

Universidade Federal do Espírito Santo
Centro de Ciências Humanas e Naturais
Programa de Pós-graduação em Oceanografia Ambiental

**Identificação e Dinâmica de Agregação de Meros
(*Epinephelus itajara*) na Região Sul do Brasil e Registro
de Anomalias Ósseas**

Leonardo Schlögel Bueno

**Tese submetida ao Programa de Pós-
Graduação em Oceanografia Ambiental (Área de
Concentração em Sistemas Costeiros e
Marinhos) da Universidade Federal do Espírito
Santo como requisito parcial para a obtenção
do grau de Doutor em Oceanografia Ambiental**

Vitória, ES
Outubro, 2014

Universidade Federal do Espírito Santo
Centro de Ciências Humanas e Naturais
Programa de Pós-graduação em Oceanografia Ambiental

**Identificação e Dinâmica de Agregação de Meros
(*Epinephelus itajara*) na Região Sul do Brasil e Registro
de Anomalias Ósseas**

Leonardo Schlögel Bueno

Orientador: Dr. Mauricio Hostim Silva

Vitória, ES
Outubro, 2014

Agradecimentos

Este trabalho resultou da conjugação de esforços, apoio, renúncias, realizações, incentivos e, portanto, devo muitos agradecimentos:

Ao meu orientador Professor, Dr. Mauricio Hostim Silva, cujo apoio foi fundamental para a concretização dos meus objetivos, tanto na realização acadêmica quanto na participação no projeto Meros do Brasil, que me aproximou de reconhecidos pesquisadores e do conhecimento prático, além do suporte, conhecimentos científicos, seu bom humor e generosidade;

Ao Professor Pós-Doutor Pedro Carlos Pinheiro, amigo que acompanhou meu caminho acadêmico e sempre me incentivou a buscar novos conhecimentos;

Aos demais professores do Programa de Pós-Graduação em Oceanografia Ambiental das UFES que transmitiram seus conhecimentos e colaboraram na construção desse trabalho;

Aos meus pais, Ivan e Rosane, pelo carinho, esforço, incentivo, amor e principalmente pelas pessoas maravilhosas que são;

À Fernanda Vollrath, minha noiva, pelo incentivo e compreensão pelos momentos de ausência;

Aos meus avós, Fridolim e Mary, cujos exemplos influenciaram minhas escolhas e trajetória;

À tia Elayne, pela paciência na orientação da revisão e normalização dos originais;

À prima Renata Cunali, pelas ótimas revisões e dicas sobre a escrita em inglês;

Ao Alexandre, Da Graça e Carol pelos ótimos momentos vívidos no Espírito Santo ;

Aos demais familiares, especialmente, Carolina, Cynthia, Alex e Rafa;

À família Beninca por me acolher de forma tão agradável e pela amizade;

Aos grandes companheiros Felipe Daros (Batatinha), Áthila A. Bertoncine, Leonardo Machado, Matheus O. Freitas pela experiência de mergulho e conhecimentos que me possibilitaram aperfeiçoar minhas técnicas de coleta. Também pela amizade, os bons mergulhos e muitas risadas;

Aos amigos companheiros de mergulho que me ajudaram na coleta dos dados e despenharam papel importantíssimo para realização deste trabalho: Jonas, Thiago (Abu), Jonhatas (Jony), Diogo (Kid);

Aos amigos Lucio e Paty pela longa e boa amizade, ajuda e incentivo em momentos difíceis;

À toda equipe do Projeto Meros do Brasil pelos bons momentos, discussões científicas e grande aporte de conhecimento;

Aos companheiros do Instituto COMAR, Jonhatas Alvez, Thiago Souza, Douglas Macali e Diogo Moreira por segurarem as pontas na minha ausência neste período;

Ao equipe da Sub-Marine, em especial, o Fabio pela ajuda e suporte logístico;

Ao Cesar da Lancha Furacão cuja a parceria e amizade possibilitaram as amostragens e realização do trabalho;

À Associação Catarinense de Pesca Submarina na aproximação da pesquisa com os pescadores sub, parceria a qual vem gerando bons frutos;

Ao Chineque (Rafael Luiz Velo) companheiro de mar e sua fiel companheira “pesca sub sul” que me apresentaram muitos dos pontos de mergulhos utilizados nessa tese, além de prover muitas das informações sobre a ocorrência de meros na região;

À Vania, que fez os meus momentos vividos em São Mateus se tornarem muito mais agradáveis e por todo carinho na recepção em sua casa;

À família Koenig/Coleman pela grande acolhida e generosidade na minha empreitada nos EUA ,além dos grandes ensinamentos;

Ao casal Chris e Jess por me acolherem em seu lar e pelas grandes lições de língua inglesa, boas risadas e imersão na cultura norte americana;

À toda equipe do Reef Fish Ecology Lab (FSU) minha gratidão pelo companheirismo, amizade e os divertidos dias de campo;

A FAPES e a CAPES pelas bolsas concedidas.

SUMÁRIO

1 INTRODUÇÃO GERAL	11
1.1 O Mero (<i>Epinephelus itajara</i>)	11
1.2 Agregações reprodutivas	15
REFERÊNCIAS	18

Capítulo 1 - The reproductive period for Goliath Grouper *Epinephelus itajara* in southern Brazil..... 21

Abstract	22
INTRODUCTION.....	23
MATERIAL AND METHODS.....	24
Study Area	24
Data Collection	26
UVC Surveys	27
Catching surveys	27
Data analysis	28
RESULTS.....	29
Diving Survey	29
Gonad samples	33
DISCUSSION.....	35
Needs to management and enforcement.....	38
REFERENCES.....	40

Capítulo 2- Goliath Grouper local change or disappearance of spawning aggregation and dislocation among these sites at south Brazil..... 43

Abstract	44
1 Introduction	45
2 Material and methods	46
2.1 Study area.....	46
2.2 Data Acquisition.....	49
2.2.1 Dive surveys.....	49
2.2.2 External tagging.....	49
2.2.3 Data analysis.....	50
3 Results	50
3.1 Dive surveys.....	50
3.2 External tagging.....	54
4 Discussion	57
References	61

Capítulo 3 - First records of pughead and short-tail skeletal deformities in Goliath Grouper, *Epinephelus itajara*, (Perciformes: Epinephelidae) registered during spawning aggregations.....65

Abstract	66
INTRODUCTION.....	67
MATERIAL AND METHODS.....	67

RESULTS	67
DISCUSSION	68
REFERENCES	70

LISTA DE FIGURAS

Capítulo 1 - The reproductive period for Goliath Grouper *Epinephelus itajara* in southern Brazil

- Fig. 1.** Map of Brazil indicating the study area on the left an expanded view of the study sites on the right including Balsa Norte (BN), Marine Artificial Reefs (RAM) and Monobóia (MB)..... 25
- Fig. 2.** Sketches and photographs of artificial reefs - studied sites (RAM; Monobóia and Balsa Norte). Drawing - Rosane Schlögel and Photos - Leonardo S. Bueno. 26
- Fig. 3.** Monthly variation average SPUE (sightings-per-unit-effort) of Goliath Grouper (*Epinephelus itajara*) determined by roving diver surveys conducted from 2007-2014 at Monobóia, RAM and Balsa Norte in southern Brazil. Error bars represents the maximum value found and black dots represented means. 31
- Fig. 4.** Size class (TL) distributions of Goliath Grouper (GG) from south Brazil relative to seasons, measured *in situ* using laser metrics, hook and line samples and donated specimens from 2007-2014. The dotted line marks the division between size classes of immature and mature Goliath Grouper, according to Bullock et al. (1992)..... 32
- Fig. 5.** Photomicrographs of ovarian histology of Goliath Groupers (*Epinephelus itajara*) sampled in south Brazil magnification 10 x. A) female, 144 cm TL sampled during July showing RT phase – MB = muscle bundle, PG = primary growth oocyte; B) female, 230 cm TL - (December, 27th 2012) showing reproductive phase: spawning capable – CA = cortical alveolar, PG = primary growth, Vtg = primary vitellogenesis oocyte, Vtg3 = tertiary vitellogenic oocyte, GVM = germinal vesicle migration; C) female, 159 cm TL showing SC phase in actively spawning subphase – H = hydrated oocytes; D) female 205 cm TL (January, 10th 2014) in the reproductive phase postspawning – POF = postovulatory follicle complex. 34
- Fig. 6.** Variations in SPUE (sightings-per-unit-effort) of Goliath Grouper (*Epinephelus itajara*) along lunar phases, for pooled months (November, December, January and February) from 2007-2013 in south Brazil. 35

Capítulo 2 - Goliath Grouper local change or disappearance of spawning aggregation and dislocation among these sites at south Brazil

- Fig. 1.** Study area pointed out in the Brazil map. Natural rock reefs (pentagon) and artificial reefs (triangles). 47
- Fig. 2.** Goliath Grouper using artificial reef areas. At site Monobóia (A) and at site RAM (B) located in south Brazil..... 48
- Fig. 3.** Means of Goliath Grouper abundance by sampled sites (MB = Monobóia, RAM = Recifes Artificiais Marinhos, BN = Balsa Norte, BS = Balsa Sul, TA = Tamboretas, AG = Arquipélago das Graças, LL = Laje dos

Lobos) located at south Brazil. RDT samples data grouped from 2007 to 2014.	53
Fig. 4. Goliath Groupers abundance by season in all sampled sites divided into artificial group (MB, RAM, BN and BS) and natural group (TA, AG, LL), during the years of 2007 to 2014 sites located at south Brazil.....	53
Fig. 5. Goliath Groupers abundance distributed by years in all sampled sites divided into artificial (MB, RAM, BN and BS) and natural group (TA, AG, LL), sites located at south of Brazil.	54
Fig. 6. Cluster analysis using Goliath Grouper abundance data at south Brazil by sites. Dendrogram from clustering using the legends: BN= Balsa Norte, BS= Balsa Sul, MB= Monoboia, RAM= Recifes Artificiais Marinho, TA= Tamboretes, AG= Arquipélago das Graças, LL= Laje dos Lobos. The triangles (▲) represent artificial reefs and lozenges (◆) represent natural reefs. Cut line defined at 67 % of similarity.	55
Fig. 7. Percentage of adult Goliath Grouper, <i>Epinephelus itajara</i> (n=67) regarding distance travelled in south Brazil from original offshore tagging site.....	56
Fig. 8. Movement tagged adult Goliath Grouper (<i>Epinephelus itajara</i>) at south Brazil. The Arabic numerals post at each site represent the number of Goliath Grouper tagged in that sites, arrows = adult movement, Roman numerals = number of Goliath Grouper resight at the tagged site.	56

Capítulo 3 - First records of pughead and short-tail skeletal deformities in Goliath Grouper, *Epinephelus itajara*, (Perciformes: Epinephelidae) registered during spawning aggregations

Fig. 1. (a) Normal Goliath Grouper (<i>Epinephelus itajara</i>) (b) Pughead Goliath Grouper observed off Jupiter, Florida, USA, (b inset) Close-up of the deformity, (c) Pughead Goliath Grouper at southern Brazil (d) “short-tail” Goliath Grouper at Paraná state, south Brazil. Note: split dorsal fin was the result of sampling fin rays for age determination.	68
---	----

LISTA E TABELAS

Capítulo 1 - The reproductive period for Goliath Grouper *Epinephelus itajara* in southern Brazil

- Table 1.** Characteristics of three primary study sites off southern Brazil. 29
- Table 2.** Seasonal differences in SPUE for Goliath Grouper (*Epinephelus itajara*) encountered on artificial habitats in southern Brazil, showing **N samples** = number of dive surveys, maximum abundance (**max**), minimum abundance (**min**), mean, standard deviation (**SD**) and variance (**var**), unit effort = 30 minute survey. 30
- Table 3.** Pair-wise *a posteriori* comparison of seasonal differences in Goliath Grouper (*Epinephelus itajara*) sightings-per-unit-effort (SPUE). **t** = t-statistic on pseudo-F values; **p** = *p* value; ns = not significant, * = significant at the 0.05 level. 30
- Table 4.** Seasonal differences in the mean size and standard deviation (**SD**) of all Goliath Grouper (*Epinephelus itajara*) measured with lasers and caught from study sites off southern Brazil (pooled catches Balsa Norte, RAM, and Monobóia). 33

Capítulo 2 - Goliath Grouper local change or disappearance of spawning aggregation and dislocation among these sites at south Brazil

- Table 1.** Sites differences in SPUE for Goliath Grouper (*Epinephelus itajara*) encountered on artificial and natural reefs in southern Brazil, showing **Samples** = number of dive surveys, maximum abundance (**max**), **mean**, standard deviation (**SD**) and frequency of occurrence (%) unit effort = 30 minutes survey. 51
- Table 2.** Multiple comparison using data on goliath grouper abundance presenting *p*-value for the studied sites (MB = Monobóia, RAM = Recifes Artificiais Marinhos, BN = Balsa Norte, BS = Balsa Sul, TA = Tamboretetes, AG = Arquipélago das Graças, LL = Laje dos Lobos). The sites are distribute according to reef types (Artificial or Natural). 52

APRESENTAÇÃO

O mero, *Epinephelus itajara* ou “senhor das pedras” como é conhecido popularmente, pertence ao grupo das grandes garoupas e é classificado pela International Union for Conservation of Nature (IUCN) como uma espécie criticamente em perigo de extinção e, possivelmente, já extinta em alguns lugares do mundo antes mesmo que se tenha informações sobre sua biologia e ecologia. Devido à sua lenta taxa de crescimento, maturação tardia (cerca de 5 anos), alta longevidade (até 37 anos), formação de agregados reprodutivos (>100 ind.) em áreas rasas (<50 m) e a alta fidelidade ao habitat fazem com que essa espécie se torne altamente suscetível à sobrepesca. Trata-se de um dos maiores peixes ósseos encontrados no oceano Atlântico, podendo alcançar mais de 2,5 metros de comprimento e pesar até 400 quilos. Grande predador apresenta papel ecológico importante, modelando as comunidades dos ambientes em que vive e exercendo efeito positivo na abundância e biodiversidade de peixes recifais dessas áreas.

A presente tese de doutorado foi elaborada a partir de dados do Projeto Meros do Brasil - Pesquisa e Conservação, que visa aumentar e divulgar o conhecimento sobre essa espécie de peixe. Os dados utilizados para este trabalho são provenientes da região sul do Brasil, compreendendo os estados do Paraná e Santa Catarina, cujos ambientes estudados abrangem áreas recifais naturais e artificiais localizadas em mar aberto. Também foram usadas informações procedentes de amostragens realizadas na Flórida, mediante parceria com o *Reef Fish Ecology Laboratory (Florida State University)*. Para obtenção dos dados foram utilizadas quatro diferentes metodologias: censos visuais subaquáticos, empregando o método de busca intensiva; capturas (anzol e linha de mão) de exemplares devolvidos vivos; marcações (*tags* externos); e amostragens de exemplares encontrados mortos. Os dados obtidos são provenientes de série amostral de 7 anos (2007 a 2014), totalizando 316 amostras (censo visual), 17 exemplares capturados e 67 exemplares marcados com *tags*.

Descreve-se, de forma inédita, a dinâmica espaço temporal das agregações reprodutivas de meros na região sul do Brasil. A tese foi dividida em introdução geral e três capítulos compostos por manuscritos em formato de artigos científicos. A introdução geral versa sobre a espécie e agregações reprodutivas. Descrevem-se, no capítulo 1, a época e os locais de reprodução do mero na região de estudo, bem como o tamanho dos meros presentes nas agregações reprodutivas. O capítulo 2 abrange o uso dos recifes artificiais pela espécie e discute o desaparecimento de agregações reprodutivas relatadas no passado, revelando dados sobre a movimentação de meros adultos no sul do Brasil. O último capítulo trata da descrição das anormalidades ósseas encontradas em meros presentes em agregações reprodutivas, tanto no Brasil quanto na Flórida.

Os dados gerados por esse estudo fazem parte das primeiras informações sobre esta espécie no Brasil, trazendo assim conhecimento base para fomentar questões de pesquisa e metodologias a serem empregadas em futuros estudos. Espera-se que os dados aqui apresentados possam contribuir no fomento de medidas para conservação desta espécie.

1 INTRODUÇÃO GERAL

1.1 O Mero (*Epinephelus itajara*)

O mero, *Epinephelus itajara* (Lichtenstein, 1822) ou “senhor das pedras”, como é conhecido popularmente (HOSTIM-SILVA *et al.*, 2005), pertence ao grupo das garoupas. Foi descrito por Lichtenstein em 1822, como *Serranus itajara* e estava enquadrado inicialmente na família Serranidae. Posteriormente, em 1884, Bloch propôs a inclusão do mero no gênero *Epinephelus*, que passou a pertencer à sub-família Epinephelinae e adquiriu o nome científico de *Epinephelus itajara* (FERREIRA *et al.*, 2012). Em estudo publicado em 2007, Craig e Hastings, baseados em análises genéticas realizaram uma revisão e sugeriram elevar o status da subfamília Epinephelinae, sendo criada a família Epinephelidae à qual o mero pertence.

E. itajara pode alcançar mais de 2,5 metros de comprimento e pesar até 400 quilos (BULLOCK *et al.*, 1992; SADOVY & ECKLUND, 1999). Apresentam maturação tardia e alta longevidade, delongam cerca de 6 a 8 anos para se tornarem sexualmente ativos e podem viver até 37 anos (BULLOCK *et al.*, 1992). Apesar de seu grande tamanho, o mero se alimenta em nível trófico baixo, predando em grande parte crustáceos (ex.: caranguejos, lagostas e camarões), peixes lentos, normalmente associados ao substrato (ex.: Arraias, Bagres, Peixe-sapo, Baiacu Espinho e Peixe Cofre), dificilmente se alimentando de Lutjanídeos (caranhas) ou Epinefilídeos (garoupas) (FERREIRA *et al.*, 2012).

Seu ciclo de vida inicia-se a partir da reprodução realizada em grandes agregados reprodutivos, com ovos e larvas pelágicas. O desenvolvimento larval ocorre no plâncton, durante o período de 35 a 80 dias, dando origem a pequenos juvenis com aproximadamente 15 mm de comprimento padrão (SADOVY DE MITCHESON & COLIN, 2012). Os juvenis são bentônicos e inicialmente ocupam áreas de mangue. Assumem residência em meio as raízes de mangue, durante sua prolongada fase juvenil, que pode durar de 5 a 6 anos (KOENIG *et al.*, 2007). Após o término da fase juvenil deixam as áreas de manguezal e migram para áreas recifais costeiras, criando distinção

ontogenética em relação ao uso do habitat. Os juvenis utilizam áreas estuarinas e manguezais, enquanto os adultos preferem áreas recifais em mar aberto, não havendo sobreposição de nicho entre eles (KOENIG *et al.*, 2007). No sul do Brasil, meros adultos são encontrados frequentemente em ambientes recifais de mar aberto, mas também podem ser observados em ambientes estuarinos, em geral, habitando fundos rochosos com profundidades de até 30 metros.

O mero vive em águas tropicais do oeste ou leste do oceano Atlântico. Na parte oeste, habita as águas da Carolina do Norte nos Estados Unidos da América (EUA) até o sul do Brasil, incluindo o Golfo do México e o Mar do Caribe. Na parte leste do Atlântico, sua distribuição se estende desde o Senegal até o Congo, embora seja raro nas Ilhas Canárias (FERREIRA *et al.*, 2012) e esteja possivelmente extinto na costa oeste da África (CRAIG *et al.*, 2009). Acreditava-se que *E. itajara* ocorria na costa oeste do Oceano Pacífico. No entanto, Craig et al. (2009) mediante análises genéticas descobriram que os meros que ocorrem no oceano Pacífico são geneticamente diferentes dos encontrados no Atlântico. Essa descoberta provocou a separação dos meros em duas espécies, sendo aqueles que ocorrem no Pacífico nomeado de *Epinephelus quinquefasciatus*, mantando-se a nomenclatura original de *Epinephelus itajara* para os que habitam o Atlântico, o Golfo do México e o Mar do Caribe.

O mero foi classificado como hermafrodita protogínico mas não há evidências concretas de que a espécie de fato seja hermafrodita funcional (FERREIRA *et al.*, 2012). Durante as agregações reprodutivas apresentam razão sexual entre fêmeas macho de 1,1:1 diferenciando do padrão para hermafroditas protogínicos. O aumento no número de hermafroditas com o passar das semanas durante o período reprodutivo, indicando que a mudança de sexo inicia-se e continua durante esse período na costa leste da Flórida. A porção da população que apresenta hermafroditismo mostra-se significativamente baixa, compreendendo 16 % dos meros estudados em agregações na Flórida onde, meros machos, fêmeas e bissexuais ocorrem em todas as classes de tamanhos entre 120 cm e 239 cm TL (KOENIG & COLEMAN, 2013).

O mero forma grandes agregados reprodutivos em águas rasas, agrupando centenas de exemplares em um único recife no mesmo dia. Demonstra alta fidelidade ao habitat, permanecendo longos períodos de tempo na mesma região durante os períodos de agregação (BULLOCK *et al.*, 1992; SADOVY & ECKLUND, 1999; FRIAS-TORRES, 2006). A alta fidelidade aos locais de desova e sua periodicidade foi demonstrada por Koenig & Coleman (2013) que evidenciaram que a desova de meros ocorre com maior frequência na lua nova, havendo a grande taxa de retorno (65 %) dos meros aos locais de reprodução onde foram marcados em anos anteriores no mesmo período.

As agregações reprodutivas realizadas pela espécie enquadra-se no tipo transitório (NEMETH, 2012), ocorrendo a movimentação dos exemplares de sua área de vida até as áreas de reprodução. Segundo Koenig & Coleman (2013) a distância média de movimentação dos meros até as áreas de reprodução é de 6,74 km, com a máxima distância percorrida de 437,8 km em 10 dias (43,7 km/dia), não havendo diferença na movimentação entre machos e fêmeas, mas havendo mais movimentação de indivíduos maiores que menores.

A previsibilidade em tempo e lugar das agregações reprodutivas de meros pelos pescadores, aliadas à maturação tardia e longo período de vida, tornaram a espécie altamente suscetível à sobrepesca. Na década de 50, no Brasil, foram registradas pescarias com elevado número de capturas de meros (Souza, 2000) e se obtiveram os primeiros números de estatística pesqueira de meros nos Estados Unidos da América (EUA). Em apenas poucas décadas de pesca ocorreu grande declínio populacional, que quase levou ao desaparecimento do mero, ao ponto de ser classificado pela International Union for Conservation of Nature (IUCN) como uma espécie criticamente em perigo de extinção. A primeira ação para reverter esse quadro foi a proibição da pesca do mero nas águas territoriais dos EUA em 1992. No Brasil, a pesca do mero está proibida desde 2002. Apesar da ausência de indícios de recuperação dos estoques corre-se o risco da reabertura da pesca no ano de 2015 sem o necessário conhecimento sobre a espécie para o estabelecimento de medidas de manejo. Além da pesca predatória, as populações de meros são ameaçadas por fatores como a poluição de áreas estuarinas e a supressão de áreas de manguezal, ameaçando principalmente as formas jovens.

Em 1994 ocorreu a primeira inclusão do mero na lista de espécies ameaçadas, ou seja, Lista Vermelha da União Internacional de Conservação da Natureza (FRIAS-TORRES, 2006). No Brasil, a evidência de ameaça aos estoques populacionais de mero levou o Instituto Brasileiro do Meio Ambiente e dos Recursos Renováveis (IBAMA), em 20 de setembro de 2002, a publicar a Portaria IBAMA nº 121, que proíbe a pesca dessa espécie em todo o território nacional (BRASIL, 2002). Essa foi uma das primeiras tentativas de restaurar o equilíbrio populacional de *E. itajara* e fornecer tempo para que cientistas estudem seus remanescentes populacionais. Contudo, as sucessivas inclusões em listas de espécies ameaçadas internacionais (TAK-CHUEN & FERREIRA, 2007) e nacionais (ABILHÔA & DUBOC, 2002), a conhecida degradação de ambientes necessários ao seu ciclo de vida, juntamente com a carência de informação sobre sua abundância e distribuição em águas brasileiras, levaram à prorrogação da moratória da pesca do mero no Brasil por mais cinco anos mediante a Portaria IBAMA nº 42/2007 (BRASIL, 2007). No ano de 2012, após o fim da portaria do IBAMA, ocorreu a mobilização da sociedade brasileira e grande esforço dos pesquisadores e da equipe do Projeto Meros do Brasil visando a volta da proibição da pesca. O resultado dessa movimentação junto aos Ministérios do Meio Ambiente e da Pesca e Aquicultura foi o estabelecimento da Instrução Normativa Interministerial de número 13, publicada em 16 de outubro de 2012, que proíbe a pesca e comercialização da espécie por mais 3 anos (BRASIL, 2012). Devido às características peculiares da espécie, essa continua classificada como criticamente em perigo em vários locais do mundo assim como no Brasil.

Apesar da proibição de sua pesca, comércio e transporte, o mero continua sendo ameaçado no Brasil pela pesca ilegal, além de sofrer com a degradação do habitat necessário ao seu ciclo de vida, tais como áreas estuarinas e manguezais que vem sendo poluídas e suprimidas devido ao crescente desenvolvimento imobiliário, industrial e portuário. Sendo assim, estudos envolvendo a bioecologia e mapeamento de áreas prioritárias para o seu ciclo de vida vem ao encontro da necessidade de dados científicos para fomentar medidas de manejo, ordenamento e preservação dessa espécie.

1.2 Agregações reprodutivas

Agregações reprodutivas são espetáculos da natureza, eventos biológicos extravagantes, conhecidos pela ocorrência em muitas espécies de peixes recifais. Tais agregações representam fator chave na regeneração das populações ao mesmo tempo que são oportunidades extremamente atraentes de pesca (SADOVY DE MITCHESON & COLIN, 2012).

Muitas espécies de peixes recifais agregam-se para a reprodução em locais e em épocas específicas (JOHANNES, 1978; CARTER & PERRINE, 1994; SADOVY *et al.*, 1994; DOMEIER & COLIN, 1997), mas as mais estudadas são as agregações de espécies com alto valor ou interesse comercial e/ou que já apresentam declínio populacional aparente, como é o caso do mero (*Epinephelus itajara*) (BULLOCK *et al.*, 1992; COLIN, 1990; EKLUND & SCHULL, 2001; SALA *et al.*, 2003; GERHARDINGER *et al.*, 2007).

No grupo das garoupas de grande porte, o mero é uma das poucas espécies que formam agregações reprodutivas em águas rasas (<50 m) utilizando recifes de mar aberto (COLEMAN & KOENIG, 2003). Preferem especialmente recifes rochosos, naufrágios e recifes artificiais que contenham grandes buracos, cavernas e alto relevo.

Agregações reprodutivas são definidas como: uma repetida concentração de animais marinhos da mesma espécie, reunidos com o propósito de reprodução. A densidade/número de indivíduos participantes dessas agregação é pelo menos quatro vezes maior que o encontrado em períodos não reprodutivos. Agregação reprodutiva devem resultar em uma fonte pontual e massiva de prole (DOMEIER, 2012) e podem ser constituídas de centenas de exemplares ou mais. Essas agregações são espacialmente e temporalmente consistentes, isto é, ocorrem no mesmo período ano após ano e os peixes apresentam grande interanual fidelidade aos locais de reprodução (SADOVY DE MITCHESON & COLIN, 2012). Segundo Colin *et al.* (2003) os principais critérios a serem observados para a identificação de agregações reprodutivas são: o aumento repentino no número de indivíduos em determinado local; características físicas dos exemplares, tais como padrões de cor e abdômen dilatado; características histológicas das gônadas como, por exemplo, fêmeas com óvulos hidratados ou com folículos pós-ovulatórios

presentes, ou ainda, a visualização do evento de liberação dos gametas na coluna d'água.

Domeier & Colin (1997) subdividiram agregações reprodutivas em dois tipos distintos, as residentes e as transitórias, baseados em: 1) a frequência com que a cada agregação reprodutiva ocorre; 2) o tempo de duração da agregação; 3) a especificidade do local da agregação, e 4) a distância que cada exemplar de peixe percorre para alcançar o local de agregação. Em termos gerais, nessas agregações os exemplares de peixes migram para locais além de seu território. Agregações reprodutivas transitórias frequentemente ocorrem em época específica do ano durando cerca de dois meses. Uma única agregação desse tipo pode representar o esforço total de reprodução dos exemplares daquela espécie por ano (SADOVY DE MITCHESON & COLIN, 2012).

Agregações são fenômenos altamente dinâmicos que representam um desafio para a realização de estudos tais como: efetuar a contagem de grande número de peixes em curto período de tempo, cujo número varia a cada hora ou dia; trabalhar em pontos de mergulho profundos e/ou com forte correnteza; e realizar longas jornadas de mergulhos consecutivos durante os ciclos lunares específicos (SADOVY DE MITCHESON & COLIN, 2012).

O rápido declínio encontrado em muitas espécies que realizam agregações por todo o mundo enfatiza a urgência de aumentar rapidamente o entendimento de como essas agregações funcionam nos recifes de corais e outros ambientes (NEMETH, 2009). Sabe-se muito pouco sobre o funcionamento e as implicações ecológicas das agregações reprodutivas de peixes recifais no Brasil, assim como os locais de ocorrência dessas agregações. Em relação às agregações de meros, a maior parte dos estudos foi realizada tendo como fonte de dados o conhecimento ecológico local. Deve-se salientar que apenas o estado de Santa Catarina contava com estudos que indicavam a ocorrência de agregações reprodutivas de mero. No entanto, devido à pesca predatória, quando foram registrados tais indicativos já havia grande declínio populacional, podendo inclusive parte dessas agregações terem desaparecido (GERHARDINGER *et al.*, 2007; GERHARDINGER *et al.*, 2009).

A presente tese teve como objetivo aprofundar o conhecimento científico sobre a biologia, ecologia e comportamento do mero (*Epinephelus itajara*), buscando fornecer informações para embasar medidas de manejo e conservação da espécie, bem como gerar embasamento teórico para futuros estudos e subsidiar novas questões de pesquisa.

REFERÊNCIAS

ABILHOA, V.; DUBOC, L. F. Peixes. In: MICKITH, S. B.; BÉRNILS, R. S. (eds.) **A lista vermelha da fauna ameaçada do Paraná**. Curitiba: Instituto Ambiental do Paraná, 2004. 763 p.

BRASIL. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). **Portaria 42, de 19 de setembro de 2007**. Prorroga por período de cinco anos, a proibição da captura da espécie (*Epinephelus itajara*) nas águas jurisdicionais brasileiras. Disponível em: <https://www.ibama.gov.br/category/40?download...42-2007-.p> Acesso em: 26/08/2014.

BRASIL. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). **Portaria 121, de 20 de setembro de 2002**. Proíbe a captura nas águas jurisdicionais brasileiras, transporte e comercialização da espécie *Epinephelus itajara*. Disponível em: http://www.pesca.sp.gov.br/leg_121.php Acesso em: 26/08/2014.

BRASIL. Ministério da Pesca e Aquicultura (MPA). Instrução Normativa Interministerial nº 13, de 16 de outubro de 2012. Proíbe a captura nas águas jurisdicionais brasileiras, transporte e comercialização da espécie *Epinephelus itajara* no período de 3 anos. **Diário Oficial [da] República Federativa do Brasil**, Brasília, 17 de outubro de 2012. Seção 1, p. 122-123.

BULLOCK, L. H.; MURPHY, M. D.; GODCHARLES, M. F.; MITCHELL, M. E. Age, growth, and reproduction of jewfish *Epinephelus itajara* in eastern Gulf of México. **Fishery Bulletin**, v. 90, p.243-249, 1992.

CARTER, J.; PERRINE, D. A spawning aggregation of dog snapper, *Lutjanus jocu* (Pisces: Lutjanidae) in Belize, Central America. **Bull. Mar. Sci.**, v.55, p.228-234, 1994.

COLEMAN, F. C.; KOENIG, C. C. **Population density, demographics, and predation effects of adult goliath grouper**. Saint Petersburg, FL: National Oceanic and Atmospheric Administration, 2003. (Final Report MARFIN Grant Number 0548-0175).

COLIN, P. L. Preliminary investigations of reproductive activity of the Jewfish, *Epinephelus itajara* (Pisces: Serranidae). In: GULF AND CARIBBEAN FISHERIES INSTITUTE, 43rd, Miami, 1990. **Proceedings...** Miami, Florida: Gulf and Caribbean Fisheries Institute, 1990. p.188-147.

COLIN, P. L.; SADOVY, Y. J.; DOMEIER, M. L. **Manual for the study and conservation of reef fish spawning aggregations**. Fallbrook, CA: SCRFA, 2003. 98 p. (Society for the Conservation of Reef Fish Aggregations Special Publication, 1).

CRAIG, M. T.; HASTINGS, P.A. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. **Ichthyol Res.**, v.17, p.1–17, 2007.

CRAIG, M. T.; GRAHAM, R. T.; TORRES, R. A.; HYDE, J. R.; FREITAS, M. O.; FERREIRA, B. P.; HOSTIM-SILVA, M.; GERHARDINGER, L. C.; BERTONCINI, A. A.; ROBERTSON, D. R. How many species of goliath grouper are there? Cryptic genetic divergence in a threatened marine fish and the resurrection of a geopolitical species. **Endanger Species Res.**, v.7, p.167–174, 2009

DOMEIERS, M. L.; COLIN, P. L. Tropical reef fish spawning aggregations: defined and reviewed. **Bull. Mar. Sci.**, v.60, p.698-726, 1997.

DOMEIERS, M. L. Revisiting spawning aggregations: definitions and challenges. *In*: SADOVY DE MITCHESON, Y.; COLIN, P.L. **Reef fish spawning aggregations: biology, research and management**. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, 2012. v. 35.

EKLUND, A. M.; SCHULL, J. A stepwise approach to investigating the movement patterns and habitat utilization of goliath grouper, *Epinephelus itajara*, using conventional tagging, acoustic telemetry and satellite tracking. *In*: SIBERT, J. R.; NIELSEN, J. L. (eds.). **Electronic tagging and tracking in marine fisheries**. Dordrecht, Netherlands: Kluwer Academic Publishers, 2001. p.189-216.

FERREIRA, B. P.; HOSTIM-SILVA, M.; BERTONCINI, A. A.; COLEMAN, F. C.; KOENIG, C. C. Atlantic Goliath Grouper – *Epinephelus itajara*. *In*: SADOVY DE MITCHESON, Y.; COLIN, P.L. **Reef fish spawning aggregations: biology, research and management**. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, 2012. p. 417-421 (Fish & Fisheries Series, 35).

FRIAS-TORRES, S. Habitat use of juvenile goliath grouper *Epinephelus itajara* in the Florida Keys, USA. **Endangered Species Research**, v.1, p.1-6, 2006.

GERHARDINGER, L. C.; FREITAS, M. O.; MEDEIROS, R. P.; GODOY, E. A.; MARENZI, R. C.; HOSTIM-SILVA, M. Local ecological knowledge on the planning and management of marine protected areas and conservation of fish spawning aggregations: the experience of “Meros do Brasil” project. *In*: ÁREAS aquáticas protegidas como instrumento de gestão pesqueira. Brasília: Ministério do Meio Ambiente, 2007. p. 117-139. (Áreas protegidas do Brasil, 4).

GERHARDINGER, L. C.; HOSTIM-SILVA, M.; MEDEIROS, R. P.; MATAREZI, J.; BERTONCINI, A. A.; FREITAS, M. O.; FERREIRA, B.P. Fishers’ resource mapping and goliath grouper *Epinephelus itajara* (Serranidae) conservation in Brazil. **Neotropical Ichthyology**, v.7, n.1, p.93-102, 2009.

HOSTIM-SILVA, M.; BERTONCINI, A. A.; GERHARDINGER, L. C.; MACHADO, L. F. The Lord of the rocks conservation program in Brazil: the need for a new perception of marine fishes. **Coral Reefs**, v.24, p.74, 2005.

JOHANNES, R. E. Reproductive strategies of coastal marine fishes in the tropics. **Environ. Biol. Fishes.**, v.3, p.65-84, 1978.

KOENIG, C. C.; COLEMAN, F. C.; EKLUND, A. M.; SCHULL, J.; UELAND, J. Mangroves as essential nursery habitat for goliath groups (*Epinephelus itajara*). **Bulletin of Marine Science**, v.80, n.3, p. 567–586, 2007.

KOENIG, C. C.; COLEMAN, F. C. **The recovering goliath grouper population of the southeastern US:** non-consumptive investigations for stock assessment. Saint Petersburg, FL: National Oceanic and Atmospheric Administration, 2013. 70 p. (MARFIN Project FINAL Report, NA10NMF4330123).

NEMETH, R. S. Dynamics of reef fish and decapod crustacean spawning aggregations: underlying mechanisms, habitat linkages, and trophic interactions. *In:* NAGELKERKEN, I. **Ecological connectivity among tropical coastal ecosystems.** New York: Springer, 2009 DOI 10.1007/978-90-481-2406-0.

NEMETH, R. S. Ecosystem aspects of species that aggregate to spawn. *In:* SADOVY DE MITCHESON, Y.; COLIN, P.L. **Reef fish spawning aggregations:** biology, research and management. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, 2012. p. 21-83. (Fish & Fisheries Series, 35).

SADOVY DE MITCHESON, Y. S.; COLIN, P. L. **Reef fish spawning aggregations:** biology, research and management. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, 2012. 621 p. (Fish & Fisheries Series, 35).

SADOVY, Y.; EKLUND, A. M. Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), and the jewfish, *E. itajara* (Lichtenstein, 1822). **NOAA Technical Report NMFS**, 1999. 65 p. (v.146).

SADOVY, Y.; COLIN, P. L.; DOMEIER, M. L. Aggregation and spawning in the tiger grouper (*Mycteroperca tigris* (Pisces:Serranidae). **Copeia**, n.2, p.511-516, 1994.

SALA, E.; ARBUTO-OROPEZA; PAREDES, G.; THOMPSON, G. Spawning aggregations and reproductive behavior of reef fishes in the Gulf of Califórnia. **Bulletin of Marine Science**, v.72, n.1, p.103-201, 2003.

SOUZA, H.S. **O homem da ilha e os pioneiros da caça submarina.** 2nd ed. Florianópolis: Editora Dehon, 2000 352 p.

TAK-CHUEN, C. T.; FERREIRA, B. P. *Epinephelus itajara*. *In:* International Union for Conservation of Nature (IUCN). **2007 IUCN Red List of Threatened Species.** Disponível em: www.iucnredlist.org. Acesso em: 12 de agosto de 2009.

*Em processo de análise pelo Journal of the Marine Biological Association of the
United Kingdom*

CAPÍTULO 1

**The reproductive period for Goliath Grouper *Epinephelus itajara* in
southern Brazil**

Abstract

Fishing for the critically endangered Goliath Grouper (GG) (Epinephelus itajara) has been prohibited in Brazil since 2002. However, this prohibition is likely to be lifted at 2015. Despite efforts, information about their distribution, abundance, or ecology is sparse and there is no data available suggesting that populations have recovered. In this study, we have gathered sightings-per-unit-effort (SPUE) data on three sites in southern Brazil using scuba diving surveys and examining seasonal differences in size distribution and reproductive condition of specimens collected by us or donated by law enforcement officers. Based on the obtained data we observed that the SPUE differed significantly with season ($p < 0.05$), increasing in late spring and peaking during the summer months. A significant effect was observed in the number of fish relative to lunar cycle ($p < 0.05$). All females sampled during the summer were considered reproductively ready to spawn and postspawning, while all those sampled during other seasons were either regressing or dormant. What these data strongly infer is that we have located Goliath Grouper spawning aggregation sites south the state of Paraná and north of the state of Santa Catarina and identified summer as the most likely spawning season. We have also evaluated size frequency distributions, abundance, reproductive characteristics and correlation with environmental parameters. These data may provide information useful for stock assessments and therefore the management and conservation of this fish. This is the first step involved in raising new questions and planning further studies with GG in Brazil.

Running Head: Reproduction of Goliath Grouper in south Brazil.

Keywords: Artificial Reefs, Spawning Aggregations, Reef Fish, Epinephelidae, south Atlantic, Endangered Species, Marine Conservation.

INTRODUCTION

Goliath Grouper, *Epinephelus itajara* (Lichtenstein, 1822), the largest reef fish in the western Atlantic, is considered critically endangered throughout its range (IUCN, 2013). In the western Atlantic, it ranges from North Carolina to southern Brazil, including the Gulf of Mexico and the Caribbean Sea. In the eastern Atlantic, its distribution extends from Senegal to Congo, although it is rare in the Canary Islands (Ferreira *et al.*, 2012) and is believed to be extinct in the eastern Atlantic from Senegal to Congo (Craig *et al.*, 2009). *E. itajara* belongs to the family Epinephelidae, can reach over 2.5 meters total length and weigh up to 400 kilograms with late maturation around 6 to 8 years (males between 110 to 115 cm TL, and females between 120 to 135 cm) and high longevity up to 37 years (Bullock *et al.*, 1992; Sadovy & Eklund, 1999).

The Goliath Grouper forms reproductive aggregations in shallow water (< 50 m) (Ferreira *et al.*, 2012), with a preference for high-relief rocky and/or artificial reefs (Koenig & Coleman, 2009). Their spawning aggregations may consist of more than one hundred individuals (Bullock *et al.*, 1992; Sadovy & Eklund, 1999; Koenig *et al.*, 2007; Koenig *et al.*, 2011). Domeier & Colin (1997) defined reproductive aggregations as single species groups that gather at specific times at specific locations at densities or numbers that are significantly higher than those found at the same site during non-reproductive times, suggesting that an increase in fish density greater than three-fold constitutes a spawning aggregation. These aggregations generally occur at the same time and same site annually (Johannes, 1978; Carter & Perrine, 1994; Sadovy *et al.*, 1994; Domeier & Colin, 1997).

Gerhardinger *et al.* (2009) using fishermen's local ecological knowledge suggested the *E. itajara* spawning aggregation might occur during the summer and on the full moon at south Brazil, which coincides with spawning times in the northern hemisphere (Bullock *et al.*, 1992). Knowledge and protection of these spawning aggregations are key factors to the species' persistence (Sadovy de Mitcheson & Colin, 2012). Fishers threaten this persistence by targeting spawning aggregations to increase catch per effort.

In Brazil, Goliath Groupers are protected by a fishing moratorium instated in 2002 for 5 years (2002-2007), renewed in 2007 for another 5 years (2008-

2012), and again in 2012 for additional three years (2012-2015). However the illegal catches still ongoing and are frequent (Giglio *et al.*, 2013), combined with later maturity may be are impacting the recovery of these populations. Despite the fact that there are no signs of stock recovery and little understanding of Goliath Grouper ecology, full protection for this species will likely be lifted in 2015.

Our main objective in this study was to determine whether Goliath Grouper spawning aggregations still occur in southern Brazil, with focus on determining their location, describing their physical and biological characteristics, and the time at which they are actively reproducing. The results of this study provide critical data relevant for the management and conservation of this species, and represent a first step to new discussions and to continue our research efforts in Brazil.

MATERIAL AND METHODS

Study Area

The study area is located in southern Brazil, between 25° and 27° south latitude in the western Atlantic (Fig. 1). The region has distinct seasonality with summer (late December through late March) being the warmest. During this time, moderate east and northeast winds predominate, bringing warm (up to 27°C) clear waters from the east. The weather is more variable in the fall (late March through late June), and spring (late September through late December) with an increase in large eastern and southeastern swells and coincident decrease in underwater visibility. The winter (late June through late September) is dominated by cold fronts that bring very large swells from the south and southeast, decreasing water temperature (18°C) and increasing turbidity in coastal waters. The surface currents (3.6 meters) in this region in summer and spring direct currents toward the coast and parallel to the coast in the fall and winter (Stein & Noernberg, 2013).



Fig. 1. Map of Brazil indicating the study area on the left an expanded view of the study sites on the right including Balsa Norte (BN), Marine Artificial Reefs (RAM) and Monobóia (MB).

Three artificial reefs were chosen as sampling sites: two at the state of Paraná – “Balsa Norte” (BN), and Marine Artificial Reefs (RAM) – and one offshore the state of Santa Catarina – “Monobóia” (MB). These three sites were selected after 7 years of dive samples at 8 different reef areas (see chapter 2). The selection criteria were frequency and abundance of Goliath Grouper.

The ship Balsa Norte was intentionally sunk in January 2001 to form an artificial reef and is located ~38 km offshore at a depth of 27 m. The ship is 76.3 m long x 11.4 m wide x 5 m high with large features and dark crevices (Fig. 2). The RAM site is composed by two reef areas, separated by ~ 1 km, each one composed by 30 concrete structures, block forms, and reef balls, sunk in June 2000. The structures are 1.5 m high spread on the sand in an area of approximately 30 m x 30 m (Fig. 2). These structures occur ~12 km offshore at a depth of 18 m over sand bottom. Since both areas composed one single site they were sampled in the same field day.

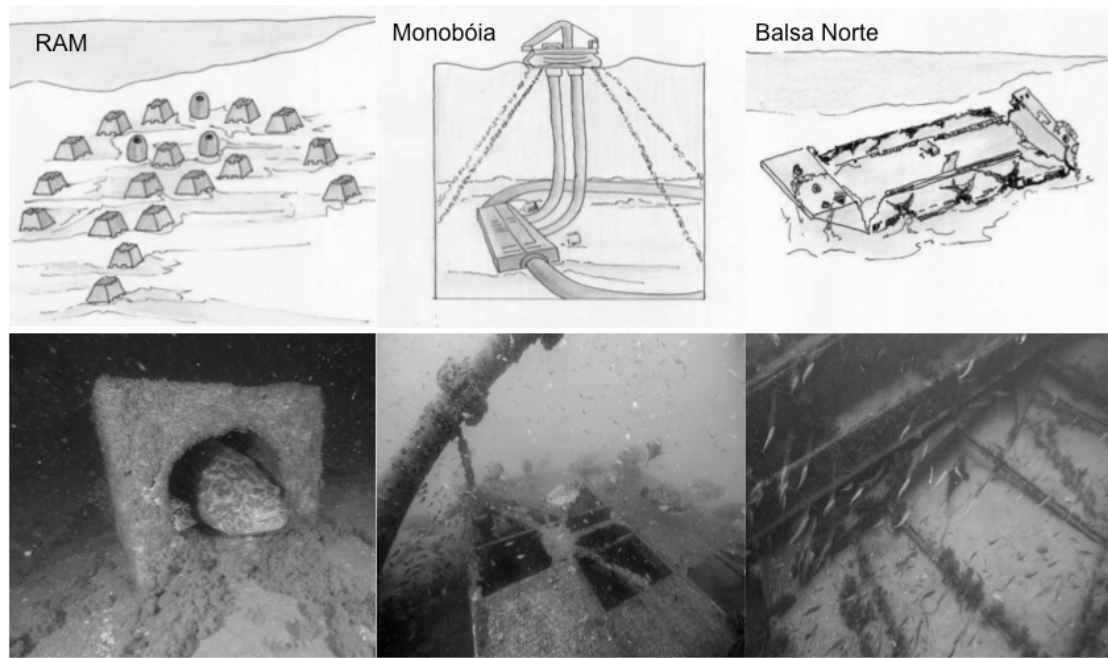


Fig. 2. Sketches and photographs of artificial reefs - studied sites (RAM; Monobóia and Balsa Norte). Drawing - Rosane Schlögel and Photos - Leonardo S. Bueno.

The Monobóia is an artificial reef, formed by pipelines, manifold and some concrete and metallic structures, installed in the 1970s. This reef is attached to a single point mooring buoy located 8 km offshore, where tankers moor to unload oil. The main artificial reef area is about 50 x 50 m and is composed of diverse materials spread on the sand at 25 m deep. The main structure is a metallic protection with 9 m long by 8 m wide and 2 m height being on top of the manifolds. The main structural characteristics of this structure are the vertical flexible pipelines and 6 large chains connecting it to the bottom that gives this site a vertical profile reaching the surface (Fig. 2).

Data Collection

Data was collected from (1) underwater visual census (UVC) on SCUBA; (2) photographs of Goliath Grouper taken during UVC; (3) live specimens obtained through hook-and-line and (4) dead specimens that we either found on site or were donated by law enforcement officers. All activities were authorized by license: SISBIO 15080-2 and SISBIO 31719-1.

UVC Surveys

Between 2007 and 2014 we conducted UVC on Goliath Grouper at all three study sites during each season, with greater emphasis during summer. We utilized the Roving Diver Technique (RDT) (Jones & Thompson, 1978), with the surveys conducted only when visibility exceeded 3 m. During UVC each site was thoroughly surveyed including all crevices and the surrounding perimeter. Temperature, visibility, and number of Goliath Grouper encountered were registered. During this study, we were forbidden of performing diving surveys at the Monobóia, the most important spawning site after the summer of 2012. This restriction was imposed by the organization responsible for the maintenance of this structure. Such restriction compromises significantly the development of further studies about Goliath Grouper spawning in this area of south Brazil.

Goliath Grouper were photographed to determine size, with size estimations conducted using two parallel laser pointers 25 cm apart when fish were perpendicular to the beams. The photograph, laser dots appearing on the fish be used as a metric to determine the fish's total length (TL) according to a centimeter size class (Koenig *et al.*, 2011).

Catching surveys

In addition to the diving surveys, we collected Goliath Grouper with hook-and-line. After hooked the fish was brought slowly to surface, pulled gently into the boat where it was strapped onto a stretcher, vented and had the eyes covered with wet towel (to avoid eye damage from the sun) and gills were irrigated with clean sea water from a pump. The fishes were tagged using conventional tags for the purpose of identify de individual during subsequent UVC or recapture; TL was measured by metric tape. We examined reproductive condition and sex by obtaining gonad biopsies. This technique required that we insert one end of a plastic tube (0.7 cm diam.) through the gonoduct into the gonad, while attaching the other end to a manually operated vacuum pump. The pump was used to suck tissue into an in-line vial (135 mL) obtaining solid pieces of gonads; the contents were immediately preserved in 10 % formalin for 24 h, and then transferred to 70 % ethyl alcohol. After each fish was sampled they were released on the same site. We also obtained gonad samples from

dead fish that were either found or donated by law enforcement officials. These samples were processed in the same manner as those captured from study sites.

Tissue samples were then stained in hemotoxylin-eosin, embedded in paraffin, sectioned to 4-6 micrometers, and then examined under a compound microscope to determine sex and reproductive condition. For gonad analysis, we used five developmental phases, following Brown-Peterson *et al.* (2011): Immature (IM), Developing (DV), Capable of Spawning (SC), Regressing (RG), and Regenerating (RT). The IM phase corresponds to fish that have never spawned, characterized histologically in females by the presence of oogonia and primary growth oocytes through the perinuclear stage (Grier *et al.*, 2009), as well as little space among oocytes in the lamellae and ovarian wall generally thin. In DV females, the ovary is beginning to develop, but not ready to spawn. The SC fish are developmentally and physiologically able to spawn; RG represents the cessation of spawning, and the RT phase corresponds to a sexually mature but reproductively inactive individual.

Data analysis

Sightings data obtained during diving surveys were transformed into sightings-per-unit-effort SPUE (Equation 1), thus taking into account the different sampling efforts of dive time (in minutes) for each survey. For the purposes of this index we used 30 min as the standard time effort, since this was the average time of surveys.

$$\text{Equation 1: } \text{SPUE} = N * (T/t)$$

Where: SPUE = sightings per unit effort, N = number of fish observed, T= duration in minutes of each survey and t is the 30 min to standardization efforts.

We used a three-factor PERMANOVA (season x site x lunar phase) design to evaluate differences in sightings per unit effort by season. A one-way PERMANOVA was applied evaluate differences in sightings per unit effort by months. Linear regression was used to evaluate the relationship between water temperature and SPUE, as well as the relationship between water visibility and SPUE. For the definition of an aggregation, were utilized the means observed

monthly and seasonally and if the means increase was greater than 4-fold among periods it was considered a spawning aggregation period (Domeier, 2012). Extreme abundance values, characterized by the peak of abundance of Goliath Grouper fund, were also used to characterize the spawning aggregations. The seasons were defined by the Gregorian calendar. Lunar phases were defined as lunar time (new moon, full moon, 1st quarter, 2nd quarter) + and - 3 days. Thus, for the present study a lunar phase comprehended a 7-d period. The total length classes were created using the Sturges's formula (Vieira, 2003).

RESULTS

Diving Survey

Between 2007 and 2014, we performed 107 RDT surveys distributed over the three study sites totalizing 3040 minutes (50.6 hours) sampling effort (Table 1). Goliath Groupers were sighted more frequently during surveys at Monobóia (at least 1 GG present at 96.2 % of the samples) followed by RAM (59.3 %) or Balsa Norte (28.5 %). Monobóia was also the site where the highest number of Goliath Grouper was observed during a single survey (N = 54 for Monobóia, 32 for RAM and 8 for Balsa Norte).

Table 1. Characteristics of three primary study sites off southern Brazil.

Characteristics	Balsa Norte	RAM	Monobóia
Site area (m ²)	~870	~900	~2500
Height of site (m)	5	1.5	25
No. surveys	25	30	52
Total time (min)	635	761	1644
Mean time (min) (+-SD)	29.2(5.8)	26.5 (8.7)	28.8 (8.4)

SPUE of Goliath Grouper was higher in the summer at Monobóia, RAM and Balsa Norte. High values were also observed during spring at Monobóia and RAM. Comparing the seasonal mean abundance, spring and summer

presented means values (15 and 12 Goliath Grouper) more than 4 times higher than fall and winter (1.5 and 2.9 Goliath Grouper) (Table 2).

Table 2. Seasonal differences in SPUE for Goliath Grouper (*Epinephelus itajara*) encountered on artificial habitats in southern Brazil, showing **N samples** = number of dive surveys, maximum abundance (**max**), minimum abundance (**min**), mean, standard deviation (**SD**) and variance (**var**), unit effort = 30 minute survey.

Season-months	N samples	max	min	mean	SD	var
Spring	23	22	8	15.2	3.06	9.39
Summer	47	54	0	12.6	12.82	164.29
Fall	27	4	0	1.5	1.42	2.03
Winter	10	5	1	2.9	1.46	2.12

PERMANOVA analyses of Goliath Grouper SPUE showed significant differences among seasons ($F=33.53$; $p<0.05$). Pair-wise comparisons showed differences among all seasons, except for winter and fall (Table 3). Furthermore, summer had many extreme values with high abundances overcoming others seasons. Spring showed high abundances in mid-December few days before the summer.

Table 3. Pair-wise *a posteriori* comparison of seasonal differences in Goliath Grouper (*Epinephelus itajara*) sightings-per-unit-effort (SPUE). **t** = t-statistic on pseudo-F values; **p** = p value; ns = not significant, * = significant at the 0.05 level.

Season	t	p
Summer x Winter	4.42	*
Summer x Fall	3.19	*
Summer x Spring	2.53	*
Winter x Fall	7.79	ns
Winter x Spring	20.75	*
Fall x Spring	14.65	*

The months that have shown greatest maximum values in abundances were February (N = 54) followed by January (N = 42), December (N = 34) and November (N = 20). Comparing sightings among months, January had the highest mean SPUE followed by February, December and November respectively (Fig. 3). Moreover, were compared abundances between the months in which Goliath Grouper mean SPUE started to increase on sites: from October (8.0) through February (13.1). A sudden drop in abundances occurred in March, reaching its lowest value in July. The PERMANOVA one-way test of SPUE by months showed that November, December, January and February, each showed significant differences from the other months ($p < 0.05$).

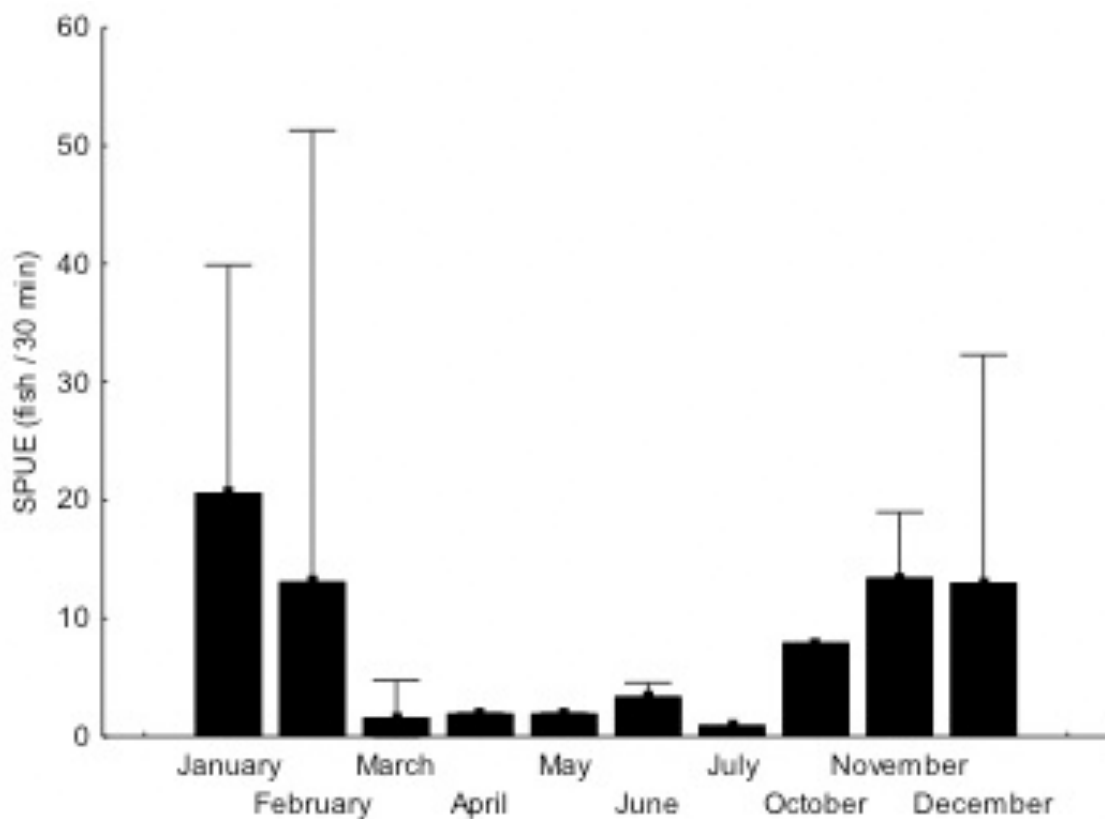


Fig. 3. Monthly variation average SPUE (sightings-per-unit-effort) of Goliath Grouper (*Epinephelus itajara*) determined by roving diver surveys conducted from 2007-2014 at Monobóia, RAM and Balsa Norte in southern Brazil. Error bars represents the maximum value found and black dots represented means.

We examined size-class distributions for the Goliath Grouper measured

by lasers (N = 126) and catches (N = 10) by season (Fig. 4). Sizes ranged from 50 to 230 cm TL. The most abundant size classes for summer were respectively 98-121, 146-169 and 170-193 cm being the only season with specimens larger than 194 cm.

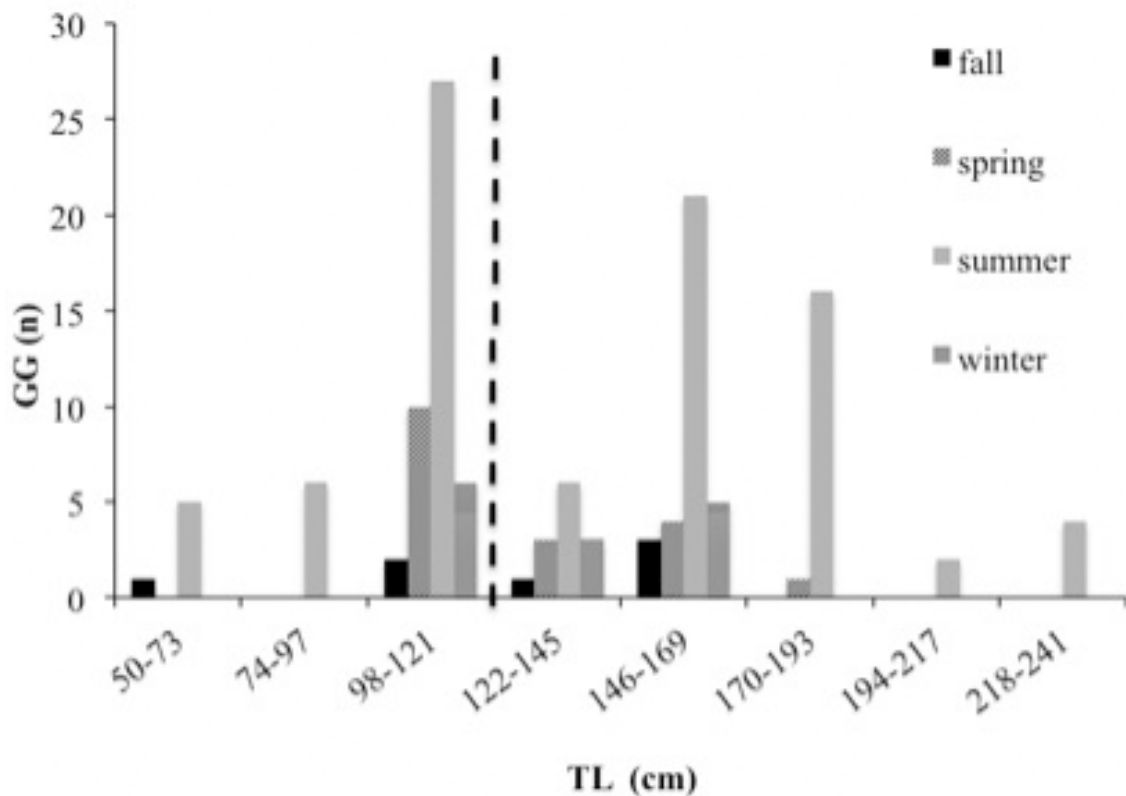


Fig. 4. Size class (TL) distributions of Goliath Grouper (GG) from south Brazil relative to seasons, measured *in situ* using laser metrics, hook-and-line samples and donated specimens from 2007-2014. The dotted line marks the division between size classes of immature and mature Goliath Grouper, according to Bullock et al. (1992).

While the mean size of fish appeared to differ little among seasons (Table 4), the greatest range in size and the largest fish were recorded during summer (Fig. 4) while the smallest GG (50 cm) was observed during fall.

Table 4. Seasonal differences in the mean size and standard deviation (**SD**) of all Goliath Grouper (*Epinephelus itajara*) measured with lasers and caught from study sites off southern Brazil (pooled catches Balsa Norte, RAM, and Monobóia).

Season	N	mean	SD
fall	7	121.4	31.9
spring	18	125.0	24.3
summer	87	137.2	42.6
winter	14	126.4	24.1
Total	126	133.4	38.4

Gonad samples

Gonad samples were obtained from 17 GG (fourteen females and two males): ten collected from our study sites, one that was found dead, and six obtained from law enforcement officers. We sampled donated specimens (N = 6) obtained during winter (July, 16th 2011) from areas close to our study sites. Four of these were females (132, 144, 148 and 180 cm TL) and two were males (136 and 147 cm TL). All females were in the regenerative stage (Fig. 5-A), indicating that they were not reproductively active. Among the Goliath Groupers collected for us, seven females (100 to 195 cm TL) obtained from RAM during December and January of 2013 and January and February of 2014 were at SC phase (Fig.5-B). A partially decayed Goliath Grouper found on December, 27th 2012, was sampled revealing a female (230 cm TL), also at SC phase. A female (159 cm TL) captured by February, 4th 2014 at site RAM presented SC phase with the actively spawning subphase with hydration (hydrated oocytes) (Fig.5-C). Other two female captured at same site at February, 14th 2013 (119 cm TL) and January, 10th 2014 (205 cm TL) presented postspawning phase showing POFs (postovulatory follicle complex) (Fig. 5-D).

Correlation with environmental parameters and moon phases

Assuming that November through February is the spawning season, we

analyzed the factors that may influence aggregating behavior to this period. SPUE of Goliath Groupers showed no correlation with either water temperature ($R^2 = 0.02$; $p > 0.05$) or visibility ($R^2 = 0.003$; $p > 0.05$).

Comparing the abundances among lunar phases, the full and new moons had higher SPUE means and extreme values when compared to others phases. The maximum mean value of SPUE was associated with the new moon phase (Fig. 6). The PERMANOVA by SPUE showed that the moon phase has a significant effect on Goliath Grouper aggregation ($p < 0.05$). The pair-wise test showed that full moon is significantly different from new moon, and second quarter. Second quarter is also significantly different from new moon .

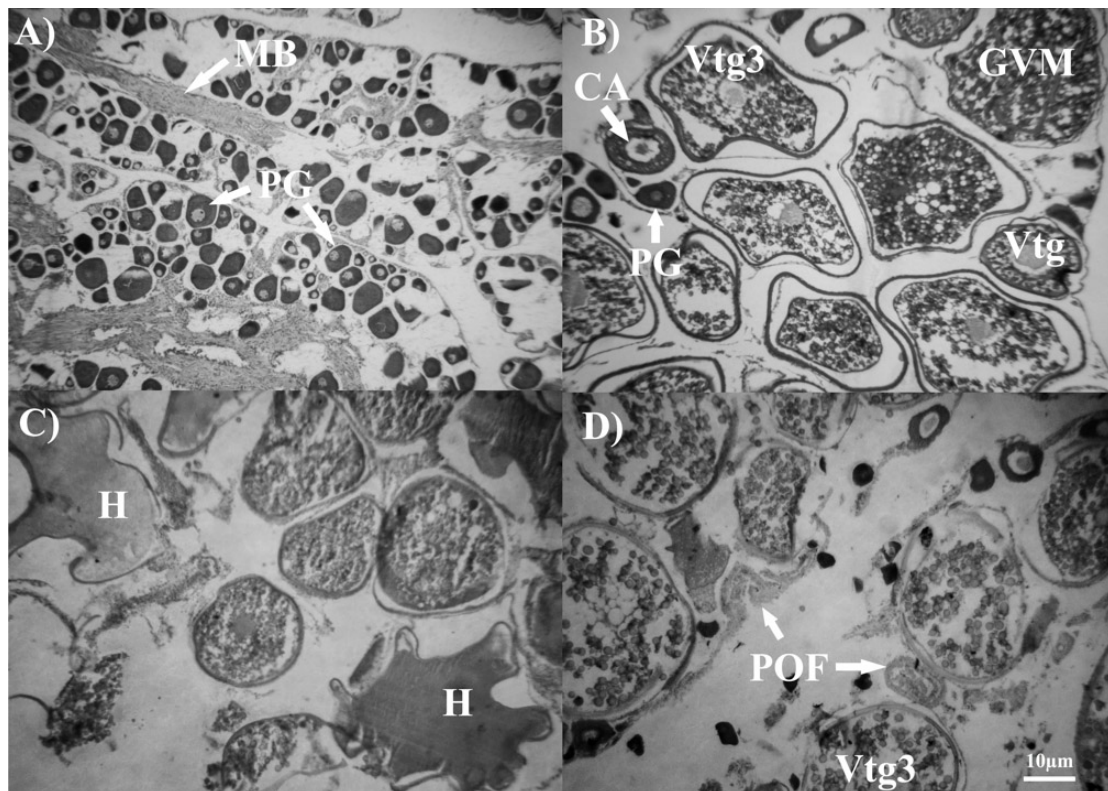


Fig. 5. Photomicrographs of ovarian histology of Goliath Groupers (*Epinephelus itajara*) sampled in south Brazil magnification 10 x. A) female, 144 cm TL sampled during July showing RT phase – MB = muscle bundle, PG = primary growth oocyte; B) female, 230 cm TL - (December, 27th 2012) showing reproductive phase: spawning capable – CA = cortical alveolar, PG = primary growth, Vtg = primary vitellogenesis oocyte, Vtg3 = tertiary vitellogenic oocyte, GVM = germinal vesicle migration; C) female, 159 cm TL showing SC phase in actively spawning subphase – H = hydrated oocytes; D) female 205 cm TL (January, 10th 2014) in the reproductive phase postspawning – POF = postovulatory follicle complex.

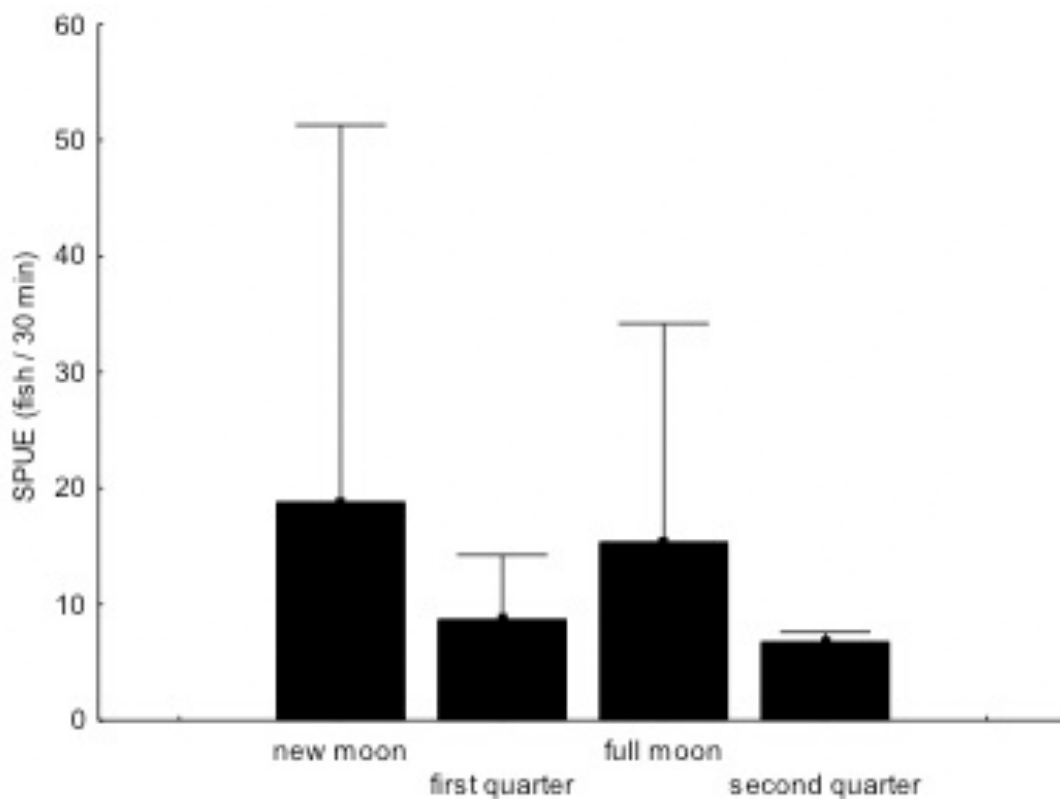


Fig. 6. Variations in SPUE (sightings-per-unit-effort) of Goliath Grouper (*Epinephelus itajara*) along lunar phases, for pooled months (November, December, January and February) from 2007-2014 in south Brazil.

DISCUSSION

According to Sadovy *et al.* (2008), spawning aggregations of many species have been severely disrupted by overexploitation and loss of habitat, to the point of disappearing from traditional sites. This is a global phenomenon that brings a sense of urgency to our need to better understand how aggregations function wherever they occur (Nemeth, 2009). In southern Brazil in the 1950s, for instance, Goliath Grouper aggregations were quite large and also heavily fished (Souza, 2000; Gerhardinger *et al.*, 2006). Nowadays, most of the aggregations known from anecdotal references have disappeared without being documented. Historically, little was known about the dynamics of reproductive aggregations of reef fish or the locations and timing of spawning in southern Atlantic ocean. This study represents pioneer dicription of spawing aggregations of Goliath Grouper in south Brazil, based on seasonal abundance

and histological data taken from diver surveys and fisheries-dependent data describing the artificial reefs sites where the aggregations occur.

Based on the definition of Domeier (2012), our abundance data indicate that spawning aggregations of Goliath Grouper form on or near artificial reefs. Moreover, the data also suggest that there is a seasonal component for aggregation formation, with highest abundance values found in summer months. Therefore, the extreme values and significant differences observed between the seasons are also strong evidence for spawning aggregations. According to Colin *et al.* (2003), to identify a spawning aggregation site, the area must meet two main criteria: (1) a sudden increase in the number of individuals in a certain location and certain time, and (2) that the physical characteristics of the fish suggest imminent reproduction including changes in color patterns, distended abdomens, or the presence of hydrated eggs, post-ovulatory follicles, or viewing the release of gametes in the water column. Spawning of Goliath Grouper occurs at night according to Mann *et al.* (2009), and since our surveys were conducted during the day, we did not observe spawning. However, we observed behaviors that are expected to be related to courtship and spawning such as “stacking behavior” and coloration changes, both of which were reported by Colin (1990) in his description of Goliath Grouper on spawning sites. In the present study, these two criteria were found: 1- The number of Goliath Grouper increased significantly during the summer, from November through February, and 2- we also confirmed that at that time, fishes were reproductively active (spawning capable, actively spawning and postspawning phases) through gonad biopsies, providing evidence that this is the spawning season. Even with a limited number of gonad samples it is possible to support the assertion that summer is the spawning period, as previously pointed by Gerhardinger *et al.* (2006; 2009) for the north State of Santa Catarina, confirming anecdotal evidence that Goliath Grouper spawn in summer. Similar results were observed in southeastern of United States by Bullock *et al.*, 1992, Colin 1990, Eklund & Schull 2001, and Koenig *et al.*, 2011.

The three investigated sites are located near large estuarine areas (Paranaguá, Guaratuba and Babitonga bay). This proximity may be a factor for *E. itajara* choosing these as spawning areas, since juveniles are mangrove-

dependent during their first years (Koenig *et al.*, 2007). This hypothesis is reinforced by the fact that a surface current flows toward the coast in this region during spring and summer (Stein & Noernberg, 2013) probably taking the eggs to these mangrove habitats.

Among the sites, Monobóia presented the highest abundances of Goliath Groupers during aggregation time and during the year, this may be related to two characteristics. 1- The site has the more structures including many artificial reefs, anchors, chains, pipelines and concrete. 2 - Because the vertical relief of Monobóia reef extends from the bottom to the surface, there may be some advantages to the fish, in other words, they use the vertical structure as guidelines to reach warmer water. Corroborating this hypothesis, Goliath Grouper were observed using the vertical pipeline structures during a time when a thermocline was present.

Fifty-five percent of individuals observed during our dives were over 130 cm TL. According to Bullock *et al.* (1992) and Koenig *et al.* (2011), these may represent adults. The majority of the fishes observed during summer were in size class 98 - 121 cm TL, suggesting that most fishes documented during the spawning season probably participated of spawning aggregation event before the mature age estimated by Bullock *et al.* (1992). Two females sampled presented spawning capable and postspawning reproductivity phases with 100 and 119 cm (TL) therefore smaller than those described by the authors. This factor can be related to overfishing (Dayton *et al.*, 2003) that have occurred few years ago associated to illegal ongoing fishing activity (see next topic). These findings, combined with our estimate of size structure, provide data required for stock assessment models, while replication of our visual surveys across sites and over time could provide an index of abundance within the study area (*e.g.*, Pórch & Eklund, 2004, Pórch *et al.*, 2006.)

Our studies and those of others (Mann *et al.*, 2009, Gerhardinger *et al.*, 2006 and 2009) suggest a relationship between spawning aggregation and lunar phases, according to which lunar phases control the spawning timing during summer months. Our data showed the lunar phases had influenced the Goliath Grouper aggregation being the new moon and full moon the phases that have higher SPUE. That fact can be related of the influence of moon phases in

the tide amplitude and current what may be helps to bring the eggs close to shore.

Based on our experience and findings, a combination of long-term and intensive short-term monitoring strategies is recommended to fully characterize trends in seasonal abundance and habitat use for Goliath Grouper. Environmental parameters should be submitted to long term monitoring at spawning areas for a better comprehension of inherent timing and distribution variations. The use of new technologies, such acoustic telemetry and bioacoustics, is recommended to expand knowledge on aggregations sites and adjacent migratory corridors.

Needs to management and enforcement

Illegal fishing of Goliath Grouper in Brazil (Giglio *et al.*, 2013), and more specifically in our study area, is very common, but apprehensions and punishment, such as the one carried out by Federal Maritime Police Special Core-NEPOM/SDF during our research, are rare. Since enforcement operations are uncommon, illegal fishing finds no restrains in the region. However, two actions that could prove effective are the establishment of marine protected areas around these presumed spawning areas and increased enforcement during spawning period. It was created at 20th June 2013, a marine protection area to cover part of the studied sites (RAM), called National Park of Currais Islands.

Enforcement is an important deterrent to illegal fishing, however raising awareness of the public about the value of Goliath Grouper aggregations for dive tourism may be equally effective. Monetary benefits of ecotourism dive at Nassau Grouper aggregations were described by Sala *et al.* (2003), and are also being experienced by dive boat operators at southeastern Florida (USA) since multiple dive boats are ferrying divers out to see Goliath Grouper aggregations nearly every day and the business is expanding (Koenig, personal communication).

Thus, when government agencies realize that more money can be earn from observing healthy populations and aggregations of Goliath Grouper, the incentive for protection will be even more powerful.

In addition to illegal fishing, pollution and mangrove habitat destruction

threaten the survival of Goliath Grouper populations in Brazil. Koenig *et al.* (2007) have clearly demonstrated the importance of mangrove habitat to juvenile Goliath Grouper in the southeastern US. High water quality standards in mangrove and coastal habitats must be maintained or the consequences could be dire, not only for Goliath Grouper, but for many other estuary-dependent species as well.

This paper provides evidence on the formation of spawning aggregations of Goliath Grouper in southern Brazil, and provides a starting point for additional research into the ecology and behavior of this endangered species over a broader area. We intend to raise awareness of the importance of the areas described in this paper as significant to the recovery of Goliath Grouper populations throughout southern Brazil. We expect that through this awareness management agencies will continue to take effective conservation measures that will lead to population recovery and therefore benefit both a limited fishery and a dive tourist industry.

ACKNOWLEDGMENTS

We are grateful to Jonhathas A. Alves, Thiago F. Souza, Felipe Darros, Rafael L. Velo and Leonardo F. Machado for their active participation in this research. We also thank all the team of Meros of Brazil Project, which is sponsored by Petrobras through the Programa Petrobras Ambiental, and all the team of Fish Ecology Lab (FSU). L.S.B. has received a scholarship from FAPES (Fundação de Amparo a Pesquisa do Espírito Santo) and Sandwich scholarship by CAPES Foundation, Ministry of Education of Brazil from PDSE program. Thanks to Pedro Carlos Pinheiro, André Cattani and Diogo Augusto Moreira. We would like to acknowledge the support from COMAR Institute, Lancha Furacão and Submarine Serviços for providing important technical help for dive operations.

REFERENCES

Brown-Peterson N.J., Wyanski D.M., Saborido-Rey F., Macewicz B.J., Lowerre-Barbieri S.K. (2011) A standardized terminology for describing reproductive development in fishes. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 3, 52–70.

Bullock L.H., Murphy M.D., Godcharles M.F., Mitchell M.E. (1992) Age, growth, and reproduction of jewfish *Epinephelus itajara* in eastern Gulf of México. *Fishery Bulletin* 90, 243-249.

Carter J. and Perrine D. (1994) A spawning aggregation of dog snapper, *Lutjanus jocu* (Pisces: Lutjanidae) in Belize, Central America. *Bulletin of Marine Science* 55, 228-234.

Colin P.L. (1990) Preliminary investigations of reproductive activity of the Jewfish, *Epinephelus itajara* (Pisces: Serranidae). In Goodwin M.H. and Waugh G.T. (eds) *Proceedings of the 43rd Gulf and Caribbean Fisheries Institute*. Miami, Florida: GCFI pp.188-147.

Colin P.L., Sadovy Y.J., Domeier M.L. (2003) *Manual for the study and conservation of reef fish spawning aggregations*. Fallbrook, CA: SCRFA, p.98 [Society for the Conservation of Reef Fish Aggregations Special Publication, no. 1].

Craig M.T., Graham R.T., Torres R.A., Hyde J.R., Freitas M.O., Ferreira B.P., Hostim-Silva M., Gerhardinger L.C., Bertoncini A.A., Robertson D.R. (2009) How many species of goliath grouper are there? Cryptic genetic divergence in a threatened marine fish and the resurrection of a geopolitical species. *Endanger Species Research* 7, 167–174.

Dayton P.K., Thrush S., Coleman F.C. (2003) *Ecological effects of fishing in marine ecosystems of the United States*. Arlington, Virginia: Pew Oceans Commission 44 p.

Domeier M.L. and Colin P.L. (1997) Tropical reef fish spawning aggregations: defined and re-viewed. *Bulletin of Marine Science* 60, 698 -726.

Domeier M.L. (2012) Revisiting spawning aggregations: definitions and challenges. In Sadovy de Mitcheson Y., Colin P.L. (eds) *Reef fish spawning aggregations: biology, research and management*. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, pp. 1-20 [Fish & Fisheries series, no. 35].

Eklund A., Schull J. (2001) A stepwise approach to investigating the movement patterns and habitat utilization of goliath grouper, *Epinephelus itajara*, using conventional tagging, acoustic telemetry and satellite tracking. In Sibert J.R. and Nielsen J.L. (eds) *Electronic tagging and tracking in marine fisheries*. Dordrecht, Netherlands: Kluwer Academic Publishers, pp.189-216.

Ferreira B.P., Hostim-Silva M., Bertoncini A.A., Coleman F.C., Koenig C.C. (2012) Atlantic Goliath Grouper – *Epinephelus itajara*. In Sadovy de Mitcheson Y., Colin P.L. (eds) *Reef fish spawning aggregations: biology, research and management*. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, pp. 417-422 [Fish & Fisheries series, no. 35].

Gerhardinger L.C., Medeiros R., Marenzi R.C., Bertoncini A.A., Hostim-Silva M. (2006) Local ecological knowledge on the Goliath Grouper *Epinephelus itajara*. *Neotropical Ichthyology* 4(4), 441-450.

Gerhardinger L.C., Hostim-Silva M., Medeiros R.P., Matarezi J., Bertoncini A.A., Freitas M.O., Ferreira B.P. (2009) Fishers' resource mapping and goliath grouper *Epinephelus itajara* (Serranidae) conservation in Brazil. *Neotropical Ichthyology* 7(1), 93-102.

Giglio V.J., Bertoncini A.A., Ferreira B.P., Hostim-Silva M., Freitas M.O. (2013) Historical, illegal landings and seizures of goliath grouper, *Epinephelus itajara*, in Brazil: how effective is the fishery moratorium? *The Brazilian Journal of Nature Conservation* (submitted).

Grier H.J., Uribe-Aranzabal U.C., Patiño R. (2009) The ovary, folliculogenesis, and oogenesis in teleosts. In Jamieson B.G.M. (eds) *Reproductive biology and phylogeny of fishes (agnathans and bony fishes)*. Science Publishers, pp. 25–84.

IUCN (International Union for the Conservation of Nature) (2013) IUCN Red List of Threatened Species. Available from: www.iucnredlist.org (accessed 25 Ago 2013).

Johannes R.E. (1978) Reproductive strategies of coastal marine fishes in the tropics. *Environmental Biology of Fishes* 3, 65-84.

Jones R.S. and Thompson M.J. (1978) Comparison of Florida reef fish assemblages using a rapid visual technique. *Bulletin of Marine Sciences* 28, 159-172.

Koenig C.C., Coleman F.C., Eklund A.M., Schull J., Ueland J. (2007) Mangroves as essential nursery habitat for goliath groups (*Epinephelus itajara*). *Bulletin of Marine Science* 80(3), 567–586.

Koenig C.C. and Coleman F.C. (2009) Population density, demographics, and predation effects of adult goliath grouper. *National Oceanic and Atmospheric Administration, Final Report MARFIN Grant Number 0548-0175*, 96 pp.

Koenig C.C., Coleman F.C., Kingon K. (2011) Pattern of recovery of the Goliath Grouper *Epinephelus itajara* population in the southeastern US. *Bulletin of Marine Science* 87, 000-000. doi:10.5343/bms. 2010.1056.

Mann D.A., Locascio J.V., Coleman F.C., Koenig C.C. (2009) Goliath grouper (*Epinephelus itajara*) sound production and movement patterns on aggregation sites. *Endangered Species Research* 7, 229–236.

Nemeth R.S. (2009) Dynamics of reef fish and decapod crustacean spawning aggregations: underlying mechanisms, habitat linkages, and trophic interactions. In Nagelkerken I. (eds) *Ecological Connectivity among Tropical Coastal Ecosystems*. New York: Springer pp.613.

Porch C.E. and Eklund A.M. (2004) Standardized visual counts of goliath grouper off south Florida and their possible use as indices of abundance. *Gulf of Mexico Science* 2, 155–163.

Porch C.E., Eklund A.M., Scott G.P. (2006) A catch-free stock assessment model with application to goliath grouper (*Epinephelus itajara*) off southern Florida. *Fishery Bulletin* 104, 89-101.

Sadovy Y. Colin P.L., Domeier M.L. (1994) Aggregation and spawning in the tiger grouper, *Mycteroperca tigris* (Pisces:Serranidae). *Copeia* 2, 511-516.

Sadovy Y. and Eklund A.M. (1999) Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), and the jewfish, *E. itajara* (Lichtenstein, 1822). *NOAA Technical Report NMFS* 146, pp.65.

Sadovy de Mitcheson Y.S. and Colin P.L. (2012) *Reef fish spawning aggregations: biology, research and management*. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, p.620.

Sadovy Y.M., Cornish A., Domeier M.L., Colin P., Russell M., Lindeman K. (2008) A global baseline for spawning aggregations of reef fishes. *Conservation Biology* 22(5), 1233–1244.

Sala E., Arbuto-Oropeza, Paredes G., Thompson G. (2003) Spawning aggregations and reproductive behavior of reef fishes in the Gulf of Califórnia. *Bulletin of Marine Science* 72(1), 103-201.

Souza H.S. (2000) *O homem da ilha e os pioneiros da caça submarina*. 2nd edition. Florianópolis: Editora Dehon, 352 pp.

Stein E.A.M., Noernberg M.A. (2013) Variabilidade oceanográfica da plataforma continental interna da região sul do embaçamento de São Paulo-Paraná. *Revista Brasileira de Geofísica*, accepted.

Vieira S. (2003) *Bioestatística: tópicos avançados*. Rio de Janeiro: Campus, pp. 212.

CAPÍTULO 2

**Goliath Grouper local change or disappearance of spawning aggregation
and dislocation among these sites at south Brazil**

Abstract

Goliath Groupers (GG) form spawning aggregations with 100 or more fishes. Disappearance or local change of these aggregations may occur due to: fishing pressure and attraction for artificial reefs, combined or isolated. This paper has gathered sightings-per-unit-effort (SPUE) data on 3 natural and 4 artificial reefs sites in southern Brazil, comparing them to previous works data from the same sites aiming to assess previous and present location of GG aggregations. GG abundance differed significantly between artificial and natural reefs. In natural reefs no aggregations were found (GG max number = 2), however in artificial reefs aggregations with 54 GG were observed. Based on the obtained data we found strong evidence suggesting GG have changed their spawning sites and are using artificial structures to aggregate. We will also discuss the impact of the loss of GG aggregations over the rock reefs environment.

Highlights:

- We found strong evidences suggesting that Goliath Groupers changed they spawning sites
- Goliath Groupers are using artificial structures to spawning aggregations
- Goliath Groupers abundance on artificial was significantly higher than natural reefs

Keywords: Artificial Reefs, Reef Fish, Epinephelidae, South Atlantic, Endangered Species, *Epinephelus itajara*, Coastal zone, Effects-fish.

1 Introduction

The Goliath Grouper (GG) is a large grouper threatened throughout its distribution range and likely to be extinguished in the African coast (Ferreira et al., 2012; Craig et al., 2009) being classified as critically endangered by the IUCN Red List (IUCN, 2013). Goliath Grouper is a large predator and can reach over 2.5 meters total length weighing more than 300 kilos (Bullock et al., 1992), eating mostly crabs and slow moving fishes, it plays a very important role shaping reef community within their range (Koenig et al., 2011). Being one of the few grouper that spawn offshore in shallow reefs until 50 m (Coleman and Koenig, 2003) using rock reefs, coral reefs, and artificial reefs (Koenig et al., 2011) Goliath Grouper spawning aggregations can be formed by 100 or more fishes. These aggregations are spatial and temporally consistent spawning almost in the same period year after year, presenting strong interannual fidelity to spawn site (Sadovy de Mitcheson and Colin, 2012; Koenig et al., 2011).

Groupers like Goliath Grouper, as well as many reef fish, migrate to form annual spawning aggregations during specific periods of the year and have strong site fidelity (Domeier and Colin, 1997; Sadovy and Eklund, 1999), showing variable longevity of site use (Sadovy de Mitcheson and Colin, 2012) and plausibly representing a learned reproductive strategy (Helfman and Schultz, 1984). However, the spawning site may change or the aggregation may disappear for reasons such as: fishing pressure (Aguilar-Perera, 2006) and the attraction factor for artificial reefs, suggested in this paper, combined or isolated.

The presence of artificial reefs on the sea can cause diverse types of impact to the marine environment (Brickhill et al., 2005). One of these impacts is the attraction of large reef fish by these structures that was studied and discussed by many authors (Bohnsack et al., 1997; Bohnsack, 1989; Lindberg, 1997). However few of them took into account the influence and environmental impact of artificial reefs on the natural reefs in the region of installation (Osenberg et al., 2002).

The attraction exerted by artificial reefs is clearly observed on Goliath Grouper being registered by many authors (Koenig et al., 2011, Bohnsack et al., 1999; Sadovy and Eklund, 1999; Gerhardinger et al., 2006; Gerhardinger et al.,

2009; Coleman and Koenig, 2003; Mann et al., 2008). Nevertheless none of the studies focused on discussing its impact over this specie or natural environment. Marine habitats and the fisheries may be modified by these human-made physical structures placed in the sea as artificial reefs (Seaman, 2002). These structures serve a variety of functions ranging from mariculture, tourism, marine resource conservation (stop trawling nets), petroleum industry, and hydraulics engineer.

The use of artificial reefs for several purposes along the Brazilian coast has increased over the last years, encouraging researches on this subject (Simon et al., 2011). However the impacts of these artificial structures on nearby natural environments have been assessed only for a few studies in Brazil, e.g., Simon et al. (2011).

The present work aims to evaluate the disappearance of reported Goliath Grouper aggregations from natural rock reefs and the possible relation with the installation of artificial reefs in the studied area. We will also discuss the impact of the loss of Goliath Grouper aggregations over the rock reefs environment and how this change in distribution may affect the specie.

2 Material and methods

2.1 Study area

The study area is located in southern Brazil, between 25° and 27° south latitude in the western Atlantic. The region has distinct seasonality with summer (late December through late March) being the warmest period. During this time, moderate east and northeast winds predominate, bringing warm (up to 28 °C) clear waters from the east. The weather is more variable in the fall (late March through late June), and spring (late September through late December) with an increase in large eastern and southeastern swells and coincident decrease in underwater visibility. Winter (late June through late September) is characterized mainly by cold fronts that bring large swells from the south and southeast, decreasing water temperature (18 °C) and increasing turbidity in coastal waters.

Seven reefs were selected to compose the sampling area, three natural rock reefs and four artificial reefs (Fig. 1): Balsa Sul (BS), Balsa Norte (BN), Recifes Artificiais Marinhos (RAM) and Monobóia (MB) are the artificial reefs

and Arquipélago das Graças (AG), Tamborettes (TA) and Laje dos Lobos (LL) the natural reefs.



Fig. 1. Study area pointed out in the Brazil map. Natural rock reefs (pentagon) and artificial reefs (triangles).

Among the sampled areas the sites Balsa Sul, Balsa Norte and RAM are artificial reefs installed to foment fishing tourism and stop trawling nets. BS and BN are 5 meters high and ~ 75 meters longer by ~11 meters wide, barges that were intentionally sunk at 21/01/2001, 27 meters deep in sand bottom at ~32 km offshore. The study site RAM is part of a big artificial reef program that has installed several artificial reef areas (~40) formed by concrete blocks and reef balls with structures about 1.5 meters high to the bottom (Fig. 2), ~12 km offshore. For the present study two of those artificial reef areas were selected (sites: Parque dos Meros and RAM G4). These two reefs are about 1 km far apart each other are considered of the same site (RAM) to abundance analyses. Both of them were sunk at 09/06/2000 in a depth of 18 meters and in 2013 were included at a new marine protect area called National Park of Currais Island.

Monoboia is a single point mooring buoy installed at 1977 in a depth of 25 meters, composed by a vertical bottom/surface structure and many structures in the bottom such as pipelines, concrete, large anchors and chains in approximately 500 meters radius, ~8 km offshore. The artificial sites offer great rest shelter areas to Goliath Groupers (Fig. 2). The three natural sites (AG, TA and LL) comprehend rock reefs close to coastal islands with max depths at 19 m and present similar structural complexity with different relief forms inside each one of them. The longer distance between sites is from Laje dos Lobos to Balsa Norte ~91 km, the closest sites are Monobóia and Arquipélago das Graças ~11km distance.

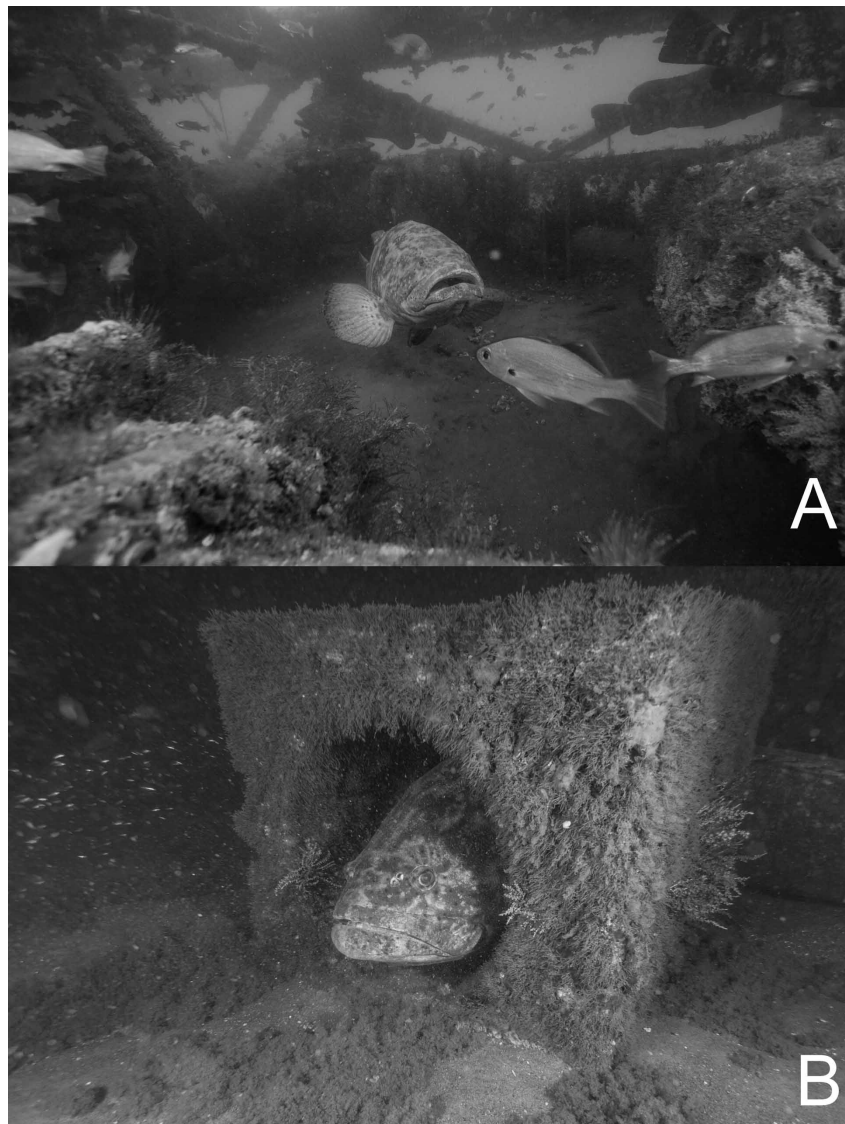


Fig. 2. Goliath Grouper using artificial reef areas. At site Monobóia (A) and at site RAM (B) located in south Brazil.

2.2 Data Acquisition

2.2.1 *Dive surveys*

Between October 2007 and March 2014 we have conducted dive surveys on Goliath Grouper at all seven study sites during each season, with greater emphasis during summer aiming the suppose reproductive period (Bullock et al., 1992; Koenig et al., 2011; Gerhardinger et al., 2009). The utilized method was the Roving Diver Technique (RDT) (Jones and Thompson, 1978). Each dive survey, was conducted only when visibility was great than 3 m, and consisted of a search of approximately 30 minutes, throughout the site (including all crevices and the surrounding perimeter) performed by two divers that were recording the number of Goliath Grouper encountered.

To the natural reefs the RDTs samples focused on Goliath Groupers aggregation sites reported by local experts spearfishing and in Gerhardinger et al. (2009). Local spearfishermen's report about Goliath Grouper occurrence and aggregations sites was a result of an information network created during this sampling period. This network is formed by ten spearfisherman that dive frequently in the study area. The RDTs samples were made randomly inside the indicated aggregations reef sites, covering more than one point by field day.

The artificial reefs RDTs samples we take in count the reef configuration to try cover the most part of the reef for sample. For each shipwreck (BS and BN) one dive was necessary to sampled all its structure, on site RAM we sampled always the same two close points by field day check all reef areas. In the MB site we checked the main structure settled in the bottom, vertical and horizontal pipelines.

2.2.2 *External tagging*

Goliath grouper were tagged underwater using SCUBA with dart tags (Floy Tag - BFIM-96) employing modified spearguns. During each dive, Goliath Groupers were tagged as much as possible. Each diver was trained to identify tagged individuals during the surveys as well as to record color pattern and distinguishing body characteristics. External tagging technique was used to analyze the movement of Goliath Groupers between the sites.

2.2.3 Data analysis

Sightings data obtained during RDT diving surveys were transformed into sightings-per-unit-effort SPUE (Equation 1), thus taking into accounts the different sampling efforts of dive time (in minutes) for each survey. For the purposes of this index, we used 30 min as the standard time effort, since this was the average time of surveys.

$$\text{Equation 1: } \text{SPUE} = N * (T/t)$$

Where:

SPUE = sightings per unit effort, N = number of fish observed, T = duration in minutes of each survey and t is the 30 min to standardization efforts.

To compare the abundance between the area and between artificial and natural reefs the non-parametric test of Kruskal-Wallis was used. When significant differences ($p < 0.05$) were observed the de Mann-Whitney posteriori test was used (Sokal and Rohlf, 1995).

The data were transformed by $\text{Log}(x+1)$ to created a hierarchical cluster analyses used to represent the average similarity (Bray-Curtis) between the sites sampled. The consistence of visually defined groups in cluster was tested through analysis of similarities (ANOSIM).

3 Results

3.1 Dive surveys

Were performed 316 RDT surveys distributed over the seven sites with approximately 192 hours diving, 160 RDTs samples at artificial reefs and 156 at natural reefs between 2007 and 2014. The high SPUE was observed at Monobóia with 54 Goliath Grouper, followed by RAM (32), Balsa Norte (30), Balsa Sul (10), Tamborettes (2), Laje dos Lobos (2) and Arquipélago das Graças (1). The natural reefs combined showed 11 % of frequency of occurrence of Goliath Grouper against 88 % frequency of occurrence at artificial reefs with means higher than 4 specimens by SPUE (Table 1).

Table 1. Sites differences in SPUE for Goliath Grouper (*Epinephelus itajara*) encountered on artificial and natural reefs in southern Brazil, showing **Samples** = number of dive surveys, maximum abundance (**max**), **mean**, standard deviation (**SD**) and frequency of occurrence (%) unit effort = 30 minutes survey.

Sites/Reef Type	Samples	Max	Mean	SD	%
Monobóia	52	54	12.55	10.88	96
RAM	62	32	7.73	10.18	74
Balsa Norte	30	30	4.72	5.07	100
Balsa Sul	16	10	4.09	3.21	63
Tamboretas	65	2	0.26	0.60	22
Arq. Graças	40	1	0.02	0.14	3
Laje dos Lobos	51	2	0.06	0.32	4
Artificial Reef	160	54	8.37	9.67	88
Natural Reef	156	2	0.14	0.45	11
Total	316	54	4.54	8.25	64

The abundance of Goliath Groupers on artificial reefs sites was significantly different than the natural reefs. Comparing the means between the groups (natural and artificial) we found great difference. Max values showed huge discrepancy, the artificial reefs group being more abundant than the natural reefs group (Fig. 3). Exception made to Monoboia and RAM in artificial reefs group, no statistically significant differences were observed among the sites of each group. However between both groups there were statistically significantly differences (Table 2).

Comparing data from natural and artificial groups by seasons the Goliath Grouper was more abundant in artificial reefs in all season. The artificial reef areas proved to be more abundant during spring and summer with great drop during fall and winter; on the other hand in the natural reef sites the abundance had low variance during seasons (Fig. 4).

Table 2. Multiple comparison using data on Goliath Grouper abundance presenting p-value for the studied sites (MB = Monobóia, RAM = Recifes Artificiais Marinhos, BN = Balsa Norte, BS = Balsa Sul, TA = Tamboretas, AG = Arquipélago das Graças, LL = Laje dos Lobos). The sites are distribute according to reef types (Artificial or Natural).

Groups	Sites	MB	RAM	BN	BS	TA	AG	LL
Artificial	MB		0.0091	1.0000	1.0000	0.0000	0.0000	0.0000
	RAM	0.0091		1.0000	1.0000	0.0000	0.0000	0.0000
	BN	1.0000	1.0000		1.0000	0.0000	0.0000	0.0000
	BS	1.0000	1.0000	1.0000		0.0023	0.0003	0.0002
Natural	TA	0.0000	0.0000	0.0000	0.0023		1.0000	1.0000
	AG	0.0000	0.0000	0.0000	0.0003	1.0000		1.0000
	LL	0.0000	0.0000	0.0000	0.0002	1.0000	1.0000	

Comparing the abundance between natural and artificial sites during the sampling years, there was no variance in the natural abundance being never higher than two Goliath Groupers. For the artificial reefs some variation in the abundance of Goliath Grouper was verified being the greater means and max values observed at 2011 and 2013. At artificial reef sites the mean of abundance was always higher than 7 Goliath Groupers for all sampling years (Fig. 5).

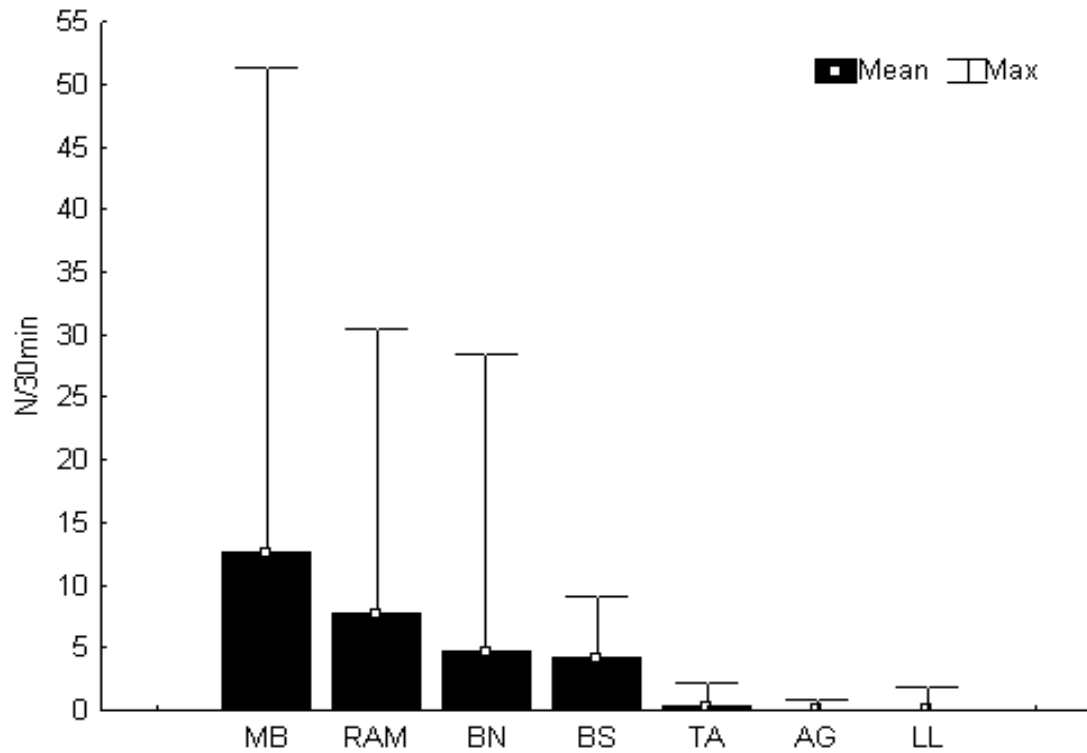


Fig. 3. Means of Goliath Grouper abundance by sampled sites (MB = Monobóia, RAM = Recifes Artificiais Marinheiros, BN = Balsa Norte, BS = Balsa Sul, TA = Tamboretetes, AG = Arquipélago das Graças, LL = Laje dos Lobos) located at south Brazil. RDT samples data grouped from 2007 to 2014.

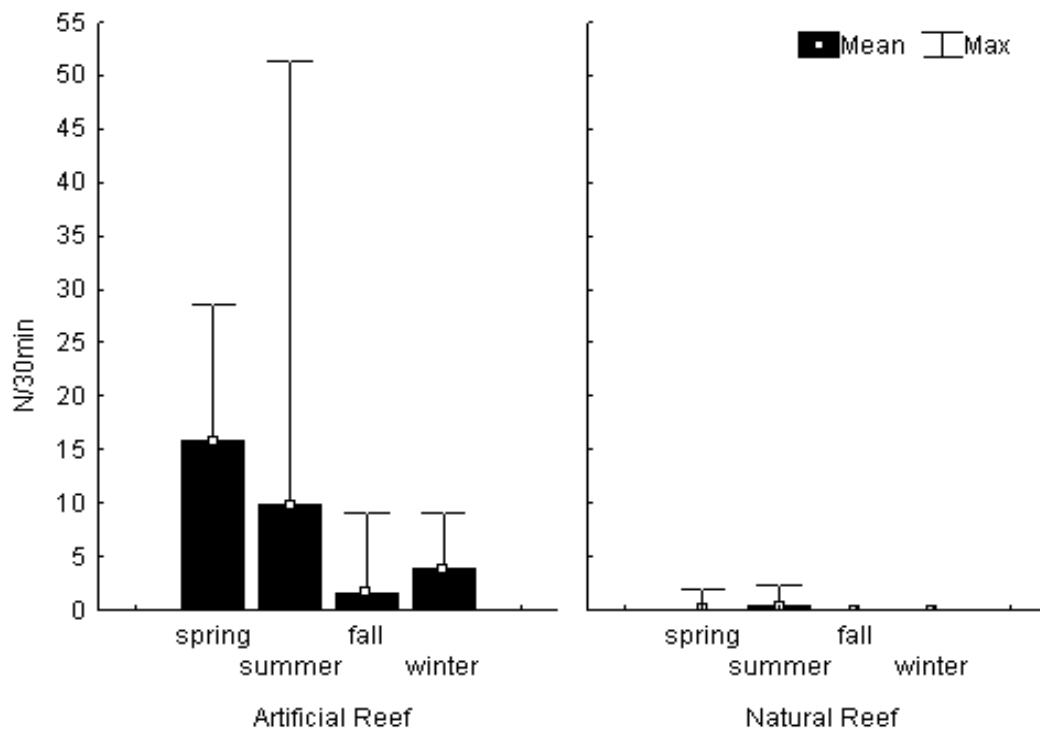


Fig. 4. Goliath Groupers abundance by season in all sampled sites divided into artificial group (MB, RAM, BN and BS) and natural group (TA, AG, LL), during the years of 2007 to 2014 sites located at south Brazil.

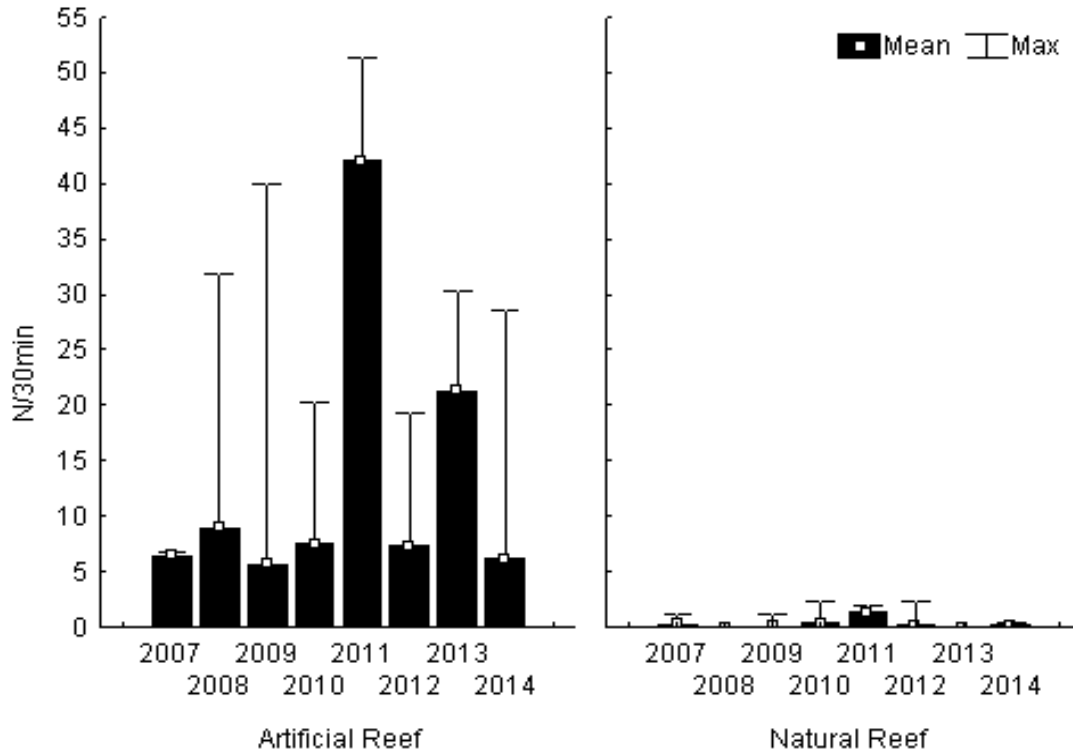


Fig. 5. Goliath Groupers abundance distributed by years in all sampled sites divided into artificial (MB, RAM, BN and BS) and natural group (TA, AG, LL), sites located at south of Brazil.

Cluster analysis resulted on the formation of two groups (Fig. 6) with the cut line in 67 % of similarity. First group was composed by all artificial reefs and the second by all natural reefs. The artificial group was significantly different from the natural reefs (ANOSIM, $R = 1$ $p = 0.029$).

3.2 External tagging

A total of 67 Goliath Groupers were tagged and there were 25 resight (36 %), the time between liberty and resight varying between a few hours until 1825 day after tagging. The maximum distance traveled was 60.5 km with resight about 5 years later. This fish showed up in two different sites, Parque dos Meros (1825 days at liberty) and RAM G4 (69 days latter), was a male with 1.57 m total length.

Sixty percent of resighted fishes were registered in the same site of tagged, 36 % were found 1 km far from the tagging place and 4 % at a distance of more than 10km from tagging site (Fig. 7). The Goliath Groupers resight at the same site where they were tagged had the resight between hours until 39 days after tagging. We had 10 Goliath Grouper that traveled between sites Parque dos Meros e RAM G4 distant about 1 km (Fig. 8), 6 of these showed were using this two close area for more than one month (47 until 69 days) during the summer of 2013/2014.

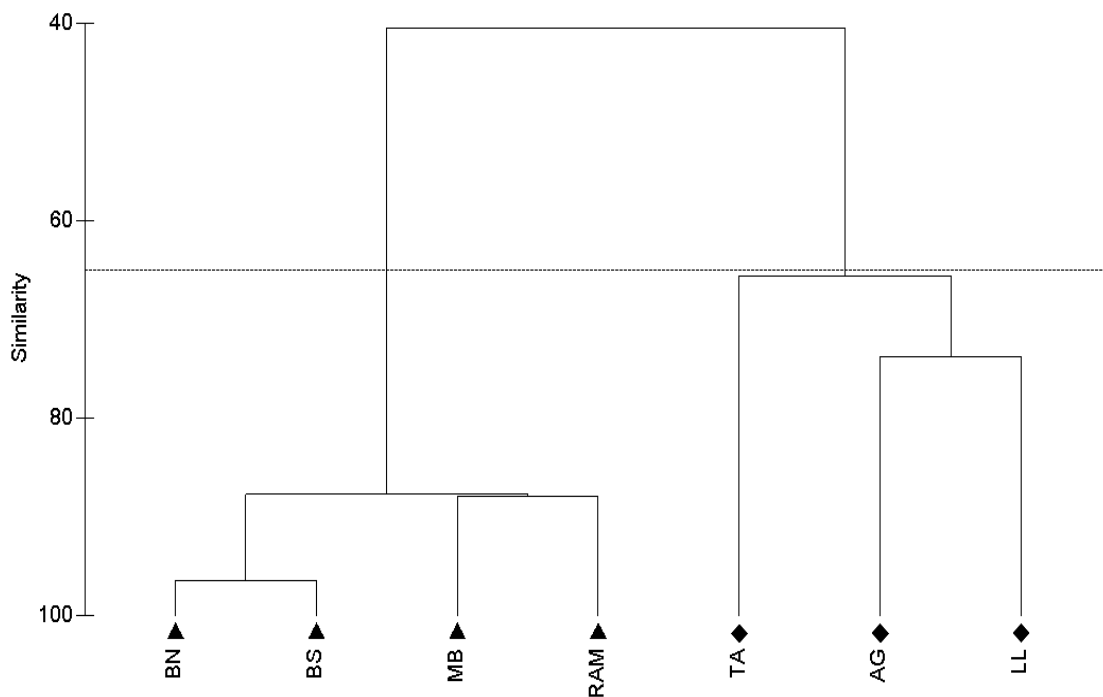


Fig. 6. Cluster analysis using Goliath Grouper abundance data at south Brazil by sites. Dendrogram from clustering using the legends: BN = Balsa Norte, BS = Balsa Sul, MB = Monoboia, RAM = Recifes Artificiais Marinho, TA = Tamboretetes, AG = Arquipélago das Graças, LL = Laje dos Lobos. The triangles (▲) represent artificial reefs and lozenges (◆) represent natural reefs. Cut line defined at 67 % of similarity.

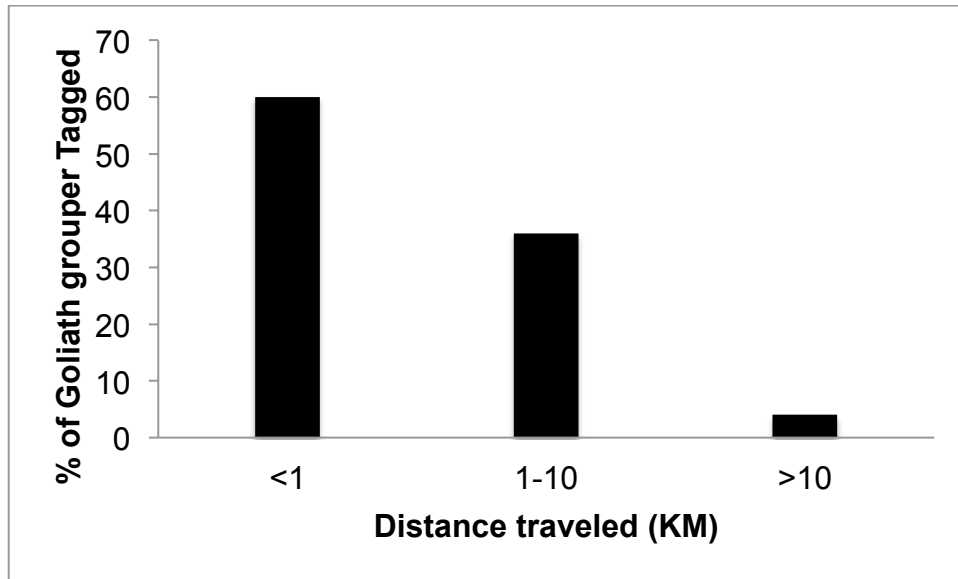


Fig. 7. Percentage of adult Goliath Grouper, *Epinephelus itajara* (n=67) regarding distance travelled in south Brazil from original offshore tagging site.

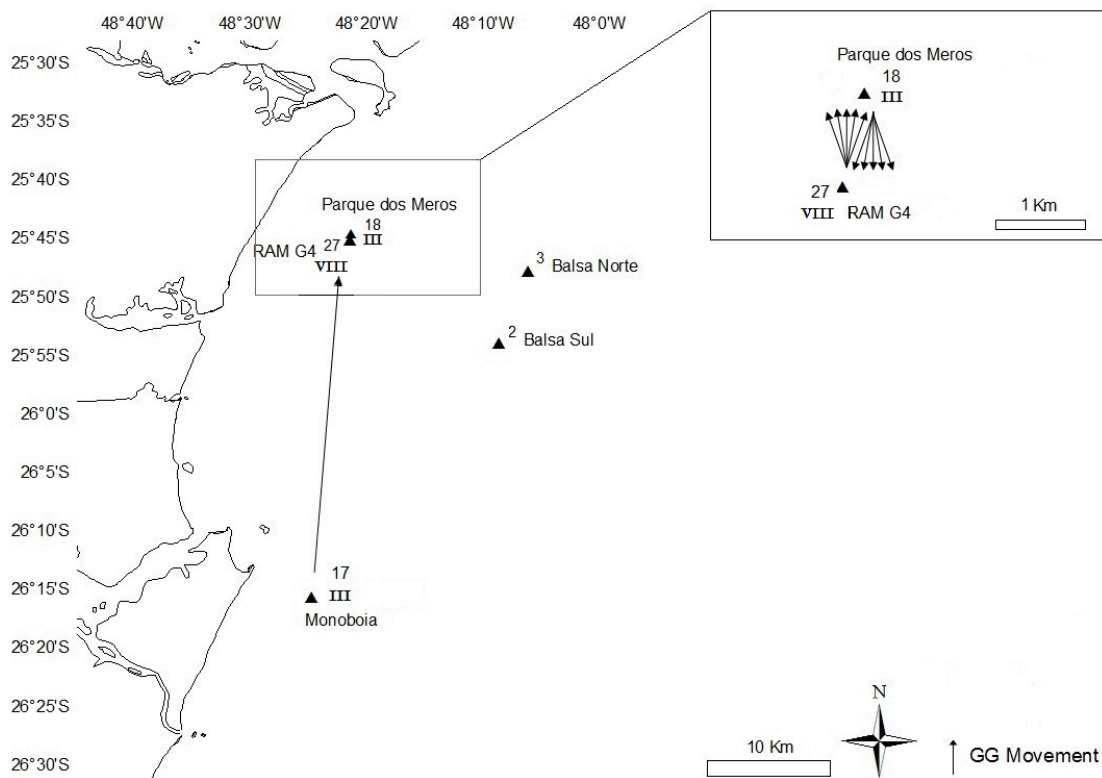


Fig. 8. Movement tagged adult Goliath Grouper (*Epinephelus itajara*) at south Brazil. The Arabic numerals post at each site represent the number of Goliath Grouper tagged in that sites, arrows = adult movement, Roman numerals = number of Goliath Grouper resight at the tagged site.

4 Discussion

Our data have demonstrated that Goliath Grouper changed the aggregate areas preferring the artificial reefs and using less the natural reefs in the last decades. The aggregations cited by fishermen and spearfishermen in Gerhardinger et al. (2009) were not found anymore. According to their work the sites Laje dos Lobos, Tamboretas and Arquipélago das Graças were Goliath Grouper spawning aggregation sites gathering more than sixty Goliath Grouper on one site in a single day. Authors have listed Laje dos Lobos as the site with higher abundance of Goliath Groupers (>60 GG) followed by Tamboretas (~15 GG), and Arquipélago das Graças (~5 GG), and the presence of Goliath Groupers was reported as very frequent in these natural reefs. On the other hand in our surveys these sites (LL, TA and AG) had abundance lower than 3 Goliath Groupers and low frequency of occurrence even during reproductive period (summer and spring), which does not characterize a spawning aggregations. During the seven years of study, in addition to the surveys, we have also received information from a network of active experienced spearfishermen (10) and still there was no report about more than 2 Goliath Grouper sighted in the studied natural reefs. Moreover in other study performed by Daros et al. (2012) from October 2008 to August 2009 in two natural areas close to site RAM, aiming at producing a reef fish check list through dive surveys, none Goliath Grouper was registered. Alves and Pinheiro (2011) found similar results, while performing a checklist of the fishes in the Laje dos Lobos and other close natural reef site at 2006, and no Goliath Grouper was found at these sites. This may indicate that the use of natural environment by Goliath Grouper has decrease in the past years.

Greater abundance in the artificial reefs was observed during summer and spring, coinciding with the reproductive period of this specie in the studied region as previously mentioned in Chapter 1, corroborating data from other studies performed at different sites along the world (Bullock et al., 1992; Colin, 1990; Eklund and Schull, 2001; Sala et al., 2003; Koenig et al., 2011). That information combined to the absence of aggregation in natural reefs may suggest that the Goliath Grouper have changed the spawning areas and are currently spawning at artificial reefs. This change may cause an expressive

impact on the natural areas with the loss of an important top predator and by ceasing the input of a big seasonal energy through spawning.

Koenig et al. (2011) suggest that the indigenous Goliath Grouper has a positive effect on the biodiversity and abundance of associated reef fishes, a result expected from principles of evolutionary ecology. Since Goliath Grouper adults may dominate the biomass on the reefs where they reside, it is expected that they play an important role shaping reef communities within their range. Furthermore, high concentrations of spawning adults or fish eggs may attract a wide diversity of predators attempting to take advantage of this temporary and predictable food source (Nemeth, 2012). Still according to this author, since fishes migrate through their catchment area and converge on the spawning site, this temporary concentration may gather hundreds or thousands of fishes during reproductive periods providing a potentially important mechanism to interlink and possibly influencing food webs. The Goliath Grouper also play an active role through their excavating behavior, resulting in a positive influence on species richness at a local scale (Coleman et al., 2010). In fact by excavating *E. itajara* exposes high relief reefs that were buried by storms, thereby making them available for a great number of other species (Koenig et al., 2011).

The abandonment of natural reefs by Goliath Grouper may result from different factors such the need to scape from heavy fishing pressure exerted in the past as well as due to the attraction for artificial reefs. This attraction or the preference by the artificial reefs can be related to the structural complexity that favors the Goliath Grouper as shown in Fig. 2. Hackradt et al. (2011) have studied the influence of habitat structure on fish assemblage at the same area studied in the present work (reefs from the RAM program). They observed that Goliath Grouper have a positive correlation with quadrilateral blocks artificial reef (Fig. 2) due to their low structural complexity when compared to natural rock reefs and other types of artificial reefs.

Creation of artificial reefs by itself can affect the distribution of the ichthyofauna, attracting reef fishes that originally would stay in natural reef areas (Osenberg et al., 2002). Simon et al. (2011) highlight the fact that artificial reefs collaborate to remove large predator fishes from their natural areas, which may alter important ecological processes such as predation and competition. The exit of large predators, such as Goliath Grouper, from natural areas and

consequent loss their functional role generates ecological impacts to the natural reefs. Neutralization of this functional role may compromise the ecological stability on the ecosystem generating lower resilience and lower resistance to invasions by exotics species when compared with balanced ecosystems (Stallings, 2009).

Tagging data show that Goliath Groupers are sedentary and have site fidelity during spawning season, with 60 % of tagged species travelling less than 1 km. This corroborates with Koenig et al. (2011) study information according to which 82 % traveled less than 1 km. One individual has shown up in different years (2009, 2013, 2014) in three different sites travelling about 60.5 km. Furthermore some of them were tagged in the morning and a few hours later were sighted at a site one kilometer far, indicating that the Goliath Grouper can use all studied sites and move easily between them. Therefore it is possible to assume that Goliath Grouper can easily move to natural area but it prefers to stay at artificial reefs. Koenig et al. (2011) suggest that this preference is related to low availability of food resources in natural areas. Crabs and other crustaceans constitute the primary food of Goliath Grouper (Koenig and Coleman, 2009), and the low abundance of these animals combined to the extreme rugosity of natural reefs hamper the access to the prey justifying the preference for artificial reefs where the low structural complexity make easier to capture the prey. Simon et al. (2011) indicate the attraction of snappers and grouper to artificial reefs is related to a high prey concentration and lower energy cost of encounter food on artificial areas.

Probably the Goliath Groupers found in natural reefs are in their functional migration area moving from their catchment area to staging areas (Nemeth, 2012). Considering that there are a great number of artificial reefs set close to the studied sites, it is possible to presume that they may are being temporary occupied during migration, a role previously played by natural reefs. The flux of fish biomass from feeding grounds to spawning aggregation sites and subsequent spawning by aggregating species, provide an important and largely overlooked ecological component of connectivity within marine ecosystems, due to fish movements, habitat use and interspecific interactions as well as the energy transfer resulting from feeding and defecation (Nemeth, 2012). Although little information exists on predator-prey dynamics at

aggregation sites, it is known that Goliath Grouper feed at aggregation sites and probably along migratory pathways (Koenig et al., 2011).

It has been argued that artificial reefs may not represent the solution or the tool to increase fishing production (Osenberg et al., 2002; Bohnsack, 1989). Instead, they may simply affect distribution, by attracting fish from natural reefs and concentrating them upon artificial ones. If true, then resources currently spent on artificial reef programs may be misdirected, if not harmful, to natural reef systems (Osenberg et al., 2002). Artificial reefs also attract fishermen and the fishing industry resulting on high mortality rate of fishes that may cause a decline on the fish population in long term (Bohnsack, 1989).

Artificial reefs with good planning and functional effective protection may favor some species such as Goliath Grouper to improve reproductive success. However, most of the studied artificial reefs do not have any kind of law or regulation to protect these species against fishery, and in those that have enforcement is low and infrequent. Unplanned installation of artificial reefs without enforcement or management can be a huge problem bringing risk to species that are attracted or that are spawning in these areas. Epinephelidae and Lutjanidae are main targets of fishermen in Brazil and therefore more affected by this problem. The same low structural complexity of artificial reefs isolated in homogenous substrate (sand bottom), that attract GG and large top predators may also become a trap where large fishes can be easily found and caught by fishermen.

Marine environments suffer several variations that may influence fish dynamics and distribution. To better understand these dynamics and processes, long term monitoring is required in order to expand the study area and analyze more factors. Considering the GG dislocation and the distance among spawning sites, combined to difficulties regarding water visibility and weather, it is recommended the application of new techniques, such as passive acoustic telemetry, and expansion of spearfishermen collaboration network through participatory research programs.

ACKNOWLEDGMENTS

We are grateful to Jonas Rodrigues Leite, Felipe Darros, Rafael L. Velo and Leonardo F. Machado for their active participation in this research. We also thank all the team of Meros of Brazil Project, which is sponsored by Petrobras through the Programa Petrobras Ambiental and all the team of Fish Ecology Lab (FSU). L.S.B. has received a scholarship from FAPES (Fundação de Amparo a Pesquisa do Espírito Santo) and Sandwich scholarship by CAPES Foundation, Ministry of Education of Brazil from PDSE program. Thanks to André Cattani and Diogo Augusto Moreira. We would like to acknowledge the support from COMAR Institute, Lancha Furacão and Submarine Serviços for providing important technical help for dive operations.

References

- Aguilar-Perera A.** (2006) Disappearance of a Nassau grouper spawning aggregation off the southern Mexican Caribbean coast. *Marine Ecology Progress Series* 327, p.289-296.
- Alves A. A. and Pinheiro P. C.** (2011) Peixes recifais das ilhas costeiras do Balneário Barra do Sul – Santa Catarina – Brasil. *Revista CEPSUL - Biodiversidade e Conservação Marinha* 2(1), 10-21.
- Bohnsack, J. A.** (1989) Are high densities of fishes at artificial reefs the result of habitat limitation or behavioral preference? *Bulletin of Marine Science* 44, 631-645.
- Bohnsack J. A., Ecklund A. M., Szmant A. M.,** (1997) Essay: artificial reef research: is there more than the attraction-production issue? *Fisheries* 22, 14-16.
- Bohnsack J.A., McClellan D.B., Harper D.E., Davenport G.S., Konoval G.J., Eklund A.M., Contillo J.P., Bolden S.K., Fischel P.C., Sandorf G.S., Javech J.C., White M.W., Pickett M.H., Hulsbeck M.W., Tobias J.L., Ault J.S., Meester G.A., Smith S.G., Luo J.** (1999). *Baseline data for evaluating reef fish populations in the Florida Keys*. NOAA Tech. Memo. NMFS-SEFSC, no.427.
- Brickhill M. J., Lee S. Y., Connolly R. M.** (2005) Fishes associated with artificial reefs: attributing changes to attraction or production using novel approaches. *Journal of Fish Biology* 67, pp. 53–71.
- Bullock L.H., Murphy M.D., Godcharles M.F., Mitchell M.E.** (1992) Age, growth, and reproduction of jewfish *Epinephelus itajara* in eastern Gulf of México. *Fishery Bulletin* 90, 243-249.

Coleman, F. C. and Koenig, C. C. (2003). *Population density, demographics, and predation effects of adult goliath grouper*. Silver Spring, MD: National Oceanic and Atmospheric Administration [Final Report MARFIN Grant Number 0548-0175].

Coleman F.C., Koenig C.C., Scanlon K., Heppell S., Miller M.W. (2010) Benthic habitat modification through excavation by red grouper *Epinephelus morio* (Valenciennes) in the northeastern Gulf of Mexico. *Open Fisheries Science Journal* 3, 1–15.

Colin P.L. (1990) Preliminary investigations of reproductive activity of the Jewfish, *Epinephelus itajara* (Pisces: Serranidae). In Goodwin M.H. and Waugh G.T. (eds) *Proceedings of the 43rd Gulf and Caribbean Fisheries Institute*. Miami, Florida: GCFI pp.188-147.

Craig M.T., Graham R.T., Torres R.A., Hyde J.R., Freitas M.O., Ferreira B.P., Hostim-Silva M., Gerhardinger L.C., Bertoncini A.A., Robertson D.R. (2009) How many species of goliath grouper are there? Cryptic genetic divergence in a threatened marine fish and the resurrection of a geopolitical species. *Endanger Species Research* 7, 167–174.

Daros F. D., Bueno L. S., Vilar C.C., Passos A. C., Spach H. L. (2012) Archipelago and Itacolomis Island, Paraná state, Brazil. *Check List Journal of Species List and Distribution* 8, 349-354.

Domeier M.L. and Colin P.L. (1997) Tropical reef fish spawning aggregations: defined and re-viewed. *Bulletin of Marine Science* 60, 698 -726.

Eklund A.M. and Schull J. (2001) A stepwise approach to investigating the movement patterns and habitat utilization of goliath grouper, *Epinephelus itajara*, using conventional tagging, acoustic telemetry and satellite tracking. In Sibert J.R. and Nielsen J.L. (eds) *Electronic tagging and tracking in marine fisheries*. Dordrecht, Netherlands: Kluwer Academic Publishers, pp.189-216.

Ferreira B.P., Hostim-Silva M., Bertoncini A.A., Coleman F.C., Koenig C.C. (2012) Atlantic Goliath Grouper – *Epinephelus itajara*. In Sadovy de Mitcheson Y., Colin P.L. (eds) *Reef Fish spawning aggregations: biology, research and management*. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, pp. 417-422 [Fish & Fisheries series, no. 35].

Gerhardinger L.C., Medeiros R., Marenzi R.C., Bertoncini A.A., Hostim-Silva M. (2006) Local ecological knowledge on the Goliath Grouper *Epinephelus itajara*. *Neotropical Ichthyology* 4(4), 441-450.

Gerhardinger L.C., Hostim-Silva M., Medeiros R.P., Matarezi J., Bertoncini A.A., Freitas M.O., Ferreira B.P. (2009) Fishers' resource mapping and goliath grouper *Epinephelus itajara* (Serranidae) conservation in Brazil. *Neotropical Ichthyology* 7(1), 93-102.

Hackradta C. W., Félix-Hackradta F. C., García-Charton J. A. (2011) Influence of habitat structure on fish assemblage of an artificial reef in southern Brazil. *Marine Environmental Research* 72, 235-247.

Helfman, G. S. and Schultz, E. T. (1983) Social transmission of behavioural traditions in a coral reef fish. *Animal Behaviour* 32, p.379-384 DOI: 10.1016/S0003-3472(84)80272-9.

IUCN (International Union for the Conservation of Nature) (2013) IUCN Red List of Threatened Species. Available from: www.iucnredlist.org (accessed 25 Dec. 2013).

Jones R.S. and Thompson M.J. (1978) Comparison of Florida reef fish assemblages using a rapid visual technique. *Bulletin of Marine Sciences* 28, 159-172.

Koenig C.C. and Coleman F.C. (2009) Population density, demographics, and predation effects of adult goliath grouper. *National Oceanic and Atmospheric Administration, Final Report MARFIN Grant Number 0548-0175*, 96 pp.

Koenig C.C., Coleman F.C., Kingon K. (2011) Pattern of recovery of the Goliath Grouper *Epinephelus itajara* population in the southeastern US. *Bulletin of Marine Science* 87, 000-000. doi:10.5343/bms. 2010.1056.

Lindberg J. W. (1997) Can science resolve the attractio-production issue? *Fisheries* 22, 10-13.

Mann D.A., Locascio J.V., Coleman F.C., Koenig C.C. (2009) Goliath grouper (*Epinephelus itajara*) sound production and movement patterns on aggregation sites. *Endangered Species Research* 7, 229–236.

Nemeth R. S. (2012) Ecosystem aspects of species that aggregate to spawn. In Sadovy de Mitcheson Y. and Colin P.L. (eds) *Reef fish spawning aggregations: biology, research and management*. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University. pp 21-55 [Fish & Fisheries series, no. 35].

Osenberg C. W., St. Mary C. M., Wilson J. A., Lindberg J. W. (2002) A quantitative framework to evaluate the attraction–production controversy. *Journal of Marine Science* 59, S214–S221.

Sadovy Y. and Eklund A.M. (1999) Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), and the jewfish, *E. itajara* (Lichtenstein, 1822). *NOAA Technical Report NMFS* 146, pp.65.

Sadovy de Mitcheson Y.S. and Colin P.L. (2012) *Reef fish spawning aggregations: biology, research and management*. Corvallis, USA: Fisheries & Wildlife Department, Oregon State University, p.620.

Sala E., Arbutto-Oropeza, Paredes G., Thompson G. (2003) Spawning aggregations and reproductive behavior of reef fishes in the Gulf of California. *Bulletin of Marine Science* 72(1), 103-201.

Seaman W. (2002) Unifying trends and opportunities in global artificial reef research, including evaluation. *ICES Journal of Marine Science* 59,S14–6.

Simon T., Pinheiro H. T., Joyeux J. C. (2011) Target fishes on artificial reefs: Evidences of impacts over nearby natural environments. *Science of the Total Environment* 409, 4579–4584.

Sokal R. R., Rohlf F. J. (1995) *Biometry*. [s.l.]: W. H. Freeman and Company, 859 p.

Stallings C. D. (2009) Fishery-independent data reveal negative effect of human population density on Caribbean predatory fish communities. *PLoS ONE* 4,e5333.

CAPÍTULO 3

First records of pughead and short-tail skeletal deformities in Goliath Grouper, *Epinephelus itajara*, (Perciformes: Epinephelidae) registered during spawning aggregations

Abstract

Here we report new records of two types of skeletal deformity, "pughead" and "short tail" in adult Goliath Grouper (Epinephelus itajara). We used two different sources to register: photographs during dives surveys and live specimens that were caught using hook-and-line. The skeletal deformities were observed just during spawning aggregations. "Pughead" was registered in Brazil and Florida and "short tail" was just registered in Brazil.

Running head: First record of skeletal deformities on Goliath Grouper

Keywords: Endangered Species, Reef Fish, Spawning Aggregations, Florida, South Brazil

INTRODUCTION

Goliath Grouper, *Epinephelus itajara* (Lichtenstein, 1822), is the largest reef fish in the western hemisphere, attaining a weight of over 300 kg and a length of over 2.5 m. The species has been vastly overfished throughout its range, which extends from North Carolina, USA to southern Brazil, and is now considered critically endangered (IUCN, 2013). They are recovering in Florida under full protection since 1990 (Koenig *et al.*, 2011), but their populations remain at relatively low levels elsewhere (Ferreira *et al.*, 2012).

This paper reports observations of skeletal abnormalities of pugheadedness and short-tail for Goliath Grouper during studies of spawning aggregations by the authors. Skeletal abnormalities have been described for a variety of species (Cobcroft *et al.*, 2001), but to date none have been described for Goliath Grouper. The causes of skeletal abnormalities are unknown and typically rare, appear to be initiated during early development, and may be related to dietary deficiencies, pollutants, adverse temperatures, inappropriate light conditions or oxygen depletion (Scott, 2001, Vågsholm & Djupvik, 1998).

MATERIAL AND METHODS

The records reported in this paper are from studies conducted at the east coast of Florida and south of Brazil, and were obtained from two different sources: photographs of Goliath Grouper taken during surveys using a Nikon D5100 camera with Ikelite underwater housing by the senior author and live specimens that were caught using hook-and-line. These records were registered during spawning aggregations of Goliath Grouper.

RESULTS

A regular Goliath Grouper (Figure 1a) photographed at Jupiter, Florida, USA and the pughead (Figure 1b) that was captured, sampled and released on 16 July 2012 at 'Three-holes' natural reef 7.5 km north of Jupiter Inlet, Jupiter, Florida, USA. This adult male (174 cm total length) showed no overt signs of stress or malnutrition. The same fish was sighted on 2 September 2012, 7.2 km southeast of 'Three-holes' on 'Sun Tug' artificial reef and was photographed

underwater. Another pughead (Figure 1c) was observed on 15 February 2011 at 'Monobóia' artificial reef about 11 km off Santa Catarina state, and an individual with 'short-tail' skeletal anomaly (Figure 1d) was observed nearby on 'RAM' artificial reef off Paraná state, south Brazil.

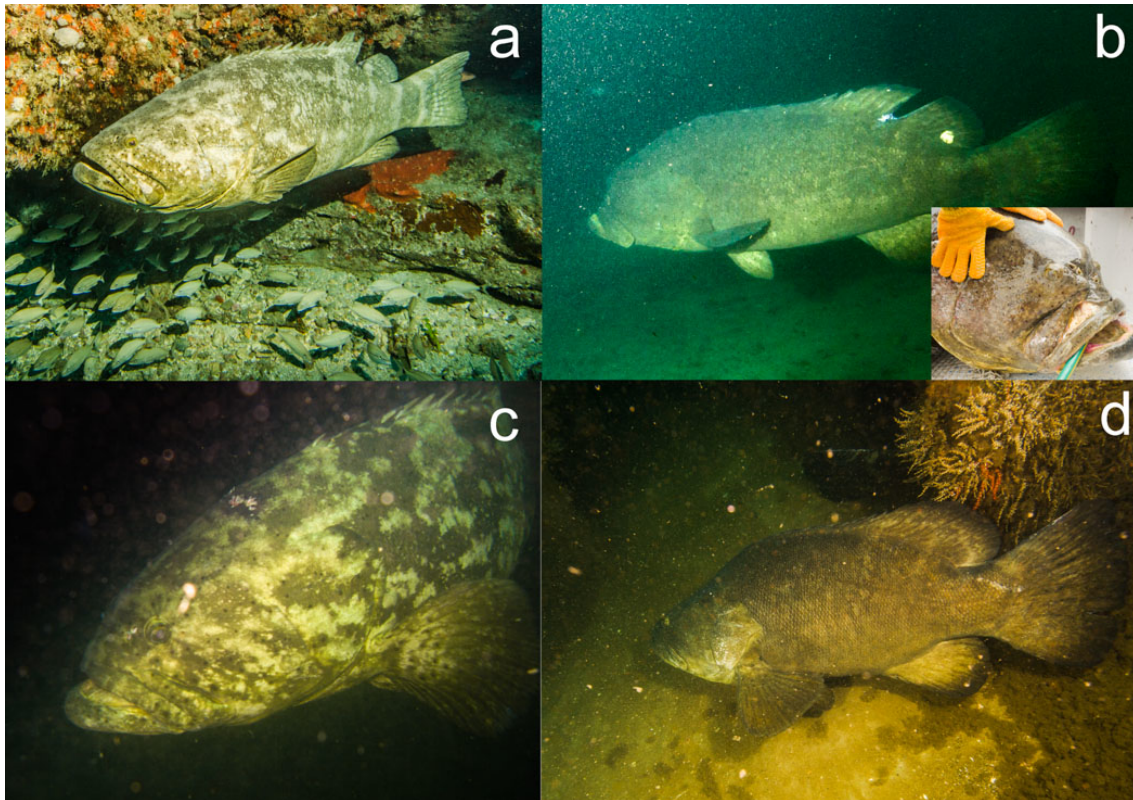


Fig. 1. (a) Normal Goliath Grouper (*Epinephelus itajara*) (b) Pughead Goliath Grouper observed off Jupiter, Florida, USA, (b inset) Close-up of the deformity, (c) Pughead Goliath Grouper at southern Brazil (d) "short-tail" Goliath Grouper at Paraná state, south Brazil.

Note: split dorsal fin was the result of sampling fin rays for age determination.

DISCUSSION

Pugheadedness is characterized by an antero-posterior compression of the upper jaw resulting in apparent protrusion of the lower jaw. "Short-tail" is associated with malformations of vertebrae including fusion of vertebrae, distortion and underdevelopment of single vertebrae, and alterations of the intervertebral spaces (Gavaia *et al.*, 2002, Vågsholm & Djupvik, 1998).

The causes of the observed deformities in Goliath Grouper are unknown, but they probably arise in early development (Cobcroft *et al.*, 2001). Deformities typically have been observed in very low frequencies in wild populations. In the

research done in Florida on Goliath Grouper, we have captured and sampled nearly 600 adults and over 2500 juveniles. We have observed over three times that many underwater on dives, but have only observed pugheadedness once. So, although pugheadedness is widespread among species it is rare within any one species in wild populations as observed by Gudger, 1937; Mansueti, 1960; Cheek, 1965; Isaacson, 1965; Briggs, 1966; Dahlberg, 1970; Bortone, 1971; Riehl & Schmitt, 1984 and others. However, hatchery-reared fish typically show a much greater frequency of skeletal abnormalities, including pugheadedness, than wild fish (e.g., Grinstead, 1971; Komada, 1980; Cobcroft *et al.*, 2001). In the sheltered conditions of a hatchery, several causes have been implicated including overcrowding (Shariff *et al.*, 1986), xenobiotics (Haga *et al.*, 2003), nutrition (Cobcroft *et al.*, 2001), inbreeding (Sadler & King, 2001), and dietary deficiencies (Takeuchi *et al.*, 1998). Higher rates of survival of deformed fish under the controlled conditions in a hatchery would also contribute to the observation of higher frequencies than in the wild.

It is unknown whether the observed deformities in Goliath Grouper reduced survival potential. The pugheaded and short-tail individuals showed no overt sign of poor health, so feeding was apparently unhindered by the condition. Goliath Grouper feed on slow-moving fishes and crustaceans, so once located, prey are easily ingested via suction feeding. Avoidance of predators was also not likely hindered by the observed maladies because the fish remain close to structure such as reefs and wrecks as adults (Koenig *et al.*, 2011) and mangrove shorelines and undercuts as juveniles (Koenig *et al.*, 2007) and are quick to dive for cover when potential predators appear. The fact of this individuals were in spawning aggregations may indicated that they can be capable to reproduce but is unknown what influences the deformities cause in reproductions displays or reproductive success.

ACKNOWLEDGMENTS

The authors acknowledge the grants received in Brazil from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Espírito Santo (FAPES) and Meros do Brasil Project with financial support of Programa Petrobras Ambiental – Petrobras, and grants in the USA from NOAA MARFIN Program.

REFERENCES

- Bortone, S.J.** (1971) Pugheadedness in the Vermilion Snapper, *Rhomboplites aurorubens*, in the Northern Gulf of Mexico. *Transactions of the American Fisheries Society* 1971(2): 366-368.
- Briggs, P. T.** (1966) A pugheaded tautog. *California Fish and Game Journal* 13:236–237.
- Cheek, R. P.** (1965) Pugheadedness in the American shad. *Transactions of the American Fisheries Society* 94:97–98.
- Cobcroft, J.M., Pankhursta P.M., Sadler J., Hart P.R.** (2001) Jaw development and malformation in cultured striped trumpeter, *Latris lineata*. *Aquaculture* 199:267-282.
- Dahlberg, M.D.** (1970) Frequencies of abnormalities in Georgia estuarine fishes. *Transactions of the American Fisheries Society* 1:95–97.
- Ferreira B.P., Hostim-Silva M., Bertoncini A.A., Coleman F.C., Koenig C.C.** (2012) Atlantic goliath grouper – *Epinephelus itajara*. In Mitcheson Y.S., Colin P.L. (eds) *Reef fish spawning aggregations: biology, research and management*. Fisheries & Wildlife Department, Oregon State University, Corvallis, USA For further. v.35. pp. 417-422.
- Gavaia P.J., Dinis M.T., Cancela M.L.** (2002) Osteological development and abnormalities of the vertebral column and caudal skeleton in larval and juvenile stages of hatchery-reared Senegal sole (*Solea senegalensis*). *Aquaculture* 211: 305 – 323.
- Grinstead, B.G.** (1971) Effects of pugheadedness on growth and survival of Striped Bass, *Morone saxatilis* (Walbaum), introduced into Canton Reservoir, Oklahoma. *Proceedings of the Oklahoma Academy of Science* 51: 8-12.
- Gudger, E.W.** (1937) A pugheaded two-lined dab, *Lepidopsetta bilineata*, the only known pug-headed flatfish. *American Museum Novitates* 959: 1-5.
- Haga, Y., Suzuki T., Kagechika H., Takeuchi T.** (2003) A retinoic acid receptor-selective agonist causes jaw deformity in the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* 221:381–392.
- Isaacson, P. A.** (1965) Pugheadedness in the black perch, *Embiotoca jacksoni*. *Transactions of the American Fisheries Society* 94:98.
- IUCN** (International Union for the Conservation of Nature) (2013) *IUCN Red List of Threatened Species*. Available from: www.iucnredlist.org (accessed 25 Dez 2013).
- Koenig C.C., Coleman F.C., Eklund A.M., Schull J., Ueland J.** (2007) Mangroves as essential nursery habitat for goliath grouper (*Epinephelus itajara*). *Bulletin of Marine Science* 80(30): 567-586.
- Koenig C.C., Coleman F.C., Kingon K.** (2011) Pattern of recovery of the goliath grouper, *Epinephelus itajara*, population in the southeastern US. *Bulletin of Marine Science* 87(0). 2011 doi:10.5343/bms.2010.1056
- Komada, N.** (1980) Incidence of gross malformations and vertebral anomalies of natural and hatchery *Plecoglossus altivelis*. *Copeia* 1980:29-35.

- Mansueti, R.J.** (1960) An unusually large pug-headed striped bass, *Roccus saxatilis*, from Chesapeake Bay, Maryland. *Chesapeake Science* 1(2): 111-113.
- Riehl, R. and P. Schmitt.** (1984) The skull in normal and pugheaded females of the mosquitofish *Heterandria formosa* Agassiz, 1853 (Teleostei, Poeciliidae). *Gegenbaurs Morphologisches Jahrbuch* 131:261–270.
- Sadler, J. P. and King P. M.** (2001) High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture* 198:369–386.
- Scott D.** (2001) Chemical pollution as a factor affecting the sea survival of Atlantic salmon, *Salmo salar* L. *Fisheries Management and Ecology* 8:487–499.
- Shariff, M., Zainuddin A.T., Abdullah H.** (1986) Pugheadness in bighead carp, *Aristichthys nobilis* (Richardson). *Journal of Fish Diseases* 9:457–460.
- Takeuchi, T. D., Haga J., Seikai Y., Watanabe T.** (1998) Effect of vitamin A compounds on bone deformity in larval Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 169:155–165.
- Vågsholm I., Djupvik H.O.** (1998) Risk factors for spinal deformities in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 21: 47–5