

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

**BIOLOGIA REPRODUTIVA DAS RAIAS
Sympterygia acuta GARMAN, 1877 E *S. bonapartii*
MÜLLER & HENLE, 1841
(CHONDRICHTHYES, RAJOIDEI) NA
PLATAFORMA SUL DO BRASIL**

ALEXANDER BASALLO MELO

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Orientadora: Dra. Maria Cristina Oddone

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RESUMO

Sympterygia acuta e *S. bonapartii* são duas espécies de raias ovíparas endêmicas da costa Sul - Ocidental do Oceano Atlântico. No Brasil, ambas as espécies completam seu ciclo de vida nas águas costeiras do Sudeste e Sul. O estudo da reprodução das espécies em questão foi realizado três décadas atrás na região Sul do Brasil, constituindo uma valiosa referência para a avaliação do potencial reprodutivo destas espécies durante muito tempo. Porém, além de nunca ter sido publicado, os parâmetros reprodutivos nele estimados encontram-se desatualizados e possivelmente não se aplicam mais ao estado das populações atuais. Estudos reprodutivos em *S. bonapartii* referentes às populações da costa da Argentina e do Uruguai. Foram realizados posteriormente estudos que demonstraram mudanças no potencial reprodutivo de raias através do tempo, sendo a pressão pesqueira um dos fatores que atuam sobre estes processos. O presente estudo teve como objetivo providenciar um panorama atual sobre a biologia da reprodução de *S. acuta* e *S. bonapartii* no sul do Brasil, em termos de desenvolvimento sexual no nível macro e micro-anatômico. Um total de 262 exemplares foram coletados entre fêmeas e machos das duas espécies, provenientes de cruzeiros de pesquisa e cruzeiros da frota comercial de pesca de arrasto de fundo no sul do Brasil. Foram coletados e registrados dados de diferentes parâmetros reprodutivos, e também os tratos reprodutivos de ambas as espécies foram extraídos para análises de histologia. Os resultados obtidos através das análises dos parâmetros reprodutivos incluíram a descrição do desenvolvimento sexual, as relações morfométricas e cálculo CT_{50} de maturidade. Este último resultou em valores de 44,7 e 46,1 cm em fêmeas e machos de *S. acuta*, respectivamente, *S. bonapartii* apresentou valores de CT_{50} de 59,9 e 58,4 cm, indicando mudanças através do tempo para o tamanho de maturidade.

Foram identificados os sete estágios da espermatogênese das espécies em questão, validados através de estudos realizados em outras espécies de elasmobrânquios. Além foi descrita a micro-anatomia através de análises histológicas dos tratos reprodutivos e gônadas de machos de ambas as espécies estudadas. A descrição dos estágios da espermatogênese permitiu o cálculo do CT₅₀ histológico, correspondendo a 44,7 e 54,6 cm em *S. acuta* e *S. bonapartii*, respectivamente. Estes valores, quando comparados com CT₅₀ morfológico, apresentaram diferenças em ambas as espécies estudadas, com valores menores de tamanho de maturidade com o método de CT₅₀ histológico. A análise histológica do trato reprodutivo masculino, em ambas as espécies, mostrou diferenças durante o desenvolvimento sexual.

ABSTRACT

Sympterygia acuta and *S. bonapartii* are two species of oviparous skates endemic to the coast of the Western Atlantic Ocean. In Brazil, both species make their life cycle in the coastal waters of Southeast and South. A study on the reproduction of these species had been conducted three decades ago in southern Brazil, constituting a valuable reference for the evaluation of the reproductive potential. However, besides never being published, such estimates of the reproductive parameters are outdated and may no longer apply to the current population status. Further studies were performed on the reproduction of *S. bonapartii* referring to populations from the coast of Argentina and Uruguay. Reproductive studies on different rajoids, case demonstrated changes in the reproductive potential over time, and the fishing pressure would be the most important factor that influence these processes. The present study aimed to provide a current study on the reproductive biology of *S. acuta* and *S. bonapartii* in southern Brazil, in terms of sexual development at the macro and micro-anatomical scale. A total of 262 males and females of *Sympterygia* spp were collected, from research and commercial bottom trawl cruises operating in southern Brazil. Data on different reproductive parameters were obtained, and also the reproductive tracts of both species were extracted for histological. results on reproductive parameters included description of the sexual development, morphometric relationships and the total length at 50% maturity estimate (TL₅₀), Were observed through time in the size of maturity, being found TL₅₀ values of 44.7 and 46.1 cm in females and males of *S. acuta*, respectively. For *S. bonapartii*, TL₅₀ was 59.9 and 58.4 cm, respectively. Seven stages of the spermatogenesis of the species in question were identified and validated with studies carried out in other elasmobranchs. Was described micro-anatomy

through histological analysis of the gonads and reproductive tracts of males in both species. The description of the stages of spermatogenesis allowed the calculation of histological TL₅₀, which corresponded to 44.7 and 54.6 cm in *S. acuta* and *S. bonapartii*, respectively. These values, when compared with the morphological TL₅₀ showed differences in both species, with lower values for the histological method. Histological analysis of the male reproductive tract in both species showed differences during sexual development.

1. INTRODUÇÃO

A Classe Chondrichthyes está formada por todos os peixes de esqueleto cartilagenoso, agrupando de modo geral as quimeras, tubarões e raias. Estes dois últimos pertencem à Subclasse Elasmobranchii. Entretanto, dentro do grupo das raias, ou Batoidea, está contida a subordem Rajoidei, que inclui somente espécies com modo reprodutivo ovíparo (Hamlett *et al.*, 2005).

O gênero *Sympterygia* (Müller & Henle, 1837) é formado por quatro espécies ovíparas endêmicas das costas sul da América do Sul. Nas águas costeiras do Sul do Chile, no Oceano Pacífico, ocorrem exclusivamente as espécies *Sympterygia brevicaudata* (Cope, 1877) e *Sympterygia lima* (Poepfig, 1835) (McEachran & Miyake, 1990). Na costa sul - Ocidental do Oceano Atlântico são encontradas as espécies *Sympterygia acuta* (Garman, 1877) e *Sympterygia bonapartii* (Müller & Henle, 1841), sendo endêmicas dessa região (McEachran, 1982).

A raia *S. acuta* encontra-se distribuída entre 22° e 43°20' S, em águas costeiras rasas em profundidades que variam entre a linha da costa até 40 m (Menni, 1973; Menni, & Stehmann, 2000). Por outro lado, Meneses & Paesch (2003) afirmam que *S. acuta* é encontrada em águas costeiras até 188 m de profundidade, sendo comumente encontrada na isóbata de 50 m de profundidade (Massa *et al.*, 2004). No Brasil, *S. acuta* completa todo seu ciclo de vida na zona costeira da plataforma Sul, ocorrendo ao longo do ano sem variações sazonais na sua abundância (De Queiroz, 1984; Vooren, 1997). É uma espécie presente em ambientes relacionados com estuários ao longo de todo o ano (Díaz Andrade *et al.*, 2009), e constitui um recurso econômico importante na maior parte da sua área de distribuição (De Queiroz, 1984; Massa *et al.*, 2004).

A espécie *S. bonapartii* apresenta uma distribuição espacial restrita para a costa sul - Ocidental do Atlântico, sendo encontrada desde a latitude de 32° S, no Rio Grande do Sul, até a latitude 44° S, no norte da Argentina. Registros de ocorrência de *S. bonapartii* no Estreito de Magalhães (Chile) são considerados duvidosos (M. C. Oddone, obs. pess.). A espécie é encontrada até a profundidade de 100 m (Menni & Stehmann, 2000; Massa & Lamilla, 2004) sendo mais abundante em áreas estuarinas na plataforma Argentina, com um pico de abundância observado entre a primavera e o verão (Mabragaña *et al.*, 2002).

Os rajoídeos, assim como os peixes cartilaginosos em geral, caracterizam-se por apresentar uma baixa taxa de fecundidade anual, uma maturação sexual tardia e alta longevidade (Dulvy *et al.*, 2000). Estas características, somadas a uma alta taxa de exploração pesqueira nos últimos anos, levaram ao declínio de várias populações tanto de raias como de tubarões no mundo. No caso dos rajoídeos, a vulnerabilidade à pesca excessiva é tal que existem casos confirmados de extinção local para *Dipturus laevis* (Mitchill, 1818), *D. batis* (Linnaeus, 1758), *D. oxyrinchus* (Linnaeus, 1758) e *Rostroraja Alba* (Lacepède, 1803) (Dulvy *et al.*, 2000; Dulvy & Reynolds, 2002), e possivelmente outras. Dentro da classificação global da Lista Vermelha de espécies em perigo de extinção, da União Internacional para a Conservação da Natureza – UICN, *S. acuta* está catalogada como “vulnerável” (VU) (Massa & Hozbor, 2004) e *S. bonapartii* como “dados insuficientes” (DD) (Massa & Lamilla, 2004).

A quantidade de batoídeos desembarcados nos portos dos Estados do Rio Grande do Sul e Santa Catarina vem aumentando, alcançando valores de 776 t e 1458 t por ano, respectivamente, estes valores representam entre 19% e 34%, dos

elasmobrânquios demersais desembarcados para estas regiões (Oddone, 2003; Casarini, 2006). No Sudeste e Sul do Brasil, os rajoídeos são comumente capturados com arrasto de fundo (arrasto de parelha e de portas) e também com redes de emalhe de fundo, principalmente na plataforma continental interna (Casarini, 1999; 2006).

Estudos relacionados à distribuição, dieta e biologia reprodutiva das espécies *S. acuta* e *S. bonapartii* foram realizados em décadas passadas na plataforma continental do Rio Grande do Sul (De Queiroz, 1984; De Queiroz, 1995; Pires, 1987). Na costa da Argentina e Uruguai foram realizados estudos de reprodução e abundância em *S. bonapartii* (Mabragaña *et al.*, 2002; Oddone & Velasco, 2004), desenvolvimento embrionário em *S. bonapartii* (Jañez & Sueiro, 2006, 2009) e aspectos histológicos do trato reprodutor feminino em *S. acuta* e *S. bonapartii* (Díaz Andrade *et al.*, 2009, 2011; Galíndez & Estecondo, 2008; Galíndez *et al.*, 2010). Aspectos relacionados à biologia reprodutiva de elasmobrânquios, tal como de outros peixes, podem mudar ao longo do tempo, como é o caso da diminuição do tamanho da primeira maturidade sexual, desenvolvimento precoce das gônadas, entre outras (Paesch & Oddone, 2008).

Devido ao forte endemismo das raias *S. acuta* e *S. bonapartii* na zona costeira do Sul do Brasil, e pelo fato de serem estas espécies um recurso alvo da pesca da região, é de se esperar uma diminuição das suas populações nos próximos anos, como consequência da sobreexploração que vem sofrendo (Dulvy *et al.*, 2000). As raias em geral precisam de uma idade avançada para amadurecer, o que as torna um recurso vulnerável, pois a pesca afeta fortemente aqueles indivíduos que ainda não alcançaram a maturidade sexual (Dulvy & Reynolds, 2002; Iglesias *et al.*, 2009).

A pressão de pesca influencia diretamente as características reprodutivas dos elasmobrânquios, podendo afetar o ingresso de novos indivíduos nas populações e ainda fazer com que as populações respondam com menor tamanho na primeira maturidade sexual (Walters & Martell, 2004). Por tal motivo, estudos atuais que avaliem parâmetros reprodutivos nas espécies *S. acuta* e *S. bonapartii* são necessários para garantir o correto manejo destas populações.

2. OBJETIVOS

2.1 GERAL

Estudar os parâmetros da reprodução de *Sympterygia acuta* e *S. bonapartii* na plataforma sul do Brasil.

2.2 ESPECÍFICOS

Para ambos os sexos de *Sympterygia acuta* e *S. bonapartii*:

2.2.1 Reconhecer dimorfismo sexual relacionado aos parâmetros morfométricos.

2.2.2 Analisar o desenvolvimento sexual com relação às diferentes estruturas reprodutivas e aos estágios de maturidade estabelecidos.

2.2.3 Determinar o tamanho da primeira maturidade sexual.

Para fêmeas de ambas as espécies

2.2.4 Determinar a fecundidade ovariana.

Para machos das duas espécies em questão:

2.2.5 Caracterizar histologicamente os diferentes estágios da espermatogênese.

2.2.6 Analisar os aspectos micro-anatômicos do tecido testicular e do trato reprodutor masculino que incluem o epidídimo, glândula de Leydig, ducto deferente e vesícula seminal.

2.2.7 Determinar o tamanho de maturidade através de técnicas histológicas e comparar com o calculado através das estruturas morfológicas.

2.2.8 Identificar os graus de maturidade estabelecidos de acordo com as características micro-anatômicas do trato reprodutor masculino.

3. METODOLOGIA

3.1 Amostras e área de estudo

As amostras de *Sympterygia* spp. foram obtidas através de duas fontes:

i) cruzeiros na lancha "Larus", do Instituto de Oceanografia da Universidade Federal do Rio Grande, de um dia de duração, durante maio, junho, julho e agosto de 2011.

ii) dois embarques na frota de pesca comercial que atua no Rio Grande do Sul, o primeiro realizado entre 22 e 30 de setembro de 2011, e o segundo entre 30 de janeiro e 10 de fevereiro de 2012. A área de estudo está localizada na plataforma continental interna do Sul do Brasil, entre Conceição e Chui nas latitudes de 31° 43'S e 33°45'S, em profundidades entre 15 e 142 m.

3.2 Morfometria e biometria

Para cada indivíduo coletado foram registrados os seguintes dados:

Comprimento total (CT), medido desde o focinho até a extremidade distal da nadadeira caudal; e largura do disco (LD), medido como o comprimento máximo entre os extremos distais das nadadeiras peitorais; peso total (PT), peso eviscerado (PE), peso do fígado (PF) e peso das gônadas (PG).

Nos machos foram registrados os seguintes dados: comprimento do cláspere e da glândula cláspere, número de espinhos alares e número de fileiras de espinhos alares. Foram registrados espinhos alares considerados como "em desenvolvimento", identificados como uma marca translúcida circular na epiderme mais escura (*sensu* Oddone & Vooren, 2005). O estado de calcificação do cláspere foi avaliado manualmente e classificado como "flexível" ou "rígido".

Nas fêmeas foram registrados os seguintes dados: altura das glândulas ovidutais (AGO), de acordo com Serra Pereira *et al.* (2011), número de folículos vitelogênicos maiores que 1 cm (Mabragaña *et al.*, 2002) e menores do que 1 cm (Díaz Andrade *et al.*, 2011), diâmetro e cor do maior folículo ovariano, e presença ou ausência de cápsulas ovígeras no útero e na cloaca (Oddone & Vooren, 2005).

De acordo com o desenvolvimento das estruturas reprodutivas acima mencionadas em relação ao CT, os indivíduos foram classificados em três categorias de maturação: imaturo, adolescente e maduro (Oddone *et al.*, 2007).

3.3 Análise dos dados

Para ambas as espécies e sexos foram estabelecidas as relações morfométricas entre CT e PT, CT e PE e LD e CT. Os dados foram transformados (Log10) para se

tornarem lineares e foi utilizado o teste F (Souza, 1998) na comparação das curvas entre sexos.

A equação logística foi utilizada para estimar o tamanho da maturidade sexual, conforme: $PCT = 1 / (1 - e^{-(a + b CT)})$, onde:

PCT é a fração de indivíduos maduros para cada classe de CT; a e b são parâmetros.

O CT_{50} , ou o tamanho para o qual 50% da população é madura, e dado pela razão de a / b (Restrepo & Watson, 1991).

O teste t de Student foi aplicado para testar a simetria no número de espinhos alares e de fileiras de espinhos alares entre nadadeiras, e na comparação entre o número de folículos ovarianos entre as fêmeas maduras ovadas e as não ovadas (Sokal & Rohlf, 2012). O teste de F foi utilizado para avaliar a presença de dimorfismo sexual nas relações CT-LD, CT-PT e CT-PE (Souza, 1998). Os parâmetros reprodutivos foram expressos em termos de média e desvio padrão (média \pm SD). Em todos os casos, "n" é o tamanho da amostra. Consideram-se para todos os testes aplicados um nível de significância de 0,05.

Como complemento das análises histológicas aplicadas aos machos, foi calculado o índice gonadossomático (IGS) para machos de ambas as espécies estudadas, de acordo com King (1995), como $IGS = (PG / PE) * 100$. Os valores do IGS foram comparados com os valores de CT e de tamanho do cláster para cada estágio de maturidade e em ambas as espécies.

O CT_{50} histológico foi calculado aplicando-se a equação logística anteriormente descrita à proporção de machos que apresentaram todas as fases da espermatogênese no

testículo. O valor obtido foi contrastado com o CT₅₀ morfológico, tal como descrito por Nolan *et al.* (2002).

3.4 Preparo das amostras para análises histológicas

Os tratos reprodutivos de machos de *S. acuta* e *S. bonapartii* foram extraídos imediatamente após a captura e fixados com formalina 10% durante 24 horas. Posteriormente, as amostras foram armazenadas em álcool 70%. Os testículos (testículo e órgão epigonal) foram seccionados transversalmente na região anterior do segundo quarto da metade das gônadas (ICES, 2010). Secções de tecido orientadas no plano transversal foram retiradas da região superior do epidídimo e da glândula de Leydig (Jones & Hamlett, 2006). Também foram obtidos cortes orientados no plano coronal para os ductos deferentes e vesícula seminal.

Para as análises histológicas, as amostras foram submetidas a um processador de tecidos (Leica ASP-200). Posteriormente, o tecido foi embebido em Paraplast Xtra (Sigma P3808), e cortes histológicos de 6 µm foram obtidos utilizando um micrótomo rotativo automatizado (Leica RM2255). Os cortes histológicos foram corados com Hematoxilina e Eosina (HE), a reação do Ácido Periódico de Schiff (PAS) e Alcian Blue (AB) pH 1,0 e 2,5 (Carson e Hladík, 2009).

A classificação histológica dos estágios da espermatogênese foi realizada utilizando os parâmetros propostos por Maruska *et al.* (1996). Os ductos masculinos foram avaliados tomando em consideração o tipo de epitélio interno e a presença de reatividade para os corantes PAS e AB pH 1,0 e 2,5.

3.5 Aquisição de imagens

As imagens foram capturadas através de um microscópio de campo claro Olympus BX 51 com câmera de alta resolução Olympus DP72, em diferentes aumentos.

4. RESULTADOS

4.1 Relações morfométricas em *S. bonapartii* e *S. acuta*

Foram analisados 92 exemplares machos de *S. bonapartii*. O comprimento total (CT) variou entre 47,2 e 76,8 cm. Indivíduos imaturos apresentaram CT de 47,2-62,5 cm; adolescentes apresentaram CT de 55-71,6 cm, enquanto que nos exemplares maduros o CT variou de 58,6 até 76,8 cm. A relação entre o LD e CT nos machos foi linear ($LD = 2,213 + 0,6252 * CT$; $R^2 = 0,911$).

Um total de 125 exemplares fêmeas de *S. bonapartii* foi analisado. O comprimento total (CT) variou de 30,8 a 79,1 cm. Fêmeas imaturas apresentaram variação de 30,8 a 67,5 cm no CT. Adolescentes apresentaram CT entre 36,8 e 73,3 cm. Em fêmeas maduras o CT variou de 58,0 a 79,1 cm, enquanto que o CT em fêmeas ovadas variou entre 68,0 e 71,2 cm. A relação apresentada entre LD e CT em fêmeas foi linear ($LD = 1,921 + 0,6488 * CT$; $R^2 = 0,890$).

Diferenças significativas ($p < 0,05$) foram detectadas entre os sexos para as relações CT-LD, CT-PT e CT-PE para *S. bonapartii*.

Um total de 17 exemplares machos de *S. acuta* foi registrado, com CT variando entre 29,5 e 56,5 cm. Os valores de CT para os indivíduos imaturos variaram entre 29,5 e 47,0 cm. Adolescentes apresentaram CT entre 43,8 e 46,5 cm. O CT de indivíduos

machos maduros variou entre 47,0 e 56,5 cm. A relação entre o LD e CT nos machos foi linear ($LD = 2,275 + 0,5069 * CT$; $R^2 = 0,956$).

Entre de 28 fêmeas analisadas de *S. acuta*, o CT variou de 21,0 a 60,0 cm. Fêmeas imaturas apresentaram CT de 21,0-51,4 cm; adolescentes de 48,9-52,7 cm, e fêmeas maduras de 49,6-60,0 cm. Fêmeas ovadas apresentaram CT entre 47,7 e 58,0 cm. A relação entre o LD e CT em fêmeas foi linear ($LD = 1,295 + 0,5181 * CT$; $R^2 = 0,970$). Em *S. acuta* as relações entre CT-LD, CT-PT e CT-PE foram sexualmente dimórficas.

4.2 Desenvolvimento sexual de machos de *Sympterygia bonapartii*

O comprimento do cláspere em indivíduos imaturos variou entre 3,8-11,3 cm. Adolescentes apresentaram comprimento do cláspere entre 9,5 e 15,4 cm e em machos maduros estes valores variaram entre 13,5 e 18,1 cm. O início da calcificação do cláspere aconteceu no CT de 55,2 cm. O CT_{50} foi estimado em 53,2 cm ($R = 0,81$, $n = 92$).

O comprimento da glândula do cláspere em indivíduos imaturos variou entre 1,8 e 3,6 cm. Em adolescentes este comprimento variou de 3,1 a 6,7 cm e em indivíduos maduros variou entre 5,5 e 9,0 cm. O ponto de inflexão da relação glândula do cláspere - CT foi estimado em 57,3 cm ($R = 0,60$; $n = 92$).

Lóbulos testiculares foram visíveis macroscopicamente a partir do CT de 47,2 cm. Indivíduos imaturos tinham lóbulos testiculares com diâmetros variando entre 0,1 e 0,5 cm. O diâmetro do lóbulo em adolescentes variou entre 0,3 e 0,7 cm. Machos maduros tinham lóbulos testiculares com diâmetros entre 0,3 e 0,8 cm.

O peso testicular variou de 1,5 a 21,1 g em imaturos, entre 8,4 e 32,1 g em machos adolescentes, e entre 10,2 a 34,7 g em maduros.

O número de fileiras de espinhos alares variou de 1 a 6 em cada nadadeira peitoral, sem diferença significativa entre nadadeira peitoral esquerda e direita ($p= 0,91$). O número de espinhos alares variou entre 6 e 88 na nadadeira direita e 6 e 94 na esquerda, sem diferença significativa entre ambas as nadadeiras ($p= 0,98$). O número de espinhos alares em desenvolvimento variou de 6 a 24 e de 6 a 20 para as nadadeiras peitorais direita e esquerda, respectivamente. Não houve diferença significativa entre ambas as nadadeiras ($p= 0,82$). Indivíduos imaturos com espinhos em desenvolvimento apresentaram CT menor que 62,5 cm. Indivíduos adolescentes com CT entre 50 e 71,5 cm apresentaram espinhos em desenvolvimento, e também foram registrados em dois exemplares maduros com CT de 59,6 e 61,3 cm, respectivamente. O CT_{50} foi estimado em 58,4 cm ($R= 0,98$; $n= 92$) para os machos de *Sympterygia acuta*.

4.3 Desenvolvimento sexual de machos de *Sympterygia acuta*

O comprimento do cláster variou entre 0,9 e 3,7 cm, para indivíduos imaturos, entre 7,6 e 9,4 cm em exemplares adolescentes e entre 7,3 e 13 cm em indivíduos maduros. O início da calcificação do cláster ocorreu no CT de 43,8 cm. O ponto de inflexão da curva logística aplicada à relação CT - comprimento do cláster foi de 43,9 cm ($R = 0,69$; $n = 17$).

Indivíduos com CT entre 43,8 e 52,5 cm apresentaram glândula do cláster com comprimento entre 2,3 e 5,2 cm. O ponto de inflexão para relação da CT - glândula cláster, descrita por uma curva sigmóide, foi 52,65 cm ($R = 0,58$; $n = 11$).

O peso testicular variou entre 0,55 e 1,38 g em indivíduos imaturos, entre 5 e 10 g nos exemplares adolescentes, e entre 6 e 13,9 g nos machos maduros.

O número de fileiras de espinhos alares variou entre 4 e 6 para as nadadeiras peitorais direita e esquerda, sem diferença significativa entre as duas fileiras ($p = 0,75$). O número de espinhos alares variou entre 16 a 75 no lado direito e entre 14 a 68 na nadadeira peitoral esquerda, sem diferença estatística entre estes ($p = 0,86$). O CT_{50} para machos de *S. acuta* foi de 46,1 cm ($R = 0,99$; $n = 17$).

4.4 Desenvolvimento sexual de fêmeas de *Sympterygia bonapartii*

Fêmeas com peso de ovários entre 1,2 e 1,9 g foram consideradas imaturas, sem atividade vitelogênica. Fêmeas adolescentes apresentaram folículos ovarianos correspondentes a dois tipos: brancos e amarelos, com um peso gonadal variando entre 0,5 e 16,2 g. Fêmeas com peso de ovário entre 4,8 e 108,5 g, foram consideradas maduras apresentando folículos vitelogênicos amarelos. Folículos vitelogênicos em fêmeas ovadas pesavam entre 28,1 e 72,2 g.

O diâmetro de folículos brancos foi de 0,1-1 cm e foram registrados em indivíduos imaturos e adolescentes com CT de até 72,5 cm. A vitelogênese foi macroscopicamente evidenciada quando os folículos atingiram um diâmetro de 0,4 cm. A menor fêmea com folículos vitelogênicos amarelos apresentou CT de 36,8 cm. O diâmetro dos folículos vitelogênicos para fêmeas sexualmente maduras variou entre 0,4 e 4 cm. Fêmeas ovadas apresentaram folículos de diâmetro entre 2,3 e 2,9 cm.

Indivíduos imaturos apresentaram largura do útero (LU) entre 0,2-2,2 cm, enquanto que em exemplares adolescentes foi observado LU de 0,4 a 2,4 e LU de 1,0 a 3,8 cm nos exemplares de fêmeas maduras. Fêmeas ovadas apresentaram LU entre 2,1 e 3,3 cm.

A AGO variou entre 0,4 e 1,9 cm nas fêmeas imaturas e entre 0,4 e 1,9 cm nas fêmeas adolescentes. Fêmeas maduras apresentaram AGO variando entre 1,3 e 3,5 cm, enquanto nas fêmeas ovadas, a AGO variou entre 2,1 e 3,3 cm. A fecundidade ovariana variou entre 9 e 74 folículos. O número de folículos vitelogênicos amarelos <1 cm ocorreu em exemplares adolescentes e maduros, variando entre 4 e 54. Em fêmeas ovadas o número de folículos variou entre 21 e 35. Não houve diferença significativa entre o número de folículos vitelogênicos >1 cm e o número de folículos para fêmeas (teste t para amostras independentes). O CT₅₀ para *S. bonapartii* foi calculado em 59,9 cm (R= 0.99; n= 125).

4.5 Desenvolvimento sexual de fêmeas de *Sympterygia acuta*

Fêmeas de *S. acuta* que apresentaram peso do ovário entre 0,4 e 3,0 g foram consideradas imaturas. Fêmeas adolescentes apresentaram folículos tanto brancos e amarelos, com ovários entre 3,8 e 8,5 g. Fêmeas estavam maduras, apresentando folículos amarelos e ovários pesando entre 8,2 e 35,0 g. Fêmeas ovadas apresentaram CT entre 52 e 58 cm, correspondendo a pesos ovarianos entre 20,8 e 40 g.

A LU em fêmeas imaturas variou de 0,1 a 0,6 cm. Fêmeas adolescentes apresentaram LU entre 0,6 e 2 cm e as fêmeas maduras registraram LU entre 0,4 e 1,7 cm. Em fêmeas ovadas esta variável oscilou entre 2,6 e 3,6 cm.

Fêmeas imaturas com CT de até 51,4 cm apresentaram diâmetro de folículos brancos variando entre 0,1 e 0,5 cm. A vitelogênese foi evidenciada macroscopicamente quando os folículos apresentaram um diâmetro de 0,6 cm. A menor fêmea observada com presença de folículos vitelogênicos amarelos media 48,9 cm de CT. O diâmetro dos

folículos vitelogênicos para fêmeas maduras variou entre 0,6 e 2,4 cm e nas fêmeas ovadas variou entre 1,5 e 2,5 cm.

A AGO variou entre 0,15 e 0,6 cm para fêmeas imaturas, entre 1,3 a 1,7 cm em fêmeas adolescentes e entre 2 a 3,1 cm nas fêmeas maduras. Fêmeas ovadas apresentaram AGO entre 2,04 e 2,7 cm. O CT_{50} foi calculado em 44,77 cm ($R= 0,90$; $n= 28$) para as fêmeas de *S. acuta*.

4.6 Anatomia e histologia da gônada masculina de *S. acuta* e *S. bonapartii*

Os testículos de ambas as espécies apresentaram características semelhantes em sua estrutura anatômica macroscópica. Ambos os testículos são simétricos e funcionais, com forma lobulada, localizado dorsalmente na cavidade peritoneal. As gônadas masculinas são formadas pelo órgão epigonal (OE) e testículo em associação íntima, com elevada vascularização do tecido (Artigo 2. Fig. 1.).

As análises macroscópicas em indivíduos imaturos mostram lóbulos testiculares de pequeno tamanho e uma coloração pálida. Dutos nestes espécimes não apresentam nenhuma diferenciação. O OE é formado na maior parte por células granulosas (leucócitos) e granulócitos eosinófilos. Na região medial do tecido gonadal foram detectados dutos, com o epitélio cúbico simples e sem presença de atividade secretora, que corresponde ao ducto eferente (DE). Foi revelada, nesta mesma região, um tecido conjuntivo intratesticular que apresentou atividade secretora, com presença de glicoproteínas positivas para a coloração histológica PAS (Fig.2).

Observações microscópicas indicam uma relação OE/ tecido testicular germinal, diminuindo gradualmente o primeiro com o avanço do processo de maturação (Fig. 2).

4.7 Estágios da espermatogênese em *S. acuta* e *S. bonapartii*

4.7.1 Estágio I (EI)

Esta fase inicial da espermatogênese é caracterizada pela presença de células germinativas dispersas, formando um tecido solto, sem a delimitação de uma membrana. Estas células indicam o ponto de partida da espermatogênese direcionada diametricamente (Fig. 3a, b).

4.7.2 Estágio II (EII)

Este estágio é caracterizado pela presença de espermatogônias, resultado das divisões mitóticas consecutivas das células germinais. Nesta fase, os espermatocistos apresentam internamente uma camada de células de Sertoli com espermatogônias migrando periféricamente, dispostos em torno do lúmen central, delimitado pela membrana basal (Fig. 3c).

4.7.3 Estágio III (EIII)

Depois de passar por divisões mitóticas, as espermatogônias se transformam em espermatócitos primários. Isto inclui uma redução do material genético através da primeira divisão meiótica dando origem posteriormente a espermatócitos secundários. Os espermatocistos deste estágio contêm células esféricas volumosas, com núcleos grandes em relação aos estágios anteriores (Fig. 3d).

4.7.4 Estágio IV (EIV)

Esta fase caracteriza-se pela presença de espermátides devido à segunda divisão meiótica sofrida pelos espermátócitos secundários. Morfologicamente, as espermátides possuem um corpo celular pequeno, os núcleos arredondados (Fig. 3e).

4.7.5 Estágio V (EV)

O aparecimento de espermátides na fase anterior marca o início da espermiogênese. Durante a maturação das espermátides, as células de Sertoli migram para a região periférica do espermatocisto. O espermatozoide imaturo, que aparece neste estágio é encontrado em forma desorganizada dentro do espermatocisto (Fig. 3f).

4.7.6 Estágio VI (EVI)

Os espermatozoides maduros são organizados em pacotes, circulando os espermatocistos periféricamente ao lado das células de Sertoli. As cabeças dos espermatozoides maduros ou espermatozeugmata apresentam uma forma de espiral típico, voltado para a periferia do espermatocisto. Um amplo lúmen comunica os espermatozoides maduros para os dutos coletores, onde posteriormente se comunicam com o DD. Partículas de secreção PAS positivas, de natureza glicoproteica, são observadas neste estágio. Dutos próximos ao OE registraram essas mesmas substâncias (Fig. 3g).

4.7.7 Estágio VII (EVII)

Os espermatocistos neste estágio sofrem deformação, adotando formas achatadas, com pouca ou nenhuma presença de esperma. O colapso dos espermatocistos causa um deslocamento de células de Sertoli, encontrando-se dispersas e particularmente visível. Espermatocistos nesta fase são degradados, indicando com isso o final da espermatogênese (Fig. 3h).

4.8 Histologia do ducto reprodutor masculino de *S. acuta* e *S. bonapartii*

4.8.1 Epidídimo

Anatomicamente, o epidídimo é constituído por um túbulo espiral comprimido, que mostra uma forma alongada, dorso-ventralmente achatado, fixado à parede dorsal da cavidade abdominal. Os machos de *S. acuta* e *S. bonapartii* foram histologicamente semelhantes em relação à micro-anatomia do epidídimo. Em secção transversal, o epidídimo é caracterizado por um epitélio colunar simples ciliado, com atividade secretora do mesmo e caracterizado por uma membrana basal fortemente positiva com PAS, AB pH 1,0 e 2,5. Indivíduos maduros apresentaram um epidídimo vascularizado com quantidades abundantes de matriz seminal, cúmulos de espermatozóides compõem a espermatozeugmata (Fig. 4a, b).

4.8.2 Glândula Leydig

Macroscopicamente em *S. acuta* e *S. bonapartii* a glândula Leydig (GL) está localizada na região posterior do epidídimo em vista ventral. Do ponto de vista histológico, em secção transversal, observa-se que o tecido da GL reveste a área dorsal do epidídimo. A GL é caracterizada por epitélio colunar simples ciliado com células

secretoras PAS positivas, formando massas de secreção dentro do túbulo da glândula, sendo achadas dentro da matriz seminal do epidídimo (Fig. 4c, d).

4.8.3 Duto deferente

O duto deferente na porção anterior encontra-se formando uma estrutura em espiral visível a olho nu. Nos machos maduros de *S. acuta* e *S. bonapartii*, o duto deferente mostrou um epitélio colunar simples. Além disso, foi verificada a presença de uma matriz com espermatozeugmata PAS positiva. Inserida nas faces laterais do duto deferente, no tecido conjuntivo circundante, formam-se glândulas tubulares simples que produzem grandes quantidades de secreções que são fortemente positivas com PAS, AB pH 1,0 e 2,5 (Fig. 4d, e).

4.8.4 Vesícula seminal

A vesícula seminal é formada por um epitélio colunar simples ciliado, caracterizado por apresentar quantidade elevada de matriz seminal, preenchida com conglomerados de espermatozeugmata, partículas eosinófilas e secreções PAS +. Lateralmente dispostos observa-se o tecido glandular, semelhante ao observado nos dutos deferentes, contendo no interior destes grandes quantidades de secreções, com mucopolissacarídeos ácidos sulfatados e carboxilados que apresentam reatividade positiva a AB pH 1,0 e 2,5, respectivamente. Adicionalmente, foi verificada a secreção de glicoproteínas e substâncias neutras com PAS no tecido glandular adjacente à vesícula seminal (Fig. 4g, h).

4.9 Comparação do tamanho de maturidade 50% (CT₅₀) morfológico e histológico de *S. acuta* e *S. bonapartii*

Valores de CT₅₀ avaliados para os parâmetros morfométricas de machos de *S. acuta* e *S. bonapartii* correspondem a 46,1 cm (R= 0,99, n= 17) e 58,4 cm (R= 0,98, n= 92), respectivamente. No entanto, valores de CT₅₀ avaliados para os parâmetros histológicos que considera a proporção relativa de espermatocistos contendo espermatozoides maduros, foi estimada em 44,7 cm (R=0,99, n= 16) e 54,68 cm (R= 0,98 n= 79) para *S. acuta* e *S. bonapartii*, respectivamente (Fig. 5).

O cálculo do CT₅₀ histológico indicou um tamanho menor para a maturidade em ambas as espécies analisadas, quando comparado com o CT₅₀ morfológico (Fig. 5).

4.10 Índice gonadosomático e relações morfométricas dos machos de *S. acuta* e *S. bonapartii*

Para machos de *S. acuta* com CT entre 29,5 e 47,0 cm, os valores para IGS correspondem a 0,39 e 0,52. Valores de índice gonadosomático (IGS) entre 1,17 e 2,31 correspondem a exemplares adolescentes com CT entre 43,0 e 46,0 cm. Em exemplares maduros com 47,0 até 56,0 cm, o IGS variou entre 0,93 e 2,68 (Fig. 6a, c).

Para *S. bonapartii*, indivíduos imaturos com CT entre 47,2 e 63,5 cm apresentaram IGS entre 0,18 e 1,81. Adolescentes com CT entre 55,0 e 71,6 cm apresentaram IGS variando entre 0,74 e 2,60. Machos maduros com CT entre 58,6 e 76,8 cm apresentaram IGS com valores entre 0,76 a 1,87. Os maiores valores de IGS observados em machos de *S. bonapartii* corresponderam a indivíduos adolescentes.

Foram observadas diferenças nos valores de comprimento do cláster para cada estágio de maturidade proposto nas duas espécies estudadas (Fig. 6c, d).

5. CONCLUSÕES

As raias *Sympterygia acuta* e *Sympterygia bonapartii* da plataforma costeira do Sul do Brasil apresentaram dimorfismo sexual, definido através da relação dos parâmetros morfométricos observados, como comprimento total e largura do disco; comprimento total e peso total; e comprimento total e peso eviscerado.

Fêmeas e machos da espécie *S. acuta* e fêmeas da espécie *S. bonapartii*, apresentaram tamanho de primeira maturidade sexual menor do que o registrado anteriormente na região sul do Brasil, a partir de análises de parâmetros reprodutivos morfométricos.

A forte pressão que exercida pela atividade pesqueira da frota de arrasto de fundo na região sul do Brasil poderia estar relacionada com mudanças observadas nos parâmetros reprodutivos de *S. acuta* e *S. bonapartii* ao longo do tempo, assim como as mudanças no CT_{50} .

As análises histológicas do trato reprodutor masculino em *S. acuta* e *S. bonapartii* revelaram informações importantes a nível celular, permitindo um maior entendimento acerca da reprodução destas espécies. Estas por tanto, podem ser utilizadas em conjunto com parâmetros morfométricos, como o tamanho do cláster, grau de calcificação, entre outros para validar processos reprodutivos estudados através dos métodos mais tradicionais (morfológicos).

Aspectos micro-anatômicos do tecido testicular e do trato reprodutor masculino em *S. acuta* e *S. bonapartii* são compartilhados por outras espécies de rajiódeos.

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7. ANEXOS

7.1. Artigo 1. Reproductive biology of the skates *Sympterygia acuta* Garman, 1877 and *S. bonapartii* Müller & Henle, 1841 (Chondrichthyes: Rajoidei) in South Brazil

Reproductive biology of the skates *Sympterygia acuta* Garman, 1877 and *S. bonapartii* Müller & Henle, 1841 (Chondrichthyes: Rajoidei) in south Brazil

Alexander Basallo¹ and Maria Cristina Oddone²

¹ Universidade Federal do Rio Grande, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Oceanografia Biológica, Av. Itália, Km 8 s/n, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil, alexbiology8@hotmail.com

² Universidade Federal do Rio Grande, Instituto de Ciências Biológicas, Fisiologia Animal Comparada, Av. Itália, Km 8 s/n, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil.

The present study analyzed sexual development and sizes at 50% maturity and morphometric relationships for both sexes of *Sympterygia acuta* and *S. bonapartii* endemic of south-western Atlantic Ocean. Specimens were obtained through research cruises and commercial fishing trips during 2011 and 2012 along southern Brazilian coast in latitudes ranging from 34°28'S to 31°29'S and, at depths between 15 and 142 m. Significant differences with values ($p < 0.001$) in *S. bonapartii* and ($p = 0.015$; 0.006 and 0.006) in *S. acuta* between sexes for the relationships total length (cm) - disc width (cm) and total length - total/eviscerated weight (g) respectively, demonstrated sexual dimorphism during the development in both species. The values estimated for maturity

sizes were 46.1 and 44.7 cm for *S. acuta* and 58.4 and 59.9 cm for *S. bonapartii* (for males and females respectively). The results for mature individuals indicated temporal changes in the composition of TL₅₀ values for the two species of genus *Sympterygia*. A decrease in maturity sizes for both sexes was observed in *S. acuta* and females of *S. bonapartii* of the southern coast of Brazil.

O presente estudo analisou os tamanhos de maturidade e relação do comprimento total com diferentes parâmetros reprodutivos, para ambos os sexos das espécies *Sympterygia acuta* e *S. bonapartii* endêmica do sudoeste do Oceano Atlântico. Os indivíduos foram coletados em cruzeiros de investigação e viagens de pesca comercial durante 2011 e 2012 ao longo da costa do Sul do Brasil em latitudes que variam de 34°28'S a 31° 29'S, e em profundidades entre 15 e 142 m. Houve diferenças significativas em ambos os sexos com valores ($p < 0,001$) para *S. bonapartii* e ($p = 0,015, 0,006$ e $0,006$) em *S. acuta* para as relações comprimento total (cm) - largura do disco (cm) e comprimento - peso total / eviscerado (g) respectivamente, mostraram dimorfismo sexual durante o desenvolvimento. Os valores estimados para tamanhos de maturação de *S. acuta* foram de 46.1cm para os machos e 44.7 cm para as fêmeas; *S. bonapartii* apresentou valores de 58,4 e 59,9 (para machos e fêmeas, respectivamente). Os resultados para indivíduos maduros indicaram mudanças temporais nos valores de CT₅₀ para as duas espécies do gênero *Sympterygia* endêmico do Oceano Atlântico Sul – Ocidental. A diminuição nos tamanhos de maturidade para ambos os sexos foi observada em *S. acuta* e fêmeas de *S. bonapartii* da costa Sul do Brasil.

Key words: Maturity, Reproduction, Oviparity, Endemic, Morphometric.

Introduction

The genus *Sympterygia* Müller & Henle, 1837 (Chondrichthyes, Rajiformes) constitute a taxonomical stable clade within the Suborder Rajoidei and includes four neotropical oviparous skate species; *S. lima* (Poepfig, 1835), *S. brevicaudata* (Cope, 1877), *S. acuta* (Garman, 1877) and *S. bonapartii* (Müller & Henle, 1841) (McEachran, 1982; Ebert & Compagno, 2007). The latter two are endemic to the western South Atlantic Ocean shelves (Figueiredo, 1977; McEachran & Aschliman, 2004) and represent an important economic resource in their whole distribution area (Mabragaña *et al.*, 2002; Menni & Stehmann, 2000; Paesch & Domingo, 2003). In southern Brazil (South and Southeast), *S. acuta* and *S. bonapartii* complete their life cycle in the inner-shelf waters (De Queiroz, 1986; Vooren, 1997; Vooren *et al.*, 2005).

Like other elasmobranchs, the rajoids present relatively low annual fecundity, late sexual maturity and high longevity. These characteristics, together with fish exploration, have lead to population decreases worldwide, even with confirmed cases of local extinction (Dulvy *et al.*, 2000; Dulvy & Reynolds, 2002; Iglésias, 2009). The Bignose Fanskate *S. acuta* is (globally) considered by the IUCN Red List of Threatened species as “Vulnerable”. On the other hand, the Smallnose Fanskate, *S. bonapartii* is classified as “Data Deficient” (Massa & Hozbor, 2004; Massa & Lamilla, 2004). However, due to the high fishing pressure to which both species are subjected, the mentioned categories may change regionally in a short-term period.

Studies on the reproductive ecology, size-at-maturity and abundance of *S. bonapartii* were carried out in the coasts of Argentina and Uruguay over the last decade,

demonstrating an increasing concern for the fisheries effects upon the populations as well as the need for biological data (especially on reproduction) in order to get the stocks responsibly managed (Mabragaña *et al.*, 2002; Oddone & Velasco, 2004).

De Queiroz (1986) analyzed the reproductive biology of *Sympterygia* spp. in the southern Brazilian shelf. Apart from this data being part of an unpublished thesis, they were collected in the period 1981-1984, *i.e.*, almost 30 years ago. Moreover, it is a well-known fact that in the rajoids reproductive parameters such as the size-at-maturity are able to change when the species is subject to the pressure of excessive fishing (Walters & Martell, 2004).

It is, therefore necessary, to provide an updated study on the biology of the reproduction and seasonality traits for genus *Sympterygia* in south Brazil, that may aid to management decision takers and/or conservation agencies in the establishment of conservation; actions such as closed areas and minimum landing sizes. The aim of this study was to provide updated information on the reproductive parameters of *S. bonapartii* and *S. acuta* in the southernmost continental shelf of Brazil between Conceição (31° 43'S) and Chui (33°45'S).

Material and Methods

Samples of *Sympterygia* spp. were obtained through two sources: cruises aboard the research vessel “Larus” (Instituto de Oceanografia, Universidade Federal de Rio Grande) during one-day-long research cruises, in May, June, July and August of 2011 and two commercial fishing trips carried out along the coast of Rio Grande do Sul State

(South Brazil) from September 22nd to 30th 2011 and from January 30th to February 10th 2012. The studied area was located between the latitudes of 34°28'S and 31°29'S at depths between 15 and 142 m (Fig. 1).

Of each specimen, the following data were registered: total length (TL) from the snout to the extremity of the tail, the width of the disc (DW) measured as the length between opposite pectoral fin tips, total weight (TW), eviscerated weight (EW), liver weight (LW) and gonad weight (GW) (measured values in cm and g, respectively) (*sensu* Oddone *et al.* 2007a).

For the males, the clasper and clasper gland length, the number of alar thorns and rows of alar thorns were recorded. Alar thorns considered as “developing” were registered as a circular light mark on a darker epidermis, being evident laterally on the dorsal region of both pectoral fins (*sensu* Oddone & Vooren, 2005). The calcification of the clasper was manually assessed and classified as "flexible" or "rigid". In females, the height of the oviducal glands according to Serra Pereira *et al.* (2011), number of vitellogenic follicles larger than 1 cm (Mabragaña *et al.*, 2002) and smaller than 1 cm (Díaz Andrade *et al.*, 2011), uteri width, diameter and colour of the largest ovarian follicle, and presence or absence of egg capsules in the uterus and in the cloaca were registered (Oddone & Vooren, 2005). According to the development of the above-mentioned reproductive structures in relation to TL, the individuals were classified in the three maturity categories: immature, adolescent and mature (Oddone *et al.*, 2007a). Morphometric measurements of both species and sexes were compared. Total length-width disc, total length-total weight and total length-eviscerated weight were log-

transformed to become linear and the F-test was used to establish comparisons (Souza, 1998). The logistic equation was used to estimate the size of sexual maturity, as

$$PTL=1/ (1-e^{-(a+bTL)})$$

where, PTL is the fraction of mature individuals for each class of TL, a and b are parameters. The TL_{50} , or the size at which 50% of the population is mature, is given by the ratio a/b (Restrepo & Watson, 1991). The Student's t-test was applied to compare the mean number of alar thorns and mean number of rows of alar thorns and establishing differences between the number of ovarian follicles in mature females and egg-bearing females (Sokal & Rohlf, 2012). Reproductive parameters were expressed in terms of mean and standard deviation as mean \pm S.D. In all cases "n" is the size of the sample. A level of significance of 0.05 was considered for all the applied tests.

Results

Morphometric relationships in *S. bonapartii* and *S. acuta*

A total of 92 males of *S. bonapartii* were obtained and analyzed. Total length ranged between 47.2 cm and 76.8 cm. Immature males had TLs of 47.2-62.5 cm (mean=56.12; SD=4.41; n=14); adolescents of 55.0-71.6 cm (mean=61.38; SD=4.78; n=19) and in mature specimens TL varied from 58.6 to 76.8 cm (mean=65.46; SD=4.39; n=59). The relationship between DW and TL in males was linear (Fig. 2a) (Table 1).

A total of 125 females of *S. bonapartii* were analyzed. Total length varied between 30.8 and 79.1 cm. Values in immature females varied from 30.8 to 67.5 cm (mean=56.2; SD=12.55; n=17). Adolescents had total length ranging from 36.8 to 73.3 cm

(mean=65.1; SD=9.99; n=12). In mature females, TL varied from 58.0 to 79.1 cm (mean=68.8; SD=4.20; n=96) and egg-bearing females TL corresponded from 68.0 to 71.2 cm (mean=69.3; SD=1.35; n=4). The relationship presented between DW and TL in females was linear (Fig. 2a) (Table 1).

According to F-test results significant differences were detected between sexes for to the relationship TL-DW, TL-TW and TL-EW for *S. bonapartii* (Table 1, Fig. 2a-c).

A number of 17 male *S. acuta* were recorded. Total length varied from 29.5 to 56.5 cm. The values of TL for immature individuals ranged between 29.5 and 47.0 cm (mean=41.5; SD=8.19; n=4). Adolescents ranged from 43.8 to 46.5 cm long (mean=45.15; SD=1.90; n=2). Mature specimens had TLs varying between 47.0 and 56.5 cm (mean=50.25; SD=2.70; n=11). The relationship between DW and TL in males was linear (Fig. 2d) (Table 1).

A total of 28 females of *S. acuta* were analyzed, distributed in TLs between 21.0 and 60.0 cm. Immature females had TLs of 21.0-51.4 cm (mean=33.07; SD=11.08; n=7); adolescents of 48.9-52.7 cm (mean=50.76; SD=1.46; n=5); in mature specimens TL varied from 49.6 to 60.0 cm (mean=53.65; SD=3.08; n=11). Egg-bearing females had values between 47.7 and 58.0 cm TL (mean=53.14; SD=3.74; n=5). The relationship between DW and TL in females was linear (Fig. 2d) (Table 1).

In *S. acuta* the relationships between TL-DW, TL-TW and TL-EW were sexually dimorphic (Table 1, Fig. 2 d-f).

Sexual development of the male *Sympterygia bonapartii*

The clasper length in immature individuals varied from 3.8 to 11.3 cm (mean=8.73; SD=2.16; n=14). Adolescents had clasper length ranging from 9.5 to 15.4 cm (mean=12.26; SD=1.73; n=19) and in mature males these values varied between 13.5 and 18.1 cm (mean=15.53; SD=1.05; n=59). The onset of clasper calcification was observed at TL=55.2 cm. The inflexion point of the clasper length-TL relation was estimated to be at 53.2 cm (R=0.81; n=92) (Fig. 3a).

The clasper gland length in immature individuals ranged between 1.8 and 3.6 cm (mean=2.94; SD=0.59; n=14). In adolescents this length ranged from 3.1 to 6.7 cm (mean=4.63; SD=0.94; n=19) and in mature individuals from 5.5 to 9.0 cm (mean=6.99; SD=0.77; n=59) (Fig. 3b). The inflexion point of the clasper gland-TL relationship was estimated at 57.3 cm (R=0.60; n=92).

Testicular lobules become macroscopically visible at TLs of 47.2 cm onward. Immature individuals had testicular lobules with diameters varying between 0.1 and 0.5 cm (mean=0.3; SD=0.14; n=14). The adolescent lobule diameter ranged between 0.3 and 0.7 cm (mean=0.47; SD=0.12; n=19). Mature males had testicular lobules with diameters between 0.3 and 0.8 cm (mean=0.52; SD=0.12; n=59) (Fig. 3c).

Testicles weight varied from 1.5 to 21.1 g (mean=11.34; SD=6.91; n=14) in immature, from 8.4 to 32.1 g (mean=22.14; SD=7.19; n=19) in adolescents and from 10.2 to 34.7 g (mean=20.45; SD=4.96; n=59) in mature males (Fig. 3d).

The number of alar thorns rows varied from 1 to 6 on each pectoral fin. No significant difference was detected between left and right pectoral fins regarding the mean alar thorn rows number (mean=2.49, SD=1.38, n=77; mean=2.46, SD=1.47, n=77, respectively) ($t=0.11$; d.f.=76; $p=0.91$). The number of alar thorns ranged between 6 to

88 on the right fin and the 6 to 94 on the left one (mean=30.64, SD=19.84, n=77; mean=30.57, SD=20.60, n=77, respectively) with no significant differences between both fins ($t=0.019$; d.f.=76; $p=0.98$). The number of developing thorns varied from 6 to 24 and from 6 to 20 for right and left pectoral fins respectively (mean=11.05, SD=4.22, n=20; mean=10.75, SD=4.12, n=20, respectively) (Fig. 3e). Differences between fins were not significant ($t=0.22$; d.f.=38; $p=0.82$).

Immature individuals with developing thorns had TLs smaller than 62.5 cm. An adolescent individual with TL between 50.0 and 71.5 cm showed developing thorns, that were also recorded in two mature specimens with TLs of 59.6 and 61.3 cm respectively (Fig. 3e).

According to the logistic curve fitted to the proportion of mature males in relation to TL classes, TL_{50} was estimated at 58.4 cm ($R=0.98$; n=92) for male *S. bonapartii* (Fig. 4).

Sexual development of the male *Sympterygia acuta*

The measures of clasper length in immature individuals of *S. acuta* varied 0.9 to 3.7 cm (mean=2.8; SD=1.29; n=4). In adolescents from 7.6 to 9.4 cm (mean=8.5; SD=1.27; n=2), and in mature individuals from 7.3 to 13.0 cm (mean=9.5; SD=1.92; n=11). The onset of calcification occurred when males attained a TL of 43.8 cm. The inflection point of the curve clasper length vs. Total length was 43.9 cm ($R=0.69$; n=17) (Fig. 5a). Individuals with TL between 43.8 and 52.5 cm, had values of clasper gland length between 2.3 and 5.2 cm. (Fig. 5b).

The testicular weight of immature individuals varied between 0.5 and 2.0 g (mean=1.39; SD=0.61; n=4). The testicles weight in adolescents varied between 5.0 and 10.0 g

(mean=7.5; SD=3.53; n=2). Mature males had testicular weights between 6.0 and 13.9 g (mean=10.79; SD=2.24; n=11) (Fig. 5c).

The number of alar thorns rows varied between 4 and 6, on both right and left pectoral fins (mean=5, SD=0.63, n=11; mean=4.9, SD=0.70, n=11, respectively) with no significant differences between both fins ($t=0.31$; d.f.=10; $p=0.75$). The number of alar thorns varied from the 16 to 75 on the right and from 14 to 68 left pectoral fin (mean=29.36, SD=21.03, n=11; mean=27.91, SD=18.8, n=11, respectively) (Fig. 5d) with no differences between them ($t=0.17$; d.f.=10; $p=0.86$) (Fig. 5d). The size at 50% maturity for *S. acuta* males (TL_{50}), was 46.1 cm ($R=0.99$; n=17) (Fig. 4).

Sexual development of the female *Sympterygia bonapartii*

Females with ovary weights between 1.2 and 10.2 g, being immature with no vitellogenic activity (mean=6.8; SD=2.81; n=13). Adolescent females had ovarian follicles corresponding to two types, whites and yellow, with gonad weight ranging from 0.5 to 16.2 g (mean=8.7; SD=3.87; n=12). Females were mature and had ovaries weighing between 4.8 and 108.5 g, bearing yellow vitellogenic follicles (mean=29.2; SD=18.69; n=92). Vitellogenic follicles in egg-bearing females occurred with corresponding ovarian weights between 28.1 and 72.2 g (mean=46.9; SD=18.61; n=4) (Fig. 6a).

White follicles diameter ranged between 0.1- 1.0 cm (mean=0.4; SD=0.27; n=17) and were recorded in immature individuals and adolescents of up to $TL=72.5$ cm. The onset of vitellogenesis occurred when follicles attained a diameter of 0.4 cm. The smallest female with vitellogenic yellow follicles was 36.8 cm TL. The diameter of vitellogenic

follicles in mature females varied between 0.4- 4.0 cm (mean=2.0; SD=0.83; n=97).

Egg-bearing females had follicles diameter between 2.3 and 2.9 cm (mean=2.6; SD=0.25; n=4) (Fig. 6b).

Mean values for uterus width in females varied among all the maturity stages analyzed.

Immature individuals had uteri ranging from 0.2 to 2.2 cm wide (mean=1.0; SD=0.62; n=17). Meanwhile, adolescent females presented uteri with widths of 0.4 to 2.4 cm (mean=1.5; SD=0.55; n=12). In mature individual, uteri width varied from 1.0 to 3.8 cm (mean=2.5; SD=0.48; n=92). Within this group, egg-bearing females had uteri ranging between 2.1 and 3.3 cm wide (mean=3.8; SD=0.43; n=4) (Fig. 6c).

The Oviducal gland height (OGH) varied from 0.4 to 1.9 cm (mean=1.1; SD=0.47; n=13) in immature females and from 0.4 to 1.9 cm (mean=1.2; SD=0.51; n=10) in adolescents.

Mature females had OGHs varying from 1.3 to 3.5 cm (mean=2.1; SD=0.42; n=91), while egg-bearing females had OGHs ranging from 2.1 to 3.3 cm

(mean=2.6; SD=0.52; n=4) (Fig. 6d). Ovarian fecundity varied from 9 to 74

(mean=29.81; SD=10.85; n=82). The number of vitellogenic yellow follicles < 1 cm,

occurred in individuals classified as adolescent and mature, varying from 4 to 54

follicles (mean=23.16; SD=15.46; n=13). In egg-bearing females, the number of

follicles varied from 21 to 35 (mean=29.3; SD=6.02; n=4). No difference was observed,

between the number of vitellogenic follicles larger than 1 cm and the number of follicles

in egg-bearing females (t-test for independent samples, $t=0.10$; d.f.=84; $p=0.91$) (Fig.

6e). The TL_{50} estimate for *S. bonapartii* was 59.9 cm ($R=0.99$; n=125) (Fig. 4).

Sexual development of the female *Sympterygia acuta*

Ovaries of immature females weighted between 0.4 and 3.0 g (mean=1.46; SD= 1.10; n= 5). Adolescents had both white and yellow follicles, with ovaries weights between 3.8 and 8.5 g (mean=6.04; SD=2.18. n=5). Mature females bore yellow follicles and had ovaries weighting between 8.2 and 35.0 g (mean=21.71; SD=8.96; n=7). Ovaries of egg-bearing females weighted between 20.8 and 40.0 g (mean=33.95; SD=9.07; n=4) (Fig. 7a). Uteri width in immature females varied from 0.1 to 0.6 cm (mean=0.31; SD=0.19; n=7). Adolescent females had uteri ranging from 0.6 to 2.0 cm wide (mean=1.38; SD=0.50; n=5). In matures females, uteri width varied from 0.4 to 1.7 cm (mean=0.81; SD=0.55; n=11) and egg-bearing females uteri from 2.6 to 3.6 cm (mean=3.08; SD=0.37; n=5) (Fig. 7b).

Immature females with TL up to 51.4 cm had white follicles diameter varying from 0.1 to 0.5 cm (mean=0.20; SD=0.2; n=5). Vitellogenesis was macroscopically evident when follicles attained a diameter of 0.6 cm. The smallest female with vitellogenic yellow follicles was 48.9 cm long. The diameter of vitellogenic follicles in mature females varied between 0.6 and 2.4 cm (mean=1.6; SD=0.51; n=14) and in the egg-bearing females from 1.5 and 2.5 cm (mean=2.04; SD=0.43; n=4) (Fig. 7c).

OGH varied from 0.1 to 0.6 cm (mean=0.41; SD=0.19; n=4) in immature females; from 1.3 to 1.7 cm (mean=1.52; SD=0.17; n=4) in adolescents and from 2.0 to 3.1 cm (mean=2.48; SD=0.27; n=11) in mature females. Egg-bearing females had OGHs varying from 2.0 to 2.7 cm (mean=2.36; SD=0.28; n=5) (Fig. 7d). The TL₅₀ was estimated at 44.7 cm (R=0.90; n=28) for female *S. acuta* (Fig. 4).

Discussion

The results on morphometrics obtained in *S. bonapartii* and *S. acuta* for the relations TL-DW, TL-TW and TL-EW indicated differences between males and females in both analyzed species. Sexual dimorphism in these relationships has been described for *S. bonapartii* (Mabragaña *et al.*, 2002) and for other SW Atlantic rajoids (Mabragaña & Cousseau, 2004; Oddone & Vooren, 2004; Oddone & Amorim, 2007; Orlando *et al.*, 2011).

The relationship TL-DW was sexually dimorphic in *S. bonapartii*, what had been recorded for a congeneric species, *S. lima*, endemic of the coast Chile, Southeast Pacific Ocean by Lamilla *et al.* (1984).

The TL-DW relationship in *S. acuta* indicated that immature males presented higher size when compared with females in this first stage of development. However, in adolescents and mature individuals just the opposite was observed. Such morphometrics differences throughout the ontogeny were also recorded in *Atlantoraja cyclophora* (Regan, 1903) and *Rioraja agassizii* (Muller & Henle, 1841) in southern Brazil (Oddone & Vooren, 2004; Oddone *et al.*, 2007a). On the other hand, the growth pattern in *S. bonapartii* demonstrated that, in this species, females had larger DW than males for any TL considered. Maybe these dimorphic traits possibly occur like a reproductive strategy of the species.

Minimum TL at first maturity was found to be similar (or close) in both sexes for *S. bonapartii*. Mabragaña *et al.* (2002) observed similar situation in the same specie of the coast off Argentina. Possibility there would no intersexual differences in the size of first maturity. However, according to Mabragaña *et al.* (2002) differences at the interpopulational level could exist, due to differences in the minimum size at maturity

for the individuals captured off Argentina compared with measurements obtained in this present study for these species that occur off southern Brazil.

The length of clasper in relation to the TL in males of *S. bonapartii* described a sigmoid curve, typically observed for the sexual development of rajoids and most elasmobranchs. Studies in other species of the family Arhynchobatidae (Fowler, 1934) demonstrated that the morphometric values for total clasper length and clasper gland, fitted themselves in the sigmoid growth curve, at least in the genus *Atlantoraja* and *Rioraja agassizii* (Oddone & Vooren 2005; Oddone *et al.*, 2007a; Oddone & Amorim 2008; Oddone *et al.*, 2008). This type of logistic growth curves also was observed in four species of the family Rajidae (Blainville, 1816), namely, *Leucoraja ocellata* (Mitchill, 1815), *Leucoraja erinacea* (Mitchill, 1825), *Amblyraja radiata* (Donovan, 1808) and *Malacoraja senta* (Garman, 1885), distributed off the east coast of Canada (McPhie & Campana, 2009).

The number of rows of alar thorns in males of *S. bonapartii* varied between 1 and 6, in agreement with data reported by Mabrugaña *et al.* (2002), and also similar to the pattern observed in *A. cyclophora* (Oddone & Vooren, 2005) and *Bathyraja albomaculata* (Ruocco *et al.*, 2006). This sexual character present in males can vary among species, being an important parameter used for maturity staging.

S. bonapartii showed a high level of overlap among values of three stages of sexual development, being detected regarding the following variables: testicle weight, diameter of lobule, clasper gland length, number of rows of thorns and alar thorns. However, mean values of these variables indicate differences between each stages of sexual development.

Males *S. bonapartii* were found to mature with TL of 58.4 cm. On the other hand, De Queiroz (1986) recorded a value of 52 cm for this parameter, indicating that TL_{50} may have increased in 3.3 % (6.4 cm) over a ~30 years period. However, methodological differences may also be the result of differences in the maturity estimates (Oddone & Velasco, 2004). De Queiroz (1986) clasper recording methodology (based on Hubbs & Ishiyama, 1968) differed from the one used in the present paper, though he based in virtually the same reproductive variables for male sexual maturity staging. For male *S. bonapartii*, TL_{50} also is different from those calculated from other south-western Atlantic areas, such as Argentina 65 cm and Uruguay 57 cm (Mabragaña *et al.*, 2002; Oddone & Velasco 2004), though values reported by Oddone & Velasco (2004) were very close to those obtained in the present study. This similarity can be a consequence of the proximity of the area of the sampled specimens (in the extreme south of Brazil), with the border area between the coasts of Brazil and Uruguay.

In males *S. acuta* the inflection point of the curve fitted for the relationship between clasper length and TL coincided with the value of minimum TL for the clasper calcification. Therefore the estimation of maturity solely through the TL-clasper length relationship would subestimated. Similarities among these measures were also observed in *S. bonapartii*, demonstrating the importance in the manual record of the clasper calcification degree used for determination the maturity in males of chondrichthyes.

Adult males of *S. acuta* bore a maximum of 6 rows of alar thorns, just like that observed for *S. bonapartii*. Other studies have also demonstrated a similarity in this number maximum of rows of alar thorn, for other species of the same family (Mabragaña *et al.*, 2002; Oddone & Vooren, 2005; Ruocco *et al.*, 2006).

Conversely, for males of *S. acuta* a decreased in TL₅₀ was observed, comparing with the value obtained by De Queiroz (1986) for the same region, corresponding to 3.0 % (5.9 cm).

In females analyzed by De Queiroz (1984) ovary weight in mature *S. bonapartii* varied from 25 to 170 g. For *S. acuta* the same author recorded ovaries weight of 10 to 50 g. In this study ovary weight for mature females showed to have decreased when compared with that of De Queiroz (1984). These kind of changes in the reproductive variables over time may directly affect the reproductive parameters. Likely, females of both species may develop at least some degree of reproductive plasticity, reflected in this case in a decrease of gonadal size of mature individuals, which could be a result of over fishing in the region during the last decades.

Ovarian follicles larger than 1.0 cm were considered vitellogenic by Mabragaña *et al.* (2002) for *S. bonapartii*. However, De Queiroz (1984) observed that the vitellogenesis starting to 1.0 and 1.5 cm in *S. bonapartii* and *S. acuta*, respectively. Moreover, Diaz Andrade *et al.* (2011) examined ovarian follicles smaller than 1.0 cm in mature females of *S. bonapartii* through the histological analysis of the ovary. They concluded that the traditional macroscopic assessment of the follicular vitellogenesis can turn into a subjective parameter of analysis, hindering classification of the development stage.

The analysis performed by Diaz Andrade *et al.* (2009) in females of *S. acuta*, revealed that at an histological level, vitellogenesis would start at 0.55 cm. This value is similar to that obtained in the present study, where vitellogenesis was macroscopically detected from follicles with diameters of 0.6 cm onward. A more recent study by Diaz Andrade *et al.* (2011) demonstrated that vitellogenic follicles with diameter larger than 0.3 cm

are characteristic of mature females of *S. bonapartii*. In the present study the diameter of vitellogenic follicles found was starting from 0.4 cm in this same species.

Serra Pereira *et al.* (2011) analyzed the relationship of various measures of oviducal gland, including height, width and thickness, in *Raja clavata* (Linnaeus, 1758) in relation to maturity stages (i.e., “in development”; “capable spawning”; “actively spawning”), finding differences among stages. Differences in the OGH were also detected in *S. bonapartii* and *S. acuta*, which increased during sexual development.

The size of maturity obtained for females *S. bonapartii* and *S. acuta*, when compared with those values reported by De Queiroz (1986) in southern Brazil, indicated a considerable decrease in the size of maturity over the last 30 years. Such decline represented 7 and 4.4 % or (10 and 8.3 cm) for female *S. bonapartii* and *S. acuta*, respectively, and may be caused by overfishing which focuses heavily in the southern region of Brazil (Walters & Martell, 2004; Paesch & Oddone, 2008). The TL_{50} estimates obtained for both sexes of *S. bonapartii* demonstrated differences to the regional level. The restricted distribution of the species could indicate the possibility of the existence of two different populations of *S. bonapartii*, the first occupying the higher latitudes of the south-western Atlantic Ocean in the coast off Argentina and a second population composed by individuals that transit between the coastal zone of Uruguay and southern Brazil. This could be attributed to the transport of water masses from the Brazil Current and the Malvinas Current, accentuated mainly during the period of summer and winter respectively (Miloslavich *et al.*, 2011). Differences in size of maturity were discussed for *S. bonapartii* by Mabrugaña *et al.* (2002) and assigned to

the presence of populations that attain sexual maturing at different size and populations with sexes having the same size at maturity.

The genus *Sympterygia* from the south-western Atlantic may have suffered a decline as a consequence of strong fishing pressure upon their populations over the decades.

Characteristics related to reproduction such as the maturity size may change in oviparous skates that are affected by fishing (Ebert *et al.*, 2008; Paesch & Oddone, 2008; Orlando *et al.*, 2011). As it is the case of *Sympterygia* spp., it is fundamental to count with relative current data on reproduction. Furthermore, future research should also be focused on the reproductive cycle both temporally and spatially. Significant decrease in the size at maturity as a result of fishing pressure was documented for *Zearaja chilensis* (Paesch & Oddone, 2008) in the south-western Atlantic Ocean.

Data on the reproductive biology of the genus *Sympterygia* endemic to the south-western Atlantic Ocean presented in this study, may provide tools for the evaluation of the stocks, in order to properly conserve and manage the populations of *Sympterygia* spp. that are used for human consumption and exploited unrestrictedly as fishery resources.

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Table (s)

Table 1. Relationships analyzed for *S. bonapartii* and *S. acuta*. TL= total length (cm), DW= disc width (cm), TW= total weight (g), EW = eviscerated weight (g) for males (M) and females (F) with the respective potential regression equation; correlation coefficient (R^2) and sample number (n).

Curve	Sex	Equation	R^2	n	Comparison
<i>S. bonapartii</i>					
TL-DW	M	DW=2.213+0.6252*TL	0.911	92	F=3.106;d.f.=(2,213);p<0.001
	F	DW=1.921+0.6488*TL	0.890	125	
TL-TW	M	TW=0.019968TL ^{2.71552}	0.852	87	F=3.314;d.f.=(2,203);p<0.001
	F	TW=0.043169TL ^{2.5428}	0.745	120	
TL-EW	M	EW=0.022659TL ^{2.66553}	0.879	87	F=3.174;d.f.=(2,203);p<0.001
	F	EW=1.30685TL ^{2.95601}	0.743	120	
<i>S. acuta</i>					
TL-DW	M	DW=2.275+0,5069 *TL	0.956	17	F=4.449;d.f.=(2,39);p=0.015
	F	DW=1.295+0.5181*TL	0.970	26	
TL-TW	M	TW=0.901592TL ^{2.15908}	0.922	17	F=3.438;d.f.=(2,41);p=0.006
	F	TW=0.041639TL ^{2.43972}	0.754	28	
TL-EW	M	EW=0.002462TL ^{3.1534}	0.883	17	F=3.461;d.f.=(2,41);p=0.006
	F	EW=0.048513TL ^{2.36878}	0.761	28	

Figure(s) legend(s)

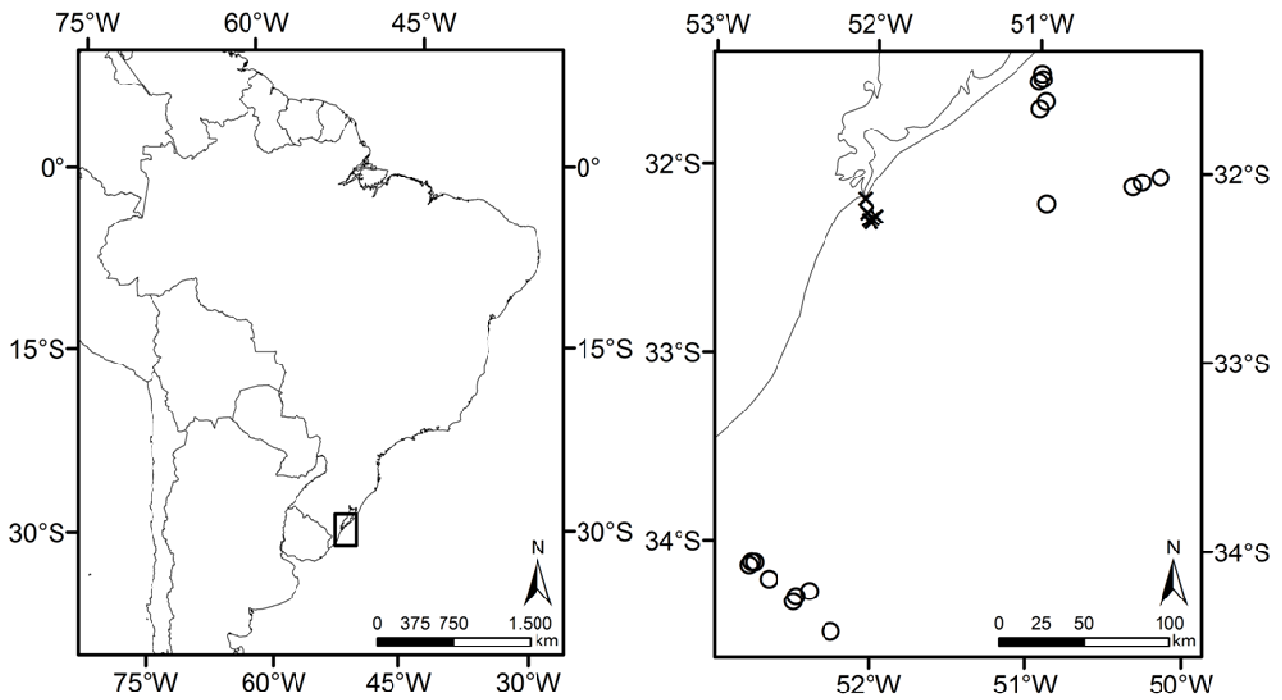


Fig. 1. Map of the study area. Left map represents South America. The square is a detail of the study area. The right map represents south of Brazil indicating the trawling stations where the specimens of *Sympterygia* were captured; research cruises of the research vessel “Larus” (triangles) and commercial bottom trawl fishing trips (circles).

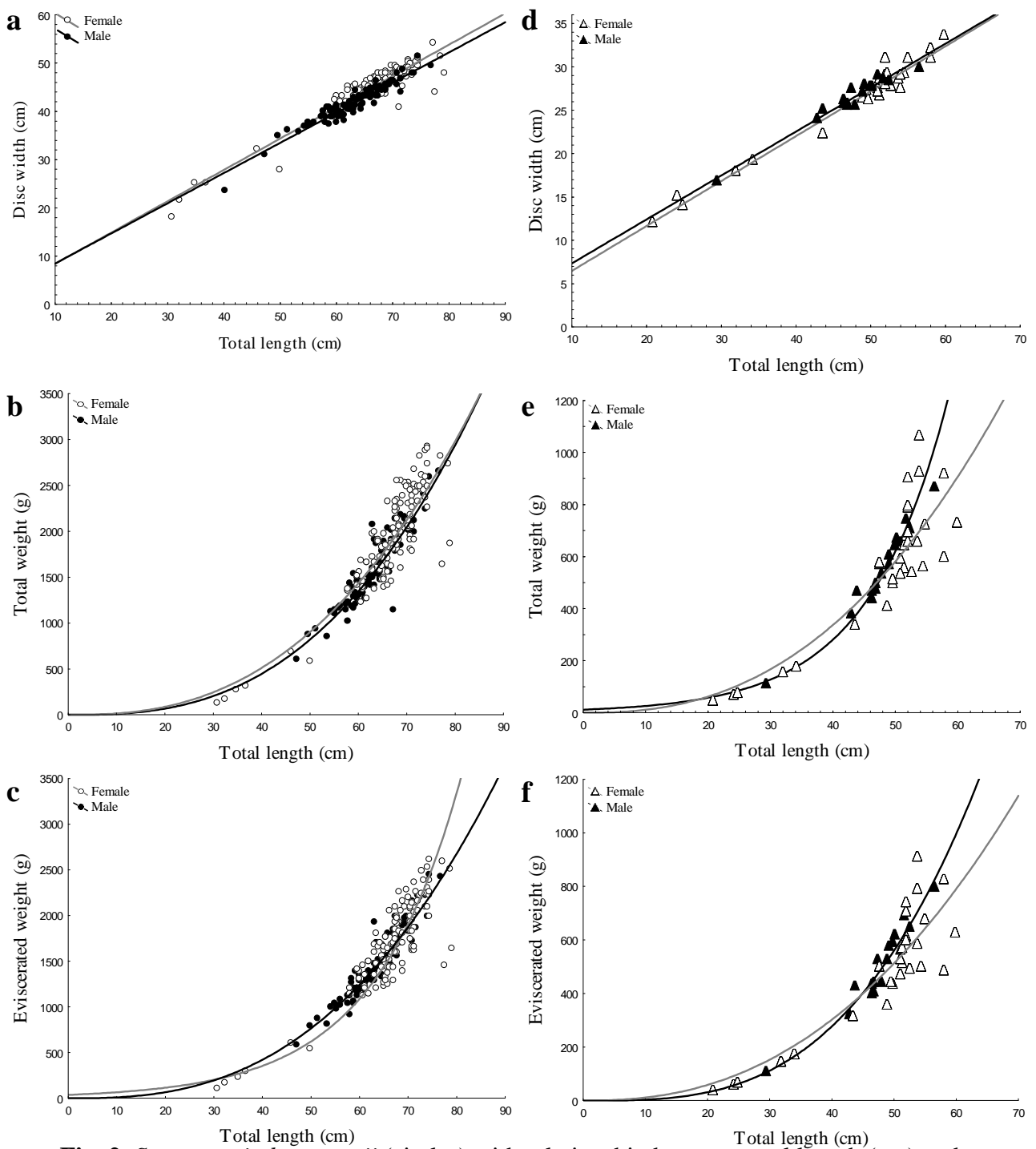


Fig. 2. *Sympterygia bonapartii* (circles) with relationship between total length (cm) and (a) disc width (cm), (b) total weight (g) and (c) eviscerated weight (g) (by sex).

Sympterygia acuta (triangles) with relationship between total length (cm) and (d) disc width (cm), (e) total weight (g) and (f) eviscerated weight (g).

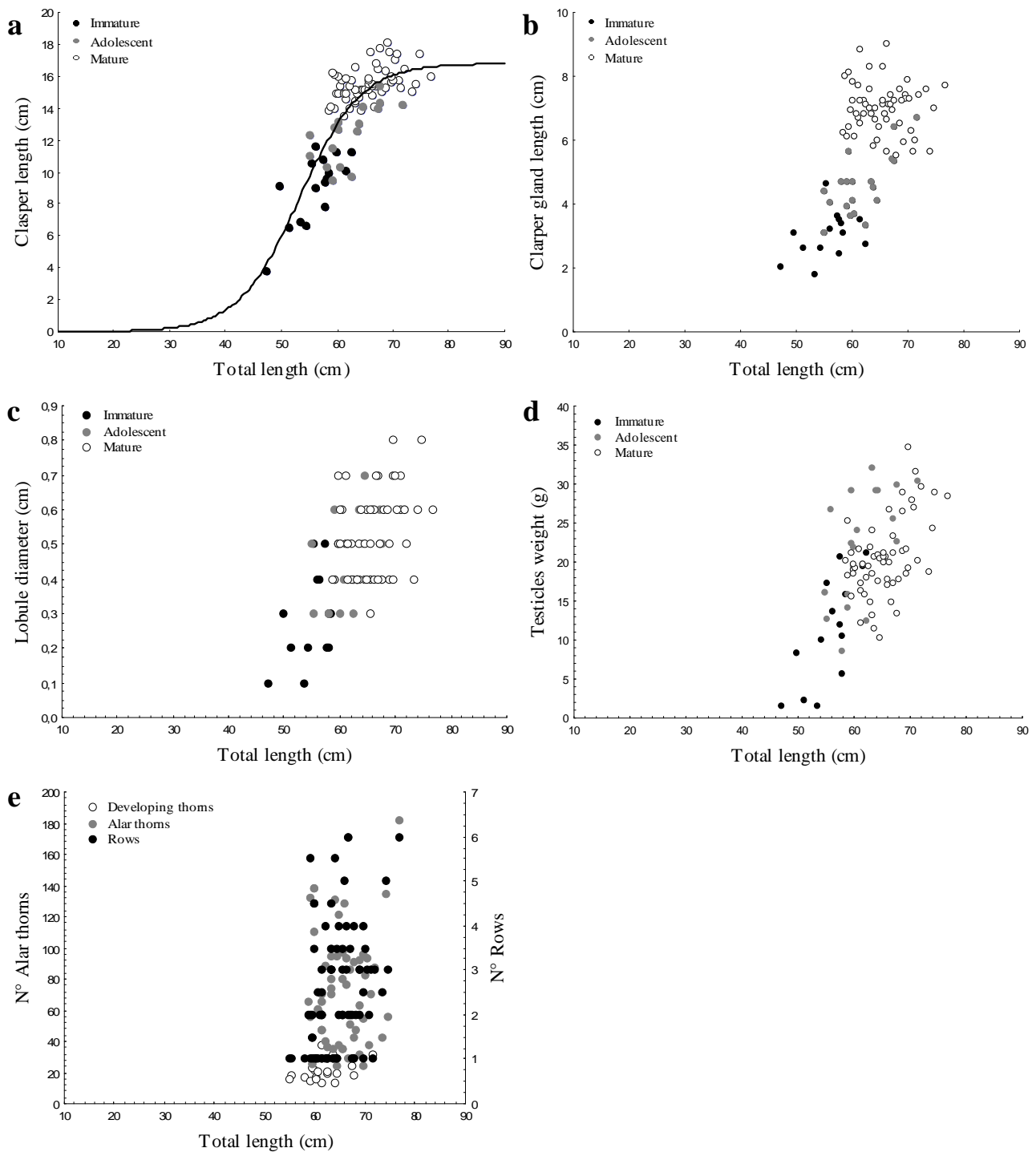


Fig. 3. *Sympterygia bonapartii*. Relationship between total length (cm) and (a) clasper length (cm), (b) clasper gland length (cm), (c) diameter of the testicular lobule (cm), (d) testicles weight (g) and (e) number of alar thorns rows; developing thorns and number of rows of alar thorns.

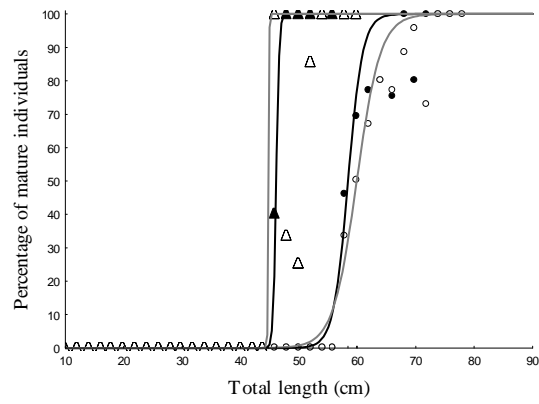


Fig. 4. Percentage of mature individuals by total length class (TL₅₀) for males and females *Sympterygia acuta* (triangles) and *Sympterygia bonapartii* (circles). Bold symbols represent the males and empty symbols represent the females.

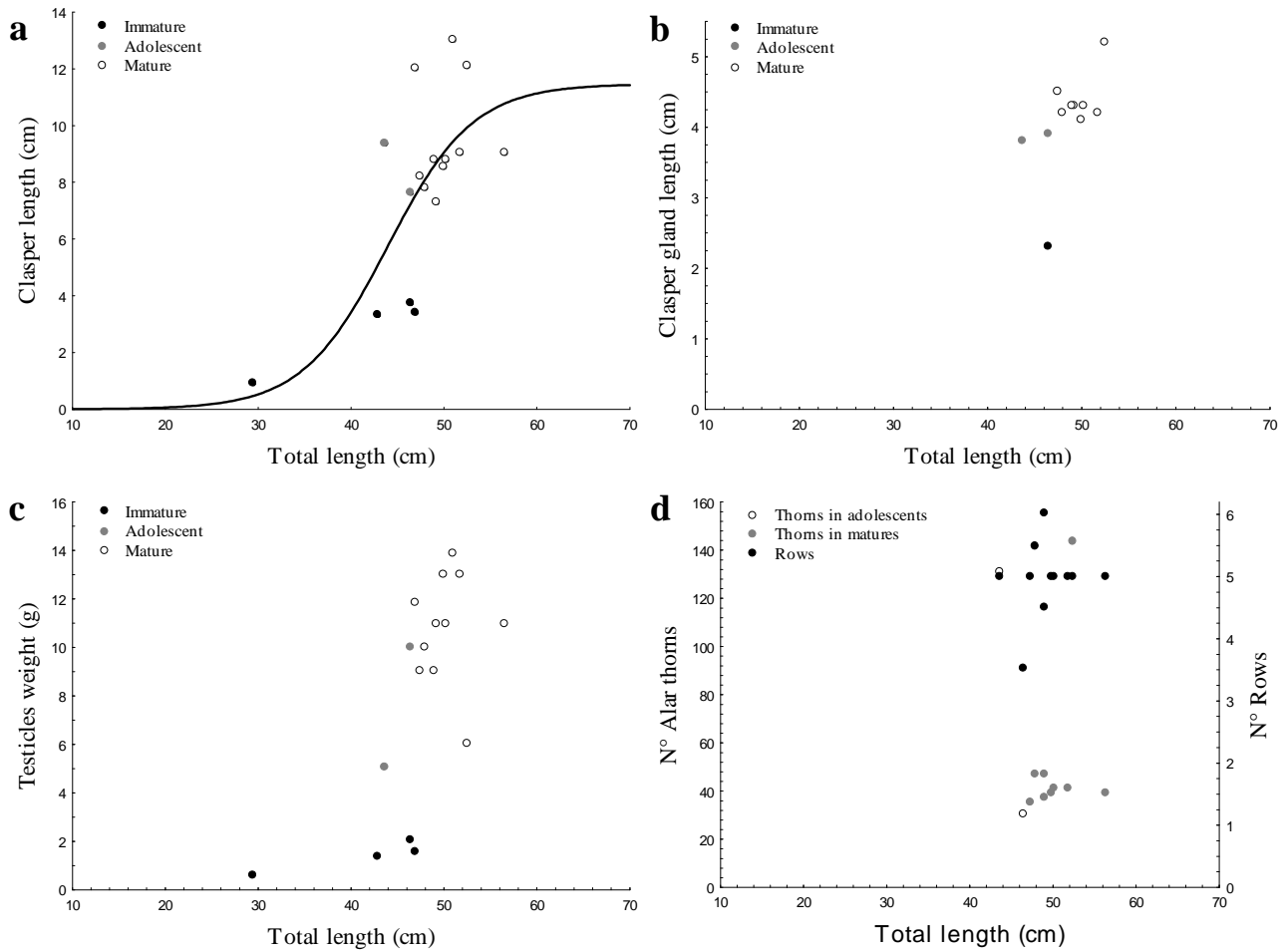


Fig. 5. *Sympterygia acuta*. Relationship between total length (cm) and (a) clasper length (cm), (b) clasper gland length (cm), (c) testicles weight (g) and (d) number of alar thorns rows and number of rows of alar thorns.

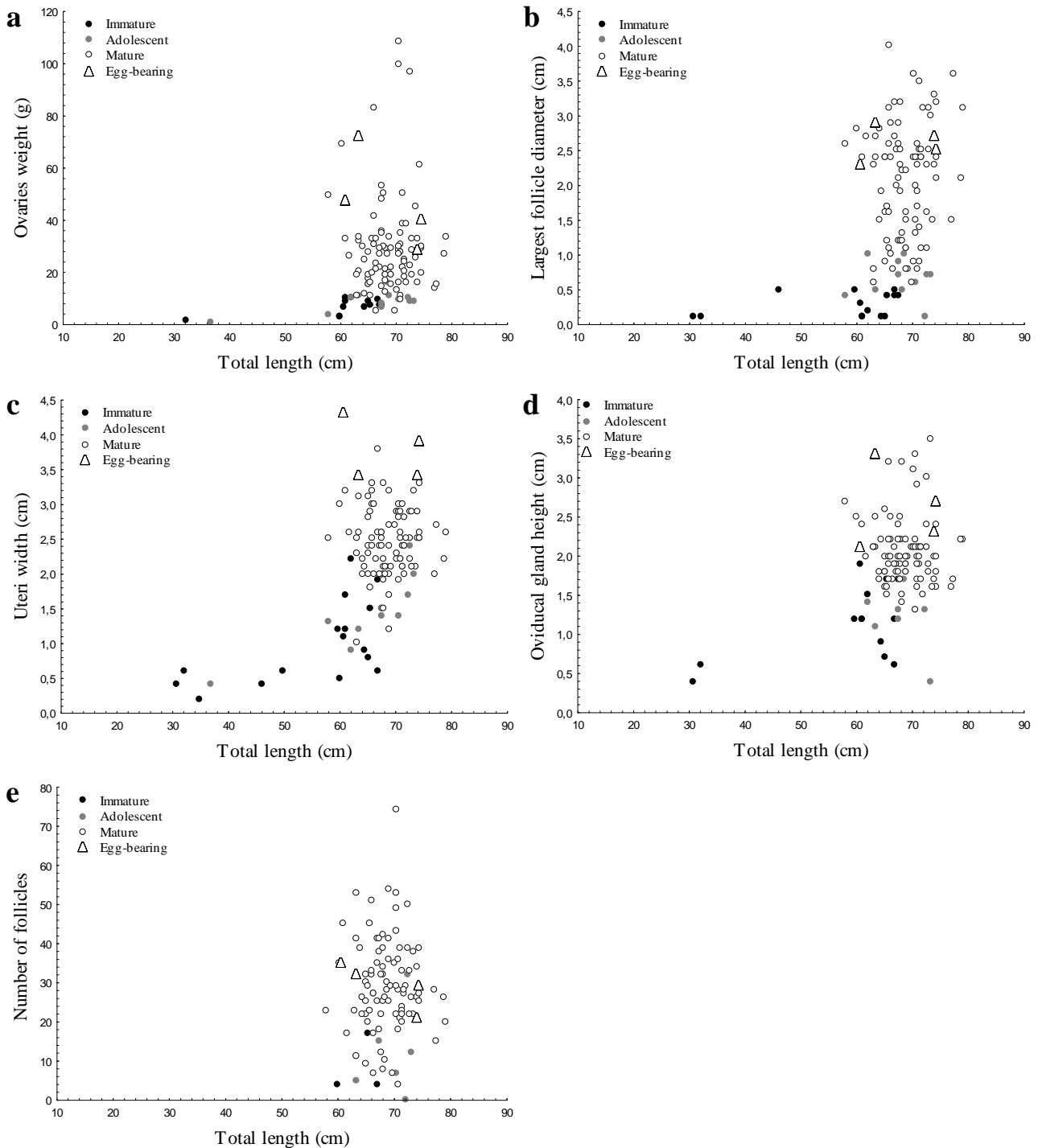


Fig. 6. Sexual development for females of *Sympterygia bonapartii*. Relationship between total length (cm) and (a) ovaries weight (g), (b) diameter of the largest follicle (cm), (c) uteri width (cm), (d) oviducal gland height (cm) and (e) number of follicles.

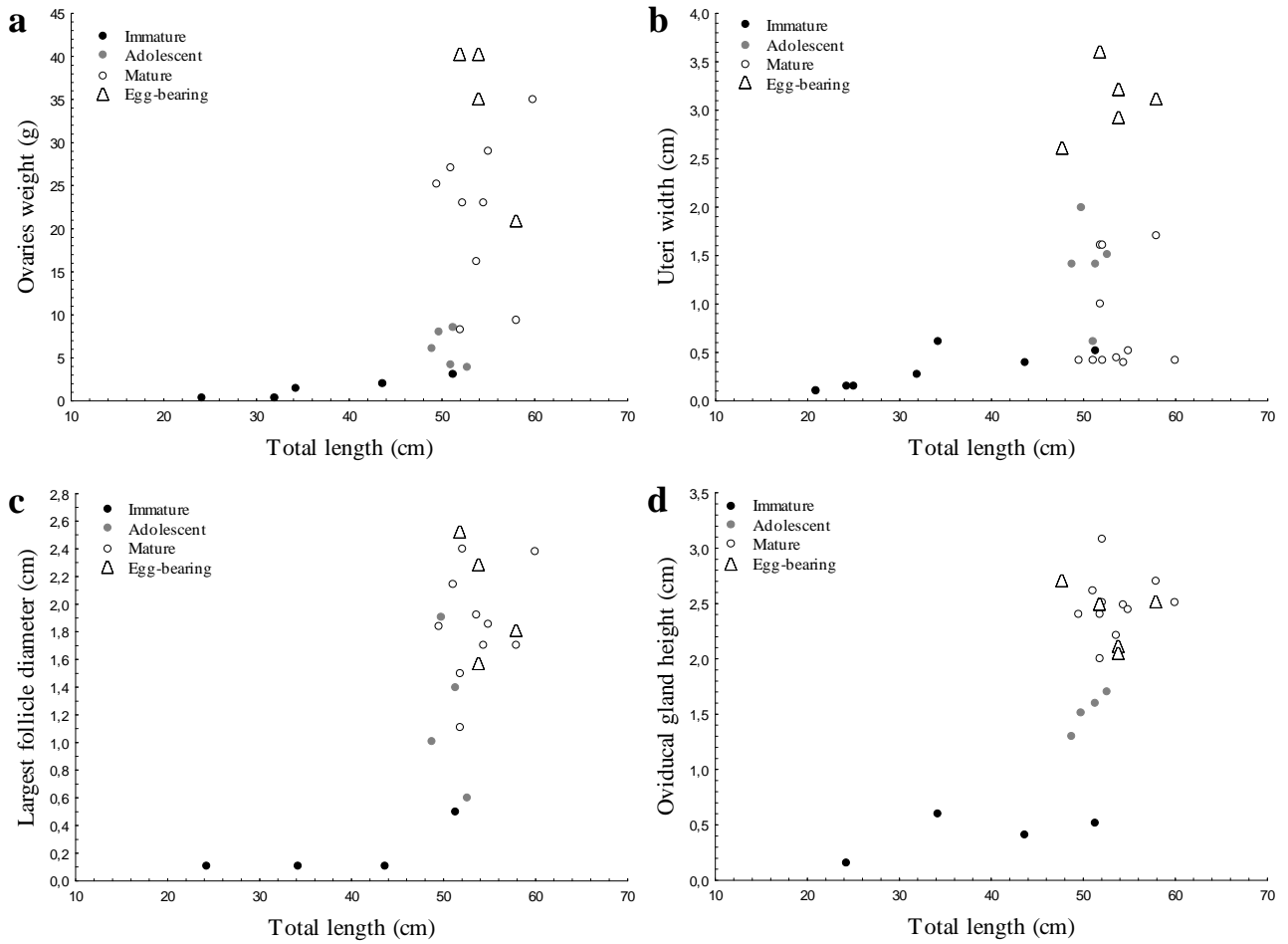


Fig. 7. Sexual development for females of *Sympterygia acuta*. Relationship between total length (cm) and (a) ovaries weight, (b) uteri width (cm), (c) diameter of the largest follicle (cm) and (d) oviducal gland height (cm).

7.2 Artigo 2. Histology of the testicles and male reproductive tract of the skates *Sympterygia acuta* Garman, 1877 and *S. bonapartii* Müller & Henle, 1841 (Chondrichthyes: Rajoidei) in the western south Atlantic Ocean

HISTOLOGY OF THE TESTICLES AND MALE REPRODUCTIVE TRACT OF THE SKATES *SYMPTERYGIA ACUTA* GARMAN, 1877 AND *S. BONAPARTII* MÜLLER & HENLE, 1841 (CHONDRICHTHYES: RAJOIDEI) IN THE WESTERN SOUTH ATLANTIC OCEAN

Alexander Basallo¹, Antonio Sergio Jr. Varela² and Maria Cristina Oddone³

¹ Universidade Federal do Rio Grande, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Oceanografia Biológica, Av. Itália, Km 8 s/n, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil, alexbiology8@hotmail.com

^{2,3} Universidade Federal do Rio Grande, Instituto de Ciências Biológicas, Laboratório de Histologia. Av. Itália, Km 8 s/n, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil.

ABSTRACT

The male reproductive tract in chondrichthyan is composed of testicles, including epigonal organ, and reproductive ducts. The latter include efferent ducts, epididymis, Leydig gland, deferent duct and seminal vesicle. These structures perform different functions related to male reproduction. The skates *S. acuta* and *S. bonapartii* are endemic to the south-western Atlantic Ocean. Specimens were obtained from research cruises and fishing trips carried out throughout 2011 and 2012. The fishing trawls occurred in the area between 34° 28'S and 31° 29'S, southern Brazil, at depths between

15 and 142 m. The reproductive tracts were fixed in 10% formalin and preserved in 70% alcohol. Histological techniques were applied to optical microscopy, using staining with HE, PAS and AB pH 1.0 and 2.5, with tissue sections of 6 μm . Histological stages of spermatogenesis were described in both species, providing macro and micro-anatomical descriptions of the different reproductive structures. In addition, the morphological TL₅₀ (total length at 50% maturity) was compared with the histological TL₅₀, demonstrating that the latter corresponded to lower TL values. This fact is probably related to a delay in the spermatogenic activity during sexual development in the males *S. acuta* and *S. bonapartii* in relation to the establishment of macroscopical reproductive structures that reveal sexual maturity.

RESUMO

O aparelho reprodutor masculino em condriotes é composto por os testículos, incluindo órgão epigonal e dutos reprodutivos. Estes últimos incluem dutos eferentes, epidídimo, glândula de Leydig, ducto deferente e vesícula seminal. Estas estruturas desempenham diferentes funções relacionadas à reprodução nos machos. As raias *S. acuta* e *S. bonapartii* são endêmicas do Oceano Atlântico Sul - ocidental. As amostras foram obtidas a partir de cruzeiros de pesquisa e viagens de pesca realizadas ao longo de 2011 e 2012. A pesca com redes de arrasto ocorreu na área entre 34° 28'S e 31° 29'S, sul do Brasil, em profundidades entre 15 e 142 m. Os tratos reprodutivos foram fixados em formol a 10% e conservados em álcool 70%. Foram aplicadas técnicas histológicas para microscopia óptica, utilizando coloração com HE, PAS e AB pH 1,0 e 2,5, com cortes de tecidos de 6 μm . Foram descritos os estágios histológicos da espermatogênese em ambas as espécies estudadas, proporcionando descrições macro e micro-anatômicas das

diferentes estruturas reprodutivas. Além disso, o CT₅₀ morfológico (comprimento total 50% de maturidade) foi comparado com o CT₅₀ histológico, demonstrando que este último correspondeu valores menores de CT. Este fato provavelmente está relacionado a um atraso na atividade espermatogênica durante o desenvolvimento sexual nos machos de *S. acuta* e *S. bonapartii* em relação ao estabelecimento das estruturas reprodutivas macroscópicas que revelam a maturidade sexual.

Descriptors: Reproduction, Spermatogenesis, Histology, Microanatomy, Sexual development.

Descritores: Reprodução, Espermatogênese, Histologia, Micro-anatomia, Desenvolvimento sexual.

INTRODUCTION

The chondrichthyan fishes include sharks, rays, skates and chimaeras. These cartilaginous fishes first appeared almost 400 million years ago and are characterized by being rather diverse, especially in their reproductive modes (WALKER, 2005). The skates *Sympterygia acuta* (GARMAN, 1877) and *Sympterygia bonapartii* (MÜLLER; HENLE, 1841) are endemic the western-south Atlantic Ocean, being found in coastal waters from Brazil to Argentina (McEACHRAN; ASCHLIMAN, 2004). In South and Southeast Brazil, *S. acuta* and *S. bonapartii* complete their life cycles in inner-shelf waters (VOOREN, 1997; VOOREN et al., 2005).

The testicular structure in Chondrichthyes is composed by testis and epigonal gland. In male Chondrichthyes, some of the principal functions this organ is spermatogenesis and steroidogenesis (WALKER, 2005). The basic testicular unit in Chondrichthyan are structures called spermatocysts (HAMLETT, 1999). The union of

several spermatocysts constitutes a larger physical structure, known as testicular lobule. Three types of histological testicular organization were described in cartilaginous fishes; diameter, radial and compound, the latter being common in batoids, e.g. rays and skates (PRATT, 1988). Compound testes are characterized by a germinal zone (GZ) situated on the ventral testicular surface, from where lobes develop radially and migrate diametrically (HAMLETT, 1999). This type of arrangement can be observed across the width of the testis towards the efferent ducts, final destination of mature spermatozoa (PRATT, 1988; JAMIESON, 2005).

During the sexual development of the male gonads, mature spermatozoa produced within the testis are transported through the genital ducts, i.e., efferent ducts, epididymis, deferent duct and seminal vesicle (CONRATH, 2005; WALKER, 2005). The sperm travels within a rich matrix secretions produced at these sites and in glands annexed, e.g. in Leydig and alkaline gland (WALKER, 2005; JONES; HAMLETT, 2006). The analysis of the testicle to the microscopic level, clearly allows determining the stage of maturity to the individual level, being possible to associate this with the total length of the individual or any other reproductive parameter (MARUSKA et al., 1996).

Histological studies on reproductive organs may help revealing aspects of the physiology and the mechanisms that regulate reproduction in Chondrichthyes. Also, the application of histological techniques performed in reproduction studies allows understanding the different aspects of the life history of these fishes. The analysis of the micro-anatomy of the reproductive tract reveals valuable insights concerning the sexual development of this animal group. NOLAN et al. (2002), for instance, calculated the

histological TL₅₀ in males *Raja montagui* (FOWLER, 1910), based on the gonadal histologic analysis for staging an individual in terms of maturity (apart from the traditional morphological TL₅₀).

Studies of the gonads and reproductive tract in Chondrichthyans in general under a histological perspective, for the west South Atlantic Ocean are scarce, especially for rajoids. The information presented in this paper may contribute to better understand aspects of reproduction in skates. The present study aims analyze and characterize the microanatomy of the gonads and male reproductive tract of the oviparous skates *S. acuta* and *S. bonapartii*.

MATERIAL AND METHODS

Specimens of *S. acuta* and *S. bonapartii* were collected during one-day long research cruises carried out on May, June, July and August 2011 and from two commercial fishing trips that occurred from September 22nd to 30th 2011 and from January 30th to February 10th 2012. The study area was situated between latitudes 34°28'S and 31°29'S, at depths between 15 and 142 m, in southern Brazil. The individuals collected had their total length (TL) (cm) from the snout to the extremity of the tail, the clasper length (CL) and testes weight (TW) (g) recorded. Maturity stages considered (immature, adolescent and mature), criteria and details on maturity staging followed by ODDONE et al. (2007) and size-at-50%-maturity estimates for males of *S. acuta* and *S. bonapartii* are in agreement with BASALLO (unpublished data). Gonadosomatic index (GSI) was calculated according to KING (1995), but considering eviscerated weight instead of total weight (*sensu* PERES; VOOREN, 1991), as $GSI =$

$(W_g / W_e) * 100$, where W_g = gonadal weight (g) and W_e = eviscerated fish weight (g). Values corresponding to GSI were expressed in terms of mean and standard deviation for each maturity stage in both species.

In order to perform the histological analysis, the complete reproductive tract of *S. acuta* and *S. bonapartii* males was removed; fixed in 10% formalin for 24 hours and then preserved in ethanol 70%. The testicle was sectioned in the second quarter of the previous half gonad (ICES, 2010). Pieces of tissue were removed from the upper region of the epididymis (JONES; HAMLETT, 2006). Deferent ducts and seminal vesicle cuts were oriented in coronal section. The nomenclature of the reproductive tract is in agreement with HAMLETT (1999).

Samples of gonads (including the epigonal organ) and reproductive tract were processed for histology using a tissue processor Leica ASP-200. Subsequently, the tissue was embedded in Paraplast Xtra (Sigma P3808) and sectioned to a thickness of 6 μm using an automated Rotary Microtome (Leica RM2255). The histological sections were stained with Hematoxylin and Eosin (HE), the reaction Periodic Acid Schiff (PAS), Alcian Blue (AB) pH 1.0 and 2.5 (CARSON; HLADIK, 2009). Images were acquired through a brightfield microscope Olympus BX 51 with high resolution camera (Olympus DP72). The classification of the histological stages of the spermatogenesis was performed using the parameters proposed by MARUSKA et al. (1996).

The histological TL_{50} was calculated considering individuals that had all stages of spermatogenesis detected in their testicles. The logistic equation $PTL = 1 / (1 - e^{-(a+bTL)})$ was applied to the proportion of mature individuals (PTL) by TL class, where a and b are equation parameters. Histological TL_{50} value was compared with the morphological

TL₅₀ value (calculated for this same skate sample) such as described by NOLAN et al. (2002).

RESULTS

Anatomy and Histology of Male Gonads

The testes of both species *S. acuta* and *S. bonapartii* showed similar characteristics in their anatomical macroscopical structure. Both testes were perfectly symmetrical and functional, with lobular shape, located dorsally within the peritoneal cavity (Fig. 1). The male gonads are formed by the epigonal organ and testis in intimate association, indicating the high vascularisation of this tissue. Macroscopical analyses of the individuals classified as immature in both species based on the morphology showed small testicular lobules in size and pale coloration. Ducts in these specimens had still no differentiation. The gonads were constituted principally by the epigonal organ (EO) and germinal testicular tissue. The later showed a highly vascularized tissue in mature males (Fig. 1). The EO was conformed mostly by granular cells (leucocytes) and eosinophilic granulocytes (Fig. 2a). In the middle region of the gonadal tissue, ducts with simple cubic epithelium and no secretory activity were detected, corresponding to the efferent duct (ED). Positive reactivity positive for PAS technique was revealed in this region, surrounded by connective tissue an intratesticular secretion of glycoproteins (Fig. 2b).

Microscopic observations showed that the ratio EO/germinal testicular tissue gradually and considerably diminished with the maturation progress (Fig. 2c). Also, males classified as adolescents and mature for both species analyzed, had a reduced EO with respect to the germinal testicular tissue, macroscopically represented by the presence of the largest lobules situated on the dorsal-anterior testis zone (Fig. 2d). The

mature sperm produced in the testicles is carried around in the spermatogenic matrix through the efferent ducts passing later through the epididymis, Leydig gland, deferens duct and finally stored in seminal vesicle (Fig. 1).

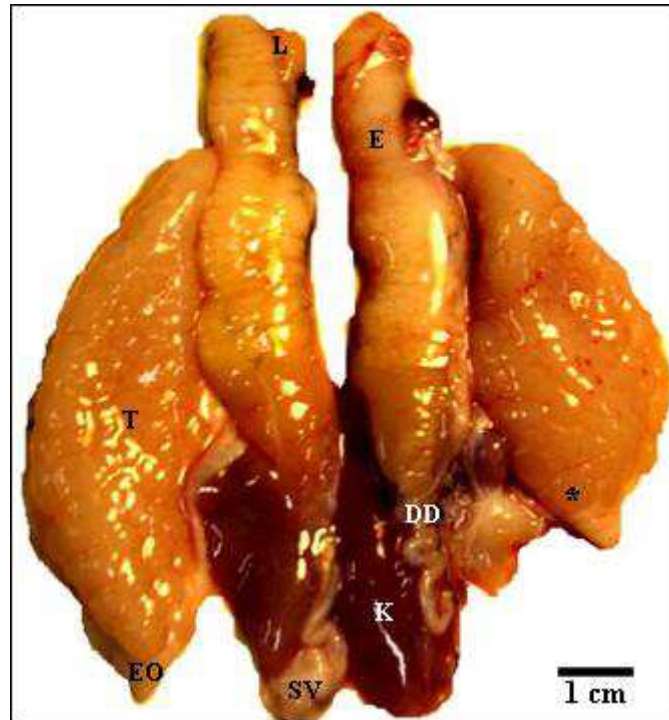


Fig. 1. Ventral view of the testes and complete male reproductive tract of a mature male of *Sympterygia acuta* showing the testes (T) connected to the epigonal organ (EO), the asterisk points one singular testicular lobule. The male reproductive tract initiates with efferent ducts (not visible in the figure), followed by epididymis (E) and Leydig gland (L) and deferens duct (DD), converging in the seminal vesicle (SV). In the posterior portion of these ducts the kidneys (K) can be seen.

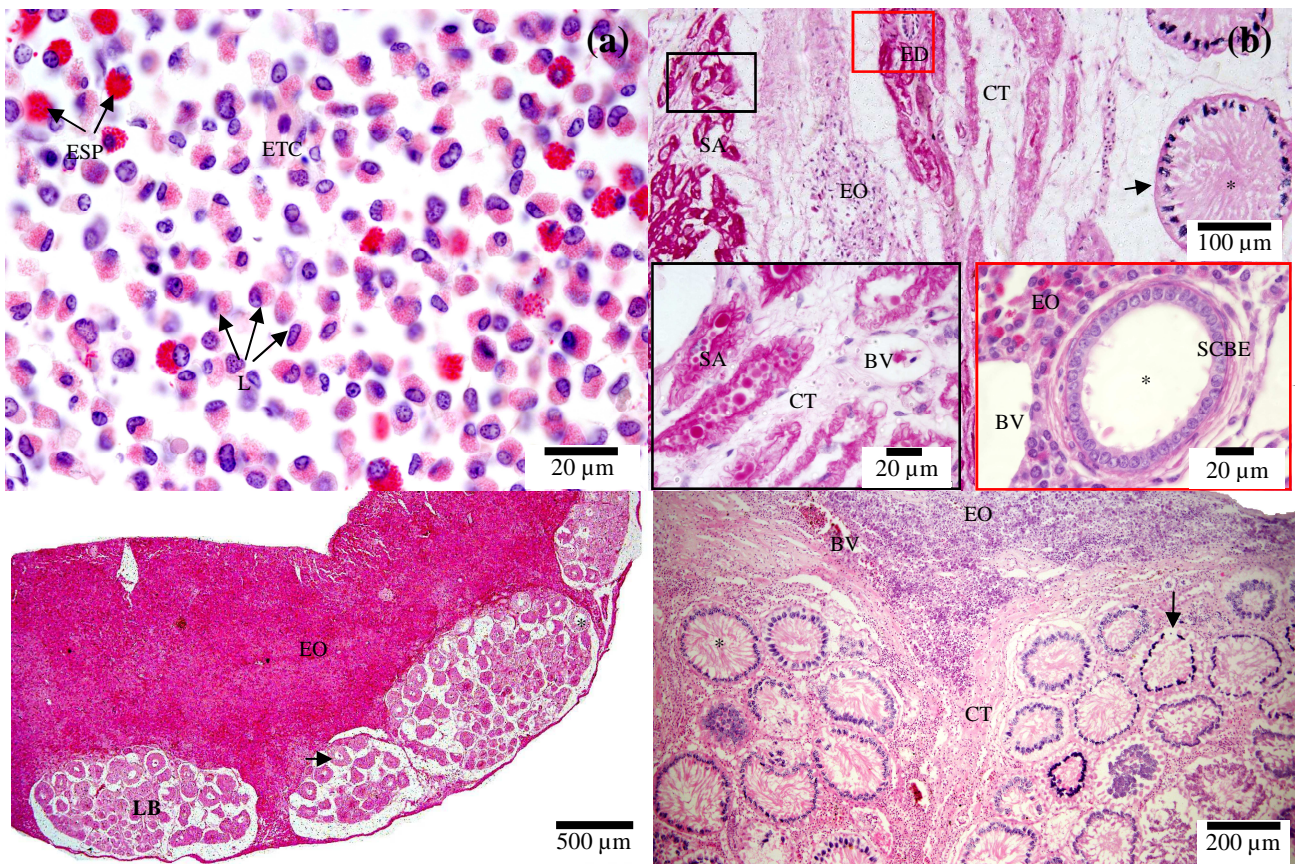


Fig. 2. Cross sections of gonads of *S. acuta* (a; c) and *S. bonapartii* (b; d); (a) epigonal organ (EO) with lymphomyeloid tissue, arrows indicate eosinophils granulocytes (ESP) and lymphocytes (L) and a single erythrocyte (ETC); (b) testis and EO, the black square shows a detail of the testicle with duct secretory activity (SA), along with connective tissue (CT) and blood vessels (BV); red square shows a detail of efferent duct (ED) without secretory activity, composed of simple cubic epithelium (SCBE), arrow showing spermatocyst and the asterisk represents the lumen; (c) gonads of an immature individual with clustered spermatocysts within a lobule (LB); (d) gonad of a sexually mature individual. Figures (a-c) stained with PAS and (d) stained with HE.

Stages of Spermatogenesis

In a cross section of testis in mature male *S. acuta* and *S. bonapartii*, it was possible to see differentiated spermatocysts, indicating the progression of spermatogenesis (Fig. 3a). Differences were observed in the stages of spermatogenesis for each proposed stage of maturity in both species studied. Immature males of *S. acuta* presented the stages of spermatogenesis stages SI and SII. Adolescents and mature males showed all stages of spermatogenesis from SI to SVII. While the testis of immature males of *S. bonapartii* showed a predominance of stages SI and SII, some immature showed transitional stages between phases SI-SVI. Adolescent males had testes ranging from phase SII to SVII, with predominance of stages SIV and SVI. In mature individuals all stages of spermatogenesis were present, from SI to SVII. In addition, a predominance of stages SVI and SVII were observed in the gonads of mature individuals (Fig. 3a).

Stage I (SI)

In this initial phase, the spermatogenesis is characterized by the presence of dispersed germinal cells, forming a loose tissue without the delimitation of a membrane. These cells indicated that beginning spermatogenesis is irradiated diametrically. Small germinal cells with a grouping tendency were also observed. The Sertoli cells were observed in association with the germinal cell. Subsequently, spermatocysts in early development stage with spermatogonias presenting a large nucleus were formed (Fig. 3b).

Stage II (SII)

Spermatogonias result from the consecutive mitotic divisions of the germinal cells. At this stage, the spermatocysts presented internally a layer of spermatogonias with Sertoli cells migrating peripherally, arranged around the central lumen, with basement membrane delimiting these spermatocysts (Fig. 3c).

Stage III (SIII)

After undergoing mitotic divisions, the spermatogonias convert to primary spermatocytes. This includes a reduction of genetic material through the first meiotic division giving rise to secondary spermatocytes. The spermatocysts contains voluminous spherical cells, with large nuclei compared to previous stages (Fig. 3d).

Stage IV (SIV)

This phase is characterized by the presence of spermatids due to the second meiotic division by the secondary spermatocytes. Morphologically, the spermatids have a small cell body, round nuclei. There was a high density of spermatids occupying a large area of the spermatocyst, evidencing a lumen reduction in some cases (Fig. 3e).

Stage V

The appearance of spermatids in the previous stage marked the beginning of the spermiogenesis. During spermatids maturation, Sertoli cells migrate toward the periphery delimiting the spermatocysts, allowing in subsequent stages a radial growth of spermatozoa. The immature sperm that is formed in this phase was found in a disorganized form (Fig. 3f).

Stage VI (SVI)

The mature spermatozoa are organized in packages, circling the peripherically spermatocysts beside the Sertoli cells. The heads of the mature sperm or

spermatozougmata present a typical spiral shape, oriented toward the periphery of spermatocyst. A broad lumen communicates mature sperm toward the collecting ducts where it subsequently is communicated with the efferent vessels (Fig. 2b). Particles PAS + secretion in nature glycoprotein were observed in this stage, being observed near the EO ducts containing these same substances (Fig. 3g).

Stage VII (SVII)

The spermatocysts suffer deformation at this stage, adopting flattened forms; with slight or no sperm. The collapse of the spermatocysts caused a displacement of Sertoli cells from their initial position, being dispersed and being particularly visible. Spermatocysts in this stage were observed evacuated and in process of degradation (Fig. 3h), indicating the end of spermatogenesis.

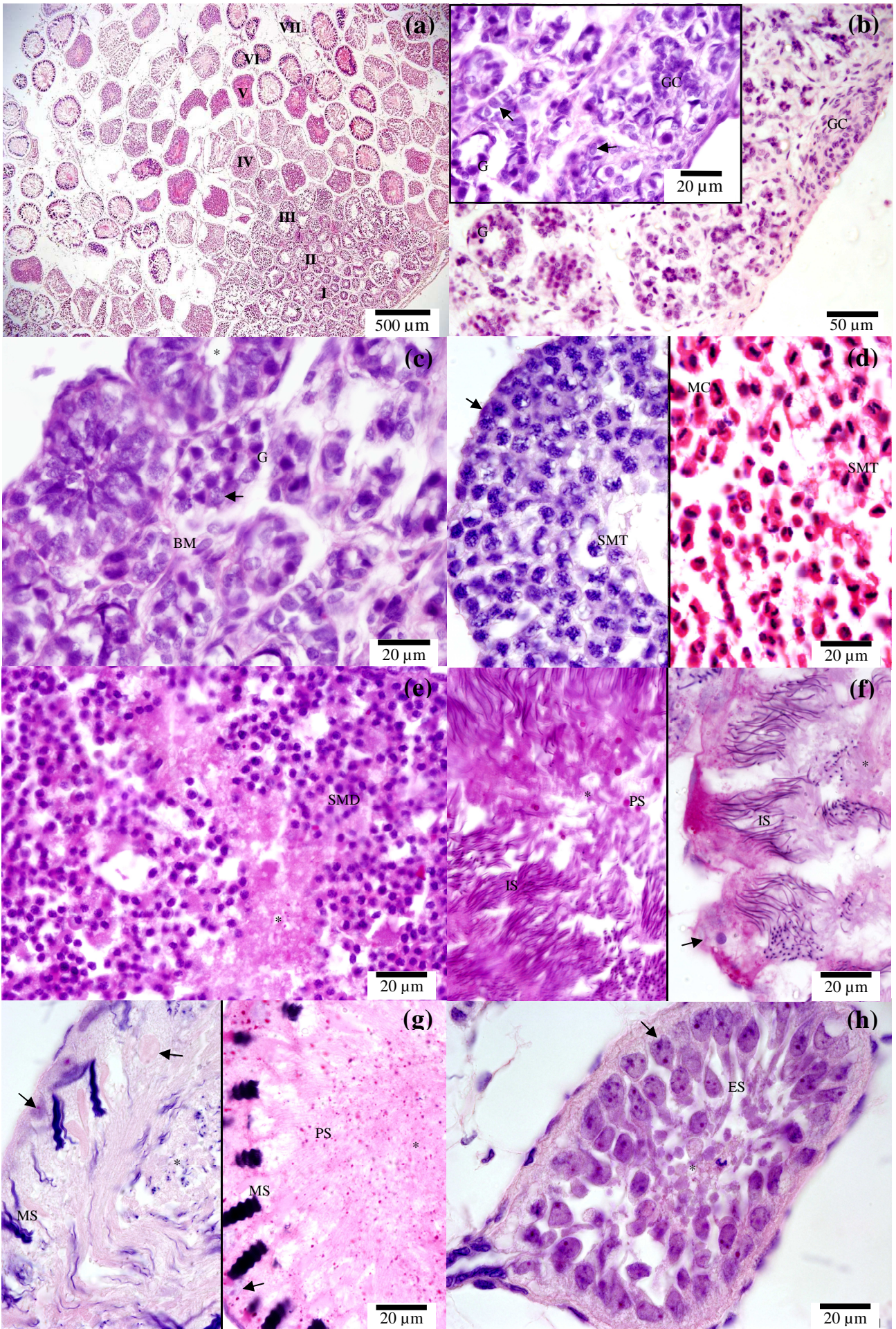


Fig. 3. Spermatogenesis in *S.acuta* (a-d) and *S. bonapartii* (e-h). Testis in cross-section; (a) progression of diametric stages of spermatogenesis, stained with HE; (b) Stage I: indicating in detail germinal cells (GC), spermatogonia (G) next to Sertoli cells indicated by the arrow, stained with HE; (c) Stage II: spermatogonias with large nucleus, besides basement membrane (BM) at the peripheric spermatocysts stained with AB pH 2.5; (d) Stage III: formed by spermatocytes (SMT) with Sertoli of cells migrating to the periphery, mitotic cells (MC) are also observed, right micrograph stained with AB pH 1.0 and left stained with PAS; (e) Stage IV: spermatids (SMD) with small round nuclei, stained with PAS; (f) Stage V: immature sperm (IS) within the disorganized spermatocyst, showing that particle secretion (PS), stained with PAS; (g) Stage VI: mature spermatozoa (MS) packaged in the periphery, on the left micrograph the evacuation of sperm into the lumen is indicated, stained with HE, On right micrograph indicated a strong presence of particle secretion (PS) is indicated, stained with PAS; (h) Stage VII: spermatocyst in degradation with large amounts of Sertoli cells (arrow), without presence of spermatozoa, stained with AB pH 2.5.

Epididymis

Anatomically, the epididymis is comprised of a coiled and compressed tubule, showing an elongated shape, dorsoventrally flattened, attached to the dorsal wall of the abdominal cavity (Fig. 1). The epididymis was externally covered by a thin membrane formed by connective tissue.

The males of *S. acuta* and *S. bonapartii* were histologically similar in relation to the micro-anatomy of the epididymis. In cross section, the epididymis demonstrated to

be a duct lined by a ciliated simple columnar epithelium. This tissue bore cells with secretory activity, characterized by a basement membrane positive with PAS; AB pH 1.0 and 2.5 (Fig. 4). In immature males, the epididymis appeared as a practically closed duct, with a pretty small lumen and with no sperm (Fig. 4a). Paradoxically, mature individuals had a large and vascularised epididymis with abundant amounts of seminal matrix within this cavity, forming a dense fluid with clusters of sperm in form of spermatozeugmata (Fig. 4b). Also the glycoprotein particles PAS positive similar to those found within mature spermatocysts stage VI in the testis, were present integrating the seminal matrix.

The Leydig Gland

Macroscopically in *S. acuta* and *S. bonapartii* the Leydig gland is located in the posterior region of the epididymis in ventral view. From the histological point of view, in a cross-section, the tissue of the Leydig gland was observed lining dorsally to the area of epididymis. This tissue was characterised by the presence of a simple ciliated columnar epithelium PAS +. This epithelium showed secretory cells characterised by a light supranuclear (LS); also cell with nucleus near the apex (Fig. 4c). Tubules of the Leydig gland in immature males showed scanty or absent secretory activity of the epithelium; although there was strong reactivity of the basement membrane, evidenced with the staining PAS and AB (Fig. 4d).

Ductus Deferens

The anterior portion of the ductus deferens is located immediately after the epididymis. This ductus appears forming a spiraled structure visible to the naked eye (Fig. 1). In mature males of *S. acuta* and *S. bonapartii*, the ductus deferens showed a

duct lined by a columnar epithelium simple. Within this duct the presence of a seminal matrix with spermatozeugmata PAS+ was detected. On its lateral sides the deferent duct inserts itself into the surrounding connective tissue, forming simple tubular glands highly positive with PAS, AB 1.0 and 2.5 pH. These glands were composed by a simple cuboidal epithelium with goblet cells, indicating that the secretion of this epithelium was associated with sperm inside the ducts.

Seminal Vesicle

The seminal vesicle is formed by a ciliated simple columnar epithelium, characterized by the high amount of seminal matrix, conformed by clusters of sperm or spermatozeugmata, eosinophils particles and PAS+ secretions. Willing sideways observed the glandular tissue similar to the findings in the deferent ducts, containing inside these tubules large amounts of secretions of sulfated and carboxylated acid mucopolysaccharides (AB pH 1.0 and 2.5 +) additionally proved whether secretion of glycoproteins and neutral substances with (PAS +).

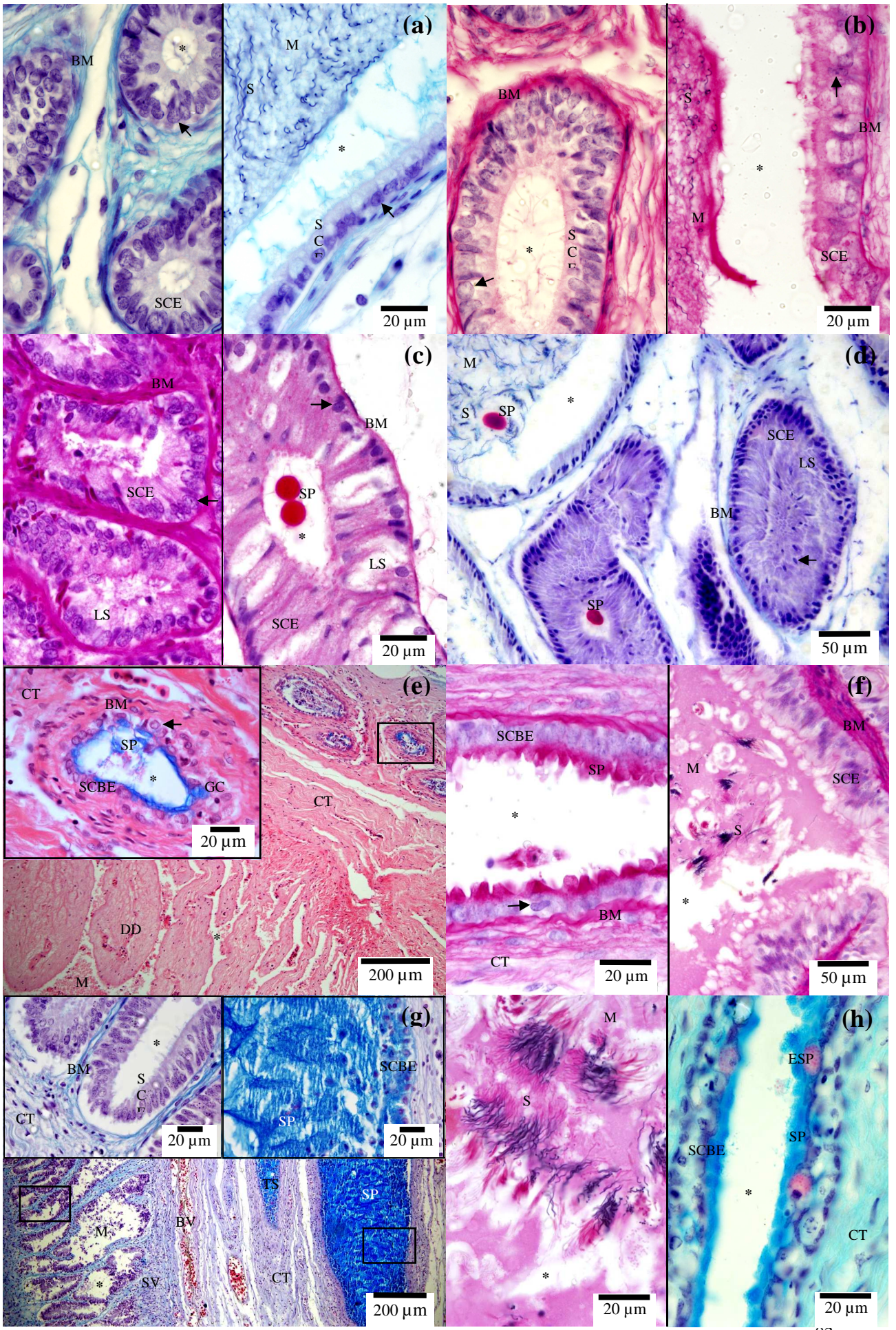


Fig. 4. Male reproductive tract of *S. acuta* (a, b, c, f, h) and *S. bonapartii* (d, e, g); (a) left epididymis of an immature individual, showing a duct with simple columnar epithelium (SCE), the arrow indicates the cells nucleus, basement membrane (BM) and the small empty lumen (asterisk), on the right side, a tubule of the epididymis of a mature individual is indicated, showing a matrix (M) with spermatozeugmata (S) staining with AB pH 2.5; (b) presence of glycoproteins in the matrix (M) and SCE, verified with PAS staining applied in epididymis of immature and mature males, on the left and right respectively; (c) Leydig gland with simple columnar epithelium (SCE) with supranuclear light (LS), basement membrane (BM) of the epithelium together with basal nucleus (arrow), production of secretions (SP) accumulated in the lumen; stained with PAS . (d) Leydig gland contributing with secretory products (SP) in the proximity of epididymis, arrow indicates apical nuclei; staining AB pH 2.5; (e) dutus deferens (DD) with simple columnar epithelium (SCE) and matrix (M); square in black color indicates detail of glandular ducts annexes, composed of simple cubic epithelium (SCBE) and goblet cells (GC), stained with AB pH1.0 and 2.5. (f) On the left ducts annexed to DD with production of secretions (SP) PAS positive, in the right ductus deferens with SCE and a matrix with clusters of spermatozeugmata; (g) detail of the seminal vesicle (square on the upper left) composed of a simple columnar epithelium (SCE) and basement membrane (BM), inside the seminal vesicle there is a matrix with spermatozeugmata; the square on top on the right indicates large secretory tubules (TS), abundant secretory products (SP), rich in mucopolysaccharides acids, sulfated and carboxylated, evidenced by the AB pH 1.0 and 2.5 staining and (h) clusters of spermatozeugmata (micrograph on the left), stained with PAS, on the right, a secretory

tubule containing granulocytes eosinophils (ESP) and secretory products (SP) AB positive. Figures (a and b) correspond to as much immature and mature individuals; figures (c-h) correspond to mature individuals.

Comparison of the Morphological and Histological Size-at-50%-Maturity (TL₅₀)

Values of morphometrical TL₅₀ for male *S. acuta* and *S. bonapartii* were 46.1 cm (R = 0.99; n = 17) and 58.4 cm (R = 0.98, n = 92), respectively (Fig. 5). However, histological TL₅₀ considering the relative proportion of spermatocysts containing mature sperm (Fig. 5b, d) was estimated to lie in 44.7 cm (R= 0.99, n= 16) and 54.68 cm (R= 0.98 n= 79) for *S. acuta* and *S. bonapartii* respectively (Fig. 5).

The histological method considers males that have completed all stages of spermatogenesis, the histological TL₅₀ in both species analyzed was lower than the morphological TL₅₀ (Fig. 5).

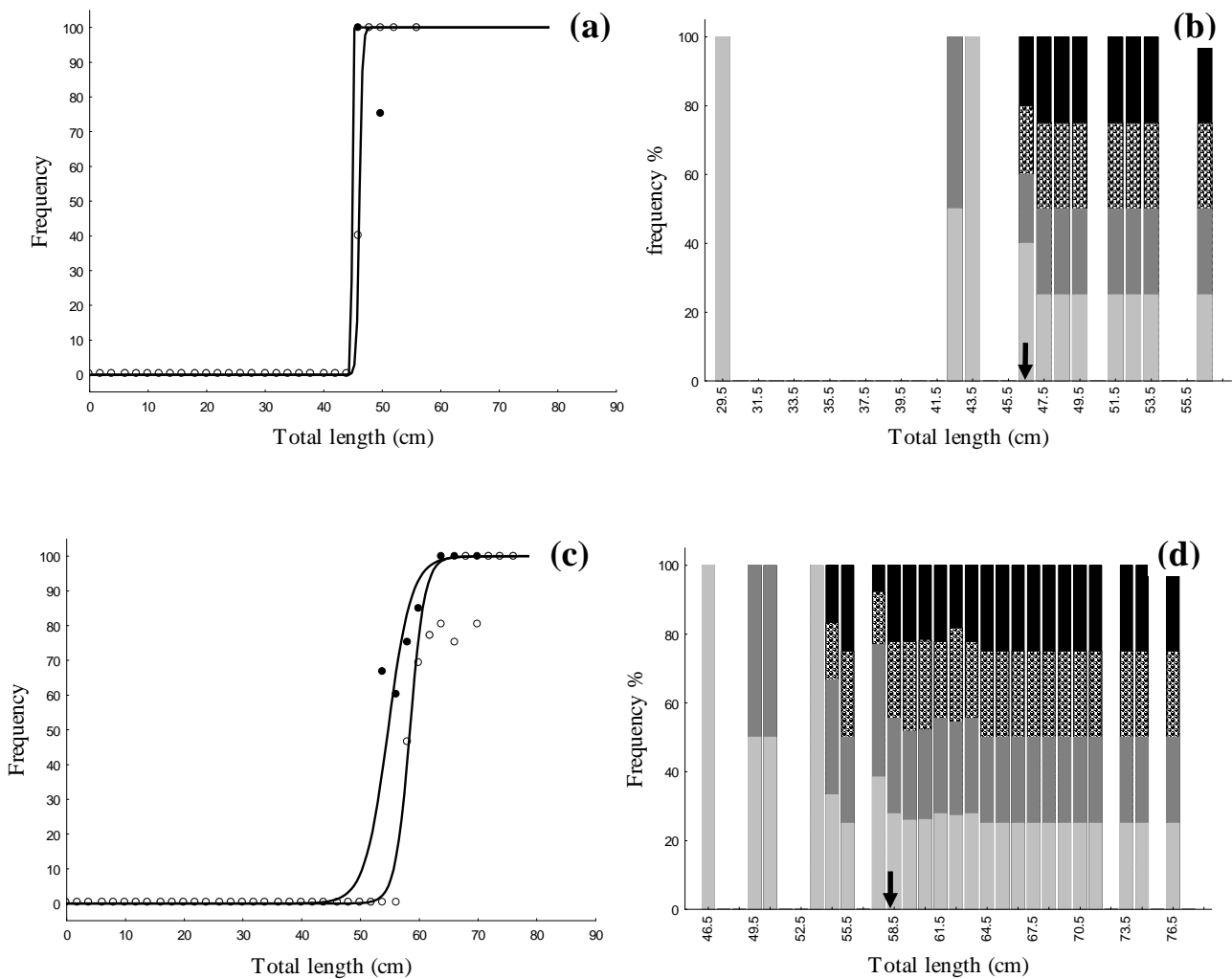


Fig 5. Total length-at-50%-maturity estimates for *Sympterygia acuta* (a) and *Sympterygia bonapartii* (c): percentage of mature males by total length, empty symbols represent those cases when maturity was assessed through the traditional method based on morphological characteristics of the reproductive tract and gonads; bold circles represent histological TL_{50} proposed by NOLAN et al. (2002). The relative abundance of the stages of spermatogenesis detected [(spermatogonia (light gray), spermatocyt (dark gray), spermatids (black dots) and spermatozoa (black))] and the total length (cm) for *S. acuta* (b) and *S. bonapartii* (d). The arrow indicated the size corresponding to the morphological TL_{50} .

Gonadosomatic Index and Morphometric Relationships of male *S. acuta* and *S. bonapartii*

For immature males of *S. acuta* with TLs between 29.5 and 47.0 cm, GSI values ranged between 0.39 and 0.52 (mean=0.46; SD=0.05; n=4). Those TLs between 43.0 and 46.0 cm corresponded to adolescents specimens, for which GSI ranged from 1.17 to 2.31 (mean=1.74; SD=0.81; n=2). In mature specimens between 47.0 and 56.0 cm GSI varied between 0.93 and 2.68 (mean=1.90; SD=0.49; n=11) (Fig. 6a).

For *S. bonapartii* immature individuals with TLs between 47.2 and 63.5 cm corresponded to GSI varying from 0.18 to 1.81 (mean=1.02; SD=0.53; n=14). Adolescents with TLs between 55.0 and 71.6 cm had GSI ranging from 0.74 to 2.60 (mean=1.66; SD=0.48; n=19). Mature, on the other hand, with TLs between 58.6 and 76.8 cm had GSI values varying from 0.76 to 1.87 cm (mean=1.30; SD=0.28; n=59) (Fig. 6c). The highest values of GSI in males *S. bonapartii* corresponded to adolescent individuals (Fig. 6c-d). Clasper length values showed differences among the mean values for each stage of maturity in both species (Fig. 6b,d).

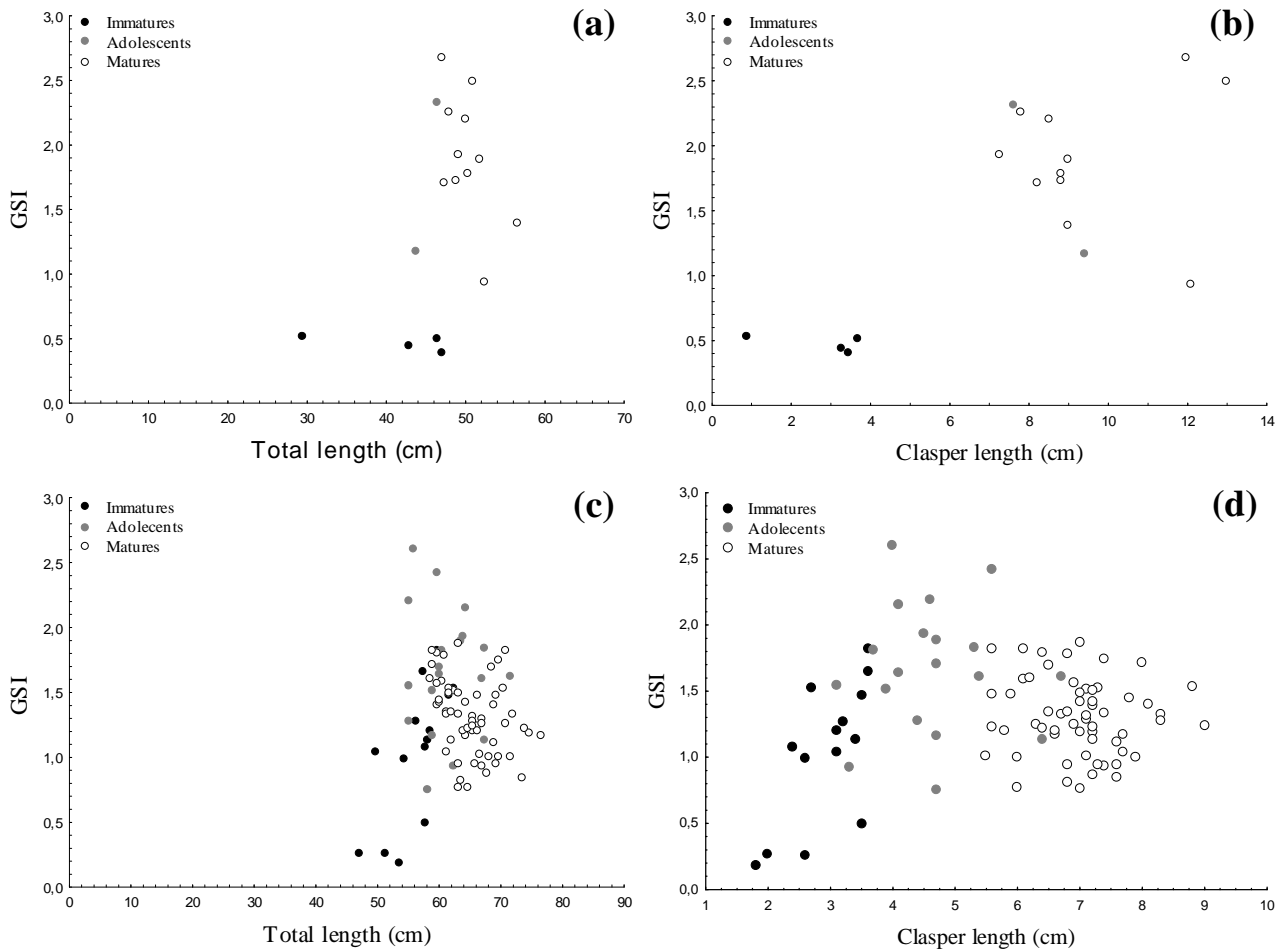


Fig. 6. *Sympterygia acuta*. Relationship between gonadosomatic index and (a) total length (cm), (b) clasper length (cm). *Sympterygia bonapartii* (c) and (d).

DISCUSSION

Mature males of *S. acuta* and *S. bonapartii* showed compound testicular organization, with lobules developing from germinal zones irradiating diametrically when viewed in cross section, being consistent with the classification of PRATT (1988) for elasmobranch gonads. The EO in both species analyzed showed the typical lymphomyeloid tissue of this organ, which controls functions and processes related with hemopoiesis and immune system. The EO is composed of eosinophils granulocytes cells, such as described for batoids by HINE and WAIN (1987), with lymphocytes and

erythrocytes in the case of the rajoid *Raja eglanteria* (BOSC, 1802) (WALSH; LUER, 1998), being these cells characterized in other elasmobranchs by WALSH and LUER (2004).

Changes in the degree of differentiation of testicular tissue were found in the stages of spermatogenesis during the sexual development in males of *S. acuta* and *S. bonapartii*. Other studies in skates indicated changes in the phases of spermatogenesis associated with maturation stages used in *Sympterygia* spp., in *Raja clavata* (LINNAEUS, 1758) for instance (SERRA-PEREIRA et al., 2011). Such differentiation of spermatogenesis stages during sexual development were observed in *Leucoraja wallacei* (HULLEY, 1970) and *Dipturus pullopunctatus* (SMITH, 1964) (WALMSLEY-HART et al., 1999), *Leucoraja ocellata* (MITCHILL, 1815) (SULIKOWSKI et al., 2005). Histological observations on testis of *Urobatis halleri* (COOPER, 1863) were studied by MULL et al. (2008) associated with changes in spermatogenesis during reproductive development.

Histological differences were found between the epididymis of *S. acuta* and *S. bonapartii* during the sexual development. However, the micro-anatomical characteristics recorded in both species may be used as a reference parameter in identifying the maturity of stage. The epididymis had the presence of seminal fluid with particles of glycoproteins (PAS +), accompanied by clusters of spermatozeugmata in both species. These secretions were also detected by HAMLETT (1999) in the epididymis of *Leucoraja erinacea* (MITCHILL, 1825). Studies on the oviparous shark *Heterodontus portusjacksoni* (MEYER, 1793) showed a relation of these secretions

with the increase of concentration of protein in fluid the lumen of the deferens duct (JONES; LIN, 1993).

The Leydig gland in mature males of *S. acuta* and *S. bonapartii* the production of PAS + secretions that form masses of material accumulated in the glandular lumen. These same secretions were demonstrated to be produced in the Leydig gland of the skate *Leucoraja erinacea* (HAMLETT, 1999; JONES; HAMLETT, 2006). The nature of the secretions produced by the Leydig gland was associated with that of the particles found in the seminal fluid of the epididymis and in other parts of the male reproductive tract in deferens duct and seminal vesicle of *S. acuta* and *S. bonapartii*.

The secretory tubules observed in the deferent duct and seminal vesicle, located laterally, possibly provide elements related to the maintenance and storage of sperm in the seminal vesicle before the copula. The histochemical studies made by JONES and HAMLETT (2002) described processes in glycosylation during the production of secretions along the genital tract, being important for the maturation and transport of sperm in *Leucoraja erinacea*.

The histochemical analysis performed in each duct of genital tract of *S. acuta* and *S. bonapartii* are in agreement with histological descriptions carried out in other species of skates (HAMLETT, 1999; JONES; HAMLETT, 2002, 2006; SERRA-PEREIRA et al. 2011), in the oviparous shark *Heterodontus portusjacksoni* (JONES et al., 1984) the viviparous shark *Centroscymnus coelolepis* (BOCAGE; CAPELLO, 1864) (MOURA et al., 2011) and the chimera *Callorhynchus milii* (VINCENT, 1823) (HAMLETT et al., 2002). This was particularly valid in terms of the internal epithelia,

type of cells present, tissue reactivity using the stains PAS and AB and presence of spermatozeugmata in the ducts.

Different methodologies are commonly used to assess the maturity in chondrichthyes (WALKER, 2005). In the present study, two techniques for the assessment of maturity in elasmobranches were compared for *S. acuta* and *S. bonapartii*; the morphological and the histological TL₅₀. From this comparison, it was possible to establish differences between these two techniques for the assessment of maturity, particularly for *S. bonapartii*, where the value of histological TL₅₀ was lower than the morphological. This can be explained as a result of the addition of individuals considered as adolescents from the morphological point of view, that actually had mature characteristics when histologically analyzed assessing and confirming the presence of all the stages of spermatogenesis.

Further, it was verified that some individuals that had been classified as adolescents through to morphological method and therefore with a small clasper, had on the others high IGS values. In these cases and for both species, it was demonstrated through histology that the testicles bore mature spermatocysts corresponding to stages SV and SVI. However in these males there was no presence of sperm in the seminal ducts. EBERT et al. (2008) observed the same in males of *Raja binoculata* (GIRARD, 1854) and *R. rhina* (JORDAN; GILBERT, 1880), record the clasper being developed later in relation to the spermatocysts in the gonads, a fact also documented by SULIKOWSKI et al. (2005, 2006, 2007), in three species of the Gulf of Maine, *Leucoraja ocellata*, *Amblyraja radiata* (DONOVAN, 1808) and *Malacoraja senta* (GARMAN, 1885).

The presence of spermatozoa associations recorded within the epididymis, deferens duct and seminal vesicle of *S. acuta* and *S. bonapartii* are in agreement with what PRATT and TANAKA (1994) described as spermatozeugmata. The abundance of spermatozoa in different parts of the reproductive tract in both species might be related to events previous or after the copula in mature males (SIERRA-PEREIRA et al., 2011). Further studies on the reproductive cycle are required for better understanding these processes. However, it is well known that skates follow continuous reproduction throughout the year, which may or not include the presence of peaks of reproductive activity (WOURMS, 1977, ODDONE & VELASCO, 2008)

BARONE et al. (2007) showed the presence of resting gonads in terms of gametogenesis and with reduced size in mature males of *Raja asterias* (DELAROCHE, 1809) that had fully developed claspers. These males had fully developed claspers big and drive with reduced gonads. A similar observation was done in *S. bonapartii*, with some individuals had mature gonads but with low weight for the maturity stage considered, what was clearly reflected through the values of GSI. In addition, considering clasper size, these individuals also had fully developed claspers (in terms of size and calcification, gonads with mature spermatocysts and no sperm in the reproductive tracts.

Posterior studies carried out for the species for which resting gonads had been reported allowed including which individual within a different reproductive stage (SERRA-PEREIRA et al. 2011). Histological studies represent an accurate tool for the maturity staging in male chondrichthyan fishes. However, it is necessary to integrate these results

with morphological ones, in order to get a more holistic understanding of the reproduction.

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