

**UNIVERSIDADE FEDERAL DO RIO GRANDE
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**ACANTOCEFALLOSES E NEMATODÍASE GASTROINTESTINAL
EM *ARCTOCEPHALUS AUSTRALIS* (MAMMALIA: PINNIPEDIA):
EXISTE DIFERENÇA ENTRE GÊNERO SEXUAL, CONDIÇÃO
SEXUAL, HISTOPATOLOGIA E SAZONALIDADE?**

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Quando pensamos saber todas as repostas,

vem a vida e muda todas as perguntas.

(Santo Agostinho)

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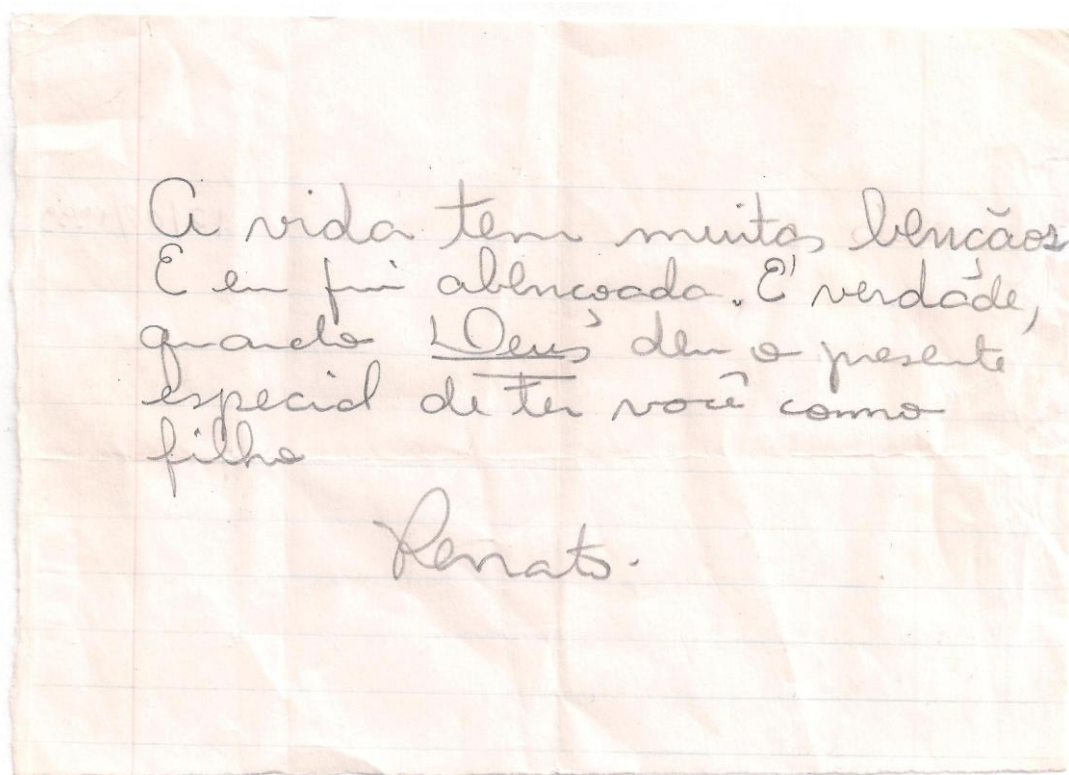
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RESUMO GERAL

A tese analisa as influências do gênero sexual e da condição reprodutiva de *Arctocephalus australis* sobre os índices parasitológicos de algumas helmintíases gastrointestinais, assim como descreve e tipifica as alterações causadas por helmintos do tubo gastrointestinal de hospedeiros encalhados mortos no litoral sul do Brasil. Os índices parasitológicos analisados compreendem a Prevalência (P%) e a Intensidade Média de Infecção (MI) das corinosomíases (*Corynosoma australe*, *C. cetaceum* e *Bolbosoma* sp.) e da contracequíase (*Contracaecum* sp.) considerando os gêneros sexuais e condições reprodutiva dos hospedeiros. Outras variáveis parasitológicas são também avaliadas para a espécie hospedeira, tais como Abundância Média de Infecção (MA), a Amplitude de Infecção (Ax) e o Padrão de Agrupamento (CRD – “Crowding”). A sazonalidade destas helmintíases é analisada entre dois grandes grupos: Primavera-Verão (S-S) e Outono-Inverno (A-W). Variáveis para as lesões da contracequíase são adaptadas daquelas parasitológicas (L-P%, L-MI, L-MA e L-Ax) e analisadas igualmente para os hospedeiros, seus gêneros e condições sexuais. As intensidades de infecção parasitárias (PII) são analisadas para todas as helmintíases com relação ao comprimento dos hospedeiros (TL) machos e fêmeas. As intensidades de lesão (PIL) relacionadas à contracequíase são também analisadas com relação ao TL dos hospedeiros machos e fêmeas. Tecidos gastrointestinais (normal e anormal) e tecido gonadal (masculino e feminino) foram submetidos ao protocolo histológico de rotina para inclusão em parafina e montagem permanente em bálsamo. Tecidos gastrointestinais foram descritos, mensurados sob microscopia e comparados. Tecidos gonadais foram analisados para a condição sexual dos hospedeiros (filhotes, pré-púberes, adultos) de acordo com a gametogênese, luteogênese e luteólise. Lesões por contracequíase foram contadas e mensuradas de acordo com o tipo. *Corynosoma cetaceum* representa uma infecção acidental refletida pelos baixos valores de

P%, MI, MA e Ax, somadas as formas juvenis encontradas. As infecções se iniciam nos hospedeiros ainda filhotes. As PII, nas corinosomíases (por *C. australe* e *Bolbosoma* sp.) alcançam valores mais altos e mais rapidamente nos hospedeiros machos, enquanto que na contracequíase ocorre o contrário. De modo geral, as P% e as MI nos hospedeiros machos são mais elevadas, mas não necessariamente diferentes dos valores das fêmeas. Neste sentido, hospedeiros machos podem ser interpretados como estoques parasitários para *C. australe*, pois demonstram extrema distância entre média e variância para esta parasitose quando comparados aos valores apresentados pelas fêmeas hospedeiras. A bolbosomíase é a única que demonstra diferenças os gêneros sexuais dos hospedeiros. Os valores de MI e MA mostram que *C. australe* infecta predominantemente a metade caudal do intestino delgado, enquanto que *Bolbosoma* sp. infecta predominantemente a metade cranial, constituindo assim uma guilda. Nenhuma helmintíase demonstrou padrões sazonais marcados pela P% e MI, todavia CRD aponta para diferenças entre os agrupamentos sazonais da bolbosomíase. As polimorfíases não causam lesões inflamatórias nos hospedeiros, mas a contracequíase causa cinco tipos de lesão gástrica entre inflamatórias e não-inflamatórias. *Corynosoma australe* apresenta três padrões de ancoragem na mucosa intestinal (diferentes graus de aprofundamento corporal na mucosa intestinal), assim como, cavidades côncavas crateróides (desancoragem). Hospedeiros machos e fêmeas se assemelham no modo quantitativo de aquisição de lesões gástricas por contracequíase, conforme crescem e maturam sexualmente. Das lesões por contracequíase dois tipos não são inflamatórias (locais de penetração na parede estomacal com o parasito parcialmente exposto na luz gástrica e os locais de ancoragem-desancoragem pontual do parasito adulto na superfície mucosa); dois são severamente inflamatórias (úlceras e piogranulomas) e outra compreende cicatrizes (principalmente dos processos inflamatórios). Todavia, todos os tipos de lesão, sejam das polimorfíases ou da contracequíase, causam alterações significativas na estrutura das paredes dos órgãos afetados,

tal como a redução da espessura da mucosa e deformações nas estruturas glandulares estomacais e das criptas intestinais. As lesões inflamatórias são marcadas por severa infiltração imunocelular (macrófagos, células gigantes de corpo estranho, neutrófilos, eosinófilos), necrose e fibrose. Não houve diferença entre machos e fêmeas hospedeiros, assim como entre suas condições reprodutivas para os índices lesionais. Todavia as dimensões em comprimento e largura das úlceras (maiores nos hospedeiros machos) diferiram entre os gêneros sexuais.

PALAVRAS-CHAVE: corinosomíase, bolbosomíase, contracequíase, gênero sexual do hospedeiro, condição sexual do hospedeiro, Arctocephalinae, lobo-marinho-do-sul, histopatologia, guilda.

GENERAL ABSTRACT

The thesis analyzes the influence of gender and reproductive condition of the host on the parasitological indices of some gastrointestinal helminths, as well as describes and typifies the helminth-etiological changes in the gastrointestinal tract of *Arctocephalus australis* stranded dead on the Southern coast of Brazil. The parasitological indices examined include the Prevalence (P%) and Mean Intensity of Infection (MI) of corynosomiasis (*Corynosoma australe*, *C. cetaceum* and *Bolbosoma* sp.) and contracaeciasis (*Contracaecum* sp.) for host's genders and sexual conditions. Other parasitological variables are also evaluated for the host species, such as Mean Abundance of Infection (MA), the Range of Variation (Ax) and the Crowding Pattern (CRD). The seasonality for the helminthiasis is analyzed between two major groups: Spring-Summer (S-S) and Autumn-Winter (A-W). Concerning the contracaeciasis, lesional variables (L-P%, L-MI, L-MA and L-Ax) are adapted from those parasitological and analyzed also for the hosts, their gender and sexual condition. The Parasite Intensities of Infection (PII) are analyzed for all helminthiasis in relation to the male and female host's length (TL). Concerning the contracaeciasis, the Parasite Lesional Intensities (PIL) are analyzed with respect to the male and female host's TL. Gastrointestinal tissues (normal and abnormal) and gonadal tissue (male and female) were submitted to routine histological protocol for embedding in paraffin and permanent mounting in balsam. Gastrointestinal tissues were histologically described, measured and compared. Gonadal tissues were analyzed for the sexual condition of the hosts (young, prepubertal, adult) according to gametogenesis, luteogenesis and luteolysis. Lesions were counted and measured, according to their types. *Corynosoma cetaceum* represents an accidental infection expressed by low values of P%, MI, MA and Ax, added with only juvenile forms founded. The infection begins in the hosts when they are still pups. For corynosomiasis (*C. australe* and *C. cetaceum*), the PII reaches higher and earlier values in the male hosts, whereas the opposite

occurs concerning the contraeciasis. In general, the P% and MI are higher for male hosts but they are not different from the female hosts. In this sense, male host's can be interpreted as parasitic stocks for *C. australe*, because they show extreme distance between mean and variance for this parasitosis, when compared to the values presented by the female hosts. The Bolbosomiasis is the only one to demonstrate host's gender difference in P%. The values of MI and MA show that *C. australe* infects predominantly the caudal half of the small intestine, whereas *Bolbosoma* sp. infects predominantly cranial half, thus forming a guild. No helminthiasis showed marked seasonal patterns of P% and MI, however CRD may explain differences between seasonal crowding for bolbosomiasis. The polymorphiasis do not cause inflammatory lesions in the hosts, but there were five types of gastric contraeciasis-related inflammatory lesions as well as non-inflammatory. *Corynosoma australe* shows three attachment patterns in the intestinal mucosa (degrees of body depth intestinal attachment) as well as crater-like concave holes (detachment). Concerning the number of contraeciasis-related lesions, male and female hosts are similar as they grow and mature. The contraeciasis-related lesions comprise two non-inflammatory types (local embedding of the parasite within the stomach wall with parasite's body partially hanging out toward the stomach lumen and locals of punctual parasite's attachment-detachment on the mucosal surface); two types are severely inflammatory lesions (ulcers and piogranuloma) and other comprises scars (mainly from inflammatory processes). However, all kinds of lesions, that came from the polymorphiasis or from the contraeciasis cause significant alterations in the structure of the wall of the affected organs, such as mucosal thickness reduction and deformations in glandular structures of the gastric and intestinal crypts. The inflammatory lesions are characterized by severe immune cell infiltration (macrophages, multinucleated foreign-giant cells, neutrophils, eosinophils), necrosis and fibrosis. There was no difference between male and female hosts, as well as between their reproductive conditions for lesional

indices. However the length and width of ulcers (higher in male hosts) differed between the genders.

KEY WORDS: corynosomiasis, bolbosomiasis, contracaeciasis, host's gender, host's sexual condition, Arctocephalinae, South American fur seal, histopathology, guild.

PREFÁCIO

O extremo sul do Brasil abriga o Estuário da Lagoa dos Patos que demonstra alta produtividade, influenciando a distribuição e ocorrência de mamíferos marinhos, como cetáceos e pinípedes. O grupo de pinípedes mais comum no Brasil, compreende os otarídeos, com duas espécies predominantes, os lobos-marinhos-do-sul *Arctocephalus australis* e os leões-marinhos-do-sul *Otaria flavescens*, que utilizam a área costeira como zona de descanso e alimentação. Não existem colônias de reprodução no litoral brasileiro. Estes animais, por serem restritos a costa, principalmente devido ao modo de vida anfíbio, são mais facilmente estudados e conhecidos. Leões-marinhos tendem a interagir fortemente com a atividade pesqueira local, o que gera uma relação conflituosa, resultando em mortalidade por causas antrópicas. Em contrapartida, lobos-marinhos são considerados como animais esquivos com relação à atividade de pesca, o que pode indicar que sua mortalidade está mais relacionada a causas não-antrópicas. Todavia muitos aspectos ligados à biologia dos otarídeos, em especial aos lobos-marinhos, tais como sua composição de presas, helmintofauna e índices parasitológicos são pobremente estudadas, assim como as histopatologias causadas por helmintos. Principalmente em comparação com investigações mais midiáticas, tais como aquelas para o crescimento populacional e mortalidade por atividade antrópica das espécies de pinípedes ao redor do mundo.

Esta tese compõe cinco artigos independentes redigidos em língua inglesa (Anexos I-V) e precedidos por uma primeira parte, escrita em língua portuguesa, onde são apresentados a introdução, os objetivos e hipóteses do estudo, descrição breve do material e métodos, a síntese dos resultados e as conclusões.

A distribuição dos Anexos segue a apresentação da ecologia e da histopatologia dos acantocéfalos no hospedeiro (Anexos I a V). Os Anexos I e II compreendem os processos parasitológicos das acantocefaloses no hospedeiro, como por exemplo, suas distribuições e

ocupações de nichos. Consequentemente, no Anexo III é apresentado o estudo da relação do padrão de distribuição de corinosomíases e bolbosomíase com os processos de ancoragem-desancoragem destas helmintíases, interligando e reforçando os resultados do Anexo I, através do estudo histopatológico no entendimento da guilda parasitária. O Anexo IV, expõe um caso anormal de corynosomíase, tratado separadamente para não conflitar com a caracterização das lesões que ocorrem na interação normal hospedeiro-parasito, como exposto no Anexo III. O Anexo V expõe os processos histopatológicos e parasitológicos da contracequíase, tipicamente gástrica, no hospedeiro.

1. INTRODUÇÃO

1.1. Parasitologia: visão geral

Estar parasitado é uma condição inerente à vida em todos os grupos animais (Bush et al. 2001, Pizzi et al. 2009). Neste panorama, há enorme descaso quanto à importância dos parasitos no ecossistema, tendo em vista que eles exercem forte pressão seletiva sobre suas populações hospedeiras, ou seja, na dinâmica populacional, competições interespecíficas, fluxo de energia e biodiversidade (Bush et al. 2001, Hudson et al. 2006). A mídia, em todos os seus níveis, quando trata de espécies ameaçadas de extinção geralmente o faz em função da fauna (ou megafauna) de importância econômica ou que apresentem algum efeito “carismático” sobre o ser humano; todavia, as espécies parasitas que serão eliminadas conjuntamente com este ecossistema-hospedeiro faunístico são totalmente ignoradas (Rózsa 1992, Windsor 1995, 1997, 1998, Jairajpuri 2005, Hudson et al. 2006, Pizzi et al. 2009).

Os hospedeiros representam o ecossistema ou microecossistema do qual os parasitos retiram seus recursos para sobreviver e completar, adequadamente, seus ciclos de vida numa relação complexa hospedeiro-parasito e com diversos graus de associação patofisiológica (Mettrick & Podesta 1974, Bush et al. 2001, Horwitz & Wilcox 2005). Estas associações

patofisiológicas (=consequências produzidas pelas doenças, neste caso as helmintíases, em funções orgânicas) respeitam traços filogenéticos coevolutivos (principalmente bioquímicos) que permitem o adequado assentamento e/ou recrutamento do parasito. Estas questões coevolutivas, que demonstram a aptidão parasitária são regidas, também, por fatores intrínsecos do hospedeiro e fornecem a base exploratória para a Teoria da Rainha Vermelha (Dybdahl & Storter 2003, Boeger et al. 2005), do Rei Suicida (Dybdahl & Storter 2003) e da Ultradispersão (“Overdispersion”) Parasitária (Munger et al. 1989, Wakelin 1994, Von Zuben 1997, Bush et al. 2001, Cattadori et al. 2008). A ultradispersão parasitária (ou distribuição agregada ou Abordagem de Crofton) representa um fenômeno natural onde poucos hospedeiros albergam a maior proporção da população de parasitos, enquanto que a maioria dos hospedeiros tende a albergar poucos parasitos (Crofton 1971, Von Zuben 1997, Bush et al. 2001). A abordagem de Crofton (“Crofton’s Approach) indica que é nestes poucos hospedeiros (com cargas parasitárias altas) que processos parasitários denso-dependentes exercem influência na sobrevivência ou fecundidade dos hospedeiros, denotando que tais hospedeiros constituem o “pool” gênico que pode ser retirado da população hospedeira sem afetar esta mesma população (Holmes 1979, Von Zuben 1997). Os processos de seleção, por parte do ecossistema hospedeiro, sobre os parasitos podem ser rigorosos o suficiente para gerar ecótipos (=fenótipos) parasitários ou reduzir seu potencial reprodutivo (George-Nascimento & Marin 1992, George-Nascimento et al. 1992, Wilson et al. 2002, Khokhlova et al. 2010) devido a influências imunológicas, tendo em vista que parasitos são antígenos (Klein 2004, Morales-Montor et al. 2004, Nava-Castro et al. 2011). Adicionalmente, as próprias relações parasitárias intra e interespecíficas na formação de guildas e ocupação de nichos (Zander 2001) regem mecanismos que modificam drasticamente as relações numéricas (=densidade e/ou intensidade parasitária e/ou prevalência), morfologia e distribuição espacial por influência do efeito do agrupamento (“Crowding effect”) sobre os recursos ambientais

limitantes (do ecossistema hospedeiro), tais como espaço e nutrição (Von Zuben 1997, Bush & Lotz 2000, Read 2000, Roberts 2000, Poulin 2001). Desta forma, diversos e mesclados fatores heterogênicos regem a dinâmica populacional parasitária (Von Zuben 1997, Wilson et al. 2002, Cattadori et al. 2008)

Também eventos cíclicos podem afetar a interação hospedeiro-parasito, principalmente em grupos parasitos que demonstram ciclos de vida heteroxênicos (Margolis et al. 1992, Brooks & McLeannan 1993, Bush et al. 2001). Altizer et al. (2006) ponderam sobre a importância de distinguir ciclos anuais de doenças infecciosas daquelas derivadas de parâmetros epidêmicos (ou epizootiológicos) sazonais. O entendimento do regime sazonal do parasitismo colabora na explicação de como hospedeiro e parasito se relacionam e como e quando se deve acionar o controle parasitário. Além disto, também permite ponderar sobre quais os riscos que mudanças antropogênicas e climáticas podem trazer na propagação de doenças sazonais (Altizer et al. 2006). Isto é especialmente importante com relação a transformações ambientais que ocorrem em longo prazo, como as causadas pelo aquecimento global e complexos eventos como o “El Niño”/Oscilação Sul (ENOS) (Lalli & Parsons 2004, Garrison 2010). Estes tipos de fenômeno podem alterar a sazonalidade e os padrões de dispersão de parasitos (Altizer et al. 2006), e a dinâmica de populações de seus hospedeiros intermediários e definitivos (Poulin 1993, 1998, Bush et al. 2001, Wilson et al. 2002, Soto et al. 2004).

Analisando a relação hospedeiro-parasito, Brooks & McLeannan (1993) elucidam o quanto artificial é o estudo da parasitologia se tratada como um grupamento animal a ser entendido. Os autores apontam que este modo de vida homoplásico, em praticamente todos os grupos animais, deve ser estudado e entendido ao nível da particularidade do taxa parasítico e ainda mais, da espécie-alvo parasita e seu hospedeiro-alvo, pois cada espécie parasita responde aos seus próprios padrões ecológicos de seleção. Em suma, compartilhar um modo

de vida não infere em ser selecionado pelo ambiente (hospedeiro) pelos mesmos mecanismos. Concomitantemente a isto, Margolis et al. (1982) percebem que estudar todos os parâmetros populacionais parasitológicos dentro do macro ecossistema é um processo virtual, principalmente com relação a espécies que apresentam ciclo de vida heteroxênico e que por consequência ocorrem em diferentes níveis tróficos. Desta forma os autores cunham os termos infrapopulação e infracomunidade para as porções parasitárias contempladas em um hospedeiro-alvo. A partir deste princípio, 17 anos mais tarde, pensando sobre o entendimento da ferramenta da terminologia e dos índices parasitários no estudo da fauna parasita, Bush et al. (1997) sob a forma de um comitê buscam entender e padronizar terminologias empregadas na parasitologia, tais como Prevalência, Intensidade Média de Infecção e Abundância Média. Mais recentemente, Zander (2001) repensa sobre a estrutura das comunidades parasitárias e cimenta a utilização da Guilda como instrumento ecológico parasitário. Estudos como de Wirsing et al. (2007) mostram que os índices parasitológicos e suas interpretações variam amplamente se a carga de parasitos é tratada agrupada (múltipla = todas as espécies) ou se é tratada isolada (por espécie), com relação a cada espécie hospedeira e/ou seus gêneros sexuais, reforçando a necessidade do estudo isolado das espécies parasitas devido suas características filogeneticamente determinadas. Neste contexto de análise, os índices parasitários são ferramentas que mostram a aptidão hospedeiro-parasito e inspiram diversas biomodelagens da dispersão e ocorrência de parasitos, que raramente é normal, devido a todos os fatores heterogênicos, rapidamente apontados acima (Rózsa et al. 2000, Reiczigel et al. 2005). No contexto geral do entendimento da dinâmica das infrapopulações, outros parâmetros são pensados e utilizados, tal como o Padrão de Agrupamento (“Crowding”) e o efeito deste agrupamento sobre as infrapopulações (Roberts 2000, Rózsa 2000, Reiczigel et al. 2005). Neste panorama, Wirsing et al. (2007) refletem sobre a possibilidade de interpretar

hospedeiros como estoques para infrapopulações com altas distâncias relativas entre variância e Abundância Média.

1.2. Helmintíases e mamíferos aquáticos

Helmintíases são registradas ao longo do tempo em diversas populações de mamíferos aquáticos (Ridgway 1972, Poulin 1993, Valente et al. 1997, Béron-Vera et al. 2007, Morgades et al. 2006, Carvalho 2009, Rocha 2010, Pereira 2012). Em contrapartida, os processos de investigação de alterações teciduais causadas por helmintos geralmente não contemplam os objetivos das pesquisas.

Os estudos helmintológicos iniciais para os mamíferos marinhos buscavam entender principalmente os processos massivos de encalhe em cetáceos que apresentam estrita relação social e formação de manadas, pois se acreditava que os mesmos ocorriam devido à afetabilidade do sistema de ecolocação do espécime líder (Si-Kwang Liu & Eduard 1971, Ridgway & Dailey 1972, Dailey & Stroud 1978, Geraci 1978, Geraci & Aubin 1979, Colongue et al. 1985). Os encalhes massivos sempre retiveram especial atenção política, midiática e conservacionista com relação às espécies afetadas, seu aspecto “carismático” com o público e as possíveis consequências da “saúde” do oceano nestes eventos (O’Brien et al. 1993, Gulland & Hall 2007, Jennings et al. 2001, Reddy et al. 2001ab, Reddy & Ridgway 2003, Bossart 2006, 2007, Bogomolni et al. 2010).

Outra questão importante que deve ser apontada são as flutuações temporais da pesquisa mastofaunal marinha, devido tendências tecnológicas e de fomento para pesquisa. Gulland & Hall (2007) mostram que existem picos de produção e pesquisa com relação aos mamíferos aquáticos, refletindo, por exemplo, em altas quantidades de produção de pesquisa helmintológica nos anos 1970 e 1980 (pelos motivos acima discutidos), mudando para picos de produção de estudos com contaminantes e poluentes entre 1980-1990; picos de estudos de

epizootologias virais (devido ao avanço da biologia molecular) entre 1990-2000, de acordo com interesses político, econômico e sanitário. Assim as pesquisas estão intimamente relacionadas ao interesse dos órgãos de fomento na aprovação de projetos considerados de alto valor tecnológico em detrimento de pesquisas básicas que alicerces estas tecnologias. Existem diversas lacunas de informação sobre as doenças antropogênicas ou simplesmente parasitológicas e/ou o quanto estes dois fatores se autoinfluenciam com relação aos mamíferos marinhos. Isto pode estar relacionado ao valor histórico direcionado aos animais domésticos em detrimento do conhecimento da vida selvagem assim como fatores econômicos em detrimento da conservação das espécies não-domésticas (Gulland & Hall 2007).

Porém, atualmente as necessidades de conservação e manejo de estoques de mamíferos marinhos sob influência de intensa atividade pesqueira (Jennings et al. 2001) tem acionado a parasitologia como ferramenta de incremento no entendimento da formação de estoques (Aznar et al. 1994, Balbuena et al. 1995, Valente et al. 1997, Marigo et al. 2002, Morgades et al. 2006, Rocha 2010, Iñiguez et al. 2011, Pereira 2012) concomitante com estudos morfológicos (Ramos 2001).

1.2.1. Macroecossistema, microecossistema e infrapopulações

Regiões estuarinas representam regiões de alta produtividade do ecossistema marinho contendo alta biomassa devido ao enriquecimento pela drenagem e descarga fluvial de nutrientes e aos padrões de circulação da água e regimes de vento (Lalli & Parsons 2004, Garrison 2010). Esta alta produtividade influencia a cadeia trófica próxima às regiões estuarinas, incluindo aquela onde vivem os mamíferos marinhos. Os lobos-marinhos, como demais organismos aquáticos, estão intimamente ligados aos padrões espaço-temporais de suas fontes de alimento e apresentam variações regionais, sexuais, circadianas e ontogenéticas

para a dieta (Carey 1992, Gales et al. 1993, Horning & Trillmich 1999, Naya et al. 2002, Casper et al. 2006, Ciaputa & Sicinski 2006, Willis et al. 2008, Drago et al. 2009, Boren 2010). Para hospedeiros definitivos de helmintos gastrointestinais como os mamíferos marinhos, a predação é via de infecção (Poulin 1993, 1998, 2001, Bush et al. 2001, Wilson et al. 2002).

O pinípede mais comumente encontrada encalhada no litoral do extremo sul do Brasil é o lobo-marinho-do-sul, *Arctocephalus australis* Zimmermann 1783 (Pinedo 1990, Pinedo et al. 1992, Silva 2004, Mäder et al. 2006). A espécie é tipicamente Sul Americana e sua distribuição é ampla, com registros desde o Rio de Janeiro – Brasil (Oceano Atlântico) rumo ao sul para a Terra do Fogo (transição Atlântico-Pacífico) e depois rumo ao norte para Lima – Perú (Bonner 1981, Vaz-Ferreira 1982, Gentry & Kooyman 1986, Ponce de León 2000). Como demais otarídeos, *A. australis* compreende uma espécie polígama com formação de haréns e extrema expressão fenotípica para o dimorfismo sexual exacerbado em machos, apresentando, a espécie forte seleção sexual (Vaz-Ferreira 1982, Bonner 1981, Cassini 1998). A maturidade sexual nas fêmeas é alcançada entre dois e quatro anos e nos machos entre cinco e seis anos. Todavia, machos demonstram as habilidades comportamentais necessárias para a manutenção de haréns entre sete e oito anos de idade (Bonner 1981). Para águas uruguaias, Katz et al. (2009) registram filhotes em amamentação entre 60-80 cm, fêmeas filhotes desmamadas entre 68-94 cm e fêmeas pré-púberes entre 90-115 cm de comprimento. O período de amamentação da espécie varia de seis a 16 meses (Vaz-Ferreira & Ponce de León 1987, García 2008). A formação de haréns se dá com a chegada antecipada das fêmeas nas zonas de reprodução, geralmente durante novembro para: os partos, cópula e retardo na implantação do blastocisto, alimentação, atendimento materno ao filhote recém-nascido (amamentação). Variações latitudinais nos períodos reprodutivos são observadas como, por exemplo, nas colônias do Oceano Pacífico (Perú) onde o período é mais amplo (outubro-

dezembro), do que no Uruguai (final de novembro e início de dezembro). Machos alcançam comprimentos de até 1,95 m e peso de 140-159 Kg e fêmeas alcançam comprimentos de 1,4 m e 48,5-60 Kg de peso (Bonner 1981, Vaz-Ferreira 1982, Vaz-Ferreira & Ponce de León 1987, Pinedo et al. 1992).

Para a costa do Atlântico, *A. australis* é hospedeiro de diversos *taxa* parasíticos, mais bem conhecidos em espécimes do Uruguai (Morgades et al. 2006), todavia ao nível de identificação e registro, sem contemplar índices parasitológicos. No Brasil, hospedeiros como *A. australis*, que apresentam ampla distribuição latitudinal (e neste caso particular também longitudinal) tendem a demonstrar alta riqueza de espécies (Bush et al. 2001). Entretanto, pesquisas sobre a dieta deste hospedeiro (Gentry & Kooyman 1986, Santos & Haimovici 2001, Naya et al. 2002) demonstram os processos de ligação trófica, podendo explicar os padrões de infecção gastrointestinal, ainda são pobremente explorados. Pesquisas direcionadas ao estudo das parasitoses de mamíferos marinhos em águas brasileiras e os consequentes resultados destas parasitoses, como as alterações teciduais causadas por helmintos, podem ser consideradas escassas ou inexistentes (p.e. Ruoppolo 2003, Silva & Cousin, 2004, 2006ab, Motta et al. 2008). Escassos trabalhos contemplam a helmintologia de otarídeos em águas brasileiras (Pereira 2012), com referência aos índices parasitários que podem explicar a aptidão parasitária sobre um dado hospedeiro (Anderson & Gordon 1982, Anderson & May 1982, Gregory & Blackburn 1991, Bush et al. 2001) em contraposição ao importante papel dos helmintos na ecologia da biosfera (Windsor 1995, 1997, 1998, Jairajpuri 2005) e saúde de seus hospedeiros (Thomson 1983). Os cetáceos compreendem um grupo de mamíferos melhor investigado quanto às helmintíasis no litoral Sul Americano (Aznar et al. 1994, Balbuena et al. 1995, Valente et al. 1997, 2001, Silva & Cousin 2004, 2006ab, Béron-Vera et al. 2007, Motta et al. 2008, Carvalho 2009, Rocha 2010).

O lobo-marinho-do-sul *A. australis* se alimenta sobre uma ampla área da plataforma continental, predando sobre peixes Engraulidae (anchoíta *Engraulis anchoita*, Manjuba *Anchoa marinii*), Sciaenidae (pescada-olhuda *Cynoscyon striatus*, maria-mole *C. gatlucupa*, castanha *Umbrina canosai*), Scombridae (cavala *Scomber japonicus*), Stromateidae (palometa *Peprilus* sp.) e Trichiuridae (peixe-espada *Trichiurus lepturus*), assim como invertebrados (cefalópodes, bivalves, gastrópodes e crustáceos) (Vaz-Ferreira 1982, Santos & Haimovici 2001, Naya et al. 2002, Martínez 2009). Em águas uruguaias (Ilha dos Lobos), o principal item alimentar são os cefalópodes e esta dieta muda quantitativamente interanualmente, provavelmente, devido a mudanças na disponibilidade de peixes e cefalópodes (Naya et al. 2002). Para o litoral brasileiro, os cefalópodes *Loligo sanpaulensis* e *Argonauta nodosa* perfazem importantes itens da dieta de *A. australis* (Santos & Haimovici 2001).

Ambos acantocéfalos e nematóides anisaquídeos, em linhas gerais, apresentam ciclos de vida heteroxênicos que apresentam especificidade em sua relação hospedeiro-parasito. Esta relação é complexa e abrange diferentes níveis tróficos em seus estágios de vida infectantes rumo ao sucesso reprodutivo (estágio adulto) dentro de um hospedeiro definitivo, porém passando por hospedeiros intermediários (Petrochenko 1971, Parshad & Crompton 1981, Nickol 1985, Combes 1997, Bush et al. 2001, Roberts & Janovy Jr. 2008).

Espécies de acantocéfalos no meio aquático utilizam microcrustáceos (geralmente Amphipoda, Copepoda, Isopoda ou Ostracoda) como hospedeiros intermediários primários e peixes como hospedeiros intermediários secundários (ou hospedeiros paratênicos). Vertebrados endotermos (mamíferos e aves aquáticas) são os hospedeiros definitivos (Petrochenko 1971, Parshad & Crompton 1981, Nickol 1985, Bush et al. 2001). Seus estágios de desenvolvimento são os acântores (nos hospedeiros primários), as acantelas (nos hospedeiros intermediários secundários ou paratênicos) e os cistacantos e/ou adultos em hospedeiros endotermos definitivos que são infectados de modo passivo (ingestão do estágio

infestante) (Petrochenko 1971, Nickol 1985). Acantocéfalos adultos geralmente se assentam e recrutam na luz intestinal de seus hospedeiros endotermos definitivos para completar seus ciclos de vida adequadamente (Crompton 1973, Parshad & Crompton 1981, Nickol 1985), mas exceções podem ser encontradas para espécies que assentam e recrutam na luz gástrica de mamíferos aquáticos (Valente et al. 1997, Aznar et al. 2001, Silva & Cousin, 2006b, Rocha 2010). Formas acantela de *Corynosoma* sp., *C. australe* e *Bolbosoma* sp. são registradas na pescada-olhuda *C. quatuorcupa* (Sardella et al. 2005), no peixe-espada *T. lepturus* (Santos et al. 2008), na cavala *S. japonicus* (Rego & Santos 1983) e na corvina *Micropogonias furnieri* (Pereira Jr. & Neves 1993, Alarcos & Etchegoin 2010). Devido à importância trófica dos cefalópodes para mamíferos e aves marinhas ao redor do globo (Gales et al. 1993, Clarke 1996, Santos & Haimovici 2001, Libertelli et al. 2004, Boyle & Rodhouse 2005, Xavier et al. 2011) e da ocorrência de bolbosomíase em Ziphiidae (teutófagos), a contribuição dos cefalópodes para a bolbosomíase em *A. australis* deve ser considerada.

Alguns grupos de acantocéfalos, como *Corynosoma* spp., que parasitam mamíferos e aves marinhas são melhor conhecidos em diversos aspectos de sua biologia, tais como anatomia (Zdzitowiecki 1986a, 1989, Aznar et al. 1999a, Nickol et al. 2002), sistemática (Zdzitowiecki 1984a, Aznar et al. 1999b) e ecologia do parasitismo (Zdzitowiecki 1986b, Zdzitowiecki 1984b, 1985, Nickol & Valtonen 2002, Aznar et al. 2001, 2004, Sinisalo et al. 2004, Rocha 2010) do que *Bolbosoma* spp. (Measures 1992, Amim & Margolis 1998). Há evidência de que os padrões de distribuição e ancoragem de *C. australe* em diferentes porções do tubo intestinal de *A. australis* estão relacionados com processos ontogenéticos, nutricionais e reprodutivos do parasito (Aznar et al. 2004). Também há evidência de que a morfologia do tronco de *Corynosoma* spp., que pode ser usado como um ancorador acessório (Aznar et al. 1999a) ajude a explicar os processos migratórios e histopatológicos causados por *C. australe* no hospedeiro definitivo. Normalmente acantocéfalos são considerados parasitos de baixo

potencial patológico aos mamíferos marinhos (Ridgway 1972, Bonner 1990, Vlasman & Campbell 2003). Porém, toda interação hospedeiro-parasito é, no mínimo, espoliativa (Mettrick & Podesta 1974, Bush et al. 2001). Alterações teciduais severas, assim como outros processos inflamatórios relacionados com acantocefalose em mamíferos são registrados, tais como abscessos intestinais por bolbosomíase (Silva & Cousin 2006a), peritonite séptica por profilicóliase (Kreuder et al. 2003), neoplasias, enterite crônica, perfuração intestinal e granuloma eosinofílico por macracantorrincose (Taman 2009).

O ciclo de vida dos nematóides anisaquídeos segue o mesmo modelo geral de hospedeiros intermediários primários, secundários (ou paratênicos) e definitivos dos acantocéfalos (Bush et al. 2001). Todavia, existem particularidades para os nematóides. Anisakidae como *Anisakis* spp., *Contracaecum* spp. e *Pseudoterranova* spp. apresentam sucesso na colonização de seus hospedeiros (Raga et al. 2002). Durante o percurso parasitário dentro dos seus hospedeiros, as larvas de anisaquídeos mudam (sofrem ecdise e metamorfose) quatro vezes, gerando quatro estágios larvais (L-1 ao L-4) até atingirem o estágio adulto no trato gastrointestinal de vertebrados endotermos (Bush et al. 2001, Dziekonska-Rynko & Rokicki 2007, Roberts & Janovy Jr. 2008, Dziekonska-Rynko et al. 2010). Os anisaquídeos detêm ampla variação genética dentro dos estoques mundiais de seus hospedeiros mamíferos (Nader et al. 2000, Mattiucci et al. 2003, Kijewska et al. 2009, Iñiguez et al. 2011). Anisaquídeos são tipicamente gástricos em cetáceos (Dailey & Stroud 1978, Abollo et al. 1998, Valente et al. 2001, Silva & Cousin 2004, Jaber et al. 2006, Motta et al. 2008, Rocha 2010), pinípedes (Si-Kwang Liu & Edward 1971, Fagerholm & Gibson 1987, Spraker et al. 2003, Morgades et al. 2006) e aves aquáticas (Si-Kwang Liu & Edward 1971, Kuiken et al. 1999, Kanarek & Rolbiecki 2006, Dziekonska-Rynko & Rokicki, 2008, Rokicki et al. 2011). Também são organismos extremamente deletérios aos tecidos gastrointestinais de seus hospedeiros por causar processos inflamatórios severos (Spraker et al. 2003, Silva & Cousin

2004, Jaber et al. 2006, Dziekonska-Rynko & Rokicki, 2008, Motta et al. 2008, Rokichi et al. 2011), assim como outros nematóides parasitos de peixes, para a estrutura gastrointestinal de seus hospedeiros (Iversen & Kelley 1974, Meguid & Eure 1996). Estes processos contemplam a ação concomitante do aparelho bucal e das enzimas digestórias para penetração e/ou histofagia por estes helmintos (McKerrow 1989, Pascual et al. 2000, Ros-Moreno et al. 2000, Bush et al. 2001, Dziekonska-Rynko et al. 2003, Dzik 2006). Diversos *taxa* de hospedeiros, tais como, peixes (Iversen & Kelley 1974, Obiekezie et al. 1992, Meguid & Eure 1996), antigo grupamento reptil (McAllister et al. 1993, Taiwo et al. 2001, Mihalca et al. 2007), aves e mamíferos (Abollo et al. 1998, Kuiken et al. 1999, Pascual et al. 2000, Silva & Cousin 2004, Jaber et al. 2006, Kierdorf et al. 2006, Motta et al. 2008, Rokichi et al. 2011), desenvolvem alterações teciduais causadas por nematóides. O anisaquídeo registrado para *A. australis* da costa Uruguiaia, é *Contracaecum* sp. (Morgades et al. 2006) e *Contracaecum ogmorhini* é registrado para o simpátrico *Otaria flavescens* do extremo sul do Brasil (Pereira 2012). Hospedeiros intermediários ou paratênicos que abrigam *Contracaecum* sp. na zona de distribuição estudada para ambos otarídeos são o peixe-espada *Trichiurus lepturus* (Lent & Freitas 1948) e a cavala *S. japonicus* (Rego & Santos 1983).

1.3. Gênero sexual do hospedeiro e parasitoses

Em aves e mamíferos tem sido registradas tendências de maior mortalidade relacionadas ao gênero masculino em espécies que apresentam fenótipos dimórfico-sexuais com machos maiores do que as fêmeas (Clutton-Brok et al. 1985, Clinton & Le Boeuf 1993, Nager et al. 2000). Nestes casos, a característica mais marcante é a aquisição de massa muscular masculina, ou seja, um volume corporal exacerbado ao atingir a maturidade sexual (Ferguson & Larivière 2008). Estas características de mortalidade estão relacionadas ao estresse constante na busca de recursos (nutricionais e reprodutivos) que podem afetar a expressão

imune e a taxa de exposição dos machos aos patógenos (Clutton-Brok et al. 1985, Olsen & Kovacs 1996, Nager et al. 2000, Klein 2004). Por outro lado, a manifestação da tendência de maior mortalidade em fêmeas é sustentada para espécies onde as fêmeas são maiores que os machos (Clutton-Brok et al. 1985, Torres & Dummond 1997). O fenótipo dimórfico-sexual exacerbado em machos está relacionado à manutenção de dispositivos anatômicos que permitam acesso às fêmeas e que garantam, perante outros machos, maiores chances de compartilhamento genético (seleção sexual) (Ralls 1977, Gittleman 1986, Kunz et al. 1996, Fairbairn 1997). Entre os lobos-marinhos a agressividade do macho tende a ser predominante sobre a oportunidade de escolha da fêmea por um reprodutor nas áreas de reprodução (Bonner 1981). Todavia fêmeas de lobos-marinhos também sofrem estresse demográfico-reprodutivos nos períodos pré e pós-parto na busca por lugares mais adequados (microhabitats praianos) para o atendimento maternal de seus filhotes (Acevedo et al. 2003, Pavés et al. 2005, Fernández-Juricic & Cassini 2007). Esta pressão social sobre as fêmeas está relacionada desde a implantação retardada do blastocisto (Trillmich 1986ab, Atkinson 1997), os crescentes conflitos intrasexuais femininos que se refletem no comportamento agressivo (agonístico) durante a gestação e amamentação para defender áreas de parto e bem-estar do filhote (Pavés et al. 2005, Fernández-Juricic & Cassini 2007) e a lactação, que influi direta e fortemente sobre o número de retornos da fêmea ao mar para alimentação (Goldsworthy 1999).

Diferentes mecanismos heterogênicos intrínsecos ao hospedeiro, como idade, gênero sexual, maturidade sexual, imunidade e genética, assim como fatores intra e interespecíficos nas relações parasitárias tendem a influenciar os índices parasitários (principalmente a prevalência e a intensidade de infecção) ou o padrão de dispersão parasitária de modo que os hospedeiros machos apresentem maiores valores parasitêmicos (níveis parasitários) do que as fêmeas (Poulin 1996, Wilson et al. 2002, Klein 2004). *Sensu lato* artropodíases, nematodíasis

e acantocefaloses tendem a ser macho-prevalente (Poulin 1993, 1996, 1998, 2001, 2005, Barriga & Al-Khalidi 1991, Wilson et al. 2002, Klein 2004, Cowan et al. 2007, Ferrari et al. 2007, Khokhlova et al. 2010). Desta forma, a imunocompetência é considerada inferior no gênero masculino (Olsen & Kovacs 1996, Klein 2004, Stoehr & Kokko 2006). Todavia poucos são os estudos que buscam investigar se existem diferenças ligadas ao gênero sexual dos hospedeiros (Wirsing et al. 2007). Porém os padrões de dominância parasitários, se é que existe algum, demonstram ser difíceis de prever (Wilson et al. 2002, Morales-Montor et al. 2004).

Uma hipótese recorrente é a de que os machos de mamíferos são mais suscetíveis a infecções parasitárias do que fêmeas com base nos valores de prevalência e intensidade de infecção (Klein 2004, Wilson et al. 2002), preconizando desta forma um paradigma da supremacia feminina sobre as parasitoses e doenças, o qual é contestado por Morales-Montor et al. (2004). Existem evidências de que estão envolvidas neste contexto complexas e flutuantes interações imuno-endócrinas relacionadas às diferenças inter-sexuais de comportamento e de concentração dos hormônios esteróides (sexuais) e glicocorticóides (cortisol ou corticosterona) rumo à vida adulta sexual que, via de regra, são imunossupressores célula-humorais (Olsen & Kovacs 1996, Klein 2004, Morales-Montor et al. 2004, Stoehr & Kokko 2006). Há uma dicotomia imunológica entre os gêneros sexuais de vertebrados, que regulam de modo diferente a ativação imuno-celular, a infiltração (exudação) celular e a produção de citocinas (Messingham et al. 2001, Müller et al. 2005, Bird et al. 2008). Devido às progestinas, fêmeas de mamíferos tendem a ficar mais suscetíveis a parasitoses durante seus períodos gestacionais, devido à supressão imunocelular (anti-abortiva), mas com a manutenção imunohumoral (anti-inflamatória) (Olsen & Kovacs 1996, Morales-Montor et al. 2004, Stoehr & Kokko 2006, Medeiros et al. 2007, Cabrera-Muñoz et al. 2010). Todavia existem evidências de que helmintíases também podem ser mais

prevalentes e intensas em hospedeiros fêmeas, principalmente as cestodíasis (Barriga & Al-Khalidi 1991, Escobedo et al. 2004, Morales-Montor et al. 2004) e algumas nematodíases (Rossin et al. 2010). Cestodíases tendem a ser fêmea-prevalente devido a propriedades intrínsecas do grupo parasito na conversão hormonal androgênica-estrogênica no hospedeiro macho (mais custosa energeticamente ao parasito) ou da facilidade da utilização de estrógenos (progesterona) disponíveis nos hospedeiros fêmeas, para o desenvolvimento dos cisticercos ou maturação das proglotides (Escobedo et al. 2004, Morales-Montor & Larralde 2005, Vargas-Villavicencio et al. 2005, Valdéz et al. 2006). Wirsing et al. (2007) suportam a inexistência de diferenças entre os gêneros sexuais de hospedeiros do grupo dos mamíferos carnívoros, e atentam para a importância de interpretar as tendências de gênero sexual como indicadores de que aquele gênero sexual age como estoque para a espécie parasita em questão, porque a susceptibilidade de infecção masculina não é onipresente em mamíferos. Em se tratando de macho-estoque parasitário, o comportamento ativo de forrageio de roedores fossoriais machos e os processos de dispersão de nematóides parasitos para as fêmeas passivas em amamentação nas galerias subterrâneas, implica nesta questão (Ferrari et al. 2007). Também implicações ecológicas como as do cuidado parental masculino e dietas diferenciadas entre machos e fêmeas podem fortemente influenciar no parasitismo (Reimchen & Nosil 2001). Assim, empiricamente tem se demonstrado que machos são mais responsáveis pela disseminação e manutenção dos ciclos parasitários do que as fêmeas, devido a diferenças na suscetibilidade e taxa de exposição, o que culmina na ultradispersão parasitária (Reimchen & Nosil 2001, Skorpíng & Jensen 2004). Morales-Montor et al. (2004), avaliando o paradigma da supremacia feminina mediante as parasitoses, chegam a conclusões dúbias sobre o tema e reforçam a especificidade dos casos na relação hospedeiro-parasito, não havendo, desta forma, um consenso universal de fragilidade masculina ou de supremacia feminina para este tema.

Também estudos têm demonstrado que existe dimorfismo sexual quanto às capacidades do sistema imune em lidar com traumas, septicemias e hemorragias devido a ação dos hormônios sexuais sobre a imunidade celular e humoral (anti e/ou pró-inflamatório) tanto em vertebrados (Angele & Faist 2000, Schneider et al. 2003, Stoehr & Kokko 2006, Nunn et al. 2012) quanto em invertebrados (Nunn et al. 2012, Ruiz-Guzmán et al. 2012 *in press*). O dimorfismo imune também está refletido sobre as capacidades de cicatrização (reparação dos traumas = feridas, por exemplo) de cada gênero sexual (Messingham et al. 2001, Bird et al. 2008). Também existem evidências de que após traumas e hemorragias fêmeas de mamíferos (inclusive humanas) mantêm seus níveis de citocinas imuno-mediadoras altas ou constantes, enquanto machos apresentam níveis deprimidos destes comunicadores celulares (Angele & Faist 2000). Este dimorfismo imune está expresso de forma inata (Moxley et al. 2002), assim como na resposta às vacinas (Cook 2008). Desta forma, os processos de desenvolvimento e cura, assim como os tipos e dimensões das lesões inflamatórias causadas pelos helmintos poderiam também estar moduladas pelo dimorfismo sexual imune do hospedeiro.

Com base nestes antecedentes, deveria se esperar que os machos de *Arctocephalus australis*, rumo à idade reprodutiva, com maiores dimensões, maiores níveis de androgênios e maiores requerimentos nutricionais para a manutenção das características sexuais secundárias apresentassem maiores índices helmintológicos; maiores quantidades e/ou dimensões e/ou tipos de lesões causadas pelos helmintos, do que as fêmeas. Todavia, fêmeas maduras sexualmente (ou em estágio reprodutivo) poderiam também demonstrar prevalências helmínticas maiores ou iguais aos machos devido à influência imunossupressora dos hormônios sexuais. Mais além, em não havendo diferenças entre os gêneros sexuais, as tendências de prevalência e intensidade de infecção podem indicar qual gênero sexual pode compreender o estoque da espécie-alvo parasita na continuidade do seu ciclo. Todas estas questões reforçam a artificialidade da parasitologia (apontada no início desta Introdução)

como ciência única e a fragilidade da aplicação universal dos seus métodos no entendimento daquilo que deve ser tratado num âmbito muito mais particular.

1.4. Objetivos e hipóteses

A tese objetiva avaliar através dos índices parasitológicos (Prevalência, Intensidade Média de Infecção, Abundância Média de Infecção) das variáveis parasitológicas (Amplitude de Variação e Padrão de Agrupamento) e índices lesionais (Prevalência de Lesão, Intensidade Média de Lesão e Abundância Média de Lesão), a influência da sazonalidade, do gênero sexual e da condição reprodutiva do hospedeiro sobre as acantocefaloses e a nematodíase gastrointestinal de *A. australis* do extremo sul do Brasil, assim como sobre as lesões causadas por helmintos.

Objetivos Específicos

Os objetivos específicos visam responder ao menos uma hipótese ecológica, como segue abaixo:

- 1.4.1. Estimar os índices parasitológicos (Prevalência, Intensidade Média de Infecção e Abundância Média) e as variáveis parasitológicas (Amplitude de Variação e Padrão de Agrupamento) gastrointestinais para as acantocefaloses e a nematodíase em *A. australis* do extremo sul do Brasil de acordo com o gênero sexual e a condição reprodutiva do hospedeiro (Anexos I, II e V).
- 1.4.2. Avaliar possíveis diferenças de gênero sexual e de condição reprodutiva sobre as infrapopulações gastrointestinais de acantocéfalos e nematoides através dos índices e variáveis parasitológicos (Anexos I, II e V).

1.4.3. Avaliar tendências de Prevalência de acordo com o gênero sexual do hospedeiro (Anexos I, II e V).

Hipótese 1 – A Prevalência e Intensidade Média de Infecção para todas as helmintíases são semelhantes entre os gêneros sexuais dos hospedeiros.

Hipótese 2 – A Prevalência e Intensidade Média de Infecção são semelhantes entre as condições sexuais dos hospedeiros.

1.4.4. Avaliar padrões sazonais de Prevalência e Intensidade Média de Infecção para as acantocefaloses e a nematodíase em *A. australis* do extremo sul do Brasil (Anexos I, II e V).

Hipótese 3 – Infrapopulações apresentam diferenças sazonais para as Prevalências e Intensidade Média de Infecção.

1.4.5. Avaliar a ocupação de nichos intestinais pelos acantocéfalos em *A. australis* (Anexos I, II, III, IV e V).

1.4.6. Avaliar a histopatologia como ferramenta para explicar a ocupação de nichos intestinais pelos acantocéfalos de *A. australis* (Anexo I).

Hipótese 4 – As características da ancoragem refletem a ocupação de nichos intestinais pelos acantocéfalos.

1.4.7. Descrever e caracterizar histologicamente a condição normal do estômago e intestinos de *A. australis* (Anexos III e V).

1.4.8. Avaliar a relação entre as acantocefaloses e a nematodíase com as lesões inflamatórias gastrointestinais em *A. australis*, (Anexos III, IV e V).

1.4.9. Caracterizar macro e microscopicamente as lesões gastrointestinais relacionadas às acantocefaloses e a nematodíase em *A. australis* (Anexos II, III, IV e V).

Hipótese 5 – As lesões causadas pelos helmintos diminuem a espessura das mucosas nas porções gastrointestinais envolvidas.

1.4.10. Avaliar o processo de aquisição das lesões gástricas entre os gêneros sexuais e as condições sexuais do hospedeiro (Anexo V).

Hipótese 6 – Machos e fêmeas de *A. australis* respondem igualmente às lesões causadas por helmintos.

2. MATERIAL E MÉTODOS: LINHAS GERAIS

2.1. Amostragem dos Hospedeiros

Trinta e dois *A. australis* (16machos: 16fêmeas) foram coletados entre a Praia do Cassino (ca. 32°11'14.23"S; 52°09'21.70"W) e o Chuí (ca. 33°44'35.96"S; 53°22'12.70"W) no litoral do Rio Grande do Sul – Brasil (Licença IBAMA - SISBIO nº 17529-1) entre agosto de 2008 e dezembro de 2010, para triagem *extra situ*. As amostragens foram tratadas dentro de dois grandes períodos anuais: Primavera-Verão (S-S) e Outono-Inverno (A-W). A identificação e sexagem dos hospedeiros foi efetuada *sensu* Pinedo et al. (1992). Medidas do comprimento total (TL) de cada hospedeiro foram tomadas linearmente (*sensu* Dierauf 1994).

A condição de conservação das carcaças utilizadas para necropsia seguiu Dierauf (1994). Somente carcaças códigos 2 e 3 foram utilizadas neste estudo. Carcaças com código 2 foram utilizadas para: coleta de tecidos para procedimento histológico (q.v. 2.2. Histologia e histopatologia), contagem e mensuração de lesões gástricas e para coleta de parasitos. Carcaças com código 3 foram utilizadas somente para coleta de parasitos e contagem e mensuração das lesões gástricas. Adicionalmente aos apontamentos amostrais de Dierauf (1994) as carcaças também foram observadas para outras alterações *post-mortem* (*sensu* Thomson 1993), como o *rigor mortis*, o *livor mortis*, a expansão abdominal por gases de decomposição e a ausência de entomofauna decompositora.

Os procedimentos de necropsia foram desenvolvidos no Laboratório de Necropsia da Universidade Federal do Rio Grande (FURG) do Instituto de Ciências Biológicas (ICB) para retirada e análise do tubo digestório e das gônadas (direita e esquerda) masculinas (testículos extra-cavitários na região inguinal) e femininas (assim como do útero) de acordo com

Ridgway (1972) e Stewardson et al. (1999) para as relações anatômicas. Os procedimentos de abertura das cavidades torácica e abdominal de cada hospedeiro foram efetuados *sensu* Dierauf (1994) para isolamento do tubo digestório e glândulas anexas e também dos órgãos reprodutivos. O tubo digestório foi dividido em suas porções anátomo-funcionais (esôfago e estômago, intestinos delgado e grosso) devidamente laqueadas, etiquetadas, ensacadas e congeladas para triagem histológica, histopatológica e parasitológica. O intestino delgado foi separado do mesentério, linearmente mensurado e dividido em uma porção cranial e outra caudal para averiguar diferenças na distribuição dos parasitos.

Macroscopicamente, as lesões relacionadas à contracepção foram contadas, descritas e mensuradas de acordo com cada porção anátomo-funcional (*sensu* Silva & Cousin 2004).

Também foram coletados dos hospedeiros os sincrânios e dentes, bigodes, unhas, tecido muscular e conteúdo gastrointestinal (otólitos, peixes inteiros e bicos de Cephalopoda) quando presentes. Os sincrânios foram tratados para descarnificação por cozimento (Auricchio & Salomão 2002). Este material foi entregue ao Laboratório de Tartarugas e Mamíferos Marinhos (LTMM) do Instituto de Oceanografia (IO) da FURG, devidamente referenciado de acordo com sistema utilizado pelo LTMM – IO, contemplando outras pesquisas multidisciplinares não tratadas neste estudo.

2.2. Histologia e histopatologia

Tecidos gastrointestinais normais e anormais, assim como os gonadais, foram submetidos ao protocolo de rotina para impregnação em parafina e montagem permanente em bálsamo do Canadá.

Os tecidos gastrointestinais normais e anormais (lesões e/ou com parasitos ancorados) foram fixados em líquido de Bouin para serem submetidos aos protocolo histológico de rotina para inclusão em parafina e montagem permanente em bálsamo do Canadá. Os blocos de

parafina foram microtomizados a 7µm de espessura e corados com Hematoxilina-Eosina (H-E) e Alcian-Blue (A-B) para histoquímica do muco (*sensu* Silva & Cousin, 2004, 2006ab).

Histometrias foram utilizadas para comparação entre condição normal e anormal (q.v. Análise Estatística) da camada da parede gastrointestinal na área afetada pelo parasito-alvo com aquela normal. Esta comparação foi efetuada com relação à espessura da camada da parede gastrointestinal em condições normais com aquelas anormais, respeitando cada porção anátomo-funcional gastrointestinal (estômago e intestinos). As histometrias dos preparados foram efetuadas com ocular métrica calibrada com lâmina micrométrica (Nikken – Tokio; 0,01mm) em diferentes magnificações (4x10, 10x10, 20x10), seguindo Silva & Cousin (2004, 2006ab). As características macroscópicas e as microscópicas foram somadas para tipificar a severidade dos padrões teciduais anormais encontrados (Thomson 1983).

Para análise da maturidade sexual (gametogênese) dos hospedeiros, os tecidos gonadais seguiram o mesmo tratamento histológico empregado para os tecidos gastrointestinais (exceto para o A-B). As gônadas masculinas foram extraídas conjuntamente com os epidídimos e uma fatia foi retirada transversalmente ao nível central para averiguar a histomorfologia da espermatogênese. As gônadas femininas foram submetidas à análise estereomicroscópica da foliculogênese, luteogênese ou luteólise. Após, os ovários foram separados das tubas uterinas e cortados em seções de 1-2mm para serem submetidos ao protocolo histológico. Os procedimento de preparação gonadal dos hospedeiros foi efetuado *sensu* Atkinson (1997), Bukovsky et al. (2005), Wistuba et al. (2007), Colegrove et al. (2009), Katz et al. (2009), Lucacin & Pinto-Neto (2009) e Palma-Cerda et al. (2011). De acordo com a espermatogênese, os hospedeiros machos podem ser categorizados como: filhotes (espermatogônia e espermátocitos), pré-púberes (espermátides), púberes (espermatozoides na luz dos túbulos seminíferos – espermatozoides testiculares) e reprodutivos (espermatozoides na luz do epidídimo) (Wistuba et al. 2007). As fêmeas hospedeiras podem ser categorizadas

em suas condições sexuais, de acordo com a população folicular do estroma ovariano, como: filhotes (principalmente folículos primários), pré-púberes (folículos antrais e Graafianos presentes) e reprodutivas-reprodutoras (Corpo Lúteo – Corpo Albicans presentes) (Katz et al. 2009, Palma-Cerda 2011).

2.3. Coleta e preparação parasitária gastrointestinal

Inicialmente, os parasitos foram coletados das porções anátomo-funcionais via observação direta, lavagem da superfície luminal e retirada de espécimes ancorados na parede gastrointestinal. A lavagem luminal foi efetuada dentro de bacias e o volume do lavado foi peneirado em malhas de 0,5-1mm². O peneirado foi repassado a placas de Petry para triagem estereomicroscópica para separação em grupos taxonômicos maiores de parasitos (Acanthocephala e Nematoda) e conservação. Os grupos parasitos foram separados em infrapopulações (*sensu* Margolis et al. 1982, Bush et al. 1997) para contagem, triagem (gênero e/ou espécies) de espécimes destinados a fixação e conservação e ao protocolo de coloração e montagem permanente de parasitos em bálsamo do Canadá (*sensu* Amato et al. 1991). Antes da triagem e fixação em AFA (Alcool-Formol-Ácido Acético), os acantocéfalos foram mantidos em água refrigerada por 24 horas para extroversão da probóscide que constitui importante instrumento taxonômico (Petrochenko 1971). Os nematóides foram fixados também em AFA e Líquido de Bouin. Espécimes de ambos os grupos parasitos foram corados com Carmum de Semichon e Eosina. Os nematóides foram também clarificados em Lactofenol de Aman para montagem temporária e diagnose taxonômica.

Os acantocéfalos foram identificados *sensu* Petrochenko (1971), Zdzitowiecki (1984b, 1989), Measures (1992), Pereira Jr. & Neves (1993), Braicovich et al. (2005) e Sardella et al. (2005) respeitando os critérios para: oncotaxia da probóscide (estrutura e padrão de distribuição dos ganchos), forma do corpo, tronco (pressoma e metassoma), espinhos

(estrutura e padrão de distribuição dos espinhos somáticos) e organologia. Acantocéfalos foram observados quanto a seus estágios ontogenéticos, i.e., formas cistacanto (estágio infectante final encontrado em hospedeiros intermediários rumo a um hospedeiro definitivo adequado) e formas adultas (*sensu* Nickol 1985). Fêmeas foram investigadas quanto a fertilização e condição da maturidade sexual (acasaladas e não-acasaladas) *sensu* os critérios de Bates & Kennedy (1990), Sinisalo et al. (2004) e Valtonen et al. (2004) para os ovos (embrionados com acantores) dentro da cavidade corporal das fêmeas. Fêmeas fertilizadas foram interpretadas como sendo aquelas que continham acantores desenvolvidos ou em desenvolvimento. Fêmeas maduras compreendem aquelas que contêm gônadas, útero e estruturas vaginais completamente formadas.

Os nematóides foram identificados *sensu* Yamaguti (1926), Yorke & Maplestone (1926), Travassos et al. (1928), Anderson et al. (1974), Vicente et al. (1985), Fagerholm & Gibson (1987) e Paoletti (2009). Nematóides anisaquídeos foram investigados para os estágios ontogenéticos: larva recém-eclodida [larva estágio 3 (L-3) para o estágio 4 (L-4) com fina bainha cuticular corporal, esôfago diferenciado, ventrículo, apêndice ventricular e ceco intestinal e “dente” de eclosão], juvenil (principalmente para fêmeas sem ovos dentro dos úteros convolutos bem desenvolvidos), adultos [fêmeas (ovadas) e machos (espículas bem desenvolvidas e papilas pré e pós-cloacais proeminentes)] *sensu* Anderson et al. (1974), Möller & Anders (1986), Fagerholm & Gibson (1987), Kanarek & Rolbiecki (2006), Dziekonska-Rynko & Rokicki (2007), Paoletti (2009) e Dziekonska-Rynko et al. (2010).

2.4. Análises estatísticas

Cada parasito foi analisado pro gênero sexual *sensu* Wirsing et al. (2007) de diferentes formas para estimativas dos índices parasitários. Índices parasitológicos, como Prevalência (P%), Intensidade Média de Infecção (MI) e Abundância Média (MA) foram determinados

(*sensu* Bush et al. 1997) e analisados *sensu* Rózsa et al. (2000) no programa “Quantitative Parasitology – QP 3.0”. A P% (Teste do Chi-quadrado, X^2) e a MI (Intervalo de confiança por “Bootstrap”, BC_a , $p < 0,05$) foram comparados entre os gêneros sexuais (machos versus fêmeas) e a condição reprodutiva do hospedeiro (machos filhotes versus machos pré-púberes, machos filhotes versus fêmeas filhotes, machos pré-púberes versus fêmeas reprodutivas-reprodutoras). A P% fornece informações sobre os tamanhos relativos de hospedeiros infectados e não infectados, enquanto que a MI varia independente da P%, o que não causa redundância da informação. Todavia, MA não foi comparada, pois carrega a mesma informação que MI e informação parcial da P%, resultando em redundância na informação. A Amplitude de Variação (Ax) parasitária foi expressa conforme Bush et al. (2001).

O padrão de distribuição, i.e., ao acaso, uniforme ou agregado (Crowded – CRD) dos parasitos foi efetuado *sensu* Von Zuben (1997), Rózsa et al. (2000), Bush et al. (2001) e Reiczigel et al. (2005) entre hospedeiros machos e fêmeas para aptidão (“fitness”) parasitária respeitando os gêneros sexuais dos hospedeiros e suas condições reprodutivas [Teste do Chi-quadrado (X^2)]. O padrão de distribuição respeita a Média ($\bar{x}$) e a Variância (s^2) e relação Média/Variância ($\bar{x}/s^2$), onde: $s^2 \approx \bar{x}$, representa a distribuição ao acaso; $s^2 < \bar{x}$, representa uma distribuição uniforme e $s^2 > \bar{x}$, representa uma distribuição agregada (CRD) (Von Zuben 1997, Rózsa et al. 2000, Bush et al. 2001, Reiczigel et al. 2005).

Para os parasitos constituintes da comunidade do intestino delgado, as variáveis parasitológicas P%, MI, MA, Ax e CRD foram também estimadas para as porções cranial e caudal do órgão. As comparações estatísticas ficaram restritas ao gênero sexual do hospedeiro e entre as porções intestinais, no mesmo padrão acima descrito para P%, MI e CRD.

Os valores histométricos, em espessura, da mucosa afetada de cada divisão anátomo-funcional dos órgãos foi comparada pela Análise de Variância (Teste de Tukey, $p < 0,05$) (*sensu* Silva & Cousin 2004, 2006ab).

A Análise de Regressão foi aplicada para a Intensidade de Infecção Parasitaria (PII) para hospedeiros machos e fêmeas e seus TL, rumo à maturidade sexual, para avaliar o ângulo das Linhas de Tendência ($y=a+bx$), o Coeficiente de Determinação (r^2) e o Coeficiente de Correlação de Pearson (r) (Vieira 1981, Silva & Pinto 2011). Para as lesões gástricas, a Intensidade de Lesões relacionada com o Parasito (PLI) foi submetida a Análise de Regressão como para PII.

As estações agrupadas (S-S e A-W) tiveram suas P%, MI e CRD comparadas com os mesmos critérios utilizados para os gêneros sexuais dos hospedeiros.

3. SÍNTESE DOS RESULTADOS

3.1. Hospedeiros: amplitude de comprimento, gênero sexual e condição reprodutiva

Os hospedeiros amostrados compreenderam 32 espécimes de *A. australis* na proporção de 16 machos: 16 fêmeas.

A amplitude dos comprimentos dos hospedeiros (TL) variou entre $0,84m \geq TL \leq 1,76m$ para os machos e entre $0,8m \geq TL \leq 1,34m$ para as fêmeas.

Os hospedeiros foram classificados de acordo com a gametogênese (q.v. Material e Métodos) e dentro das TL como machos filhotes ($0,84m \geq TL \leq 1,03m$), fêmeas filhotes ($0,8 \geq TL \leq 0,98m$), machos pré-púberes ($1,32m \geq TL \leq 1,76m$) e fêmeas reprodutivas-reprodutoras ($1,33m \geq TL \leq 1,34m$).

3.2. Parasitologia

Considerando as helmintíases com relação ao crescimento e maturação dos gêneros sexuais dos hospedeiros (Anexos I, II e V), todas elas indicaram que: (1) as infecções se iniciam ainda quando filhotes, tanto nos hospedeiros machos quanto nos hospedeiros fêmeas; (2) as intensidades (DTL) de infecção das acantocefaloses por *Corynosoma australe* e por

Bolbosoma sp. aumentaram de modo semelhante nos dois casos, todavia com os hospedeiros machos atingindo mais precocemente intensidades de infecção maiores do que as fêmeas hospedeiras e, (3) em contrapartida, a contraqueíase atinge níveis mais altos e mais rapidamente (DTL) nas fêmeas hospedeiras do que nos machos hospedeiros. Com relação à infecção por *C. cetaceum* (Anexo II), todos os índices parasitológicos e os estágios de desenvolvimento imaturos e infecundos encontrados nos hospedeiros demonstram que se trata de uma infecção acidental. Isto se reflete na amplitude de variação extremamente baixa deste parasito, não permitindo assim o mesmo tipo de análise desenvolvido para as demais polimorfíases e para a contraqueíase. Diferentemente, também, várias coortes (cistacantos, formas juvenis e formas adultas em reprodução) de *C. australe* e *Bolbosoma* sp. e de *Contracaecum* sp. (larvas estágio 3: L-3 e estágio 4: L-4, formas juvenis e formas adultas em reprodução) foram encontradas na luz gastrointestinal dos hospedeiros, denotando processos de reinfecção constantes ao longo da vida dos hospedeiros e também a aptidão desta espécie hospedeira para abrigar esta espécie parasita.

Uma hipótese pode ser considerada para explicar a infecção acidental de *C. cetaceum*: a inadequada formação de microhabitat do estômago não compartimentalizado dos pinípedes (Ridgway 1972, Stewardson et al. 1999) em comparação com o estômago policompartimentalizado dos cetáceos (Ridgway 1972, Harrison et al. 1970, Smith 1972, Yamasaki et al. 1974). Outra hipótese pode explicar o porquê deste parasito se assentar na região pilórica do estômago de *A. australis*: a característica mucogênica do tipo glandular da região pilórica, que também é compartilhada pelo compartimento pilórico do estômago dos cetáceos (Harrison et al. 1970, Smith 1972, Yamasaki et al. 1974, Chaves & Silva 1988, Silva & Cousin 2006b). Esta diferenciação em microhabitas está de acordo com evidências de adequado desenvolvimento e maturação de *C. cetaceum* em espécies hospedeiras com estômagos policompartimentalizados e com compartimentos pilóricos de natureza

mucogênica como em *Pontoporia blainvillei* (Valente et al. 1997, Aznar et al. 2001, Sardella et al. 2005) e *Tursiops truncatus* (Aznar et al. 1999b). Em contrapartida, espécies hospedeiras de estômago monocompartimentalizados como *A. pusillus* apresentam estes parasitos imaturos (Aznar et al. 1999b) semelhantemente a *A. australis* (Sardella et al. 2005 e Anexo II).

Considerando os índices parasitológicos das infecções não acidentais em *A. australis*, as polimorfíases (por *C. australe* e *Bolbosoma* sp.) e a contracequíase demonstram particularidades quanto ao gênero sexual do hospedeiro e suas categorias de maturidade sexual (Anexos I e V). A corinosomíase (Anexo I) e a contracequíase (Anexo V) não demonstram diferenças estatísticas entre gêneros sexuais e categorias de maturidade sexual do hospedeiro. Todavia, a bolbosomíase difere em P% entre os gêneros sexuais, mas não difere entre categorias de maturidade sexual (Anexo I). Outra observação que deve ser ressaltada é a de que mesmo sem diferenças estatísticas observáveis, hospedeiros machos (todos ou somente os filhotes) claramente apresentam valores helmínticos maiores do que as fêmeas hospedeiras (todas ou somente as filhotes) (Anexos I e V). Desta forma, observando os altos valores da variância (s^2) com relação a media ($\bar{x}$) apresentados por *C. australe* (Anexo I) e os apontamentos ecológicos de Wirsing et al. (2007) para consideração de estoques parasitários ligados ao gênero sexual dos hospedeiros, no mínimo para esta espécie parasita, os *A. australis* machos podem ser sugeridos como estoques de ciclo de vida parasitários. No entanto, a contracequíase demonstra que as fêmeas hospedeiras reprodutivas-reprodutoras apresentam maiores valores do que os machos hospedeiros pré-púberes (Anexo V). Estas particularidades de infecção podem estar relacionadas a diferentes fatores (heterogeneidades) comportamentais e reprodutivos dos hospedeiros (Wilson et al. 2002) e que podem reger cada interação hospedeiro-parasito de modo extremamente particular, principalmente em espécies dimórfico-sexuais como os lobos-marinhos (Kunz et al. 1996, Bonner 1981, Fairbairn 1997,

Ferguson & Larivière 2008). Tais heterogenidades podem conduzir ao consumo diferenciado de hospedeiros intermediários ou paratênicos de cada espécie parasita, segundo características próprias de cada gênero sexual do hospedeiro à medida que crescem (Carey 1992, Horning & Trillmich 1999, Naya et al. 2002, Casper et al. 2006, Ciaputa & Sicinski 2006, Willis et al. 2008, Drago et al. 2009, Boren 2010), de acordo com mudanças nos requerimentos energéticos ligados à reprodução (Trillmich 1986ab, Atkinson 1997) e a influência dos hormônios sexuais sobre o sistema imune do hospedeiro (Messingham et al. 2001, Klein 2004, Morales-Montor et al. 2004, Bird et al. 2008).

Os polimorfídeos *C. australe* e *Bolbosoma* sp., que cohabitam o intestino delgado, demonstram constituir uma guilda e infectam, predominantemente, porções diferentes do órgão (de acordo com os valores de MI e MA). Apesar de não haverem diferenças estatísticas na distribuição destes parasitos (para P% e MI) ao longo das porções intestinais investigadas, graficamente os valores de MI e MA indicam que *C. australe* infecta principalmente a metade caudal do intestino delgado, enquanto que *Bolbosoma* sp. infecta predominantemente a porção cranial do órgão (Anexo I). Para o intestino grosso diferenças foram observadas para os valores do CRD da corinosomíase, mas foram interpretadas como reflexo dos altos valores da intensidade de infecção do intestino delgado (Anexo I). Curiosamente, *Bolbosoma* sp. agrupa diferentemente no intestino delgado de hospedeiros machos e fêmeas e também difere entre as porções cranial e caudal do intestino delgado dos hospedeiros machos. Neste contexto, pode-se hipotetizar que além da diminuição da competição dos parasitos por recursos e espaço (Mettrick & Podesta, 1974, Bush & Lotz 2000, Read 2000, Roberts 2000), assim como os processos de migração intraluminal de *C. australe* de acordo com as pressões e mecanismos de reprodução do parasito (Parshad & Crompton 1981, Aznar et al. 2004), é provável que existam diferenças no consumo de hospedeiros intermediários ou paratênicos deste parasito (Carey 1992, Gales et al. 1993, Horning & Trillmich 1986ab, 1999, Naya et al.

2002, Casper et al. 2006, Ciaputa & Sicinski 2006, Willis et al. 2008, Drago et al. 2009, Boren 2010). Outros fatores, como particularidades anatômicas (Aznar et al. 1999a) que resultam no modo de ancoragem e desancoragem (mais ou menos cabíveis) (Aznar et al. 1999a, Zdzitowiecki 1985, Dezfuli et al. 2002) de cada espécie analisada também podem estar envolvidas (Anexo I e III).

Nenhuma das espécies parasitas analisadas demonstrou diferenças sazonais quanto aos índices de P% e MI (Anexos I, II e V). Todavia *Bolbosoma* sp. foi a única espécie parasita que mostrou um padrão de agrupamento (CRD) sazonal diferenciado entre P-V e O-I (Anexo I). A ausência de padrões sazonais pode estar relacionada com a presença das diferentes coortes parasitas encontradas para as espécies investigadas, tais como *C. australe*, *Bolbosoma* sp. e *Contracaecum* sp. A ausência de sazonalidade para *C. cetaceum* pode ser explicada pelas evidências de infecção accidental por este parasito nesta espécie hospedeira.

Os resultados demonstram que o entendimento da uma comunidade parasita componente (ou assembleia parasita) de um hospedeiro deve ser espécie-específico, ou seja, relativo à espécie-alvo parasita, não sendo adequado fazer generalizações. Isto reforça as discussões e entendimentos de Margolis et al. (1982), Brooks & McLennan (1993), Zander (2001), Zander et al. (2002) e Wirsing et al. (2007), por exemplo. Desta forma, os trabalhos demonstram que algumas espécies parasitas, neste caso *Bolbosoma* sp., podem apresentar diferenças parasitológicas com relação ao gênero sexual do hospedeiro (*Hipótese 1*) que a condição sexual dos hospedeiros pode não influenciar nos níveis parasitêmicos de um hospedeiro (*Hipótese 2*) e que helmintíases podem não apresentar marcado período sazonal (*Hipótese 3*).

3.3. Histopatologia

As histopatologias causadas pelas polimorfíases (Anexos II, III e IV) e pela contracequíase (Anexo V) foram classificadas em não-inflamatórias e inflamatórias. Todas as histopatologias relacionadas com as polimorfíases em condição normal de assentamento parasitário (Anexos II e III) e em condição anormal de assentamento parasitário (Anexo IV) não são inflamatórias. Todavia a ação dos acantocéfalos sobre os tecidos gastrointestinais gera processos de diminuição significativa da espessura da mucosa dos órgãos nos assentamentos parasitários normais. Em suma, estes afinamentos das mucosas são marcados por degeneração das estruturas glandulares gástricas e pilóricas, assim como das criptas intestinais de Lieberkühn. A degeneração destas estruturas demanda grande quantidade de tecido necrótico e deformação glandular das áreas periféricas à ancoragem parasitária (Anexos II e III).

A histopatologia mostra que *C. australe* apresenta quatro padrões lesionais à mucosa intestinal (intestino delgado e grosso) (Anexo III). Três padrões estão diretamente relacionados à capacidade de ancoragem na mucosa em diferentes graus de aprofundamento corporal na mucosa intestinal (BDINA-1 ao 3) e que geram diferentes graus de anormalidade tecidual (sumarizado acima): O BDINA-1 é exclusivo do intestino delgado e o parasito ancora nas vilosidades; no BDINA-2 o parasito afeta as criptas de Lieberkühn em diversas profundidades e no BDINA-3 o parasito alcança a submucosa, destruindo toda a mucosa. O quarto tipo lesional relacionado ao *C. australe* compreende cavidades côncavas crateróides (CLCH) que representam locais de desancoragem do parasito. Estas evidências corroboram o estudo de Aznar et al. (2004) que explicam a capacidade migratória desta espécie parasita dentro da luz intestinal do hospedeiro. A histopatologia da ancoragem anormal de *C. australe* na serosa intestinal (Anexo IV) demonstra a importância da topografia da mucosa intestinal para a adequada ancoragem parasitária. Espécies de acantocéfalos com pressomas altamente

modificados e de invasão profunda nos tecidos de seus hospedeiros tendem a não desancorar (Zdzitowiecki 1985, Dezfuli et al. 2002). Ambos Anexos (III e IV) reforçam a influência da utilização do metassoma como aparato ancorador secundário, corroborando a hipótese de Aznar et al. (1999a) e a alta adaptação dos helmintos gastrointestinais ao seus nichos ao longo do tubo digestório (Hayunga 1991).

A contracequíase, entre as helmintíases analisadas neste trabalho (Anexos I, II, III e IV), foi a única que gerou nos hospedeiros lesões inflamatórias (Anexo V). Todavia foram encontrados cinco tipos principais de lesões nas regiões do corpo-fundo e píloro do estômago. Dois tipos de lesão não são severos: (1) locais de penetração do *Contracaecum* sp. na parede estomacal (CESW) onde o parasito pode estar parcialmente exposto fora da parede estomacal causando necrose e erosão da mucosa e (2) os locais de ancoragem-desancoragem pontual do *Contracaecum* sp. (CPADS) que apresenta um molde oral quitinoso na forma de cápsula com entalhes dos lábios do parasito (OPCA). A OPCA mostra que a estrutura do colar cefálico de *Contracaecum* sp. pode estar envolvida no processo de ancoragem dos parasitos adultos à mucosa gástrica. Outros dois tipos de lesão são inflamatórias (severas): as úlceras e os piogranulomas. As lesões inflamatórias apresentam severa exudatação celular (macrófagos, células gigantes de corpo estranho, linfócitos, eosinófilos e neutrófilos), fibrose, necrose da mucosa e submucosa e túneis de deslocamento parasitário na submucosa. As cicatrizes representam o último tipo lesional encontrado e demonstram extremo grau de fibrose cicatricional. Compreendem os processos de cura das lesões inflamatórias e algumas vezes ainda em estado de cura incompleta podem apresentar alguma infiltração celular imune. Todas as lesões inflamatórias resultaram em redução significativa da espessura da mucosa gástrica. A distribuição das lesões com o crescimento dos hospedeiros sugere que a aquisição destas lesões, respeite o mesmo padrão entre hospedeiros machos e fêmeas. A ausência de diferenças entre os gêneros sexuais quanto a quantidade de lesões gástricas reforça os

resultados da aquisição igualitária das lesões entre os gêneros. Todavia, somente machos desenvolveram piogranulomas. Com relação às dimensões das úlceras, a largura e o comprimento são maiores em machos hospedeiros do que em fêmeas hospedeiras.

Estes resultados demonstram que a ocupação de diferentes nichos intestinais por *C. australe* e *Bolbosoma* sp. pode ser analisada e discutida em bases histopatológicas (*Hipótese 4*) e que os gêneros sexuais e a maturidade sexual dos hospedeiros não influenciaram nos padrões quantitativos das lesões gástricas causadas por *Contracaecum* sp. (*Hipóteses 5 e 6*). Todavia a possibilidade de que diferentes tipos de lesões possam ocorrer entre os gêneros sexuais do hospedeiro (como por exemplo piogranulomas em machos) reforça a necessidade de se continuar avaliando os efeitos histopatológicos da contracequiose com relação ao gênero sexual dos hospedeiros.

4. CONCLUSÕES

Esta pesquisa permite concluir que:

- ▶ Índices parasitários gastrointestinais em *A. australis* respondem, diferentemente às influências do gênero sexual do hospedeiro (Anexos I, II e V).
- ▶ A condição sexual de *A. australis* não diferenciou as P% e as MI de *Corynosoma australe* e de *Contracaecum* sp., tanto dos hospedeiros machos como das fêmeas (Anexos I, II e V).
- ▶ Não houveram padrões sazonais observáveis para as P% e as MI das helmintíases analisadas, todavia outros índices parasitológicos como o Padrão de Agrupamento (CRD) podem ser considerados para estes parâmetros ecológicos em parasitoses (Anexos I, II e V).
- ▶ O parasito *C. cetaceum* compreende uma infecção acidental neste hospedeiro (Anexo II).

- ▶ Os parasitos *C. australe*, *Bolbosoma* sp. e *Contracaecum* sp. compreendem parasitos gastrointestinais não acidentais de *A. australis* (Anexos I e V).
- ▶ Hospedeiros machos adquirem Intensidades de Infecção maiores e mais precocemente de *C. australe* e *Bolbosoma* sp. do que fêmeas hospedeiras, conforme ambos crescem e maturam sexualmente (Anexo I).
- ▶ Fêmeas hospedeiras adquirem Intensidades de Infecção maiores e mais rapidamente de *Contracaecum* sp. do que machos hospedeiros, conforme ambos crescem e maturam sexualmente (Anexo V).
- ▶ Hospedeiros machos podem ser considerados estoques para *C. australe*.
- ▶ Os parasitos *C. australe* e *Bolbosoma* sp. constituem uma guilda na luz gastrointestinal e infectam nichos predominantemente diferentes do intestino delgado (Anexo I).
- ▶ A histopatologia constitui uma ferramenta para o entendimento de processos ecológicos de assentamento e distribuição parasitários (Anexo II).
- ▶ Corinosomíases não geram processos inflamatórios severos em *A. australis*, mas causam alterações teciduais significativas nas porções gastrointestinais afetadas (Anexos II, III e IV).
- ▶ Machos e fêmeas hospedeiros desenvolvem lesões por contracequíase de modo semelhante conforme ambos crescem (Anexo V).
- ▶ Lesões ligadas à contracequíase são de caráter inflamatório e não-inflamatório, todas causando alterações significativas na parede gástrica do hospedeiro (Anexo V).

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6. ANEXOS

ANEXO I

***Corynosoma australe* and *Bolbosoma* sp. (Acanthocephala, Polymorphidae) in
Arctocephalus australis (Mammalia, Pinnipedia) from Southern Brazilian coast:
parasitological indices, seasonality and host gender influences**

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Abstract - The parasitological variables [Prevalence (P%), Mean Intensity (MI), Mean Abundance (MA), Range of Variation (Ax) and Crowding value (CRD, s^2/x] of *Corynosoma australe* and *Bolbosoma* sp. in *Arctocephalus australis* are presented based on the necropsy of 30 specimens stranded dead on the shore of Rio Grande do Sul State – Brazil from August-2008 to September-2010. Differences for P% (Chi-square test, X^2) and MI (Bootstrap Confidence Interval, BC_a , $p < 0.05$) were analyzed for each parasite species: (1) for host's genders and host's reproductive status [males (pups, prepubertals) and females (pups)]; (2) for the two portions of the small intestine (anterior half and posterior half); (3) for the large intestine for host's genders and, (4) for the grouped seasons (Spring-Summer: S-S; Autumn-Winter: A-W). CDR values were analyzed as in 2 and 3. Hosts' gonads were histologically examined for sexual maturity according to spermatogenesis or folliculogenesis, luteogenesis and luteolysis. The corynosomiasis as well as bolbosomiasis, occurred along the sampling period. These acanthocephalosis showed higher values always for male hosts within all analyzed categories (gender, reproductive status, intestine portion) and their increased towards the host's adulthood. The two parasite species form an intestinal guild and occupy predominantly different intestinal parts with *Bolbosoma* sp. harboring within the first half and *C. australe* harboring within the last half. *Corynosoma australe* shows different infective cohorts uniformly distributed within the small intestine lumen and without seasonal pattern. There was gender-related difference for *C. australe*-CRD for the large intestine. This difference could be simply a result from the parasite's predominant niche occupation within the second half of the small intestine. Unlike, *Bolbosoma* sp. shows difference in the P% for host's genders and for host's gender-related small intestine. There are *Bolbosoma*-related CDR differences for host's genders, between first and last small intestine parts and grouped analyzed season, as well. The *Bolbosoma*-related differences may results of gender-related

diet differences, parasite's anatomic characteristics (detach-related incapacities) and by parasite's cohort-related maturational characteristics.

Key words: Acanthocephalosis, Polymorphidiasis, Mammalian host, Arctocephalinae, Southern Brazilian coast

Introduction

Acanthocephala as well as several parasite groups have heteroxenous life-cycle that, generally, comprises a specific host-parasite relationship. This host-parasite relationship is complex and encircles different trophic levels and their infective life-cycle stages, towards the reproductive success within an adequate site in the definitive host (Combes, 1997; Bush *et al.*, 2001). The helminth's small size, usually, difficult biological studies despite their extreme diversity and great importance to the health of their hosts (Raga *et al.*, 2002; Bush *et al.*, 2001).

Adult Acanthocephala normally settles and recruits within the intestinal lumen of endothermic vertebrates to finish adequately their life cycles (Crompton, 1973; Nickol, 1985; Bush *et al.*, 2001; Aznar *et al.*, 2004). Pinnipedia such as the South American fur seal *Arctocephalus australis* is among these vertebrates (Zimmermann, 1783) (Raga *et al.*, 2002; Morgades *et al.*, 2006). Exceptions for the Acanthocephala site of settlement and recruitment exist (Valente *et al.*, 1997; Aznar *et al.*, 2001). Concerning to the marine mammals that occur in Brazilian waters little is known about their parasitosis, parasitosis-related mortality and other parasite-host relationship aspects (Ruoppolo, 2003; Silva and Cousin, 2004; 2006a, b; Carvalho, 2009). Parasitosis is also recognized as a significant factor in massive strandings and mortality of Pinnipedia (Ponce de León, 2000; McKenzie *et al.*, 2005) but acanthocephalosis tends to be considered with low negative impact in Pinnipedia (Ridgway,

1972; Bonner, 1990; Vlasman and Campbell, 2003). Although the parasitism is a homoplastic life-style in several animal groups within several animal Phyla (or practically in any one) all parasites derive resources from their hosts (Bush *et al.*, 2001).

The South American fur seal *A. australis* is the Pinnipedia species that most often strand on the Southern Brazilian coast (Pinedo, 1990; Silva, 2004; Mäder *et al.*, 2006). This definitive mammalian host species is widely distributed in South America from the Atlantic Ocean (Rio de Janeiro – Brazil), southward for the Pacific Ocean (Tierra del Fuego) and northward to Lima – Peru (Bonner, 1981; Vaz-Ferreira, 1982). Despite the strong commercial exploitation suffered by the Arctocephalinae during the XVIII-XIX centuries (Bonner, 1981; Vaz-Ferreira and Ponce de Leon, 1987) and *a posteriori* focused studies for population dynamics (Wynen *et al.*, 2000; Ferreira *et al.*, 2008) there are several gaps about this mammalian group. Concerning to *A. australis*, the diet (Santos and Haimovici, 2001; Naya *et al.*, 2002) and the parasitism (Morgades *et al.* 2006) and parasite-related tissue changes are scarcely contemplated. Hosts with wider demographic ranges, such as *A. australis*, tend to demonstrate higher parasite's species richness (Bush *et al.*, 2001). Parasitosis such as arthropodiasis, nematodiasis and acanthocephalosis are driven by host's intrinsic features such as age and sex as well as by intra- inter-specific parasite relationships and tend to be male-biased according to the parasitological indices (infection levels) or parasite distribution patterns (pattern of parasite spread within the host population) on several animal host groups (Klein, 2004; Cowan *et al.*, 2007; Ferrari *et al.*, 2007). However other helminthiasis such as cestodiasis (Barriga and Al-Khalidi, 1991; Escobedo *et al.*, 2004, Morales-Montor *et al.*, 2004) or even some nematodiasis (Rossin *et al.*, 2010) tends to be female-biased .

For the Pinnipedia-related acanthocephalosis, the infection of *A. australis* by *Corynosoma* spp. appears to be the best studied group in ecological and parasitological aspects (George-Nascimento and Marín, 1992; Aznar *et al.*, 1999a, b; 2004; Nickol *et al.*,

2002; Sinisalo *et al.*, 2004; Valtonen *et al.*, 2004; Sardella *et al.*, 2005) but other Acanthocephala, such as *Bolbosoma* sp., are poorly contemplated (Morgades *et al.*, 2006). On the other hand, is scarce the number of studies that contemplate parasitological indices in Pinnipedia.

The parasitological indices of *C. australe* and *Bolbosoma* sp. in *A. australis* are presented for host's gender, host's sexual maturity status and seasonality.

Materials and Methods

Hosts sampling: The hosts specimens (15male: 15female) were collected from the Cassino Beach (ca. 32°11'14.23"S; 52°09'21.70"W) to Chui (ca. 33°44'35.96"S; 53°22'12.70"W) - Rio Grande do Sul – Brazil. The sex of the hosts was determined by external examination and their total lengths (TL) was measured linearly in meters (m) (*sensu* Dierauf 1994). Samplings for hosts were performed within two major grouped seasons, *i.e.*, Autumn-Winter (A-W) and Spring-Summer (S-S) from August-2008 to September-2010.

The conservation status of carcasses utilized followed Dierauf (1994). Only carcasses between Dierauf's code 2 and 3 were utilized in this study. Additionally to the Dierauf's procedures for hosts specimens samplings, other *post-mortem* alterations were observed, such as *rigor-mortis*, *livor-mortis*, abdominal expansion by decomposition gases and absence of decomposers (as larval forms of insects) (Thomson 1983).

The necropsy procedure for the opening of the thoracic and abdominal cavities, for the removal of digestive system, for the removal of the reproductive organs and for the gross analyses of the organs, followed Dierauf (1994). Thus, the digestive tube was divided in its anatomical parts (esophagus and stomach, small and large intestines). These parts were tied, labeled, bagged and frozen for the parasite screening. The small intestine was separated from

the mesentery, linearly measured and divided in a cranial portion and other caudal portion for analyze the parasite's distribution.

Host's sexual maturation: Male gonads (both left and right) with epididymis and the entire reproductive tract of females were extracted for analysis of sexual maturity. The stereomicroscopy in the female gonads was performed to observe ovarian follicles, luteogenesis and the luteolysis *sensu* Bukovsky *et al.* (2005), Katz *et al.* (2009), Lucacin and Pinto-Neto (2009) and Palma-Cerda *et al.* (2011). Ovaries (both left and right) were separate from the uterus and ovarian sections (1-2mm) were performed and observed for folliculogenesis. The histomorphology of the spermatogenesis followed Wistuba *et al.* (2007).

Histological protocols: Histological sampling of the testicles was made transversally in the center of the gonad in order to sample the epididymis. Midsagittal sections from both ovaries were submitted to histological routine. Tissue samplings from the intestines and gonads, were fixed (Bouin's fluid) for histological routine for paraffin-embedding; sectioning (7µm thickness); staining (Hematoxylin-Eosin) and permanent mounting in balsam (*sensu* Silva and Cousin 2004, 2006ab). According to the spermatogenesis, male host's can be categorized as: pups (spermatogonia and spermatocytes), prepubertal (spermatids); pubertal (spermatozoa within seminiferous tubules = testicular spermatozoa) and adult reproductive (spermatozoa within epididymis) (Wistuba *et al.* 2007). Host's female sexual maturity can be categorized by the follicle-type population within ovarian stroma as: pups (mainly primary follicles presence); prepubertal (Antral and Graafian follicles presence) and adult reproductive-breeding females (corpus luteum-corpus albicans presence) (Bukovsky *et al.* 2005, Katz *et al.* 2009, Lucacin and Pinto-Neto 2009, Palma-Cerda *et al.* 2011).

Parasite protocols: The entire intestine was extracted from the abdominal cavity. The small and large intestines were separated. Each one was linearly measured in meters (m). The small intestine was cut in two parts of equal lengths. Each small intestine half was divided in

subsets to be opened and washed into a plastic bowl. The parasites were detached from the intestine wall according to each subset from each small intestine half. The content from the plastic bowl for each subset from each intestine half was sieved and observed under stereomicroscope for parasite's screening. Parasites were collected and counted according to each half of the intestine as well as for the large intestine.

The parasites were prepared for permanent mounting in balsam and staining (Semichon's Carmim and Eosin) following and adapting from Amato *et al.* (1991) and Silva and Cousin (2006a, b). Parasite identification was performed *sensu* Petrochenko (1971), Pereira Jr. and Neves (1993) and Sardella *et al.* (2005) holdfast criteria: proboscis oncotaxia (structure and distribution pattern of the hooks), body shape, trunk (presoma and metasoma) spine (somatic spine's structure and distribution pattern) and organology. For all hosts, the parasites were observed for their ontogenetic stage, *i.e.* cystacanth forms (the final infective stage found in intermediated host towards a potential definitive host) and adult forms *sensu* Nickol (1985). Females were observed for fertilization and maturity status (mated; non-mated) *sensu* Sinisalo *et al.* (2004) and Valtonen *et al.* (2004) criteria for eggs (sheltered acanthors) within body cavity. Fertilized females were interpreted as those containing developing-developed acanthors and mature females as those containing gonads, uterine and vaginal structures completely formed.

The hosts individual Parasite Intensity of Infection (PII), *i.e.*, the raw parasite values of infection (small+large intestine) by each host, was performed for male and female hosts' length (Regression Analysis) to provide the Dispersion Tendency Line (DTL) ($y=a+bx$; for the slope value) for each host gender (Vieira, 1981; Silva and Pinto, 2011).

Parasitological indices such as Prevalence (P%), Mean Intensity (MI) and Mean Abundance (MA) (Bush *et al.*, 1997) were determined and analyzed following Rózsa *et al.* (2000) by the "Quantitative Parasitology Program - QP 3.0 Program" for total sampled hosts,

host's gender, host's gender sexual maturity status, small intestine, large intestine, small intestine anterior half and small intestine posterior half. The P% (Chi-square test, X^2) and MI (Bootstrap confidence interval, BC_a , $p < 0.05$) were compared between host's gender (total males vs. total females), host's gender maturity (male pup vs. prepubertal males; male pups vs. female pups) for the small as well as for large intestines. The small intestine halves were compared for host's genders and among themselves. Female pups and reproductive-breeding females as well as prepubertal males and reproductive-breeding females were not compared due to low number of reproductive-breeding females in sample. The Range of Variation (Ax) of the parasite was expressed as Bush *et al.* (2001).

The distributional patterns, *i.e.* random, uniform or aggregated, of *C. australe* and *Bolbosoma* sp. were performed *sensu* Von Zuben (1997), Rózsa *et al.* (2000), Bush *et al.* (2001) and Reiczigel *et al.* (2005) for intestines (small and large) and small intestine portions (anterior and posterior halves) between males and females hosts. The distributional pattern respects the Mean ($\bar{x}$), Variance (s^2) and Variance/Mean ($s^2/\bar{x}$) ratios, where: $s^2 \approx \bar{x}$, represents a random distribution; $s^2 < \bar{x}$, represents a uniform distribution and $s^2 > \bar{x}$, represents an aggregated distribution (Crowded – CRD). The statistical significance for goodness of fit was assessed by Chi-square test (X^2) (Von Zuben, 1997; Rózsa *et al.*, 2000; Bush *et al.*, 2001; Reiczigel *et al.*, 2005).

Seasonal variations for the P% and MI were compared between A-W and S-S grouped seasons for all hosts and sampling periods.

Results

Hosts' Infrapopulations characteristics

The hosts' TL range was: $0.84\text{m} \geq \text{TL} \leq 1.76\text{m}$ for males and $0.8\text{m} \geq \text{TL} \leq 1.34\text{m}$ for females.

The *C. australe* infection, through the sample period, was marked by high numbers of cystacanth forms concomitantly with adult forms for both sexes what represents uninterrupted host re-infections. Females comprised mated and non-mated specimens. *Bolbosoma* sp. comprised cystacanth forms and adult forms, as well. The cystacanth forms/adult forms ratio was not considered for both parasites.

Corynosoma australe was found in great number free in the intestinal lumen unlike of *Bolbosoma* sp. found, mainly, deeply attached within the intestinal mucosa by the bulb-like presoma (foretrunk). As result of *Bolbosoma* sp. deeply attachment, this parasite was extracted from the intestinal mucosa by utilization of pincers.

Parasites distribution according to host's TL

The size of male and female hosts infected with *C. australe*, were $0.95\text{m} \geq \text{TL} \leq 1.76\text{m}$ and $0.87\text{m} \geq \text{TL} \leq 1.34\text{m}$, respectively. For *Bolbosoma* sp. the hosts' lengths were $0.95\text{m} \geq \text{TL} \leq 1.76$ for males and 0.98m and 1.33m for the two infected females, respectively. Thus, hosts with $\text{TL} \leq 0.94\text{m}$ (for male) and $\text{TL} \leq 0.86\text{m}$ (for females) do not harbor these Acanthocephala species (Figs. 1 and 2).

Both parasites species show similar pattern of distribution according to the hosts' TL. There are two observed patterns of parasite distribution. Pups of both sexes have a similar initial infection pattern with higher number of parasites within a 0.9m - 1.0m TL range. On the other hand, prepubertal males ($\text{TL} > 1.32\text{m}$) tend to show the higher parasite distributional values within all sampled hosts (Figs. 1 and 2).

Concerning to the DTL for the PII, male hosts show the highest values for both *C. australe* ($b=8240.3$) and *Bolbosoma* sp. ($b=90.58$). For female hosts the *C. australe* value comprised $b=1971.9$ and for *Bolbosoma* sp. $b=26.32$. These values show that the PII in male hosts tends to increase several times more than in female hosts. *Corynosoma australe*

infection increased four times more in males of *A. australe* than females, while *Bolbosoma* sp. infection increasing in males hosts is almost 3.5 times more than in female hosts.

Gender-related parasitological indices

Sampled hosts comprised males and females pups as well as prepubertal males and reproductive-breeding females. The parasitological variables [(P%, MI, MA, Ax and Crowding value (s^2/x)] for the total sampled hosts are showed in the Table 1. *Corynosoma australe* shows higher values for all parasitological variables.

Concerning to the hosts' genders and their analyzed categories of sexual maturity status, males always showed higher values for P%, MI, MA and Ax for both parasite species, resulting in a male-biased infection pattern, *i.e.*, males harbor more parasites than females. There were no gender-related differences for P% and MI concerning to *C. australe* infection, however there were gender-related differences for P% for the *Bolbosoma* sp. infection (Table 2).

Intestine-related parasitological indices

Analyzing parasites species concerning to the small and large intestines and the two portions of the small intestine (anterior half and posterior half), *C. australe* did not show differences between hosts' genders concerning to the P% as well as for MI for the analyzed intestinal categories. However *Bolbosoma* sp. shows gender-related difference for P% for the small intestine. Moreover, P%, MI, MA and Ax were several times higher for the small intestine as well as its portions than for large intestine (Table 3).

On the other hand, graphically MI and MA show a male-biased pattern of infection. Whether for only infected hosts (MI) or the entire sampled hosts (MA), male hosts harbor higher number of each parasite species than female hosts according to intestines as well as small intestine portions. Female hosts tend to maintain lowest parasite loads for both parasite species. Moreover, MI and MA show that *C. australe* and *Bolbosoma* sp. harbor

predominantly in different small intestine portions. *Corynosoma australe* shows its MI and MA higher values (higher harboring) for the posterior half of the small intestine (small intestine half-2) while *Bolbosoma* sp. shows this parasitological indices with higher values for the first half of the small intestine (small intestine half-1) (Figs. 3-6) occupying relatively well-established intestinal niches.

Corynosoma australe does not demonstrate gender-related differences for crowding value (CRD) for the small intestine and their portions and its aggregations (harboring) are higher for these digestive tube areas in male hosts. On the other hand, for the large intestine *C. australe* showed differences for crowding value (s^2/x) according to the hosts' genders. Moreover, *Bolbosoma* sp. showed gender-related difference for the small intestine and for the two small intestine portions of male hosts (Table 4).

Seasonally, there were no differences for P% or MI between A-W and S-S for both parasites, denoting that the infection occurs along the year. Moreover, the Crowding value (CRD) for *Bolbosoma* sp. shows seasonal differences, inferring that this parasite settles and recruits more robustly during the A-W period (Table 5).

Discussion

Epidemiologists (and epizootiologists) recognize across a range of vertebrate hosts and parasite taxa, as well as humans, that male hosts tend to harbor higher rates of parasitism and diseases than females (Wilson *et al.*, 2002; Klein, 2004). The parasitological indices as well as the number of parasite species that a host species can harbor normally vary (Bush *et al.*, 2001). This universal feature results from several heterogeneities such as dispersion of infective stages, parasite-host fitness (genetic and immunity), intra- and inter-specific parasite interactions, host body condition, host sex, host age, host behaviour and environmental stochastic phenomena (Crompton, 1973; Combes, 1997; Wilson *et al.*, 2002; Dybdahl and

Storter, 2003; Klein, 2004; Poulin, 2001; 2005). All analyzed parasitological variables (PII distribution, P%, MI, MA, Ax, and CRD) for all intestinal categories in this work indicate that corynosomiasis (= *C. australe*) and bolbosomiasis are male-biased in *A. australis*. Other studies for other parasitic groups corroborate the male-biased tendency of parasitism (Klein, 2004; Cowan *et al.*, 2007; Ferrari *et al.*, 2007) as well as for *C. australe* (Aznar *et al.*, 2004).

The aggregated distributional PII of both parasites within the pups probably is related with the weaning and the beginning of the ingestion of solid food. The lactation in Otariidae is prolonged (4-36 months) reflecting in ample ways of pups' maternal attendance with a gradual weaning and gradual development of aquatic skills by pups to forage on their own towards definitive weaning according to body mass acquisition (David and Rand, 1986; Doidge *et al.*, 1986; Trillmich, 1986a, b; Baylis *et al.*, 2005; Gastebois *et al.*, 2011). Vaz-Ferreira and Ponce de León (1987) record for *A. australe* 6-12 months of lactation. For *A. australis* from Uruguayan coast, Katz *et al.* (2009) record 60-80cm of TL range for infant (sucking) specimens, 68-94cm of TL range for weaned female specimens and 90-115cm for immature (prepubertal) females. For the present study, these reproductive and ontogenetic works corroborate the TL range and reproductive status findings for sampled specimens within pup category. Aznar *et al.* (2004) record that *A. australis* lesser than one year old were already infected and calves would be infected in the first ingestion of solid food as shown in this work.

For vertebrates the energy requirements increase proportionally with the body mass and the intestinal length can indicate the metabolic rate of an organism according to the food resource, *i.e.*, herbivore, carnivore or omnivore (Odum, 1988; Pough *et al.*, 1993; Schmidt-Nielsen, 2002). If ageing implies in increasing of the body size (until the asymptotic maximum), older animals are potentially more exposed to re-infections than young animals because they feed for longer life time and they possess larger surface areas (allometrically, in

this case, gastrointestinal surface) what can contribute for higher infection loads (Wilson *et al.*, 2002; Cowan *et al.*, 2007). Otariidae comprises a mammalian group with male-biased sexual dimorphism in body size and/or body weight (Kunz *et al.*, 1996; Atkinson, 1997) toward the hyperallometry of organs and structures associated with harem dominance under strong male sexual selection (Clinton and Le Boeuf, 1993; Fairbairn, 1997; Isaac, 2005) as other Carnivora (Gittleman, 1986; Ferguson and Larivière, 2008). Studies on mammals show association between sexual size dimorphism and sexual differences for diseases (Moore and Wilson, 2002; Klein, 2004). The tendency for higher *C. australe*-related and *Bolbosoma*-related parasitic loads in males of *A. australis* according to the growth toward adulthood stasis (=ageing), *i.e.*, as the growth continues for males and ends for females corroborate the observations above. On the other hand, the acquired immunity developed in response to accumulated antigen contact, *i.e.*, parasite constant contact acts to decrease parasite establishment, survival, reproduction and/or maturation (Combes, 1997; Wilson *et al.*, 2002; Dybdahl and Storter, 2003). Female hosts exhibited lower (P%) and stable parasite loads (DTL, MI and MA) probably due to their more efficient immune system than males (Klein, 2004) considering the gender-related growth patterns and implications as discussed for male hosts above.

Parasitological indices, such as P%, explain the parasite fitness upon a host species (Gregory and Blackburn, 1991; Bush *et al.*, 2001) as a variable to help the natural selection pressure (Anderson and Gordon, 1982; Anderson and May, 1982). The higher parasitological indices (P%, MI and MA), Ax and Crowding values (aggregation or harboring) presented in this work corroborate the adequate fitness of *C. australe* for *A. australis*. Moreover the presence of parasite's different cohorts reinforce previous studies that consider this parasite as part of the helminth gastrointestinal community for this mammalian host *sensu* the structure of parasite's population and its recruitment patterns (Aznar *et al.*, 2004). *Corynosoma*

australe has been reported in sympatrically species of marine mammals from the Southern Brazilian coast, such as *Pontoporia blainvillei* (Cetacea) (Valente *et al.*, 1997) and *Otaria flavescens* (Pereira 2012). Moreover, as well as Morgades *et al.* (2006) other Acanthocephala are recorded here for this Arctocephalinae such as *Bolbosoma* sp. and *C. cetaceum*. Qualitative (number of parasite species) and/or quantitative (parasitological values) differences could be related with the short range time of sampling developed by Aznar *et al.* (2004) only for 1-2 days of August (austral winter), unlike the present work and Morgades *et al.* (2006) with interannual higher sample range.

The gender-related host's ontogeny (pup-toward-prepubertal male or pup-toward-breeding-reproductive female) of *A. australis* demonstrates that none of Acanthocephala species differ for the analyzed parasitological indices. Similar results, *i.e.*, no observed differences for *C. australe*-related and *B. turbinella*-related MI in sub-adults and adults (mainly males) of *Otaria flavescens* (Pinnipedia, Otariidae) were recorded by Pereira (2012). This could suggest that *C. australe* and *Bolbosoma* sp. infect similarly these sympatric hosts. It can also be considered that both Otariidae hosts share in their diets at least intermediate (or paratenic) host species of these two Acanthocephala. *Arctocephalus australis* forages over a wide area of the continental shelf and beyond preying on Engraulidae (Argentine anchovy *Engraulis anchoita*, anchovy *Anchoa marmorata*), Sciaenidae (*Cynoscyon striatus*, weakfish *C. gatlucupa*, Argentine croaker *Umbrina canosai*), Scombridae (chub mackerel *Scomber japonicus*), Stromateidae (harvestfish *Peprilus* sp.) and Trichiuridae (*Trichiurus lepturus*) fishes as well as invertebrates (Cephalopoda, Bivalvia, Gastropoda and Crustacea) (Vaz-Ferreira, 1982; Santos and Haimovici, 2001; Naya *et al.*, 2002; Martínez, 2009). For the Uruguayan coast (Isla de Lobos), the main preys were Cephalopoda and the diet quantitatively changes between years may due to availability of fish species and Cephalopoda (Naya *et al.*, 2002). For Brazilian coast, the Cephalopoda *Loligo sanpaulensis* and *Argonauta*

nodosa comprise important prey for *A. australis*. Prey items such as *C. striatus*, *T. lepturus*, *E. anchoita* and *L. sanpaulensis* are overlapped with the sympatric *O. flavescens* (Falco, 2008; Drago *et al.*, 2009). Isotope evidence suggests that benthic preys are more consumed from post-weaning to adulthood in both sexes of *O. flavescens* (Drago *et al.*, 2009). *Otaria flavescens*-related prey such as whitemouth croaker *Micropogonias furnieri* and Argentine croaker *U. canosai* harbor acanthella of *Corynosoma* sp. and *C. australe* (Pereira Jr. and Neves, 1993; Anderson and Amato, 1998; Alarcos and Etchegoin, 2010). For Brazilian coastal fish species, there are report of occurrences of *Corynosoma* (=Polymorphus) spp. and *Bolbosoma* sp. for *C. guatucupa*, *T. lepturus*, *S. japonicus* and *M. furnieri* (Rego and Santos, 1983; Pereira Jr. and Neves, 1993; Sardella *et al.*, 2005; Santos *et al.*, 2008; Alarcos and Etchegoin, 2010). Southern Ocean predator such as marine mammals and birds widely depend on Cephalopoda (Clarke, 1996; Santos and Haimovici, 2001; Xavier *et al.*, 2011). Cephalopoda prey millions of tons of Euphausiidae and forms essential prey for top marine predators (Clarke, 1996). Measures (1992) and Dailey and Brownell Jr. (1972) discuss several Mysticeti Copepoda- Euphausiidae-specialists as typically harboring *Bolbosoma* spp. Moreover, Ziphiidae Cephalopoda-specialists are recorded for bolbosomiasis (Dailey and Brownell Jr., 1972). Thus, the Cephalopoda contribution for bolbosomiasis in *A. australis* should be considered.

Concerning to the entire small intestine, the parasitological variables values for P%, MI, MA and CRD show that *C. australe* is widely distributed along of the small intestine of both host's genders. Conversely, *Bolbosoma* sp. shows gender-related (male-biased) differences for P% and CRD and these results could reflect different ratio of predation between males and females hosts on intermediate or paratenic hosts that harbor *Bolbosoma* sp. cystacanth forms (Rego and Santos, 1983; Pereira Jr. and Neves, 1993; Sardella *et al.*, 2005; Santos *et al.*, 2008) that will settle and recruit in *A. australe*. Changes in proportionalities of prey items due

to variables such as seasonality, lunar cycle and diving skills, locations are recorded for Arctocephalinae such as *A. pussillus* (Gales *et al.*, 1993), *A. forsteri* (Carey, 1992; Boren, 2010), *A. galapagoensis* (Horning and Trillmich, 1999) and *A. gazella* (Ciaputa and Sicinski, 2006).

In a general point of view, the mammalian intestine can be divided in two principal anatomic and functional components: the small and large intestine (Gartner and Hiatt, 1999). For the studies of helminthiasis in aquatic mastozoological fauna, generally, the small intestine is divided in subsets to research the pattern of parasite's dispersion along the intestinal lumen (Valente *et al.*, 1997; Aznar *et al.*, 2004; Rocha, 2010; Pereira, 2012). The small intestine can be divided in two subsets, the jejunum and ileum, each one with a main (but not exclusive) physiological digestive function (Pácha, 2000). This intestinal physiology can influence the dispersion pattern of intestinal parasites according to parasite nutritional and maturational requirements (Mettrick and Podesta, 1974; Bush *et al.*, 2001) and corroborating observed distributional pattern for both Acanthocephala within entire small intestine. There is histological evidence that *C. australe* is able to attach-detach several times life-long from the intestine mucosa exhibiting a gradual pattern of mucosal embedding, corroborating studies for intestine luminal reproductive, nutritional and crowding patterns of parasite migration jejunum-to-ileum-ward (Mettrick and Podesta, 1974, Parshad and Crompton, 1981; George-Nascimento and Marin, 1992; Aznar *et al.*, 2004). Anatomical and histological studies for Polymorphidae holdfast apparatus (Aznar *et al.*, 1999a) reinforce attach-detach capacities in *Corynosoma australe* and this parasite feature should be considered to explain absence of differences for parasitological variables between small intestine portions. Similar result is reported by Pereira (2012) for small intestine (divided in three portions) of *O. flavescens* infected by *C. australe*. Regarding the intestinal parasite niche, MI and MA for both parasites and CRD for *Bolbosoma* sp., show that *C. australe* and *Bolbosoma* sp. harbor predominantly,

but not exclusively, in different intestine portions (*i.e.*, physiological portions) and forming an intestinal guild (*sensu* Bush *et al.*, 2001; Zander, 2001). Concerning to the intestinal division proposed by Aznar *et al.* (2004), it can be observed that *C. australe* harbor more intensely in the second half of the small intestine of *A. australis*. Similar pattern was observed in this work. Pereira (2012) records that *Bolbosoma turbinella* harbored mainly in the first two thirds of the small intestine of *O. flavescens*. This distributional tendency for both parasite's species can mean a way of avoid (or attenuate) the competition by host's resources as well as observed for several gastrointestinal parasite groups and their spatiotemporal requirements such as nutrition, gaseous exchanges, growing and reproduction (Mettrick and Podesta, 1974; Von Zuben, 1997; Bush and Lotz, 2000; Roberts, 2000; Poulin, 2001, 2005; Wilson *et al.*, 2002; Cattadori *et al.*, 2008).

Attachment anatomic-related mechanisms (holdfast apparatus) for Acanthocephala as well as intestinal physiological characteristics could also influence the distributional pattern within intestinal length. Acanthocephala was ecologically selected toward a successful performance of the attachment mechanism (Petrochenko, 1971; Taraschewski *et al.*, 1989; Taraschewski, 1990; Aznar *et al.*, 1999a). The morphology of some Acanthocephala species permits deep penetration within the digestive tube wall what causes extensive tissue damage (Petrochenko, 1971; Dezfuli *et al.*, 2002). Unlike attach-detach capacities described above for *C. australe*, *Bolbosoma* spp. attach very severely and deep in the host's tissues due to anatomical configuration of the presoma (foretrunk) (Petrochenko, 1971). Dailey *et al.* (2000) record for *Eschrichtius robustus* (Cetacea, Eschrichtiidae) multifocal transmural abscesses caused by encapsulated broke proboscis region of *B. balanae* along the ileum. Deep bulb-like presoma intestine mucosal embedding of *B. turbinella* is reported for *Balaenoptera musculus* (Measures, 1992). Other Acanthocephala such as *Profilicolis antarcticus* (Profilicolidae) from with a globular proboscis from *Clionis alba* (Zdzitowiecki, 1985) and *Pomphorhynchus laevis*

(Pomphorynchidae) with presomal rivet-like mode of attachment from the European chub *Leuciscus cephalus* (= *Squalius cephalus*) (Cyprinidae) (Dezfuli *et al.*, 2002), show anatomic peculiarities that avoid detachment. Thus the non-detaching evidence for *Bolbosoma* sp. can result in a early settlement and recruitment within the first meters of the small intestine and reduced or null migration capacity along the intestine lumen.

The large intestine host's gender-related differences observed for *C. australe* can be merely proportional. If there are more parasites within the small intestine of males is expected that more parasites also occurs within the large intestine. *Corynosoma australe* as well as other infrapopulations of Acanthocephala species are female-biased (Poulin, 1997, Aznar *et al.* 2004). Aznar *et al.* (2004) show that the fifth portion of the small intestine has the highest intensities of *C. australe* gravid females then the specimens present in the large intestine could represent mainly females on final reproductive cycle.

Concerning to the seasonality, for *C. australe* the results show that this parasite is non-seasonal, *i.e.*, occurs throughout the year, reinforcing the uniformity of harboring for host's genders according to the different cohorts within the intestinal lumen, P%, MI, MA and CRD values. *Bolbosoma* sp., on the other hand, shows difference for CRD values between grouped seasons, reinforcing the seasonality of this parasite in *A. australis* which could be reflected in the uniformity of the sampled parasite's cohort within the intestinal lumen of *A. australis*.

In summary, male hosts harbor the highest parasites loads than female hosts; *C. australe* is non-seasonal and does not differ according to host's genders; *Bolbosoma* sp. is seasonal and differs according to host's genders and small intestine portions in male hosts and; the two parasites occupy different small intestine portions with *Bolbosoma* sp. predominantly anteriad-half and *C. australe* posteriad-half.

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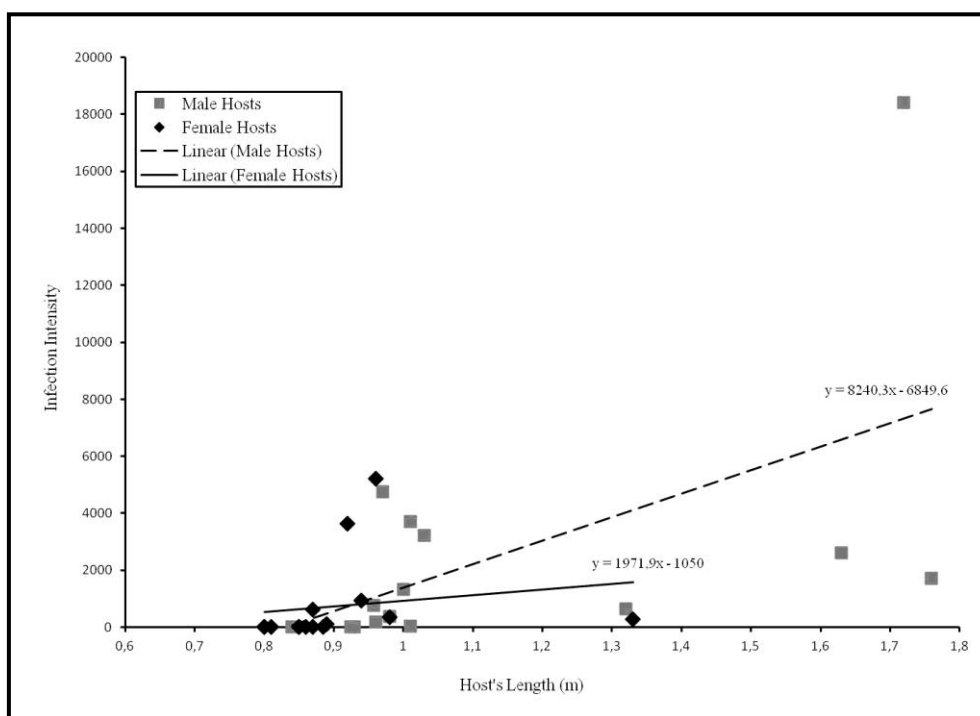


Figure 1. Distribution of the Infection Intensity (PII) of *Corynosoma australe* in *Arctocephalus australis* (n=30; 1male:1female) from Southern Brazilian coast according to host's gender length.

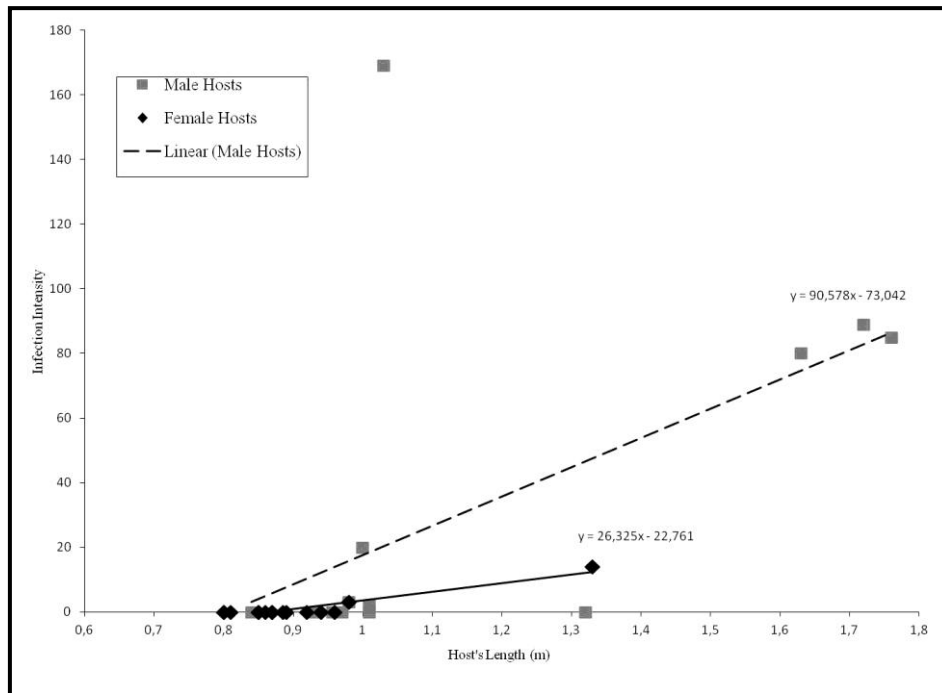


Figure 2. Distribution of the Infection Intensity (PII) of *Bolbosoma* sp. in *Arctocephalus australis* (n=30; 1male:1female) from Southern Brazilian coast according to host's gender length.

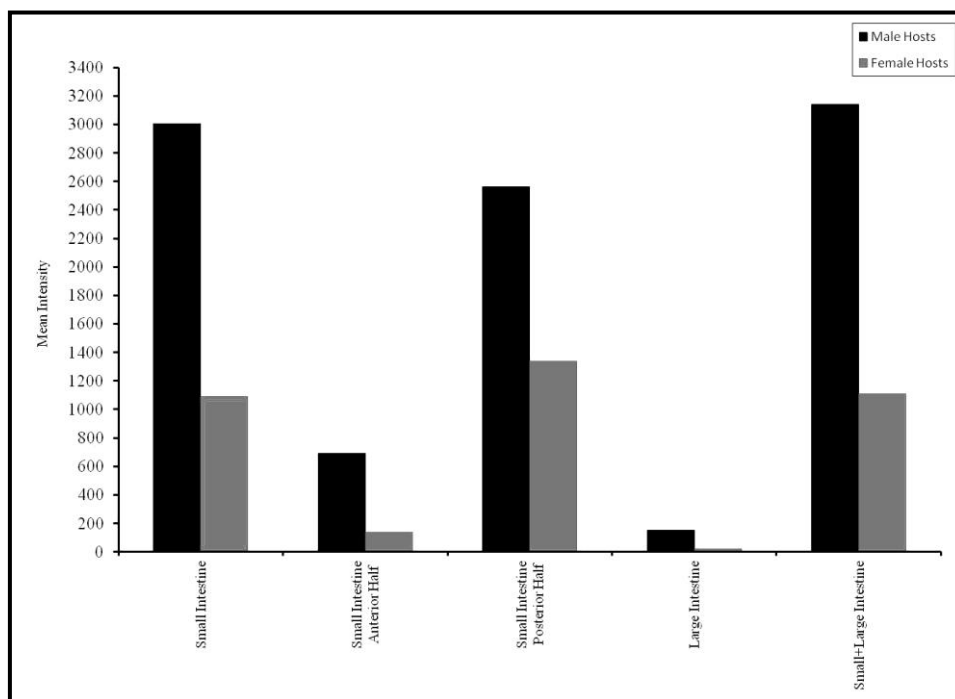


Figure 3. Mean Intensity of *Corynosoma australe* within intestines (small+large), small intestine and small intestine halves (anterior and posterior) and large intestine of *Arctocephalus australis* from Southern Brazilian coast.

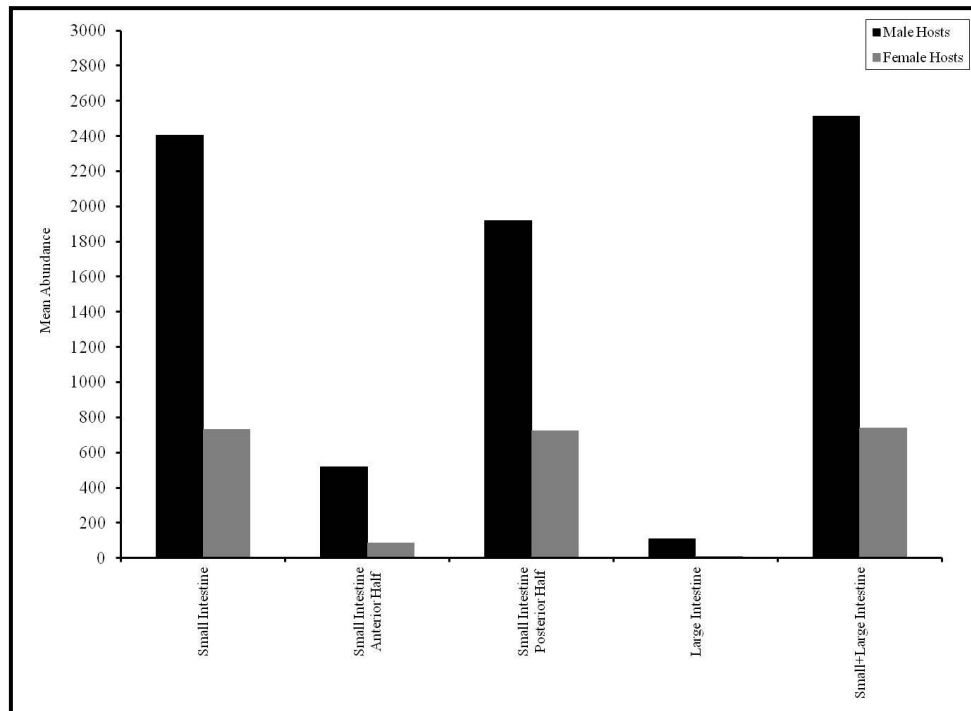


Figure 4. Mean Abundance of *Corynosoma australe* within intestines (small+large), small intestine and small intestine halves (anterior and posterior) and large intestine of *Arctocephalus australis* from Southern Brazilian coast.

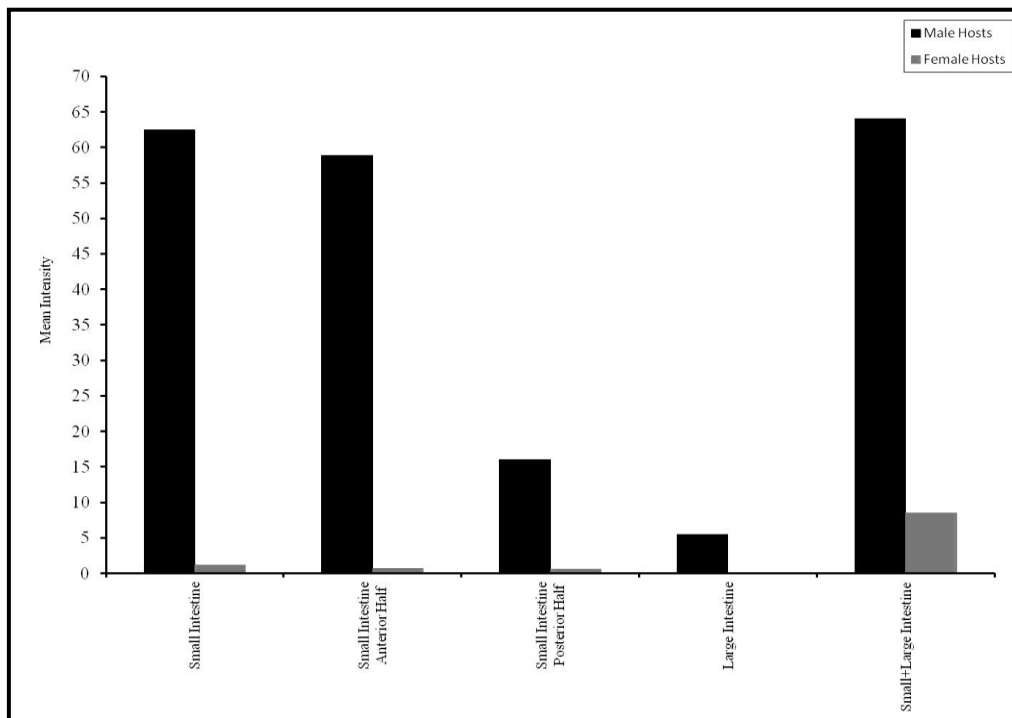


Figure 5. Mean Intensity of *Bolbosoma* sp. within intestines (small+large), small intestine and small intestine halves (anterior and posterior) and large intestine of *Arctocephalus australis* from Southern Brazilian coast.

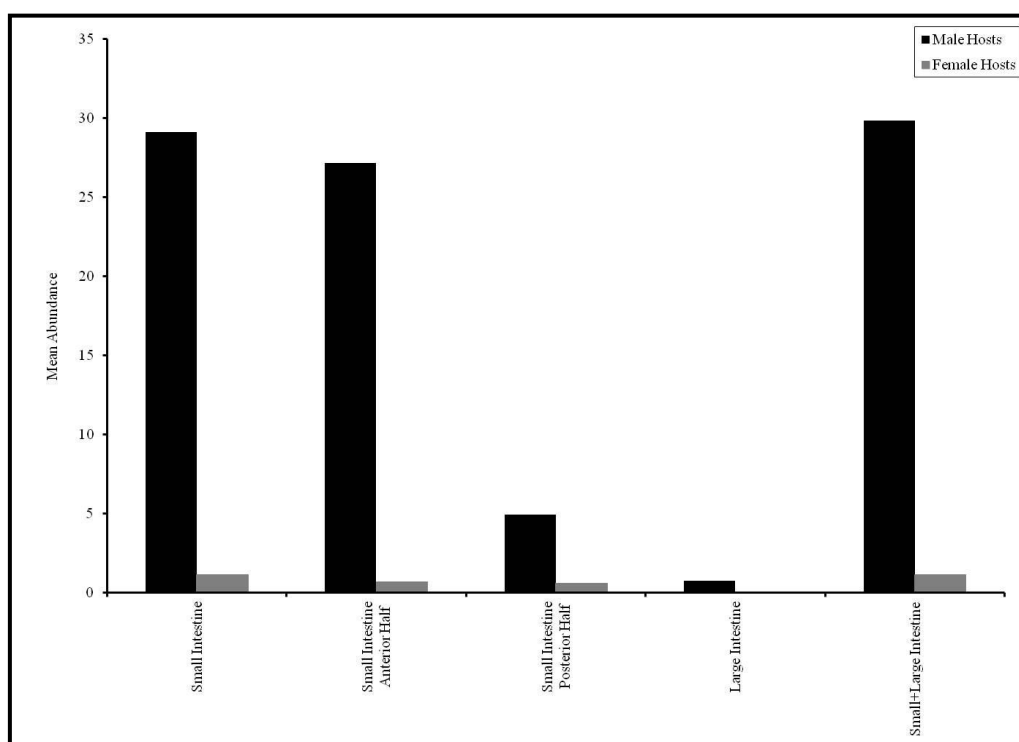


Figure 6. Mean Abundance of *Bolbosoma* sp. within intestines (small+large), small intestine, small intestine halves (anterior and posterior) and large intestine of *Arctocephalus australis* from Southern Brazilian coast.

Table 1. Parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA), Range of Variation (Ax) and Crowding Value robustness (CRD - s^2/x) for *Corynosoma australe* and *Bolbosoma* sp. from the intestines (small+large) of *Arctocephalus australis* stranded on the Rio Grande do Sul – Brazil coast.

Intestinal Parasite Species	Parasitological Indices				
	P%	MI	MA	Ax	CRD
<i>Corynosoma australe</i>	73.3	2217.45	1626.13	1-18399	7639.54
<i>Bolbosoma</i> sp.	30	51.67	15.5	2-169	96.81

For P% values: numbers within parenthesis (helminthiasis-related cases/number of analyzed host) are discriminating the ratio for *Corynosoma australe*- and *Bolbosoma*-related cases within the sampled hosts.

Table 2. Parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA) and Range of Variation (Ax) for *Corynosoma australe* and *Bolbosoma* sp. from the intestines (small+large) according to the gender and sexual condition of *Arctocephalus australis* stranded on the Rio Grande do Sul – Brazil coast.

Intestinal Parasite Species	Host Sexual Parameters	Host Sex							
		Male*				Female**			
		P%	MI	MA	Ax	P%	MI	MA	Ax
<i>Corynosoma australe</i>									
	Exclusively	80 (12/15)	3140.58	2512.47	32-18399	66.7 (10/15)	1109.7	739.8	1-5217
	Pups	72.7 (8/11)	1792.75	1303.82	32-4754	64.3 (9/14)	1203.89	773.93	1-5217
	Prepubertal*	100	5836.25	5452.5	639-18399	-	-	-	262-262
	Reproductive-Breeding**	(4/4)							
<i>Bolbosoma</i> sp.									
	Exclusively	46.7 ^a (7/15)	64	29.87	2-169	13.3 ^b (2/15)	8.5	1.13	3-14
	Pups	36.4 (4/11)	48.5	17.63	2-169	7.7	3	0.23	3-3
	Prepubertal*	75	84.67	63.5	80-89	-	-	-	14-14
	Reproductive-Breeding**	(3/4)							

For P% values: numbers within parenthesis (helminthiasis-related cases/number of analyzed host) are discriminating the ratio for *Corynosoma australe*- and *Bolbosoma*-related cases within the total observed cases for each host's gender and for gender-related sexual maturation status.

Different superscript letters represent differences for expressed parasitological indices (p<0.05).

Reproductive-Breeding females were not computed.

Table 3 - Parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA) and Range of Variation (Ax) for *Corynosoma australe* and *Bolbosoma* sp. from the small and large intestines and two great portions of the small intestine (first and second halves) of *Arctocephalus australis* stranded on the Rio Grande do Sul – Brazil coast.

Parasite Species and Parasite Crowding Variables	Male Hosts				Female Hosts			
	Intestine or Intestine Portion				Intestine or Intestine Portion			
	Small			Large	Small			Large
	Entire	First Half	Second Half		Entire	First Half	Second Half	
<i>Corynosoma australe</i>								
P%	80 (12/15)	75 (9/12)	75 (9/12)	73.3 (11/15)	66.7 (10/15)	61.5 (8/13)	53.8 (7/13)	46.7 (7/15)
MI	3003	693.11	2560.22	150.09	1090.2	135.5	1339.86	19.29
MA	2402.4	519.83	1920.17	110.07	730.8	83.38	721.46	9
Ax	26-17405	1-3919	25-13486	1-994	1-5193	1-620	3-4913	8-53
<i>Bolbosoma</i> sp.								
P%	46.7 ^a (7/15)	46.2 (6/13)	30.8 (4/13)	13.3 (2/15)	13.3 ^b (2/15)	15.4 (2/13)	7.7 (1/13)	0
MI	62.43	58.83	16	5.5	8.5	4.5	8	0
MA	29.13	27.15	4.92	0.73	1.13	0.69	0.62	0
Ax	2-167	2-129	5-38	2-9	3-14	3-6	8-8	0

For P% values: numbers within parenthesis (helminthiasis-related cases/number of analyzed host) are discriminating the ratio for *Corynosoma australe*- and *Bolbosoma*-related cases within each intestinal division.

Different superscript letters represent differences for expressed parasitological indices (p<0.05).

Table 4 – Crowding values (s^2/x) analysis (X^2) for *Corynosoma australe* and *Bolbosoma* sp. for the intestines (small and large) and half portions of small intestine of *Arctocephalus australis* stranded in Southern Brazilian coast.

Parasite Species and Parasite Crowding Variables	Male Hosts*				Female Hosts*			
	Intestine or Intestine Portion				Intestine or Intestine Portion			
	Small			Large	Small			Large
	Entire	First Half	Second Half		Entire	First Half	Second Half	
<i>Corynosoma australe</i>								
N*	15	12	12	15	15	13	13	15
$\bar{x}$	2402.4	519.83	1920.17	110.07	730.8	83.38	721.46	9
s^2	19568202.54	1235875.79	14609802.7	72411.64	2387085.46	36808.42	2271690.77	210.14
s^2/x	8145.27	2377.45	7608.61	657.89 ^a	3266.4	441.43	3148.73	23.35 ^b
<i>Bolbosoma</i> sp.								
N*	15	13	13	15	15	13	13	15
$\bar{x}$	29	27.15	4.92	0.73	1.13	0.69	0.62	0
s^2	2547.7	1932.47	116.58	5.5	13.27	3.23	4.92	0
s^2/x	87.45 ^a	71.17 ^b	23.68 ^b	7.49	11.71 ^a	4.67	8	0

* hosts utilized on analysis for each intestine or intestine portion by gender.

For *Corynosoma australe* different superscript letters represent differences ($p < 0.05$).

For *Bolbosoma* sp. equal superscript letters represent differences between equal intestinal categories ($p < 0.05$).

Table 5. Seasonal (Autumn-Winter: A-W; Spring-Summer: S-S) parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA), Range of Variation (Ax) and Crowding values (CRD, s^2/x) and statistical results for P% (X^2) MI (Bootstrap confidence interval, BC_a , $p < 0.05$) and CRD (X^2) for *Corynosoma australe* and *Bolbosoma* sp. from intestines of *Arctocephalus australis* (n=30) stranded on the Rio Grande do Sul – Brazil coast for 2008-2010 period.

Parasitological Variables	Parasite Species			
	<i>Corynosoma australe</i>		<i>Bolbosoma</i> sp.	
	Grouped Seasons		Grouped Seasons	
	A-W	S-S	A-W	S-S
P%	77.8 (14/18)	66.7 (8/12)	42.1 (8/19)	9.1 (1/11)
MI	2509.36	1706.63	57.88	2
MA	1951.72	1137.75	24.37	0.18
Ax	32-18399	1-4754	3-169	2-2
CRD	9653.23	2813.12	89.91 ^a	2 ^b

For P% values: numbers within parenthesis () are helminthiasis-related cases/number of analyzed host ratio for *Corynosoma australe* and *Bolbosoma* sp.

Different superscript letters represent significant differences ($p < 0.05$).

ANEXO II

***Corynosoma cetaceum* Johnston & Best, 1942 (Acanthocephala, Polymorphidae) in
Arctocephalus australis Zimmermann, 1783 (Mammalia: Pinnipedia):
histopathology, parasitological indices, seasonality and host gender influences**

Renato Z. Silva, João Carlos B. Cousin and Joaber Pereira Jr.

(Normatizado e submetido ao *Estudos de Biologia*)

***Corynosoma cetaceum* Johnston & Best, 1942 (Acanthocephala, Polymorphidae) in *Arctocephalus australis* Zimmermann, 1783 (Mammalia: Pinnipedia): histopathology, parasitological indices, seasonality and host gender influences**

Corynosoma cetaceum (Acanthocephala: Polymorphidae) em *Arctocephalus australis* (Mammalia: Pinnipedia): histopatologia, índices parasitológicos, sazonalidade e influência do gênero sexual do hospedeiro

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Abstract

The parasitological indices [Prevalence (P%), Mean Intensity (MI), Mean Abundance (MA) and the Range of Variation (Ax)] of *Corynosoma cetaceum* in *Arctocephalus australis* and the histopathology related with the this Acanthocephala are presented based in the necropsy of 32 specimens stranded dead on the shore of Rio Grande do Sul – Brazil from 2008 to 2010. Differences between sexes and reproductive status [males (pups, prepubertals) and females (pups, reproductive-breeding)] were analyzed for P% (Chi-square test, X^2) and MI (Bootstrap Confidence Interval, BC_a , $p < 0.05$) (Quantitative Parasitology - QP 3.0) and between genders for season groups (Spring-Summer: S-S; Autumn-Winter: A-W). Abnormal tissue samplings from the hosts' stomachs (parasite's attached histopathology) and the hosts' gonads (for sexual maturity) were submitted to the histological protocol for paraffin embedding and permanent mounting in balsam. The infection occurred along the sampling period with low P%, MI and MA (respectively) for sexes (males: 37.5, 2.83 and 1.5 and females: 31.3, 3 and 1), for the reproductive status (males pups: 25, 1.33 and 1; prepubertal males: 75, 4.33 and 5; female pups: 28.6, 2.5 and 1; reproductive-breeding females: 50, 5 and 5) and for season groups (S-S: 16.7, 4 and 0.67 and A-W: 45, 2.78 and 1.25). There are no parasitological indices-related differences between sexes and sexual maturation status. There are no season-related differences for P% and MI. The results corroborate an accidental infection despite the absence of severe inflammatory processes added with the parasite's inadequate development and reinforce the food plasticity and uniformity for the genders and analyzed sexual maturity status of Arctocephalinae.

Key words. Stomachal abnormalities. Parasite attachment. Corynosomiasis. Arctocephalinae. Accidental infection

Resumo

Os índices parasitológicos [Prevalência (P%), Intensidade Média de Infecção (MI), Abundância Média (MA) e Amplitude de Variação (Ax)] de *Corynosoma cetaceum* em *Arctocephalus australis* e a histopatologia causada pelo *Acanthocephala* são apresentados baseados no exame de 32 espécimes encontrados encalhados mortos no

litoral do Rio Grande do Sul – Brasil entre 2008 e 2010. Os sexos e condições reprodutivas [machos (filhotes, pré-púberes) e fêmeas (filhotes, reprodutivas-reprodutoras)] foram analisados para P% (Qui-quadrado, X^2) e MI (Intervalo de Confiança via “Bootstrap”, BC_a , $p < 0.05$) (“Quantitative Parasitology” - QP 3.0) e entre os sexos para os grupos sazonais (Primavera-Verão: P-V; Outono-Inverno: O-I). Amostras teciduais estomacais anormais (ancoragem parasitária) e as gônadas dos hospedeiros (maturidade sexual) foram submetidas ao protocolo histológico de inclusão em parafina e montagem permanente em bálsamo. A infecção ocorreu durante todo o período amostral com baixas P%, MI e MA (respectivamente) para os sexos (machos: 37,5; 2,83 e 1,5 e fêmeas: 31,3; 3 e 1), para as condições reprodutivas de cada sexo [machos filhotes: 25; 1,33 e 1; machos pré-púberes: 75; 4,33 e 5; fêmeas filhotes: 28,6; 2,5 e 1; fêmeas reprodutivas-reprodutoras: 50; 5 e 5) e para os grupos sazonais (P-V: 16,7; 4 0,67 e O-I: 45; 2,78 e 1,25). Não há diferenças parasitêmicas sexuais ou nas categorias de maturidade sexual. Grupos sazonais não diferem em P% ou MI. Os resultados sugerem tratar de uma infecção acidental apesar da ausência de processos inflamatórios severos em adição com o desenvolvimento inadequado dos parasitos e reforçam a uniformidade alimentar dos Arctocephalinae entre os sexos e categorias de maturidade sexual.

Palavras-chave: Anormalidade estomacal. Ancoragem parasitária. Corinosomíase. Arctocephalinae. Infecção acidental

Introduction

Helminthiasis is commonly found in marine mammals populations (Geraci & Aubin, 1979) and there is no doubt that the gastrointestinal helminths are highly successful groups to adapt to their microenvironment (Hayunga, 1991). Definitive hosts, as aquatic mammals, have tissue abnormalities caused by helminths (Dailey & Stroud, 1978; Silva & Cousin, 2004; 2006a, b; Motta et al. 2008) caused by several parasite mechanisms such as oral apparatus actions, body holdfasts and/or enzymes (Thomson, 1983; Hayunga, 1991). In aquatic mammals, pathologies *sensu lato* are generally recorded as macroscopic necropsy findings of tissue alterations, (Abollo et al. 1998; Liskins 2002; Mazzariol et al. 2007) and there is paucity to microanatomical analysis

(Parsons & Jefferson, 2000; Spraker et al. 2003; Jaber et al. 2004, 2006; Silva & Cousin, 2004; 2006a, b; Motta et al. 2008; Colegrove et al. 2009).

The reports for marine mammals' diseases are technologically biased showing peaks according to temporal die-offs of host species (i.e., helminths, biotoxins, viruses, bacteria, human interactions) and temporal technologic advances (Gulland & Hall, 2007). The marine mammals' helminthological studies were focused to understand the die-offs in mass strandings of species with closer social behavior to formation of herds mainly in the 1970s and 1980s (Ridgway & Dailey, 1972; Dailey & Stroud, 1978; Geraci, 1978, Geraci & Aubin, 1979; Colongue et al. 1985). Large scale die-offs command considerable attention and concern by scientists, politicians, managers and public thinking about ocean "health" deteriorating because "charismatic" marine mammal species are affected (Jennings et al. 2001; Reddy et al. 2001a, b; Reddy & Ridgway, 2003; Bossart, 2006, 2007; Gulland & Hall, 2007). After intense investigations for parasite-related massive die-offs of marine mammals, parasites were broadly utilized for investigate the social structure and stock identity from middle of the 1990s (Balbuena et al. 1995; Valente et al. 1997).

Several Pinnipedia are definitive hosts to *Corynosoma* spp. (Acanthocephala, Polymorphidae) (Van Cleave, 1952; Zdzitowiecki, 1984, 1986; Raga et al. 2002; Valtonen et al. 2004) including the South American fur seal *Arctocephalus australis*, the most common stranded species in Southern Brazilian shore (Pinedo, 1990; Silva, 2004). Although Ridgway (1972), Bonner (1990) and Vlasman and Campbell (2003) consider Acanthocephala as a low pathological parasite group in Pinnipedia, there are evidences of Acanthocephala-related mortality for mammals (Ponce de León, 2000; Kreuder et al. 2003; McKenzie et al. 2005; Taman, 2009). There is lack of information, whether anthropogenic-related or parasitosis-related, concerning to the true incidence of marine mammals' diseases. This is probably a consequence of the historical focus on domestic animal health rather than wildlife diseases as well as the economic implications rather than conservation (Gulland & Hall, 2007). Several ecological studies were carried out for *Corynosoma* spp. (George-Nascimento & Marín, 1992; Aznar et al. 2001, 2004; Sinisalo et al. 2004; Valtonen et al. 2004; Sardella et al. 2005) but histopathological aspects of corynosomiasis on mammalian hosts as well as

parasitological indices for *A. australis* are scarcely contemplated (Silva & Cousin, 2006b).

The gastric histopathological pattern and parasitological indices of *Corynosoma cetaceum* in *Arctocephalus australis* are presented.

Materials and methods

Hosts sampling: The specimens of *A. australis* (16males: 16 females) were collected from Cassino Beach (ca. 32°11'14.23"S; 52°09'21.70"W) to Chui (ca. 33°44'35.96"S; 53°22'12.70"W) Rio Grande do Sul State – Brazil. The total lengths (TL) of the hosts were measure linearly in meters (m) (following Dierauf 1994) and their sex were determined by external examination. Normal and abnormal (attached *C. cetaceum*) tissue samplings were taken from the stomachs (fundic and pyloric regions). Samplings for hosts were performed within two major seasons grouping, i.e., Autumn-Winter (A-W) and Spring-Summer (S-S) from 2008 A-W to 2010 A-W.

The conservation status of carcasses utilized in this study followed Dierauf (1994). Concerning to this author, only carcasses code 2 and 3 were collected for necropsy procedures. Additionally other post-mortem alterations were observed, such as *rigor-mortis*, *livor-mortis*, abdominal expansion by decomposition gases and absence of decomposers (as larval forms of insects) (Thomson 1983).

The opening of the thoracic and abdominal cavities for the removal of the digestive system, reproductive organs and gross analyses of the organs were performed according to Dierauf (1994). Thus, the digestive tube was divided in its anatomical parts (esophagus and stomach, small and large intestines). These parts were tied, labeled, bagged and frozen for the parasite screening. The small intestine was separated from the mesentery and form the large intestine, linearly measured and divided in a cranial portion and other caudal portion for analyze the parasite's distribution.

Host's sexual maturation: The entire reproductive system of the females was extracted for analysis of the sexual maturity as well as the male gonads (both left and right) with epididymis. The stereomicroscopy in the female gonads was performed to observe ovarian follicles, luteogenesis and the luteolysis *sensu* Bukovsky et al. (2005), Katz et al. (2009), Lucacin and Pinto-Neto (2009) and Palma-Cerda et al. (2011). Ovaries (both left and right) were separate from the uterus and ovarian sections (1-

2mm) were performed and observed for folliculogenesis. The histomorphology of the spermatogenesis followed Wistuba et al. (2007).

Histological protocols: Histological samplings of the testicles were made transversally in the center of the gonad in order to sample the epididymis. Midsagittal sections from both ovaries were submitted to histological routine. Tissue samplings from stomachs and gonads, were fixed (Bouin's fluid) for histological routine for paraffin-embedding; sectioning (7µm thickness); staining (Hematoxylin-Eosin) and permanent mounting in balsam (Silva and Cousin, 2004, 2006a, b). According to the spermatogenesis the male host's can be categorized as: pups (spermatogonia and spermatocytes), prepubertal (spermatids); pubertal (spermatozoa within seminiferous tubules = testicular spermatozoa) and adult reproductive (spermatozoa within epididymis) (Wistuba et al. 2007). Host's female sexual maturity can be categorized by the follicle-type population within ovarian stroma as: pups (mainly primary follicles presence); prepubertal (Antral and Graafian follicles presence) and adult reproductive-breeding females (corpus luteum-corpus albicans presence) (Bukovsky et al. 2005, Katz et al. 2009, Lucacin & Pinto-Neto 2009, Palma-Cerda et al. 2011).

Parasite protocols: The host's stomachs were opened and washed into a plastic bowl. The small and large intestines were separated. The small intestines, to be linearly measured, were separated from the mesentery. They were divided in two equal portions (first and last halves) of equal lengths. Each small intestine half was divided in subsets to be opened and washed into a plastic bowl. The parasites were detached (when no free within the intestine lumen) from the intestine wall according to each subset from each small intestine half. The content from the plastic bowl for the stomach as well as for each subset from each intestine half was sieved and observed under stereomicroscope for parasite's screening. Parasites were collected and counted according to stomach and each half of the small intestine as well as for the large intestine. They were prepared for permanent mounting in balsam and staining (Semichon's Carmim and Eosin) following and adapting from Amato et al. (1991). Parasites were identified *sensu* Petrochenko (1971) and Sardella et al. (2005) holdfast criteria: proboscis oncotaxia (structure and distribution pattern of the hooks), body shape, trunk (presoma and metasoma) spine (somatic spine's structure and distribution pattern) and organology. Infrapopulations were analyzed for cystacanth and adult forms (*sensu* Nickol 1985). Females were

observed for fertilization and maturity status (matted; non-matted) *sensu* Sinisalo et al. (2004) and Valtonen et al. (2004) criteria for eggs (sheltered acanthors) within body cavity. Fertilized females represent those containing developing/developed acanthors and mature females as those containing gonads, uterine and vaginal structures completely formed.

Parasitological indices such as Prevalence (P%), Mean Intensity (MI) and Mean Abundance (MA) *sensu* Bush et al. (1997, 2001) were determined and analyzed *sensu* Rozsa et al. (2000) by the “Quantitative Parasitology Program - QP 3.0 Program”. The P% (Chi-square test, X^2) and MI (Bootstrap confidence interval, BC_a , $p < 0.05$) were compared between host’s gender (total males and females), host’s gender maturity (pup and prepubertal males; male pups and female pups; female pups and reproductive-breeding females and prepubertal males and reproductive-breeding females).

Seasonal variations for the P% and MI were analyzed comparing the grouped seasons, i.e., Autumn and Winter (A-W) and Spring and Summer (S-S) as for all hosts and for all sample period.

Results

Lengths of infected hosts and host’s sexual maturity: Samples comprised pups (n=12) to prepubertal males (n=4) as well as pups (n=14) and reproductive/breeding females (n=2). Corynosomiasis occurred in all categories for males and females hosts.

Hosts’ TL ranges were: $0.84\text{m} \geq \text{TL} \leq 1.76\text{m}$ to males and $0.8\text{m} \geq \text{TL} \leq 1.34\text{m}$ to females. Male and female hosts infected by *Corynosoma cetaceum* comprised $0.97\text{m} \geq \text{TL} \leq 1.76\text{m}$ $0.87\text{m} \geq \text{TL} \leq 1.33\text{m}$, respectively. From 32 hosts, six out of 16 males and five out of 16 females showed *C. cetaceum*-related (n=11) corynosomiasis (Table 1).

Infection sites and parasite reproductive status: The parasite was present within the stomachal lumen and/or within the duodenal lumen of the small intestine. Corynosomiasis exclusively within the stomach were five out of 11 (45.45%) and exclusively within the initial duodenum were four out of 11 (36.36%). Corynosomiasis within both gastrointestinal regions (stomach and duodenum) were two out of 11 (18.18%). There were no fertilized (no-gravid) females.

The stomachal corynosomiasis shows the pyloric region as the main site of the cases of infection ($P\% = 80$), thus the fundic region (closer to fundus-pylorus transitional region) as the lesser site of the cases of infection. Few parasites were superficially attached to the stomachal mucosa and, in these cases a concavity formed by the parasite's body could be noted, after parasite's removal. This concavity comprised the presoma (foretrunk) and metasoma (hindtrunk) of the parasite. The duodenal corynosomiasis comprised only free specimens within the intestinal lumen.

Seasonality: *Corynosoma cetaceum* infects *A. australis* throughout the years and seasons but with low intensities for both host's genders (Table 1) and with low parasitological indices (Table 2). Males were mainly infected between 2008 and 2009. *Corynosoma cetaceum* occurred in male hosts throughout the 2008 (A-W and S-S) and only during A-W of the 2009, summing 83.33% of the cases. Only 16.66% of the cases occurred isolated in A-W of 2010. Concerning to the year (2010), there was an inversion of infective pattern. Females were the principal gender infected in 2010. Only 20% occurred during the A-W of 2008 and 80% of the infection occurred throughout the 2010 (A-W and S-S). There are no season-related differences for $P\%$ and MI (Table 2).

Gender and reproductive status: The parasitological indices of *C. cetaceum* infrapopulation of total host sampling are: $P\% = 34.4$, $MI = 2.91$ and $MA = 1.0$.

There are no significant differences between genders or sexual maturation status for $P\%$ and MI (Table 3).

Histopathology: The parasites attach superficially (middle-outward) within the fundic mucosa and form a concavity with their body as result of the holdfast embedding: proboscis hooks, foretrunk (presoma) spines and hindtrunk (metasoma) spines. Inner layers of the stomach wall, i.e., submucosa, muscular layer and serosa are not mechanically affected by the parasite.

Within the fundic stomachal mucosa *C. cetaceum* causes necrosis, erosion and reduction in length of the simple or branched tubular gastric glands. The glandular erosion occurs in the middle-apical region, degenerating the gastric crypts and glandular neck, reaching the middle of the glandular body and releasing cell remains from glandular epithelial cells and from the papillary- or finger-like lamina propria. The affected glands by parasite's holdfast show mainly kariorexis and kariolysis of the

muciparous neck cells, chief (zymogenic) cells and parietal (oxyntic) cells. The retraction of the proboscis deforms and curves the gastric glands (Fig. 1).

All abnormal features of pyloric mucosa were similar for the fundic mucosa. However, it is important to elucidate that this region is composed exclusively by simple or branched tubular muciparous glands (pyloric glands) and the necrotic features are related exclusively with muciparous cells.

Discussion

Helminthiasis in *A. australis* is poorly recorded and generally this parasitosis is focused investigate the biological parameters of a parasite-target species (Aznar et al. 2004; Sardella et al. 2005). In Uruguayan waters, the parasite assemblage is recorded and compared by similarity index for the sympatric *A. australis* and *Otaria flavescens* (Shaw, 1800) but parasitological indices as P%, MI, MA and range of variation (Ax) are not available (Morgades et al. 2006). The influence of the same host's species on the morphology (ecotype) and/or fecundity of intestinal *Corynosoma* sp. (George-Nascimento & Marin, 1992) and nematode parasite (George-Nascimento et al. 1992) were also analyzed. Morgades et al. (2006) report *Corynosoma* sp. for pups and *Corynosoma* spp. for juveniles and adults *A. australis*. Morgades et al. (2006) provides a clue to think that *Corynosoma* spp. most likely are both *C. cetaceum* and *C. australe* each in its proper gastrointestinal niche space, i.e., within the stomach and intestines respectively, as record Aznar et al. (2004) and Sardella et al. (2005). *Corynosoma* sp. analyzed by George-Nascimento and Marín (1992) from intestine of *A. australis* is most likely *C. australe*. The pup's infection reinforce the first intake of solid food as a way to allows the parasite settlement (Vaz-Ferreira & Ponce de León, 1987) but little is known about the food habits of the host that strand in Brazilian shores (Santos & Haimovici, 2001).

Normally adult Acanthocephala settles and recruits within the intestinal lumen of endothermic vertebrates (Crompton, 1973; Nickol, 1985; Bush et al. 2001; Aznar et al. 2004; Sinisalo et al. 2004), but some species represent exceptions (Aznar et al. 2001; Morgades et al. 2006; Muñoz & Olmos, 2008). *Corynosoma cetaceum* is recorded with an atypical settlement site within the gastrointestinal tract of *Pontoporia blainvillei* (Cetacea, Pontoporiidae). Within this polygastric (=polychambered) (main stomachal

chamber, connecting channel and pyloric stomachal chamber) (Yamasaki et al. 1974) host the parasite shows its higher P% and MA in the pyloric stomach lumen followed by the duodenal ampulla (small intestine) (Valente et al. 1997; Aznar et al. 2001). Also, the fecundity proportional ratio for the females of *C. cetaceum* in the pyloric stomachal chamber of *P. blainvillei* shows that it is the appropriated gastrointestinal niche space to the parasite reaches its sexual maturation, thus this parasite constitute part of the helminth community for this host (Valente et al. 1997; Aznar et al. 1994, 2001). The largest stomachal chamber (main stomach) of *P. blainvillei* has the lower values of *C. cetaceum* and this can be related with the lower nutritional concentrations because the digestion is in its initial process (compared with higher free nutrient values toward pyloric chamber of the stomach) and also due to the anti-mechanical protection during the breakdown of the diet items within the main stomach (Aznar et al. 2001). Other Cetacea with polychambered stomachs such as *Cephalorhynchus eutropia* (Delphinidae) and *Phocaena spinipinnis* (Phocoenidae) are recorded typically harboring *C. cetaceum* within their pyloric stomachal chamber (Muñoz & Olmos, 2008).

On the other hand, the histochemical characteristics of the mucosa of the chambers and portions of the stomach surface should be considered. The anti-corrosive (anti-acid and anti-enzymatic) properties of the mucous within the pyloric region should be added with the reflections of Aznar et al. (2001). This can help to explain why *C. cetaceum* occur in the pyloric region of monogastric (monochambered) species as *A. australis* (Ridgway, 1972) and within its closer duodenum portion. The pyloric stomachal chamber of Cetacea shares the same muciparous glandular characteristic (Harrison et al. 1970; Smith, 1972; Chaves & Silva, 1988; Silva & Cousin, 2006b). Gastric corynosomiasis in the monochambered stomach of *A. australis* shows higher P% in the pyloric portion composed by mucosal muciparous glands. The parasite-host interaction level or parasite supra-population-host supra-population interaction level, *sensu* Zander (2001), is adjusted by biochemical coevolutionary factor to parasite's taxis, settlement and recruitment in/within an adequate host or host's body site to adequately finish the parasite's life-cycle (Combes, 1997; Dybdahl & Storter, 2003).

In evolutionary studies the parasitological indices, such as P%, are commonly utilized to predict and measure the "parasite load" or "parasite pressure" upon a host species (Gregory & Blackburn, 1991) as a selective pathogen (=parasite) force to help

the process of natural selection (Anderson & Gordon, 1982; Anderson & May, 1982). The weak parasite's occurrence across sampled years, the low values of the P%, and extremely low values of MI and MA as well as no-gravid (no-mated) females of *C. cetaceum* show that at least *A. australis* is an inadequate or accidental host for this parasite. According to the structure of the population and recruitment patterns, the congeneric *C. australe* is considered as typical component of the parasite's intestinal infrapopulation of *A. australis* (Vaz-Ferreira & Ponce de León, 1987; Aznar et al. 2001; Morgades et al. 2006). On another hand, *C. cetaceum* due to its parasitological indices and structure and dynamics of the population is considered component of the gastrointestinal helminth community of Cetacea (Andrade et al. 1997, Béron-Vera et al. 2007).

The *C. cetaceum* fitness to Cetacea species perhaps is due to the gastric anatomy (polychambered) that permits the anatomical isolation by well-defined chambers forming well-defined microhabitats (Berón-Vera et al. 2007). These well-defined microhabitats help to avoid or diminishes inter-specific competitions with other gastrointestinal parasite infrapopulations and/or infracommunities (Von Zuben, 1997; Poulin, 2001). The monochambered stomach condition, typical of Pinnipedia and other Carnivora, perhaps can contribute for the inter-specific parasite competition. *Arctocephalus australis* shows well-defined helminths species according to gastrointestinal niche space, *i.e.*, anatomic division of the digestive tube (Morgades et al. 2006). Hosts, as *A. australis*, with wider demographic ranges tend to demonstrate higher parasite's species richness (Bush et al. 2001) as result from the higher variety of preys (Naya et al. 2002; Casper et al. 2006). The interactions between concomitant parasite infrapopulations in individual hosts will determine how many parasites species can coexist within single hosts, and ultimately in the parasite component community (Bush et al. 1997, 2001) in a maximum parasite's load (Bush & Lotz, 2000; Read, 2000; Roberts, 2000; Cattadori et al. 2008).

Concerning to the genders and/or sexual maturity status, the absence of differences for the parasitological indices can be related with the common high food plasticity or gender-related food particularities within Arctocephalinae (Gales et al. 1993; Georges et al. 2000; Harcourt et al. 2002; Casper et al. 2006; Ciaputa & Sicinski, 2006; Boren, 2010) and Otariinae (Drago et al. 2009). The typical sexual dimorphism of

mammals (Fairbairn, 1997; Isaac, 2005; Ferguson, 2008) implies in possible differences in the parasitic loads due to gender-related heterogeneities concerning to the behaviour, endocrinology, morphology (length and body mass = hyperallometry), energetic requirements and genetic of the hosts (Cassini, 2000; Klein, 2004; Morales-Montor et al. 2004; Cattadori et al. 2008) as well as environmental selective pressure (Ferguson, 2008). The absence of heterogeneity in exposure (or no differences in susceptibility) can rapidly results an aggregated distribution of parasites but until the present is unclear what is the relative significance of these different mechanisms, and the importance of their interactions in accentuating individual differences in parasite loads (Wilson et al. 2002).

Puberty is not fixed in Pinnipedia. It occurs more similarly over a range of age among females of Phocidae and Otariidae and it is more variable within Pinnipedia males (Atkinson, 1997). The greater variation within Pinnipedia males is partially due to the need to gain behavioural experience and body mass to sustain successfully the energetic costs to compete for breeding rookeries (Atkinson, 1997). This results in a sexual selection according to high degree of sexual dimorphism in polygynous Pinnipedia, such as Otariidae, it is generally marked by the larger body size of the male several times heavier than females, as an advantage in intrasexual dominance in male-male combat for breeding territory (Clinton & Le Bouef, 1993). Moreover, the higher male body mass is correlated with the increasing of the home range (Ferguson, 2008). Within the sampled specimens, the male hosts were very closer to female hosts according to the TL. This corroborate the absence of hyperallometric growth of organs and structures [or higher body mass according to Bergmann's rule influence *sensu* Ferguson and Larivière, (Ferguson, 2008)] associated with combat or display in males (Fairbairn, 1997). This can explain the absence of differences to the P% and MI concerning to sampled sexes, at the least for *C. cetaceum*, probably due to the similar energetic requirements and/or superposition of foraging areas within the specimens without hyperallometric sexual dimorphism, reflecting in food uniformity between genders within the sampled length classes.

On the other hand, the season-related equalities for P% and MI and the low MA and Ax should be considered as reinforce for the diet plasticity and uniformity for the hosts, i.e., Otarioidea are very flexible foragers (Casper et al. 2006; Georges et al. 2000;

Harcourt et al. 2002; Ciaputa & Sicinski, 2006; Drago et al. 2009; Boren, 2010). Moreover these results corroborate accidental occurrence of this parasite in this host species as previously discussed. Food plasticity can result in the crowded (in patches) pattern of occurrence of gastrointestinal parasite groups within a host trophic level, generally secondary and definitive hosts (this latter such as marine mammals) (Bush et al. 2001; Rósa et al. 2000; Reiczigel et al. 2005). The crowded parasite pattern to definitive hosts is related with stochastic environmental changes that can influence the dynamic of intermediate hosts' population such as Russel cycle and primary production (Lalli & Parson, 2004) (for the oceanographic environment), the host's susceptibility (influenced by the immunology, genetic and behaviour), host's intra- and/or inter-specific parasite competition and differences between host's trophic levels (ontogeny-associated) food necessities (Von Zuben, 1997; Bush & Lotz, 2000; Read, 2000; Roberts, 2000; Cattadori et al. 2008). The host immunological efficiency aspects (Von Zuben, 1997; Klein, 2004) can explain the observed low parasitological indices, the immaturity of the parasites within gastric lumen and the low inflammatory effect of this parasite.

Histopathologically, the stomachal attachment of *C. cetaceum* in *A. australis* is similar from that of *Pontoporia blainvillei* (Cetacea, Pontoporiidae) (Silva & Cousin, 2006b). However other polymorphidiasis can result in different host tissue reaction such as *Bolbosoma*-associated intestinal colonic granuloma (Silva & Cousin, 2006a). Nevertheless acanthocephalosis are recorded in mammalian hosts contributing to mortalities via inflammatory processes as septic peritonitis associated with the *Profilicolis* spp. (Profilicolidae) migration in *Enhydra lutris* (Kreuder et al. 2003) and *Macracanthorhynchus*-associated (Macracanthorhynchidae) neoplasia, chronic enteritis, intestinal perforation and intestinal eosinophilic granuloma in *Hemiechinus auritus* (Taman, 2009). Some authors (Ridgway, 1972; Vlasman & Campbell, 2003), maybe from a strict pathological point of view, consider the acanthocephalosis as not significant for the Pinnipedia mortalities, but parasites influences their host's health from an ample range of level (p.e., physiological and behavioural) (Thomson, 1983; Bush et al. 2001).

Conclusion

Corynosoma cetaceum is found mainly within the pyloric region of the stomach and early duodenum; it occurs throughout the year for both genders of *A. australis* in very low parasitological indices; there are no genders-related differences for P% and MI as well as for the analyzed sexual maturation categories (male pups, prepubertal males, female pups and breeding females); there are no season-related differences for P% and MI and these results indicate that the occurrence of this parasite is accidental in *A. australis* despite the absence of severe inflammatory processes in the host's stomach.

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Table 1. Seasonal distribution of *Corynosoma cetaceum*-related cases within season grouping (Autumn-Winter: A-W; Spring-Summer: S-S) in *Arctocephalus australis* stranded on the Rio Grande do Sul – Brazil coast.

Host's Gender	Year and Seasonal Grouping					
	2008		2009		2010	
	A-W	S-S	A-W	S-S	A-W	S-S
Male (n=6/16)		5			1	
Female (n=5/16)	1					4

Table 2. Parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA) and Range of Variation (A_x) for season (Autumn-Winter: A-W; Spring-Summer: S-S) and statistical results for P% (X^2) and MI (Bootstrap confidence interval, BC_a , $p < 0.05$) for *Corynosoma cetaceum* from the stomachs of *Arctocephalus australis* stranded on the Rio Grande do Sul – Brazil coast for 2008-2010 period.

Stomachal Parasite	Grouped Seasons							
	A-W				S-S			
	P%	MI	MA	A_x	P%	MI	MA	A_x
<i>Corynosoma cetaceum</i>	45 (9/20)	2.78	1.25	1-7	16.7 (2/12)	4	0.67	1-7

For P% values: numbers within parenthesis () are helminthiasis-related cases/number of analyzed host ratio for *Corynosoma cetaceum*.

Table 3. Parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA) and Range of Variation (Ax) for *Corynosoma cetaceum*-related cases (n=11) from *Arctocephalus australis* (n=32) stranded on the Rio Grande do Sul – Brazil coast.

Host Sexual Parameters	Host Sex							
	Male*				Female**			
	P%	MI	MA	Ax	P%	MI	MA	Ax
Exclusively	37.5 (6/16)	2.83	1.5	1-7	31.3 (5/16)	3	1	1-7
Pups	25 (3/12)	1.33	1	1-2	28.6 (4/14)	2.5	1	1-7
Prepubertal*/Reproductive-Breeding**	75 (3/4)	4.33	5	1-7	50 (1/2)	5	5	5

For P% values: within parenthesis numbers () are helminthiasis-related cases/number of analyzed host ratio for *Corynosoma cetaceum*.

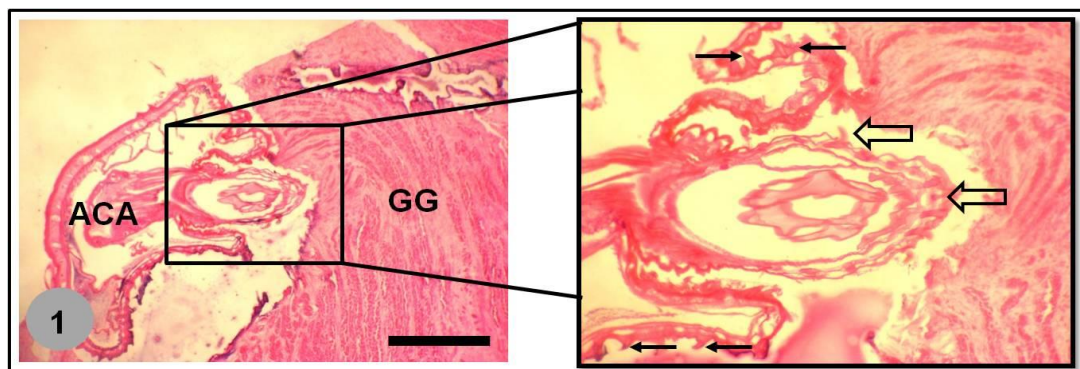


Fig. 1. Microphotograph of the attachment of *Corynosoma cetaceum* within the stomach mucosa of *Arctocephalus australis*. Observe the concavity harboring the parasite (ACA) and the erosion and deformities of the gastric glands (GG) from the mucosa. The detail on right shows the parasite's proboscis and cute proboscis hooks (open arrows) and trunk spines (solid arrows). Stain: H-E. Scale bar: 560 μ m.

ANEXO III

**Histological patterns of the intestinal attachment of *Corynosoma australe*
(Acanthocephala: Polymorphidae) in *Arctocephalus australis* (Mammalia: Pinnipedia)**

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Histological patterns of the intestinal attachment of *Corynosoma australe* (Acanthocephala: Polymorphidae) in *Arctocephalus australis* (Mammalia: Pinnipedia)

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Short title: Attachment of *Corynosoma australe* in *Arctocephalus australis*

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Abstract The mucosal attachment pattern of *Corynosoma australe* in the intestines of *Arctocephalus australis* is described. Normal and abnormal tissue were sampled from 32 hosts to be submitted to histological routine protocol to embedding in paraffin and permanent mounting in balsam. *Corynosoma australe* shows three different degrees of body depth intestinal attachment (BDINA-1 to 3). BDINA-1: it is exclusive of the small intestine and the parasite attaches on the villi; BDINA-2: parasite affects the Lieberkühn crypts in several depth levels and, BDINA-3: the parasite reaches the submucosa. These attachment patterns alter the mucosa by degeneration and dysfunction due to necrosis of mucosal structure, great quantities of cellular debris and significant reduction of the mucosal thickness. Other aspects are crater-like concave holes (CLCH) as sites where *C. australe* could be attached-detached several times according to adult migratory processes within luminal intestine space. The submucosa shows edema probably due to the local mucosal alterations resulting in homeostatic break. There is no severe inflammatory response by host but BDINA-1 to BDINA-3 and CLCH

could represent foci to secondary opportunistic infections and significant areas of malabsorption in severely infected hosts contributing to increase clinical signs of preexistent pathologies.

Key words. Intestinal histopathology, Parasite attachment pattern, Corynosomiasis, Mammalian host

Introduction

The host-parasite relationship is complex, occurring at different trophic levels and may cause tissue changes as observed in definitive hosts (Silva and Cousin 2004). Otariidae mammals, as South American fur seal *Arctocephalus australis* (Zimmermann 1783), comprise definitive hosts to Acanthocephala to finish their life cycles adequately (Bush et al., 2001; Raga et al. 2002). Despite some studies on the biology of *A. australis* as well as other aquatic mammals in South America, there is a lack or little information to helminthiasis and to helminthiasis-associated histopathologies (Silva and Cousin 2004). There are a greater number of histopathological and parasitic pathogenicity studies to Pinnipedia species in the North Hemisphere (Ridgway 1972; Lauckner 1985). Maybe this can be the result from an alternative data utilization of marine mammals stocks for researches during historical hunting or commercial fishery bycatch (Crespo and Hall 2001; Jennings et al. 2001).

Parasitosis is recognized as a significant factor in massive strandings and mortality of Pinnipedia (Ponce de León 2000; McKenzie et al. 2005) but Acanthocephala is considered a group of low negative impact in Pinnipedia (Ridgway 1972; Vlasman and Campbell 2003). However attachment sites of Acanthocephala can represent locals for secondary opportunistic infections and the effect of parasites in their hosts is at least spoliative (Bush et al. 2001). *Corynosoma australe* Johnston 1937 (Acanthocephala, Polymorphidae) is commonly found in *A. australis* but histopathological aspects caused by this parasite on mammalian host is absent or scarcely contemplated in the literature.

The mucosal attachment pattern of *Corynosoma australe* in the intestines of *Arctocephalus australis* is described.

Material and Methods

For this study the carcasses (15males: 15females) of *A. australis* were collected from Cassino Beach (ca. 32°11'14.23"S; 52°09'21.70"W) to Chui (ca. 33°44'35.96"S; 53°22'12.70"W) – Rio Grande do Sul State – Brazil. The total length (TL) of the hosts was linearly measured in meters (m) (*sensu* Dierauf 1994) and the sex was determined by external examination (Pinedo et al. 1992). Only carcasses respecting the conservation status code 2 and 3 from Dierauf (1994) were utilized for tissue and parasite's samplings.

Normal and abnormal (attached *C. australe*) tissues samplings from small and large intestines were extracted for the histological routine protocol for embedding in paraffin and permanent mounting in balsam. Tissues samples were fixed (Bouin's fluid). Paraffin blocks were sliced (microtomy) under 7µm in thickness and the histological slides were stained with Hematoxylin-Eosin and Alcian-Blue *sensu* Silva and Cousin (2006a; b). Under stereomicroscopy, portions of the intestine were analyzed to detect and characterize attachment patterns of *C. australe*. The collection and preparation of parasites for permanent mounting in balsam and staining (Semichon's Carmim and Eosin) followed and adapted from Amato et al. (1991) and Silva and Cousin (2006a; b). Parasites were identified *sensu* Petrochenko (1971), Zdzitowiecki (1984), Pereira Jr. and Neves (1993) and Sardella et al. (2005) holdfast criteria: proboscis oncotaxia, body shape, trunk (presoma and metasoma) spine (structure and distribution pattern) and organology.

Histometric statistical comparisons (Variance Analysis; Tukey's Test; $p < 0.05$), according to the thickness, was done only between normal and abnormal layer affected (=mucosa) by *C. australe* per each intestine anatomical division (small and large intestine). The histometries were performed with a metric eyepiece lens calibrated with a micrometric slice (Nikken – Tokio; 0.01mm) according to the different magnitudes (4x10, 10x10, 20x10), following Silva and Cousin (2004; 2006a; b). The gross and microscopic aspects of the lesions were utilized to typify the severity of the injury (*sensu* Thomson 1983).

Results

In the sampling the hosts' TL range were: $0.84\text{m} \geq \text{TL} \leq 1.76\text{m}$ to males and $0.8\text{m} \geq \text{TL} \leq 1.34\text{m}$ to females. Male and female hosts infected by *Corynosoma australe* comprised $0.95\text{m} \geq \text{TL} \leq 1.76\text{m}$ $0.87\text{m} \geq \text{TL} \leq 1.34\text{m}$, respectively.

Macroscopically, the attachment of *C. australe* within IN showed three different degrees of body depth intestinal attachment (BDINA): 1 - outer or superficial (proboscis attached on IN intervilli spaces), 2 - medial (presoma IN-embedding) and 3 - inner or deepest (metasoma partially IN-embedding). Mainly in cases two and three delicate borderline elevations or demarcations in the attachment site with a deep central hole, housing the proboscis, were noted. There were no color intestine changes around the *C. australe*-attachment site. Other lesional features associated with *C. australe* are crater-like concave holes (CLCH) as sites where *C. australe* could be detached.

Microscopic aspects of corynosomiasis are presented below.

BDINA-1: Proboscis attachment occurs on intervilli spaces not invading the Lieberkühn crypts. There are villi folding, broking and erosion. The villi can be absent. The Lieberkühn crypts show necrotic features and architectural disorganization. The submucosa is edematous and shows dilated lymphatic vessels added with the non-modeled dense connective tissue with collapsed collagen fibers. The large intestine has no villi, thus BDINA-1 pattern is absent (Fig. 1A).

BDINA-2: Parasite can reach the Lieberkühn crypts causing mucosal damage in several depths proceeding toward, probably, BDINA-3 or CLCH patterns. Beyond the hooks of the proboscis, the mucosal damage is exacerbated by the action of the spines of the presoma. There is total erosive destruction of villi and different alterations of the Lieberkühn crypts according to parasite's mucosal depth. Thus, great quantities of cells debris (mainly pyknosis from cylindrical epithelium and loose connective tissue) occur near the attachment site and around the parasite. The muscularis mucosae is not eroded. Villi on periphery of lesion are strongly pushed laterally and this confers to the lesion a parasite's body mold-like aspect. The submucosa is edematous as in BDINA-1, shows low lymphocyte infiltration and foreign-body-like giant cells were not present. There are hypertrophy of the Lieberkühn crypts due to folding and/or erosion of the villi. The Lieberkühn crypts are filled with remnants of mucous and enterocytes remains (Figs. 1B and 2A-B).

BDINA-3: It is a deeper lesion feature caused by parasite's holdfast. There is total mucosal destruction beyond the MM. *Corynosoma australe* reaches the submucosa, sometimes causing a slight concave depression on it. Cell and tissue alterations observed are similar that found in BDINA-1 and BDINA-2 (Figs. 1C and 2C).

CLCH: Similar to BDINA-3 but without the parasite, resembling sites where *C. australe* detached. The submucosa is exposed directly to intestine lumen. In some cases occurs incomplete re-epithelization of the affected area of the mucosa (Fig. 2D).

In BDINA-2 and BDINA-3 the spines of trunk (presoma and metasoma) of *C. australe* are strongly attached on the intestine tissues as a parasitic proboscis auxiliary mechanism to mucosal embedding (Fig. 2E). The parasite can folds the area between proboscis and presoma utilizing neck retractors muscle bundles what increases the body attachment (surface contact) area. The attachment pattern observed leads to a significant reduction of the thickness of the mucosa of the small as well as of the large intestine, where the parasite was attached (Table 1). There were no *Corynosoma australe*-related inflammatory processes (Figs. 1A-C and 2A-E).

Discussion

Corynosomiasis, in this study, did not show inflammation as in *Pontoporia blainvillei* (Cetacea, Pontoporiidae) stomachs by *C. (=Polymorphus) cetaceum* infection (Silva and Cousin 2006b) but differed from intestinal *Bolbosoma*-associated colonic granuloma in this host (Silva and Cousin 2006a). Nevertheless Acanthocephalosis are recorded in mammalian hosts causing *Profilicolis*-associated (Profilicolidae) septic peritonitis by migration in *Enhydra lutris* (Kreuder et al. 2003) and *Macracanthorhynchus*-associated (Macracanthorhynchidae) neoplasia, chronic enteritis, intestinal perforation and intestinal eosinophilic granuloma in *Hemiechinus auritus* (Taman 2009), thus contributing to mortalities. In *Halichoerus grypus* Fabricius 1791 (Pinnipedia, Phocidae) *Corynosoma*-associated intestinal “ulcerations” are recorded (Bergman et al. 2003).

Several ecologic pressures influenced many aspects of the functional morphology of the Acanthocephala species and selected these parasites to an effective attachment mechanism (Petrochenko 1971) but there are scarce studies investigating the relationship between Acanthocephala morphology and attachment performance (Petrochenko 1971; Taraschewski et al. 1989; Taraschewski 1990; Aznar et al. 1999). The histopathological pattern from BDINA-1 to CLCH may represent a parasite attachment progressive mechanism (APM). APM could begins with BDINA-1 (as an initial attachment phase to newly-recruited, newly-adulthood and adult parasites) toward CLCH (to senescent-died or newly-detached parasites), according to *C. australe* biological needs. APM is supported by Acanthocephala’s migration capacity within intestinal lumen due to reproductive

processes (maturation, copulation and oviposition) while they move mainly jejunum-to-ileum-ward (George-Nascimento and Marin 1992; Aznar et al. 2004). Parasite's migratory processes can also be influenced by circadian and seasonal cycles such as food's availability, host's food intake rigor, food bulk intestinal flow, food's nutritional composition and intra- inter-specific relationships as crowding effect (Mettrick and Podesta 1974; Nickol 1985). Males of Acanthocephala are more active in copulation than females; they move seeking for females to mate with as many as possible (Parshad and Crompton 1981). On the other hand, within taxa such as Acanthocephala the infrapopulation sex ratios are commonly female-biased (Poulin 1997; Aznar et al. 2001, 2004) and females *Corynosoma* spp. have longer life-span than males (Aznar et al. 2001) and they migrate intestine posteriad according to ova maturation (Aznar et al. 2004). Thus, sexual selection can influence Acanthocephala's spatial distribution (Sinisalo et al. 2004) and attach-detach rates. Male-to-female attachment's ratio was not studied here but there are evidences that *C. australe* could attach-detach several times lifelong at different intestinal length levels (Parshad and Crompton 1981; George-Nascimento and Marin 1992; Aznar et al. 2004), conducting to several CLCH by each specimen within host's intestine.

In Corynosomosis, the parasite's gradual and chronic mucosal embedding can be explained by the use of the trunk as a secondary holdfast due to the anatomy of the powerful muscles that allow precise movements forward and backward (Hayunga 1991; Aznar et al. 1999). The morphology of some Acanthocephala groups permits deep penetration within the digestive tube wall, what causes extensive tissue damage (Dezfuli et al. 2002). An example of this mechanism can be observed concerning to *Bolbosoma* spp. (Polymorphidae, Acanthocephala) that embed its trunk disc deeply within intestinal mucosa of mammalian definitive hosts (Petrochenko 1971; Measures 1992; Silva and Cousin 2006a). The penetration depth of this parasite group and the density of helminths comprise the two principle factors responsible by Acanthocephala pathogenicity as records Taraschewski (2000). *Pomphorhynchus laevis* (Pomphorynchidae) infecting the European chub *Leuciscus cephalus* (= *Squalius cephalus*) (Cyprinidae), shows four gradual attachment-embedding patterns involving different parasite's body parts (proboscis, neck, trunk) in different depths within the intestine gut wall (mucosa, submucosa, muscular layer and serosa) (Dezfuli et al. 2002). The involvement of different parasite's body parts for attach in a surface of the digestive tube is similar to that recorded in this survey. *Pomphorhynchus laevis* shows a rivet-like mode of attachment that trespass the intestine wall and sometimes reaches the liver and pancreas (Dezfuli et al. 2002), whereas *C. australe* shows a body mold-like aspect does not trespassing the totally the intestine wall (*i.e.*, the attachmen and damage is restrict to the mucosa).

Although severe inflammatory responses were not observed, the new areas of attachment (reattached specimens) and CLCH of *C. australe* can also represent foci of secondary infection and dysfunctional areas (Thompson 1983; Carlton and McGavin 1998). Concerning to the dysfunctionality, the abnormal mean area of attachment of 1.5mm² by each *C. australe* (Aznar et al. 2004), addicted with edema of the submucosa and luminal exposition, could be considered as an important factor to nutrients malabsorption in hard infected hosts. It is important to maintain the isotonicity of the luminal contents because hypertonic solutions within lumen cause structural and functional damage on the mucosa (Mettrick and Podesta 1974; Pácha 2000). Intestinal epithelium promotes strong exchange of water and electrolytes with body's extracellular fluids and disturbances in this orderly intestinal secretion-reabsorption function can quickly promote severe body fluid depletion (Mettrick and Podesta 1974). The alterations in enterocytes can promotes disturbances in the boundary between apical and basolateral membrane (=tight junctions) that prevents free movements of water and solutes through the lateral intercellular spaces, affecting water channels of the aquaporin family recently identified in enterocytes and salts channels absorption (sodium chloride, potassium, calcium and phosphate) (Pácha 2000) what explain the observed edema in the submucosa could to lead malabsorptions. Malabsorption syndrome can be caused by gastrointestinal parasites (Mettrick and Podesta 1974; Horn Jr. and Fine 1977). Moreover, in severally infected hosts, the malabsorption by the host (as results of mucosal reduction as observed in Table 1) added with the host-parasite nutritional competition [due to a complex syncytium in the body wall with several pores and canals to absorption in Acanthocephala (Hayunga 1991; Bush et al. 2001)] can increase clinical signs of preexistent pathologies or contributes to break of the homeostasis in the host (Mettrick and Podesta 1974; Thompson 1983; Carlton and McGavin 1998). Dezfuli et al. (2002) record *Pomphorhynchus*-related catarrhal enteritis and strong infiltration of granulocytic cell series. For *A. australis*, the catarrhal enteritis was Cestoda-related [Diphyllbothriosis and Anoplocephalosis (RZ Silva et al. unpublished data)] but granulocyte infiltration was not present.

Conclusion

Corynosoma australe does not cause inflammatory reactions in the intestines of *A. australis* but its attachment-detachment sites during adult life stage show a chronicle and continuous pattern of mucosal embedding until reach the intestinal submucosa.

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Table 1 Variance Analysis (Tukey's test; $p < 0.05$) for histometries (thickness) of the normal and abnormal mucosa (μm) to small and large intestines of *Arctocephalus australis* parasitized by *Corynosoma australe*.

Intestine Layer	Intestine Portion			
	Small Intestine		Large Intestine	
	Normal dimension (μm)	Abnormal dimension (μm)	Normal dimension (μm)	Abnormal dimension (μm)
Mucosa	1116.75 $\pm$ 380.82 ^a	313.27 $\pm$ 239.69 ^b	880.81 $\pm$ 40.62 ^a	280.04 $\pm$ 257.72 ^b

Different letters mean significant differences between normal and abnormal mucosa according to each intestine. Variance Analysis (ab) $p < 0.05$

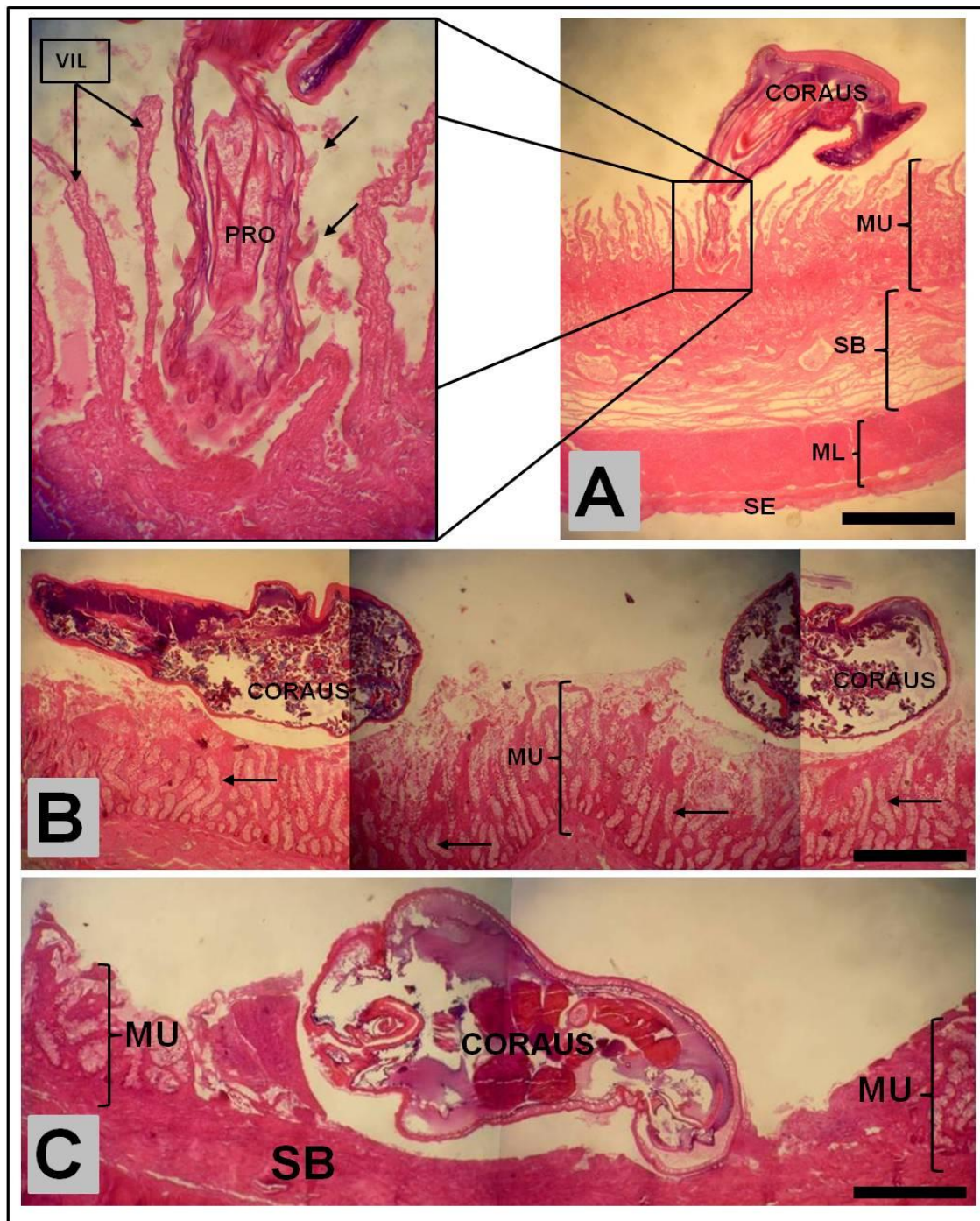


Fig. 1 Microphotographs of body depth intestinal attachment (BDINA) pattern of *Corynosoma australe* (CORAUS) within small intestine of *Arctocephalus australis*. MU – Mucosa; SB - Submucosa; ML - Muscular layer; SE – Serosa. A: BDINA-1. Note the villi (VIL) attachment of the armed (arrows) proboscis (PRO) and the edema of the submucosa. Stain: H-E. Scale bar: 270µm. B: BDINA-2 in initial phase. Observe the absence of villi in the attachment site and the cellular remains. Parasite is reaching the Lieberkühn crypts (arrows). Stain: H-E. Scale bar: 220 µm. C: BDINA-3. Parasite destroys completely the mucosa and reaches submucosa. Stain: H-E. Scale bar: 140 µm.

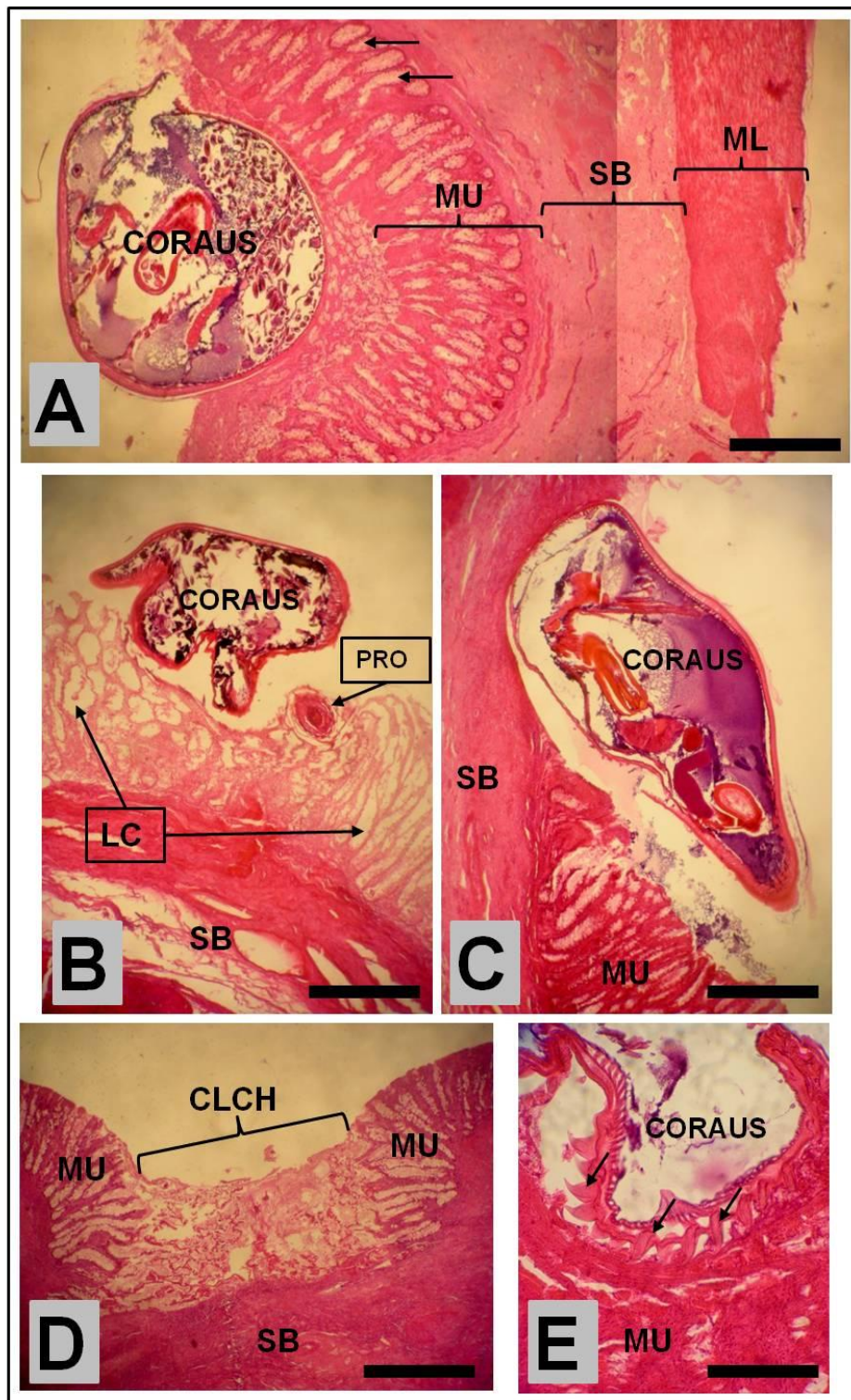


Fig. 2 Microphotographs of body depth intestinal attachment (BDINA) and crater-like concave holes (CLCH) pattern of *Corynosoma australe* (CORAUS) within large intestine of *Arctocephalus australis*. MU – Mucosa; SB - Submucosa; ML - Muscular layer; SE – Serosa. A-B: BDINA-2. In (A) note the direct contact of the parasite with the intestine wall, the cellular debris between parasite and intestine and (in B) the proboscis (PRO) attachment in the Lieberkühn crypts and the edema of the submucosa. Stain: H-E. Scale bar: 520µm. C: BDINA-3. Mucosa is completely destroyed and parasite reaches the submucosa. Stain: H-E. Scale bar: 260µm. D: CLCH. Observe the absence of parasite within mucosal lesion, the mucosal removal and the re-epithelization process. Stain: H-E. Scale bar: 260µm. E: Parasite trunk as a secondary holdfast. Note the trunk spines (arrows) anchoring the intestine mucosal tissue. Stain: H-E. Scale bar: 130µm.

ANEXO IV

Unusual intestinal attachment of *Corynosoma australe* in *Arctocephalus australis*

Renato Z. Silva, Joaber Pereira Júnior and João Carlos B. Cousin

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CASE REPORT

Unusual intestinal attachment of *Corynosoma australe* in *Arctocephalus australis*

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ABSTRACT

Background: In endothermic vertebrates adult Acanthocephala normally settles and recruits within the intestinal lumen, but can be found gastric enigmatic exceptions. Modification in the normal parasite's microhabitat installation generally causes strong host's defensive mechanisms. *Corynosoma* spp. Lühe, 1904 and other Acanthocephala are common parasite found in Pinnipedia such as the South American fur seal *Arctocephalus australis*, but little is known about parasitemia in marine mammals that occur in Brazilian waters.

Case: Unusual attachment site of *Corynosoma australe* in *A. australis* is reported during the necropsy proceedings for sampling tissues and parasites in one male *A. australis* from the southern Brazilian coast. The parasite was attached outside the small intestine. The parasite was identified according to the intraluminal infrapopulation *sensu* criteria for proboscis

oncotaxia, body shape, trunk (presoma and metasoma) spine (structure and distribution pattern) and organology according to the infrapopulation collected from the intestine lumen that were treated and prepared for permanent mounting in balsam and staining with Semichon's carmin or Eosin. The unusual specimen was excised for histological routine protocol for embedding in paraffin and permanent mounting in balsam. Stereomicroscopy for gross describing was performed before histological sampling. Macroscopically, the parasite was within a serosal non-perforated concavity without encapsulation and other host's defensive mechanisms. Histologically, there was an intraluminal attached *C. australe* specimen and almost opposite there was the unusual outside specimen on the serosa. The submucosa and muscular layer were compressed between parasites. The mucosal specimen caused erosion of the intestinal villi and Lieberkühn crypts. The serosal specimen was very superficially deposited and causes serosal erosion and deposition of cellular debris in the parasite-host interface. There was no fibrosis of the intestine wall layers. There was no parasitic encapsulation as well as other inflammatory processes. The muscular layer showed a bagpipe-like folded feature. Scars and necrosis by parasitic migration across intestinal layers were not observed.

Discussion: Acanthocephala shows an evolutionary selection toward an affective attachment performance within the gastrointestinal lumen of the definitive hosts. The serosal concavity formed by the parasite's trunk corroborates studies about Acanthocephala anatomical adaptations for attachment. The gross concavity aspect corroborates the microscopical findings and the parasite probably uses the trunk as a secondary holdfast. The concavity can also represent a way to avoid the parasite detachment by intestinal peristaltic movements. The serosal non-invasive attachment can mean that the epithelial features of the intestinal mucosa and its topography are important to the adequate attachment. The serosal characteristics (loose surface, reduced thickness, lamina propria's loose connective tissue) and

non-epithelial nature of the muscular layer can also difficult the parasite's attachment. The bagpipe-like folding added with the serosal concavity can represent a compensatory way to the lesser available contact surface for attachment in comparison with the intestinal mucosa. The absence of inflammation could be a result of the acuteness of the parasite installation on the serosa. Added to this, there was no depth injury caused by the parasite outside-to-inside the intestine, probably as inadequate serosa topography to holdfast attachment.

Key words: intestinal histopathology, unusual parasite attachment, corynosomiasis, mammalian host, Arctocephalinae.

Descritores: histopatologia intestinal, ancoragem parasitária anormal, corinosomíase, hospedeiro mamífero, Arctocephalinae.

INTRODUCTION

Acanthocephala as well as several parasite groups have heteroxenous life-cycle that, generally, comprises a specific host-parasite relationship. This relationship occurs within different trophic level, according to the fitness to finish adequately their infective life-cycle stages, towards adult reproductive stage within an adequate site in the definitive host [7,9].

In endothermic vertebrates adult Acanthocephala normally settles and recruits within the intestinal lumen [7,4,10,17], but can be found gastric enigmatic exceptions for some species [3,34]. Modification in the normal microhabitat installation, i.e., unusual site to settle and recruit, generally causes strong host's defensive mechanisms, such as inflammatory response and parasite encapsulation [17,23,32]. This is due to parasite-host specificity molded by co-evolutionary processes [13] that results in a parasite's migratory movements within

host's body commanded by biochemical characteristics from host's synthesized products (cytokines) or components (fibers, extracellular matrixes) by target tissues or organs [9,11,32].

Corynosoma spp. Lühe, 1904 and other Acanthocephala are common parasites found in Pinnipedia [22] such as the South American fur seal *Arctocephalus australis* (Zimmermann, 1783) [16], but little is known about parasitemia in marine mammals that occur in Brazilian waters [8,24,26].

CASE REPORT

Unusual attachment site of *Corynosoma australe* (Acanthocephala, Polymorphidae) in *Arctocephalus australis* is reported during necropsy proceedings to sampling normal and abnormal gastrointestinal tissues and parasites in one male *A. australis* (total length = 1,63m) out of from 32 specimens (16male:16female) sampled. It was perceived an Acanthocephala attached outside the small intestine (ileum portion) serosa. The hosts were collected from Cassino Beach (ca. 32°11'14.23"S; 52°09'21.70"W) to Chui (ca. 33°44'35.96"S; 53°22'12.70"W) – Rio Grande do Sul – Brazil (IBAMA: SISBIO licence 17529-1). The outside attached parasite was identified as *Corynosoma australe* according to the infrapopulation within intestine lumen of the hosts. The criteria for analysis and identification, i.e., proboscis oncotaxia, body shape, trunk (presoma and metasoma) spine (structure and distribution pattern) and organology follow Petrochenko [20], Zdzitowiecki [35], Pereira Jr. & Neves [19] and Sardella et al. [25]. The intraluminal parasites were treated and prepared *sensu* Amato et al. [1] to permanent mounting in balsam and staining with Semichon's carmin or Eosin. The unusual attached specimen and the vicinity intestine tissue area were excised to be fixed (Bouin's fluid) for histological routine protocol to embedding in paraffin under cutting

thickness of 7µm, stained with Hematoxylin-Eosin and permanent mounting in balsam [27,28]. Before histological sampling the unusually attached specimen and intestine vicinity were observed under stereomicroscopy.

Macroscopically, the intestine outside parasite was within a concavity without encapsulation and other defensive mechanisms, as calcification, by the host. The concavity was not a perforated hole seen to be a fold-like of the intestinal serosa.

Histologically, this abnormal region suffers two injuries ways of parasitic attachment. There is a *C. australe* specimen attached within the mucosa (intestine lumen-to-serosa-ward, i.e., inside-to-outside) and almost opposite there is another specimen attached on the serosa (visceral cavity-to-intestine lumen-ward, i.e., outside-to-inside) within a concave fold-like of the serosa. There were two compressed intestinal layers between these two attached specimens: the submucosa and muscular layer. The submucosa is thinner than the rest of histological slide. The specimen attached within the mucosa caused mucosal erosion of the villi and Lieberkühn crypts. Concerning the outside specimen, it was very superficially deposited. It was noted that the serosa is eroded and cellular debris from the mesothelium and loose connective tissue from the lamina propria were deposited in the parasite-host interface. The erosion of the serosa displays the muscular layer directly to abdominal cavity. There was no fibrosis of the serosa or of other intestine wall layer. There was no parasitic encapsulation as well as inflammatory processes as abscess- or granuloma-like starting. The muscular layer was, *a priori*, intact and scars and necrosis by parasitic migration across intestinal layers above (=intestinal lumen-to-serosa-ward) are not noted. The outer muscular layer is bagpipe-like folded (Figure 1).

The stereomicroscopical fold-like aspect of the serosa was confirmed under optic microscopy and the parasite showed that anterior portion of the trunk, possibly, acting as a secondary holdfast, providing and/or maintaining the fold of serosa.

DISCUSSION

Acanthocephala suffered several selective ecologic pressures to excel the biological mechanism toward an affective attachment performance [2,13,20,30,31]. The concavity formed with help of the anterior portion of the trunk of *C. australe* corroborates similar study to understand external (=body spines) and internal anatomy of trunk characteristics of *C. cetaceum* that acts concomitantly with the proboscis hooks to maintain the parasite attached in its hosts [2]. The concavity can also represent a way to avoid the detachment by intestinal peristaltic movements that externally, i.e., in the visceral cavity, led to attrition between intestinal handles [12].

The superficial attachment (no depth embedding) on the serosa can mean that the epithelial features of the mucosa and its topography, i.e., villi and Lieberkühn crypts are important to the adequate attachment of the parasite in the intestinal mucosa (as the adequate site for cystacanth's settlement and recruitment). This approach is considered by Hayunga [13] to parasite holdfast adaptation to host intestine topography. The loose surface and the thinner serosa addicted with the non-epithelial nature of other reached intestinal layers (in this case the muscular layer and the loose connective tissue of the lamina propria of the serosa) [12] can also represent a barrier toward a deeper attachment. The bagpipe-like folded outer muscular layer addicted with the concavity formation on the serosa (discussed above) can represent a compensatory way to the lesser available contact surface to attach in comparison with the higher available contact surface of the intestine mucosa [18]. Thus, the host

morphological adaptations to intraluminal intestine existence, i.e., adequate holdfast to an adequate site acquisition to adequate development by parasite [7,13,15,20] can be implied in this question (Anexos I and III).

Despite the absence of inflammation observed in this case, other studies record diverse pathological aspects related with acanthocephalosis in aquatic mammals developing inflammatory processes [5,14,27-29]. On the other hand, the acanthocephalosis is considered by some authors as causative of insignificant pathological effects in Pinnipedia [6,23,33] but studies indicate high mortalities rates in newborn Arctocephalinae partially due to parasitosis [21]. The absence of inflammation as observed here could result from the acuteness of the parasite installation on the serosa, but mistakes to parasite site of settlement (be acute or chronic) conduct to severe immunological response forming granuloma and abscesses [27,32].

In conclusion, the isolated unusual way of attachment of *C. australe* outside the intestine wall of *A. australe* does not cause inflammatory processes in the host. It might be a result of acuteness of the parasite installation on the serosa, added to this there was no depth injury caused by the parasite outside-to-inside the intestine probably as inadequate serosa topography to holdfast attachment.

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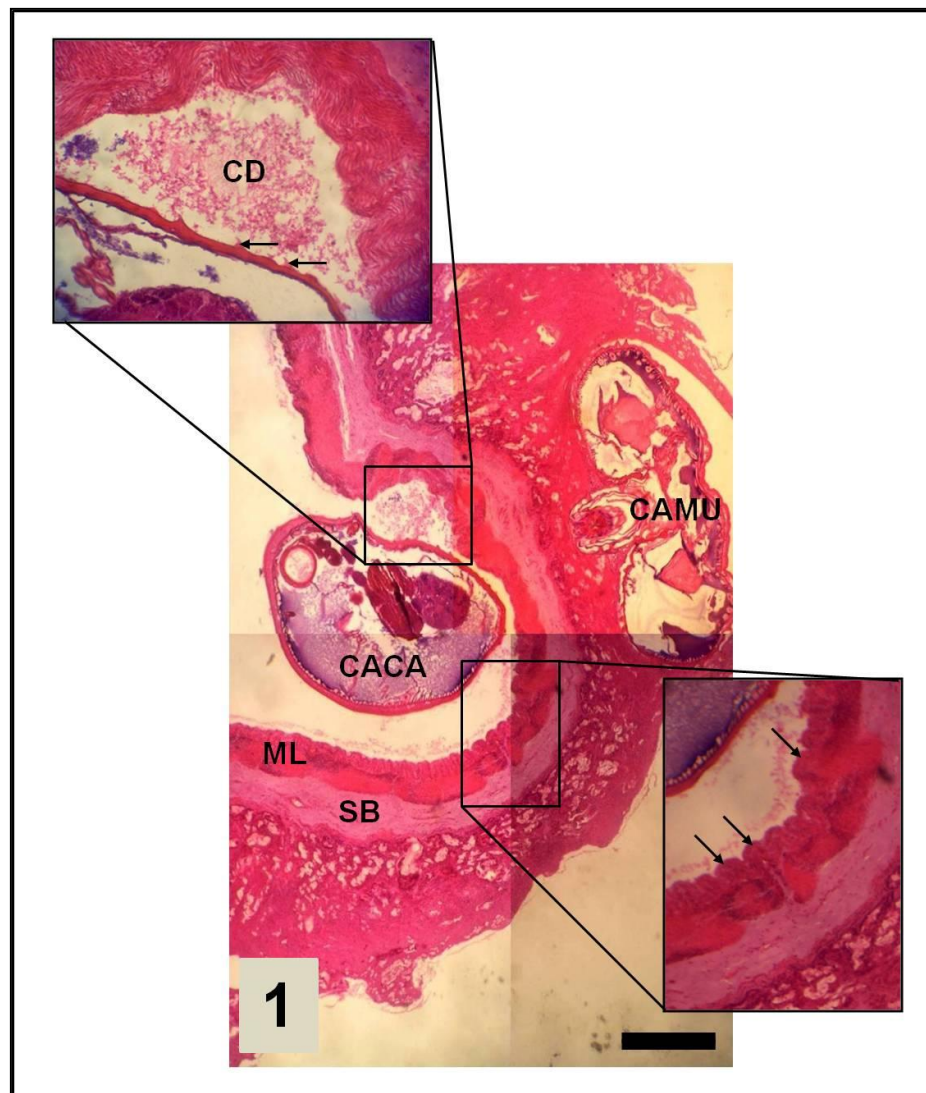


Figure 1. Microphotograph of unusual settles of the *Corynosoma australe* in the intestine of *Arctocephalus australis*. Note that there is one parasite adequately attached (CAMU) within the intestine mucosa and other attached outside intestinal lumen (inadequately on the visceral cavity space) (CACA) within a concavity by folding of the intestine wall (Muscular layer – ML and Submucosa – SB). **Superior left detail:** interface of parasite-external intestine host wall showing cellular debris (CD) from serosa and parasite's trunk spines (arrows). **Inferior high detail:** interface of parasite-external intestine host showing the bagpipe-like folded (arrows) outer longitudinal muscular. Stain: H-E. Scale bar = 260µm.

ANEXO V

***Contracaecum* sp. (Nematoda, Anisakidae) in *Arctocephalus australis* (Mammalia, Pinnipedia) from Southern Atlantic coast: histopathology, parasitological indices, seasonality and host gender influences**

Renato Z. Silva, João Carlos B. Cousin and Joaber Pereira Jr.

(Normatizado de acordo com *The Latin American Journal of Aquatic Mammals*)

***Contracaecum* sp. (Nematoda, Anisakidae) in *Arctocephalus australis* (Mammalia, Pinnipedia) from Southern Brazilian coast: histopathology, parasitological indices, seasonality and host gender influences**

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Abstract – The contraeciasis-related parasitological variables [Prevalence (P%), Mean Intensity (MI), Mean Abundance (MA), Range of Variation (Ax) and Crowding value (CRD, s^2/x)] as well as the histopathology and lesional variables (L-P%, L-MI, L-MA and L-Ax) are presented based in the necropsy of 32 specimens stranded dead on the shore of Rio Grande do Sul – Brazil from 2008 to 2010. Differences for P% (Chi-square test, X^2) and MI (Bootstrap Confidence Interval, BC_a , $p < 0.05$) were analyzed for host's genders and reproductive status [males (pups, prepubertals) and females (pups)] and for host's genders in grouped seasons (Spring-Summer: S-S; Autumn-Winter: A-W). L-P% and L-MI were analyzed as P% and MI. Normal and abnormal mucosa were compared (Variance Analysis, Tukey's test, $p < 0.05$). Lesions were counted and measure. It was found five *Contraecium*-related main types of stomach lesions in the body-fundus and pylorus of *A. australis*. Two were non-severe: *Contraecium*-related embedding within the stomach wall (CESW) with partially hanging out mucosa parasite's body that causes mucosal erosion and necrosis, and *Contraecium*-related punctual attached-detached site (CPADS) that showed and sklerotized chitinous-like oral mold-like capsule of parasite attachment (OPCA) with parasite's labia intaglio. Two severe inflammatory lesions: ulcers and piogranuloma (with severe immune cell exudation (macrophages, multinucleated giant foreign-cells, lymphocytes, eosinophils and neutrophils), fibrosis, mucosal and submucosal necrosis and submucosal parasitic tunnels of displacement and scars (healing lesions). Inflammatory lesions caused significant mucosal reduction in thickness. Only male hosts presented piogranuloma. Genders showed similar pattern for acquisition of the stomachal lesions but ulcer's dimensions differed between genders. Female hosts reach their parasite's burden earlier than males, toward the adulthood. There was no gender-related difference for P%, MI, L-P% and L-MI concerning to the total host's and pups, but males showed the higher parasite's burden. Reproductive-breeding females showed lower lesion numbers than prepubertal males, but there were no gender-related numerical

differences. Contraecaeciasis is non-seasonal and this is reinforced by different parasite's cohort found within gastric lumen. Male and female hosts could be responding differently to gastric lesion formation and number and dimension toward the adulthood may due to gender-related immunological heterogeneities.

Key words: Contraecaeciasis, Mammalian host, Arctocephalinae, Southern Brazilian coast

Introduction

Hosts are the ecosystems (or microecosystems) where parasites derive their resources to survive in a complex host-parasite relationship with several pathophysiological degrees (Mettrick and Podesta, 1974; Poulin 1996; 1998; Bush *et al.* 2001; Jairajpuri, 2005). This insight led Margolis *et al.* (1982) to postulate concepts as infrapopulation and infracommunity. For marine mammals populations, the helminthiasis comprise commons findings (Colongue *et al.*, 1985; Measures, 1992; Valente *et al.*, 1997; Abollo *et al.*, 1998b; Aznar *et al.*, 1994; 2004; Nickol *et al.*, 2002; Béron-Vera *et al.*, 2007; Morgades *et al.*, 2006), showing that gastrointestinal helminths are strongly adapted to their microenvironment (Mettrick and Podesta, 1974; Hayunga, 1991; Dybdahl and Storter, 2003). In definitive hosts such as aquatic organisms, the complex host-parasite relationship can result in tissue abnormalities (Dailey and Stroud, 1978; Ruopplo, 2003; Silva and Cousin, 2004; 2006a, b; Motta *et al.*, 2008).

Due to the oral apparatus added with digestive enzymes for host's tissue penetration and/or histophagy (McKerrow, 1989; Ros-Moreno *et al.*, 2000; Dzickonska-Rynko *et al.*, 2003; Dzik, 2006), Nematoda are very pathological for their hosts causing severe tissue gastrointestinal alterations in several hosts *taxa* such as fishes (Obiekezie *et al.*, 1992; Meguid and Eure, 1996), reptiles (McAllister *et al.*, 1993; Taiwo *et al.*, 2001; Mihalca *et al.*, 2007),

birds (Si-Kwang Liu and Edward, 1971; Rokicki *et al.*, 2011;) and mammals (Si-Kwang Liu and Edward, 1971; Spraker *et al.*, 2003; Abollo *et al.*, 1998a; Silva and Cousin, 2004; Jaber *et al.*, 2006; Motta *et al.*, 2008). However, there is broad paucity concerning to microanatomical analysis of lesions caused by helminths found in aquatic mammals (Parsons & Jefferson, 2000; Ruopplo, 2003; Spraker *et al.* 2003; Jaber *et al.* 2004, 2006; Silva & Cousin, 2004; 2006a, b; Motta *et al.* 2008).

Parasitic Nematoda shows a wide diversity and plasticity (body size, cuticle ornamentations, oral apparatus, stoma or oesophagostom and annexes, sensory structures, spines, papillae and other features) for parasitizing all animal groups (Jairajpuri, 2005; Bush *et al.*, 2001). Concerning to Vertebrata, it is observed, at least, that each species is the definitive host for two Nematoda parasitic species, then 100.000 species of Nematoda can be stipulated parasitizing this animal taxon (Jairajpuri, 2005). Moreover, Dobson *et al.* (2008) estimate 75.000 to 300.000 helminth species parasitizing Vertebrata. Within parasitic Nematoda, Anisakidae such as *Anisakis* spp. presents zoonotic and economic implications, mainly due to the higher raw fish consume in a globalized world (Kagei *et al.*, 1978; Carvajal and Rego, 1985; Barriga *et al.*, 1999; Rosales *et al.*, 1999). Moreover reviews such as that done by McCarthy and Moore (2000) and Dorny *et al.* (2009) record *Contracaecum* spp. as etiological agent of human zoonoses and this should be considered to Brazilian waters.

The most common Pinnipedia species to strand in the Southern Brazilian coast is the South American fur seal *Arctocephalus australis* (Pinedo, 1990; Silva, 2004; Mäder *et al.*, 2006). *Arctocephalus australis* is widely distributed in South America from the Atlantic Ocean (Rio de Janeiro – Brazil), southward for the Pacific Ocean (Tierra del Fuego) and northward to Lima – Peru (Bonner, 1981; Vaz-Ferreira, 1982; Ponce de León, 2000) and comprises a definitive host for several gastrointestinal helminths (Morgades *et al.*, 2006). Researches about several aspects of the biology of this mammalian species such as diet

(Santos and Haimovici, 2001; Naya *et al.*, 2002) and the parasitism (Morgades *et al.* 2006) as well as parasite-related tissue changes should be considered as scarcely contemplated. Hosts with wider demographic ranges, such as *A. australis*, tend to demonstrate higher parasite's species richness (Bush *et al.*, 2001). Scarce numbers of works contemplate parasitological indices in Pinnipedia in Brazilian coast (Pereira, 2012), despite the parasite's importance on the ecology of the biosphere (Windsor, 1998).

Several host's intrinsic features such as age, sex and host's immunity as well as by intra- inter-specific parasite relationships and tend to be male-biased according to the parasitological indices (infection levels) or parasite distribution patterns (pattern of parasite spread within the host population) on several animal host groups drives parasitosis such as arthropodiasis, nematodiasis and acanthocephalosis (Mettrick and Podesta, 1974; Barriga and Al-Khalidi, 1991; Wilson *et al.*, 2002; Klein, 2004; Cowan *et al.*, 2007; Ferrari *et al.*, 2007; Khokhlova *et al.*, 2010). On the other hand, female-biased helminthiasis such as cestodiasis (Barriga and Al-Khalidi, 1991; Escobedo *et al.*, 2004, Morales-Montor *et al.*, 2004) and nematodiasis (Rossin *et al.*, 2010), are recorded.

The normal gastric histology and the *Contracaecum*-related lesion types and histopathology and parasitological indices are presented for host's gender, host's sexual maturity status and seasonality in *A. australis* from the Southern Brazilian coast.

Materials and Methods

Hosts sampling: The hosts specimens (16male:16female) were collected from the Cassino Beach (ca. 32°11'14.23"S; 52°09'21.70"W) to Chui (ca. 33°44'35.96"S; 53°22'12.70"W) - Rio Grande do Sul State – Brazil. They were sexed by external examination and the total lengths (TL) were measure linearly in meters (m) following Dierauf

(1994). Samplings were performed within two great seasons grouping, *i.e.*, Autumn-Winter (A-W) and Spring-Summer (S-S) from August-2008 to September-2010.

The host's carcasses utilized in this study were observed for their conservation status according to Dierauf (1994). Only carcasses between Dierauf's code 2 and 3 were utilized to necropsy procedures. Other *post-mortem* alterations were observed, such as *rigor-mortis*, *livor-mortis*, abdominal expansion by decomposition gases and absence of decomposers (as larval forms of insects) (Thomson 1983) in addition to Dierauf's procedures for hosts specimens samplings,

The opening of the thoracic and abdominal cavities, for the removal of digestive system, for the removal of the reproductive organs and for the gross analyses of the organs, followed Dierauf (1994) for necropsy procedures. The digestive tube was divided in its anatomical parts (esophagus and stomach, small and large intestines). Each part was tied, labeled, bagged and frozen for the parasite screening, tissue samplings and parasite-related lesion observation. The stomach was separated from the intestines, opened and washed (for parasite collection) and observed for attached parasites and lesions (more details below: Parasite protocols).

Host's sexual maturation: The sexual analysis for the host's sexual maturation was performed according to the gametogenesis of the testicles (both left and right) with epididymis and the ovaries. The female gonads were observed under stereomicroscopy to ovarian follicles, luteogenesis and the luteolysis *sensu* Bukovsky *et al.* (2005), Katz *et al.* (2009), Lucacin and Pinto-Neto (2009) and Palma-Cerda *et al.* (2011). Ovaries (both left and right) were separate from the uterus and ovarian sections (1-2mm) were performed and observed for folliculogenesis. The histomorphology of the spermatogenesis followed Wistuba *et al.* (2007).

Histological protocols: Samples of normal and abnormal (lesion type inflammatory areas, parasite wall embedded *in situ* and parasite detached areas) were extracted from the body-fundus and pyloric (pylorus) regions of the stomach. Testicular tissue samples were extracted transversally in the center of the gonad in order to sample the epididymis as well as midsagittal tissue sections from both ovaries. The stomach's tissue samples were fixed (Bouin's fluid) for the histological routine protocol for embedding in paraffin; cutting (7µm thickness); staining (Hematoxylin-Eosin and Alcian-Blue) and permanent mounting in balsam (Silva and Cousin, 2004).

The gonadal tissues were fixed, cut, stained (Hematoxylin-Eosin) and mounted as well as the stomach tissue samples. According to the spermatogenesis the male host's were categorized as: pups (spermatogonia and spermatocytes), prepubertal (spermatids); pubertal (spermatozoa within seminiferous tubules = testicular spermatozoa) and adult reproductive (spermatozoa within epididymis) (Wistuba *et al.* 2007). Host's female sexual maturity was categorized by the follicle-type population within ovarian stroma as: pups (mainly primary follicles presence); prepubertal (Antral and Graafian follicles presence) and adult reproductive-breeding females (corpus luteum-corpus albicans presence) (Bukovsky *et al.* 2005, Katz *et al.* 2009, Lucacin and Pinto-Neto 2009, Palma-Cerda *et al.* 2011).

The thickness of the stomach normal and abnormal layer (=mucosa) affected by *Contracaecum* sp. was compared by Variance Analysis (Tukey's test; $p < 0.05$). It was utilized a micrometric slice (Nikken – Tokio; 0.01mm) to calibrate an eyepiece lens according to the different magnitudes (4x10, 10x10, 20x10), following Silva and Cousin (2004; 2006a; b). The gross and microscopic aspects of the lesions were utilized to typify the severity of the injury (*sensu* Thomson 1983).

Parasite protocols: Each stomach was opened and washed into plastic bowl for parasite collection. The content of the plastic bowl was sieved to be observed under stereomicroscope for the screening of the parasites. The stomach wall was observed for attached parasites and lesions (more details below: Gross lesion treatment). Parasites were collected, counted and screened for mounting. The entire intestine was extracted from the abdominal cavity. The small and large intestines were separated. Each one was linearly measured in meters (m). The small intestine was cut in two parts of equal lengths. Each small intestine half was divided in subsets to be opened and washed into a plastic bowl (same stomach's procedure) for Nematoda collection.

They were prepared for permanent mounting in balsam and staining (Semichon's Carmim and Eosin) following and adapting from Amato *et al.* (1991) and Silva and Cousin (2004). Semi-permanent mounting were accomplished in Aman's lactophenol for parasites previously fixed in Bouin's fluid. Parasite identification was performed *sensu* Yorke and Maplestone (1926), Anderson *et al.* (1974), Vicente *et al.* (1985); Fagerholm and Gibson (1987), Paoletti (2009).

The helminths were observed for their ontogenetic stage: newly hatched larva [(stage 3 larva (L-3) to stage 4 larva (L-4) with larval loose-fitting cuticle, differentiated esophagus, ventriculus, ventricular appendix and intestinal caecum and boring tooth)], juvenile (mainly for female specimens without ova within the very well developed convoluted uterus) and adults [female (uterine mass ova) and males (well developed spicules, presence, form and distribution of prominent precloacal and postcloacal papillae) *sensu* Anderson *et al.* (1974), Fagerholm and Gibson (1987), Kanarek and Rolbiecki (2006), Paoletti (2009) and Dziekonska-Rynko *et al.* (2010).

The host's individual Parasite Infection Intensity (PII), *i.e.*, the raw parasite values of infection (stomach+intestines) was performed for male and for female hosts' length

(Regression Analysis) to provide the Dispersion Tendency Line (DTL) ($y=a+bx$; for the b-slope value) and the Coefficients of Determination (r^2) and Pearson's Coefficient of Correlation (r) (Vieira, 1981; Silva and Pinto, 2011). The parasites from the stomach and intestines were analyzed together for PII because only larval *Contracaecum* sp. forms were found within intestine lumen and because anisakidosis, including contraeciasis, is typically gastric in endothermic hosts (Valente *et al.*, 1997; Abollo *et al.*, 1998b; Spraker *et al.*, 2003; Kanarek and Rolbiecki, 2006; Morgades *et al.*, 2006; Dziekonska-Rynko and Rokicki, 2008).

Parasitological indices such as Prevalence (P%), Mean Intensity (MI) and Mean Abundance (MA) (Bush *et al.*, 1997) were determined and analyzed *sensu* Rózsa *et al.* (2000) by the "Quantitative Parasitology Program - QP 3.0 Program" for total sampled hosts, host's gender and host's gender sexual maturity status. The P% (Chi-square test, X^2) and MI (Bootstrap confidence interval, BC_a , $p<0.05$) were compared between host's gender (total males vs. total females), host's gender maturity status (male pup vs. prepubertal males; male pups vs. female pups; prepubertal males vs. reproductive-breeding females and female pups vs. reproductive-breeding females). The Range of Variation (Ax) of the parasite was expressed as Bush *et al.* (2001).

The distributional patterns, *i.e.* random, uniform or aggregated, of *Contracaecum* sp. were performed *sensu* Von Zuben (1997), Rózsa *et al.* (2000), Bush *et al.* (2001) and Reiczigel *et al.* (2005) between males and females hosts. The statistical significance for goodness of fit was assessed by Chi-square test (X^2) (Von Zuben, 1997; Rózsa *et al.*, 2000; Bush *et al.*, 2001; Reiczigel *et al.*, 2005) for host's gender and host's gender sexual status as well as for P%.

Seasonal variations for the P% and MI were compared between A-W and S-S grouped seasons such as for all hosts and for all sample period.

Gross Lesion treatment: Lesion type-related measurements were effected in lesion's length and width. Lesion-related indices were utilized *sensu* Abollo *et al.* (1998) that apply to the gastric ulcers of Cetacea species the Abundance indices. Thus Lesion Prevalence (L-P%), Lesion Mean Intensity (L-MI), Lesion Mean Abundance (L-MA) and Lesion Range of Variation (L-Ax) were utilized. These lesion-related indices were adapted and extended to Bush *et al.* (1997) to be analyzed *sensu* Rózsa *et al.* (2000) by the "Quantitative Parasitology Program - QP 3.0 Program for L-P% and L-MI as for the P% and MI. The lesion's length and width, as well as the lesion's number, were compared between host's genders (Variance Analysis, Tukey's test, $p < 0.05$). The host's individual Parasite-related Lesion Intensity (PLI), *i.e.*, the raw severe inflammatory *Contracaecum*-related lesion was performed for male and for female hosts' length according to their PII.

Results

Gross lesions characteristics

Macroscopically, contraeciasis-related gastric lesions for both, body-fundus and pyloric, stomach analyzed regions showed two main no-severe inflammatory lesions and three main severe inflammatory types.

The non-severe contraeciasis-related gastric lesions types were dispersed within the stomach lumen. These lesions were isolated and mainly *Contracaecum*-related embedding within the stomach wall (CESW) unlike the pale *Contracaecum*-related punctual attached-detached site (CPADS) of swallows oral attachment by adult forms (Fig. 1A). Concerning to the CESW, it is observed that the parasite's body portion hanging out from the mucosa wall causing a low relief print on the mucosa surface. CPADS are low relief trilobulated printed areas with the intaglio of the adult Nematoda labia. This latter mucosal alteration can be observed in stomachs recently opened with several adult parasite already free as well as

during the procedures for lesions searching that result, sometimes, in natural detachment in real-time. They are perceptible by the touch as rigid topographic modification on the mucosal surface. Contraecaeciasis, in sampled *A. australis*, did not form attached parasite clusters on the stomachal mucosa. Edematous and hyperemic mucosa (gastritis-like) was observed as well as multifocal hemorrhage points mainly in specimens with higher number of stage 3 larvae.

The contraecaeciasis-related severe inflammatory lesions were ulcers (nearly opened ulcers), piogranuloma and scars (healing ulcers). The ulcers were acute or semi-acute lesions that show well demarked edges, star-like shape to well circular aspect concerning to the normal gastric tissue around, bright and smooth surface from brownish to deep dark and they are generally associated with Nematoda mucosal embedding (Fig. 1B). The scars and ulcers were present in the body-fundus as well as in the pylorus region. The scars comprised healing chronic lesions, very well delimited, pale, smooth and depressed areas (crater-like) on the stomach mucosal surface (Fig. 1C). Both scars and ulcers occurred in the gastric folds top as well as in the gastric folds base (between gastric folds) and in their vicinities can be observed features of the non-severe CPADS lesions. The piogranuloma were palpable protuberances and pus leaks from the lesion under manipulation. They occurred only in the body-fundus mucosa in the gastric folds top. Nematoda specimen was deep embedded by the lesion pore where the pus leaked. Gastric ulcers are the predominant lesion type, followed by gastric scars and piogranuloma (in only one male pup, TL=0.97m).

Values for total lesions and lesion type-related indices for the total sampled hosts are presented in the Table 1.

Gender-related lesional characteristics according to host's TL

Host's genders shows similar pattern of PLI slope increasing toward the adulthood (DTL) (male hosts: $r^2=0.2793$; $r=0.5284$; $b=35.242$ and female hosts: $r^2=0.4564$; $r=0.6756$;

b=31.751) according to their gender-related maximum length (Figure 2). However there was no PLI-related difference between host's genders for gastric lesions (male hosts mean PLI: 9.13 ± 20.56 and female hosts mean PLI: 4.31 ± 7.75 ; $p > 0.05$) as well as for host's genders or analyzed host's sexual maturity status L-P% and L-MI, the lesional variables were male-biased for almost all of them. Host's genders developed contracaeciasis-related lesions similarly (Table 3).

Concerning to the lesion's dimensions, they were male-biased, *i.e.*, male hosts showed the highest values for length and width. There were gender-related differences for ulcers dimensions; however scars' dimensions did not differ between host's genders (Table 4).

Concerning to the contracaeciasis-related lesions, male pups acquired the lesions slightly latter than female pups ($0.95m \geq TL \leq 1.76m$ and $0.87m \geq TL \leq 1.34m$, respectively) but in both cases the lesions occur with the parasite's presence (Figs. 2 and 6).

Histological lesions characteristics

CESW: Comprises mainly larvae and juvenile specimens that perforated the stomach wall and reached the mucosa and submucosa in several degrees toward the serosa. Thus the lesion's diameter is very close to that of parasite's diameter. There was no piogeny. The necrosis of the host's gastric tissues is close to the embedded parasite's body. The immune cell exudation comprised characteristics shared with the ulcers, as described below. The mucosal erosion is caused by the hanged out parasite's body remain that is in contact with the stomach's luminal surface (Figs. 3A and 3B).

CPADS: Comprises *Contracaecum* sp. adult-related forms of very small (punctual) sclerotized areas with oral mold-like parasitic capsule of attachment (OPCA) (Fig. 3C). OPCA were concave, as a bell-like format, with intaglio of the parasite's labia and inter-labia. They were strongly eosinophilic and resemble chitinous-like structures may as excreted or secreted by the parasite and merging with the epithelial tissue of the stomach's mucosa. Their

superior margins showed a serrate silhouette very close related with the parasite's cephalic collarete (Figs. 4A and 4B). In healing gastric areas these structures were mixed with the granulation tissue and they were surrounded by high quantities of macrophages and multinucleated foreign-giant cell, lymphocytes, neutrophils and histiocytes. Bacterial masses could be observed within its concavity mixed with cell remains.

Ulcers: The parasite deep embedding was ulcerogenic and this lesion reached the mucosa and submucosa in several depth degrees as described for the CESW above. Concerning to the mucosa there was the necrosis of the gastric or pyloric glands with total or partial loss of the normal mucosal architecture. The most striking feature of the necrosis was the pyknosis and karyorrhexis of the glandular cell population, lamina propria and muscularis mucosae, according to the stomach region and mucosal deep of affection. In gastric glands apically necrosis-affected there was the formation of hyaline-like secretion contents, mainly if the apical necrotic region was non-sequestered as these glands were blocked. The non-sequestered necrotic region was H-E-negative and maintains a black color in the preparations (Fig. 5A). Mixed within the cell necrotic debris are CPADS remains.

Concerning to the submucosa it was observed the same pattern of necrosis on the connective tissue elements, vascular and nervous elements. There was edema and vasodilatation. Parasites formed migratory channels within the submucosa matrix. These channels were slightly delimited by a fibrous capsule. The total loss of mucosal architecture exposes the submucosa and mucosal sequestration could be present. Thus necrotic feature also extends to the muscularis mucosae.

The vicinity of the necrotic tissue for the mucosa and submucosa was infiltrated by macrophages and/or multinucleated foreign-body giant cells, lymphocytes, neutrophils and histiocytes mixed with several degrees of fibroplasias and forming a granulation tissue. This granulation tissue was very thick around the parasite's body portion that penetrates the

stomach wall, mainly around the parasite's oral region. The granulation tissue can fully replace the mucosa in some cases.

The muscular layer and serosa were not affected by the mucosal and submucosal extension of the gastric ulcers.

Piogranuloma: Resembles strongly the CESW but it was observed pyogenia and fibrosis in the affected submucosa. The parasites builded similar migratory channels for the ulcers (Fig.5B).

Scars: This lesion is marked by the extreme regression or absence of the gastric mucosa. Sometimes the thinner submucosa is covered by a thin non-muciparous flattened epithelium. Granulation tissue, fibrosis and inflammatory cell exudation (in some regions in lower quantities), can be observed in lesions not totally healed similar to the pattern observed for ulcers.

The lesions decrease the thickness of the mucosa as recorded in the Table 2.

Host's parasite infrapopulation characteristics

Among the sampled specimens, the hosts' TL range was: $0.84\text{m} \geq \text{TL} \leq 1.76\text{m}$ to males and $0.8\text{m} \geq \text{TL} \leq 1.34\text{m}$ to females.

The contraeciasis was marked by high numbers of larval forms (L-3 and L-4) concomitantly with adult forms for both parasite genders. The ratio for larval forms and adult forms was not performed. Nematoda females comprised mated and non-mated specimens. This represents uninterrupted host re-infections throughout the sample period.

The host's values of the parasitological variables for contraeciasis are presented in the Table 5. The CRD value show that *Contraecum* sp. demonstrate an aggregate pattern to the host's stomach.

Parasite's distribution according to host's TL

Concerning to the histological analysis of the gametogenesis, the sampled hosts comprised males and females pups as well as prepubertal males and reproductive-breeding females. *Contracaecum* sp. occurred in male and female hosts with initial TL ($0.84\text{m} \geq \text{TL} \leq 1.76\text{m}$ and $0.87\text{m} \geq \text{TL} \leq 1.34\text{m}$, respectively). Pups of both host's genders showed the higher values for PII. Male pup hosts tended to show the highest values, however there was no gender-related difference between pups ($p > 0.05$). On the other hand, female hosts showed higher values for the DTL slope ($b = 318.04$) than male hosts ($b = 31.43$) toward the adulthood, concerning to the harboring of *Contracaecum* sp. These values showed that the PII in female hosts tended to increase several times (near 10 times) more than in male hosts toward the adulthood. Prepubertal males ($\text{TL} > 1.32\text{m}$) tended to show parasite's distributional intermediate values when compared to the reproductive-breeding females ($1.33\text{m} \leq \text{TL} \leq 1.34\text{m}$) (Fig. 6).

Gender-related parasitological indices

There was no gender-related statistical differences for the parasitological indices, according to host's ontogeny (pup-toward-prepubertal male or pup-toward-breeding-reproductive female), but if it is observed the total number of males with total numbers of females, the results are male-biased for P%, MI, MA and Ax, i.e., male hosts showed a tendency to harbor more parasites than female hosts. The pups from both genders showed similar pattern for male-tendency for parasite's harboring (Table 6). However, there were no gender-related differences for P% and MI for these cases. On the other hand, comparing the reproductive-breeding females to larger prepubertal males, the values for MI and MA reflect a female-biased tendency to harbor more *Contracaecum* sp. (Figs. 6-8). In light of these results, even through reproductive-breeding females harbor more *Contracaecum* sp., they develop fewer number of gastric lesions (Figs. 9 and 10).

Seasonally, there were no differences for P%, MI or CRD between A-W and S-S (Table 7).

Discussion

Anisakidae like *Anisakis* spp., *Contracaecum* spp. and *Pseudoterranova* spp. are Nematoda with success in the colonization process of their hosts in a broad range of environments (Raga *et al.*, 2002). It is widely recorded for fishes (Obiekezie *et al.*, 1992; Meguid and Eure, 1996), birds (Si-Kwang Liu and Edward, 1971; Rokicki *et al.*, 2011), reptiles (McAllister *et al.*, 1993; Taiwo *et al.*, 2001; Mihalca *et al.*, 2007) and mammals (Bishop, 1979; Abollo *et al.*, 1998a; Spraker *et al.*, 2003; Silva and Cousin, 2004; Jaber *et al.*, 2006; Motta *et al.*, 2008), both macro and microscopic gastrointestinal alterations caused by parasitic Nematoda. The oral apparatus of Nematoda are potentially detrimental for their host's tissues (Bush *et al.*, 2001; Roberts and Janovy Jr., 2008). Adult forms of *Contracaecum* sp. that infect *A. australis* presents swallow punctual oral mechanisms of parasite's attachment (CPADS-related) as a hyaline oral mold-like parasitic capsules of attachment (OPCA) that is apparently merged with the gastric host's mucosa. This feature was observed for other contraeciasis in piscivorous birds (Si-Kwang Liu and Edward, 1971; Rokicki *et al.*, 2011) and as well as other Pinnipedia species (Bishop, 1979; Spraker *et al.*, 2003). In all latter cited works, the authors described extremely similar oral attachment capsule's shape and histochemistry (as sclerotized and chitinous-like eosinophilic aspect). Sclerotic aspect feature for punctual attachment sites on the gastric mucosa was reported for *Anisakis typica* (Anisakidae) infecting *Pontoporia blainvillei* (Cetacea), but there was no OPCA-related features (Silva and Cousin, 2004). In all reports for contraeciasis, under optic microscopy, these oral attachment capsules showed the same serrate internal border and the parasite's labia intaglio as well as in the present work. Rokicki *et al.* (2011) mention the parasite's anterior

striation as responsible for this capsule's feature and Si-Kwang Liu and Edward (1971) as well as Bishop (1979) do not infer about this. Moreover, Rokicki *et al.* (2011) do not attribute the adequate *nomina propria* for the parasite's anterior (cephalic) anatomy and its link with the serrate border because authors could not obtain attached specimens. This could be a result of crescent contemporary studies that contemplate mainly internal Nematoda organology and molecular biology and systematic (Mattiucci and Nascetti, 2007; Kijewska *et al.*, 2009) rather the basic external characteristics (Fagerholm and Gibson, 1987). On the other hand, Si-Kwang Liu and Edward (1971), showed extremely closer histopathological image concerning to that from the present work for CPADS-OPCA. Thus, it was observed that the serrate border of the OPCA corroborate the Nematoda cephalic collarette (*sensu* Lent and Freitas, 1948; Anderson *et al.*, 1974; Tiekotter, 1981) interdigitations with oral capsule. Other Nematoda attachment patterns were present such as for *Camallanus oxycephalus* and *Spinitectus carolini* (Camallanidae) in intestinal mucosa of *Lepomis cyanellus* (Meguid and Eure, 1996). *Camallanus oxycephalus*-related adult attachmental mechanism formed peduncle-like intestine fibrous mucosal expansion toward its armed mouth. Unlike, *Spinitectus carolini* embedded deeply within the intestine wall closer the muscular layer, apparently helped by the cuticle spines. In light of this attachment particularities, the participation of the cephalic collarette of adult forms of *Contracaecum* spp. in the mucosal attachment should be considered.

The macro- and microscopic contraeciasis-related gastric alterations, i.e., CSEW, CPDAS, ulcers, scars and piogranulomas observed in *A. australis* corroborated pathological reports in Pinnipedia [such as *Eumetopias jubatus* (=jubata) (Si-Kwang Liu and Edward, 1971), *Erignathus barbatus* (Bishop, 1979), *Callorhinus ursinus* (Spraker *et al.*, 2003)], piscivorous birds [such as *Pelecanus erythrorhynchus* (Si-Kwang Liu and Edward, 1971), *Phalacrocorax auritus* (Kuiken *et al.*, 1999), *Ph. carbo* (Rokicki *et al.*, 2011)] and other wild

and domestic animals under helminthiasis and arthropodiasis (Horn Jr. and Fine, 1977). Cetacea species showed anisakiasis-related similar ulcers and/or granuloma (Abollo *et al.*, 19998a; Silva and Cousin, 2004; Jaber *et al.*, 2006; Motta *et al.*, 2008). In these latter studies as well as in the present study the processes of immune cell exudation (such as macrophages, multinucleated foreign-giant cell, lymphocytes, neutrophils, eosinophils and histiocytes), necrosis, granulation tissue and the fibrosis (reparation and cicatrization) development were very closer. The cited processes were typical of the chronicle inflammation and the immune cell types involved represent a host's way to eliminate the antigen (Thomson, 1983; Cliffe *et al.*, 2007; Nava-Castro *et al.*, 2011). Lymphocytes are immune cells that play the fundamental process to granulocytes (such as neutrophils, eosinophils, basophils) and phagocytes (such as macrophages) immunocompetence by the presentation of decodified epitopes from the antigen added with the major histocompatibility complex molecules (MHC-I and II) and CD-protein linhages, that permits the adequate realizing of proinflammatory cytokines [such as immunoglobulins (Igs), interferons (IFNs), tumoral factors, growth factors, leukotrienes) for the antigen control (Frank, 2002; Cliffe *et al.*, 2007; Paul, 2008; Reddy, 2010; Nava-Castro *et al.*, 2011). Parasites are antigens and their permanence (recruitment) in their hosts is, partially, dependent of intrinsic factors as host-parasite immunological processes that characterize the host-parasite fitness under coevolutionary processes (Wakelin, 1994; Combes, 1997; Dybdahl and Storter, 2003; Mayzels and Yazdanbakhsh, 2003; Nava-Castro *et al.*, 2011). Jaber *et al.* (2003a, b; 2006) demonstrated that helminthiasis (anisakiasis and trematodiasis) develop strong antibody (such as Igs, IFNs and MHCs) reaction in gastric and hepatic of Cetacea. These host-parasite inflammatory aspects demonstrate that the contracaeciasis-related lesions observed in *A. australis* are severe, chronicle and strongly allergenic as well as for other mammalian or avian hosts. The allergenic (proinflammatory trigger) Nematoda properties are strongly related with digestive enzymes present in parasite species. Enzymes for penetration

(serpine-, aspartic-, cysteine-, and metalloproteinases) within host's tissues and parasite's histophagous mechanisms are present in several parasite groups (McKerrow, 1989; Dzik, 2006). Proteases, glycosilases, hyaluronidases, hydrolases and aminopeptidases are important for parasite's penetration and settlement in host's body tissues and organs until to complete the relative developmental stage avoiding their expulsion (Ros-Moreno *et al.*, 2000; Dziekonska-Rynko *et al.*, 2003; Dzik, 2006). Moreover the destruction of host's tissue, several parasite products develop different functions such as host's blood coagulation inhibition (Dzik, 2006), diminishing of host's peristaltic movements (Dziekonska-Rynko *et al.*, 2004; Dzik, 2006), inhibition of host's digestive enzymes and host's anti-inflammatory properties (Dziekonska-Rynko *et al.*, 2004, Dzik, 2006; Reddy, 2010) and facilitate the larval hatching and molting (Dzik, 2006).

The necrotic process means the dysfunctionality (or future dysfunctionality) of tissues and/or organs under inflammation and the tissue dysfunctionality is one of the pillars of inflammation, *i.e.*, the tissue is dead (Horn Jr. and Fine, 1977; Thomson, 1983). The granulation tissue and fibrosis comprise the regenerative process involved during and after the inflammatory process (Horn Jr. and Fine, 1977; Thomson, 1983). The other pillars concerning to the inflammation are the edema, hyperthermia, algesia and hypereremia (Frank, 2002; Paul, 2008). Ulcers and granulomas are very strong algesic lesions (Horn Jr. and Fine, 1977; Thomson, 1983). Thus, the ulcer's presence could contribute for inappetence and afagia under stomach's wall dilatation and consecutively lesion's area dilatation, causing strong algesia. Moreover, in study with rodents infected by *Trichiuris muris* (Nematoda, Trichiuridae) under laboratory controlled conditions, was reported that the Nematoda chronicle-related presence triggers intestinal cells apoptosis mechanisms (Cliffe *et al.*, 2007) strongly related with the proinflammatory cytokines discussed above. These latter authors report that the apoptosis was not related with the trichiuriasis-related mechanical injuries, but with the parasite's time of

permanence within the intestinal lumen, independently from the infection intensity (PII). Concerning to the *Nippostrongylus brasiliensis* in similar laboratory conditions, it is recorded the participation of mast cells released products (leukotriene) developing mucosal intestine sequels such as villus atrophy, crypt hyperplasia and destruction of nervous terminations that trigger mast cell control in rats (Gay *et al.*, 2000). Mucosal gastrointestinal nervous termination destruction can influence the luminal immunology (Stead, 1992; Gay *et al.*, 2000; Nava-Castro *et al.*, 2011). These notes reinforce the mucosal damage and thickness decreasing caused by *Contracaecum* sp. hanging out from CSEW and in contact with the mucosa. In other words, the parasite's presence is enough to cause injury, due to the parasite allergenic properties as discussed above. Moreover, these observations reinforce the probable influence of the contraecacis-related gastric lesions in important physiological digestive processes in *A. australis*.

The lesion-related indices and the absence of host's gender-related difference in *A. australis* corroborate the result for *Delphinus delphis* (Cetacea) (Abollo *et al.*, 1998a). The ulcer-related L-P% presented for some Cetacea species (*D. delphis* 15%, *Stenella coeruleoalba* 20%, *Tursiops truncatus* 28.9%, *Globicephala melas* 18.7% and *Phocoena phocoena* 29.4%) (Abollo *et al.*, 1998a) are closer that in *A. australis* in the present study (q.v. Tables 2 and 3). For Brazilian Cetacea, *Peponocephala electra*, *Kogia breviceps*, *Stenella clymene* and *Steno bredanensis*, from Ceará coast it was observed 70% of *Anisakis*-related ulcers and the distribution of ulcers was different between species, concerning to the gastric stomachal compartments (Motta *et al.*, 2008). These authors observed that the ulcers occurred within the forestomach of *S. clymene*, exclusively in the main stomach of *P. electra* (2/3) and *K. breviceps*, exclusively in the pyloric stomachal compartment of *P. electra* (1/3) and *S. longirostris* developed ulcers in the forestomach as well as pyloric stomachal compartment. For *D. delphis* there was no positive correlation between host's length and the number of

anisakiasis-related gastric ulcers (Abollo *et al.* 1998a), unlike the present study for both *A. australis* genders. Concerning to contraeciasis-related ulcers in *A. australis*, the lesions occurred in the body-fundus portion as well as in the pyloric portion of the stomach. The distributional increasing pattern of *Contracecum*-related gastric lesions observed for both *A. australis* genders toward the adulthood could be associated with the continuous injury in the stomach wall that permits to observe the presence of healing lesions (indelible scars) concomitantly with new lesions (ulcers, piogranuloma, CSEW and CPDAS) in a constant cycle of healing and pathology. This cycle of cure-pathology could be related with constant *Contracecum* sp. reinfections as could be observed by the presence of different cohorts found in *A. australis*. The parasite constant contact acts to decrease parasite establishment, survival, reproduction and/or maturation due to the acquired immunity developed in response to accumulated antigen contact (Thomson, 1983; Combes, 1997; Wilson *et al.*, 2002; Dybdahl and Stortor, 2003) what can result in parasite's peak shifts (Wilson *et al.* 2002) and the lesion-related variation should be considered. Moreover different lesion types (piogranulomas exclusively in males) and differences in ulcers dimensions could, at the least, demonstrate some gender-related differences in the immunological way to conducts the parasite injury and lesion healing (Moore and Wilson, 2002; Wilson *et al.*, 2002; Klein, 2004; Morales-Montor *et al.*, 2004) mainly for older specimens (prepubertal males and reproductive-breeding females).

It is recognized by epidemiologists (and epizootiologists) that male hosts tend to develop higher rates of parasitemia and diseases than females within several vertebrate hosts and parasite taxa (Wilson *et al.*, 2002; Klein, 2004). There is a normal variation in the parasitological indices and parasite number that a host can harbor (Bush *et al.*, 2001). Several heterogeneities such as dispersion of infective stages, parasite-host fitness (genetic and immunity), intra- and inter-specific parasite interactions, host body condition, host sex, host age, host behaviour and environmental stochastic phenomena influence this catholic feature

(Crompton, 1973; Combes, 1997; Wilson *et al.*, 2002; Dybdahl and Storter, 2003; Klein, 2004; Poulin, 2001; 2005). The ontogenetic-related PII, the aggregated distributional within the pups probably is related with the weaning and the beginning of the ingestion of solid food. Otariidae demonstrate prolonged (4-36 months) period of lactation reflecting the strong mother-pup attendance toward the definitive weaning, under gradual development of aquatic skills by pups to forage on their own, according to body mass acquisition (David and Rand, 1986; Doidge *et al.*, 1986; Trillmich, 1986a, b; Atkinson, 1997; Baylis *et al.*, 2005; Gastebois *et al.*, 2011). *Arctocephalus australis* from Uruguayan coast show evidence 60-80cm for infant (suckling) TL, 68-94cm for weaned specimens and 90-115cm for juvenile (prepubertal) females (Katz *et al.*, 2009). These ontogenetic and breast-feeding data corroborate TL range and reproductive status findings for sampled specimens within pup category in the present work. Aznar *et al.* (2004) record that *A. australis* younger than one year old were already infected by helminths and calves would be infected in the first ingestion of solid food as show this work.

Concerning to the gender-related PII, the results for DTL indicate that female host's reaches the highest values earlier than male hosts and with higher correlation. This result could be explained by the strong sexual dimorphism presented by Otariidae that comprises a mammalian group with male-biased sexual dimorphism in body size and/or body weight (male tend to be several times heavier than females) (Bonner, 1981; Ridgway and Harrison, 1981; Kunz *et al.*, 1996; Atkinson, 1997) toward the hyperallometry of organs and structures associated with harem dominance under strong male sexual selection (Clinton and Le Boeuf, 1993; Fairbairn, 1997; Molina-Schiller and Pinedo, 2004; Isaac, 2005) as other Carnivora (Gittleman, 1986; Ferguson and Larivière, 2008). For *A. australis*, the physical maturation occurs within smaller TL and age in females than in males (Molina-Schiller and Pinedo, 2004). However for Vertebrata the reproductive maturation occurs before the physical

maturation (Gittleman, 1986). Physical maturation in males of *A. australis* occurs at maximum length of 1.9m and weight of 140-159Kg, while for females the physical maturation occurs at 1.4m of length and weight of 48.5-50Kg (Vaz-Ferreira 1982; Vaz-Ferreira and Ponce de León, 1987). If ageing means in increasing of the body size, older animals are more exposed to re-infections than young animals due to their longer life time on feeding and because they posses larger surface areas (=gastrointestinal surface) what can contribute for harder parasite colonization (Poulin, 1996; 1998; Wilson *et al.*, 2002; Cowan *et al.*, 2007). Higher body masses implies proportionally in higher energetic requirements in Vertebrata (Odum, 1988; Pough *et al.*, 1993; Schmidt-Nielsen, 2002). Studies on mammals show association between sexual size dimorphism and sexual differences for diseases (Moore and Wilson, 2002; Klein, 2004). On the other hand, there are allometric differences in growth rates between organs and stomach and intestines must be treated independently. For marine mammals the intestine growth is negatively correlated with the body growth due to their higher metabolic rates, in comparison with terrestrial carnivore, *i.e.*, the intestine growth faster than body (Williams *et al.*, 2001). The South African fur seal *Arctocephalus pusillus pusillus* show that its intestine growth, in mean weight, three times more than the stomach weight (Stewardson *et al.*, 1999). Thus, the intestinal length (or volume) and their available area for parasite's colonization explain the intestine parasite's loads but does not be used, necessarily, to explain the parasitological processes for the stomach's parasites. Parasites were naturally selected to be able to colonize their hosts to finish adequately their life cycle within determinate host's niches (in this case stomach and intestine) and under certain guild relationships (Bush *et al.*, 2001; Zander, 2001). The earlier *Contracaecum*-related PII increasing in females of *A. australis* toward adulthood (=ageing), *i.e.*, as the growth ends for females and continues for males corroborate the observations above. The P% increasing for *C. rudolphii*, in the black cormorant *Ph. carbo*, is age-dependent from new-hatched chicks

(50%), 2-3-weeks-old (71-83%) and adult (100%) (Dziekonska-Rynko, 2008) and for the double-crested cormorant *Ph. auritus* infected by *C. spiculigerum*, as well (Kuiken *et al.*, 1999).

The entire sampled female hosts and female pups exhibited lower P%, MI and MA probably due to their more efficient immune system than males (Klein, 2004; Cabrera-Muñoz *et al.*, 2010). Unlike the reproductive-breeding females showed P%, MI and MA higher than prepubertal males. The sexual reproductive pressure could explain these results. Otariidae females present embryonic diapause and hard maternal attendance (Trillmich 1986a, b; Atkinson, 1997). The embryonic diapause could represent an adaptation to the marine environmental conditions such as the low nutritional concentration, low prey abundance, adequate climatic conditions for pups survival as a response to natural selection under descendents and breeding (Atkinson, 1997; Soto *et al.*, 2004). Captive Australian sea lion *Neophoca cinerea* that shows shortened gestation in captivity (Gales *et al.*, 1997). Other aspects related with the Otariidae maternal attendance are the increasing levels of intra-sexual (female-female) agonistic behaviour (aggressiveness) during the pregnancy and lactation for defend areas for their parturitions and pup welfare (Pavés *et al.*, 2005; Fernández-Juricic and Cassini, 2007). Lactation influences strongly the duration and the number of foraging trips on *A. gazella* and *A. tropicalis* (Goldsworthy, 1999). The Otariidae intra-sexual female agonistic behaviour is reported in relation with the available space (area limitation, substrate quality, water pools) in the breeding colonies with high densities (Cassini, 2000; 2001; Acevedo *et al.*, 2003; Fernández-Juricic and Cassini, 2007). Due to the energetic requirements toward reproduction and the maternal attendance stress, reproductive-breeding females of *A. australis* could be preying stronger than prepubertal males under the intermediate or paratenic host of *Contracaecum* sp. Evidences are presented concerning to the *Contracaecum*-related MI and MA for reproductive-breeding females in comparison with prepubertal males. Stressed

conditions such as intra-sexual disputes low the immune system response (Klein, 2004) as well as pregnancy and lactation advance can contribute to depress the immune response and increase parasite loads (Ngongeh *et al.*, 2011).

Parasitological indices, such as P%, can explain the parasite fitness upon a host species (Gregory and Blackburn, 1991; Bush *et al.*, 2001) as a variable to help the natural selection pressure (Anderson and Gordon, 1982; Anderson and May, 1982; Von Zuben, 1997). The *Contracaecum*-related P% for all *A. australis* sampled was higher than for *C. ogmorhini* from the sympatric *O. flavescens* (10.34%) (Pereira, 2012). On the other hand contraeciasis-related MA (34.34) and Ax (332) in *O. flavescens* (Pereira, 2012) were very close to that for *A. australis* in the present work. These parasitological values added with the CRD (aggregation or harboring) presented in this work corroborate the adequate fitness of *Contracaecum* spp. for both host's species. Moreover, the different parasite cohorts reinforce previous studies that consider this parasite as part of the gastrointestinal helminth community for this mammalian host (Morgades *et al.*, 2006).

The similarity for contraeciasis-related parasitological indices between host's genders, according to their ontogenetic development (pup-toward-prepubertal male or pup-toward-breeding-reproductive female), are recorded for sub-adults and adults of *O. flavescens* (Pereira, 2012) as well as for *A. australis* in the present study. This could suggest that both Otariidae share preys (intermediate or paratenic hosts) that harbor *Contracaecum* spp. The Engraulidae (Argentine anchovy *Engraulis anchoita*, anchovy *Anchoa marmorata*), Sciaenidae (*Cynoscyon striatus*, weakfish *C. gatlucupa*, Argentine croaker *Umbrina canosai*), Scombridae (chub mackerel *Scomber japonicus*), Stromateidae (harvestfish *Peprilus* sp.) and Trichiuridae (*Trichiurus lepturus*) fish as well as invertebrates (Cephalopoda, Bivalvia, Gastropoda and Crustacea) (Vaz-Ferreira, 1982; Santos and Haimovici, 2001; Naya *et al.*, 2002; Martínez, 2009) comprise prey for *A. australis* that forages on an ample area of the continental shelf.

Cephalopoda are important prey for Uruguayan waters (Isla de Lobos) *A. australis*, but changes in diet due to stochastic phenomena in fish and Cephalopoda could be observed (Naya *et al.*, 2002). Specimens of *A. australis*, stranded in Brazilian waters, demonstrate to prey on *Loligo sanpaulensis* and *Argonauta nodosa* (Cephalopoda) (Santos and Haimovici, 2001). Moreover, there is overlapping of fish prey for both sympatric mammalian speceis *A. australis* and *O. flavescens* such as *C. striatus*, *T. lepturus*, *E. anchoita* and *L. sanpaulencis* are overlapped with the sympatric *O. flavescens* (Falco, 2008; Drago *et al.*, 2009). Both genders of *O. flavescens* prey mainly on benthic species, according to their post-weaning toward adulthood (Drago *et al.*, 2002). *Scomber japonicus* (Rego and Santos, 1983), *Trichiurus lepturus* (Lent and Freitas, 1948) are recorded harboring *Contracaecum* sp. It is expected for hosts that show similar ecological patterns (similar niche occupation) in a given environment that parasite overburden occurs (Altizer *et al.*, 2003; Wirsing *et al.*, 2007).

Contracaecum sp. is non-seasonal. There were no differences for the parasitological analyzed variables (P%, MI, MA and CRD). The occurrence throughout the whole sampling period reinforces the idea for the uniformity of harboring for grouped seasons. This, however, should be considered with caution because it is not known the prepatent period or longevity of these parasite species. This non-seasonal pattern could be related with the presence of different cohorts (L-3, L-4, juvenile and adult forms) within the stomach. The P% was higher in A-W showing that higher percentage harbored *Contracaecum* sp.

In summary, five main types of stomach lesions contracaeciais-related in *A. australis* were found: two non-severe [*Contracaecum*-related embedding within the stomach wall (CESW) and *Contracaecum*-related punctual attached-detached site (CPADS)] and two severe inflammatory lesions (ulcers and piogranuloma) and scars (healing lesions); in CPADS the parasite develops an oral mold-like capsule of parasite attachment (OPCA); inflammatory lesions cause significant mucosal reduction in thickness for both stomach portions (body-

fundus and pylorus); only male hosts presented piogranuloma; the development of stomachal lesions is closer for both host's genders toward the adulthood; ulcer's dimensions differ between genders; toward the adulthood female hosts reaches their parasite burden earlier than males; parasitological indices such as P% and MI are higher in males concerning to total host's sampled and pups; reproductive-breeding females show higher parasite burden but lower lesional numbers; contracaeciasis is non-seasonal.

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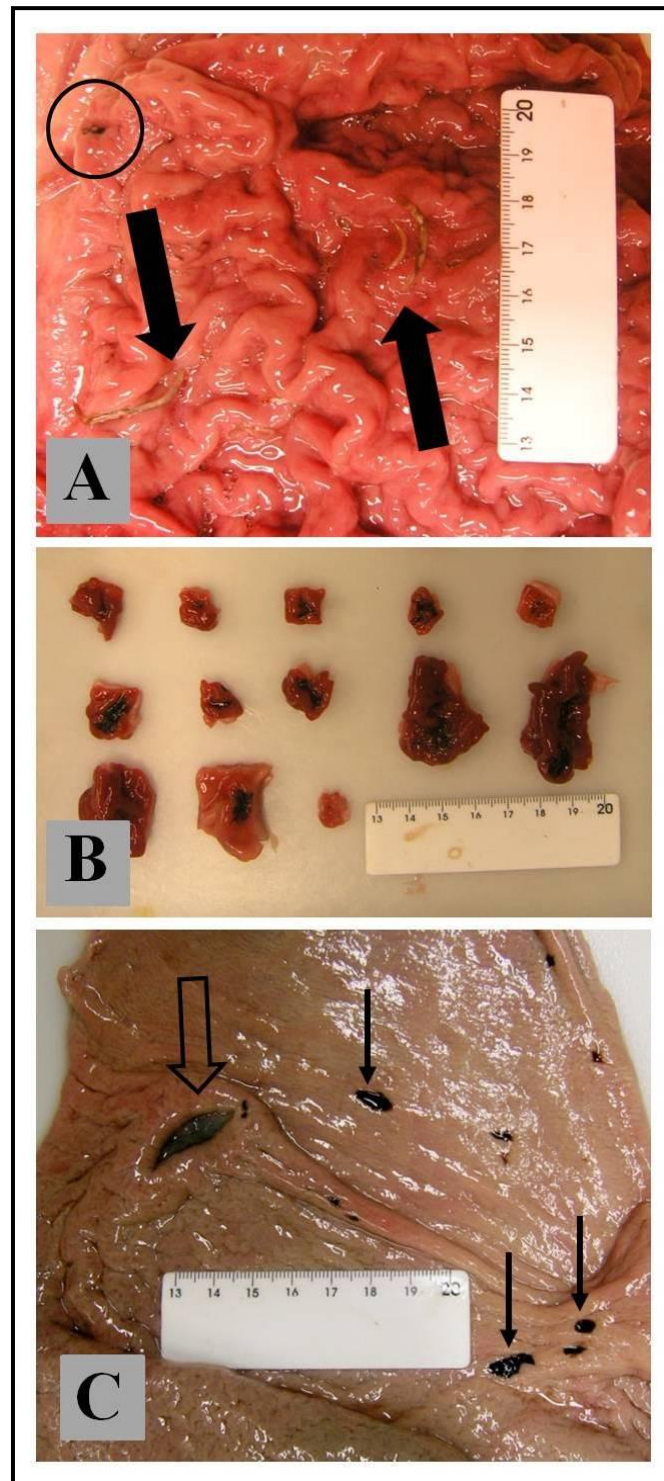


Figure 1. *Arctocephalus australis*. Gastric lesion's macroscopy. A: Stomach body-fundus portion with attached *Contracaecum* sp. on the mucosa (arrows) and small ulcer (circle). B: Extracted body-fundus ulcers. C: Pylorus with scar (opened arrow) and ulcers (thin black arrows). Scale: centimetric.

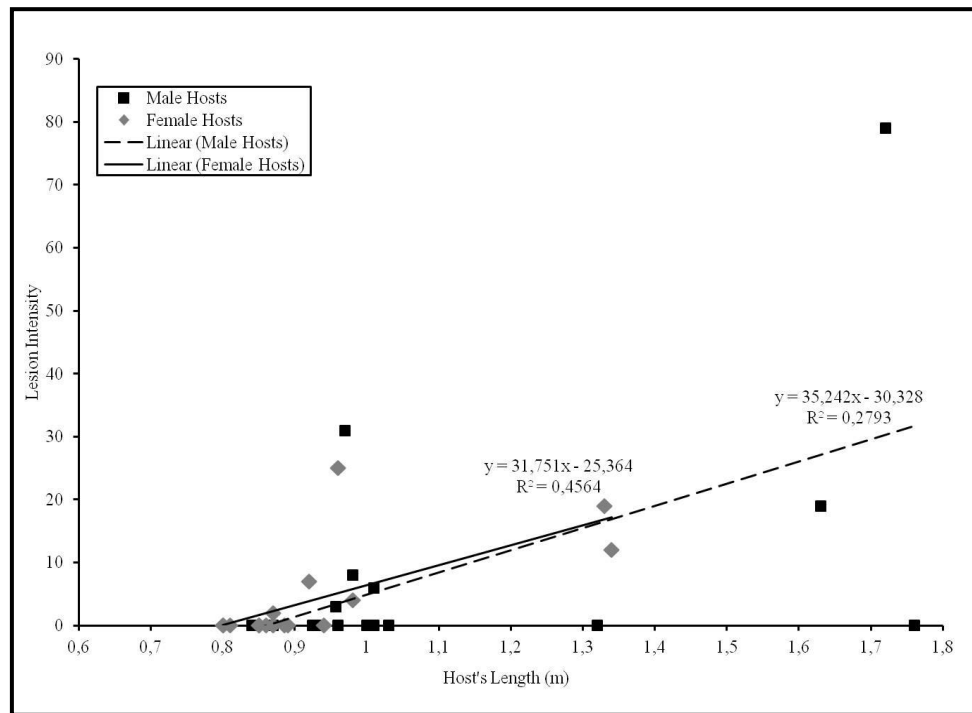


Figure 2. Distribution of the *Contracaecum*-related gastric total Lesions Intensity (PLI) in *Arctocephalus australis* (16male: 16female) from Southern Brazilian coast according host's gender length.

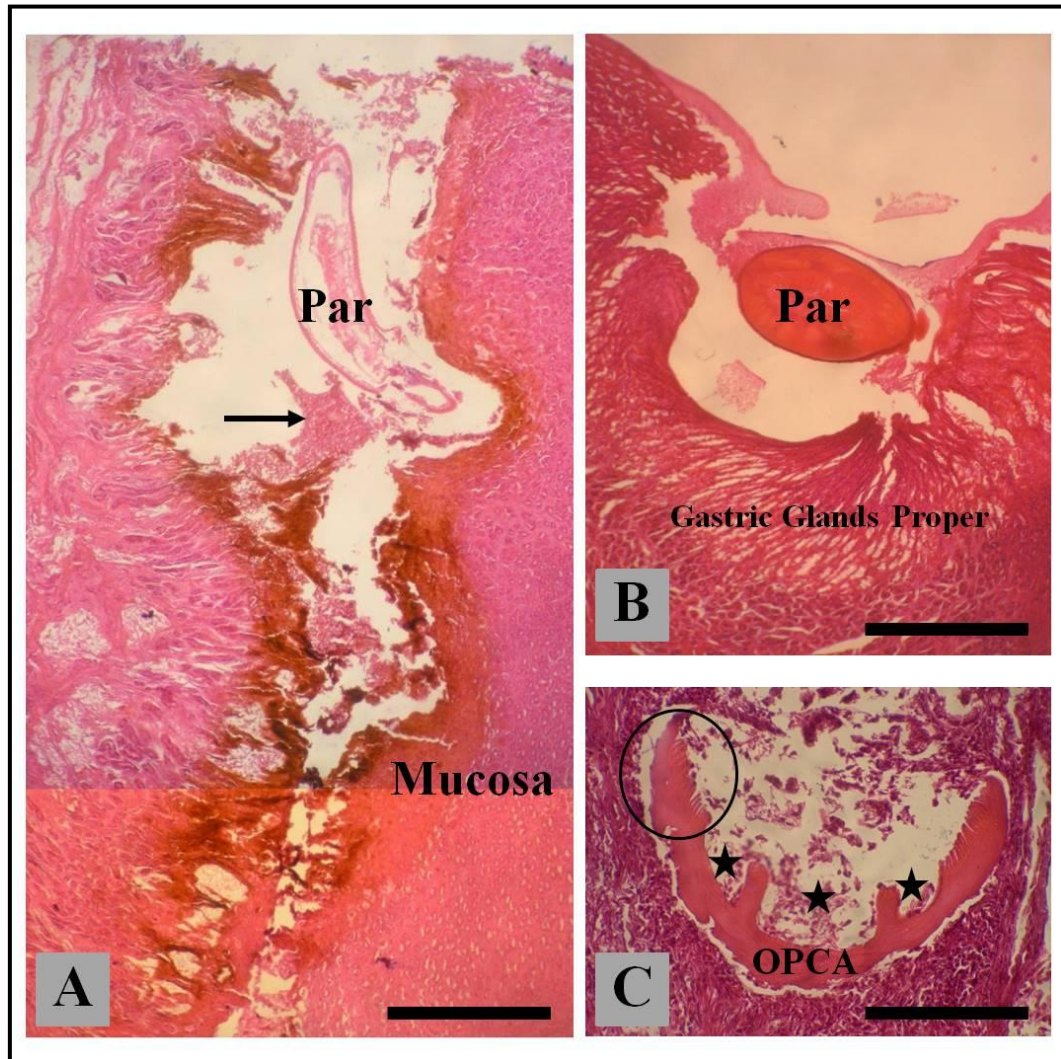


Figure 3. *Arctocephalus australis*. Microphotograph of the *Contracaecum*-related gastric lesions. A: *Contracaecum*-related embedding within the stomach wall (CESW). Note the mucosal necrotic feature caused by the parasite (Par) and the bacterial masses (arrow) Stain: H-E. Scale bar: 350 μ m. B: Mucosal hanging out parasite body (Par) transversally cut and causing mucosal erosion of the gastric glands proper. Stain: H-E. Scale bar: 350 μ m. C: Oral parasitic mold-like capsule of attachment (OPCA) with parasite's labia intaglios (stars) and the serrate border (circle). The cellular vicinity of OPCA is composed by immune cells. Stain: H-E. Scale bar: 165 μ m.

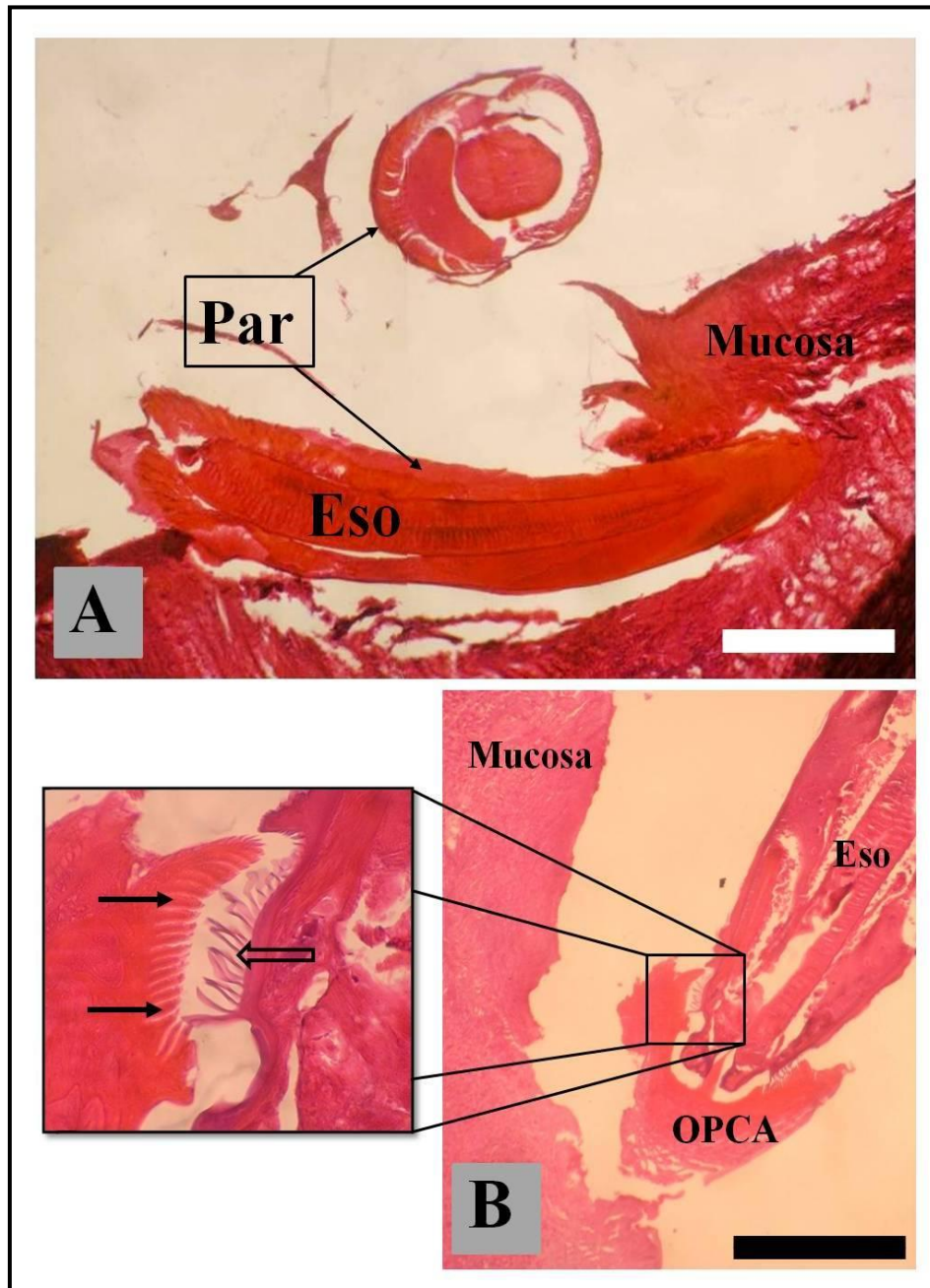


Figure 4. *Arctocephalus australis*. Microphotograph of the *Contracaecum*-related gastric lesions. A and B: *Contracaecum*-related punctual attached-detached site (CPDAS). A: Parasite with the oral region cute longitudinally and other body portion cute transversally. Observe the oral parasite's embedding on the necrotic mucosa and its esophagus (Eso). Stain: H-E. Scale bar: 560 μ m. B: Detail of the oral parasitic mold-like capsule of attachment (OPCA) and its mixing with the host's stomach mucosa. Observe the serrate border (thin black arrows) and its relationship with the ribs of the parasite's cephalic collarete (opened arrow). Stain: H-E. Scale bar: 370 μ m.

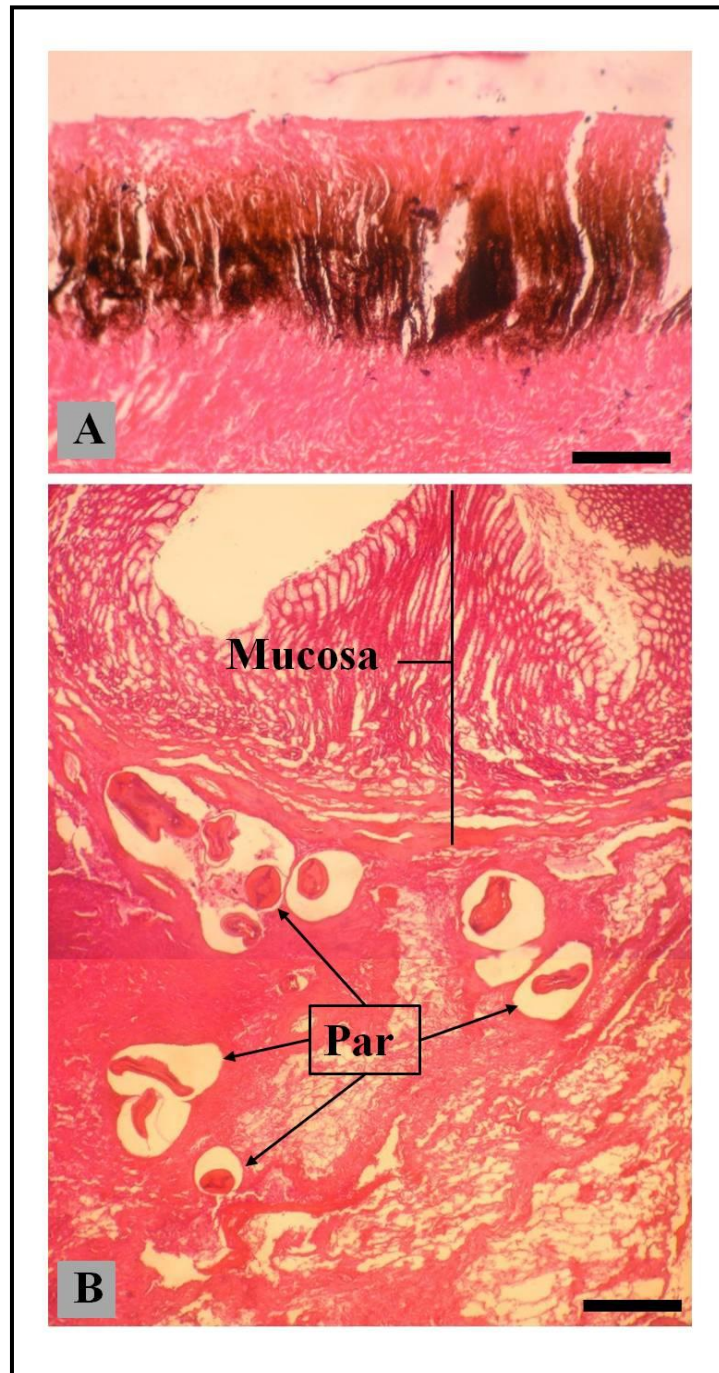


Figure 5. *Arctocephalus australis*. Microphotograph of the *Contracaecum*-related gastric lesions. A: General aspect of the necrotic gastric ulcer. Stain: H-E. Scale bar: 160 μ m. Piogranuloma aspect with several parasite larvae (Par) embedded deeply within the submucosa. Stain: H-E. Scale bar: 160 μ m.

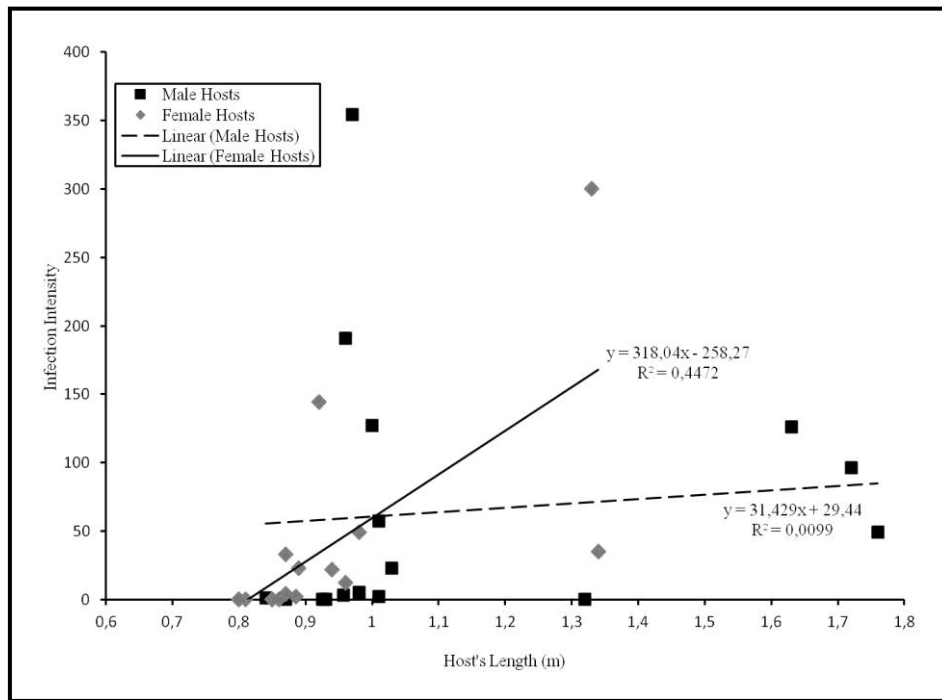


Figure 6. Distribution of the Infection Intensity of *Contracaecum* sp. in *Arctocephalus australis* (16male: 16female) from Southern Brazilian coast according host's gender length.

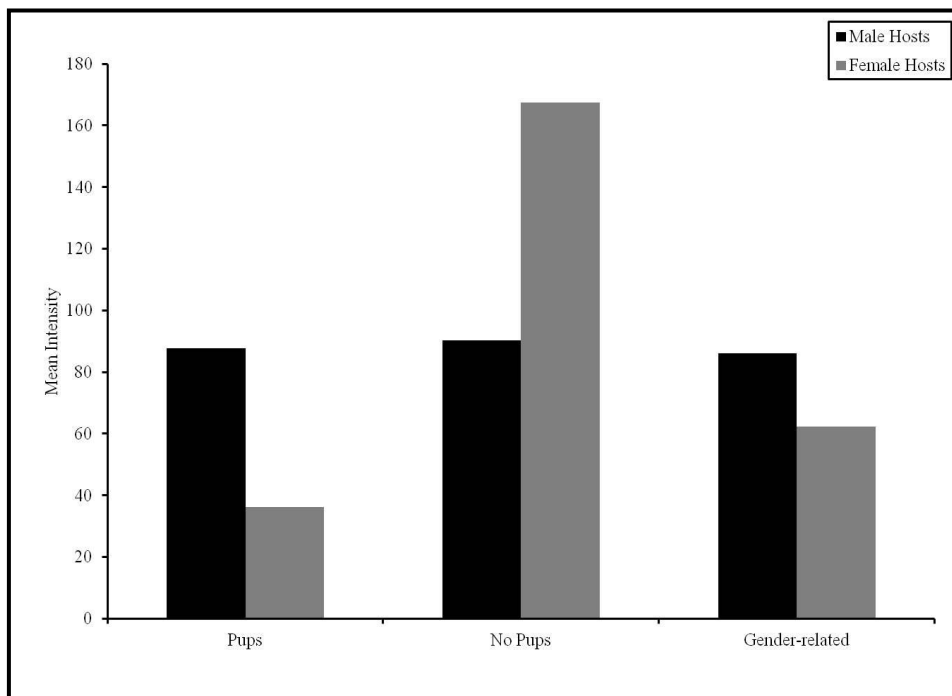


Figure 7. Mean Intensity of *Contracaecum* sp. in *Arctocephalus australis* from Southern Brazilian coast. No pups male hosts – prepubertal males; No pups female hosts – reproductive-breeding females.

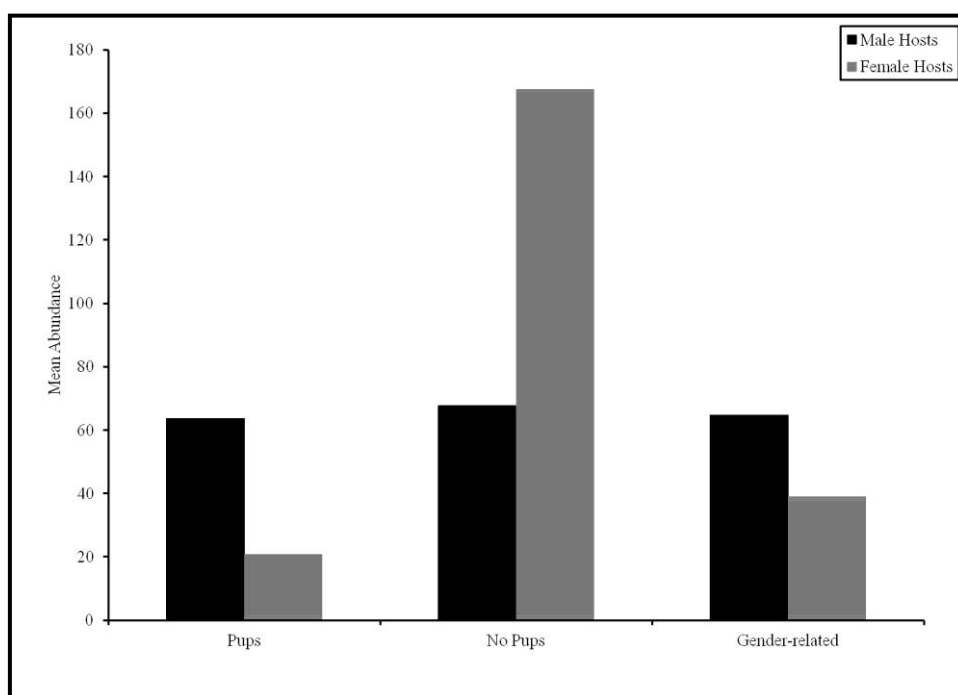


Figure 8. Mean Abundance of *Contracaecum* sp. in *Arctocephalus australis* from Southern Brazilian coast. No pups male hosts – prepubertal males; No pups female hosts – reproductive-breeding females.

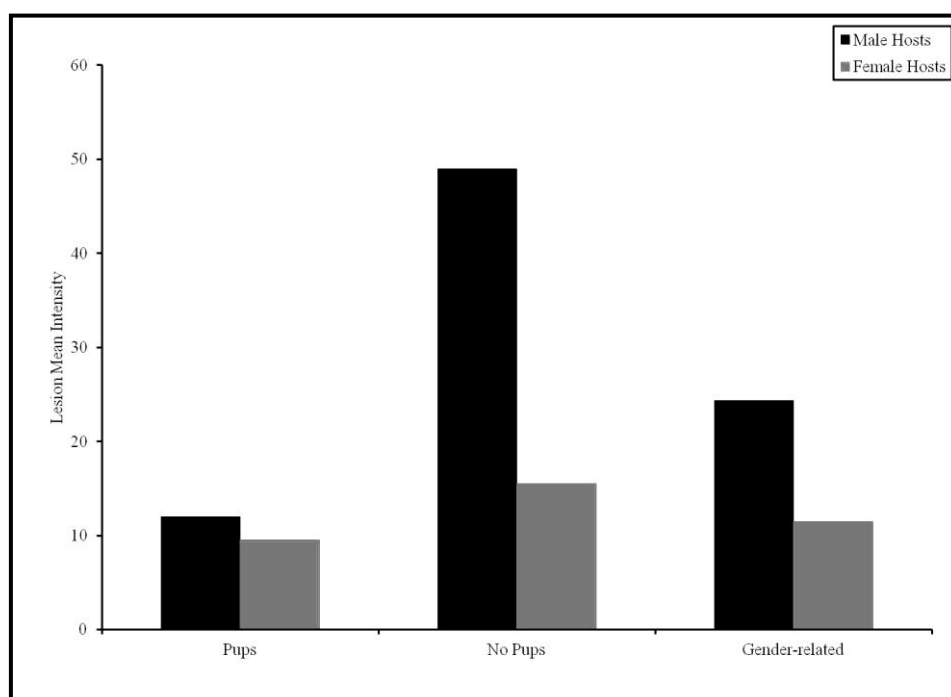


Figure 9. *Contracaecum*-related Mean Intensity of gastric lesions in *Arctocephalus australis* from Southern Brazilian coast. No pups male hosts – prepubertal males; No pups female hosts – reproductive-breeding females.

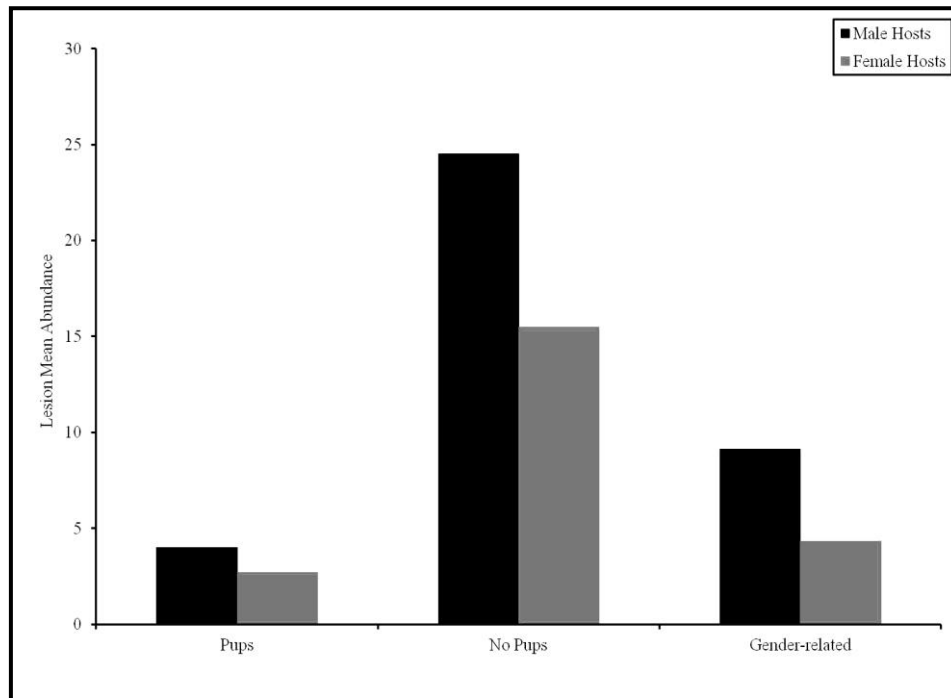


Figure 10. *Contracaecum*-related Mean Abundance of gastric lesion in *Arctocephalus australis* from Southern Brazilian coast. No pups male hosts – prepubertal males; No pups female hosts – reproductive-breeding females.

Table 1. *Contracaecum* sp. lesion-related indices (Lesion Prevalence –L-P%, Lesion Mean Intensity – L-MI and Lesion Mean Abundance –L- MA) and Lesion Range of Variation (L-Ax) from *Arctocephalus australis* stranded on the Rio Grande do Sul – Brazil coast.

Lesion-related Variable	Lesion-related Indices			
	L-P%	L-MI	L-MA	L-Ax
All Gastric Lesions	33.3 (12/36)	17.92	5.97	2-79
Gastric Lesion Type				
Ulcers	37.5 (12/32)	15.17	5.69	2-61
Scars	9.4 (3/32)	9.67	0.91	4-18
Piogranuloma	3.1 (1/32)	3	3	3-3

For P% values: numbers within parenthesis (Lesion-related variable/number of analyzed host) are discriminating the ratio for *Contracaecum* -related lesion cases within the sampled hosts.

Table 2. Variance Analysis (Tukey's test; $p < 0.05$) in thickness (μm) of the normal and abnormal mucosa (contracaeciasis-related lesion) of the stomach of *Arctocephalus australis* from Southern Brazilian coast.

Stomachal Layer	Stomach Portion			
	Body-Fundus*		Pylorus**	
	Layer condition		Layer condition	
	Normal dimension (μm)	Abnormal dimension (μm)	Normal dimension (μm)	Abnormal dimension (μm)
Mucosa	2111.59 $\pm$ 844.34 ^a	588.21 $\pm$ 410.23 ^b	1402.93 $\pm$ 494.49 ^a	794.97 $\pm$ 439.46 ^b

Different letters mean significant differences between normal and abnormal mucosa according to each intestine Variance Analysis (ab) $p < 0.005$

Table 3. Contracaeciasis-related lesional indices (Lesion Prevalence – L-P%, Lesion Mean Intensity – L-MI and Lesion Mean Abundance – L-MA) and Lesion Range of Variation (L-Ax) for *Arctocephalus australis* stranded on the Rio Grande do Sul – Brazil coast according the host's gender and sexual condition .

Infection-related Variable	Host Sexual Parameters	Host Sex							
		Male*				Female**			
		L-P%	L-MI	L-MA	L-Ax	L-P%	L-MI	L-MA	L-Ax
Gastric Lesions	Exclusively	37.5 (6/16)	24.33	5.97	3-79	37.5 (6/16)	11.5	4.31	2-25
	Pups	33.3 (4/12)	12	4	3-31	28.6 (4/14)	9.5	2.71	2-25
	Prepubertal*	50	49	24.5	19-79	100	15.5	15.5	12-19
	Reproductive-Breeding**	(2/4)				(2/2)			

For P% values: numbers within parenthesis (Infection-related variable/number of analyzed host) are discriminating the ratio for *Contracaecum* -related cases within the sampled hosts.

Table 4. Gender-related Mean values for *Contracaecum*-related gastric lesions in *Arctocephalus australis* from Southern Brazilian coast.

Gastric Lesion Type	Host's Gender			
	Male		Female	
	Lesion-related Measurements		Lesion-related Measurements	
	Length (mm)	Width (mm)	Length (mm)	Width (mm)
Ulcers	11.76 $\pm$ 8.17 ^a	6.38 $\pm$ 4.35 ^a	8.8 $\pm$ 6.66 ^b	4.79 $\pm$ 3.26 ^b
Scars	14.03 $\pm$ 8.63	8.35 $\pm$ 3.47	10.56 $\pm$ 3.34	7.10 $\pm$ 2.49
Piogranuloma	23.48 $\pm$ 3.61	15.22 $\pm$ 2.45	absent	absent

Different superscript letters represent differences ($p < 0.05$) between male and female according each lesion's dimension.

Table 5. Parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA), Range of Variation (Ax) and Crowding Value robustness (CRD - s^2/x) for *Contracaecum* sp. from *Arctocephalus australis* stranded on the Rio Grande do Sul State – Brazil coast.

Infection-related Variable	Parasitological Indices				
	P%	MI	MA	Ax	CRD
Parasite					
<i>Contracaecum</i> sp.	61.1 (22/36)	75.36	46.06	1-354	154.78

For P% values: numbers within parenthesis (Infection-related variable/number of analyzed host) are discriminating the ratio for *Contracaecum* -related cases within the sampled hosts.

Table 6. Parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA) and Range of Variation (Ax) for *Contracaecum* sp. from *Arctocephalus australis* stranded on the Rio Grande do Sul State – Brazil coast according the host's gender and sexual condition .

Infection-related Variable	Host Sexual Parameters	Host Sex							
		Male*				Female**			
		P%	MI	MA	Ax	P%	MI	MA	Ax
Parasite									
<i>Contracaecum</i> sp.									
	Exclusively	75 (12/16)	86.17	64.63	1-354	62.5 (10/16)	62.4	39	2-300
	Pups	75 (9/12)	84.78	63.58	1-354	57.1 (8/14)	36.13	20.64	2-144
	Prepubertal*	75	90.33	67.75	49-126	100	167.5	167.5	35-300
	Reproductive-Breeding**	(3/4)				(2/2)			

For P% values: numbers within parenthesis (Infection-related variable/number of analyzed host) are discriminating the ratio for *Contracaecum* -related cases within the sampled hosts.

Table 7. Seasonal (Autumn-Winter: A-W; Spring-Summer: S-S) parasitological indices (Prevalence – P%, Mean Intensity - MI and Mean Abundance – MA), Range of Variation (Ax) and Crowding values (CRD, s^2/x) for *Contracaecum* sp. from stomachs of *Arctocephalus australis* (n=32) stranded on the Rio Grande do Sul State – Brazil coast for August-2008 to September-2010.

Parasitological Variable	Grouped Seasons	
	A-W	S-S
P%	75 (15/20)	50 (6/12)
MI	69.93	97.17
MA	52.45	48.58
Ax	1-300	2-354
CRD	120.17	227.31

For P% values: numbers within parenthesis () are helminthiasis-related cases/number of analyzed host ratio for *Contracaecum* sp.