratory following standardized procedures (Garcia et al., 2007; Hoeinghaus & Davis III, 2007; Hoeinghaus et al., 2011). Samples were from individuals, though combined samples comprising multiple individuals were used for zooplankton (Garcia et al., 2007; Hoeinghaus et al., 2011). Because isotopic values of crustacean exoskeletons reflect assimilated calcium carbonate derived from the environment, only pure muscle tissue was used for stable isotope analyses. Using a sterile scalpel, muscle tissue was dissected from the anterior-dorsal region from fishes, the abdominal cavity of crustaceans, the adductor muscle for bivalves, and tentacles of cephalopods. Polychaetes were processed whole. Zooplankton individuals were separated into taxonomic groups and processed whole as combined samples for each group. All samples were inspected to remove any non-muscle material (e.g., bone or scales in fish tissue), rinsed with distilled water, and dried in sterile Petri dishes in an oven at 60°C to constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar and pestle and stored in sterile glass vials. Sub-samples were weighed to the nearest 0.01 mg using an analytical balance, pressed into Ultra-Pure tin capsules (Costech Analytical, Valencia, California), and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen isotope ratios. Results are expressed in delta

notation (parts per thousand deviation from a standard material):

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000,$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The standard material for carbon is Pee Dee Belemnite (PDB) limestone, and the nitrogen standard is atmospheric nitrogen. Standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ replicate analyses of internal standards were 0.08 and 0.11‰, respectively.

Data analyses

A total of 348 dusky grouper, 133 from the littoral zone and 215 from the neritic zone, were examined for stomach contents analyses. Of these, 78 littoral zone and 113 neritic zone individuals had non-empty stomachs and are included in data analyses. Diet items and categories were quantified using frequency of occurrence (%F; the percent of non-empty stomachs in which the diet item or category was encountered), relative abundance (%N; total number in percent of a diet item or category in relation to the total number of items or categories encountered in non-empty stomachs), and relative weight (%W; total weight in percent of a diet item or category in relation to the total weight of items or categories encountered in non-empty stomachs) following Hyslop (1980). The above parameters were subsequently used to calculate the index of relative importance (%IRI; Pinkas et al., 1971) as: $\%IRI = \%F * [\%N + \%W]$. To evaluate size-related effects on diet, dusky grouper were separated into four size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and >650 mm TL) and %IRI was calculated for all diet categories for each size class for both study locations. In addition, composition and relative biomass of diet items in individual grouper stomach contents were compared between sites and among size classes using non-metric multidimensional scaling (NMDS) calculated with the Bray–Curtis index. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in diet between sites and among size classes based on the Bray–Curtis similarity index. Factors included in the PERMANOVA model were location, size class and the location*size interaction and statistics were based on 999 permutations using the ‘adonis’ function in the R package ‘vegan’ (Oksanen et al., 2013). Average prey size and biomass were compared between sites and

among size classes using ANOVA, with pairwise comparisons performed using Tukey’s post hoc test. Normality and homogeneity of variance were assessed using Kolmogorov–Smirnov and Cochran tests (Zar, 1994), respectively, and data were log-transformed prior to analyses to meet statistical assumptions. Diet data for the littoral zone were previously analyzed by Condini et al. (2011), though not for the same size classes used in this comparison with the new neritic zone data.

Stable isotope analyses are based on carbon and nitrogen isotopic ratios of 262 dusky grouper (72 from the littoral zone and 190 from the neritic zone) and 157 samples of various consumer taxa that represent potential dusky grouper diet items and other components of the food web (58 from the littoral zone and 99 from the neritic zone). Bi-plots of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of dusky grouper and all other samples were used to visualize patterns of isotopic variation within and between study locations. The relative importance of various sources of organic carbon assimilated by consumers is indexed by relative positions of consumers on the $\delta^{13}\text{C}$ axis, whereas trophic position is indicated by relative position on the $\delta^{15}\text{N}$ axis (Peterson & Fry, 1987; Fry, 2006). Analysis of variance (ANOVA) was used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of dusky grouper between sites and among size classes. When a significant main effect was observed, pairwise comparisons were performed using Tukey’s post hoc test.

High lipid content can affect $\delta^{13}\text{C}$ (i.e., yielding less enriched values) and in such cases, chemical lipid extraction or mathematical normalization can be used to control this effect (e.g., Jardine et al., 2003; Post et al., 2007). Samples with less than 5% lipid content, corresponding to C:N ratio less than 3.5, do not need to be corrected for lipids (Post et al., 2007). We did not extract lipids from our samples because chemical lipid extraction can have undesired effects on $\delta^{15}\text{N}$ values (especially when lipid content is low; Ingram et al., 2007), and mathematical correction was deemed unnecessary because >70% of our consumers had C:N <3.5 (the majority of those with higher C:N were not direct diet items of grouper and not included in mixing models, see below).

To estimate relative contributions of primary diet items to dusky grouper growth, we used the Bayesian mixing model SIAR (version 3.0.1; Parnell et al., 2010). Importantly, SIAR incorporates uncertainties associated with variability in trophic enrichment, even

in indeterminant systems (i.e., when $n_{\text{sources}} > n_{\text{isotopes}} + 1$), and both variability (i.e., standard deviation) and trophic enrichment factors are user defined. Considering the strong influence that these factors may have on model outcomes (e.g., Bond & Diamond, 2011), as well as known variability in enrichment among trophic groups, tissue types, and other factors (Caut et al., 2009; Wyatt et al., 2010), we opted for conservative estimates of trophic enrichment factors for both carbon and nitrogen (1.0 and 3.0‰, respectively) and as well for variability (standard deviation of 0.5) that includes the range of enrichment estimates and variability across a diverse taxa representing various trophic guilds (Post, 2002; Vanderklift & Ponsard, 2003). We evaluated the sensitivity of our model results to different enrichment factors and variability estimates and found that our results were robust to reasonable variation in these parameters. Conservative enrichment factors and variability estimates also allow for potential temporal variability in isotope values of consumers and prey among our sampling periods. Mixing models were calculated for the same grouper size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and >650 mm TL) at both sites as in the stomach contents analyses, and the pool of potential diet items was restricted to include only those species recovered from grouper stomachs or identified in previous studies (Reñones et al., 2002; Linde et al., 2004; López & Orvay, 2005; Machado et al., 2008) and grouped into the same classes as used with IRI analyses (i.e., non-blue crab, blue crab, shrimp, and fish) with the exception of mollusks which were excluded due to low sample size and limited importance in the diet. Our isotope data for shrimp from the littoral zone were complemented with five additional samples from the same study area (unpublished data provided by Renata Mont'Alverne), and non-blue crab isotope values for the littoral zone were conservatively estimated as the average values for invertebrates with similar trophic ecologies collected from the same site. Mixing model results are presented as the 95, 75, and 50% credibility intervals of diet categories for each grouper size class at both sites.

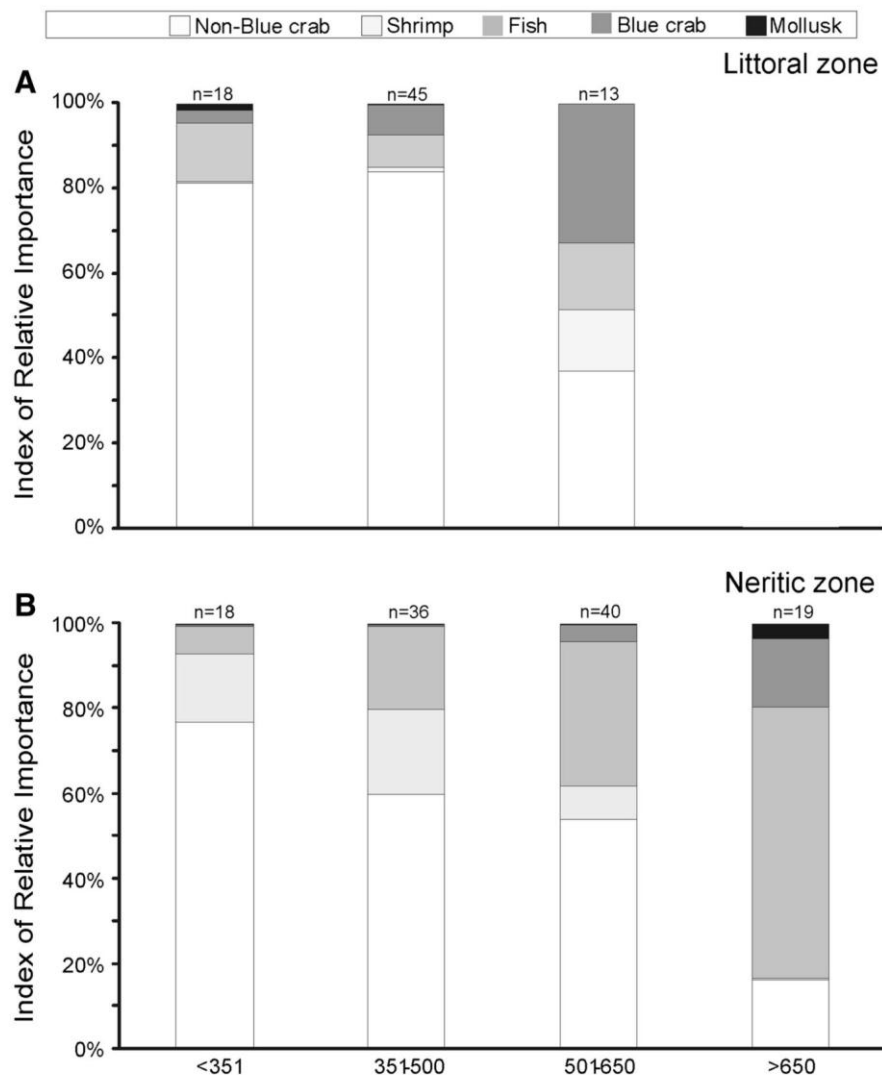
Results

Fifty-eight prey types classified at various taxonomic levels were identified from the stomach contents of

dusky grouper (Table S1). Of those, 31 and 38 prey types were recorded from stomachs of littoral zone and neritic zone samples, respectively, with only 12 shared prey categories (Table S1). Large numbers of shells, vertebrae, and otoliths were encountered but not identifiable to lower taxonomic levels due to the high degree of digestion. Non-blue crabs and fishes were the most important prey items at both sites, with blue crabs and shrimp also frequently encountered (Table S1). Based on %IRI, dusky grouper from the littoral zone had diets dominated by non-blue crabs (%IRI = 75.29) and complemented by fishes and blue crabs (%IRI = 12.56 and 9.86, respectively). In contrast, diet of dusky grouper from the neritic zone had a lower relative importance of non-blue crabs (%IRI = 52.40), increased relative importance of fishes (%IRI = 32.32) and included shrimp rather than blue crabs as the third most important category (%IRI = 10.38). The Cuban stone crab *Menippe nodifrons* and unidentified Brachyura had the highest %IRI values for non-blue crabs in the littoral zone, whereas unidentified Brachyura, unidentified Caridae, and unidentified Xanthidae had the highest %IRI values for non-blue crabs in the neritic zone. Most of the fishes encountered in stomach contents were unidentified, but the demersal whitemouth croaker *Micropogonias furnieri* was an important prey item for dusky grouper in the neritic zone (%IRI = 4.60) that was not found in stomachs of dusky grouper from the littoral zone (Table S1). Cannibalism was also recorded, with a dusky grouper of 985 mm TL consuming an individual of approximately 250 mm TL.

For both locations, %IRI for the major diet categories changed with increasing body size, most notably a decrease in importance of non-blue crabs and increase in blue crabs for the littoral zone (Fig. 2A) and an increasing importance of fish in the diet of dusky grouper from the neritic zone (Fig. 2B). Prey size and biomass increased with increasing dusky grouper body size at both sites, but the rate of increase was higher for littoral dusky grouper (all ANOVAs $P < 0.05$; Fig. 3). The large %N of shrimp in the diet of neritic dusky grouper contributed to a lower mean size and biomass of diet items for the 351–500 mm and 501–650 mm size classes compared with dusky grouper from those size classes in the littoral zone (Table S1; Fig. 2). NMDS of taxonomic composition and relative biomass of stomach contents distinguished dusky grouper primarily between sites (recognized as distinct groups along NMDS axis 1; Fig. 4)

Fig. 2 Index of relative importance (% IRI) for each of the five major dietary categories found in the stomach contents of dusky grouper *Epinephelus marginatus* in the littoral zone (A) and neritic zone (B). Data are summarized for four size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and >650 mm TL), and the respective numbers of individuals analyzed in each size class are provided above the bars



and subsequently among size classes (larger individuals from the neritic zone tend to have higher values on NMDS axis 2, whereas larger individuals from the littoral zone had higher values on axis 1, though there is much overlap of size classes for both locations; Fig. 4). PERMANOVA results were significant for both location ($F_1 = 6.5173$, $P = 0.001$) and size class ($F_3 = 1.3091$, $P = 0.030$), and marginally significant for the location by size interaction ($F_3 = 1.1990$, $P = 0.063$).

In contrast with the diet data, stable carbon and nitrogen isotope ratios of dusky grouper exhibited only minor differences between sites and among size classes (Figs. 5, 6). Mean $\delta^{13}\text{C}$ differed between sites ($P < 0.01$), though arguably not on an ecologically

relevant level (difference of approximately 0.2‰ between sites), and there was no difference between sites in $\delta^{15}\text{N}$ ($P > 0.90$). Weak trends were observed among size classes for dusky grouper at both sites, with higher variability in $\delta^{13}\text{C}$ and slightly increased $\delta^{15}\text{N}$ with increasing body size (Fig. 6). Isotopic values of large males from the neritic zone were similar to those of large females from the same region (Fig. 6).

At both sites, dusky grouper had carbon isotope values intermediate compared with $\delta^{13}\text{C}$ of other consumers in the food webs and nitrogen isotope values more enriched than all other sampled species, including other piscivorous fishes such as cutlassfish *Trichiurus lepturus* and bluefish *Pomatomus saltatrix*

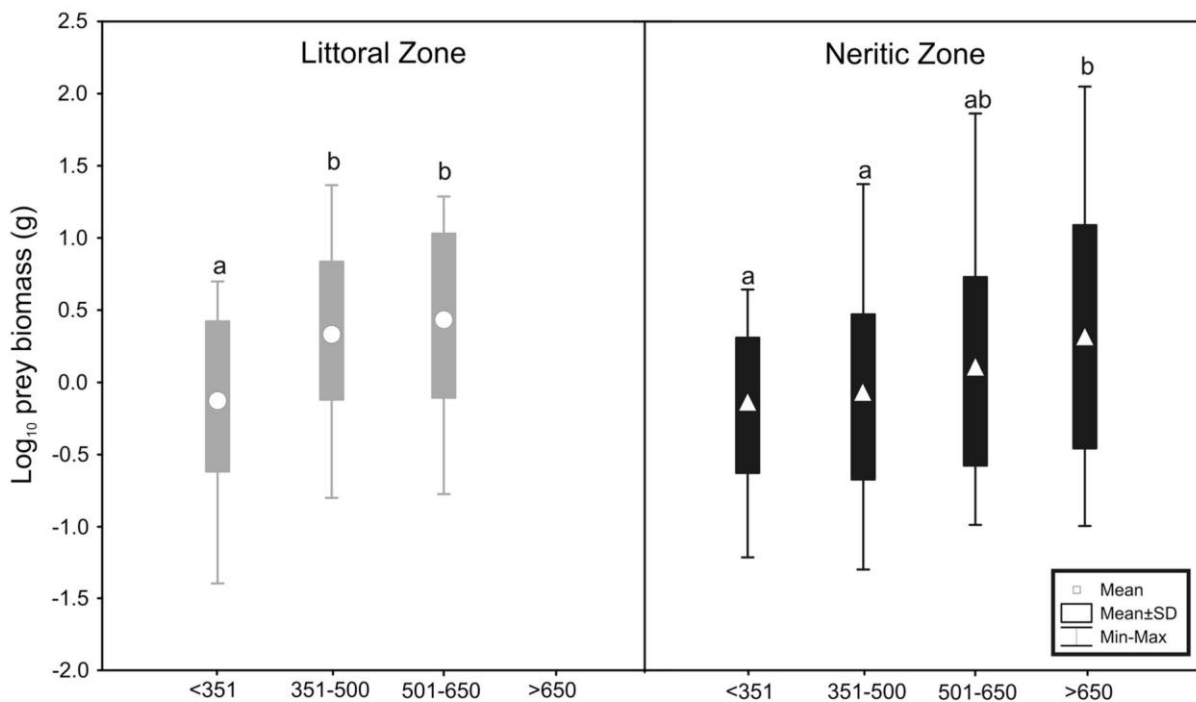
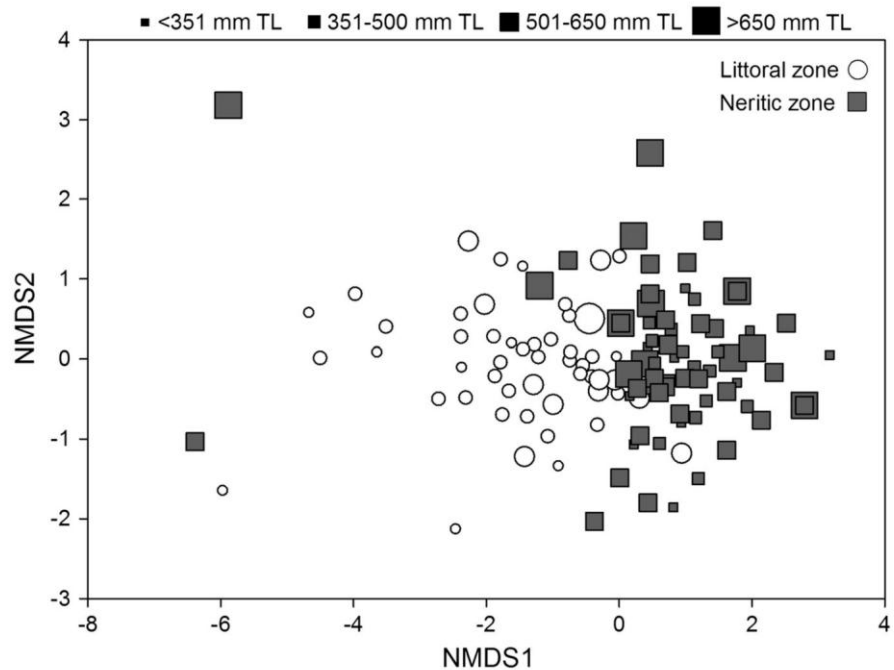


Fig. 3 Average values ($\pm$ SD) of prey biomass (g) in the stomach contents of dusky grouper *Epinephelus marginatus* in the littoral zone (A) and neritic zone (B). Data are summarized

for four size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and >650 mm TL), and shared letters above each box indicate non-significant differences among size classes

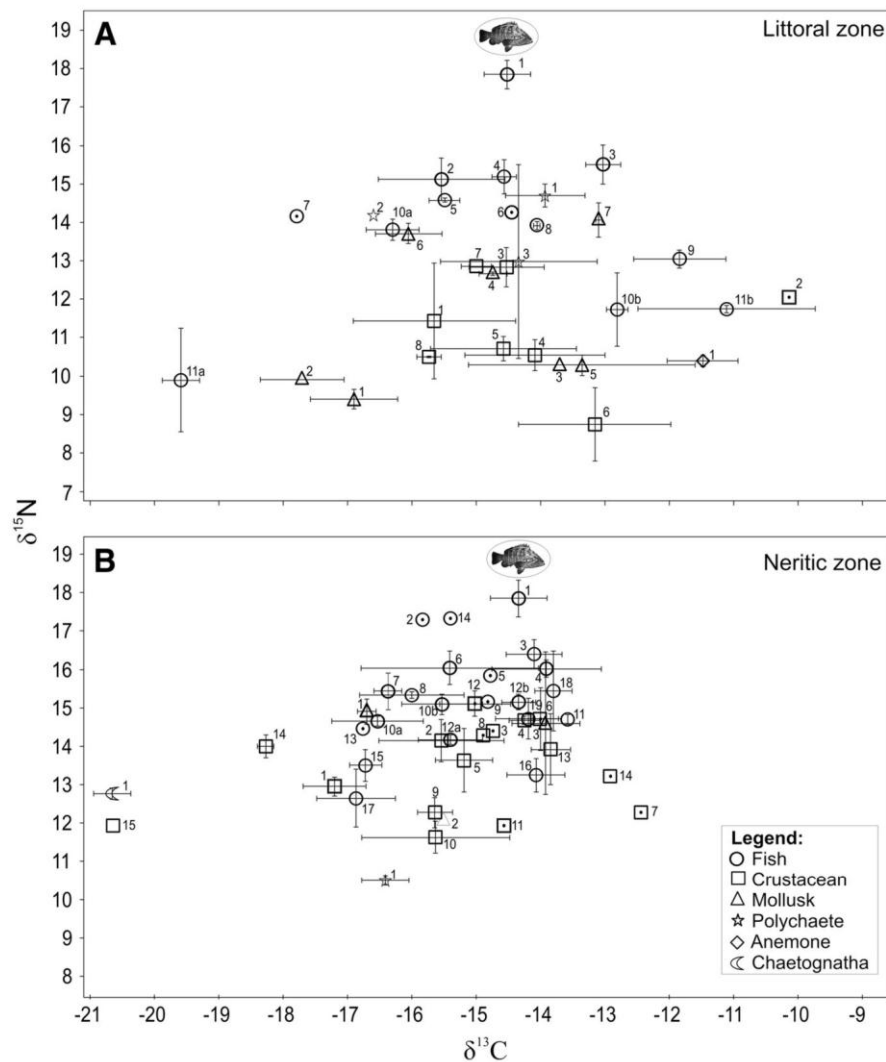
Fig. 4 Non-metric multidimensional scaling ordination (stress = 0.053) of dusky grouper diets based on prey relative biomass in littoral and neritic habitats along the southern Brazilian coast. Each point is an individual dusky grouper; symbol and shading denote location (=littoral zone; =neritic zone), and symbol size denotes size class (<351 mm TL, 351–500 mm TL, 501–650 mm TL and >650 mm TL)



collected from the neritic site (Tables S2 and S3; Fig. 5). At both sites, sciaenids (e.g., whitemouth croaker *Micropogonias furnieri*, Argentine croaker

Umbrina canosai, southern kingcroaker *Menticirrhus americanus*, barbel drum *Ctenosciaena gracilicirrus*, and striped weakfish *Cynoscion guatucupa*) and

Fig. 5 Carbon and nitrogen stable isotope bi-plots (mean $\pm$ SD) of all individuals collected in the littoral (A) and neritic (B) zones. Symbols identify major taxonomic groups, as indicated in the inset legend in B. Numbers identify species listed in Table S2 (littoral) and Table S3 (neritic); dusky grouper are fish species 1 in both bi-plots



other benthic predators (e.g., gobies, skates, predatory polychaetes) had nitrogen isotope values approximately 2–2.5‰ depleted compared with dusky grouper, whereas nitrogen isotope values of crabs (both non-blue crabs and blue crabs) and shrimp were approximately 6‰ more depleted than grouper.

Stable isotope mixing models yielded slightly different patterns of relative importance of primary diet categories than observed in stomach contents. For grouper from the littoral zone, models identified fishes as the dominant prey category across all size classes, with 95% credibility intervals of 57–84, 77–88, and 74–90% contribution in size classes <351 mm, 351–500 mm, and 501–650 mm, respectively (Fig. 7A–C). Blue crabs and non-blue crabs, in that order, completed

most of the rest of the estimated percent contribution to littoral grouper diets, and 95% credibility intervals for both decreased with increasing grouper size, concomitant with increasing contribution of fishes. In contrast, shrimp and fishes were the dominant sources identified by mixing models for grouper from the neritic zone, with shrimp decreasing in importance with increasing grouper size (95% credibility intervals for grouper <351 mm = 30–58%, and 18–46% for grouper >650 mm) and fishes becoming the most important diet category in the largest grouper size class (95% credibility interval 0.42–0.68; Fig. 7E–H). When separating the ‘fishes’ diet category into benthic omnivores and piscivores, 95% credibility intervals for benthic omnivores were 36–48% compared with 12–22% for

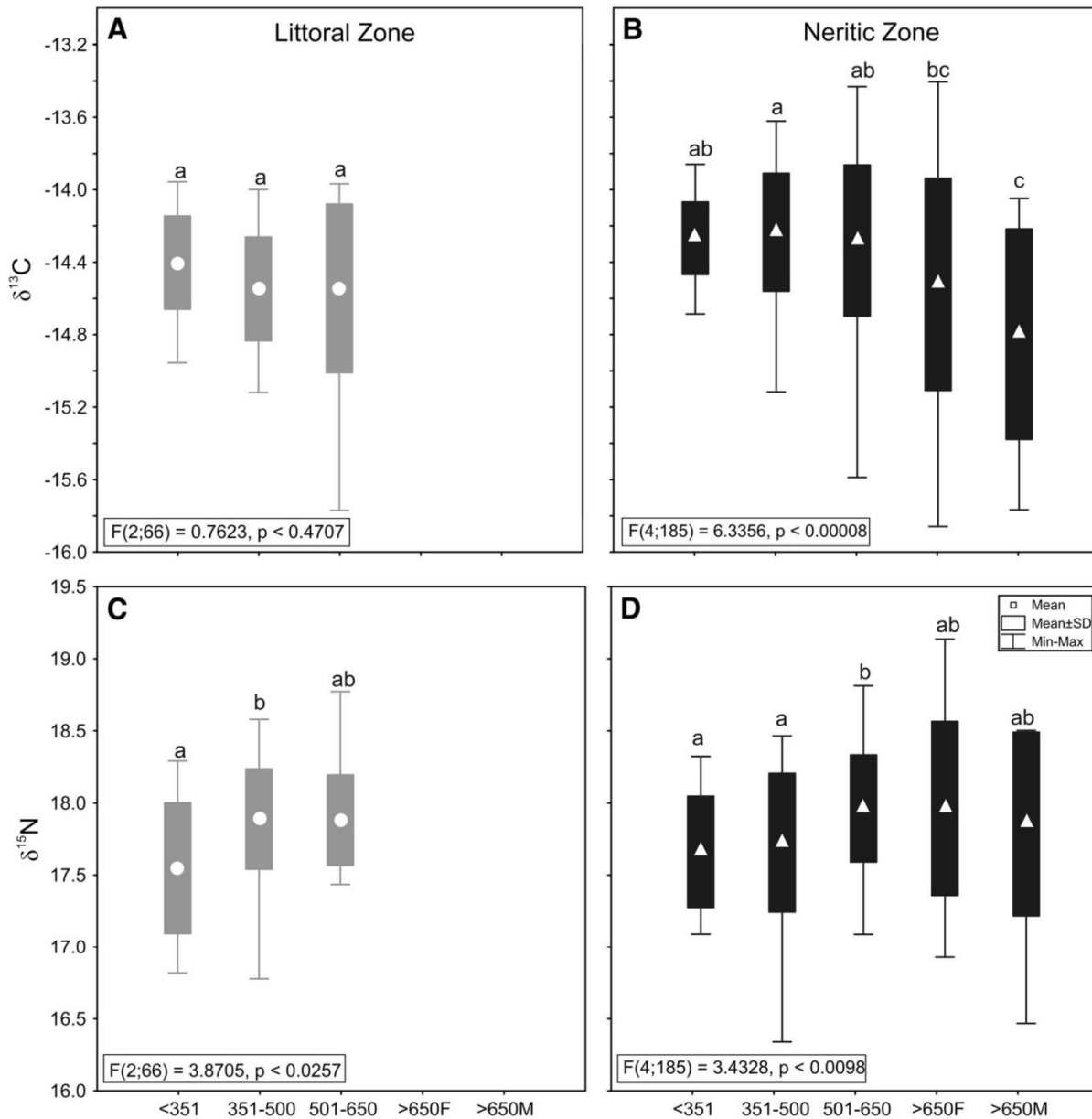


Fig. 6 Mean values ($\pm$ SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the dusky grouper *Epinephelus marginatus* in the littoral zone (A and C) and neritic zone (B and D). Data are summarized for four size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and

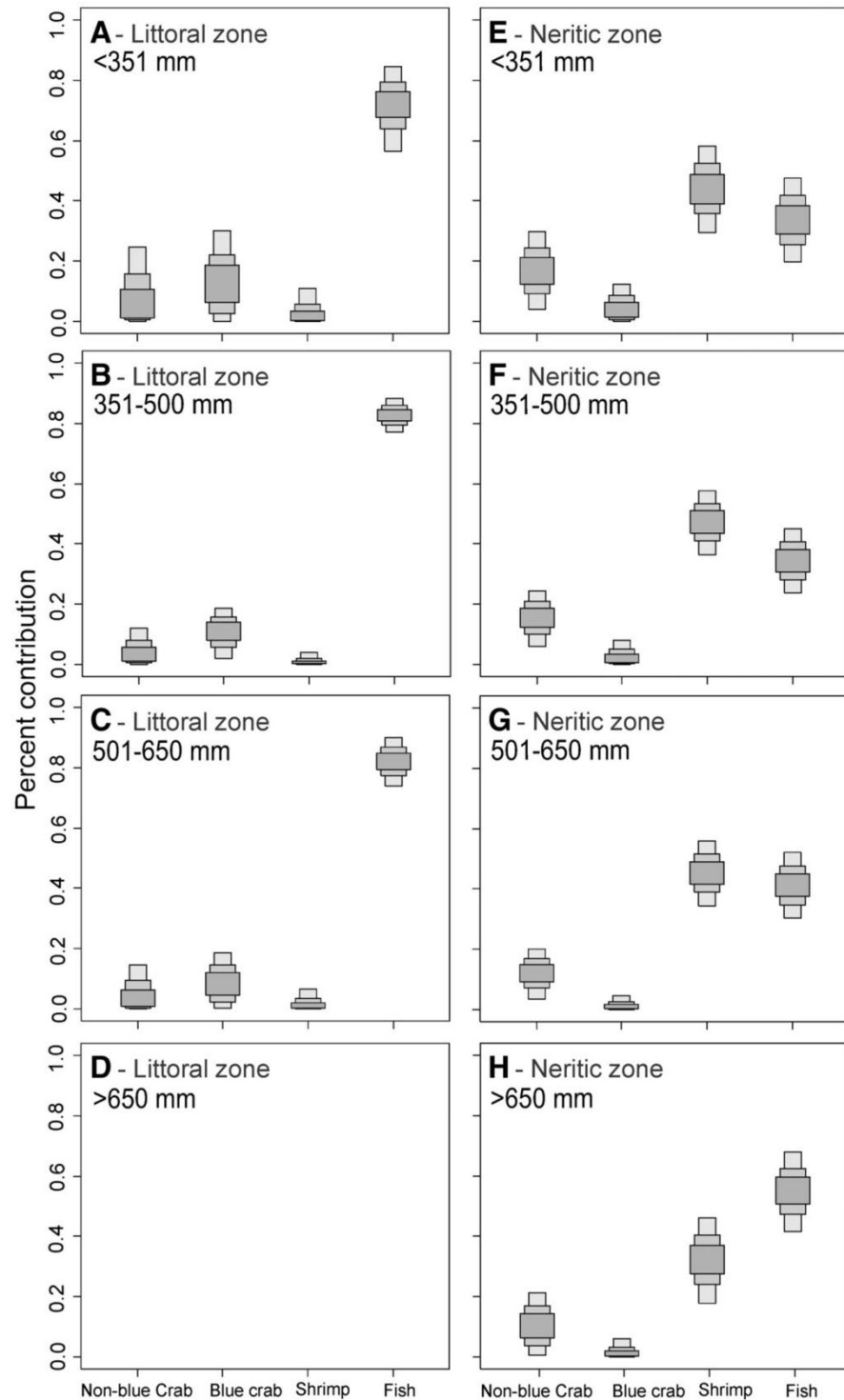
>650 mm TL), with males and females presented separately for the largest size class in the neritic zone. Shared letters above each box indicate non-significant differences among size classes

piscivores. Non-blue crabs contributed between 6 and 30% (lower and upper limits of the 95% credibility intervals across all grouper size classes), slightly decreasing in importance with increasing grouper size (Fig. 7E–H).

Discussion

Stomach contents analyses of dusky grouper from the littoral and neritic zones of southern Brazil indicated generalist predatory behavior with diets dominated by

Fig. 7 Results of SIAR mixing models showing estimated percent contribution (with 95, 75, and 50% credibility intervals) of the sources to the dusky grouper collected in the littoral (A–D) and neritic (E–H) zones. Data are summarized for four size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and >650 mm TL)



fishes, crabs, and shrimp at both sites but with a large diversity of other prey types also consumed to a lesser degree (e.g., octopi, mussels, and snails). Ontogenetic

dietary shifts were observed for grouper at both sites, from diets dominated by comparatively small non-blue crabs to increased importance of larger prey such

as blue crabs and fishes for grouper from the littoral and neritic zones, respectively, with increasing body size. Stable isotope analyses also indicated grouper at these sites are generalist upper trophic-level predators, with intermediate values for $\delta^{13}\text{C}$ and the highest observed values for $\delta^{15}\text{N}$. Stable isotope values for grouper did not differ appreciably between sites, but mixing models indicated differences in percent contribution of primary prey categories between sites, as well as among size classes for grouper from the neritic zone. Mixing models indicated a higher percent contribution of fishes to grouper growth than identified based on stomach contents analyses.

Similar to previous studies that integrated stomach contents and stable isotope analyses (e.g. Winemiller et al., 2011; Davis et al., 2012), the two approaches were complementary and the combination provided a more robust understanding of grouper diet and its role in the food webs of our study sites. Specifically, stable isotope data indicate that unidentifiable fish species in the stomach contents were likely mostly sciaenids such as whitemouth croaker, Argentine croaker, southern kingcroaker, and barbel drum. Consumption of these predatory taxa yields the observed grouper isotope values when combined with other known important diet items (i.e., crabs and shrimp) that had significantly more depleted nitrogen isotope values. Furthermore, isotope mixing models indicated that fishes are far more important to assimilated diets than suggested by stomach contents analyses alone. This difference is likely due to relatively greater amount of refractory material in crabs (e.g., carapace) versus shrimp and fishes, such that crabs may be more frequently encountered in stomach contents due to slower digestion rates but also contribute less (per unit ingested biomass) to assimilated energy. Identification of differences between ingestion and assimilation is arguably only feasible when combining stomach contents and stable isotope analyses, and is especially relevant when examining the trophic ecology of generalist/opportunistic predators that consume diverse prey types. On the other hand, stomach contents analyses provided resolution on ontogenetic diet shifts, particularly related to prey size and biomass, and differences in the relative frequency of consumption of non-blue crabs, blue crabs, and shrimp between sites and size classes that would not have been apparent from stable isotope analyses alone due to the relative similarity in carbon isotope values for

these prey types and limited assimilation. Although mixing models indicated that crabs were of only minor importance to material assimilated by grouper, the large numbers of species and individuals identified from stomach contents analyses suggest that grouper may have strong top-down effects on crab populations even if their consumption does not contribute significantly to grouper growth.

The high frequency of occurrence of crabs and fishes in diets of dusky grouper from both the littoral and neritic zones in this study is similar to findings by Machado et al. (2008) from other coastal zones in Brazil. Research in the Mediterranean (Reñones et al., 2002; Linde et al., 2004; López & Orvay, 2005) also detected high frequency of occurrence of these prey types, along with high frequency of cephalopods, principally octopuses of the genus *Octopus* and common cuttlefish *Sepia officinalis*. The importance of cephalopods in dietary studies of dusky grouper from the Mediterranean, in contrast with studies from the Atlantic (Machado et al., 2008; this study), is likely mediated by water depth. Specifically, the most common cephalopod species found in southeastern Brazil (e.g., *Illex argentinensis*, *Doryteuthis plei*, *D. sanpaulensis*, *Octopus vulgares*, and *O. tehuatlchus*) are generally encountered in deeper waters (Haimovici & Álvarez-Perez, 1990; Andriguetto & Haimovici, 1996; Santos & Haimovici, 2002) than the grouper populations sampled in this study.

Previous studies on dusky grouper identified ontogenetic diet shifts, transitioning from consumption of crustaceans at smaller sizes to gastropods and fishes for larger size classes (e.g., Reñones et al., 2002; Linde et al., 2004; Machado et al., 2008). Kulbicki et al. (2005) studied the diets of ten *Epinephelus* species and found that, in general, smaller individuals consumed mostly crabs, whereas larger individuals preferentially consumed fishes and mollusks. Ontogenetic diet shifts were also observed in this study, but with an increased importance of blue crabs for larger grouper from the littoral zone and fishes in the neritic zone. The difference in the importance of blue crabs in grouper stomach contents between sampling locations is likely influenced by higher densities of blue crabs in the littoral zone, especially as large numbers of adult female blue crabs leaving Patos Lagoon Estuary to spawn in higher salinity offshore waters must pass through this location as it provides the only connection between the estuary and the ocean. Linde et al. (2004)

suggested that ontogenetic diet shifts in grouper are associated with a change in foraging strategies, switching from an active predator preferring smaller prey (e.g., crabs) to an ambush predator targeting larger prey (e.g., fishes and cephalopods). These previous studies also noted a tendency for prey to increase in length or biomass with increasing grouper size, a pattern observed for both the littoral and neritic zones in this study. Integration of the isotope data suggests that consumption of crabs, especially by larger grouper, may be of minor importance to grouper growth. Even as such, crabs likely still provide an important link in carbon flow as prey for fishes such as whitemouth croaker that are consumed by grouper and contribute significantly to assimilated carbon.

As cited above, previous research on *Epinephelus* compared diets among species and or size classes, but information is lacking for variation in diet along a depth gradient or between comparatively shallow and deep sites. Our findings suggest that feeding behavior of dusky grouper is similar between littoral and neritic zones (in terms of general prey types and sizes), but with clear differences in the taxonomic composition of prey species. This difference in prey composition is expected to be the result of different environmental conditions between sites, as affecting prey composition and abundance, rather than intrinsic differences in foraging behavior of grouper at these sites. Specifically, the littoral site is strongly influenced by the surf zone and continental discharge from Patos Lagoon, and these conditions favor a unique suite of species such as the crabs *Menippe nodifrons* and *Callinectes danae*, whereas other species inhabit deeper marine waters, such as *Octopus* spp. and the hermit crab at the neritic study location.

Even though carbon isotope values of grouper were similar between sites, the basal carbon sources supporting secondary production of dusky grouper are expected to differ. The littoral site is strongly influenced by discharge from Patos Lagoon Estuary, and important basal carbon sources likely include saltmarsh vegetation, seagrass, macroalgae, and phytoplankton (Garcia et al., 2007; Hoeinghaus et al., 2011; Claudino et al., 2013). The neritic zone is dominated by marine influences and important basal carbon sources at this site are expected to be macroalgae, primarily Rhodophyta (Horta et al., 2008), and marine phytoplankton. Phytoplankton typically vary in $\delta^{13}\text{C}$ along salinity gradients, with values being

more depleted as the relative contribution of DIC of freshwater origin increases (Peterson et al., 1994; Canuel et al., 1995; Bouillon et al., 2000; Kaldy et al., 2005), and saltmarsh vegetation can include both comparatively depleted C_3 plants and enriched C_4 plants depending on hydrogeomorphology of the estuarine zone (Hoeinghaus et al., 2011). Due to differences in composition and relative abundance of carbon sources, and importantly carbon isotopic values of the sources, we expected that dusky grouper collected at the neritic site would have more depleted carbon isotopic values than their littoral counterparts and that carbon isotopes could thus be useful as a tracer of origin or site fidelity of dusky grouper along our coastal zone (e.g., as in Hobson, 1999; McCarthy & Waldron, 2000; Harrod et al., 2005). Contrary to expectations, sampled species at both sites were relatively enriched in $\delta^{13}\text{C}$, ranging between -20 and -10‰ . This range is consistent with marine algae providing the depleted end-member and more enriched values may include macroalgae for the neritic site, with the addition of seagrass and C_4 saltmarsh vegetation such as *Spartina alterniflora* for the littoral site (both likely through detrital pathways; Garcia et al., 2007; Hoeinghaus et al., 2011). The intermediate isotopic values of dusky grouper suggest that they integrate both marine pelagic and benthic food web pathways, with benthic carbon sources and consumers often having more enriched carbon isotope values than pelagic sources and consumers (France, 1995; Bergamino et al., 2011; Claudino et al., 2013). Isotopic analyses of dusky grouper in the Mediterranean also provided evidence of strong linkages to the benthic food web (Reñones et al., 2002). An alternative hypothesis is that dusky grouper are moving extensively between sites resulting in similar carbon isotope values for individuals at both sites. However, dusky grouper, like other grouper species, are known to be territorial and make only very limited movement during the year (e.g. Lembo et al., 2002; Pastor et al., 2009), with the exception of reproductively active adults specifically during the reproductive period (Zabala et al., 1997). Thus, it is not likely that all size classes of grouper are moving between sites with sufficient frequency to result in isotopic values that are considered a mixture from feeding at both sites.

It is important to further consider the role of animal movement on carbon dynamics and sources supporting secondary production of dusky grouper, especially

for the littoral zone study site. The littoral zone site receives not only passive transport of organic materials due to discharge from Patos Lagoon, but also is situated at the only access point through which estuarine or coastal organisms may move between systems. Animal movements across these ecosystems may provide unique carbon subsidies when an organism grows in one location and is consumed in another (e.g., Connolly et al., 2005; Garcia et al., 2007; Oliveira et al., 2014). Due to the nursery function of the estuary for many consumer species, such movements among estuarine and coastal zones are often tied to reproductive behaviors necessary to complete an organism's life-cycle, and previous research on coastal systems in this region identified significant differences in carbon isotopic values among reproductive guilds based on utilization of coastal and inshore environments (Garcia et al., 2007; Hoeinghaus et al., 2011). As mentioned above, the movement of adult female blue crabs from estuarine to higher salinity coastal zones for spawning may represent a subsidy of carbon predominantly derived from detrital *Spartina alterniflora* to coastal food webs (Hoeinghaus & Davis III, 2007). Another example can be seen in the carbon isotope signatures of the mullet *Mugil liza*. This species is a catadromous estuarine dependent, utilizing the coastal and estuarine zone for growth and migrating offshore for reproduction (Vieira, 1991). Juvenile mullets less than 30 mm TL occur in the marine environment where they feed on phytoplankton and zooplankton prior to moving inland, where larger mullets occur in the estuarine zones and shift to a diet based more heavily on detritus (Acha, 1990; Vieira, 1991). Our data from the littoral zone reflect this difference, with juvenile mullets (25 mm TL) having a mean $\delta^{13}\text{C}$ of -19.59‰ (carbon derived from marine phytoplankton) and adults (>120 mm TL) with a mean $\delta^{13}\text{C}$ of -11.11‰ (estuarine detritus). Both of these examples support interpretations of the enriched end-member of the littoral zone being at least in part comprised by estuarine-derived carbon sources such as detritus from C_4 saltmarsh grasses.

Through their role as upper trophic-level predators, grouper can exhibit strong effects on food webs of the ecosystems where they occur (Heemstra & Randall, 1993; Sluka et al., 2001). Reñones et al. (2002), studying dusky grouper populations in the Mediterranean, observed a strong correlation between $\delta^{15}\text{N}$ and

body size, corroborating the increase in trophic position with increasing body size identified from stomach contents analyses. The authors suggest that because of this correspondence between diet and isotopes, in particular the positive correlation between $\delta^{15}\text{N}$ and body size, stable isotope analyses can be particularly useful for studies in marine protected areas where fishes cannot be collected for stomach contents analyses. Unfortunately, we observed only very minor shifts in $\delta^{15}\text{N}$, even though ontogenetic dietary shifts were detected using both stomach contents and stable isotope analyses. This is likely due to grouper feeding across trophic levels at all size classes and may also reflect different $\delta^{15}\text{N}$ baselines for pelagic and benthic carbon sources. The complex nature of these combinations precludes strong shifts in $\delta^{15}\text{N}$, even though ontogenetic diet shifts were detected for prey composition and size. Our results suggest that stomach contents analyses and stable isotope analyses are highly complementary for elucidating the trophic ecology of grouper, but that it may be premature to rely solely on stable isotope analyses in the study of protected populations. When possible, it seems prudent to first validate that strong correspondence exists between ontogenetic diet shifts and stable isotope ratios (e.g., as observed in Reñones et al., 2002).

Previous research from our study system on dusky grouper age, growth, and reproduction (Seyboth et al., 2011; Condini et al., 2014) suggests that elevated beach rocks in the neritic zone are better quality habitat than the man-made rocky jetties that provide littoral habitat. Barreiros & Santos (1998) suggest that the small size of caves or crevices in the rocky jetties, rather than other factors such as prey availability, may limit the occurrence of adult dusky grouper at this site. Due in part to the lack of adults (especially males) in the littoral zone and the relative proximity of the two sites, these two rocky habitats may be part of a single metapopulation with source-sink dynamics, where the neritic population (source) exports juveniles to the littoral population (sink). Although carbon and nitrogen stable isotopes alone were not useful as tracers of habitat use between the littoral and neritic zones, inclusion of a third isotope such as sulfur could provide further resolution for quantifying connectivity between these two locations (Fry & Chumchal, 2011; Nelson et al., 2012). In addition to understanding the trophic bases of secondary production, as investigated in this study, clarification of spatial and temporal

dynamics of population connectivity is critical for protection of this endangered species, especially considering that it is targeted by both littoral and offshore fisheries. The populations in natural and man-made habitats examined here provide a relevant case study for applications to other threatened grouper populations around the world, for example, as declining populations and habitat loss result in fragmented populations or if conservation strategies are considering inclusion of artificial substrates as part of protected area or reserve designs.

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Supplemental Figure 1 Front (A) and back (B) of the Brazilian R\$100 banknote depicting the dusky grouper *Epinephelus marginatus* on the back of the banknote. Images from the Central Bank of Brazil (Banco Central do Brasil; www.bcb.gov.br).

Supplemental table 1 - Numerical abundance (%N), weight (%W), frequency of occurrence (%F) and Index of Relative Importance (%IRI = %F *[%N+%W]) for each food item found in the stomach contents of dusky grouper (*Epinephelus marginatus*) captured in littoral zone (n = 78) and the neritic zone (n = 113) of the southern Brazilian coast.

Prey items	Littoral zone				Neritic zone			
	%F	%N	%W	%IRI	%F	%N	%W	%IRI
Mollusca								
Gastropoda								
Columbellidae								
<i>Anachis isabellei</i>	1.30	0.56	0.05	0.03				
<i>Anachis spp</i>	2.60	2.22	0.23	0.25				
Gastropoda unidentified					3.54	2.00	0.14	0.32
Bivalvia								
Mytilidae								
<i>Mytilus edulis platensis</i>	2.60	1.11	0.00	0.11				
<i>Perna perna</i>	1.30	0.56	0.08	0.03				
Ostreidae								
<i>Ostrea equestris</i>	1.30	0.56	0.03	0.03				
Bivalve unidentified	2.60	1.11	0.18	0.13	3.54	1.60	0.46	0.31
Cephalopoda								
Octopodidae								
<i>Octopus vulgaris</i>					0.88	0.40	0.07	0.02
<i>Octopus tehuelchus</i>					0.88	0.40	4.04	0.17
Arthropoda								
Maxillopoda								
Balanomorpha								
Balaenidae unidentified					0.88	0.40	0.01	0.02
Malacostraca								
Anomura								
Porcellanidae								
<i>Pachycheles chubutensis</i>	1.30	0.56	0.26	0.04				
Porcellanidae unidentified					0.88	0.40	0.07	0.02
Diogenidae								
<i>Loxopagurus loxochelis</i>					0.88	0.40	0.16	0.02
Brachyura								
Majidae								
<i>Libinia spinosa</i>	5.19	2.78	0.77	0.72				
<i>Podochela gracilipes</i>					0.88	0.40	0.21	0.02
Majidae unidentified					5.31	2.80	0.58	0.76
Xanthidae								
<i>Eurypanopeus abbreviatus</i>	5.19	2.22	6.02	1.66	2.65	1.60	2.61	0.47
<i>Hexapanopeus schmitti</i>	3.90	1.67	0.34	0.30	0.88	0.40	0.07	0.02
<i>Hexapanopeus caribbaeus</i>	1.30	0.56	0.03	0.03				
<i>Menippe nodifrons</i>	18.18	12.78	21.33	24.10				

<i>Panopeus austrobesus</i>	6.49	3.89	3.98	1.98				
<i>Panopeus bermudensis</i>					3.54	2.40	0.85	0.49
<i>Eurytium limosum</i>					0.88	0.40	1.77	0.08
<i>Pilumnus caribaeus</i>					5.31	3.20	1.77	1.12
<i>Rhithropanopeus harrisi</i>					0.88	0.40	0.10	0.02
Xanthidae unidentified	7.79	3.89	1.10	1.51	14.16	10.00	10.91	12.56
Varunidae								
<i>Cyrtograpsus altimanus</i>	1.30	0.56	0.03	0.03				
<i>Cyrtograpsus angulatus</i>					7.08	5.60	2.47	2.42
<i>Neohelice granulata</i>					1.77	1.60	0.70	0.17
Varunidae unidentified					0.88	0.40	0.10	0.02
Pilumnidae								
<i>Pilumnus dasypodus</i>	5.19	2.78	0.50	0.66				
Portunidae								
<i>Arenaeus cribrarius</i>	1.30	0.56	0.06	0.03				
<i>Callinectes danae</i>	9.09	7.22	11.26	6.53				
<i>Callinectes sapidus</i>	12.99	7.22	15.67	11.55	2.65	1.20	13.01	1.60
<i>Callinectes</i> unidentified	5.19	2.22	1.32	0.71	3.54	2.80	2.73	0.83
<i>Portunus spinimanus</i>					2.65	2.00	0.73	0.31
Portunidae unidentified					5.31	4.80	1.54	1.43
Brachyura unidentified	23.38	13.89	9.34	21.10	23.01	11.20	6.40	17.18
Caridea								
Hippolytidae								
<i>Lysmata wurdemanni</i>	3.90	1.67	0.44	0.32				
Caridae unidentified	6.49	6.11	1.93	2.03	20.35	13.20	2.39	13.46
Dendrobranchiata								
Penaeidae								
<i>Farfantepenaeus paulensis</i>					1.77	1.60	0.41	0.15
Penaeidae unidentified					10.62	5.60	1.30	3.11
Solenoceridae								
<i>Pleoticus muelleri</i>	3.90	3.33	1.07	0.67				
Chordata								
Actinopterygii								
Epinephelidae								
<i>Epinephelus marginatus</i>					0.88	0.40	11.48	0.45
Ariidae								
<i>Genidens barbus</i>	1.30	1.67	4.20	0.30				
<i>Genidens</i> unidentified					0.88	0.40	0.29	0.03
Labridae								
Labridae unidentified					1.77	1.20	0.58	0.13
Sciaenidae								
<i>Micropogonias furnieri</i>					6.19	2.80	14.71	4.60
<i>Umbrina canosai</i>	1.30	0.56	0.02	0.03	0.88	0.40	0.09	0.02
Engraulidae								
<i>Lycengraulis grossidens</i>	1.30	0.56	3.37	0.20				

Batrachoididae								
<i>Porichthys porosissimus</i>	1.30	0.56	0.17	0.04	0.88	0.80	2.15	0.11
Phycidae								
<i>Urophycis brasiliensis</i>					0.88	0.40	0.02	0.02
Gobiidae								
<i>Gobionellus</i> unidentified	1.30	0.56	0.05	0.03	0.88	0.40	0.13	0.02
Blenniidae								
<i>Hypleurochilus fissicornis</i>	6.49	2.78	1.90	1.18				
Pleuronectiformes unidentified					0.88	0.40	0.26	0.02
Actinopterygii unidentified	22.08	13.33	14.26	23.67	29.20	15.60	14.69	37.52
Non-blue crabs combined	71.43	52.48	51.97	75.29	57.52	41.60	28.79	52.40
Fishes combined	32.47	17.82	20.52	12.56	37.17	22.80	44.40	32.32
Blue crabs combined	24.68	15.35	24.24	9.86	10.62	10.80	18.00	3.96
Shrimps combined	14.29	9.90	2.94	1.85	32.74	20.40	4.09	10.38
Mollusks combined	9.09	4.46	0.33	0.44	7.96	4.40	4.71	0.94

Species	Common name	Code	N	TL (mm) Mean±SD	δ ¹³ C Mean±SD	δ ¹⁵ N Mean±SD
Vertebrates						
Fish						
<i>Epinephelus marginatus</i>	Dusky grouper	1	72	461.2±97.1	-14.52±0.36	17.84±0.37
<i>Micropogonias furnieri</i>	Whitemouth croaker	2	2	212.0±11.3	-15.54±0.98	15.11±0.56
<i>Menticirrhus littoralis</i>	Gulf kingcroaker	3	2	148.0±32.5	-13.03±0.27	15.50±0.51
<i>Scartella cristata</i>	Molly miller	4	2	96.0±2.8	-14.57±0.19	15.18±0.44
<i>Odontesthes argentinensis</i>	Silverside	5	2	54.5±0.7	-15.49±0.24	14.57±0.05
<i>Umbrina canosai</i>	Argentine croaker	6	1		-14.45	14.25
<i>Platanichthys platana</i>	River Plate sprat	7	1	100	-17.79	14.15
<i>Oncopterus darwinii</i>	Remo flounder	8	2	60.0±5.7	-14.06±0.05	13.90±0.11
<i>Atherinella brasiliensis</i>	Brazilian silverside	9	2	108	-11.84±0.72	13.04±0.23
<i>Brevoortia pectinata</i>	Argentine menhaden	10a	2	39.0±4.2	-16.30±0.41	13.80±0.28
<i>Brevoortia pectinata</i>	Argentine menhaden	10b	2	78.5±3.5	-12.81±0.17	11.72±0.96
<i>Mugil liza</i>	Mullet	11a	2	25±0.0	-19.59±0.29	9.88±1.35
<i>Mugil liza</i>	Mullet	11b	2	128.5±4.9	-11.11±1.38	11.73±0.09
Invertebrates						
Crustacean						
<i>Callinectes sapidus</i>	Blue crab	1	2	132.5±10.6	-15.65±1.26	11.42±1.50
<i>Farfantepenaeus paulensis</i>	Pink prawn	2	1	75	-10.14	12.04
<i>Arenaeus cribrarius</i>	Speckled swimming crab	3	2	63.0±21.2	-14.53±0.58	12.83±0.51
<i>Sergio mirim</i>	Ghost shrimp	4	2	77.5±31.8	-14.09±1.09	10.53±0.40
<i>Ligia exotica</i>	Wharf roach	5	2	26.5±0.7	-14.58±1.13	10.71±0.32
<i>Synidotea marplatensis</i>	Isopod	6	2		-13.16±1.18	8.73±0.95
<i>Balanus improvisus</i>	Bay barnacle	7	2		-15.00±0.23	12.85±0.00
Amphipoda unidentified	Amphipod	8	2		-15.71±0.19	10.60±0.02
Mollusk						

<i>Mytilus edulis platensis</i>	Argentine mussel	1	2		-16.90±0.68	9.39±0.26
<i>Mesoderma mactroides</i>	Yellow clam	2	2	25.0±9.9	-17.71±0.65	9.90±0.00
<i>Amiantis purpurata</i>	Purple clam	3	1	65	-13.68	10.32
<i>Collisella subrugosa</i>	Acmaeid limpet	4	2		-14.74±0.22	12.65±0.04
<i>Littorina ziczac</i>	Zebra periwinkle	5	2		-12.35±0.07	10.43±0.02
<i>Olivancillaria vesica vesica</i>	Olive snail	6	2	34.0±2.8	-16.05±0.52	13.70±0.26
<i>Stramonita haemastoma</i>	Red-mouthed rock shell	7	2	61.5±4.9	-13.10±0.05	14.05±0.45
Polychaete						
<i>Diopatra sp</i>	Onuphiid polychaete	1	2		-13.93±0.62	14.69±0.30
<i>Hemipodus olivieri</i>	Glyceriid polychaete	2	1		-16.58	14.16
<i>Neanthes succinea</i>	Clam worm	3	3		-14.34±1.22	12.97±2.53
Anemone						
<i>Bunodosoma sp</i>	Sea anemone	1	2		-11.48±0.55	10.38±0.15

Supplemental table 3 Body size (total length, except crabs which are carapace width) and carbon and nitrogen stable isotope values of fishes and invertebrates collected from the neritic zone sampling location along the southern Brazilian coast.

Species	Common name	Code	N	TL (mm) Mean±SD	$\delta^{13}\text{C}$ Mean ±SD	$\delta^{15}\text{N}$ Mean±SD
Vetebrates						
Fish						
<i>Epinephelus marginatus</i>	Dusky grouper	1	190	530.9±170.0	-14.30±0.43	17.90±0.44
<i>Trichiurus lepturus</i>	Cutlassfish	2	1	1000	-15.88	17.32
<i>Ctenosciaena gracilicirrhus</i>	Barbel drum	3	3	180.3±9.0	-14.10±0.43	16.39±0.38
<i>Menticirrhus americanus</i>	Southern kingcroaker	4	3	247.0±9.6	-13.91±0.85	16.01±0.22
<i>Percophis brasiliensis</i>	Brazilian flathead	5	1	450	-14.78	15.83
<i>Micropogonias furnieri</i>	Whitemouth croaker	6	3	260.0±181.9	-15.41±1.38	16.03±0.44
<i>Cynoscion guatucupa</i>	Stripped weakfish	7	5	72.4±4.7	-16.37±0.22	15.42±0.48
<i>Trachurus lathami</i>	Rough scad	8	3	108.3±2.5	-16.00±0.81	15.32±0.09
<i>Paralichthys patagonicus</i>	Patagonian flounder	9	1	265.0±0.0	-14.82	15.15
<i>Urophycis brasiliensis</i>	Brazilian codling	10a	4	105.3±26.0	-16.53±0.71	14.64±0.13
<i>Urophycis brasiliensis</i>	Brazilian codling	10b	3	164.0±19.9	-15.53±0.63	15.08±0.27
<i>Trachinotus marginatus</i>	Plata pompano	11	2	143.0±2.8	-13.58±0.00	14.69±0.06
<i>Paralanchurus brasiliensis</i>	Banded croaker	12a	3	116.7±3.1	-15.40±0.50	14.16±0.11
<i>Paralanchurus brasiliensis</i>	Banded croaker	12b	3	181.0±5.0	-14.34±0.26	15.14±0.20
<i>Selene setapinnis</i>	Atlantic moonfish	13	1	184.0±0.0	-16.76	14.45
<i>Pomatomus saltatrix</i>	Bluefish	14	1	365.0±0.0	-15.4	17.32
<i>Anchoa marinii</i>	Marini's anchovy	15	3	94.3±3.8	-16.72±0.25	13.49±0.41
<i>Symphurus jenynsi</i>	Jenyn's tonguefish	16	4	157.3±29.2	-14.07±0.45	13.24±0.44
<i>Porichthys porosissimus</i>	Atlantic midshipman	17	3	216.0±39.2	-16.87±0.61	12.64±0.75
<i>Sympterygia acuta</i>	Bignose fanskate	18	3	445.3±117.8	-13.80±0.29	15.43±1.04
<i>Sympterygia bonapartii</i>	Smallnose fanskate	19	6	392.8±131.7	-14.19±0.51	14.70±0.53
Invertebrates						
Crustacean						
<i>Cyrtograpsus angulatus</i>	Grapsid crab	1	6	16.3±1.5	-17.20±0.49	12.94±0.25
<i>Eurypanopeus abbreviatus</i>	Lobate mud crab	2	2		-15.54±0.97	14.14±0.55

<i>Eurytium limosum</i>	Broadback mud crab	3	1		-14.74	14.39
<i>Panopeus austrobesus</i>	Brazilian mud crab	4	2	40.0±7.1	-14.25±0.24	14.67±0.10
<i>Panopeus bermudensis</i>	Strongtooth mud crab	5	2	27.0±1.4	-15.19±0.44	13.62±0.83
<i>Persephona mediterranea</i>	Mottled purse crab	6	2	39.5±0.7	-14.00±0.28	14.70±0.81
<i>Pilumnus caribaeus</i>	Coarsespine hairy crab	7	1		-12.44	12.27
<i>Platyxanthus crenulatus</i>	Purple stone crab	8	1	33	-14.85	14.24
<i>Portunus spinimanus</i>	Blotched swimming crab	9	3		-15.64±0.27	12.26±0.40
<i>Callinectes sapidus</i>	Blue crab	10	2		-15.63±1.15	11.62±0.42
<i>Farfantepenaeus paulensis</i>	Pink prawn	11	1		-14.52	11.92
Caridae unidentified	Prawn	12	4	32.5±11.9	-15.02±0.55	15.10±0.33
<i>Cirolana sp</i>	Isopod	13	2		-13.84±0.31	13.91±0.92
Amphipoda unidentified	Amphipod	14	2		-18.27±0.13	13.99±0.30
Hyperiididae unidentified	Hyperiid amphipod	15	1		-20.66	11.90
Mollusk						
<i>Illex argentinus</i>	Argentine shortfin squid	1	3	50.0±1.0	-16.70±0.14	14.89±0.32
<i>Octopus vulgaris</i>	Common octopus	2	1		-15.63	12.24
<i>Dorsanum moniliferum</i>	Nassa Mud snails	3	2	32	-13.92±0.53	14.59±1.85
Polyplacophora	Chiton	4	1	35	-12.90	13.21
Polychaete						
<i>Owenia fusiformis</i>	Spindle-shaped tubeworm	1	2		-16.41±0.37	10.49±0.07
Chaetognatha						
Chaetognatha unidentified	Arrow worms	1	2		-20.66±0.29	12.75±0.12

10. Anexo III

Prolonged estuarine habitat use by dusky grouper *Epinephelus marginatus* at subtropical latitudes revealed by otolith microchemistry

Mario V. Condini^{1*}, Sussane E. Tanner², Patrick Reis-Santos^{2,3}, Cristiano Q.
Albuquerque⁴, Tatiana D. Saint’Pierre⁵, João P. Vieira¹, Henrique N. Cabral²,
Alexandre M. Garcia¹

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Prolonged estuarine habitat use by dusky grouper *Epinephelus marginatus* at subtropical latitudes revealed by otolith microchemistry

M. V. Condini^{1,*}, S. E. Tanner², P. Reis-Santos^{2,3}, C. Q. Albuquerque⁴,
T. D. Saint’Pierre⁵, J. P. Vieira¹, H. N. Cabral², A. M. Garcia¹

¹Laboratório de Ictiologia, Instituto de Oceanografia, Universidade Federal de Rio Grande, Av. Itália Km 08, Carreiros, CEP: 96.201-900, Rio Grande, RS, Brazil

²MARE — Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Campo Grande, Lisboa, Portugal

³Southern Seas Ecology Laboratories, School of Biological Sciences, The University of Adelaide, SA 5005, Australia

⁴Departamento de Ciências Animais, Universidade Federal Rural do Semi-Árido, CEP: 59625-900, Mossoró, RN, Brazil

⁵Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro, Rua Marquês de São Vicente, 225, Gávea, 22451-900, Rio de Janeiro, Brazil

ABSTRACT: Otolith microchemistry (Sr:Ca, Ba:Ca) was used to evaluate habitat use patterns of the endangered dusky grouper *Epinephelus marginatus* in southern Brazil. Individual Sr:Ca profiles exhibited low variation, with an overall tendency to increase with age. Interestingly, individual Ba:Ca profiles presented 3 contrasting patterns: the first comprised most sampled individuals (>80%), mostly indicative of the predominant use of marine waters throughout their life history; the second pattern (~10% of all individuals) indicated that dusky grouper may use or remain in or near estuarine waters for short time periods; finally, the third identified pattern (~5%) comprised individuals remaining in estuarine waters for long periods and provided the first evidence for this species of prolonged estuarine habitat use (over a year), in particular during juvenile life stages.

KEY WORDS: Epinephelidae · Fish conservation · Life-history plasticity · Movement · Natural tags · Patos Lagoon

INTRODUCTION

Dusky grouper *Epinephelus marginatus* (Lowe, 1834) is the only large Epinephelidae species commonly found along subtropical latitudes (above 30°S) of the southwestern Atlantic Ocean, where it inhabits shelter-rich hard substrata on the continental shelf (Heemstra & Randall 1993, Sluka et al. 2001, Reñones et al. 2002). This species is highly prized by commercial fisheries and represents a significant source of income to fishermen (Condini et al. 2007, Craig et al. 2011, Sadovy de Mitcheson et al. 2013). Overfishing combined with a complex life-

history strategy (slow growth rate, late maturation, aggregative spawning behavior and sequential hermaphroditism, a trait common to several grouper species [Heemstra & Randall 1993, Craig et al. 2011]) has led to the high vulnerability of many grouper populations (Graham et al. 2009, Cheung et al. 2013, Sadovy de Mitcheson et al. 2013), and in particular to the classification of *E. marginatus* as ‘Endangered’ (EN a2d) in the IUCN Red List (Cornish & Harmelin-Vivien 2004).

Recent studies on *E. marginatus* in southern Brazil comparing littoral and neritic sites have suggested that Carpinteiro Bank (a neritic site) is the main

*Corresponding author: mvcondini@gmail.com

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growth and spawning ground for this species in the region (Condini et al. 2014a,b). In contrast, only immature individuals and resting mature females are found along rocky substrate in the littoral zone (Seyboth et al. 2011). Although comparative studies suggest that dusky grouper growth rates are higher in neritic habitats (Condini et al. 2014b), the relative importance of the different habitats for this endangered species remains unknown. A better understanding of habitat use patterns of marine fish populations is vital to evaluate their dynamics and resilience to harvest, ultimately supporting adequate management and conservation measures (Andrello et al. 2014, Davoren et al. 2015). This fundamental aspect of fish life history can be revealed through the evaluation of natural markers such as otolith microchemistry (Rooker et al. 2008, Reis-Santos et al. 2013a, Darnaude et al. 2014), as detailed analysis of the chemical composition of otoliths allows reconstruction of the environmental conditions experienced by individual fish throughout their entire life history (Campana 1999, Elsdon et al. 2008, Tanner et al. 2013). In general, strontium (Sr) and barium (Ba) concentrations in otoliths have a positive correlation with salinity and freshwater, respectively (Elsdon & Gillanders 2005, Sturrock et al. 2012, Reis-Santos et al. 2013b). Thus, fish living in marine waters present higher otolith strontium concentrations compared to fish living in freshwater, whereas the latter present conspicuously higher otolith Ba concentrations than fish living in marine waters.

The aim of this study was to investigate habitat use patterns of dusky grouper *E. marginatus* in subtropical latitudes (32°S) in the southwestern Atlantic Ocean. Findings may provide key insights on habitat use of different life-history stages which could be useful in the design of Marine Protected Areas and help to ensure the conservation of this endangered species at the southern limit of its distribution.

MATERIALS AND METHODS

Sampling was conducted at 2 locations on the southern Brazilian coast (Fig. 1). The first one is located in the littoral zone (rocky jetties, <5 m in depth), at the mouth of the Patos Lagoon estuary (~1000 km²), and is constituted of a pair of 4.5 km long rocky jetties (Fig. 1; 32°09' S, 52°05' W). Due to its position, this sampling location has varying hydrological conditions and freshwater inputs that affect its salinity profiles (Moller et al. 2001). The second sampling site is the Carpinteiro Bank (Fig. 1; 32°16' S, 51°47' W), a group of submerged rocks 20 to 30 m deep in the neritic zone approximately 16 nautical miles (~30 km) from the coast.

Dusky grouper *Epinephelus marginatus* were collected from the littoral and neritic zones in collaboration with regional fishermen. Otoliths of a total of 72 individuals of *E. marginatus* were extracted for chemical analysis; 28 of these individuals, aged between 2 and 12 yr (mean $\pm$ SD: 4.8 ± 2.4 yr), were sampled in the littoral zone (rocky jetties) and 44, aged between 2 and 40 yr (11.9 ± 10.8 yr), were sampled in the neritic zone (Carpinteiro Bank). Age determination using otoliths has already been validated for *E. marginatus* in this study area (Condini et al. 2014b). All procedures for otolith chemical analysis followed Mai et al. (2014). Each otolith section was scanned from the core to the edge using a 266 nm laser ablation system (CETAC LSX 100) coupled to an ELAN 6000 (PerkinElmer—SCIEX) inductively coupled plasma-mass spectrometer (ICP-MS), and calcium (⁴³Ca), ⁸⁶Sr and ¹³⁸Ba were measured. Blank ablations (background intensities) were measured during 50 s after every 10 otolith ablations. Strontium and calcium counts per second (cps) were subtracted from the background

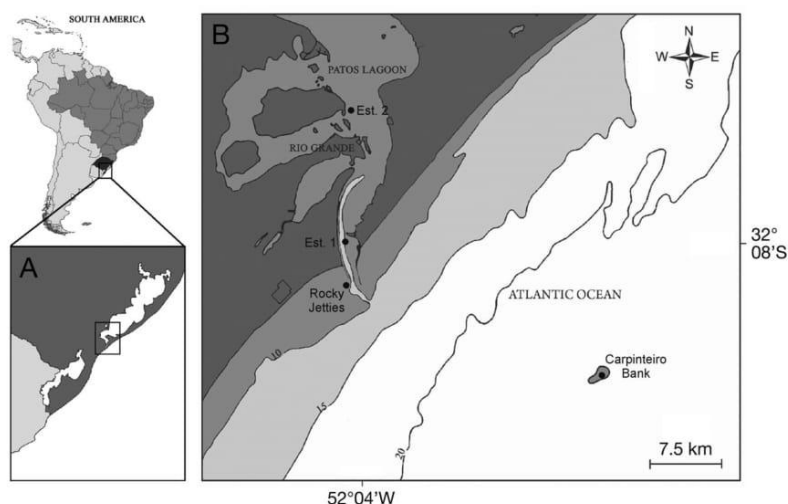
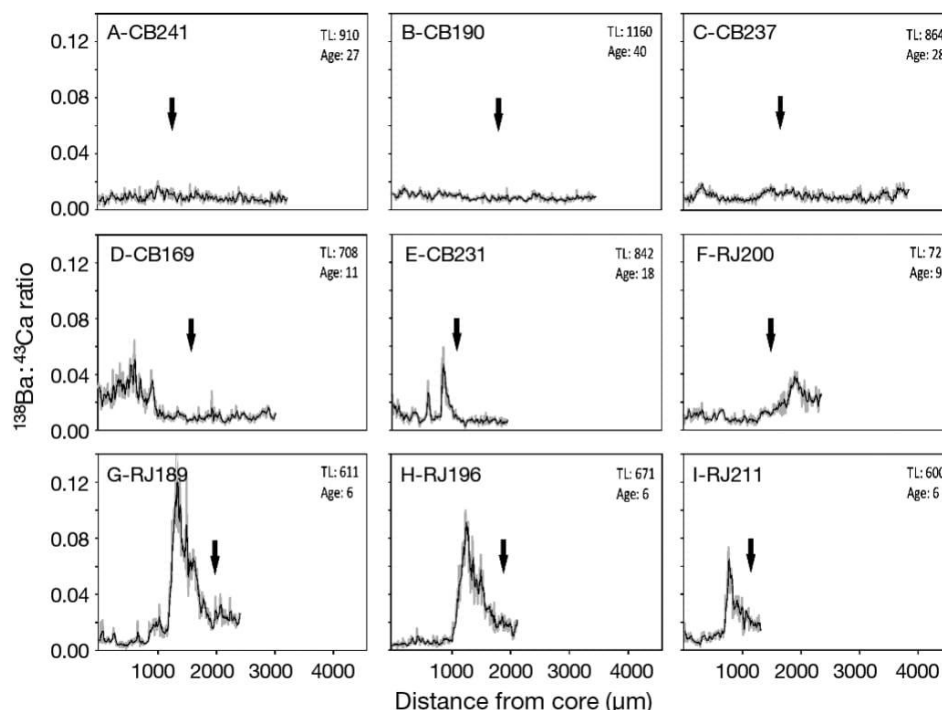


Fig. 1. Patos Lagoon (10 360 km²) and its estuarine zone in the state of Rio Grande do Sul, southern Brazil (A), showing the locations of the 2 sampling sites (rocky jetties and Carpinteiro Bank), as well as 2 sites related to salinity data (Est. 1 and Est. 2) (B). Lines along the coast denote 10 (dark gray), 15 (light gray) and 20 m (white) isobaths, respectively

Fig. 2. Ba:Ca ratio profiles along a transect from the core to the posterior edge of the otolith of *Epinephelus marginatus* collected from the littoral (RJ) and neritic (CB) zone, with age (yr) and total length (TL, mm) of each analyzed individual. All element:Ca ratios correspond to counts per second. Arrows indicate the end of the juvenile stage; before the arrow individuals are <5 yr old. The black line denotes a 3-order moving average, which was employed for better visualization of the elemental chemical profile trend exhibited by each individual otolith



level, and element:Ca ratios were then calculated for all otoliths. Before analyzing otoliths, the instrument was optimized through daily performance, with maximum analyte intensities and minimum interferences determined using oxides and double-charged ions. Repeatability was estimated as the relative standard deviation (RSD) from 10 consecutive measurements of the standard NIST 1834, resulting in relative standard deviations of 12.43, 6.91 and 3.99% for ^{138}Ba , ^{86}Sr and ^{43}Ca , respectively.

As the Carpinteiro Bank (a neritic zone) has been identified as an important spawning site for *E. marginatus* in southern Brazil (Condini et al. 2014a) and taking into consideration the close location of the littoral habitat (16 nautical miles), all individuals used in this study were assumed to belong to the same population; this assumption is corroborated by the long distances that dusky grouper larvae can disperse (>16 nautical miles; Andreello et al. 2014). To reconstruct and characterize habitat use patterns of *E. marginatus*, we analyzed the Sr:Ca and Ba:Ca life-history profiles of all 72 *E. marginatus* individuals collected from both littoral and neritic areas. In addition, otolith edge Sr:Ca and Ba:Ca ratios of individuals collected at littoral and neritic sites were compared using non-parametric Mann-Whitney *U*-tests to evaluate our ability to distinguish between sampled areas. For this comparison only fish from matching year classes (aged between 6 and 8 yr old and

born between 2000 and 2003) were used to avoid any potential influence of ontogenetic and temporal variations: specifically, 12 individuals from littoral (587.6 ± 44.9 mm TL) and 8 from neritic zones (619.0 ± 36.1 mm TL). Otolith edge chemical composition was defined as the 30 μm nearest to the edge of the otolith.

Salinity data over time were analyzed from a 40 yr time series for the Carpinteiro Bank region (specifically, from 32° to 32° 50' S and 50° to 52° W) and a 20 yr time series for littoral and estuarine areas. Data were obtained from the Coastal and Estuarine Oceanography Laboratory of Rio Grande Federal University and the Ichthyology Laboratory of Rio Grande Federal University, respectively. Salinity data were averaged by month and reported with respective standard deviations.

RESULTS AND DISCUSSION

We found 3 interesting patterns of Ba:Ca life-time profiles in *Epinephelus marginatus* that indicate differential habitat use. The first comprises most sampled individuals (>80%) and is characterized by low Ba:Ca ratios (<0.03) along an individual's entire life history (Fig. 2A–C; Fig. S1 in the Supplement at www.int-res.com/articles/suppl/n029p271_supp.pdf) and is mostly indicative of the predominant use of marine waters throughout their life history. The sec-

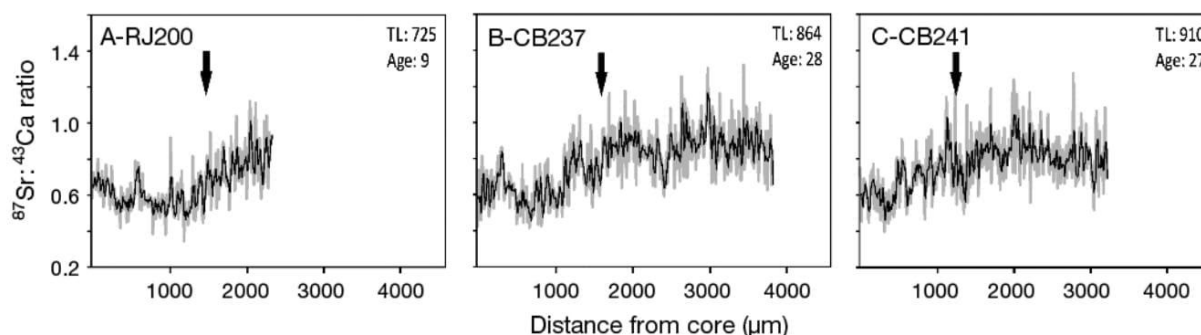


Fig. 3. Sr:Ca ratio profiles along a transect from the core to the posterior edge of the otolith of *Epinephelus marginatus* collected from the littoral (RJ) and neritic (CB) zone, with age (yr) and total length (TL, mm) of each analyzed individual. All element:Ca ratios correspond to counts per second. Arrows indicate the end of the juvenile stage; before the arrow individuals are <5 yr old. The black line denotes a 3-order moving average, which was employed for better visualization of the elemental chemical profile trend exhibited by each individual otolith

ond pattern includes juveniles and adults (~10% of all individuals) that exhibited small fluctuations in otolith Ba:Ca ratios, with peaks reaching values between 0.03 and 0.05 (Fig. 2D–F); this indicates that these fish may use or remain in, or near, estuarine waters for short time periods. Finally, the third identified pattern (~5%) comprises individuals with a steep increase in Ba:Ca ratios (up to a maximum of 0.14; Fig. 2G–I) during their juvenile stage, followed by a decrease to early life levels (<0.03). This pattern appeared mostly, though not exclusively, in fish sampled in the littoral habitat and suggests these fish remained within the Patos Lagoon estuary for extended periods of time (over 2 yr in some cases).

Our findings support the conclusions that dusky groupers have higher life-history plasticity than previously reported and that estuaries may be used as alternative habitats by some individuals during part of their lives. To our knowledge, there is no prior evidence in the literature of *E. marginatus* inhabiting brackish waters for such extended periods, though this is not entirely surprising, as juveniles of the *Epinephelus* genus (e.g. *E. striatus*, *E. coiodes*, *E. malabaricus*, *E. aeneus*, *E. itajara*) are known to use mangroves, coastal lagoons and estuaries as nursery or juvenile habitats (Heemstra & Randall 1993, Craig et al. 2011). Yet, until now, there have been only a few scattered records of occurrence of *E. marginatus* near, or within, estuarine areas (e.g. Barbanti et al. 2013, M. V. Condini unpubl. data), and no prior information on consistent use of estuaries or permanence in brackish waters.

Other studies using otolith chemistry of different species inhabiting the Patos Lagoon estuary and adjacent coastal areas (e.g. *Genidens genidens* and *Mugiliza*) showed similar ranges of otolith Ba:Ca when mi-

grating along the salinity gradient of this estuarine system. For instance, otoliths of *G. genidens* showed average Ba:Ca ratios of approximately 0.08 in the estuary, which increased to ~0.13 in the freshwater portion of the Patos Lagoon (Pereyra 2015). Ba:Ca values ranging from 0.01 to 0.14 were also reported for *Lycengraulis grossidens* and *Genidens barbus* collected along the Patos Lagoon salinity gradient (Mai et al. 2014, Avigliano et al. 2015). We recognize that inter-specific variations in otolith microchemistry may sometimes preclude comparisons between different species (Reis-Santos et al. 2008). However, as we show here, many migrant fish species presented similar ranges of Ba:Ca ratios in their otoliths along the Patos Lagoon estuary. Also, most studies indicate that water is the major source of otolith elements and that diet has little or no effect on otolith elemental composition (Walther & Thorrold 2006, Gibson-Reinemer et al. 2009, Marohn et al. 2009), which helps to discard potential confounding effects of diet in our results. In contrast to Ba:Ca, individual Sr:Ca profiles exhibited low variation, with an overall tendency to increase with age, regardless of which site individuals were collected from (Fig. 3; Fig. S2 in the Supplement at www.int-res.com/articles/suppl/n029p271_supp.pdf). Albuquerque et al. (2012) observed little or no variation in Sr:Ca ratios in the water between intermediate (~20) and high (~35) salinities along the freshwater–marine gradient in the estuarine system of Patos Lagoon. Similar results have also been observed by Phillis et al. (2011) in San Francisco Bay related to the Ca concentration of the freshwater end-member. However, higher than expected Sr or Ca end-member availability in the ambient water of the Patos Lagoon cannot be excluded, due to lack of available information (see Kraus & Secor 2004).

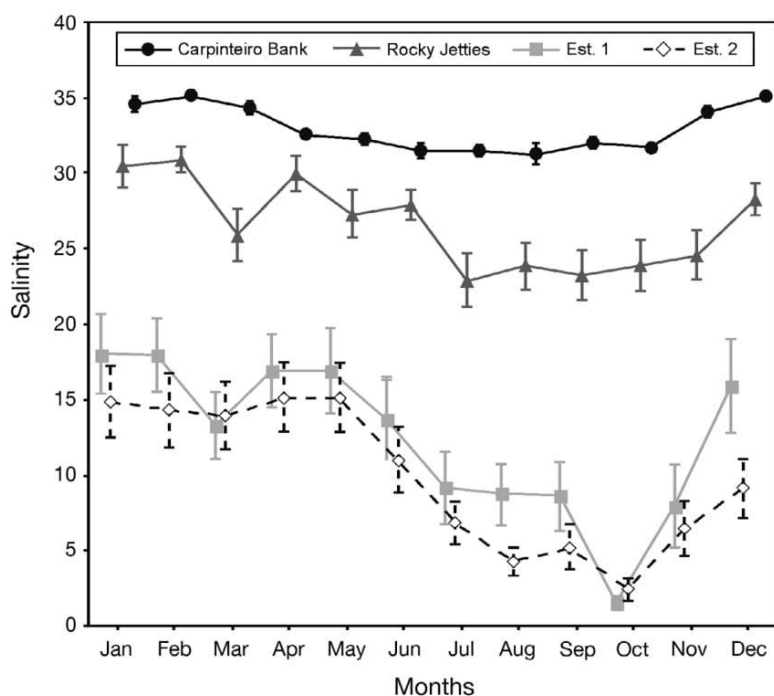


Fig. 4. Mean values ($\pm$ SE) of salinity in Patos Lagoon estuary (Est. 1 and Est. 2) and at both marine sampling sites, the littoral (rocky jetties) and neritic zone (Carpinteiro Bank)

Climatic interannual variability is considerable in the study region. For instance, the strong El Niño Southern Oscillation (ENSO) event that occurred in 2002 (birth year of some of the individuals analyzed here), led to positive rainfall anomalies in this region that increased freshwater discharge into the lagoon, which, in turn, had significant effects on fish distribution and abundance (Garcia et al. 2003, 2004). However, no generalized substantial increases in Ba:Ca ratios in *E. marginatus* otoliths were observed, nor was it possible to link these to the increased freshwater runoff produced by this or other similar climatic events. Thus, the abrupt increase in the Ba:Ca ratio in some of the analyzed individuals (Fig. 2G–I) was not related to the ENSO phenomenon, but indicative of juvenile entrance and residency for periods of at least 2 yr in brackish estuarine waters.

Otolith edge Sr:Ca and Ba:Ca ratios from individuals collected in littoral and neritic habitats were compared to determine the discriminatory power of otolith chemical signatures. The influence of the estuarine plume of the Patos Lagoon causes average salinity at the littoral site (mean = 26.5) to be lower than that at the neritic site (mean = 32.9), as well as more variable year-round (SD = 7.0 and 2.1 in littoral and neritic zones, respectively) (Fig. 4). Although there is

a known positive correlation of Sr:Ca with salinity and Ba:Ca with freshwater (Elsdon & Gillanders 2005, Sturrock et al. 2012, Reis-Santos et al. 2013b), the mean concentrations of both element:Ca ratios in the otolith edge were not significantly different between individuals of the same year class caught in the neritic and littoral zones (Mann-Whitney $U = 290$ and $p > 0.05$ for Sr:Ca; $U = 268$, $p > 0.05$ for Ba:Ca). We hypothesize that the salinity difference, as well as the freshwater input, was not large enough in this case to produce distinct otolith chemical signatures between the 2 sampled habitats, hindering our ability to use otolith chemistry to disentangle movements between these 2 proximate coastal marine areas (as pointed out by Albuquerque et al. 2012 for Sr:Ca and salinity ranges of these areas).

In conclusion, the present study revealed for the first time that individuals of *E. marginatus* can remain for prolonged time periods in a brackish system, highlighting the life-cycle

plasticity of this endangered species. Future studies should actively investigate the occurrence of dusky groupers within estuarine systems, focusing on identifying key juvenile habitats for this species via combinations of other natural tags (e.g. stable isotopes of C and O) and/or telemetry, which may also aid in resolving movements between littoral and neritic habitats of adult fish. Finally, it would also be of great interest to ascertain the physiological trade-offs related to the observed differential nursery habitat use. Overall, rocky jetties and estuarine habitats within the Patos Lagoon may be considered as potential candidates for the implementation of conservation strategies to safeguard this endangered species.

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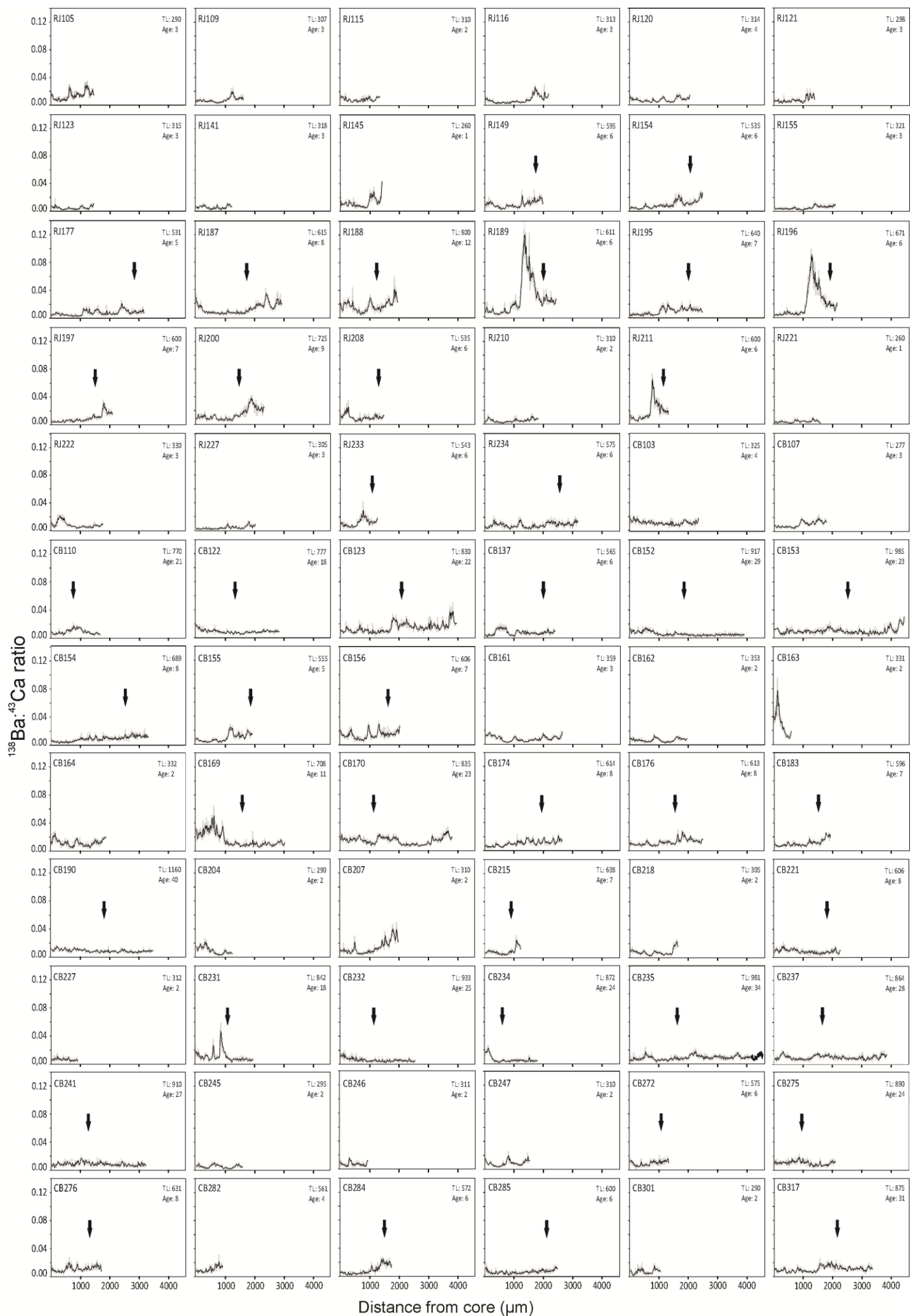
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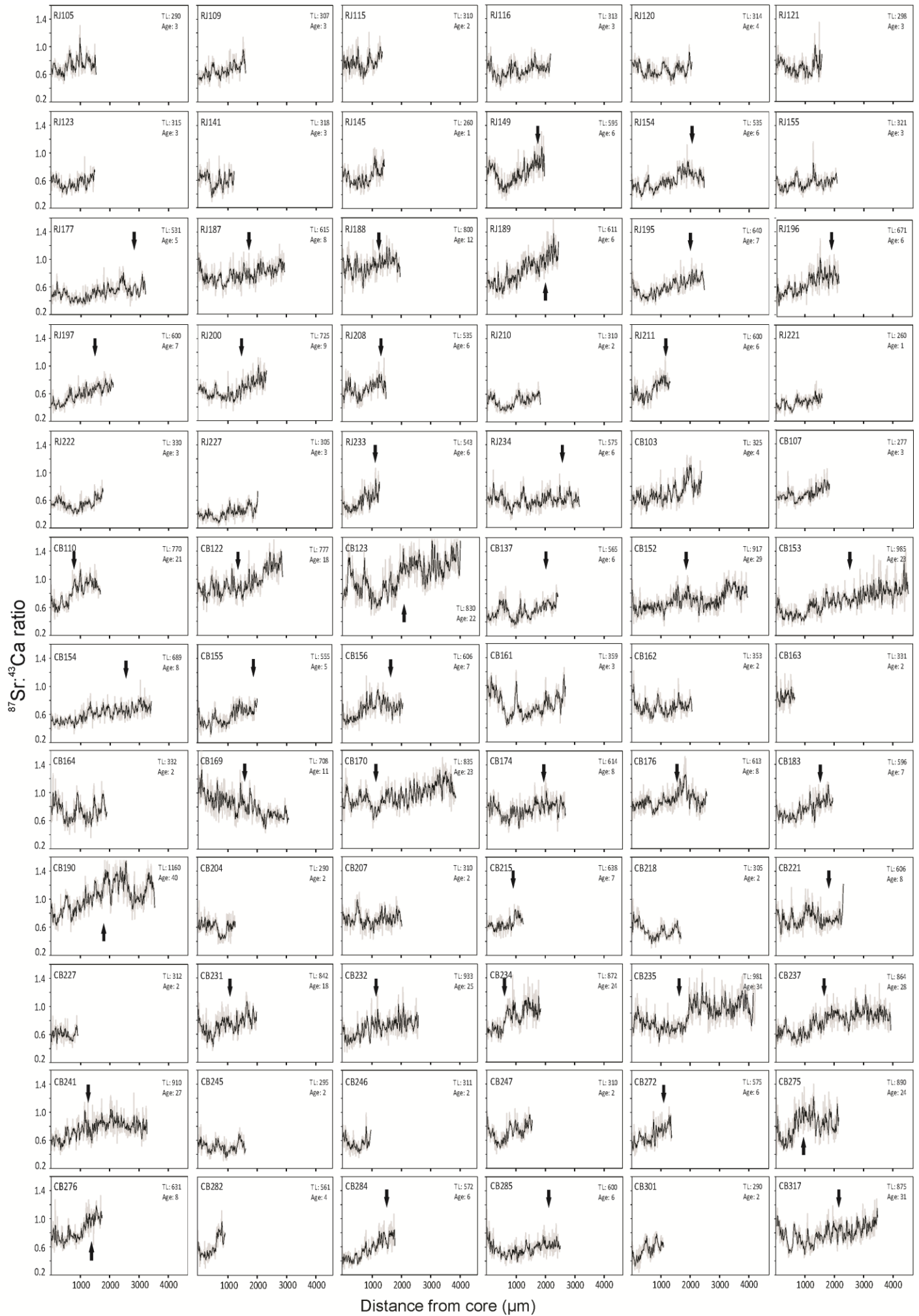
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11. Anexo IV

**Mercury concentrations in dusky grouper *Epinephelus marginatus* in littoral and neritic
habitats along the Southern Brazilian coast**

Mario V. Condini^{1*}, David J. Hoeinghaus², Aaron P. Roberts², Brianne K. Soulen² and
Alexandre M. Garcia¹

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Mercury concentrations in dusky grouper *Epinephelus marginatus* in littoral and neritic habitats along the Southern Brazilian coast

Mario V. Condini^{1*}, David J. Hoeinghaus², Aaron P. Roberts², Brianne K. Soulen² and Alexandre M. Garcia¹

Affiliations and addresses

¹Laboratory of Ichthyology, Institute of Oceanography, Federal University of Rio Grande Av. Itália Km 8, Carreiros. 96.201-900, Rio Grande, RS, Brazil.

²Department of Biological Sciences and the Advanced Environmental Research Institute, University of North Texas, 1155 Union Circle #310559, Denton, Texas 76203-5017 USA

*Corresponding author. Tel.: +55 (53) 32336539. E-mail address: mvcondini@gmail.com (M.V. Condini)

1 **Abstract**

2 Our study incorporated a comprehensive suite of parameters (i.e., body size, age,
3 diet and trophic position) to investigate mercury concentration in dusky groupers
4 *Epinephelus marginatus*. This study was carried out in rocky bottoms in littoral and
5 neritic habitats along the Southern Brazilian coast. We also determined spatial
6 variation in mercury concentrations in individuals inhabiting both zones, which may
7 provide insights into how dietary differences or potential pollution sources affect
8 bioaccumulation. A total of 244 dusky groupers was analyzed to determine total
9 mercury concentrations. Our study revealed that when considering similar body sizes,
10 individuals inhabiting littoral rocky habitats had higher concentrations of mercury
11 probably due to proximity to pollution sources associated with human activities in the
12 estuary and its drainage basin. Furthermore, large individuals (>650 mm and >8 years
13 old) showed mercury contamination levels that are potentially harmful for this
14 endangered fish species and above the acceptable limits for human consumption.

15 **Keywords:** Total mercury; trophic position; Epinephelidae; human health risk

1. Introduction

Mercury is a non-essential toxic element commonly found in aquatic ecosystems. It is released through a variety of natural and anthropogenic processes including geologic weathering, gold mining, and burning of coal (Pacyna et al., 2010). In aquatic systems, inorganic mercury can be transformed by microbial activity into organic methylmercury (MeHg) which biomagnifies in the food web and results in high concentrations of methylmercury in piscivorous species (including humans). Previous studies have shown deleterious effects of mercury on fish health at concentrations greater than 0.3 mg kg⁻¹ wet weight (Sandheinrich et al., 2011; Depew et al., 2012). These concentrations are not uncommon to aquatic food webs suggesting that health and reproduction of wild fish populations may be impacted at environmentally relevant concentrations of MeHg (Beckvar et al., 2005; Webb, 2006; Sandheinrich and Wiener, 2011). A literature review by Depew et al. (2012) of mostly freshwater fishes found that concentrations of mercury within this range results in decreases in gonadosomatic index (GSI), sex steroid production, spawning success, fertilization, fecundity, and increases in ovary apoptosis.

Large-bodied and long-lived fish species such as the dusky grouper (*Epinephelus marginatus*) are well recognized for their ability to bioaccumulate mercury (Evers et al., 2009; Tremain and Adams, 2012). Dusky grouper are exploited by fishermen in rocky bottoms along the coasts of South America (Figueiredo and Menezes, 1980; Condini et al., 2007), Europe (Heemstra and Randall, 1993) and Africa (Fennessy, 2006), and are widely consumed by human populations (Heemstra and Randall, 1993).

Mercury bioaccumulation in other epinephelids has been investigated mainly in the northern hemisphere (Hassan et al., 2007; Evers et al., 2009; Tremain and Adams, 2012; Harris et al., 2012, but see Lacerda et al., 2007 and Kutter et al., 2009), and prior studies on mercury contamination of large-bodied fishes in the southwestern Atlantic are restricted to other fish families such as scianids, trichiurids, and scombrids (Viana et al., 2005; Medeiros et al., 2008; Di Benedetto et al., 2013). These studies focused mainly on levels of mercury in fish tissues, typically muscle, and usually related contamination levels with body size (Adams and Onorato, 2005; Lacerda et al., 2007) and/or trophic position of the individual estimated by stable isotopes (Bank et al., 2007; Hassan et al., 2007; Tremain and Adams, 2012; Di Benedetto et al., 2013; Sluis et al., 2013). In several cases, these studies report that mercury concentrations in large-bodied fishes are above the proposed threshold (0.3 mg kg^{-1} wet weight) required to safeguard these wild populations (Adams and Onorato, 2005; Lacerda et al., 2007; Evers et al., 2009). In some instances, they are also above the safety levels (0.5 mg kg^{-1} wet weight, World Health Organization - WHO) for human consumption (Cai et al., 2007; Hassan et al., 2007; Harris et al., 2012; Sluis et al., 2013). Taken together, these studies provide evidence that mercury concentrations in dusky grouper are likely to occur at levels that may negatively affect fish health and pose a risk for human consumption.

Dusky grouper is an important fisheries species of conservation concern (classified by IUCN as “endangered”; Cornish and Harmelin-Vivien, 2004), thus, additional information is needed on factors (e.g. body size, age, trophic position, habitat use) that affect mercury concentrations in order to support conservation and

socio-ecological aspects of fisheries management (e.g. size limits, consumption advisories). Dusky grouper also provide a unique ecological model for the study of effects of mercury on fish populations due to a complex life-history strategy that includes slow growth rates, late maturation, aggregate spawning, and sexual reversal (Heemstra and Randall, 1993; Craig et al., 2011). The first step in the study of potential mercury effects on population dynamics is an understanding of ecological factors that mediate mercury concentrations in the individuals.

Our study provides the first account of total mercury bioaccumulation for an *Epinephelus* species in the Southwestern Atlantic, and has direct relevance for conservation of this threatened species and human health of coastal communities that rely on this fishery. In contrast with most prior studies on mercury contamination in groupers, our study incorporated a more comprehensive suite of parameters (i.e., body size, age, stage of reproductive maturation, sex, diet and trophic position) that may be expected to affect variation in mercury concentration. We also determined spatial variation in mercury concentrations in individuals inhabiting littoral and neritic zones which may provide insights into how dietary differences or potential pollution sources affect bioaccumulation. Our previous research at these sites (Condini et al., 2015) found that even though there was no relationship between body size and trophic position, dusky grouper at the littoral site had a diet dominated by fishes. Individuals at the neritic site consumed similar amounts of shrimp and fishes except for the largest size class which consumed a greater proportion of fishes. Based on this, we expected a strong positive relationship between mercury concentration and body size (length, weight) and age due to bioaccumulation, but not with trophic position (i.e.

biomagnification). Furthermore, the greater proportion of fish in the diets of dusky grouper from the littoral zone and the largest size class from the neritic zone should correspond with higher Hg accumulation rates due to longer lifespans and greater proportion of Hg associated with assimilated biomass of prey fishes versus crabs and shrimp.

2. Materials and Methods

2.1. Study area and sample collection

This study was carried out in rocky habitats located adjacent to Patos Lagoon Estuary. Patos Lagoon Estuary constitutes one of the largest and most well-studied estuarine regions in South America (see Seeliger et al. 1997 and Odebrecht et al. 2010) and has been exploited by fishermen since the nineteenth century (Von Ihering 1896). This estuary also contains the third most important Brazilian harbor that engages mainly in transport of chemical products, grains, and agricultural machines, among other potential pollutant sources (Odebrecht et al. 2010; SPRG, 2016). Mirlean et al. (2003) report mercury concentrations up to 21 mg kg^{-1} in suspended particulate matter of the shallow waters of Patos Lagoon Estuary. The authors link this contamination to domestic sewage and stormwater runoff sources. Niencheski et al. (2001) and Kutter et al. (2009) quantified mercury contamination in fishes in Patos Lagoon Estuary and adjacent marine area and found mercury concentrations $<0.3 \text{ mg kg}^{-1}$ for nearly all of species. These studies sampled primarily smaller-bodied species that feed at lower trophic positions, but also included some larger predatory species

(e.g. *Cynoscion guatcupa*, *Pomatomus saltatrix*, *Macrodon ancylodon*) and one specimen of *E. marginatus*.

Dusky grouper were sampled from two marine sites adjacent to Patos Lagoon Estuary. The first is in the littoral zone along a pair of rocky jetties (locally known as 'Molhes da Barra de Rio Grande') located at the mouth of Patos Lagoon Estuary (Fig. 1; 32°09'S, 52°05'W). The pair of rocky jetties is approximately 4.5 km long and extends from the littoral zone to the sea. They were built in the beginning of the 20th century to maintain a navigation channel to allow regular maritime access to the harbor of Rio Grande. Over time, the area was colonized by diverse marine benthic fauna (Capítoli, 1996) and by dusky grouper, mainly juveniles and immature females (Condini et al., 2007). The second study area (Carpinteiro Bank) is located in the neritic zone approximately 16 nautical miles offshore and between 20 and 30 m isobaths (Fig. 1; 32°16'S, 51°47'W) and was composed of beach rocks with a high level of cementation by recrystallized calcium carbonate (Buchmann et al., 2001; Abreu and Calliari, 2005). Both study locations represent rocky habitats within a coastal landscape that is otherwise dominated by homogeneous sandy substrates. Individuals were collected in the littoral and neritic zones between January 2008 and April 2009 and between January 2010 and May 2011, respectively.

Collected specimens were stored directly on ice and taken to the laboratory for processing. Each individual was measured for total length (TL, mm) and weighed (g). The new Hg contamination dataset presented in this study builds on previously published research conducted by the authors on maturity stages, age and trophic

ecology of *E. marginatus* in the same study area (i.e. Condini et al., 2014a, 2014b, 2015). Maturity stage and sexes of the dusky grouper were determined by histological analysis of gonads (Condini et al., 2014a) and age estimates were determined by readings of sagittal otoliths (Condini et al., 2014b). Condini et al. (2015) previously described the trophic ecology of dusky grouper at these sites based on stomach contents and stable isotope data ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The same anterior-dorsal muscle tissue samples from each individual that were used to determine stable isotopes composition are analyzed in this study for mercury concentration. The current study builds on the aforementioned previous findings for sex stage, age and trophic ecology by testing for relationships between each of these factors and muscle mercury concentration. Importantly, muscle tissue collected for these analyses is representative of the fish fillet usually consumed by humans (Adams and Onorato, 2005).

2.2. Mercury Analysis

Subsamples of dried and homogenized muscle tissue ($n = 68$ littoral, $n = 176$ neritic) were weighed to the nearest 0.3 mg using an analytical balance. Total mercury concentration was determined on a direct mercury analyzer (DMA-80; Milestone Inc., Monroe, Connecticut) using thermal decomposition, gold amalgamation, and atomic absorption spectrometry (USEPA, 1998). Calibration curves were generated using three reference materials from the National Research Council of Canada: MESS-3 (marine sediment: certified value = 91 ± 9 ng of total Hg g^{-1} of dry weight), TORT-2

(lobster hepatopancreas: certified value = $270 \pm 60 \text{ ng g}^{-1}$), and DOLT-4 (dogfish liver tissue: certified value = $2580 \pm 220 \text{ ng g}^{-1}$). Quality assurance included blanks, duplicate samples, and reference samples. Blank (empty boats) were analyzed every 20 samples with a mean Hg content of $0.0004 \pm 0.0005 \text{ mg kg}^{-1}$ (range = 0-0.0015, $n = 22$). Duplicate samples were analyzed every 20 samples with a mean relative percent difference of $3.69 \pm 2.85\%$ (range = 0.41-9.36%, $n = 15$). Reference samples (MESS-3, TORT-2 and DOLT-4) were analyzed every 10 samples with a mean percent recovery for each of: MESS-3 $101.35 \pm 3.54\%$ ($n = 8$), TORT-2 $110.01 \pm 13.63\%$ ($n = 8$), and DOLT-4 $98.90 \pm 2.44\%$ ($n = 8$). Results are presented as wet weight mercury concentrations, assuming a standard 80% moisture content of muscle. This conversion was done in order to compare our dusky grouper muscle mercury concentrations to other reported literature values as well as toxicity benchmarks which are generally determined as wet weights.

2.3. Data analyses

Individuals were pooled into four size classes (<351 mm TL, 351–500 mm TL, 501–650 mm TL, and >650 mm TL) in order to evaluate mercury contamination along the size increment of the species. These size classes were chosen based on prior studies of dusky grouper reproduction, age structure and feeding habits for the study region (Conдини et al., 2014a, 2014b, 2015, respectively). However, all statistical analyses were conducted using continuous data and ecological factors of interest, with and without the inclusion of individuals >650 mm TL from the neritic zone (no

individuals of this size were present in the littoral zone). Conducting complementary analyses with and without the largest size class is relevant for a couple reasons. First, excluding individuals >650 mm TL allows for a more direct comparison between sites with completely overlapping size distributions. Second, sexual reversal occurs at approximately the 650 mm TL threshold (Condini et al., 2014a), and this important physiological and ecological change may affect mercury accumulation differently in this largest size class compared with smaller size classes.

Relationships between factors expected to affect mercury concentration ([Hg]) (i.e. total length, weight, age and $\delta^{15}\text{N}$) were tested at the 'global' scale, i.e. all individuals from both sites, using linear regression, and relationships were compared between sites using analysis of covariance (ANCOVA). Relationships between independent variables are depicted in Fig. 2, and ANCOVAs with site as the factor and the independent variables from the global regressions as the covariate are depicted in Fig. 3. As noted above, all of the aforementioned analyses were conducted with and without individuals >650 mm TL. An additional series of ANCOVAs tested for differences in the relationship between each factor and [Hg] for individuals <650 mm TL vs. >650 mm TL from the neritic site (Hoeinghaus et al., 2006). All analyses were conducted using R version 3.1.2.

3. Results and discussion

All individuals captured from the littoral zone were females, and few males were caught from the neritic zone (94% females, 6% males). Total lengths (TL) of individuals

from the littoral zone ranged from 260 to 640 mm and total weights (TW) ranged from 0.34 to 5.23 kg (Table 1). In the neritic zone, TL ranged from 290 to 1160 mm and TW ranged from 0.31 to 25.00 kg. Approximately 53% of all individuals were below the TL of first maturation (496 mm TL), thus our sample population is approximately equally split between sexually immature and mature individuals. Importantly, the lack of large males from the littoral site is not due to sampling bias, but rather is a natural aspect of this system. Large males only occur at the neritic site, likely due to the absence of suitable habitat (i.e. large crevices) in the rocky jetties of the littoral zone and occurrence of spawning aggregations in the neritic zone (Concini et al., 2014a, 2014b).

Total mercury concentrations across both study sites ranged from 0.01 to 0.90 mg kg⁻¹ wet weight, with higher concentrations in larger, older fishes (TL, weight and age, respectively: $R^2 = 0.77$ and $R = 0.88$, both $P < 0.001$; $R^2 = 0.84$ and $R = 0.92$, both $P < 0.001$; and $R^2 = 0.81$ and $R = 0.90$, both $P < 0.001$; Fig. 3a-c). These positive relationships between mercury contamination and fish size and age reflect a well-established pattern, which occurs due to the greater assimilation rate of mercury versus the slow rate at which mercury is eliminated, i.e. bioaccumulation (McKim et al., 1976; Adams and Onorato, 2005; Evers et al., 2009; Tremain and Adams, 2012). In contrast, $\delta^{15}\text{N}$ was not correlated with [Hg] ($R^2 = 0.02$ and $R = 0.14$, both $P > 0.05$). Mercury concentrations typically correlate with $\delta^{15}\text{N}$ within and among species (Wang, 2002; Hussey et al., 2014) because nitrogen isotope ratios are useful tracers of trophic position which is also frequently correlated with body size and age in fishes (i.e. reflecting both bioaccumulation and biomagnification). Dusky grouper in this study, although representing a broad range in body sizes and ages (Fig. 2), have similar

trophic levels throughout their ontogeny (Condini et al., 2015), as evidenced by the limited difference in $\delta^{15}\text{N}$ among individuals (Table 1, Figs. 2d and 3d). That being said, dusky grouper exhibit differences in prey ingested and assimilated among sites and size classes (Condini et al., 2015). Bayesian mixing models based on carbon and nitrogen stable isotope data of dusky grouper and potential prey taxa (Condini et al. 2015) indicate greater assimilation of fishes by grouper in the littoral zone (approximately 80% contribution across size classes) compared with the neritic zone (approximately 40% for all size classes <650 mm TL, but approaching 60% for individuals >650 mm TL; shrimp were the other primary prey type, contributing 30-50% and decreasing with body size).

The aforementioned differences in diet between sites likely contribute to the significant differences in mercury accumulation rate between individuals from the littoral and neritic zones (Fig. 3a-c). Regression slopes in ANCOVA analyses for total length, weight and age were significantly steeper for the littoral site ($F= 7.746$, $d.f.= 1$, $P< 0.01$; $F= 10.045$, $d.f.= 1$, $P< 0.01$; $F= 5.993$, $d.f.= 1$, $P< 0.05$, respectively), indicating greater rate of mercury accumulation compared to the neritic site (for individuals <650 mm TL). This may be due to the greater proportion of fish in the assimilated diet of littoral zone grouper (see Condini et al. 2015), even though trophic position did not differ between sites or size classes, because the fish species primarily consumed (mainly sciaenids, e.g. whitemouth croaker *Micropogonias furnieri*, argentine croaker *Umbrina canosai*, southern kingcroaker *Menticirrhus americanus*, barbel drum *Ctenosciaena gracilicirrhus*, and stripped weakfish *Cynoscion guatucupa*) are longer lived than alternate prey (e.g. blue crabs, shrimp) and thus are expected to carry

greater total mercury in their tissues due to bioaccumulation. Similarly, Tremain and Adams (2012) investigated relationships between total mercury contamination and feeding ecology in some epinephelids and found that species feeding mainly on fish exhibited higher mean mercury concentrations than those species preying upon invertebrates.

Dusky grouper >650 mm TL from the neritic zone had a significantly higher slope in our ANCOVA analysis than smaller size classes (individuals <650 mm) ($F= 111.066$, $DF= 1$, $P< 0.001$), representing an Hg accumulation rate more than 3x faster than the smaller size classes (Fig 3a). The slopes between smaller size classes (<650 mm) from the littoral and neritic zones were not significantly different (Fig. 3a), suggesting that part of the observed increase in accumulation rate with length is due simply to bioaccumulation. This largest size class in the neritic zone exhibits a significant increase in the proportion of fish in the assimilated diet and larger prey in general (Conдини et al., 2015), but fish still contribute less to the assimilated diet than for individuals of all size classes from the littoral zone. Site specific differences in the species composition of prey may play a role (e.g. cephalopods such as *Octopus* spp. consumed only in the neritic zone; Conдини et al. 2015), though our data are insufficient to assess the importance of specific prey taxa.

Another key ecological factor that occurs only in the largest size class is sexual reversal. Several Epinephelidae species, including dusky grouper, are protogynous hermaphrodites, undergoing sex change from female to male at larger sizes (Heemstra and Randall, 1993). We are unaware of any physiological studies that have examined

potential influences of hermaphroditism (e.g. due to hormone changes) on mercury bioaccumulation. Although our sample size for comparing sexes in the largest size class is admittedly small (i.e. females 764 - 933 mm TL, $n = 11$; males 765 - 1160 mm TL, $n = 10$), we found no significant differences in total mercury concentration between genders (ANOVA: $F = 0.1096$, $P > 0.05$).

Higher mercury contamination at the littoral zone was expected since it is located in the mouth of the largest costal lagoon ($\sim 10,000 \text{ km}^2$) in Southern Brazil. This lagoon and its drainage basin ($\sim 200,000 \text{ km}^2$) is surrounded by several cities and possesses a large port with extensive traffic and industrial center located approximately 10 km from our study site (Asmus, 1998). Industrial and agricultural activities and high density urban settlements occurring in this area are associated with several contamination sources such as domestic sewage, stormwater runoff and atmospheric emissions (Seeliger and Costa, 1998; Asmus and Tagliani, 1998; Niencheski et al., 2001; Mirlean et al., 2003). All runoff and continental discharge from this lagoon reach the ocean through the estuary mouth where our study site at the rocky jetties is located (Marques et al., 2010). In contrast, the neritic population is located approximately 16 nautical miles from the mouth of the lagoon and, consequently, probably experiences far lower exposure from pollutants due to massive dilution in the marine system.

Total mercury concentrations in individuals from the littoral zone were below suggested threshold values (0.3 mg kg^{-1}) to prevent fish health impacts (Beckvar et al., 2005; Sandheinrich et al., 2011; Depew et al., 2012). A similar pattern was observed

for dusky groupers with total length less than 650 mm TL caught at the neritic zone. However, two-thirds of the individuals greater than 650 mm TL at the neritic zone ($n = 36$) had mercury concentrations above the 0.3 mg kg^{-1} toxicity threshold (Fig. 3a). Although most individuals of dusky grouper showed mercury concentration below safety levels, the majority of larger, reproductively mature individuals ($>650 \text{ mm TL}$) had higher concentrations ($>0.3 \text{ mg kg}^{-1}$) indicating potential harmful consequences for fish health and human consumption. Thus, consumption of dusky grouper larger than 650 mm TL by humans should be avoided or limited in this region of the Southwestern Atlantic to prevent exposure to mercury.

Mercury contamination values found in the present work were considerably higher than prior results reported for this lagoon by Niencheski et al. (2001) and Kutter et al. (2009). Niencheski et al. (2001) investigated only two estuarine species (*Odonthestes bonariensis* and *Micropogonias furnieri*) and all analyzed individuals had mercury concentrations below 0.3 mg kg^{-1} . In contrast, Kutter et al. (2009) analyzed several freshwater, estuarine and marine species, but only two freshwater species (*Oligosarcus jenynsii* and *O. robustus*) showed mercury contamination values above 0.3 mg kg^{-1} . Kutter et al. (2009) analyzed a single large (900 mm TL) dusky grouper specimen, which showed a lower value (215.8 ng g^{-1} wet weight, equivalent 0.22 mg kg^{-1}) than the average value observed in this study for individuals of similar body size (e.g. 0.53 mg kg^{-1} wet weight). Unfortunately, they did not report the site location where this specimen was caught, but considering its large body size and expected size distribution of this species in this region (Condini et al., 2014a), it most likely came from offshore waters and not from the rocky jetties in the littoral zone. In contrast,

Tremain and Adams (2012) determined mercury levels in several *Epinephelus* grouper species (*E. adscensionis*, *E. drummondhayi*, *E. flavolimbatus*, *E. itajara*, *E. morio* and *E. nigritus*) in the Gulf of Mexico and found mean concentrations similar to those we observed in the present study.

Many of the fish species consumed by dusky grouper (e.g., whitemouth croaker) use the estuarine regions as a feeding and growing site during their early stages of life and then migrate to the ocean to spawn. It seems plausible that the mercury concentrations observed in this dusky grouper population occur mainly due to the consumption of fish prey that spends the majority of their life in the estuarine region of Patos Lagoon. That is, fish moving from the estuary to the coastal zone are serving as a vector for mercury transport and accumulation in coastal fisheries. This is potentially significant due to the expected greater potential for mercury methylation in shallow wetland habitats of the estuary and concentrated point sources in that region. Further investigation on mercury contamination of marine estuarine-dependent fishes integrated with natural markers (e.g., otolith chemistry, stable isotopes) to reveal fish movement patterns between estuary and adjacent sea would be necessary to evaluate this hypothesis.

4. Conclusions

Our study revealed that large individuals of dusky grouper occurring in the southernmost limit of its distribution in the Southwestern Atlantic have mercury contamination levels that are potentially harmful for this endangered fish species and

also above the acceptable limits for human consumption. Mercury contamination in this species was correlated both with site locations and body sizes. Mature larger-body individuals (>650 mm and >8 years old) exhibited the highest mercury concentrations. When considering similar body sizes, individuals inhabiting littoral rocky habitats adjacent to a large coastal lagoon had higher concentrations of mercury probably due to proximity to pollution sources associated with human activities in the estuary and its drainage basin. Based on prior diet studies with this population, we hypothesized that fish consumption (mainly of estuarine-dependent fishes) was the most likely trophic link leading to mercury contamination, especially in the littoral zone. Further studies are needed to identify which human activities are the primary sources of mercury contamination and also to test our proposed hypothesis on the prevalence of estuarine-dependent fish prey as the main trophic link leading to mercury contamination in both littoral and offshore subpopulations of this endangered species.

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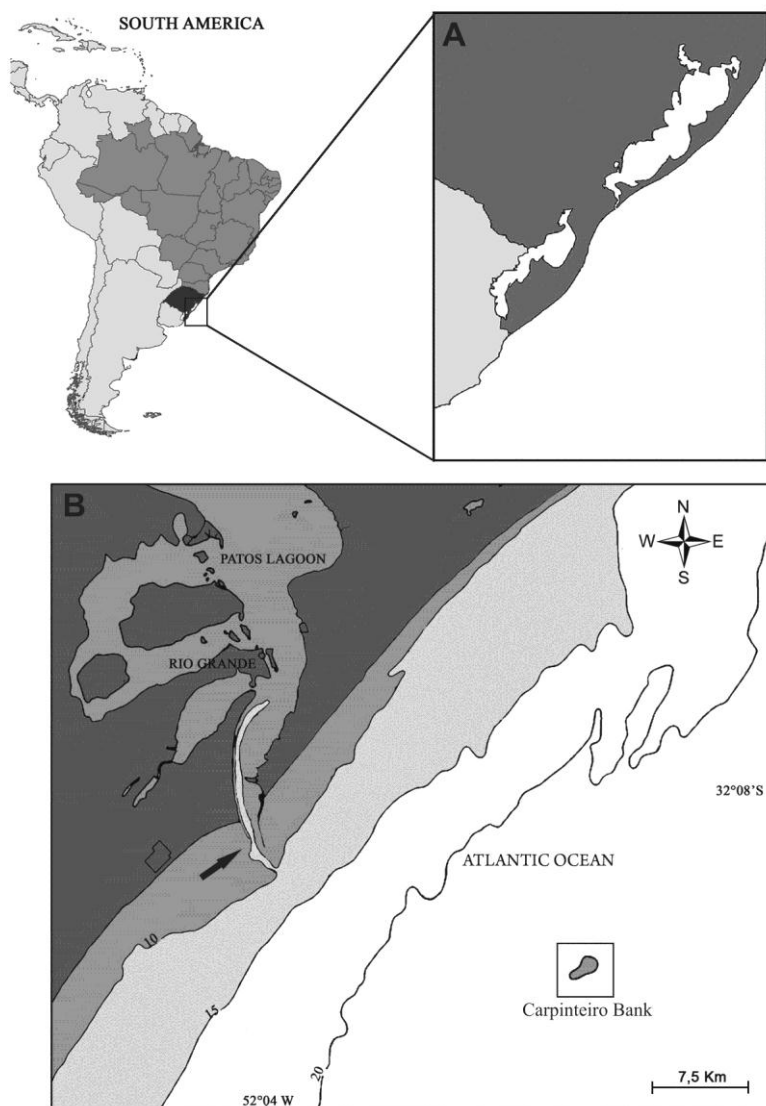


Figure 1

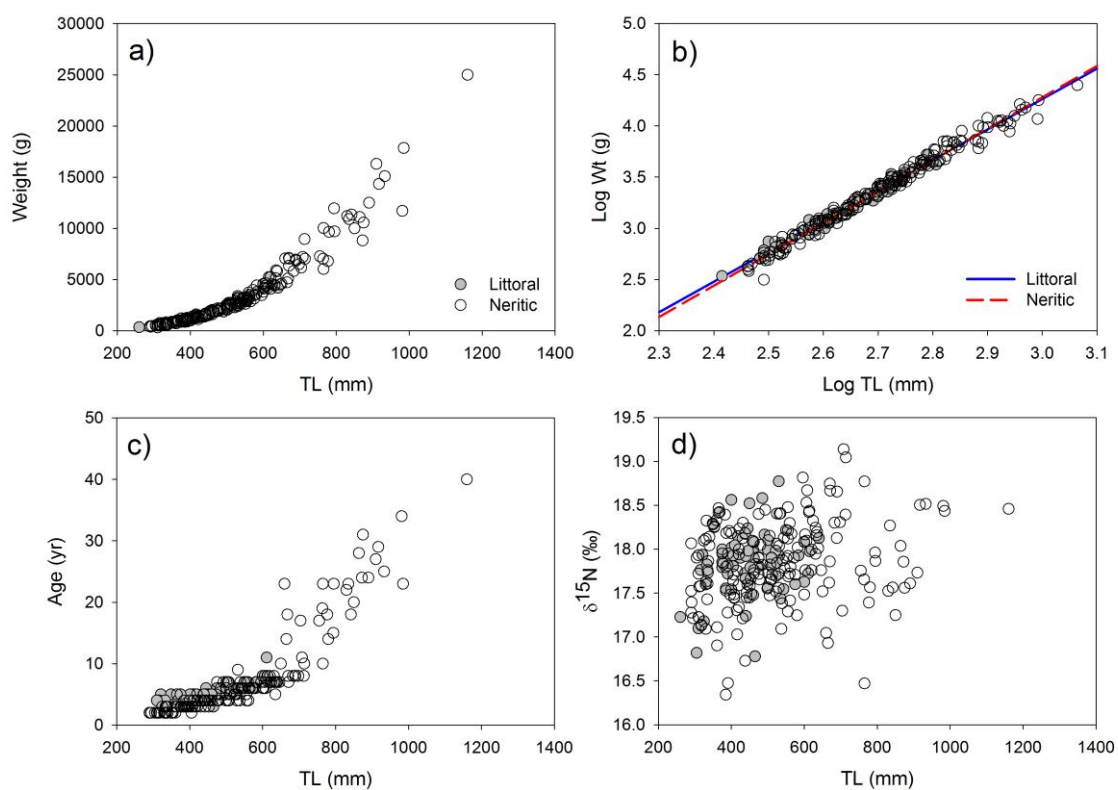


Figure 2

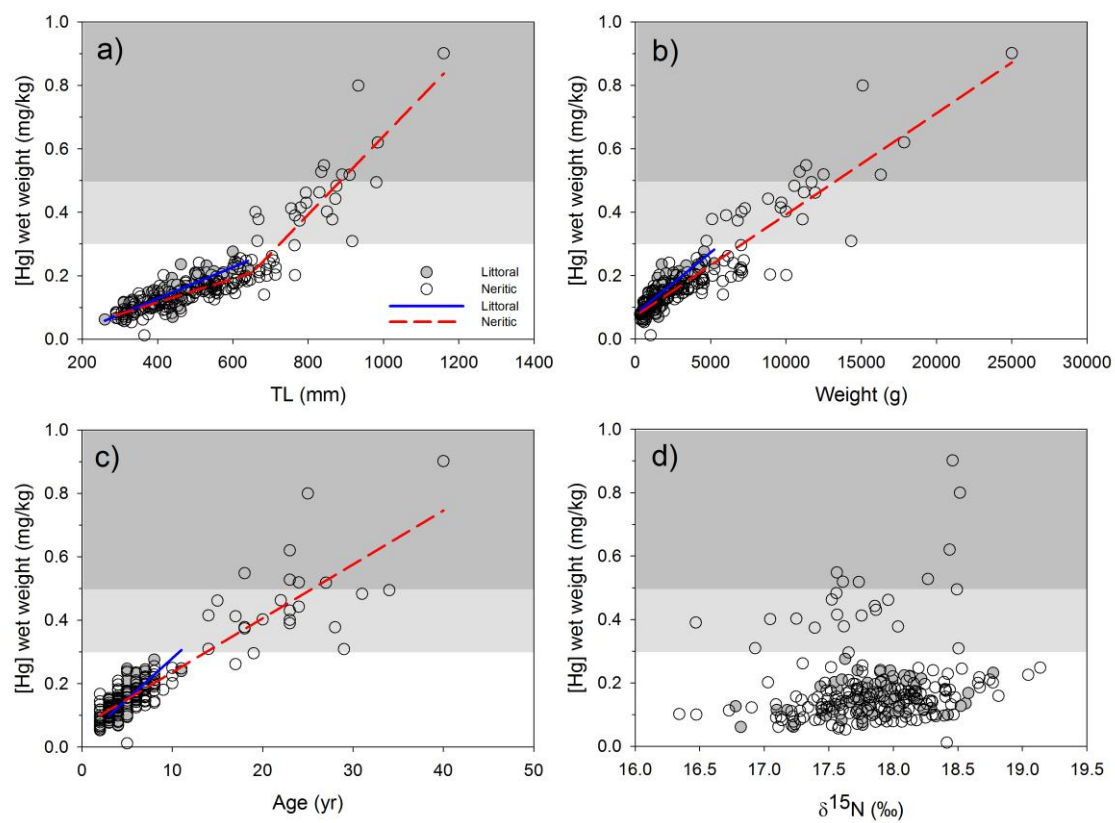


Figure 3

Table 1 - Comparison of mercury concentration (mg kg⁻¹ wet weight), total length (mm), total weight (kg), age (years) and $\delta^{15}\text{N}$ (‰) of the dusky grouper (*Epinephelus marginatus*) from littoral and neritic habitats along the Southern Brazilian coast.

Parameters	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
<351 mm	Littoral zone (n = 10)		Neritic zone (n=26)	
Hg (mg kg ⁻¹)	0.09 $\pm$ 0.03	0.06-0.12	0.08 $\pm$ 0.02	0.05-0.14
Total length (mm)	318.4 $\pm$ 24.2	260-349	318.7 $\pm$ 17.8	290-350
Total weight (Kg)	0.63 $\pm$ 0.12	0.34-0.79	0.56 $\pm$ 0.13	0.31-0.87
Age (years) *	3.9 $\pm$ 0.8	3-5	2.1 $\pm$ 0.4	2-3
$\delta^{15}\text{N}$ (‰)	17.55 $\pm$ 0.45	16.82-18.29	17.66 $\pm$ 0.39	17.09-18.32
351-500 mm	Littoral zone (n = 40)		Neritic zone (n=57)	
Hg (mg kg ⁻¹)	0.14 $\pm$ 0.03	0.07-0.24	0.12 $\pm$ 0.03	0.01-0.20
Total length (mm)	433.9 $\pm$ 37.1	368-500	419.5 $\pm$ 39.5	353-499
Total weight (Kg)	1.54 $\pm$ 0.41	0.86-2.54	1.38 $\pm$ 0.41	0.70-2.27
Age (years) **	4.7 $\pm$ 0.8	3-6	3.6 $\pm$ 1.0	2-7
$\delta^{15}\text{N}$ (‰)	17.89 $\pm$ 0.35	16.78-18.58	17.74 $\pm$ 0.48	16.34-18.47
501-650 mm	Littoral zone (n = 18)		Neritic zone (n=57)	
Hg (mg kg ⁻¹)	0.21 $\pm$ 0.03	0.15-0.28	0.18 $\pm$ 0.03	0.13-0.26
Total length (mm)	555.8 $\pm$ 42.7	501-640	567.9 $\pm$ 43.2	501-650
Total weight (Kg)	3.31 $\pm$ 0.92	2.04-5.23	3.51 $\pm$ 0.93	2.11-5.90
Age (years) ***	6.7 $\pm$ 1.4	5-11	6.2 $\pm$ 1.3	4-10
$\delta^{15}\text{N}$ (‰)	17.90 $\pm$ 0.31	17.44-18.77	17.94 $\pm$ 0.37	17.09-18.82
>650 mm	Littoral zone (n = 0)		Neritic zone (n=36)	
Hg (mg kg ⁻¹)			0.38 $\pm$ 0.17	0.14-0.90
Total length (mm)			792.8 $\pm$ 114.8	660-1160
Total weight (Kg)			9.67 $\pm$ 4.15	4.73-25.00
Age (years)			18.4 $\pm$ 8.5	7-40
$\delta^{15}\text{N}$ (‰)			17.98 $\pm$ 0.61	16.47-19.14

*Age in the littoral zone $n = 8$ and neritic zone $n = 26$; **Age in the littoral zone $n = 33$ and neritic zone $n = 51$; ***Age in the littoral zone $n = 16$ and neritic zone $n = 55$

Figure captions

Figure 1 – Patos Lagoon ($\sim 10,000 \text{ km}^2$) and its estuarine zone in the state of Rio Grande do Sul, southern Brazil (A). Rocky jetties at the mouth of the lagoon (littoral zone, arrow) and Carpinteiro Bank in the adjacent marine area (neritic zone, box highlight) (B). The lines along the coast denote 10, 15, and 20 m isobaths, respectively.

Figure 2 – Relationship between total length (mm) and weight (g), age (years) and $\delta^{15}\text{N}$ (‰) (A, C and D) and between log total length and log weight (B) of dusky grouper from littoral and neritic habitats along the Southern Brazilian coast.

Figure 3 – Relationships between total mercury concentration (mg kg^{-1} wet weight) and total length (mm), weight (g), age (years) and $\delta^{15}\text{N}$ (‰) (A through D, respectively) of dusky grouper from littoral and neritic habitats along the Southern Brazilian coast. Significant regressions are plotted for both sites for each factor. Shading indicates toxicity thresholds at 0.3 mg kg^{-1} and 0.5 mg kg^{-1} .