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Tese

Avaliação da contaminação ambiental por bactérias aeróbicas, fungos e ovos de parasitos no perímetro dos elementos tanatocenóticos de *Otaria flavescens* (Shaw, 1800) no litoral sul do Brasil

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Pelotas, 2017

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Resumo

PEREIRA, Eliane Machado**. Avaliação da contaminação ambiental por bactérias aeróbicas, fungos e ovos de parasitos no perímetro dos elementos tanatocenóticos de *Otaria flavescens* (Shaw, 1800) no litoral sul do Brasil.** 2017.102f.Tese (Doutorado em Parasitologia) – Programa de Pós-Graduação em Parasitologia. Universidade Federal de Pelotas, Pelotas, 2017.

Este estudo analisou a presença de bactérias e fungos aeróbios na superfície do corpo de *O. flavescens* em decomposição, assim como determinou o nível de contaminação do solo por estes micro-organismos e por ovos de parasitos expelidos durante o processo de decomposição da carcaça. Foram utilizados 10 espécimes de leão-marinho-do-sul, *O. flavescens*, encontrados mortos ao longo das praias do litoral do Rio Grande do Sul, sendo coletadas amostras de sedimento arenoso superficial, amostras de fezes foram aspiradas do ânus e reto. Além disso, do corpo de cada espécime foram coletadas amostras, por meio de swab estéril. Por fim, de animais vivos, foram coletadas 34 amostras de fezes da área de descanso dos leões-marinhos próximo à praia. Análise das amostras: Alíquotas de sedimento arenoso foram diluídas em água peptonada a 0,1%, semeadas em meios de cultura seletivos para bactérias e fungos e incubadas em estufa bacteriológica. As colônias bacterianas foram submetidas à coloração de Gram e identificadas utilizando o sistema ViteK®. A identificação das colônias fúngicas foi baseada nas suas características macro e micro morfológicas até seu status de gênero. Os swabs coletados do corpo do animal foram semeados por esgotamento em meios seletivos para bactérias e fungos. A identificação das colônias seguiu a mesma metodologia empregada para as amostras de sedimento arenoso. As amostras de areia foram processadas, através da técnica Caldwell & Caldwell(1995). As amostras fecais, tanto de animais vivos como de carcaças, foram examinadas através das técnicas parasitológicas de rotina. Trinta e três espécies de bactérias foram isoladas e identificadas*. Proteus, Aeromonas, Kocuria e Sphingomonas* foram encontrados em 80% dos animais examinados*. Proteus vulgaris e Sphingomonas paucimobilis f*oram as espécies encontradas em todas as amostras*,* seguidas *de Aeromonas salmonicida, Kocuria rosea e Streptococcus thoraltensis.* Apenas fungos oportunistas foram identificados*. Trichoderma* spp*., Aspergillus spp., Penicilium* spp*., Cladosporium* spp*. e Fusarium* spp*.* Nas amostras de sedimento arenoso ao redor da carcaça estavam presentes: *Aspergillus spp., Fusarium* spp*, Penicilium* spp. *e Cladosporium* spp*.* A partir de amostras fecais de animais vivos, foram encontradas fêmeas imaturas de *Corynossoma australe.* Ovos de helmintos ou oocistos de protozoários não foram encontrados em nenhuma amostra. Conclui-se que, apesar de espécimes de *O. flavescens* apresentarem altas infecções por helmintos no trato digestório, carcaças que permanecem na praia não são importantes meios de veiculação de formas infectantes de parasitos que possam colocar em risco a saúde pública. No entanto, a presença de agentes microbianos com potencial de infecção para seres humanos, seja em carcaças ou areia, a área de acesso à praia ao lado das carcaças dos animais pode ter implicações negativas na saúde pública, sendo importante o uso de equipamentos de proteção por profissionais e pesquisadores responsáveis pelo seu manuseio.

Palavras-chave: planície litorânea, sedimento arenoso, leão-marinho-do-sul, carcaças.

Abstract

PEREIRA, Eliane Machado**. Evaluation of environmental contamination by aerobic bacteria, fungi and parasite eggs al the perimeter of the tanatocenotic elements of South American sea lion, *Otaria flavescens*, in Brazilian south coastal plain.** 2017. 102f. Thesis (PhD. in Parasitology) – Graduate Program in Parasitology. Federal University of Pelotas, Pelotas.

This study analyzed the presence of aerobic bacteria and fungi on the body surface of *O. flavescens* in decomposition, as well as, the contamination level of the sandy sediment by these microorganisms and parasite eggs expelled in the feces during the process of carcass decomposition. Ten southern sea lions, *O. flavescens*, found dead along the beaches of Rio Grande do Sul coast were analyzed. Microbiological samples from the body surface of each specimen were collected using sterile swabs. Faecal samples were aspirated from the anus and the rectum of the carcass. Around each carcass, samples of superficial sandy sediment were also collected and investigated. Lastly, from live animals 34 faecal samples were collected from rocky substrate. Samples analysis: sandy sediment aliquots were diluted in peptone water 0.1%, seeded in selective culture media for bacteria and fungi and were incubated in bacteriological incubator. The bacteria were incubated at 37°C and the fungi at 25 °C. Bacterial colonies were submitted to gram staining and identified using the ViteK® system. Fungal colonies identification was based in their macro and micro morphological characteristics at the genus level. The collected swabs from the animal carcass were seeded by the streaking methodology on selective media for bacteria and fungi. The colonies identification was performed according sediment samples methodology. Sediment samples were processed, through Caldwell *&* Caldwell technique, in order to research for eggs and larvae of helminths and protozoan oocysts. Faecal samples, both from live animals and from carcasses, were examined through the parasitological techniques of routine. Thirty-three species of bacteria were isolated and identified. *Proteus*, *Aeromonas spp.*, *Kocuria spp.* and *Sphingomonas spp.,* were found in 80% of the examined animals. *Proteus vulgaris* and *Sphingomonas paucimobilis* were the most common species in all samples, followed by *Aeromonas salmonicida*, *Kocuria rosea* and *Streptococcus thoraltensis*. Only opportunistic fungi were identified. They are *Trichoderma* spp., *Aspergillus* spp., *Penicilium* spp., *Cladosporium* spp. and *Fusarium* spp. In the sandy sediment, samples around the carcass were present *Aspergillus* spp., *Fusarium* spp, *Penicilium* spp and *Cladosporium* spp. From faecal samples of live animals, immature females *of Corynossoma australe* were found. Helminthic or protozoa (oocists) eggs were not found in none sample. This study points out that, in spite of the southern sea lions show high helminthic infections, in the marine environment, the biotic and abiotic events, those occur since the death of the animal until the moment the carcass is deposited on the beach, contribute to the carcass does not be an expressive source of parasitic eggs dispersion, which may endanger public health. However, the presence of microbial agents (bacteria and fungi) with potential to infect humans, whether in carcasses or sand, the beach access area next to the animal carcass may have negative implications in public health, and the need for use of protective equipment by professionals and researchers responsible for handling them is emphasized.

Keywords: coastal plain, coastal sediment, south sea lion, carcasses,

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Sumário

1. Introdução

Os sedimentos marinhos costeiros como integrantes do ecossistema de praias estão particularmente expostos aos resíduos orgânicos e inorgânicos resultantes das atividades humanas (PEREZ-RUZAFA, 2006) assim como dos próprios animais e vegetais que integram seu biota (CRAVO-LAUREAU et al. 2014).

Sob o aspecto ecológico, vários fatores conduzem à pressão de degradação dos ecossistemas costeiros, os quais estão se tornando cada vez mais comuns, operando numa escala de tempo de anos à décadas. No entanto, estudos que descrevem a dinâmica natural dos ecossistemas de praia e impactos humanos que sofrem são escassos e fragmentados (DEFEO et al.,2009). Fezes e carcaças de animais também contribuem para o acréscimo de matéria orgânica junto ao sedimento arenoso das áreas costeiras. Os detritos são desagregados pela ação combinada de uma comunidade decompositora a qual é composta predominatemente por microorganismos e invertebrados (ANDERSON et al., 2009). Estes organsimos se alimentam dos detritos e utilizam a energia e outros nutrientes para o seu próprio crescimento (produção secundária). Eventualmente os decompositores morrem e sua biomassa entra na carga de detritos e são novamente degradados por outros decompositores (SWIFT et al., 1979). Considerando a colonização bacteriana em sedimento arenoso em áreas influenciadas por marés, Xiaofei et al (2016) verificaram que a estrutura das comunidades bacterianas se diferenciam sazonalmente e que a salinidade é o fator ambiental estruturante da comunidade microbiana no sedimento durante a sequências de marés. Por outro lado, a temperatura e concentração de nitritos são delimitantes das mudanças microbianas ao longo das estações do ano.

O corpo de um animal em decomposição é uma fonte de alta qualidade de carbono e nutrientes no solo, sendo um excelente substrato para o crescimento bacteriano e fúngico (MAIER et al., 2003; CARTER et al., 2007). O sedimento arenoso onde ocorre a decomposição é, no entanto, uma possível fonte de transmissão de microorganismos patogênicos e que muitas vezes não é avaliada quando medidas sanitárias de segurança são tomadas (MAIER et al., 2003; ANDRAUS, 2006).1.1.

Contaminação das praias

Ao longo de muito tempo, a qualidade das praias era apenas monitorada pela densidade de bactérias presentes em suas águas, pois segundo Afifi et al. (2000) a água era a principal fonte de transmissão de doenças nas praias. Nos últimos anos, vêm crescendo a preocupação em incluir outras variáveis para definir a qualidade ambiental de uma praia e sua balneabilidade, para assim preservar a saúde e o bem estar dos usuários. A qualidade das praias tem adquirido uma importância crescente por razões ambientais e de saúde pública. Existem uma série de normas, critérios, e instrumentos voltadas especificamente para analisar a qualidade ambiental das praias, são ferramentas que há décadas são utilizadas por pesquisadores e órgãos ambientais de várias partes do mundo, com o objetivo de fornecer um melhor diagnostico ambiental das áreas costeiras (ROCA et al.,2009). Esforços vêm sendo feitos para melhorar os procedimentos de avaliação e de gestão (ROCA et. al., 2009; SILVA et. al., 2009; SOUZA et al., 2015).

Os fungos são microorganismos que constituem um grupo diversificado e abundante na natureza, são onipresentes e encontrados em vegetais, animais, homem, detritos, água, ar e em abundância no solo (PAIXÃO et al., 2001). O solo, por ser um recurso natural formado por um sistema complexo de componentes é o habitat de fungos sapróbios e patogênicos, cuja dispersão é feita pelo ser humano e por outros animais, água e principalmente através dos ventos (COSTA et al., 2002). De um modo geral, os ambientes aquáticos apresentam grande diversidade de fungos e organismos zoospóricos, que juntamente às bactérias e aos detritívoros (insetos, protozoários, etc.) realizam a fragmentação e degradação de substratos orgânicos transformando-os em seus componentes originais (mineralização), o que alimenta a cadeia de detritos e estimula a ciclagem dos nutrientes (GESSNER; CHAUVET 1993).

O sedimento arenoso de praia é também um compartimento ambiental onde o ser humano poderia facilmente entrar em contato com bactérias patogênicas (MAIER et al., 2003; ROCHA, 2007; PASSOS et al., 2011). Outra via de contaminação do sedimento arenoso são as fezes de animais: Vários estudos têm sido realizados focando a contaminação da areia de praias urbanas por ovos de helmintos de animais domésticos (MATESCO et al. 2006; PEDROSA et al, 2014). A

água do mar bem como a própria areia da praia também é suscetível à contaminação fecal oriunda de efluentes da agricultura, do aporte de esgotos domésticos e dejetos dos próprios banhistas e de animais selvagens/marinhos vivos ou mortos (ALM et al., 2003). Somente um estudo que aborda a liberação de helmintos de animais marinhos e seus ovos no sedimento arenoso em praias urbanas foi encontrado na literatura (HERMOSILLA et al., 2016). Estes autores analisaram fezes de leão-marinho-do-sul, *Otaria flavescens* (Shaw, 1800) da costa chilena, mesma espécie utilizada do presente estudo.

1.2. Mamíferos Marinhos

Na classe Mammalia, existe um grupo de animais que por seu habitat comum e por suas características morfológicas e comportamentais semelhantes são denominados de uma forma genérica como mamíferos aquáticos, que Incluem três ordens: Cetacea, Sirenia e Carnivora. Pertencendo à ordem Carnivora, inclui-se os membros da sub-ordem Pinnipedia (HEWER, 1974).

Mamíferos marinhos são organismos que diferem entre si na aparência e nas estratégias de sobrevivência, constituem um grupo altamente especializado de organismos que se adaptaram e que dependem do mar para sobreviver ou pelo menos parte do seu ciclo de vida (LALLI; PARSONS, 1997). A extensa costa e a vasta rede fluvial existentes no Brasil abrigam grande número de mamíferos aquáticos (CHIARELLO et al., 2005).

Os pinípedes constituem um grupo de mamiferos adaptados à vida aquática e terrestre (SILVA, 2014). No Brasil, com a chegada do inverno e a maior influência das Corrente das Malvinas, no litoral do Rio Grande do Sul, chegam à costa águas mais frias e, com elas, uma grande riqueza de animais. Segundo Silva (2014) os pinípedes são um dos grupos de animais que frequentam esta região durante esta época do ano. São mamíferos que alternam suas vidas entre o ambiente aquático e o terrestre, em períodos de alimentação e reprodução, respectivamente. Na costa brasileira há registros de ocorrência, pelo mínimo, de sete espécies: *Otaria flavescens* (leão-marinho-do-sul), *Arctocephalus australis* (Zimmerman, 1783) (lobo-marinho-do-sul), *Arctocephalus gazella* (Peters, 1875) (lobo-marinho-antártico), *Arctocephalus tropicalis* (Gray, 1872) (lobo-marinho-subantártico), *Lobodon carcinophagus*
(Hombron & Jacquinot, 1842) (foca-caranguejeira), *Hydrurga leptonyx* (Blainville,

1820) (foca-leopardo) e *Mirounga leonina* (Linnaeus, 1758) (elefante-marinho-do-sul) (ROCHA CAMPOS, 2011). O leão-marinho-do-sul, *O. flavescens* é a espécie elemento-chave do presente estudo.

1.3. *Otaria flavescens*

Popularmente é conhecido como leão-marinho-do-sul e taxonomicamente como *Otaria flavescens* (SHAW, 1800) e *Otaria byronia* (BLAINVILLE, 1820) é comumente utilizada como sinonímia por alguns autores (PINEDO et al., 1992). Machos adultos apresentam pêlos mais longos ao redor do pescoço similar a uma juba, o que lhes conferiu o nome vulgar (Figura 1).

Concentrações do *O. flavescens* no litoral brasileiro estão no Rio Grande do Sul: o Refúgio da Vida Silvestre do Molhe Leste da Lagoa dos Patos e a Reserva Ecológica da Ilha dos Lobos, em Torres (ROSAS et al., 1994; IBAMA, 2001). Considera-se que a partir do estado de Santa Catarina até a Bahia a espécie pode aparecer ocasionalmente, através de incursões de indivíduos isolados nos meses de inverno e primavera, que se deslocam em busca de alimentação (SILVA, 2004). No Atlântico sul, colônias de reprodução ocorrem ao longo da costa do Uruguai, Argentina e Ilhas Malvinas (PINEDO et al*.,* 1992). Os machos de *O. flavescens* não reprodutores ocupam os extremos das colônias reprodutivas, sendo compostos de indivíduos de diferentes idades ou apenas de indivíduos velhos (VAZ-FERREIRA, 1981). Machos adultos atingem 2,6 m de comprimento e pesam de 300 a 350 kg; fêmeas atingem 2m e em média 150kg (Figura 2). Indivíduos da espécie são mortos regularmente por pescadores, devido aos danos nas redes de pesca e carcaças são encontradas nas praias ao longo da área de ocorrência. Seus principais predadores naturais são os tubarões e a orca (Linnaeus, 1758) (*Orcinus orca*) (Rice,1998) (VAZ-FERREIRA, 1981; SILVA, 2014).

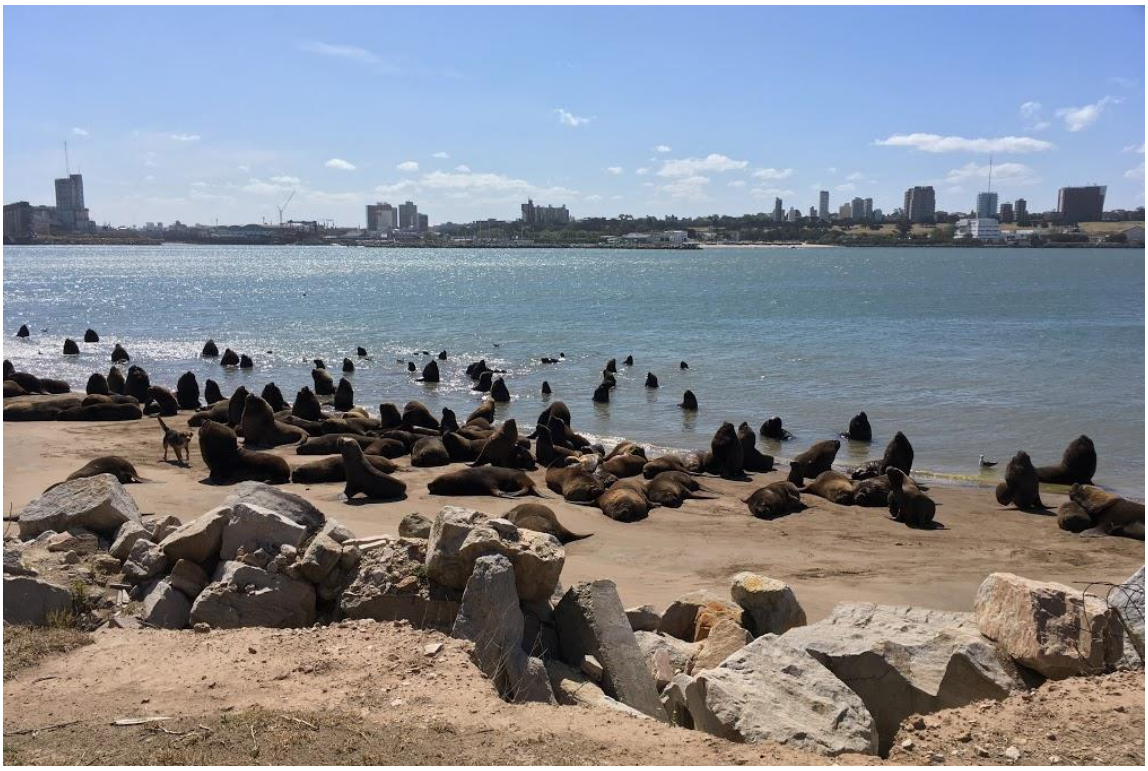

Figura1: Espécimes de *O. flavescens*, machos, em praia urbana, Mar de Plata.

Fonte: Silvia Gastal

Figura 2: Espécime de *O. flavescens*, fêmea, em Mar de Plata

Fonte: Silvia Gastal

Otaria flavescens é citada por Pinedo (1986) como a espécie de maior ocorrência na costa do Rio Grande do Sul, porém Santos et al. (1996) a menciona como a segunda espécie de pinípede mais frequente encontrada, concordando com os resultados de Silva (2004). Segundo Vaz-Ferreira (1981), o leão-marinho pode penetrar algumas vezes em estuários e rios, fato este confirmado através da ocorrência de um macho adulto no Rio Guaíba no extremo norte da Lagoa dos Patos, e de um macho juvenil na Colônia de Pesca Z3, no Município de Pelotas, RS. Considerando toda a extensão do litoral do Rio Grande do sul, os encalhes de leões-marinhos-do-sul, ocorrem todo ano, sendo reduzido nos meses de dezembro, janeiro e fevereiro (Silva 2004). A grande maioria de animais encontrados nas praias refere-se a animais mortos, classificados como machos adultos e subadultos, incluindo uma pequena quantidade de fêmeas (SILVA, 2004). A população desta espécie foi estimada em 275.000 indivíduos (SILVA, 2014).

A mortalidade é alta na costa sul do litoral do Rio Grande do Sul, onde muitos animais chegam mortos e outros morrem no local, sofrendo ali o processo de decomposição (SILVA, 2004; PEREIRA et al., 2013). Silva (2014), analisou a sequencia temporal de mortalidade de leões-marinhos nas praias do Rio Grande do Sul, entre os anos de 1993 e 2013 e observou que o índice de mortalidade anual variou de 0,11 a 0,24 leões marinhos/10 km de praia e que a estimativa anual de mortalidade varia de 80 a 181 animais. Estas carcaças não são recolhidas e permanecem por vários meses sobre a ação das marés, ventos, variações térmicas até o seu total desaparecimento, consumo por animais necrófagos ou soterramento pela areia. Considerando a conscientização ambiental e preocupação com o destino adequado dos resíduos das carcaças é possível que exista a possibilidade de contaminação do ambiente por fezes expelidas durante o processo de decomposição assim como por fungos e bactérias colonizadores da superfície da mesma.

2. Objetivos

2.1 Objetivo geral

Verificar a presença de bactérias, fungos aeróbios e ovos de helmintos na superfície do corpo de leões-marinhos em decomposição e no sedimento arenoso ao redor das carcaças.

2.2. Objetivos específicos

1. Identificar as bactérias aeróbias na superfície das carcaças;
2. Identificar as bactérias aeróbias presentes no sedimento arenoso ao redor da carcaça em decomposição;
3. Identificar os fungos que colonizam a superfície das carcaças;
4. Identificar os fungos presentes no sedimento arenoso ao redor da carcaça em decomposição;
5. Comparar os dados das comunidades bacterianas e fúngicas que cresceram na superfície da carcaça com aquelas presentes no sedimento arenoso ao redor da mesma;
6. Avaliar a dispersão de ovos de helmintos já conhecidos em *O. flavescens* da região, no sedimento arenoso ao redor das carcaças.

3. Revisão de Literatura

Considerando as carcaças de *O. flavescens* como um elemento tanato-cenótico no sedimento arenoso, aborda-se nesta revisão as patologias causadas por bactérias, fungos e helmintos em mamíferos marinhos, as quais podem apresentar importância como foco de contaminação no ecossistema de praia.

3.1. Enfermidades bacterianas

Algumas doenças emergentes que acometem os organismos marinhos são resultados de patógenos provenientes do ambiente terrestre (McCALLUM et al., 2004) e de importância para a saúde pública (MOTTA et al., 2005). Membros da família Vibrionaceae podem afetar o nicho ecológico de mamíferos marinhos, ocasionando sérias implicações para sua saúde (DUNN, 1990; DUNN et al., 2001; PEREIRA et al., 2007; Di BENEDITTO et al, 2010). Van Bressem et al. (2009) destacaram algumas doenças e agentes etiológicos emergentes e recorrentes em cetáceos, entre os quais, brucelose, lobomicose, parainfluenza, *Helicobacter* spp., *Streptococcus* spp., *Salmonella* spp. e *Mycobacterium marinum.* No Brasil, foi idendificada a presença de *Vibrio* spp. em 198 amostras oriundas de lesões em diversas áreas do organismo dos seguintes mamíferos marinhos: baleia-franca

(Desmoulins, 1822) (*Eubalaena australis*), baleia-de-bryde (Anderson, 1879) (*Balaenoptera edeni*), baleia-jubarte (Borowski, 1781) (*Megaptera novaeangliae*), baleia-minke (Lacépède, 1804) (*Balaenoptera acutorostrata*), baleia-bicuda-de-True True, 1913 (*Mesoplodon mirus*), franciscana (Gervais & d'Orbigny, 1844) (*Pontoporia blainvillei*), boto-cinza (Gervais & Deville, 1853) (*Sotalia fluviatilis*), golfinho-pintado-do-Atlântico (G. Cuvier, 1829) (*Stenella frontalis*), golfinho- comum Linnaeus, 1758 (*Delphinus delphis*) e leão-marinho-do-sul *(Shaw, 1800)* (*Otaria flavescens*) (PEREIRA et al.,2007).

Os gêneros *Staphylococcus, Campylobacter, Plesiomonas shigelloides e Aeromonadaceae,* foram identificados em 52 de 114 amostras de *P. blainvillei,* (Di BENEDITTO et al., 2010).

Devido à maioria das amostras coletadas de mamíferos marinhos ser oriunda de tanatocenose, os registros não implicam em determinar se a presença de tais microorganismos estão envolvidos na *causa mortis* dos animais ou se são oportunistas colonizadores durante biocenose.

3.2. Enfermidades fúngicas

O reino Fungi (Whittaker em 1969) inclui quatro filos: Chytridiomycota, Ascomycota, Zygomicota e Basidiomycota. O filo Ascomycota tem sido relatado como predominante em ambientes aquáticos, marinhos e com muita matéria orgânica em decomposição (PEARMAN et al. 2010). Os fungos são conhecidos popularmente como mofos e bolores (SILVA et al. 2006). São organismos com células eucarióticas, algumas espécies podem estar na forma de levedura (unicelular), na forma de fungos filamentosos (pluricelular) e outras, em espécies dimórficas, nas duas formas, que se desenvolvem dependendo das condições ambientais e do estresse encontrado (TORTORA, 2005).

Os fungos utilizam uma variedade de substratos como fontes de carbono, entretanto, alguns grupos se especializaram em degradar substratos particulares, tornando-se mais competitivos perante outros microrganismos. Por essa razão os fungos são encontrados em praticamente todos os ambientes no planeta (SILVA et al. 2006).

Exigências e características de crescimento das colônicas fúngicas são típicas de cada gênero, dos quais muitas espécies são oportunistas, podendo ser

potencialmente patogênicos, dado a um crescimento e dispersão rápidos, formação de colônias maduras, e baixa especifidade por substratos, tornando-os oportunistas. Muitas espécies são saprófitas, ou seja, vivem de matéria orgânica em decomposição no solo e às vezes são aerotransportados, e podem ter seus conídios, estruturas assexuadas reprodutivas imóveis, inalados por seres humanos (KERN e BLEVINS, 1999).

A maioria dos fungos está presente no ambiente como sapróbia ou comensal, coexistindo com animais sem afetá-los. Esporos de fungos, como de *Aspergillus* spp., por exemplo, entram frequentemente no trato respiratório de animais por inalação, mas raramente são patógenicos em animais sadios. As aves podem carregar esses fungos e não desenvolver a doença até que exista uma queda na resistência do hospedeiro devido a algum tipo de estresse (HUBALEK, 2004). Por outro lado, fungos patogênicos costumam causar sérios danos. Graves infecções fúngicas em animais silvestres que podem ser facilmente disseminadas não só para os seres humanos, mas também à outros animais (SPARAGANO; FOGETT, 2009).

Em mamíferos marinhos, os organismos fúngicos podem contribuir de maneira significativa para a mortalidade das populações (REIDARSON et al., 2001). Várias gêneros fúngicos tem sido isolados de amostras de cetáceos e pinípedes ao longo dos anos, principalmente em animais encalhados no Atlântico Norte. Dentre os associados, alguns tipos de lesões estão *Aspergillus spp.*, *Blastomyces spp.*, *Candida spp.*, *Torulopis spp.*, *Trichosporon spp.*, *Malassezia spp.* e *Fusarium spp*. (HIGGINS, 2000).

No Brasil, as informações na literatura sobre as prevalências dos agentes causais destas enfermidades ainda não permitem traçar perfis das principais espécies patógenas que acometem estes animais. Desta forma, a identificação fúngica em um processo de doença de vida livre, deve ser sempre efetuada para fins de conhecimento. Dado que a microbiota presente na superfície do corpo de *O. flavescens*, não é conhecida em animais vivos, ainda menos é conhecida em respeito àquela que o coloniza após sua morte.

3.3 Enfermidades parasitárias de mamíferos marinhos

Os mamíferos marinhos são acometidos por uma grande variedade de agentes bacterianos, micóticos, parasitários e virais, dentre eles encontram-se várias zoonoses emergentes de importância para a saúde pública e para outras espécies

animais (RUOPPOLO, 2003; STOSKOPF, 2001; REIDARSON et al., 2001). Embora a preocupação com a fauna tenha aumentado consideravelmente, a interferência humana no habitat dos animais continua acarretando sérios prejuízos ambientais e ecológicos, gerando mortes diretas e indiretas que chegam a resultar em ameaças de extinção para diversas espécies animais (CUBAS et al. 2007). Até hoje poucos ciclos de vida dos parasitos de mamíferos marinhos foram elucidados, e este conhecimento é especialmente escasso no Brasil, assim como o das causas de morbidade e mortalidade de mamíferos aquáticos que pertencem à subordem Pinnipedia (RUOPPOLO, 2003).

Os peixes albergam uma grande quantidade de parasitos, especialmente os marinhos nos quais a infecção poderá ocorrrer através da dieta, tendo os peixes como hospedeiros intermediários (HOLMES, 1990). Segundo Daszak (2000) as doenças infecciosas emergentes dos animais silvestres de vida livre podem ser classificadas em três grandes grupos: as associadas ao "derrame" de animais domésticos para populações selvagens que vivem em proximidade; os que estão diretamente relacionados à intervenção humana, via translocações de hospedeiro ou parasito; e aqueles sem envolvimento de animais ou humanos. Esses fenômenos têm implicações biológicas importantes no qual, muitas espécies selvagens são reservatórios de agentes patogênicos que ameaçam a saúde dos animais domésticos e humanos.

Muitos estudos já foram descritos causando morte de mamíferos aquáticos (MORGADES et al., 2006; AZNAR et al., 2011). Segundo Gulland et al., (2001) registros de casos de doenças de origem não infecciosa também já foram relatados em mamíferos aquáticos, como neoplasias, defeitos congênitos, assim como já foram relatado diversas espécies de parasitos acometendo diferentes órgãos como estômago, coração, intestinos, cérebro, fígado e pulmão (MARIGO, 2003; KATZ et al., 2012, PEREIRA et al. 2013). Estudos com metais pesados apontam para altas concentrações de diferentes componentes químicos em vários órgãos e tecidos (FILLMAN et al., 2007; BARAJ et al., 2009). No Atlântico, existem alguns estudos que descrevem as causas de óbitos de mamíferos marinhos no litoral do Brasil e Uruguai, descrevendo foram citadas algumas causas de óbitos que estão relacionadas com diversos fatores, sendo elas metabólicas, nutricionais, problemas no trato digestivo, urinário, e algumas síndromes (Di GUARDO et al., 2005; KATZ et al., 2012; RUOPOLLO, 2003).

3.4. Helmintos de *O. flavescens*

Alguns helmintos já são conhecidos por infectar *O. flavescens* em sua ampla área de distribuição ao longo da costa da América do Sul. Na costa Argentina, Aznar et al (2011) em estudo realizado em 252 amostras de fezes de *O. flavescens* na província de Buenos Aires, citaram a presença *de Corynossoma australe* e *Corynossoma cetaceum*.

No Uruguai, Morgades et al. (2006) estudando leões-marinhos de diversas faixas etárias encontraram *Uncinaria* sp. *Diphyllobothrium* sp. (Cestoda: Diphyllobothriidae) e *Ascocotyle (Phagicola) longa* (Trematoda: Heterohyidae). *Corynosoma australe* e *Corynosoma* sp. foram encontrados nos estômagos, intestino delgado e grosso de leões juvenis e somente no intestino delgado e grosso de animais adultos. No mesmo trabalho, os autores citam a presença de anisaquídeos não identificados e *Contracaecum* sp. coletados do estômago e intestinos de animais juvenis e adultos.

No litoral do sul do Brasil, na mesma região onde o presente estudo foi desenvolvido, Pereira et al (2012) identificaram as espécies de helmintos parasitas encontradas em espécimes de leão-marinho-do-sul. Os helmintos encontrados foram: *Bolbosoma turbinella*, *Contracaeucum ogmorhini, Stephanoprora uruguayense*, *Diphyllobothrium* sp., *Ascocotyle (Phagicola) longa* e *Stephanoprora uruguayense*, estando apenas o trato digestório destes animais parasitado. Uma alta carga parasitária, principalmente por *Corynosoma australe* (prevalência = 100% e intensidade média= 1756.04) foi detectada nos intestinos delgado e grosso.

4. Material e Métodos

4.1 Carcaças de animais (elementos tanatocenóticos)

Foram utilizados 10 espécimes de leão-marinho, *O. flavescens*, machos e fêmeas, encontrados mortos ao longo das praias do litoral do Rio Grande do sul, durante saídas de campo sistemáticas e semanais cobrindo aproximadamente 450km da costa entre a Barra do Chuí 33°44’35”S e a barra da Lagoa do Peixe 31°21’38’’S (Figura 3). As carcaças encontradas foram medidas identificadas e sexadas seguindo Pinedo et al. (1992), analisadas e classificadas segundo seu estado de decomposição seguindo os tópicos indicados em protocolo específico (IBAMA, 2005), adaptado para pinípedes. Foram amostrados somente aqueles que

se enquadraram no estágio 2 e 3 do referido protocolo, considerados frescos. Os dados foram inseridos em planilhas de campo específicas, ANEXO 1.

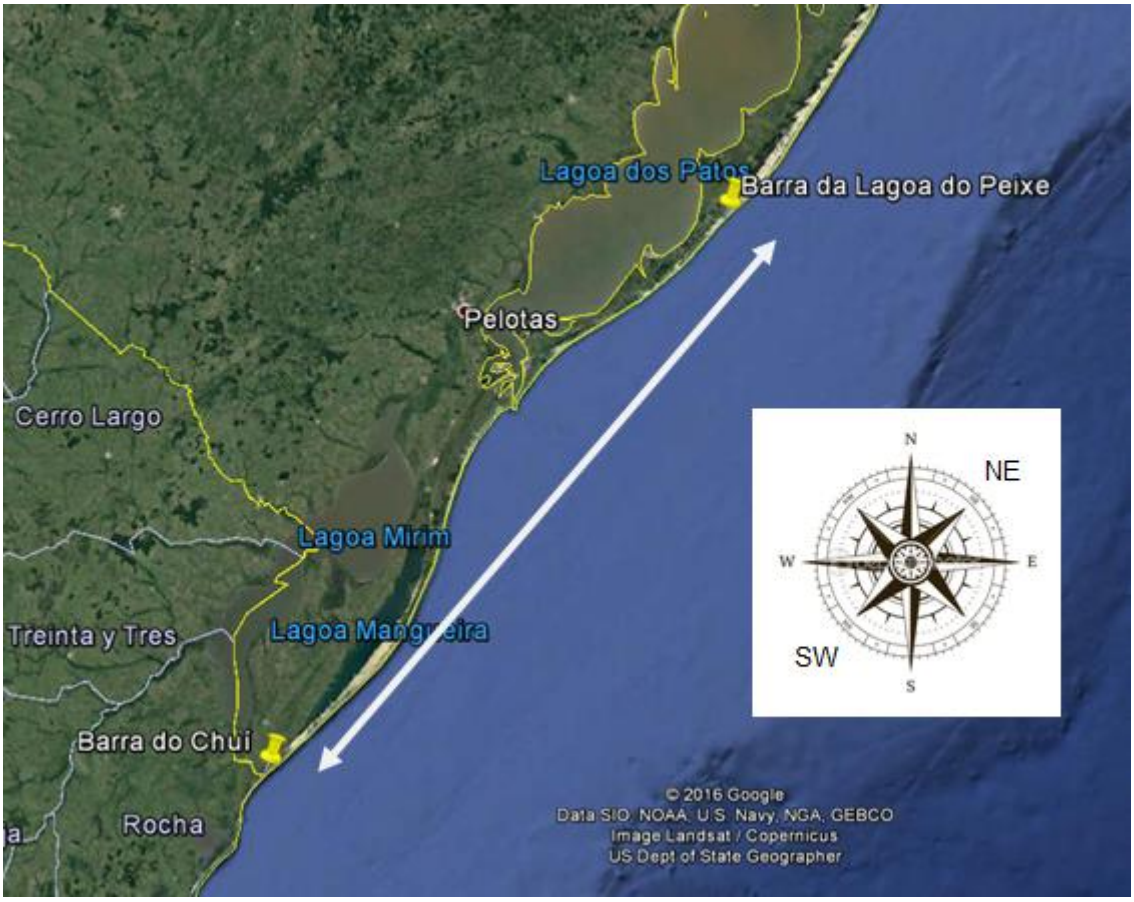

Figura 3. Área de estudo no litoral do Rio Grande do Sul, Brasil. Limites noroeste (NE) e sudeste (SW) são indicados com marcadores amarelos. Fonte Google Earth.

4.2. Amostragem

4.2.1. Amostras microbiológicas das carcaças

Foram amostrados, por meio de *swab* estéril, quatro regiões do corpo de cada espécime analisado, sendo ânus, boca, dorso e nadadeira direita. Os *swabs* foram acondicionados em caixas isotérmicas e imediatamente encaminhadas ao Laboratório de Micologia – Instituto de Biologia- Universidade Federal de Pelotas para processamento.

4.2.2. Amostras de sedimento arenoso

No perímetro de cada carcaça examinada foram amostradas quatro amostras de areia superficial, até 5 cm de profundidade com volume de 225cm$^{3.}$

O perímetro dos pontos amostrais tiveram uma distância de 10 cm das seguintes pontos do animal: boca, ânus e nadadeiras (Figura 4).

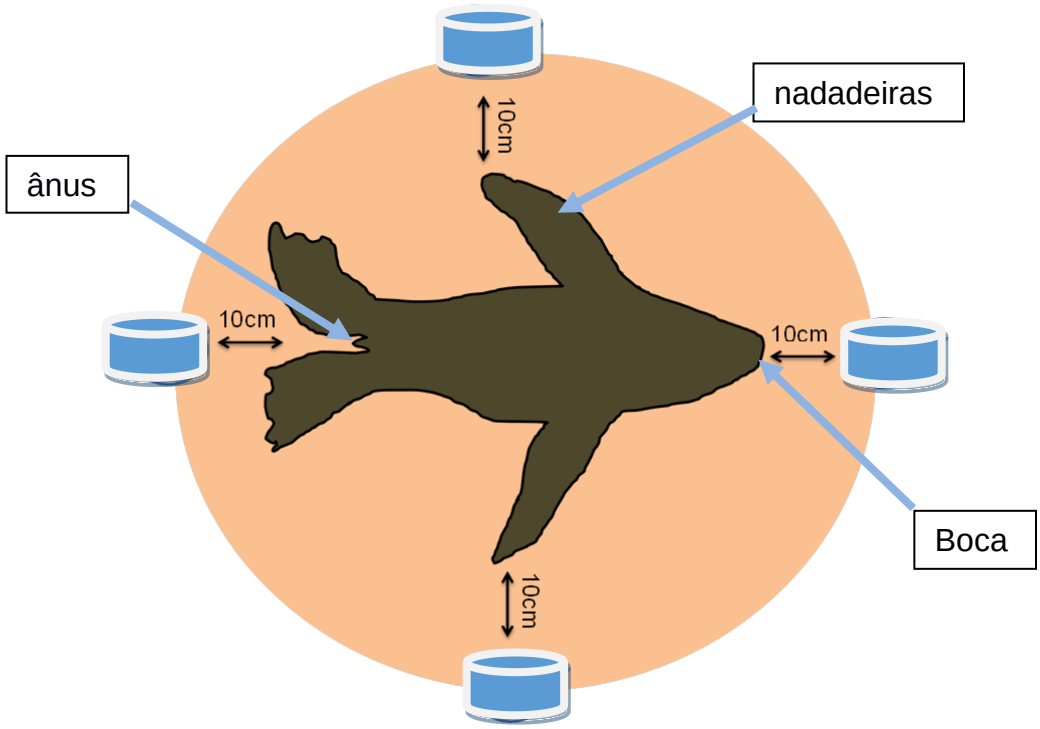

Figura 4. Esquema de vista dorsal de uma carcaça de pinípede. Os pontos de amostragem são indicados por círculos azuis e estão a uma distância de 10 cm da boca, ânus e nadadeiras, respectivamente.

Amostras controle foram coletadas ao longo do litoral sul do Rio Grande do Sul e totalizaram seis amostras de sedimento arenoso sem a presença de carcaças de *O. flavescens* ou de outros animais. Estas amostras se destinaram tanto para a análise microbiológica quanto para análise de ovos de parasitos.

4.2.3. Amostras de fezes das carcaças de *O. flavescens*

As fezes foram recolhidos por meio de sucção com seringas de 50mL do ânus e reto de *O. flavescens*. Quando não estiveram presentes ou volume reduzido, foi realizado uma lavagem do reto com AFA (Álcool-Formaldeido-Ácido-acético) desta região para posterior exame em microscópio.

4.2.4. Amostras de fezes de *O. flavescens* vivos

Foram coletadas 34 amostras de fezes de leões-marinho vivos diretamente do substrato rochoso durante duas expedições ao Refúgio do Molhe Leste. As amostras retais assim como dos animais vivos foram processadas no laboratório de parasitologia da UFPel.

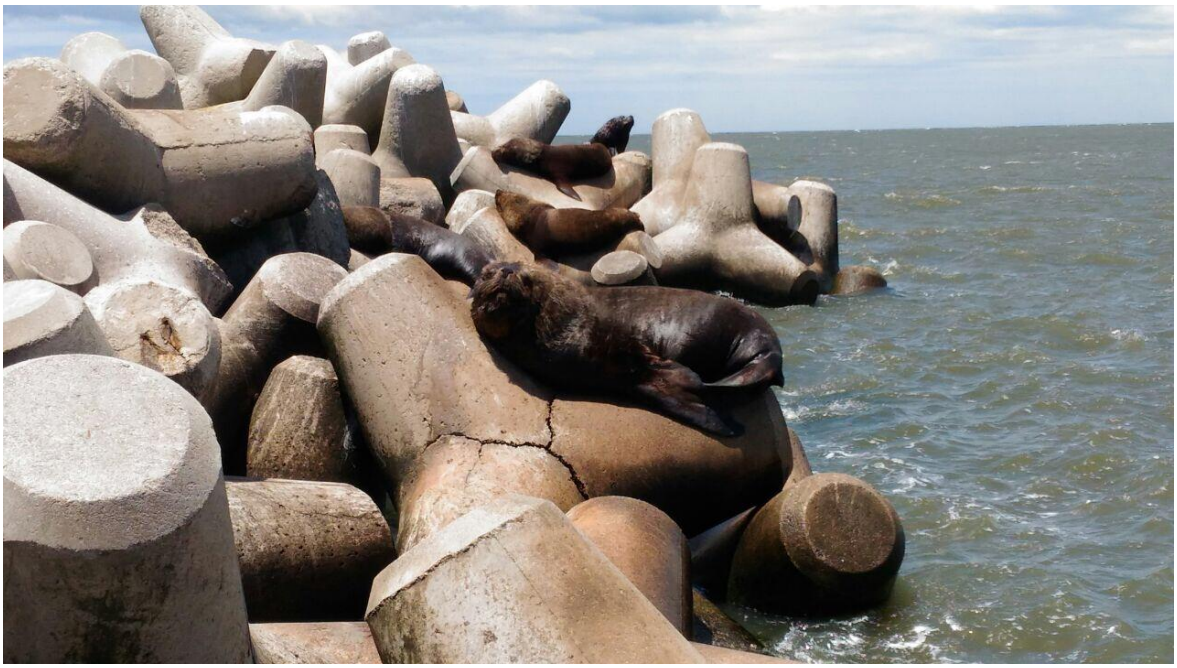

Figura 5: Leões-marinhos em descanso no Molhe Leste, Refúgio da Vida Silvestre, RS. Local das coletas de fezes dos animais vivos. Fonte: Julieses Vaz

4.3. Análises microbiológicas

4.3.1. Processamento das amostras de sedimento arenoso e superfície corpórea dos animais

Alíquotas de 25g do sedimento foram diluídas em 250mL de água peptonada a 0,1% (diluição 1:10). A partir desta primeira diluição foram realizadas diluições

seriadas, na base 10 até 10^3. Volumes de 100 µL (0,1mL) de cada uma das diluições foram semeados em meios de cultura seletivos para bactérias (Ágar *Brain Heart Infusion; Ágar MacConkey*) e fungos (*Potato dextrose Ágar).*

As placas de ágar *MacConkey* e Ágar *Brain Heart Infusion* foram incubadas em estbacteriológica, a 37ºC, durante 24 - 48 horas e as de PDA incubadas a 25ºC, durante 7 dias. Após o crescimento, todas as colônias bacterianas obtidas foram submetidas à coloração de gram e posterior identificação em gênero e espécie utilizando o sistema automatizado ViteK®. A identificação das colônias fúngicas foi baseada nas suas características macro e micro morfológicas até seu status de gênero.

Os *swabs* coletados do corpo do animal foram semeados por esgotamento em meios seletivos para bactérias (Ágar *Brain Heart Infusion*, ágar sangue, ágar *MacConkey* e meios seletivos para *Salmonella, Samonella Shigella* e fungos *Potato dextrose ágar*. Para *Salmonella* os *swabs* foram acondicionados durante 24h em Caldo Rappaport-Vassiliadis e em seguida em placas de *Salmonella-Shigella.* As placas de ágar sangue, ágar MacConkey e com meio seletivos para *Salmonella*, foram incubadas em aerobiose em estufa bacteriológica, a 37ºC, durante 24 - 48 horas e as de PDA foram incubadas a 25ºC, durante 7 dias. A identificação das colônias foi realizada conforme descrito na metodologia para as amostras de sedimento arenoso.

4.4 Análises parasitológicas

4.4.1. Sedimento arenoso

As amostras do sedimento arenoso foram processadas no laboratório de Parasitologia do Departamento de Microbiologia e Parasitologia da Universidade Federal de Pelotas, através da Técnica de Caldwell & Caldwell modificada por Corrêa (1995) para pesquisa de ovos, larvas de helmintos e oocistos de protozoários.

4.4.2. Amostras de fezes

As amostras foram examinadas através das técnicas parasitológicas: quando possível, OPG (contagem de ovos por grama de fezes), segundo a técnica de Gordon e Whitlock (1939), Faust et al. (1938) e Sheather (1923).

5. Resultados

5.1. Capítulo 1

Artigo 1. Normas de acordo com a revista Envinmental Microbiology Reports

Environmental contamination by aerobic bacteria around *Otaria flavescens* (Shaw, 1800) carcasses in Brazilian southern costal plane.

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Abstract

Anaerobic bacteria on the carcass surface and in the beach sand where carcasses of southern sea lions, *Otaria flavescens*, remained until decomposition were identified. Swabs of the mouth, anus, back and skin of the fins and sand samples around the carcasses were collected from 10 adult specimens, in moderate decomposition state, in the Brazilian southern coastal plane. The swabs were seeded on blood agar, Brain Heart Infusion, MacConkey, Rappaport and Salmonella-Shigella agar and incubated at 37°C for 24-48 hours. Aliquots of 25g of sand were diluted up to 10^3 in 250 mL 0.1% peptone water. A 100uL volume of each dilution was seeded on Ágar *Brain Heart Infusion* and MacConkey agar and incubated. The colonies were submitted to Gram stain and identified by VITEK® automated system. Thirty-three species of bacteria were isolated and identified. *Proteus*, *Aeromonas*, *Kocuria* and *Sphingomonas* were found in 80% of the examined animals. *Proteus vulgaris* and *Sphingomonas paucimobilis* were the most common species in all samples (80%), followed by *Aeromonas salmonicida* (70%), *Kocuria rosea* (60%) and *Streptococcus*

thoraltensis (60%). Due to the presence of some bacteria with potential to infect humans, the beach access next to the animal body should be avoid.

Introduction

Microorganisms are a significant component of beach sand (Pinto & Oliveira, 2011), which is an accumulating element of waste of different origins. This waste acts as substrate for the presence of parasites and pathogens that are dispersed in the environment (Lopes *et al.,* 2008; Cicero *et al*., 2012). Pathogenic microorganisms to man and fecal contamination indicators survive for long periods in beach sand due to the presence of organic matter (Vieira *et al*., 2007; Oliveira *et al.,* 2015,). As reported by the World Health Organization (WHO, 1997), bacteria, fungi, parasites and viruses have been observed in these conditions, and several genera and species of these microorganisms are potentially pathogenic to humans through contact.

The proliferation of microorganisms is determined by the availability of organic matter in the sand, which can be originate in plant biomass, animal biomass (including decomposing bodies), animal feces and other waste of anthropogenic origin (Vieira *et al*., 2001; Kinzelman *et al*., 2003; WHO, 2003). Beach vulnerability to pathogenic microorganism development is a public health hazard because this environment is susceptible to contamination, particularly by helminths and protozoa present in animal feces, including *Ancylostoma* spp. and *Toxocara* spp. (Blazius, *et al*., 2006; Cáceres *et al*., 2004; Scaini, 2003). However, imbalances caused by this contamination, risks of transmission and even the identification of most pathogenic microorganisms are still unknown. The sanity of recreational areas is primarily assessed by water quality, but studies have shown that the assessment of the microbiological quality of sand is also relevant (Alm *et al.*, 2003; Lopes *et al.,* 2008).

In this sense, sand can directly affect swimmers through causative agents of infectious diseases (Cetesb, 2009; Maier *et al*., 2003; Passos *et al*., 2011).

Decaying animal carcasses that remain in coastal areas are an important source of organic matter and pose potential environmental and zoonotic risks. Given the topographical features and limited access of some areas, animal carcasses, among which seabirds, marine mammals, fish and even pets are often found along the coastline (SW/NE) of Rio Grande do Sul, Brazil. Because of its long extension and high stranding frequency, carcasses of pinnipeds and cetaceans are beached, especially during the winter months (Pinedo *et al*., 1992). Marine mammals generally have a strong public appeal, as well as scientific and conservation interest; on account of this, close contact to the body of these animals, whether professional or moved by sheer curiosity, can pose risks to human health, including the transmission of zoonotic diseases and traumatic injuries (Waltzek *et al*., 2012).

Parasitic, fungal and bacterial diseases have been reported in many marine animals (Dunn, 1990; Dunn *et al.,* 2001; Silva, 1991; Caceres *et al*., 2004). In Brazil the presence of *Vibrio* spp. has been identified in various species of marine mammals (Pereira *et al*., 2007), especially cetaceans. Also in this group of mammals, bacteria of the genera *Staphylococcus, Campylobacter Aeromonas* and *Vibrio* have been isolated in *Pontoporia blainvillei* (Pereira *et al*., 2007; 2008). In Sirenia, Delgado (2010) recorded the presence of *Leptospira* spp. in manatee (*Trichechus inunguis*).

In pinnipeds, a wide range of bacteria, including those of public health interest, has been isolated in various geographical areas (Gonzales *et al*., 2009; Carrasco *et al.*, 2011). An example of this is tuberculosis, which has been caused by *Mycobacterium bovis* or *Mycobacterium pinnipedii* in fur seals and sea lions in captivity (Forshaw and Phelps, 1991); however, this disease has also been detected in *Arctocephalus*

australis and *Otaria flavescens* in the coastal waters of Argentina (O'Reilly and Daborn, 1995).

In southern Brazil, *Otaria flavescens* (Shaw, 1800), also known as southern sea lion, is endemic. This species is distributed along the coastal lines of both coasts of South America, grouping together on rocky sites. In the Atlantic, the northernmost grouping area of this species is southern Brazil, extending meridionally as far as Tierra del Fuego, Argentina and continuing north along the Pacific as far as Peru (Vaz-Ferreira, 1981; Rose *et al.*, 1994). The species shows significant mortality rates on the southern coast of Rio Grande do Sul State, where many animals arrive dead and others die locally, undergoing decomposition processes (Pavanato *et al.,* 2013). These carcasses are not collected and remain for several months suffering the action of the tides, winds, temperature variations until their total disappearance, consumption by scavengers or burial by sand. In the same geographical area, Pereira *et al*. (2013) reported that *O. flavescens* found dead on the beachwere highly parasitized by several species of helminths.

Considering that beach environmental quality has gained increasing importance on environmental and public health grounds (Stewart *et al.,* 2008) and that these days beach sanitary quality should not be monitored by the density of fecal coliforms present in its waters alone, this study aimed to verify the presence of aerobic bacteria on the carcass of *Otaria flavescens* in various stages of decomposition as well as in the soil surrounding the carcass deposition area.

Material and Methods

Swabs of the mouth, anus, back and skin of the fins of carcass of 10 adult specimens of *O. flavescens* found during systematic weekly field trips to the area between Torres (29 ° 20 'S) and Arroio do Chuí (33 ° 15'S), Rio Grande do Sul State, Brazil,

were collected. Carcasses were found at different stages of decomposition and only those in G2 or G3 level according to the protocol proposed by Geraci and Lounsbury (2005) adapted to pinnipeds were used. Additionally, four 225 cm^3 sand samples taken from a depth of up to 5 cm and a distance of up to 10 cm from the mouth, anus and fins were collected for each carcass. The swabs were seeded on blood agar, BHI (Brain Heart Infusion), MacConkey, Rapapport and *Salmonella Shigella* agar and incubated at 37° C for 24-48 hours. 25g aliquots of each sand sample were diluted up to 10^3 in 250 mL 0.1% peptone water. A 100uL volume of each dilution was seeded on BHI agar and MacConkey agar and incubated at 37°C for 24-48 hours. After growth, the bacterial colonies obtained were submitted to Gram stain and identified by VITEK® automated system.

Results and Discussion

At least 33 species of bacteria from carcass and sand samples were isolated and identified. Among these, bacteria of the genera *Proteus, Aeromonas, Kocuria* and *Sphingomonas* were found in 80% of the examined animals. Other genera included *Streptococcus* sp. (60%), *Staphylococcus* spp. (50%) and *Acinetobacter* (50%) (Table 1). *Proteus vulgaris* and *Sphingomonas paucimobilis* were the most common species in all samples (80%), followed by *Aeromonas salmonicida* (70%), *Kocuriarosea* (60%) and *Streptococcus thoraltensis* (60%).

Proteus vulgaris is naturally found in the digestive tract of humans and various other animal species (O'Hara *et al*., 2000). This bacteria is normally found in soil and water contaminated with fecal material and is an opportunistic pathogen to human beings known to cause infections in the urinary tract (Chow *et al.*, 1979), as well as wound contamination (Pearson *et al*., 2008). Bacteria of the genus *Proteus* have

been identified in the oral cavity of *Arctocephalus australis* undergoing rehabilitation in southern Brazil (Vargas *et al*., 2008).

Sphingomonas paucimobilis is another microbial agent typically found in soil which may occasionally cause septicemia, septic arthritis and osteomyelitis in humans (Adley & Ryan, 2010). Both bacteria were first associated with *O. flavescens* decomposition and may be originated and released during the fermentation process. Given their frequent occurrence, they stand out for their zoonotic potential through contact with mucous membranes and damaged skin, which can represent a gateway to these opportunistic microorganisms. *Aeromonas salmonicida* is a common pathogen that causes furunculosis and septicemia in fish (Kamble, 2015). This organism usually infects homeothermic vertebrate animals that live in water at low temperatures. Until recently it was thought that there was no bacterial growth at 37°C; however, a study on the microbiology of marine mammals has identified the presence of several species of *Aeromonas* in cetaceans on the Brazilian coast (Pereira *et al*., 2008). The same authors pointed out the presence of *A. caviae, A. hydrophila, A. veronii* biogroup sobria, *A. jandaei* and *A. media* in *Otaria flavescens*.The occurrence of *A. salmonicida* in this study is new for *Otaria flavescens* and, even if it cannot be ensured whether it was associated with disease in this mammal, Tewari *et al*. (2014) the occurrence of this species associated with infection in humans.

Bacteria of the genus *Kocuria* are thought to cause emerging diseases in humans (Savini *et al.,* 2010). Although the presence of species of this genus is known to be part of skin microbiota of human being, animals and marine sediment (Kim *et al*., 2004), no records have been found on marine mammals. *Kocuria rosea* was found only in the sand around carcasses in 6 out of 10 animals examined, and its presence

could not be associated with carcass decay. Regardless of its origin, this bacterium offers a contamination hazard to humans, in as much as there have been many records of infection in immuno-depressed individuals (Dunn *et al.,* 2011).

Among *Streptococcus*, *S. thoraltensis* is a newly found species that was described by Devriese *et al.* (1997) in cultures originating from the intestine and genital tract of pigs. Although the genus now accommodates more than 60 species, relatively few of these streptococcal species have been recovered from marine animals. Indeed only four species, *Streptococcus phocae* (Skaar *et al.,* 1994) and *Streptococcus halichoeri* (Lawson *et al*., 2004) from seals, *Streptococcus iniae* recovered from freshwater dolphins and *Streptococcus marimammalium* from seals (Lawson *et al*., 2005) have been so far identified. In this study, this species was mostly found in swabs collected directly from the rectum, which excludes the possibility of secondary contamination. This is therefore a new record of the species for a marine mammal. Infections in humans are not known, but that the species usually colonizes the oral cavity (Dhotre *et al*., 2013).

When bacterial growth in sand was contrasted to that in carcasses, it was observed that only seven out of 33 identified species were present in both substrates, characterizing environmental contamination: *P. vulgaris, A. haemolyticus, S. intermedius, S. paucimobilis, A. salmonicida, E. coli, and Staphylococcus warneri, Kocuria kristinae, K. rosea, Pantoea spp., Pseudomonas stutzeri, Shewanella algae, Acinetobacter ursingii, A. hydrophila, Rahnella aquatilis, Serratia marcescens, Vibrio parahaemolyticus,* and *Staphylococcus lentus.*

Of the 10 carcass analyzed the presence of bacteria of the genus *Aeromonas* species *A. salmonicida* and *A. hydrophila* was identified in 80% of samples. Species of this genus have been identified as causative agents of gastroenteritis and skin

infections in humans; in addition, they are also considered important fish pathogens (KO *et al*., 2000, Scoglio *et al.,* 2001). *A. salmonicida* was identified in both carcasses and samples of the sand around them and its occurrence seems to be directly linked to the presence of the carcass. *Aeromonas hydrophila*, despite the fact of having been isolated from carcasses of species of cetaceans and *O. flavescens* on the southern and southern coast of Brazil (Pereira *et al.*, 2008) was only identified in one sand sample of one animal. This bacterium is pathogenic and has been often isolated in samples of various origins, including water and animals, but has been mainly isolated from fish and mussels (Santos, 1998; Pereira *et al.*, 2004; Harnisz & Tucholski, 2010).

Contamination of sand on beaches is a public health hazard. *Escherichia coli* is one of the major constituents of intestinal microbiota in animals and, like thermotolerant coliforms, contaminate sand through feces (Murray, 2006). In this study the presence of *E. coli* was identified in 20% of carcasses and 10% of sand around the carcasses of *O. flavescens* studied, as well as in the sand around carcasses. Infection by *E. coli* is a disease of increasing interest, both to human and animal health, as agents belonging to the family *Enterobacteriaceae* are responsible for significant incidences of pathological processes in humans and animals (Croxen *et al.*,2010; Dho-Molin *et al.*,1999).

Bacteria with high zoonotic potential, such as *Erysipelothrix insidiosa*, *Leptospira* sp., *Brucella* sp. and *Mycobacterium bovis*, which have already been isolated from pinnipeds, (Blank *et al*., 2002) require specific methods for cultivation and identification which were not implemented in this study. Studies on the occurrence of *E. coli* associated to marine mammals are scarce (Salinas *et al*., 2010).

The genera *Streptococcus* and *Staphylococcus* were isolated in 60% and 50% of the analyzed samples, respectively. According to Thornton *et al.* (1998) these genera are commonly found on the skin of marine mammals. *Streptococcus* has subclinical signs, but may develop fever, depression, and various mastitis infections. In debilitated human it can cause respiratory symptoms, endocarditis and skin lesions (Bisno *et al*., 1996). *Staphylococcus* infection in animals can cause, in turn, skin lesions, pneumonia and eventually evolve to septicemia; in humans, it can cause abdominal pain, vomiting and diarrhea (Devriese *et al*., 2005).

Several microbiological studies have pointed out long lists of species of aerobic bacteria that grow in cultures from samples of animal carcasses on beaches (Gomes, 2014), this study, however, has extended the information on sand contamination, which may receive organic matter from other sources. Such questioning may be justified by the presence, even though less significant, of the 10 species which were found only in sediment. Considering the beach ecosystem where these carcasses were collected, a high interactivity between abiotic and biotic factors in the grounding area was found. The permanence of the carcass attracts scavengers, including birds and wild mammals, which may be responsible for a significant presence of organic matter and microorganisms on the site (Gomes, 2014). On the other hand, bacteria associated to sea lion decomposing tissues may undergo washing by the constant action of tides which, due to salinity, which could limit sand colonization.

Given the findings of this study, including the presence of microbial agents with potential to infect humans, as in carcasses as in sand, the beach access area next to the animal body may have negative implications in public health, and the need to use protective equipment by professionals and researchers responsible for handling them is emphasized.

Species	Sand	Carcasses	Gram
Aeromonas salmonicida	+	+	GN
Aeromonas hidrophila	+	-	GN
Acinetobacter haemolyticus	+	+	GN
Acinetobacter ursingii	+	-	GN
Aloiococcus otitis	-	+	GP
Citrobacter freundii	-	+	GN
Escherichia coliformes	+	+	GN
Enterococcus faecium	-	+	GP
Gamela sanguinis	-	+	GP
Kokuria rosea	+	-	GP
Kokuria varians	-	+	GP
Kokuria kristinae	+	-	GP
Leuconostoc mesenteroides spp. cremoris	-	+	GP
Pantoea spp.	-	+	GN
Pediococcus pentosaceus	-	+	GP
Pasteurella pneumotrópica	-	+	GN
Pseudomonas pseudoalcaligens	-	+	GN
Pseudomonas stutzeri	+	-	GN
Proteus vulgaris	+	+	GN
Proteus mirabilis	-	+	GN
Roseomonas gilardi	-	+	GN
Rahnella aquatilis	+	-	GN
Sphingomonas paucimobilis	+	+	GN
Staphylococcus saprophyticus	-	+	GP
Staphylococcus lentus	+	-	GP
Staphylococcus intermedius	+	+	GP
Serratia marcescens	+	-	GN
Streptococcus thoraltensis	-	+	GP
Stenotrophomonas maltophilia	-	+	GN
Staphylococcus sciuri	-	+	GP
Staphylococcus warneri	+	+	GP
Shewanella algae	+	-	GN

Table1: Identification of the aerobic bacteria species found on body surface and coastal sediment around carcass of *O. flavescens* in decomposition in Southern Brazil beaches.

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5.2. Capítulo 2

Artigo 2: Normas de acordo com a revista Aquatic Microbial Ecology

General distribution of fungal genera in thanatocenotic elements of *Otaria flavescens* (Shaw,1800) (Carnivora: Otaridae) in sediments Brazilian southern coastal plane

Distribuição de gêneros fúngicos em elementos tanatocenóticos *de Otaria flavescens* (Shaw, 1800) (Carnívora: Otaridae) em sedimentos costeiro no sul do Brasil

Abstract

The knowledge about coastal and marine ecosystems is in constant evolution, as well as about the coastal regions, those are considered transition areas between continents and oceans.Superficial samples were analyzed in 10 carcasses of *O. flavescens*, found during systematic and weekly field trips in the in Brazilian south coast. The studied carcasses were in mild to moderate state of decomposition and the used carcasses were next to the humid zone of the beach, excluding partial or total submerged carcasses. From each carcass was collected four microbiological samples, using a sterile swab, corresponding to four regions of the body: mouth, anus, back and anterior flipper (thoracic member). The swabs were stored in isothermal boxes, the material was seeded by the streaking methodology in PDA and

incubated in bacteriological incubator at 25 °C. Around each carcass, 4 samples of superficial coastal sediment, each sample with 225 cm^3 of volume. The sediment samples were strategically collected at 10 cm of distance of the following points: mouth, anus and right and left anterior flippers. Of these samples, aliquots of 25 g of sediment were diluted in 250 mL of peptone water 0.1 % and submitted to a serial dilution, in base 10, until dilution 10^3. Volumes of 100 µL of each dilution were seeded in the culture medium PDA, incubated in bacteriological incubator, at 25°C. For control, seven samples of the humid sandy sediments, collected without the presence of the carcasses, similarly to the sand collected around the carcasses. Both body surface samples and sandy sediment were submitted to fungal colonies identification. The identification was based in their macro and micro morphological characteristics until the taxonomic level of genus. Only opportunistic fungi were identified. From carcass samples were identified 5 genera of fungi: *Trichoderma* spp., *Aspergillus* spp., *Penicilium* spp., *Cladosporium* spp. and *Fusarium* spp. In the sandy sediment samples around the carcass were present 4 genera of fungi, *Aspergillus* spp., *Fusarium* spp, *Penicilium* spp and *Cladosporium* spp.

Key words: beach, fungi, American South sea lion, coastal sediment, carcasses, thanatocenosis

Introduction

The knowledge about coastal and marine ecosystems is in constant evolution, as well as about the coastal regions, those are considered transition areas between continents and oceans (Correia 2005). In these regions occur physical-chemical variations and changes in the retention rates of organic matter, pollutants, sediments and nutrients from the continent. Among the peculiar characteristics of these ecosystems, it must be considered all the natural and biological event responsible by innumerable modifications and interactions among living beings (Almeida, 2008).

The domestic residues, human and animal wastes are pollutants; those contaminate the sea and can disseminate a vast variety of pathogenic bacteria and fungi (Pinto e Oliveira 2011). Marine animals that are in contact with contaminated water can be infected, altering their physiological condition and getting sick, and, then, they are found weakened in the coastal zone (Zampiere et al., 2013). Considering the interface between the public health and marine ecology, it is questionable how the interactions between the natural microbiota of water and marine sediments change with carbon supply, due to the decomposition of marine

animal carcasses thanatocenosis with large biomass and, whether these supplies cause microbial disruption in the sandy sediment, bringing risks to human health.

Many fungal species are present in the environment as saprobes or commensals, coexisting with animals without affecting them (Gontijo et al. 2000). These fungi are part of the ecosystem and are found in high concentration and broad dispersion, in view of their great capacity of colonizing different substrates and growing in extremes environmental conditions; however, many fungi eventually can cause diseases in human beings, animals and vegetables (Zoppas et al 2011).

According to Backes et al. (2011), fungi that have aerial dispersion are named anemophilous and are the main contaminants of the air. Fungi spores can be inhaled by animals and humans, but rarely they are pathogens in healthy organisms (Souza et al. 2013), because the development of a fungal infection depends on the immune state of the host, the opportunity of interaction between the host and the fungus and the infectious dose. Other factors that can be relevant in the relative frequency the fungi can cause diseases, include the host's age, epidemiological exposures and risk factors.

In Brazil, the reports about infections caused by fungi in marine mammals are relatively rare, when compared with the data about viral, bacterial and helminthic diseases (Reidarson et al. 2001). Nevertheless, it is believed that fungal organisms can contribute, in a significant way, to the mortality of this animals group (Ruoppolo 2003).

Brazil's south coast is characterized by a rectilinear coastline with great wealth of marine fauna that include several species of birds and mammals (Villwock et al. 1984). Among the marine mammals is common the presence of pinnipeds during winter and spring months, when many of them are weakened or, even, arrive dead in the coast, conducted by marine currents (Pinedo et al. 1992, Silva 2014). *Otaria flavescens* (Shaw 1800) is one of these species those occur with high frequency in this region, because the higher concentration out of the reproductive colonies are in the "Refúgio da Vida Silvestre do Molhe Leste da Lagoa dos Patos" and in the "Reserva Ecológica da Ilha dos Lobos", both in Rio Grande do Sul, Brazil (Rosas et al. 1994, Silva 2014). Most of the animals found dead on the beach are non-breeding males, which occupy the extremes of the reproductive colonies (in Uruguay), composed by individuals of different ages or only old individuals, those can achieve a weight of 300 kg (Vaz-Ferreira 1981). Due to the topography and the urban isolation of certain coast areas, there is a high incidence of carcasses of this specie that remain on the sandy sediment until their total decomposition (Pinedo et al. 1992, Silva 2014, Pereira et al. 2012). Such events are supposed to be the cause of spreading pathogenic microorganisms, besides altering

the structure of fungal colonization in the sediment; therefore, could act as probable transmitters of diseases to the beach's visitors (Sabino et al. 2014). Thereby, zoonosis researches about marine animals are important to the health knowledge of populations, as well as, to determine ways that protect the public health through the understanding of possible transmission mechanisms of these diseases.

In literature, data about the microbial microbiota on the surface body of *O. flavescens*, that comes to Brazilian coast, are not available, as well as the microbiota that colonizes *O. flavescens* after its death and its decomposition.

This study aimed to verify the fungal microbiota of thanatocenotic elements of south sea lion, collected in south beaches of Brazil. In addition, we intended to identify the fungi in the sandy sediment and verify whether there was contamination with fungi from the carcass in decomposition.

Materials and Methods

Superficial samples were analyzed in 10 carcasses of *O. flavescens*, found during systematic and weekly field trips in the area between Torres 31°21'38''S and Arroio do Chuí (33°44'35"S), in Brazilian south coast. The studies carcasses were in mild to moderate state of decomposition (level 2 and 3 according modified protocol from Geraci et al. 2005) and the studied carcasses were next to the humid zone of the beach, excluding partial or total submerged carcasses.

From each carcass was collected four microbial samples, using a sterile swab, corresponding to four regions of the body: mouth, anus, back and anterior flipper (thoracic member). The swabs were stored in isothermal boxes, the material was seeded by the streaking methodology in Potato Dextrose Agar (PDA) and incubated in bacteriological incubator at 25°C/ 7 days. Around each carcass, 4 samples of superficial sandy sediment (maximum 5 cm of depth), each sample with 225 cm^3 of volume. The sediment samples were strategically collected at 10 cm of distance of the following points: mouth, anus, right, and left anterior flippers. Of these samples, aliquots of 25 g of sediment were diluted in 250 mL of peptone water 0.1 % and submitted to a serial dilution, in base 10, until dilution 10^3. Volume of 100 µL of each dilution were seeded in the culture medium Potato Dextrose Agar (PDA), incubated in bacteriological incubator, at 25°C, during 7 days. For a control, seven samples of the humid sandy sediments were collected without the presence of the carcasses, similarly to the sand collected around the carcasses.

Both body surface samples and sandy sediment submitted to fungal colonies identification. The identification was based in their macro and micro morphological characteristics until the taxonomic level of genus.

Results

A total of 87 samples were analyzed including the body surface, sediment around each carcass and controls. From carcass samples were identified 5 genera of fungi: *Trichoderma* spp., *Aspergillus* spp., *Penicilium* spp., *Cladosporium* spp. and *Fusarium* spp. (Figure 1) The filamentous fungi *Trichoderma* spp. and *Aspergillus* spp. were present, respectively, in 70 % and 80 % of the carcasses, observed in all points of the body. *Penicillium* spp. was identified in 20 % of the carcass, present in flippers and back, and while *Cladosporium* spp. and *Fusarium* spp. in 10 %, respectively, in mouth and flippers (Figure 2).

In the sandy sediment, samples around the carcass were present 4 genera of fungi, *Aspergillus* spp., *Fusarium* spp, *Penicilium* spp and *Cladosporium* spp. Of these fungi, *Aspergillus* spp. and *Fusarium* spp. were present, respectively, in 80 % and 50 % of the sediment samples in all evaluated points (mouth, anus and flippers). Additionally, *Penicilium* spp. was identified in 20% of samples from mouth and anus, and *Cladosporium* spp. in 60 % of samples around mouth and flippers (Figure 3).

Of the control samples were isolated the following fungi: *Aspergilus* spp., *Rhizopus* spp., *Paecilomyces* spp., *Alternalia* spp. and *Cladosporium* spp. (Figure 1).

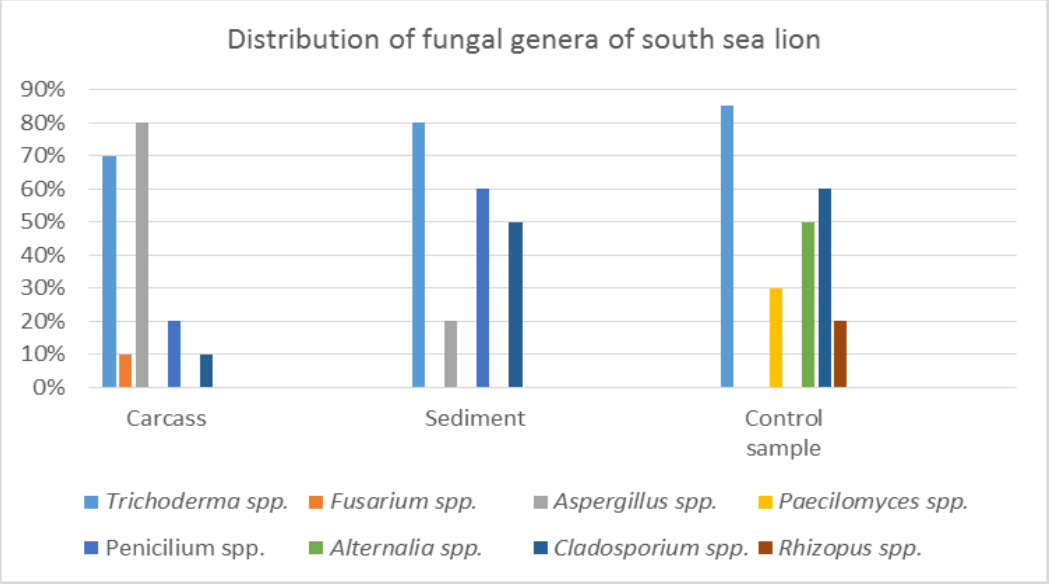

Figure 1: General distribution of fungal genera of south sea lion (*O. flavescens)* carcass, (n=10, 40 samples), sandy sediment around carcass (40 samples) and control samples (7 samples), in Southern Brazil.

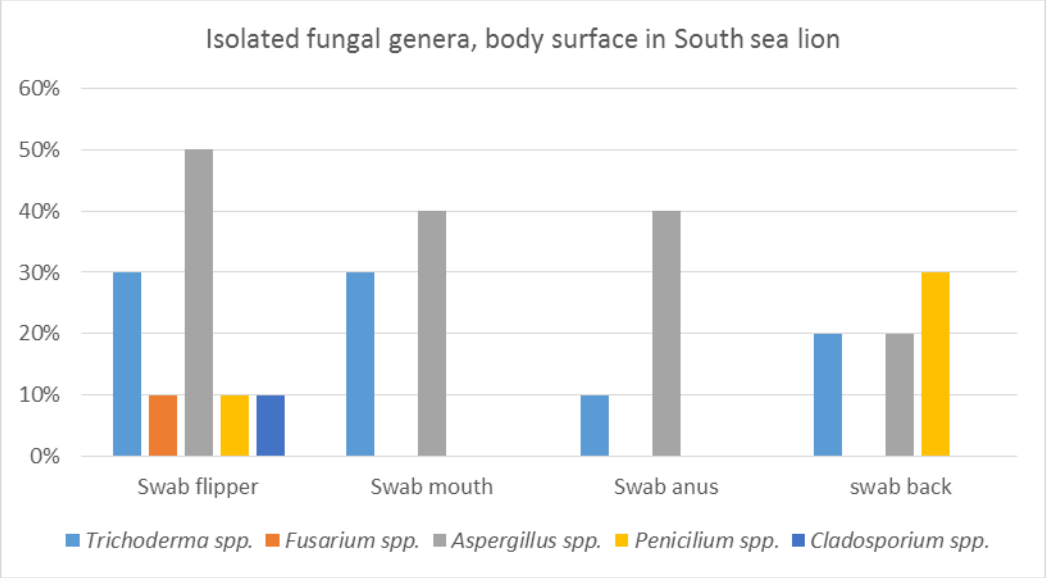

Figure 2: Isolated fungal genera, according body surface regions in South sea lion (*O. flavescens*) in decomposition (n=10, 40 samples) in Brazilian south coast.

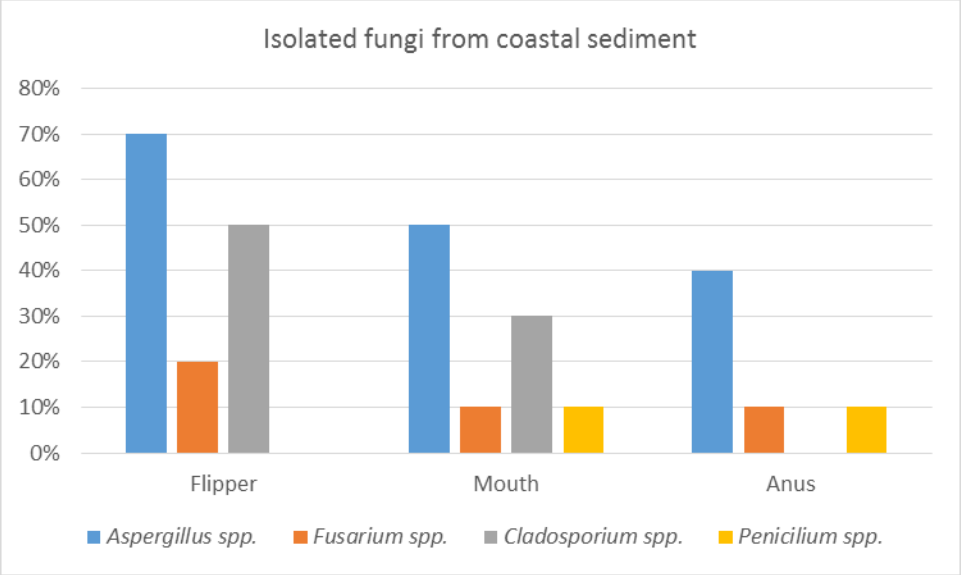

Figure 3: Isolated fungi from coastal sediment (40 samples) around carcass near to collection points of body surface of south sea lion (*O. flavescens*) (N=10), in decomposition, in Brazilian south coast.

Discussion

Few studies were performed about isolated fungi from pinnipeds. The fungal microbiota of the surface body of these animals (live or dead) is not known and little information about fungal infections in marine mammals is available in literature (Reidarson et al. 1998). On the other hand, many studies performed in this animal group use carcasses of animals; those die or arrive dead in the beaches, where there is a frequent contact with this material by the researchers (Pinedo et al. 1992, Rosas et al. 1994, Dhemer et al. 1998, Pereira et al. 2013). It is important to emphasize, that fungal organisms can contribute, in a significant manner, to the mortality of the marine mammals (Reidarson et al. 2001), can be, also, a source of contamination to humans.

Among marine mammals, the pathogenic fungi found belong to the genera, *Blastomyces*, *Candida*, *Coccidioides* and *Histoplasma*, however, only some endemic fungi are able to promote the infection in health individuals, and these fungi include *B. dermatitidis*, *C. immitis* and *H. capsulatum* (Reidarson et al. 2001).

In this, present study, *Cladosporium* spp and *Aspergillus* spp. were present in the carcass samples, in the sediment samples and in the control, suggesting the marine origin of these microorganisms, not exclusively associated with the presence in the carcass. *Cladosporium* spp. has more than 50 species; many species are cosmopolites and high

concentrate in the atmosphere, colonizing several environments and substrates, including the marine environment (Menezes et al. 2006, Zoppas 2011).

The genus *Aspergillus* spp. is responsible by aspergillosis, an opportunistic infection, severe in immunocompromised individuals (Lacaz et al. 1998, Sales, 2009). Species of this genus are found in the soil and organic matter in decomposition (Martins et al. 2005). Their spores are dispersed by the wind, the most common way of infection (Sales 2009). According to Beisswenger et al. (2012), the spores inhalation can cause pulmonary diseases as allergic bronchopulmonary aspergillosis and invasive aspergillosis, the symptoms vary from local inflammation of airways to severe infections, characterizing this pathology as an opportunistic mycosis for excellence (Carrasco et al. 2001, Fowler & Cubas, 2001). The aspergillosis manifestation is relatively uncommon to mammals, however, there are reports of the disease in dogs (Tell 2005), in felines (Tell 2005, Bentubo et al. 2006), in equines (Zangirolami Filho et al. 2008), in bovines (Juffo 2010) and in primates (Ávila et al. 2004).

Aspergilosis is one of the main causes of mortality in birds (Tell 2005) and in the marine environment is a problem, mainly in weakened birds. Cabana et al. (2012) reported aspergillosis in Magellanic penguin (*Spheniscus magellanicus*) in rehabilitation. Penguins, among other birds, are particularly susceptible, in spite of, this mycosis barely attack these marine birds in free life (Xavier et al. 2011). Aspergilosis is considered the fungal disease more frequent in captivity, corresponding to near 30 % of the mortality causes of these birds in rehabilitation centers and zoos (Abundis-Santamaria 2003, Carrasco et al. 2001, Osório et al. 2005). *Aspergillus* sp. is the organism more frequently isolated in mycotic pneumonia cases in marine mammals (Reidarson et al. 1998). The identification of this fungus in the carcass and sandy sediment reaffirms its regional distributions and the frequent infection in marine weakened birds, shows its opportunistic feature and infective capacity (Tell, 2005).

It is believed that the contamination of the carcasses by *Trichoderma* spp. can be originated by the tidal washes on them, once this genus is often found in the marine environment (Ortega et al. 2009) and citations of pathologies in pinnipeds, associated to this genus, are not reported (Reidarson et al. 1998). The exclusive presence in *O. flavescens* carcasses, as well as the differences observed in the composition of fungal communities in the sandy sediment, when compared to the control, it is sugge sted a possible suppressive action on other genera, as it was also observed by Yiying Liu et al. (2016).

According to Schoenlein-Crusius et al. (2004) in polluted waters, the incidence of soil fungi is high. Silveira et al. (2013) described in waters of the same region, where this present study was developed, 12 genera of fungi. *Aspergillus* and *Penicillium* were the more frequent

fungi. These genera were also dominant in the study of the fungal diversity conducted by Gomes et al. (2008) in the Brazilian northeast; those were associated with high salinity and alkaline pH waters. *Penicillium*, *Aspergillus* and *Trichoderma* were also isolated from sandy sediments of impacted urban beaches (Silva et al. 2003), which corroborate with the enhanced colonies growing by the supplementation of carcass organic matter.

Among the genera found in this present study, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicilium*, *Rhizopus* have already been identified in the region, and indicated with potential to cause mycotic and allergic pathology in humans (Bernardi e Nascimento, 2005). The genera *Rhizopus* spp, *Alternalia* spp and *Paecilomyces* spp., considered cosmopolites fungi, in this study were identified only in the control samples and, therefore, not related with the presence of the carcass in the sand. If on the one hand, the identified fungal genera are anemophilous, microorganisms frequently found in the environment and not considered primary pathogens, on the other hand, they are known to cause allergic processes and severe opportunistic infections in humans. Filamentous fungi as *Fusarium* spp. and *Aspergillus* spp. can cause diverse clinical manifestations, all determined by the immune response of the host. Thus, when the immune system is weakened, the probability to occur infections caused by *Aspergillus* spp. enhances, due to its opportunistic nature (Sales, 2009).

The present study is pioneer for *O. flavescens*, whose carcasses remain on the coast of the southern coast of Brazil, until their total degradation. It is believed that the fungal genera isolated from the carcasses come from the environment, those colonize the body surface of the animals, during their stay on the coast. In addition, it was verified that the carcasses do not carry fungi of zoonotic potential, except for immunologically depressed or debilitated individuals.

The structure of the fungal community of the sandy sediment seems to undergo alterations, due to the contribution of biomass, which contributes to the greater development of some genera and suppression of others. In general, the fungal community found, is opportunistic, and due to it is an environment vulnerable to the presence of several groups of fungi, the need for monitoring is evident, due to not only the presence of sea lion carcasses, but also the presence of carcasses of other species, and those remain on the coast during degradation.

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5.3. Capítulo 3

Nota científica

Otaria flavescens thanatocenotic elements of in sediments: would be an important source of environmental contamination by parasite eggs?

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Abstract

Bodies of animals in decomposition on coastal sediment of beaches represent risk to public health. This work has as aim to verify if copses of South American sea lion in decomposition at beach may be an important source of parasite eggs contamination. Were studied 10 carcasses of *O. flavescens* in mild to moderate state of decomposition, found during systematic and weekly field trips in Brazilian south coast. Carcass faeces samples were aspirated syringes, through the anus and rectum. A total of 40 sandy sediment samples around carcasses were analyzed. Additionally, 34 samples of faeces of live south sea lions were studied. Samples were processed using standard parasitological techniques. From faecal samples of live animals, immature females *of C. australe* were found. Helminthic or protozoa eggs were not found in none sample, neither in carcass nor in sandy sediment. This work points out that, in spite of *O. flavescens* show high helminthic infections, in the marine environment, the biotic and abiotic events, those occur since the death of the animal until the moment the carcass is deposited on the beach, contribute to the carcass does not be an expressive source of parasitic eggs dispersion, which may endanger public health.

Key-words: parasite eggs, South American sea lion, environmental contamination, public

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Introducion

The *Otaria flavescens* (Shaw, 1800), is a marine mammal species often found in beaches from South Brazil (Silva, 2004). The strategic location of the artificial rock barrier

($32^{0}11'S$ $-52^{0}04'W$) that protects the canal of access to Lagoa dos Patos in Rio Grande city,(Rio Grande so Sul State, Brazil) is an important rest point of adult animals (Santos et al.,1996). Although live specimens rarely are found in the beach, to the south and to the north of this canal, is common the presence of thanatocenosis on the coastal sediment, which usually remain until their total decomposition (Pereira et al., 2012). Based on the number of animals' corpses found in the beaches, Silva (2014) estimated the annual of mortality from 80 to 181 animals in South Brazil. Some beaches where carcasses are present are not inhabited, but others, such as Cassino beach, Hermenegildo and Praia do Mar Grosso are tourism references during the summer, with a great access of bathers.

Animal thanatocenotic elements represent an enormous risk to public health, since the degradation and recycling processes promoted by the decomposer invertebrates and microorganism's colonization until the total disappearance of organic wastes (thanatocenosis) are innumerable (Blauzius et al., 2006; Matesco et al., 2006).

Recently, studies in faecal samples of urban populations of *O. flavescens* in Chilean coast, revealed the zoonotic potential of several helminths, those could endanger public health (Hermosilla et al., 2016).

Gastrointestinal assemblages of helminths of *O. flavescens* in South Brazil coast are composed by *Corynosoma australe* (Acanthocephala: Polymorphidae), *Bolbosoma turbinella* (Acanthocephala: Polymorphidae), *Contracaeucum ogmorhini* (Nematoda: Anisakidae), *Stephanoprora uruguayense* (Digenea: Echinostomatidae), *Diphyllobothrium* sp. (Cestoda: Diphyllobothriidae) and *Ascocotyle (Phagicola) longa* (Digenea: Heterophyidae). Parasite load, mainly by the acanthocephalan *C. australe* (Johnston 1937), which showed 100 % of prevalence is as high as 1,756.04 worm by infected host (Pereira, 2012).

Due to the fermentative action of the *post mortem* process, elimination of retained faeces in the rectum, through the anus, can occur in consequence of the increase of pressure in

abdominal and pelvic cavities, spreading these faeces in the environment (Ferguson, 2010). This work is part of a broad project that analyzes the environmental contamination produced by carcasses of *O. flavescens* in the beaches sandy sediment, and aimed to investigate the existence of parasite eggs or, even, helminths in the sand around carcasses. Additionally, faeces of live sea lions in a sea lions rest point close to the beaches were examined as control samples.

Were studied ten carcasses of *O. flavescens* found during monitoring of the sandy beach strip, which extends to the north of the Molhes da Barra, with limit in the Lagoa do Peixe ($31^0$21'S-$51^0$02'W), and to the south of the same point, with limit in the beach of Hermenegildo, Santa Vitoria do Palmar, RS ($33^0$45'S-$53^0$23'W). Only fresh carcasses or with mild decomposition state, level 2 to 3, were used, according protocol adaptation of Geraci & Lounsbury (2005).

Carcasses faeces samples were aspirated with 50 mL syringes, through the anus and rectum. When the faeces were not present, a rectum washing with AFA (Alcohol-Formaldehyde-Acetic acid) was performed for further microscopic examination.

A total of 40 coastal sediment samples, comprising four points around each carcass were collected (e.g.: 5 cm), each one with 225 cm^3 of volume. The samples were collected strategically at a distance of 10 cm of the following points: mouth, anus and right and left anterior flippers. The samples were processed using the technique of Caldwell & Caldwell (1995) modified by Corrêa (1995) to the helminth eggs and larvae, and oocysts of protozoa research.

Additionally, 34 samples of faeces of live south sea lions deposited on the natural break water rockies (tetrapodo) in the rest point located in the Chuí artificial rock barrier ($32^0$11'S -$52^0$04'W) were collected, during two expeditions in November 2016.

The faecal samples of live and dead animals were processed, when it was possible, using the following parasitological techniques: Gordon & Whitlock technique (1939) to qualitative and quantitative diagnosis of eggs from gastrointestinal nematodes with the flotation principle. Faust technique (1938) aiming to diagnose protozoan cysts and oocysts, through centrifugal faecal flotation principle with zinc sulfate, and Sheather technique (1923) to diagnose protozoan cysts and oocysts, through centrifugal faecal flotation principle with saturated sugar solution.

The remaining content of each sample was washed in a 150 µm aperture sieves and analyzed in an optical stereomicroscope (5-40 X), in order to research helminths.

Results and discussion

The faecal analysis of live animals revealed the presence of organic fragments of food residues, such as bone, fish vertebrae, crystalline and otoliths. In only two samples, were observed the presence of *C. australe* immature females. Helminthic or protozoa (oocists) eggs were not found.

In samples of faeces, collected from the rectum of carcasses, were not found parasite eggs, as well as in the samples of sandy sediment around the carcasses. The absence of helminthic eggs in the samples of dead animals raises some questions that could be responsible for such results. The first point refers to the faeces volume, in the rectum, before the animal death. The samples varied from 1 to 6 mL, with dilution when the volume was low, while in the coprological techniques the recommended amount of faeces is around 5-10 g of faeces (De Carli, 2007). The death causes were not investigated, but according to Silva (2004) most of the South American sea lions dies due to the interaction with local fishing and, frequently, the animals have content in their digestive tracts. Other causes associated to

infectious pathologies, those could lead the animal to inappetence prior to death, are little known (Pereira et al., 2007).

The low faeces volume decreased the probability of finding parasites eggs, even so it is surprising that no egg has been found, once the studies in feacal samples of sea lions and fur seals in rehabilitation, in the same geographical region, showed mainly ascarids and acanthocephalan eggs (Jacobus et al., 2016). Therefore, the low volume of faeces could be too the responsible factor for the absence of eggs in the sandy sediment. Another factor to consider is the influence of the constant sea washings, by the waves, in the time interval from the death until the deposit of the carcass on the beach. In case the faecal ejection has occurred in total water immersion, certainly, the faecal mass would be diluted with posterior helminth eggs flotation, due to the saline concentration of the water, as simulated by the Willis-Mollay (1921) coprological technique. For that reason, would be more probable occur the beach contamination, when the death of the animals occurs on the sandy sediment.

Lastly, an intermittence of postures of helminths could happen not coinciding with the moment of samples collection, eggs degradation due to the elapsed time since the death until the collection or, still, a different strategy of helminths dispersion, not the massive release of eggs in the faeces of its host. Due to the low number of samples, there is a probability, even if low that the posture of the helminths did not happen in the moment before the death of the specimens and, then, the absence of eggs in the faeces can be explained. Once the faecal samples of the live animals were collected directed by environment, helminthic and protozoa eggs could suffer dehydration and lysis, because of solar exposition and partial dryness of the material.

On the other hand, some acanthocephalans species, such as *Acanthocephalus dirus* shows particular strategies to the dispersion of infrapopulations, being the main way of dispersion the direct elimination of the pregnant females outwards, with the faeces (Kopp et

al., 2011). This strategy is effective in order to the intermediate hosts can ingest a great number of eggs at one time, preying directly the female, that presents a whitish yellow globular body visible and attractive to crustaceans, those are part of the food chain (Zdzitowiecki, 1984). The dispersion strategies of Polymorphidae members, such as *C. australe*, were not studied, however, the presence of females, even if immature in live animal faeces suggests a new hypothesis about this topic. Studies on southern elephant seal, *Mirounga leonina*, in Antarctica reinforce this hypothesis, since entire gravid *Corynosoma* females, were present in the faeces (Silveira et al., 2014).

This work points out that, in spite of the American south sea lions show high helminthic infections, in the marine environment, the biotic and abiotic events, those occur since the death of the animal until the moment the carcass is deposited on the beach, contribute to the carcass does not be an expressive source of parasitic eggs dispersion, which may endanger public health. On the other hand, this study generates a new hypothesis regarding the dispersion process of the acanthocephalans of the genus *Corynosoma*, which is also the elimination in the faeces of intact females, containing a larger number of eggs, allowing the ingestion of these females by the intermediate hosts.

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6. Considerações finais

1. Carcaças de *O.flavescens* em leve ou moderado estado de decomposição junto ao sedimento praial, apresentam uma colonização por bacterias anaeróbicas, com presença de espécies tipicamente marinhas e outras associadas ao ambiente oxidante. Dentre estas bactérias estão espécies com potencial para infectar seres humanos, tanto nas carcaças como nas areias ao redor das mesmas.

2. A comunidade de fungos que coloniza a superficie do corpo de *O. flavescens* após a morte, é oportunista, com destaque para alguns gêneros que podem infectar o homem ou animais com o sistema imunológico deprimido, causando enfermidades.

3. Apesar de espécimes de *O. flavescens* apresentarem altas infecções por helmintos no trato digestório, carcaças que permanecem na praia não são importantes meios de veiculação de ovos de parasitos (helmintos ou protozoários) que possam colocar em risco a saúde pública.

4. Dado que tanto a colonização bacteriana como a fúngica apresentam potencial infectivo ao homem, é essencial o uso de equipamento de proteção individual (EPI) ao lidar com as carcaças.

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Anexos

8. Anexos

Artigo 1.

Revista: Envinmental Microbiology Reports

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• microbial communities: structure:function relationships and communal behavior
• microbial interactions and interactions with plants, animals and abiotic factors, including systems analysis of interactions and their component networks
• pathogen ecology and environmental epidemiology
• genomics, functional genomics, environmental genomics/metagenomics, bioinformatic analyses and comparative genomics
• responses to environmental signals and stress factors
• primary and secondary production
• element cycles and biogeochemical processes
• microbial physiological, metabolic and structural diversity
• extremophiles and life in extreme and unusual little-explored habitats
• pollution microbiology
• microbially-influenced global changes
• growth and survival
• microbes and surfaces, adhesion, biofilm biology, biofouling
• population biology and clonal structure
• microbial community genetics and evolutionary processes
• modelling and theory development
• new technological developments in microbial ecology, in particular for the study of activities of microbial communities and of non-culturable microorganisms

Interdisciplinary studies of fundamental problems are particularly appropriate.

Article Types

The types of article published in both Journals reflect the Editors' endeavour to actively promote the field of environmental microbiology, the broad scope of the subject, and the impact of socio-political, health, nutritional and economic issues and developments. Thus, in addition to the principal content of full-length (*EMI*) and compact (*EMIR*) research papers, issues may include Editorials/Opinions, Minireviews, Web Alerts, and Correspondence (general, scientific). The importance of genomics to the field is recognized by the Genomics Update feature.

Research Papers and Brief Reports

These report new original findings that significantly advance the field of environmental microbiology/microbial ecology. A principal criterion for judging the potential acceptability of a paper is that the advance reported places it in the top 10 % of research in the field. Papers may either be Full Papers or Brief Reports. In both cases, the work must be complete (preliminary communications will not be considered) and represent the indicated level of originality and accomplishment. Research papers documenting major studies should be submitted to *EMI*; Brief Reports reporting significant research in compact form should be submitted to *EMIR*. In all cases, manuscripts should be as concise as permitted by good scientific reporting. Essential material (e.g. primer sequences; strain lists, etc.) not crucial to an understanding of the main findings should be submitted as Supplementary Information.

The ultimate decision of whether a submission may be published as a Research Article or Brief Report is not dictated by the length of the initial submission, but rather by the length considered by the reviewers and Editor to be appropriate for the significance of the advance described and for its proper scientific documentation.

Minireviews

These bring to the attention of our readership exciting new developments and/or concepts in a timely fashion. They are selective in scope, focused and concise, rather than being comprehensive or historical, and may be somewhat speculative, if this is likely to provoke interesting discussions and stimulate new lines of creative experimentation. There is no strict format for minireviews, but they should include a Summary, Introduction, and Concluding Remarks, which bracket the main text. Literature citations should be balanced but not exhaustive

Most, but not all, minireviews are invited; authors wishing to submit a minireview should first contact the Minireview Editor, Juan Luis Ramos, to ascertain whether or not their topic is appropriate.

Correspondence

Submissions should contribute to discussions of topical issues in or impacting environmental microbiology, advance new hypotheses or provide new interpretations of existing hypotheses.

Exclusivity, copyright and author ethical obligations

Papers must be submitted exclusively to *EMI* or *EMIR* and are accepted on the understanding that they are entirely original and have not been, and will not be, published elsewhere. If accepted, authors will retain copyright of the article, and grant the Publisher the exclusive right to publish.

In submitting a manuscript to *EMI*/*EMIR*, the corresponding author must explicitly state in a cover letter that the content and authorship of the submitted manuscript has been approved by all authors, and that all prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected. Authors are reminded of the need to avoid plagiarism, and are strongly advised to employ one of the available plagiarism detection softwares. Failure to respect ethical norms may result in imposition of sanctions.

Conflict of Interest

EMIR requires that all authors disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise, that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. The existence of a conflict of interest does not preclude publication in this journal.

If the authors have no conflict of interest to declare, they must also state this at submission.
It is the responsibility of the corresponding author to review this policy with all authors and to collectively list in the cover letter to the Editor, in the manuscript (under the Acknowledgment section), and in the online submission system ALL pertinent commercial and other relationships.

Pre-submission English-language editing

Authors for whom English is a second language may choose to have their manuscript

professionally edited before submission to improve the English. A list of independent suppliers of editing services can be found **here**. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Presentation of manuscripts

Authors should ensure that manuscripts submitted online have line numbers.

Lengths of printed articles

Papers should be focused and succinct, and as concise as possible consistent with clarity and good scientific reporting practice. Material essential for the paper, but not for an understanding of the advance reported, such as lists of primer sequences, strain characteristics, or material too large to be part of the main text (such as multimedia adjuncts, large data sets, extra colour illustrations, bibliographies, or any other material for which insufficient space in the journals is available) etc., must be submitted as Supporting Information, for publication online. The lengths of printed articles will generally not exceed the following: Research Articles: 10 printed pages (approx. 20 double line-spaced manuscript pages with 5 displays); Brief Reports: 5 printed pages; Minireviews: 6 printed pages; Correspondence: 2 printed pages. However, longer manuscripts will be considered if the extra length is dictated by good scientific reporting practice.

Supporting information

Supporting Information can be a useful way for an author to include important but ancillary information with the online version of an article. Examples of Supporting Information include additional tables, data sets, figures, movie files, audio clips, 3D structures, and other related nonessential multimedia files. Supporting Information should be cited within the article text, and a descriptive legend should be included. It is published as supplied by the author, and a proof is not made available prior to publication; for these reasons, authors should provide any Supporting Information in the desired final format. For further information on recommended file types and requirements for submission, please visit: http://authorservices.wiley.com/bauthor/suppinfo.asp.

Title page. The title page should include: (1) a concise informative title for the work reported, (2) the names of all authors and their affiliation(s) where the work was conducted, (3) the name, full postal address, telephone and fax numbers, and email address of the corresponding author (one only), to whom all correspondence and proofs should be sent and (4) a running title of not more than 50 characters. Different current addresses of authors should appear as a footnote. Titles encapsulating the main advance ('Methylation is the initial reaction in anaerobic naphthalene
degradation') are prefered over less informative general titles ('A study of . . .' 'Characterization of . . .' 'Diversity of . . .').

Summary. All papers must include a summary, not exceeding 200 words, that succinctly describes the principal findings of the work. Background information, and descriptions of what was done and how, should be avoided as redundant, unless essential to an understanding the findings, and then should be restricted to a sentence or two.

The main text. For Research Articles, the main text should be subdivided into Introduction, Results, Discussion, Experimental Procedures, Acknowledgments, References, Table and Figure legends. The Results and Discussion sections may be combined and can include additional subheadings. New or unfamiliar experimental procedures should be described in sufficient detail to enable the experiments to be reproduced. Well documented procedures should be adequately cited. For Brief Reports, Results and Discussion are combined, there is no section on Experimental Procedures, and essential experimental details should be incorporated into the corresponding figure and table legends. The preferred position of tables and figures should be indicated at the appropriate places in the manuscript. Footnotes should be avoided.

Format

Text

The text should be formatted double-spaced, typed consistently [e.g. care taken to distinguish between '1' (one) and 'l' (lower-case L), '0' (zero) and 'O' (capital O), etc.] with no hyphenation and automatic word-wrap (no hard returns within paragraphs), and line and page numbered consecutively.

References

Authors should use the system of citing references illustrated below. Only full articles that have been published or are 'in press' may be included in the reference list. In the text, unpublished or submitted studies should be referred to as such (e.g. J. M. Smith, unpublished), or as a personal communication. It is the author's responsibility to obtain permission from colleagues to include their work as a personal communication. In the text, references should be inserted in parentheses in date order, as follows: (Pugsley, 1996; Matsunaga *et al.*, 1997). The reference list should be in alphabetical order according to the first-named author. Papers with two authors should follow those of the first-named author, arranged in alphabetical order according to the name of the second author. Articles with more than two authors should follow those of the first named author in chronological order; with multiple references from the same first author in a given year, please list the references in cited order. The title of the article must be included. For papers with up to seven authors, the names of all authors should be listed. For papers with eight or more authors, the first six names should be listed, followed by '*et al.*'. Standard abbreviations of journal titles should be used, as in the Index Medicus.

The following provide examples:

Sahm, K., MacGregor, B.J., Jørgensen, B.B., and Stahl, D.A. (1999) Sulphate reduction and vertical distribution of sulphate-reducing bacteria quantified by rRNA slotblot hybridization in a coastal marine sediment. Environ Microbiol 1: 65-74.

Madigan, M.T., Martinko, J.M., Dunlap, P.V., and Clark, D.P. (2008) Brock Biology of Microorganisms, 12th edn. New York, USA: Pearson Higher Education.

Finlay, B.I., Fenchel, T., and Embley, T.M. (1993) Methanogen endosymbiosis in anaerobic climates. In Trends in Microbial Ecology. Guerrero, R., and Pedros-Alio, C. (eds). Barcelona: Spanish Society for Microbiology, pp. 285-288.

References to material available on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Authors will be asked to provide electronic copies of the cited material for inclusion on the journal websites at the discretion of the Editors. The format for citations is as follows:

Beckleheimer, J. (1994). How do you cite URLs in a bibliography? [WWW document]. URL

www.nrlssc.navy.mil/meta/bibliography.html.

Nevertheless, as web site content and addresses are constantly changing, authors are discouraged from citing key information available only on the web.

Mathematics

In-line equations should be typed as text. The use of graphics programs and 'equation editors' should be avoided. Displayed equations are rekeyed by our typesetter.

Tables

Tables should be typed as text, using either 'tabs' or a table editor for layout. Do not use graphics software to create tables.

Figures

Please supply high quality digital versions of figures, preferably in EPS or TIFF format. TIFF files should not be produced by transferring images from a previous Powerpoint file, as this results in major loss in resolution. Photomicrographs should include a scaled bar and indicate the size (descriptions of magnification alone are not sufficient). Submitted photographic images should be scaled to publication size and must have an image resolution of 300 dpi or greater in TIFF format.

Annotated photographs, line graphs and bar charts should be generated in EPS format for best quality of reproduction. For more detailed guidelines, please refer to http://media.wiley.com/assets/7323/92/electronic_artwork_guidelines.pdf.

Provide methodological details on image acquisition and image processing, including software and operations such as colourizing and other modifications.

Authors are reminded that it is not acceptable scientific conduct to modify any separate element within an image. Sometimes adjustments of the entire image in brightness, contrast and colour balance are justified if they do not misrepresent the original, observed data. Composite figures composed of grouped images such as insets from different fields or separate parts of gels must be explained in the figure legend and differentiated by use of dividing lines or other means to make composites unambiguous.

Please ensure that electronic artwork is prepared such that, after reduction to fit across one or two columns or two-thirds page width (80 mm, 169 mm or 110 mm respectively) as required, all lettering will be clear and easy to read, i.e. no labels should be too large or too small.

Avoid using tints if possible; if they are essential to the understanding of the figure, try to make them coarse. No artwork should be incorporated into the text files. In the full-text online edition of the journal, figure legends may be truncated in abbreviated links to the full-screen version. Therefore, the first 100 characters of any legend should inform the reader of key aspects of the figure.

Authors are encouraged to use colour displays, where appropriate:

Cover Photographs

The Editors welcome proposals of images related to the content of submitted papers that may be suitable for the covers of *EMI* and *EMIR*. If such an image is accepted and used as a cover, a free pdf offprint of the cover will be provided to the author.

Conventions

Abbreviations

Standard abbreviations should be as recommended in Quantities, Units, and Symbols (The Royal Society, 1988). Abbreviations of non-standard terms should follow, in parentheses, their first full usage.

Genetic nomenclature

Standard genetic nomenclature should be used. For more detailed information, authors should consult Bachman (Microbiol Rev 47: 180-230, 1983) for E. coli K-12; Sanderson and Roth (Microbiol Rev 47: 310-453, 1983) for Salmonella typhimurium; Holloway *et al.* (Microbiol Rev 43: 73-102, 1979) for Bacillus subtilis; Perkins *et al.* (Microbiol Rev 46: 426-570, 1982) for Neurospora crassa; and the Handbook of Genetics Vol. 1 (R. C. King, ed., Plenum Press, 1974) for Saccharomyces cerevisiae.

Restriction enzymes

EMI and *EMIR* have adopted the revised convention of naming restriction enzymes without italics. Previous designations like EcoRI, KpnI HindIII, SacII, etc., are thus supplanted by EcoRI, KpnI, HindIII, SacII, etc. For more information on the updatedguidelines to naming restriction enzymes, please consult Roberts *et al.* (Nucleic Acids Res 31: 1805-1812).

Submission of Manuscripts

Cover letter

In the cover letter, (a) specify the title and authors, (b) provide a 1-2 sentence description of the advance reported and its significance, and confirm that (c) all of the reported work is original, (d) all authors have seen and approved the final version submitted, (e) all prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected, and (f) consent is given for publication in *EMI*/*EMIR*, if accepted.

Online submission

All manuscripts should be submitted online at http://mc.manuscriptcentral.com/emi. A user ID and password are required and can be obtained on the first use of the site. All file types are supported, but the following types are recommended: text in Microsoft Word or generic rich text format (RTF), figures in high resolution EPS or TIFF. Macintosh users are advised to add the correct three-letter filename suffix.

Reviewers

Authors may nominate up to five referees appropriately qualified to judge the manuscript, though the Editors may or may not ultimately select some of these. Authors may also request that one or two specified persons do not act as reviewers. Should authors feel that they have a significant conflict with another PI, they should explain this in the cover letter and this information may be taken into consideration by the assigned editor.

Manuscript revision and re-submission

There are four basic editorial decisions: Accept, Minor Revision, Reject with a possibility of submission of a new version, and Reject. A Reject decision is definitive and authors may not submit a new version of the manuscript to *EMI*/*EMIR*. A Reject with a possibility of submission of a new version requires a major re-write of the manuscript and/or inclusion of significant new data, and thus the creation of a new manuscript, which will thus be asigned a new submission date. A Minor Revision decision implies that the paper can in principle attain the required standard of the Journal without major change. Editors may or may not have a revised manuscript reviewed (generally, by the original reviewers), in order to ascertain whether changes to the original manuscript adequately respond to the criticisms. If changes

made do not result in a paper of the required standard, the revised manuscript will be definitively rejected; iterative improvements are not permitted. If a revised manuscript is accepted, the original submission date will be retained.

If any part of a study submitted to *EMI*/*EMIR* is resubmitted at a later date, it must be sent to the same handling editor, noting the EM number of the previous version of the manuscript.

Files of re-submitted manuscripts must be supplied editable formats, such as Word, for text and tables (authors should avoid embedding non-editable displays in their texts), and EPS or TIFF formats for figures. Submitted manuscripts containing non-editable files will be unsubmitted and authors will experience unnecessary delays in publication of their papers.

Copyright Assignment

Authors submitting a paper do so on the understanding that the work and its essential substance have not been published before and is not being considered for publication elsewhere. The submission of the manuscript by the authors means that the authors automatically agree to assign exclusive copyright to Wiley Blackwell if and when the manuscript is accepted for publication. The work shall not be published elsewhere in any language without the written consent of the publisher. The articles published in this journal are protected by copyright, which covers translation rights and the exclusive right to reproduce and distribute all of the articles printed in the journal. No material published in the journal may be stored on microfilm or videocassettes or in electronic database and the like or reproduced photographically without the prior written permission of the publisher.

Authors will be required to assign copyright to the Society for Applied Microbiology and John Wiley & Sons Ltd. Copyright assignment is a condition of publication and papers will not be passed to the publisher for production unless copyright has been assigned. (Government employees need to complete the Author Warranty sections, although copyright in such cases does not need to be assigned). After submission authors will retain the right to publish their paper in various medium/circumstances.

Artigo 2

Revista: Aquatic Microbial Ecology

Submission Received

Dear Dr. Eliane Pereira:

Thank you for submitting your manuscript entitled "Fungal microbiology of the body surface of *Otaria flavescens* (Carnivora: Otaridae) >decomposition on sandy sediment " to "Aquatic Microbial Ecology"; the submission number is: 4104.

Your submission will now be checked by the editorial office, and you will receive a confirmation mail from the editorial office soon. This step will also activate your personal user-id and password, enabling you to login to the system to check the status of your manuscript.

If you have any queries please send an email to: ame-ed@int-res.com.

With kind regards,

Editorial Office

Author guidelines

Acceptable manuscript types

Article type	Description	
Research Article	Describing novel research	
Review	State-of-the-art evaluation of important current research areas	
Note	Brief report on new findings not warranting a full Research Article but significant enough for publication	
Comment / Reply Comment	Critical, fair assessment of an article published in the same journal; and a reply to the Comment; see this editorial for details	
Opinion Piece**	Important, personal views on hot topics	

*Total number of words in the manuscript (including title, authors, affiliations, keywords, body text, acknowledgements, legends, tables, and literature cited)
**Previously called 'As I See It'
Theme Sections integrate expert analyses, highlighting an important research area or problem (e.g. a collection of high-quality papers stemming from a research symposium). Please approach us with your ideas for a Theme Section via email to the appropriate Managing Editor.

Submitting your manuscript

A submitted manuscript must:

- not be simultaneously submitted to any other publication outlet
- be original, i.e. not previously published whole or in part (including different languages). Inter-Research may check submissions using anti-plagiarism software
- have been approved by all immediately involved, e.g. authors, institutional authorities
- meet ethics guidelines (i.e. institutional, national and international guidelines concerning the use of animals in research, sampling of endangered species, and ethics in publication). All conflicts of interest must be declared.

If the manuscript was previously submitted to a publication outlet, this must be disclosed and a rationale for its current submission provided. Submitting a manuscript implies agreement to Inter-Research (IR) terms of publication related to Open Access, copyright and manuscript processing. Please submit your manuscript using the **online manuscript submission system** (click on the appropriate journal):

MEPS | AB | AEI | AME | CR | DAO | ESEP | ESR

In the unlikely event that you have problems, please email the submission to the **editorial office** of the journal (click on the appropriate journal):

MEPS | AB | AEI | AME | CR | DAO | ESEP | ESR

The submission must contain 2 separate files:

- a cover letter (see Cover Letter Requirements and Tips) for details
- the manuscript, which must:
    - be correctly formatted to match journal style (see Formatting Requirements and Tips for details)
    - be in one file (it includes all text, with tables and figures at the end)
    - use continuous line numbers throughout
    - be smaller than 5 MB (for accepted manuscripts, higher-resolution figures can be provided later)

Electronic supplements: material unsuitable for inclusion within the article (overlong tables, mathematical derivations, video clips, computer code, etc.) may be published on the Inter-Research website as an electronic supplement (linked to the article and abstract page). This material will not be copyedited, typeset, or checked for accuracy; responsibility for its content and presentation rests with the author(s). Figures and tables should be numbered S1, S2, S3, etc.; if references are used, the supplement must contain its own complete Literature Cited section.

After you submit: Peer-review

Manuscripts are pre-screened to ensure that the submission fits the scope of the journal and that language and formatting requirements are met. Manuscripts sent for review will usually be critically evaluated by 3 reviewers. The Contributing Editor decides on acceptance, revision, or rejection.

If your manuscript is accepted: Next Steps

You will be contacted with a request for appropriate file types if needed (send them to acceptance@int-res.com). You will receive an Acceptance Form that includes a copyright agreement and reader access options (subscriber-only or Open Access) for your article. Our open access fees can be found here. Titles of accepted manuscripts are added within a few days to 'Forthcoming publications' on the journal's web site, together with a prepress abstract and a DOI, making them citable. Proofs are sent a few weeks later. You can order offprints and, if desired, revise your access options when returning the proof. Final articles, fully paginated and citable, are published 1–3 weeks after corrected proofs have been returned by the corresponding author. The corresponding author will be sent a watermarked pdf of their article

Manuscript Formatting Requirements and Preparation Tips

1. Manuscript length

The target length of Research Articles is approximately 10 printed pages (generally about 6000 words of body text). There are additional types of manuscripts that can be submitted with different page/word targets (see Author guidelines).

2. Title page

Title: The title should be concise and informative, i.e. summarizing either the subject or the most important findings of the study rather than merely the hypothesis addressed. It should have around 100 characters (ca. 15 words), and 150 characters at most (including spaces). Avoid 'A', 'An', 'The', 'On', etc. at the beginning.

Provide a **running page head** with 3 to 6 words; e.g. 'Detection of shrimp WSSV'.

Authors and addresses: If a manuscript has several authors from different institutions:

- use superscript numerals for identification;
- provide the address of each author's institution, identifying any present address(es) if applicable. Include zip or postal code but not street address or box number;
- use an asterisk (*) to refer to a footnote that identifies the single corresponding author and provide her/his e-mail.

Key words: Supply 3 to 8 key words, listed in order of importance.

3. Text

Please use continuously numbered pages and lines, 12 point font, and double spacing. Manuscripts that do not use correct English grammar, spelling and punctuation will be returned to authors without review; if you are not a native English speaker, you should have the text edited by someone who is, before submitting your manuscript. You may also wish to consult a 'How to' book such as Day & Gastel (2011; How to write and publish a scientific paper, 7th edn. Greenwood Press, Santa Barbara, CA).

Verbosity: Please eliminate verbiage; example:

Verbose – 'The speed was chosen because past studies by Miller (1995) and Smith (1998) have shown this to be slightly greater than the maximum sustained swimming speed.'

Not verbose – 'The speed is slightly greater than the maximum sustained swimming speed (Miller 1995, Smith 1998).'

Verbose – 'It has been shown that boat noise affects whale behaviour (Smith 1994).' (and similar phrases such as 'it has been reported/found that', 'it is possible/suspected that', 'results show that')

Not verbose – 'Boat noise influences whale behaviour (Smith 1994).'

Genus and species names must be in italics; write the genus name in full at first mention in each section (Abstract, Introduction, Materials and Methods, Results, Discussion) and abbreviate whenever mentioned again in the same section. When referring to a species, do not

use the genus name alone, unless you have previously defined it that way; be precise when using 'sp.' (singular) and 'spp.' (plural).

At first mention in a section – 'The filter feeding of blue mussels Mytilus edulis was examined'.

After first mention in a section – 'Filter feeding rates of M. edulis increased with increasing temperature.'

Sequence data: Full sequence information is required when molecular methods are used. The sequences of novel primers must be given. Novel nucleotide or protein sequences must be deposited in the GenBank, EMBL or DDBJ databases and an accession number obtained.

Ocean acidification data reporting: When presenting methods and results reporting ocean acidification the 'Guide to best practices for ocean acidification research and data reporting' must be followed. Specifics for reporting ocean acidification data in scientific journals are outlined in Annex 1 of the 2015 addendum.

Equations and units: Use standard SI units. Relations or concentrations (e.g. mg per l) must be given as 'mg l–1' (not mg/l). Variables are usually italicised (except for Greek letters). Italicisation should be consistent in normal, superscript and subscripted text. Example of proper spacing: 'p < 0.05, r2 = 0.879' (not 'p<0.05, r2=0.879'); but: 'we studied organisms of size <0.5 µm'

Figures and tables

Please consult Guidelines to Authors on Figure Preparation.

Figures, tables, and their captions should be self-explanatory; e.g. abbreviations and acronyms must be defined here. For table footnotes, use superscripted lower case letters; asterisks can be used to indicate statistical significance (must be defined in the legend).

4. Acknowledgement section

Declare all sources of funding of the study. In addition, you may wish to acknowledge any assistance you received from anyone not listed as author. Include this section before your Literature Cited.

5. Literature cited

All literature used in the text must be listed, and all listed literature must appear in the text, using Harvard (Name-Year) referencing style.

Format required for citing literature (examples):

Periodicals: Use standard abbreviations according to 'BIOSIS Serial Sources' or use the style for your selected journal in a reference managing software. A list of over 30000 journal names and BIOSIS abbreviations can be found here. In addition, Endnote users may download styles for IR journals in this zip file for import into reference managing software.

- Dempster T, Holmer M (2009) Introductory editorial. Aquacult Environ Interact 1:1–5

Books: Write the title of the book in lower case, and give the publisher and place of publication. In the case of book series, give the series editor as well. Example:

- Hanski I (2005) The shrinking world: ecological consequences of habitat loss. In: Kinne O (ed) Excellence in ecology, Book 14. International Ecology Institute, Oldendorf/Luhe

Papers from books, conference reports, symposium proceedings, etc.: Give the title of the chapter, the editor(s) and title of the volume, the publisher and place of the publisher (not the location where the conference was held), and the pages of the chapter. The date cited must be the year of publication (not the year in which the conference was held). Example:

- West TL, Amrose WG (1992) Abiotic and biotic effects on population dynamics of oligohaline benthic invertebrates. In: Colombo G, Ferrari I, Ceccherelli VU, Rossi R (eds) Marine eutrophication and population dynamics. Proc 25th Eur Mar Biol Symp. Olsen & Olsen, Fredensburg, p 189–194

Dissertations: Write the title in lower case, the type of thesis / dissertation (e.g. MS / MSc / PhD), and give the university and its location. Example:

- Eve TM (2001) Chemistry and chemical ecology of Indo-Pacific gorgonians. PhD dissertation, University of California, San Diego, CA

Websites: Permanent databases such as FishBase, GenBank, or climatological sources may be included in the Literature Cited list; the access date must be given. URLs for printed publications also available online may be included with their citations. Example:

- Froese F, Pauly D (2009) FishBase. www.fishbase.org (accessed 13 Jan 2013)

  Other website references should only be cited in the body text.

6. Final checklist

Please cross-check your manuscript using this list. Consult recent IR publications as a general guide for formatting:

- Include page numbers and continuous line numbers
- Ensure that abbreviations are defined at first mention in the abstract, main manuscript Included text and figure/table legends, and that the legends are informative
- Use periods instead of commas as decimal signs
- Correctly present your statistical results (e.g. include two sets of degrees of freedom for ANOVA results and significance/p-values of regressions)
- Cite all of the references in the text and vice versa
- Correctly label your figure axes with a title and a unit where applicable
- Create legible figures: i.e. large enough font size (at least 10 pt) with sufficient resolution for pdf viewing
- Prepare the manuscript (text, figures and tables) as a single file

Anexo 3

Revista: Brazilian Journal of Veterinay Parasitology

INSTRUCTIONS TO AUTHORS

- Introduction
- Editorial Guidelines
- Ethics
- Manuscript Preparation
- Submission of manuscripts

Updated: 07/19/2016

Introduction

Brazilian Journal of Veterinary Parasitology is the official journal of the Brazilian College of Veterinary Parasitology (CBPV). The journal is a quarterly publication that covers topics on helminthes, protozoans, and arthropods as well as other related subjects. Manuscripts can be submitted in English by researchers from any country regardless of CBPV affiliation. The Brazilian Journal of Veterinary Parasitology offers free online access to all its archives dating back to 1992, which was its first year of publication.

Editorial Guidelines

The Brazilian Journal of Veterinary Parasitology accepts articles that focus primarily on animal parasites. Authors are required to send a signed cover letter that states that the entire manuscript is an unpublished original article. If the abstract of the manuscript has been presented in scientific meetings but has not been submitted for publication in other journals, this should be stated in the signed cover letter as well.

Author consent forms for manuscripts that have more than one author are required, to ensure that all authors agree with the publication. All authors should have made substantial contributions to the design of the study, acquisition of the data, analysis and interpretation of the data, and drafting of the article, and should have given final approval of the version to be submitted. Collaborators who did not actively participate in the process described above may be listed in the Acknowledgments section. Researchers who provided technical support or suggestions or a department head who made the research work possible should be acknowledged. Manuscripts with a number of authors that do not seem justifiable may have their experimental research protocol reviewed by our assistant scientific editors.

The manuscript review process will follow the journal's Editorial Guidelines and consider the editors' and/or the Ad hocreviewer'sopinions. The Editor-in-chief and assistant scientific editors may make suggestions or request changes to the manuscript but the authors are ultimately responsible for the entire text content. Articles that are submitted for publication will be reviewed by at least three anonymous reviewers, selected by the editor-in-chief and assistant scientific editors.

Brazilian Journal of Veterinary Parasitology accepts manuscripts as Full Articles, Research Notes, and Review Articles. Review Articles are submitted by experts under the editors' request. Unsolicited review articles will not be accepted but the editors or assistant scientific editors

may accept suggestions for a review topic.

Paper submission:
The articles submitted must undergo English-language revision, done by reviewers accredited by the RBPV (http://cbpv.org.br/rbpv/revisoes_traducoes.php). Likewise, the certificate of English-language revision should be sent together with the submitted article. The authors will be expected to bear the costs of the revision.

The RBPV has no cost for submitting manuscripts.

Publication fee:
After the article has been accepted, the following publication fees will be charged:
R$ 250,00 (associados do CBPV em dia com as anuidades);
R$ 500,00 (não-associados do CBPV).

For foreign authors:

US$ 92.00 (for associates of CBPV who are up-to-date with their membership dues);
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Experiments using animals should be conducted following the Brazilian College of Animal Experimentation guidelines (http://www.cobea.org.br). Articles should include the protocol number approved by the Animal Ethics Committee.

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All articles should be submitted in United States English. Always use concise and impersonal language. Footnotes should be placed at the bottom of the corresponding page and numbered with Arabic numerals in an ascending order. All manuscripts should be typed in Times New Roman font, size 12, page setup with 2.5-cm top and bottom margins, 3-cm left and right margins, and 1.5-cm line spacing. All pages should be numbered. Full Articles should have a maximum of 15 pages and Research Notes should have a maximum of 5 pages in the final layout. All tables and illustrations should be presented separately from the main text body and attached to the final manuscript without captions. The related captions should be included in the

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Description of each item of the manuscript

Original title

The full title and subtitle, if any, should not exceed 15 words. The title should not include any abbreviations, and species names and Latin words should be italicized. Titles that start with "Preliminary studies," "Notes about," and the like should be avoided. Do not use the author's name and date of citation in scientific names.

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List all authors' full name (with no abbreviations). Affiliations should include the original institution names, not their English translations, in the following order: laboratory, department, college or school, institute, university, city, state and country. Include at the bottom of the page the corresponding author information: full address, telephone number, and current e-mail.

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References will only be accepted if they are reader-friendly. References of papers published in conference proceedings will not be accepted and theses only if they are available for consultation at official websites such as the CAPES thesis bank: http://www.capes.gov.br/servicos/banco-de-teses. All cited references in the text should be carefully checked for the authors' names and dates exactly as they appear in the reference section.

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Abstracts are limited to 200 words and should be structured in a single paragraph with no indentation. The abstract should not include references. A**cronyms** or **abbreviations should be written** out in full and the **abbreviation** given in **brackets the first time they** are used in **abstract, f**or example, indirect fluorescence assay (IFA). The abstract should be informative and present the objectives, a brief description of methods, the main results, and a conclusion. All manuscripts written in English should also have the abstract and keywords written in Portuguese.

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Its content should be interpretative and based on the study results only. The discussion can be a single section or it can be presented together with the results and conclusions. It should emphasize the relevance of new findings and new hypotheses clearly supported by the results.

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Tables must be in editable format (e.g., Excel list format) and supplied in separate files. The word "Table" should precede the table title. Tables should be numbered consecutively with Arabic numerals and have a concise and descriptive title placed above them. They should be typed using double spacing and should have horizontal rules separating the header and the last row. The number of tables in the manuscript should be limited to a minimum.

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Reference to book

Levine JD. *Veterinary protozoology.* Ames: ISU Press; 1985.

Reference to book chapter
Menzies PI. Abortion in sheep: diagnosis and control. In: Youngquist RS, Threlfall WR. *Current therapy in large animal theriogenology*. 2nd ed. Philadelphia: Saunders; 2007. p. 667-680.

Reference to full article
Paim F, Souza AP, Bellato V, Sartor AA. Selective control of Rhipicephalus (Boophilus) microplus in fipronil-treated cattle raised on natural pastures in Lages, State of Santa Catarina, Brazil. *Rev Bras Parasitol Vet* 2011; 20(1): 13-16.

Reference to thesis or dissertation
Araujo MM. *Aspectos ecológicos dos helmintos gastrintestinais de caprinos do município de patos, Paraíba - Brasil* [Dissertação]. Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro; 2002.

Reference to internet URLs
Centers for Disease Control and Prevention. *Epi Info* [online]. 2002 [cited 2003 Jan 10]. Available from: http://www.cdc.gov/epiinfo/ei2002.htm.

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Two authors: authors' names and year of publication
Paim and Souza (2011) or (PAIM; SOUZA, 2011)

Three or more authors: first author's name followed by et al. and year of publication
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