UNIVERSIDADE FEDERAL DO RIO GRANDE
PÓS-GRADUAÇÃO EM AQUICULTURA

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Micropogonias furnieri na Lagoa dos Patos: um estudo da relação entre parasitismo, bem-estar de peixes e aquicultura.

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Micropogonias furnieri na Lagoa dos Patos: um estudo da relação entre parasitismo, bem-estar de peixes e aquicultura.

ANA LUIZA VELLOSO

Tese apresentada ao Programa de Pós-graduação em Aquicultura da Universidade Federal do Rio Grande, como requisito parcial à obtenção do título de DOUTOR.

Orientador: Joaber Pereira Jr.

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“Tenho esperanças de que um maior e mais profundo conhecimento do mar,
de que há milênios os homens recebem sabedoria, inspire mais uma vez
os pensamentos e as ações que preservarão o equilíbrio da Natureza e
permitirão a conservação da própria vida."

Jacques Cousteau

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Ao Wagner, por seu amor incondicional.

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RESUMO GERAL

A corvina, *Micropogonias furnieri*, representa um dos recursos pesqueiros mais importantes do Oceano Atlântico Sudoeste e apresenta potencial para aquicultura, a exemplo de outros Sciaenidae cultivados em outras partes do mundo. Um apreciável volume de informações sobre sua biologia está disponível e vários estudos foram desenvolvidos sobre parasitoses em *M. furnieri* no litoral dos estados brasileiros do Rio de Janeiro, Rio Grande do Sul, São Paulo, no Uruguai e na Argentina. Assim, a corvina pode ser um modelo biológico apropriado para o estudo das interações entre parasitismo, bem-estar de peixes e aquicultura. O objetivo deste estudo foi caracterizar a influência do parasitismo por metazoários sobre a corvina no estuário da Lagoa dos Patos, utilizando correlações entre os índices parasitológicos de Prevalência (P) e Intensidade de Infecção/Infestação (II) com índices somáticos: Índice Gônado-Somático (IGS), Índice Hepato-Somático (IHS) e Fator de Condição Relativo (Kn), que são indicadores do bem-estar. Para isso, foram estudadas amostras procedentes de dois ambientes: pré-límnico (mais distante da costa atlântica) e estuarino (mais próximo da costa), pois diferenças destes ambientes poderiam ser refletidas na fauna parasita. Além disso, foi discutida a relação entre parasitismo, bem-estar do peixe e aquicultura. Foi utilizada a definição de bem-estar animal baseada em funções, em que bem-estar significa que o animal está em boa saúde, com seus sistemas biológicos funcionando adequadamente e não sendo forçado a responder além de sua capacidade. No primeiro capítulo, é caracterizada a influência do ectoparasitismo sobre o bem-estar de *M. furnieri*. Para isso, foram necropsiados 181 espécimes de corvina e os parasitos encontrados foram: *Gauchergasilus euripedesi* (Copepoda), *Myzobdella uruguayensis* (Hirudinea), *Neomacrovalvitrema argentinensis* e *Neopterinotrematoides avaginata* (ambos Monogenoidea). A relação entre a abundância parasitária e os índices somáticos do hospedeiro foi verificada, mas nenhuma correlação significativa foi determinada, sugerindo tolerância do hospedeiro à parasitose nos valores encontrados. Além disso, a comparação das amostras nos ambientes pré-limnico e estuarino mostrou diferenças na diversidade e intensidade de espécies parasitas encontradas, sugerindo uma preferência dos parasitos por diferentes salinidades. No segundo capítulo, é estabelecida a relação entre o endoparasitismo e o bem-estar da corvina. Como ocorreu com os ectoparasitos,

não houve correlação significativa, sugerindo um equilíbrio entre hospedeiros e parasitos, o que é esperado para peixes em ambiente natural. Entre os endoparasitos encontrados estão os Aspidogastridae e Nematoda (principalmente Anisakidae). A variação na intensidade de Aspidogastridae por local amostrado pode estar relacionada à viabilidade do cíclo de vida destes parasitos. O efeito patogênico direto da presença de Nematoda é muito menos importante do que seu papel como agentes causadores de zoonoses. Além disso, as atitudes dos consumidores em relação à sua presença, especialmente pela estética do produto parasitado, pode ter um grande impacto sobre o valor de mercado do pescado, salientando a necessidade de monitoramento. No terceiro capítulo é apresentada uma revisão sobre como os efeitos do parasitismo em peixes podem ser relacionados ao bem-estar de peixes e a aquicultura, utilizando as recentes informações disponíveis sobre o tema. Para finalizar, é apresentado um conjunto de considerações que, além de estabelecer relações entre os três capítulos, sugere algumas recomendações. O impacto da salinidade sobre interações parasito-hospedeiro é um regulador potencial dos processos de sobrevivência e transmissão do parasito. Essa é uma informação importante para o cultivo de *M. furnieri*, pois sugere que o controle destes parasitos pode ser realizado por regulação dos níveis de salinidade. Deve-se considerar que as doenças são parte da natureza e, quando entendidas desta forma, torna-se mais fácil lidar com a sua ocorrência. Profilaxia e vigilância devem ser os princípios orientadores na aquicultura moderna; o tratamento deve ser usado apenas quando estritamente necessário, evitando a introdução de drogas no meio ambiente. Dessa forma, os parasitos devem ser considerados como parte deste processo, monitorá-los, mas sem a pretensão de excluí-los. Assim, pode-se dizer que é possível sustentar a interação entre os parasitos, bem-estar e a aquicultura.

GENERAL ABSTRACT

The whitemouth croaker, *Micropogonias furnieri* is one of the most important fishery resources of the South Atlantic Ocean and has potential for aquaculture, as well as other Sciaenidae cultured worldwide. An appreciable amount of information are available about their biology and several studies have been done on *M. furnieri* parasitosis in the coast of Rio de Janeiro, Sao Paulo and Rio Grande do Sul states, in Uruguay and Argentina. Therefore, the whitemouth croaker can be an appropriate biological model for the study of interactions between parasitism, fish welfare and aquaculture. The aim of this study was to characterize the metazoan parasitism influences on whitemouth croaker from the Patos Lagoon estuary, through the examination of the relationship between parasite abundance and welfare host indices (relative condition factor – Kn; hepatosomatic relation – RHS; gonadosomatic relation - RGS). These indexes are qualitative fish welfare indicators. Samples were collected in two environments of Patos Lagoon estuary: pre-limnic (farther from the Atlantic coast) and estuarine (closer to the coast), since differences in these environments could be reflected in the parasite fauna. Moreover, the relationship between parasitism, fish welfare and aquaculture was discussed. Here we adopt a function-based definition, centre on an animal's ability to adapt to its present environment. In the first chapter, the ectoparasites influences of on the welfare of *M. furnieri* are characterized. A total of 181 *M. furnieri* specimens were examined and the following parasites were found: *Gauchergasilus euripedesi* (Copepoda), *Myzobdella uruguayensis* (Hirudinea), *Neomacrovalvitrema argentinensis* and *Neopterinotrematoides avaginata* (both Monogenoidea). The relationship between parasite abundance and host indexes was verified, but no significant correlation was determined, suggesting a host tolerance to the parasite values found. Moreover, comparison of samples in the pre-limnic and estuarine areas showed differences in diversity and intensity of the parasite species found, which suggests that the parasites have a preference for different salinities. In the second chapter is established the relationship between endoparasites and whitemouth croaker welfare. There was no significant correlation, as well as the ectoparasites, which suggest a balance between hosts and parasites. This is expected for fish in the natural environment. Among the endoparasites are found Aspidogastridae and Nematoda

(mostly Anisakidae). The intensity variation in Aspidogastridae sampled from different sites could be related to their life cycle viability. The direct effect of the presence of pathogenic nematodes is much less important than their role as causative agents of zoonosis. Furthermore, consumer attitudes about the presence of nematodes in food may also have a great impact on the market value of fish products. In the third chapter we present a review, where the effects of parasites on fish are related to fish welfare and aquaculture, considering the latest available information. In the end, we present a series of considerations that establishing relation between the three chapters and provide some recommendations. The impact of salinity on host-parasite interactions is a potential regulator of the parasite survival and transmission processes. This information suggests that different saline conditions could control parasite levels. Diseases are part of nature, and when understanding like this, it is easier get along with their occurrence. Prophylaxis and surveillance should be the guiding of the work that consider modern aquaculture; treatment should only be used when strictly necessary, preventing introduction of drugs into the environment. Thus, we should consider parasites as part of this process, monitoring them, but without the claim of exclude them. So we can say that it is possible to sustain the interaction between parasites, fish welfare and aquaculture.

INTRODUÇÃO GERAL

A aquicultura é uma atividade que se caracteriza por três componentes: produção de organismos com habitat predominantemente aquático, em cativeiro, em qualquer um de seus estágios de desenvolvimento; deve existir um manejo para a produção; a criação deve ter um proprietário, ou seja, não é um bem coletivo como são as populações exploradas pela pesca (FAO 1995; Valenti 2002). Reconhecidamente, a China foi o berço da aquicultura, utilizando principalmente a carpa comum, *Cyprinus carpio* (Linnaeus, 1758), há mais de 4.000 anos (Rabanal 1988). A aquicultura moderna está condicionada à sustentabilidade e pode ser definida como a produção lucrativa de organismos aquáticos, mantendo uma interação harmônica duradoura com os ecossistemas e as comunidades locais (Valenti 2002).

A pesca de captura e a aquicultura forneceram ao mundo cerca de 142 milhões de toneladas de peixes em 2008, sendo a aquicultura responsável por 52,5 milhões de toneladas o que representa 37% da oferta total (FAO 2010). A aquicultura tem se tornado um colaborador cada vez mais importante para o desenvolvimento econômico nacional, a oferta global de alimentos e a segurança alimentar (NACE 2000). Além disso, continua a ser o setor que mais cresce para produção animal de alimentos e supera o crescimento da população, com oferta *per capita* crescente da aquicultura de 0,7 kg em 1970 para 7,8 kg em 2008, uma taxa de crescimento médio anual de 6,6% (FAO 2010).

A investigação de espécies nativas é um fator importante para a expansão da aquicultura, evitando impactos negativos da introdução de espécies exóticas (Ross *et al.* 2008). Apesar da biodiversidade existente no território brasileiro, na aquicultura nacional destaca-se a tilápia, *Oreochromis niloticus* (Linnaeus, 1758), espécie exótica, por sua viabilidade econômica devido a avançados conhecimentos de manejo e biologia (Oliveira 2009).

A corvina, *Micropogonias furnieri* (Desmarest, 1823), é uma das espécies com maior importância econômica na atividade pesqueira do Rio Grande do Sul (Vazzoler 1991; Haimovici *et al.* 2005) e surge como uma possibilidade para a aquicultura. Um apreciável volume de informações sobre sua biologia (Vazzoler 1971; Taji 1974; Vazzoler 1975; Haimovici 1977; Chao *et al.* 1982; Castello 1986; Trant & Thomas

1988; 1989; Patiño & Thomas 1990; Vazzoler 1991; Haimovici *et al.* 1993; Vazzoler 1996) e sobre as características organolépticas (Borges *et al.* 2007) está disponível.

Além disso, observações indicam que a corvina apresenta potencial para aquicultura, a exemplo de outros Sciaenidae já cultivados. Experimentos sobre cultivo (Burkert 1999) e reprodução artificial e larvicultura (Albuquerque *et al.* 2009) de *M. furnieri* têm sido realizados. O "shi drum" *Umbrina cirrosa* (Linnaeus, 1758) (similar a *Umbrina canosai* Bergh, 1895 e *U. coroides* (Cuvier, 1830), conhecidas como "castanha" no Sul do Brasil) tem potencial para a aquicultura marinha no Mediterrâneo, por seu rápido crescimento em cativeiro (Segato *et al.* 2005). *Micropogonias undulatus* (Linnaeus, 1766) é uma promissora espécie para aquicultura, devido ao sucesso obtido no cultivo dos estágios iniciais de seu desenvolvimento (Oesterling *et al.* 2004) e pode ser tão apropriada para aquicultura quanto *Sciaenops ocellatus* (Linnaeus, 1766) (Creswell *et al.* 2007). Tecnologias foram desenvolvidas para produzir alevinos de *S. ocellatus*, com os objetivos de repovoamento de águas costeiras e cultivos, intensivo e extensivo, na Florida e no Golfo do México (Ramos 1995).

Micropogonias furnieri é uma espécie costeira, de ampla distribuição geográfica, ocorrendo entre a Península de Yucatán (Golfo de México, 20ºN) e o Golfo de San Matias (Argentina, 41ºS) associada às desembocaduras de água doce (Isacc 1988). No estuário da Lagoa dos Patos as corvinas apresentam comprimento médio de primeira maturação gonadal em 18,1cm para as fêmeas e em 20,5cm para os machos (Vazzoler 1971), permanecendo no estuário até os 30 cm de comprimento (Reis *et al.* 1994). *Micropogonias furnieri* é multidesovante, liberando lotes de ovócitos na medida em que atingem maturação completa (Vazzoler 1971). Essa área ecológica é uma importante zona de produtividade biológica, conhecida por sua biodiversidade (Asmus & Tagliani 1998; Kalikoski *et al.* 2010; Seeliger & Odebrecht 2010). O perfil da salinidade no estuário da Lagoa dos Patos é caracterizado por diferentes condições estuarinas (Niencheski & Baumgarten 1997), podendo ser dividido em três regiões ecológicas distintas: 1) límnico, ao Norte 2) pré-límnico, de 31°05'S, até o limite da região estuarina e 3) estuarino, ao Sul da Lagoa, delimitada a Norte por uma linha imaginária traçada entre a "Ponta da Feitoria" (31°41'S e 52°02'O) e a "Ponta dos Lençóis" (31°48'S e 51°50'O) e ao Sul, pelos "Molhes da Barra" (32°11'S e 52°04'O) (Closs & Medeiros 1965). A entrada de água salgada do Oceano Atlântico no estuário da Lagoa dos Patos é regulada principalmente pela chuva e pela intensidade do vento (Möller *et al.* 2001). Assim, há uma predominância de água doce e água do mar nas regiões pré-límnica e estuarina, respectivamente.

No Sul do Brasil, as estimativas de biomassa desta espécie diminuíram de 200.000 t em 1996 para 70.000 t em 2002 (Haimovici & Ignácio 2005). A corvina representa um dos recursos pesqueiros mais importantes do Oceano Atlântico Sudoeste (Haimovici & Umpierre 1996), constituindo um dos principais recursos demersais da Região Sul (Vasconcelos & Haimovici 2006). Lewis *et al.* (1999) reconhecem a diminuição dos estoques, sugerindo a proibição da pesca da corvina durante o período de primavera. Os níveis de exploração posteriores a 1990 são insustentáveis e a possibilidade de uma forte redução das capturas no futuro é grande, particularmente porque continua aumentando o esforço de pesca sobre as concentrações de desovantes próximas à desembocadura da Lagoa dos Patos (Haimovici & Ignácio 2005). Atualmente, *M. furnieri* é considerada um recurso sob forte sobre-pesca (IBAMA 1995; Haimovici *et al.* 1998; Vasconcellos & Haimovici 2006).

Neste estudo, os efeitos dos parasitos sobre os peixes estão restritos à ação dos macroparasitos, o que pode ser considerado um grupo artificial de metazoários parasitas (May & Anderson 1979), sobre hospedeiros teleósteos. Vários estudos foram desenvolvidos sobre parasitoses em *M. furnieri* no litoral dos estados do Rio de Janeiro (São Clemente 1986; São Clemente *et al.* 1993; Alves & Luque 2000; 2001a; 2001b), Rio Grande do Sul (Pereira Jr. 1992; 1993; Pereira Jr. & Neves 1993; Pereira Jr. & Costa 1996; Pereira Jr. *et al.* 1996; Andrade *et al.* 1997; Pereira Jr. *et al.* 2000; 2004a; 2004b; Pereira Jr. & Boeger 2005), São Paulo (Oliveira 1985), no Uruguai (Bertullo 1965) e na Argentina (Suriano 1966; Sardella *et al.* 1995). Espécies parasitas com potencial zoonótico já foram relatadas e são comuns em *M. furnieri*, dentre as quais destacam-se *Corynosoma australe* Johnston, 1937 (Pereira Jr. & Neves 1993) e *Hysterothylacium* sp. Ward et Magath, 1917 (Pereira Jr. *et al.* 2004a). Espécies de Capillaridae, especialmente *Pseudocapillaria* (*Pseudocapillaria*) *magalhaesi* (Lent & Freitas, 1937) Moravec, 1987, são encontradas na corvina do litoral do Rio Grande do Sul (Almeida *et al.* 2008) e podem ter relevância para a saúde pública (Bair *et al.* 2001; Moravec 2001), como já demonstrado para outras espécies co-genéricas. Pelo menos quatro espécies de Capillaridae são conhecidas parasitando o homem (Cross 1992), causando infecções no intestino, fígado e pulmões, diarréias severas, perda de peso, granuloma hepático, ineficiência na absorção de proteínas, podendo levar ao óbito caso não tratadas (Paulino & Wittenberg 1973).

Endoparasitos em peixes cultivados geralmente são menos patogênicos do que ectoparasitos (Martins *et al.* 2002). Tem sido afirmado que fazendas de peixes fornecem as condições ideais (ou seja, uma alta densidade de hospedeiros em potencial) para a propagação dos macroparasitos com ciclos de vida diretos (Barker & Cone 2000). Especificamente, macroparasitos como Monogenoidea e Copepoda freqüentemente causam epizootias localizadas e graves perdas econômicas para a indústria da aquicultura (Buchmann 1988; Cone 1995). Hirudíneas podem atuar como vetores de outros parasitos (Eiras 1994) e são comumente encontradas em ambientes de água doce e salobra (Volonterio *et al.* 2004). Aspidogastrea é um antigo grupo de vermes e vem sendo estudado através de técnicas como biologia molecular e microscopia eletrônica de transmissão e de varredura (Rohde 2005). Nematóides infectam espécies de peixes em todos os tipos de ambientes e, ocasionalmente, podem causar danos substanciais ao hospedeiro (Molnár *et al.* 2006). Estágios larvais de nematóides Anisakidae podem ser patogênicos para o ser humano quando ingeridos por meio de peixe cru ou mal cozido (Chai *et al.* 2005; Lima dos Santos & Howgate 2011). Apesar de nematóides causarem relativamente poucos problemas em cultivos, são importantes em peixes de aquário (Molnár *et al.* 2006).

Os consumidores estão exigindo cada vez mais que os varejistas garantam que os peixes que eles oferecem não sejam apenas de alta qualidade e seguros para o consumo mas, também, que eles derivem de pesca sustentável (FAO 2010). Parasitas de peixes causam perdas comerciais nas indústrias de aquicultura e de pesca, podendo ter implicações tanto na saúde humana como sócio-econômicas (Barber *et al.* 2000). Os mecanismos envolvidos na resposta do hospedeiro dependem de interações entre o hospedeiro, parasito e meio ambiente (Buchmann & Lindestrom 2002). Compreender as interações ecológicas envolvidas no parasitismo pode ajudar no desenvolvimento de medidas de controle da doença, tanto em um sistema de cultivo (Guidelli *et al.* 2006) como na manutenção da pesca (Barber *et al.* 2000).

Saúde e bem-estar estão cada vez mais entre os fatores que influenciam as decisões de consumo (FAO 2010). A gestão da saúde animal é uma importante ferramenta para a prevenção de doenças na aquicultura e é vital para sustentar a produção aquícola (Harikrishnan *et al.* 2010). Portanto, os consumidores começam a exigir garantias de que seu alimento foi produzido, manipulado e vendido de forma segura à sua saúde, respeitando o meio ambiente e apontando várias outras preocupações éticas e sociais (FAO 2010). Huntingford *et al.* (2006) resumem o conceito de bem-estar em três grandes categorias: 1) Conceitos baseados em sentimentos, são definidos em termos de estados mentais subjetivos; 2) Conceitos baseados em funções, centrados na capacidade de um animal para se adaptar ao seu ambiente presente; 3) Conceitos baseados na natureza, que surgem a partir da visão de que cada espécie de animal tem uma natureza biológica inerente que deve expressar. O desenvolvimento de um melhor conjunto de indicadores de bem-estar é necessário para facilitar a compreensão desse conceito (Huntingford *et al.* 2006).

O conceito de bem-estar animal é complexo (Dawkins 1998; Appleby 1999), por isso, neste estudo foi utilizada a definição baseada em funções, em que bem-estar significa que o animal está em boa saúde, com seus sistemas biológicos funcionando adequadamente e não sendo forçado a responder além de sua capacidade (Huntingford *et al.* 2006). O Fator de Condição Relativo (Kn) é um indicador quantitativo do bem-estar dos peixes e, nas últimas décadas, tem sido utilizado como uma ferramenta para o estudo das interações parasito-hospedeiro (Ranzani-Paiva & Silva-Souza 2004; Lizama *et al.* 2006; Yamada *et al.* 2008). O Índice Gônado-Somático (IGS) e o Índice Hepato-Somático (IHS) expressam, respectivamente, o estado funcional das gônadas e o estado funcional do fígado e por conseqüência do animal (Overstreet 1983). Esses índices (Kn, IGS, IGH) são uma ferramenta para estabelecer a relação existente entre o estado físico do peixe e a intensidade de parasitos, permitindo caracterizar a influência do parasitismo sobre o hospedeiro (Velloso & Pereira Jr. 2010).

Desta forma, o objetivo geral desta tese foi verificar a viabilidade de utilização da corvina como um modelo biológico para o estudo das interações entre parasitismo, bem-estar de peixes e aquicultura. Para tanto, foi caracterizada a influência do parasitismo por metazoários sobre a corvina *M. furnieri* no estuário da Lagoa dos Patos, utilizando correlações entre os índices de Prevalência (P), Intensidade de Infecção/Infestação (II), Índice Gônado-Somático (IGS), Índice Hepato-Somático (IHS) e Fator de Condição Relativo (Kn). As amostras foram tomadas em dois ambientes distintos: pré-límnico (mais distante da costa atlântica) e estuarino (mais próximo da costa), pois essa diferença de ambientes poderia ser refletida na fauna parasita. Além disso, como objetivo final deste estudo, foi discutida a relação entre parasitismo, bem-estar do peixe e aquicultura.

O primeiro capítulo, intitulado "Influence of Ectoparasitism on the welfare of *Micropogonias furnieri*.", foi publicado na revista Aquaculture, em 2010; o segundo capítulo intitulado "The endoparasitism and welfare of *Micropogonias furnieri*." foi submetido à revista Aquaculture Research e o terceiro capítulo intitulado "Parasitism, fish welfare and aquaculture: can this interaction be sustainable?" ainda não foi submetido. No final desta tese é apresentado um conjunto de considerações que além de estabelecer relações entre os três capítulos, sugere algumas recomendações.

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CAPÍTULO I

Influence of Ectoparasitism on the welfare of *Micropogonias furnieri*.

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Influence of Ectoparasitism on the welfare of *Micropogonias furnieri*.

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ABSTRACT

The Whitemouth croaker, *Micropogonias furnieri*, is commonly found in the estuary of Patos Lagoon, RS, Brazil. Like other cultivated Sciaenidae, *Micropogonias furnieri* has potential for aquaculture. However, several difficult-to-control ectoparasites have been shown to be serious fish culture pests. In this study, the parasite influence on *M. furnieri* is evaluated through examination of the relationship between abundance and host parasitism indexes, relative condition factor (Kn), and the hepatosomatic (RHS) and gonadosomatic relations (RGS). These indexes are qualitative fish welfare indicators. A total of 181 *M. furnieri* specimens were collected from the pre-limnic and estuarine sites of the Patos Lagoon. The parasites found were *Gauchergasilus euripedesi* (Copepoda), *Myzobdella uruguayensis* (Hirudinea), *Neomacrovavitrema argentinensis* and *Neopterinotrematoides avaginata* (both Monogenoidea). The relationship between the parasite abundance and the host indexes was verified, but no significant correlation was determined. This result suggests that the found parasite levels are tolerable. On the other hand, sample area differences affected the occurrence and intensity of parasitism, which suggests that the parasites have a preference for different salinities. This result suggests that different saline conditions could determine when the estuary would be optimal for *M. furnieri* aquaculture.

KEY WORDS: Whitemouth croaker; Copepod; Leech; Monogenoidea; Relative Condition Factor (Kn); Estuary of Patos Lagoon;

1. Introduction

The whitemouth croaker, *Micropogonias furnieri* (Desmarest, 1823), is a euryhaline sublittoral species of the central western and southwestern Atlantic ocean. It is distributed from the Yucatan peninsula along the Antilles to the gulf of San Matias in Argentina (Isacc, 1988). This demersal and benthic sciaenid fish is an important resource with reported landings from 1995-2000 amounting to 28.1% of the local catch and 16% of the industrial catch in the marine coastal system of southern Brazil (Vasconcellos et al., 2007). Exploitation levels became unsustainable after 1990, which indicated a high possibility of reduced fishing in the near future (Haimovici and Ignácio, 2005). In the Patos Lagoon estuary, RS, Brazil, the mean length of whitemouth croaker in the first maturation is 18.1 cm for females and 20.5 cm for males (Vazzoler, 1971). This important ecological area is a biologically productive zone and is known for its biodiversity (Asmus and Tagliani, 1998; Kalikoski et al., 2010). The salinity profile in Patos Lagoon estuary is characterized by several estuarine conditions (Niencheski and Baumgarten, 1997).

Culture experiments suggest that *M. furnieri* shows aquaculture potential (Burkert, 1999). According to Oesterling et al. (2004), *Micropogonias undulatus* is one of the priority species for aquaculture in the United States. Creswell et al. (2007) suggest that *M. undulatus* is likely more amenable for aquaculture than the red drum (*Sciaenops ocellatus*) and other sciaenid fish. Therefore, culture of whitemouth croaker should have a positive social-ecological impact on the local fishermen community.

Fish farms provide ideal conditions (i.e., a high density of potential hosts) for effective infectious disease transmission, particularly the spread of macroparasites with direct life cycles (Barker and Cone, 2000). Specifically, Monogenoidea and Copepoda macroparasites frequently cause localized epizootics and serious economic losses to the aquaculture industry (Buchmann, 1988; Cone, 1995). *Myzobdella* spp. are piscicolid leeches that are commonly found in fresh and brackish water environments attached to the anterior and dorsal regions of fish (Volonterio et al., 2004). On the other hand, Hirudineans can act as vectors of other parasites (Eiras, 1994).

In addition to the hepato (HSR) and gonadosomatic relation (GSR) factors, the relative condition factor (Kn) is also a quantitative indicator of fish welfare (Vazzoler,

1996) and is an important tool for studying host-parasite relationships (Lizama et al., 2006; Ranzani-Paiva and Silva-Souza, 2004; Yamada et al., 2008). Understanding ecological interactions assists in the development of disease control measures in an intensive farming system (Guidelli et al., 2006). The aim of this study was to evaluate the relationships between *M. furnieri* from Patos Lagoon estuary and the metazoan ectoparasitic fauna. In addition, the parasite infrapopulation influence on the HSR, GSR and Kn of *M. furnieri* was determined.

2. Materials and methods

The Patos Lagoon estuary can be divided into three distinct ecological regions: 1) limnic, in the north 2) pre-limnic, from 31°05'S until the limit of estuarine region and 3) estuarine, in the south of the lagoon, bounded on the north by an imaginary line drawn between "Ponta da Feitoria" (31°41'S e 52°02' O) and "Ponta dos Lençóis" (31°48'S e 51°50' O) and in south, by "molhes da Barra" (32°11'S e 52°04'O) (Closs and Medeiros, 1965). The seawater input from the Atlantic Ocean in the Patos Lagoon estuary is regulated mainly by rain and wind intensity (Möller et al., 2001). Thus, there is a predominance of freshwater and seawater in the pre-limnic and estuarine regions respectively. This study included pre-limnic (the farthest from Atlantic coast) and estuarine (the nearest from the coast) areas.

A total of 181 *Micropogonias furnieri* specimens were collected from two areas: 1) pre-limnic (n=94), on 02/16/2009 and 2) estuarine (n=87), on 04/10/2009. Fish were collected in each area on the same day to minimize abiotic factor variations among the samples. The fish were frozen and examined to verify the presence of ectoparasites. Monogenoideans, leeches and copepods were collected under stereoscopic microscopy and prepared for identification according to Eiras et al. (2006). All of these procedures comply with the current laws of the country (environmental permit: IBAMA No. 17864-1).

Total weight (Wt) and total length (Lt) values of the examined whitemouth specimens were used to adjust the weight-length relationship curve. The *a* and *b* values were utilized to calculate the theoretically expected weight (We) and the relative condition factor (Kn=Wt/We). The hepatosomatic (HSR) and gonadosomatic (GSR) relationships were calculated using the following formulas: HSR=liver weight (g)/body weight (g) x 100 (Lizama et al., 2006), GSR=gonad weight (g)/body weight (g) x 100 (Vazzoler, 1996). The mean Kn values of fish infested with different parasites and fish that were not infected were compared to the standard Kn=1.0 by the student's t test. The relationship between abundance and Lt, HSR, GSR and Kn of each parasite species was verified by Spearman's rank correlation coefficient "rs" (Zar, 1996). The adopted level of statistical significance was $p \leq 0.05$.

Parasitological indexes were based on Bush et al. (1997) and compared by Quantitative Parasitology 3.0 software (Rozsa et al., 2000).

3. Results and discussion

The total length of the *M. furnieri* specimens varied between 19.1 cm and 33.3 cm, which is the length range when they reach their first maturity (Vazzoler, 1971).The total weight of the *M. furnieri* specimens varied between 58 g and 355 g. The following ectoparasites were found: *Gauchergasilus euripedesi* Montú and Boxshall, 2001; *Myzobdella uruguayensis* Mañé Garzón and Montero, 1977; *Neomacrovalvitrema argentinensis* Suriano, 1975; *Neopterinotrematoides avaginata* Suriano, 1975 (Figure 1). The analysis was performed for the two collection sites (pre-limnic and estuarine) separately and together (all estuary). However, there was no difference observed between the host indices and parasitism abundance.

Figure 1

There was a significant difference seen in the hirudinean *Myzobdella uruguayensis* prevalence, mean intensity and mean abundance values in the pre-limnic area when compared to the estuarine area (Table 1). The leech *M. lugubris* inhabits freshwater environments and is commonly found at salinity levels varying from 0 to 22 ppt (brackish to freshwater), according to Sawyer et al. (1975). The several estuarine conditions that characterize the salinity profile in the Patos Lagoon estuary are associated with high and low fluvial discharge (Niencheski and Baumgarten, 1997). In Uruguay, Volonterio et al. (2004) reported *M. uruguayensis* parasitizing *Ramdia quelen* with a prevalence of 100%. Though *M. uruguayensis* is tolerant to salinity variations, this result suggests that it prefers lower saline levels at the Patos Lagoon estuary. Similarly, the copepod *G. euripedesi* was found only in the pre-limnic area (Table 1). According to Montú and Boxshall (2002), *G. euripedesi* is widely distributed in Brazilian estuarine waters, from south of Rio Grande do Sul to the Sergipe river estuary in northeast Brazil. Many authors (Araújo and Boxshall, 2001; Montú, 1980; Thatcher and Robertson, 1984) showed that ovigerous females and males of this species are commonly found in plankton samples. This can be explained by the absence of this species in the estuarine area, where there are high salinity levels that might influence the free living stages. There are over 150 described ergasilid species, and most of them are found in freshwater; only 23 ergasilid species are found in estuarine or coastal marine habitats (Boxshall and Montú, 1997). Hence these results suggest that *M. uruguayensis* and *G. euripedesi* prefer the pre-limnic area, where less salt water is the dominant condition. This suggests that control of these parasites could be accomplished by regulation of salinity levels, which would be beneficial to culturing *M. furnieri* in the Patos Lagoon estuary.

Neomacrovalvitrema argentinensis was found on both pre-limnic and estuarine areas, with prevalences of 20.21% and 25.69%, respectively (Table 1). On the other hand, *N. avaginata* was found with P%=2.13% in the pre-limnic area and P%=14.94% in the estuarine areas (Table 1). Suriano (1975) recorded P%=59% for *N. argentinensis* and P%=11% for *N. avaginata* in Argentina, collected from Mar del Plata coast, from the same host (*M. furnieri* = *Micropogon opercularis*). In the present study, a low prevalence of *N. avaginata* in the pre-limnic area ($p=0.0017$) was found. Therefore, these results suggest a seawater preference by these monogenoideans species.

Partial or total exclusion is a case of interspecific competition and can occur in the host or among hosts (Poulin, 2007). There was absence of co-occurrence of *M. uruguayensis* with *N. argentinensis* and *N. avaginata* in the estuarine area. As shown by Poulin (2007), the ability of one species to exclude another may be a functional parasite response to competition. This is supported by Suriano (1975), which showed that *N. argentinenis* was found on the median or upper portion of gill filaments, whereas *N. avaginata* occupied the lower portion. Buchmann and Lindenstrom (2002) suggest that the host's production of immunological factors following monogenean infections may explain the acquired response. This mechanism may also be interfering with the co-occurrence of these species.

Table 1

The Kn is an important tool for studying host-parasite interactions (Lizama et al., 2006). The occurrence of each parasite group was considered independently of the others, and no difference was observed when the mean relative condition factor (Kn) values of each fish group and of the non-parasitized group were compared to the standard Kn=1.0. These results showed the balance between hosts and parasites, which was expected for wild fish. The Kn can serve as a quantitative indicator of fish welfare (Vazzoller, 1996). Low pathogenic parasites can occur in high abundance in the host without negatively influencing its Kn, and this correlation can be shown positively (Moreira et al. 2010). Frequently, the most affected hosts are not able to survive (Barber et al., 2000) and do not appear in the sample.

The relationship between parasitism abundance and total length (Lt), HSR, GSR and Kn of each parasite species was verified, but there was no significant correlation observed. Similar results were seen in several different environments by Guidelli et al. (2009), Lizama et al. (2006), Tavares-Dias et al. (2000) and Tavernari et al. (2009). The random sampling that was done in this study could potentially "dilute" the information available on the impact of parasitic diseases. Thus, it is possible to confirm that the hosts tolerate those intensities of infections. Unlike high-virulence parasites, the low parasite pathogenicity makes correlation impossible because it did not influence the Kn/parasitism ratio (Poulin, 2007). Therefore, under culture conditions, it is expected that the parasitism influence causes host damage.

Fish parasites may be useful indicators of fish and aquatic health because parasites may be sensitive to intensive fishing and pollution and show decreased abundance over time (Lafferty, 2008). Parasites can affect their host’s health in many ways, and it is not possible to draw a general conclusion about their effect on host health; however, the presence of parasites is not exclusively negative (Sures, 2008). Tools that utilize organ indicators and relative fish condition factors affecting parasitism levels are important in understanding the ecological relationship between parasites and hosts (Lizama et al., 2006). This paper provides information about *M. furnieri* ectoparasitosis tolerant levels using data collected on fish welfare.

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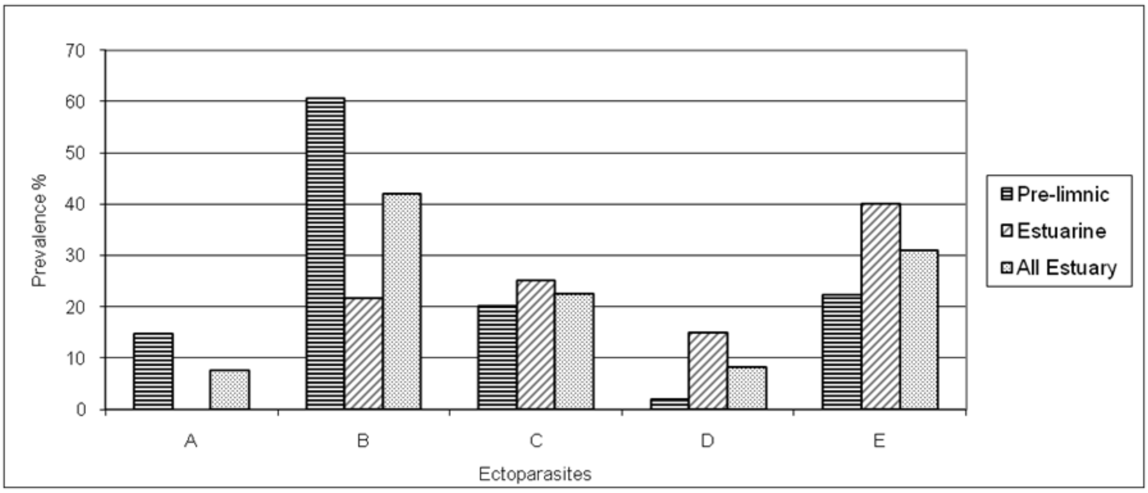
Table 1: Prevalence Values (P%), Mean Intensity (MI) and the Mean Abundance of the ectoparasitic fauna of *Micropogonias furnieri* collected at the two sites (pre-limnic and estuarine) of Patos Lagoon Estuary, RS, Brazil, and the values of both sites added together (All Estuary). *Significant values p≤0.05.

Ectoparasites	Pre-limnic			Estuarine			All Estuary		
	P%	MI	MA	P%	MI	MA	P%	MI	MA
COPEPODA									
G. euripedesi	14.89	2.79	0.41	-	-	-	7.73	2.79	0.22
HIRUDINEA									
M. uruguayensis	60.64*	2.30*	1.39*	21.84*	1.47*	0.32*	41.99	2.09	0.88
MONOGENOIDEA									
N. argentinensis	20.21	1.79*	0.36*	25.29	3.86*	0.98*	22.65	2.90	0.66
N.avaginata	2.13*	1.00	0.02	14.94*	3.69	0.55	8.29	3.33	0.28

Figure 1: Prevalence (P%) of the ectoparasitic fauna of *Micropogonias furnieri* collected from two sites (pre-limnic and estuarine) of Patos Lagoon Estuary, RS, Brazil, and the values when these areas are added together (All Estuary). A) *Gauchergasilus euripedesi*; B) *Myzobdella uruguayensis*; C) *Neomacrovalvitrema argentinensis*; D) *Neopterinotrematoides avaginata*; E) Monogenoidea (the species C and D together).



CAPÍTULO II

The endoparasitism and welfare of *Micropogonias furnieri*.

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The endoparasitism and welfare of *Micropogonias furnieri*.

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Running title: Endoparasitism and welfare of fish.

Keywords: Whitemouth Croaker; Nematoda; Anisakidae; Aspidogastridae; Relative Condition Factor (Kn); Estuary of Patos Lagoon;

ABSTRACT

The whitemouth croaker, *Micropogonias furnieri*, is a candidate species for aquaculture and is commonly found in the estuary of the Patos Lagoon, RS, Brazil. This demersal and benthic sciaenid fish is an important southern Brazilian fishery resource. In this study, endoparasitic influences on *M. Furnieri* welfare are evaluated through the examination of the relationship between parasite abundance and welfare host indices (relative condition factor – Kn; hepatosomatic relation – RHS; gonadosomatic relation - RGS). A total of 181 *M. furnieri* specimens were collected from the pre-limnic and estuarine sites of the Patos Lagoon. A relationship between parasite abundance and host indices was verified, but no significant correlation was determined, suggesting that the observed parasite levels are tolerable. On the other hand, sample area differences affected the occurrence and intensity of parasitism. Moreover, among the parasites we found in the fish are the Aspidogastridae and Nematoda (mostly Anisakidae), which may indicate the presence of zoonotic nematodes. Furthermore, consumer attitudes about the presence of nematodes in food may also have a great impact on the market value of fish products. The present work uses data about fish welfare to contribute to the body of knowledge regarding *M. furnieri* endoparasitosis tolerance levels.

1. Introduction

Fish parasites cause commercial losses in both the aquaculture and fishery industries and may have human health and socioeconomic implications in developing and developed countries (Barber *et al.* 2000). Parasitologists have closely observed the mechanisms involved in host response, which depends on interactions among the host, parasite and environment and, in several cases, on specific substances (Buchmann & Lindestrom 2002). A full understanding of the diverse effects of fish parasites on their hosts is, therefore, central to the development and maintenance of fisheries worldwide (Barber *et al.* 2000). Tools that utilize organ indicators and relative fish condition factors affecting parasitism levels are important in understanding the ecological relationships between parasites and hosts (Lizama *et al.* 2006). Therefore, in addition to the hepato (HSR) and gonadosomatic relation (GSR) factors, the relative condition factor (Kn) is also a quantitative indicator of fish welfare (Vazzoler 1996).

The whitemouth croaker, *Micropogonias furnieri* (Desmarest, 1823), is a euryhaline sublittoral species found in the central western and southwestern Atlantic Ocean. This demersal and benthic sciaenid fish is an important resource for southern Brazil (Vasconcellos *et al.* 2007). Exploitation levels of this fish became unsustainable after 1990, resulting in a high possibility of reduced fishing in the near future (Haimovici & Ignácio 2005). A part of the wild population reaches sexual maturity within the Patos Lagoon estuary, RS, Brazil (Vazzoler 1971). This important ecological area is a biologically productive zone and is known for its great biodiversity (Asmus & Tagliani 1998; Kalikoski *et al.* 2010). Experiments suggest that *M. furnieri* shows aquaculture potential (Burkert 1999). According to Oesterling *et al.* (2004), the cogeneric species *M. undulatus* (Linnaeus, 1766) is important for aquaculture in the United States. Creswell *et al.* (2007) suggest that *M. undulatus* may be more amenable for aquaculture than the red drum, *Sciaenops ocellatus* (Linnaeus, 1766), and other sciaenid fish.

The Aspidogastrea is an ancient group of flukes and has been studied extensively using experimental techniques as well as molecular biology and transmission and scanning electron microscopy (Rohde 2005). Parasitic nematodes constitute one of the earliest known groups of helminthes in fishes. They infect freshwater, marine and brackish water fish species and occasionally cause substantial damage to the host (Molnár *et al.* 2006). Larval stages of nematodes belonging to the Anisakidae can be pathogenic to humans when ingested through raw, undercooked or unfrozen fish (Chai *et al.* 2005; Lima dos Santos & Howgate 2011). Although nematodes cause relatively few problems in fish propagated in fish farms or cage cultures, they are relatively important in aquarium fish (Molnár *et al.* 2006). Understanding the ecological interactions involved in endoparasitism may assist in the development of disease control measures in an intensive farming system (Guidelli *et al.* 2006).

Endoparasites in cultivated fish are less pathogenic than ectoparasites (Martins *et al.* 2002). Velloso & Pereira Jr. (2010) studied the relationship between ectoparasites and the welfare of *M.furnieri*. Therefore, the aim of this study is to evaluate the relationships between *M. furnieri* from the Patos Lagoon estuary and the metazoan endoparasitic fauna. In addition, this study determines the influence of parasite abundance on the HSR, GSR and Kn of *M. furnieri*.

2. Materials and Methods

The Patos Lagoon estuary can be divided into three distinct ecological regions: 1) limnic, in the north; 2) pre-limnic, from 31°05'S to the limit of the estuarine region; and 3) estuarine, in the south of the lagoon. The estuarine region is bounded on the north by an imaginary line drawn between "Ponta da Feitoria" (31°41'S e 52°02'O) and "Ponta dos Lençóis" (31°48'S e 51°50'O) and in the south by "molhes da Barra" (32°11'S e 52°04'O) (Closs & Medeiros 1965). The salinity profile in the Patos Lagoon estuary is characterized by several estuarine conditions (Niencheski & Baumgarten 1997). The seawater input from the Atlantic Ocean in the Patos Lagoon estuary is mainly regulated by rain and wind intensity (Möller *et al.* 2001). Thus, there is a predominance of freshwater and seawater in the pre-limnic and estuarine regions, respectively. This study included pre-limnic (the farthest from the Atlantic coast) and estuarine (the nearest to the coast) areas.

A total of 181 *M. furnieri* specimens were collected from two areas: 1) the pre-limnic area (n=94) on 02/16/2009 and 2) the estuarine area (n=87) on 04/10/2009. Fish were collected in each area on the same day to minimize abiotic factor variations among the samples and were examined to verify the presence of endoparasites. The parasites were collected under stereoscopic microscopy and were prepared for identification according to Eiras *et al.* (2006). All of these procedures comply with the current laws of the country (environmental permit: IBAMA No. 17864-1).

Total weight (Wt) and total length (Lt) values of the examined whitemouth croaker specimens were used to adjust the weight-length relationship curve. The *a* and *b* values were utilized to calculate the theoretically expected weight (We) and the relative condition factor (Kn=Wt/We). The hepatosomatic (HSR) and gonadosomatic (GSR) relationships were calculated using the following formulas: HSR=liver weight (g)/body weight (g) x 100 (Lizama *et al.* 2006), GSR=gonad weight (g)/body weight (g) x 100 (Vazzoler 1996). The mean Kn values of fish infested with different parasites and fish that were not infected were compared to the standard Kn=1.0 by Student's t-test. The relationship between abundance and Lt, HSR, GSR and Kn of each parasite group was verified by Spearman's rank correlation coefficient "rs" (Zar 1996). The tests were applied to the groups that presented prevalence higher than 10% (Bush *et al.* 1997). The adopted level of statistical significance was $p \leq 0.05$.

Parasitological indices were based on Bush *et al.* (1997) and were compared with Quantitative Parasitology 3.0 software (Rozsa *et al.* 2000). Although Bush *et al.* (1997) emphasize that it is conceptually possible to use prevalence for other taxonomic levels (beyond species), we also adopt mean intensity and mean abundance indices for larger groups (such as Nematoda and Aspidogastridae).

3. Results and Discussion

The total length of the *M. furnieri* specimens varied between 19.1 cm and 33.3 cm, which is the length range when they reach their first maturity (Vazzoler 1971). The total weight of the *M. furnieri* specimens varied between 58 g and 355 g. The following endoparasitic groups were found in the gastrointestinal tract: Nematoda (mostly Anisakidae), Platyhelminthes (Aspidogastrea and Digenea), and Acantocephala. For statistical analysis, the endoparasites were placed into two major groups, Nematoda and

Aspidogastridae. The analysis was performed for the two collection sites (pre-limnic and estuarine) separately and together (all estuary).

There was a significant difference observed in Aspidogastridae prevalence values in the pre-limnic area compared to the estuarine area (Table 1). This difference can be explained by the life cycle of Aspidogastridae species, wherein both a mollusk and a marine teleost are required for the completion of the life cycle (Rohde 2001). Parasite responses to environmental factors are complex and do not yield a simple set of predictions (Lafferty 1997). Huspeni & Lafferty (2004) used larval trematodes that parasitize snails to evaluate a salt marsh restoration project. These authors show that snails in impacted sites had significantly fewer larval trematodes than did those from control sites located in intact salt marsh sites. Generally, parasites requiring multiple hosts to complete their life cycle are negatively influenced by disturbances that could interfere with the efficiency of transmission from one host to the next (Huspeni *et al.* 2005). For example, if the intermediate host is affected by an environmental change, such as a change in the salinity profile, it could influence parasite prevalence values in fish. Lei and Poulin (2011) reported that the impact of salinity on host–parasite interactions is a potential key regulator of parasite transmission processes in intertidal areas where trematodes are extremely common parasites of invertebrates and vertebrates. Furthermore, parasite prevalence is usually a positive function of age, due to the increased likelihood of being infected as a function of time (Poulin 2000).

Table 1

There was no difference in Nematoda prevalence between the pre-limnic and estuarine groups (Table 1). Most of these parasites were larval. The majority of nematodes reach sexual maturity through a complicated developmental cycle involving an intermediate or possibly paratenic host (Molnár *et al.* 2006). Anisakid nematodes are common parasites of the whitemouth croaker (Pereira Jr. & Costa 1996; Alves & Luque 2001; Pereira Jr. *et al.* 2004a; Pereira Jr. *et al.* 2004b). These nematodes are usually parasites of piscivorous birds, mammals or reptiles, or, less frequently, of predatory fishes (Anderson 2000). However, there was a significant difference seen in Nematoda mean intensity and mean abundance values in the pre-limnic area compared to the estuarine area (Table 1). This difference may be related to the diversity of host infrapopulations from each site. At the parasite community level, there are smooth geographical gradients in species composition (Poulin & Dick 2007). However, environmental variables affecting the density of parasite species (host availability, abiotic conditions) do not show pronounced spatial correlation (Brown 1984). In addition to the density of suitable intermediate hosts, the presence of alternate definitive hosts can be a key element in the local abundance of a parasite species (Poulin & Dick 2007). Because of a complicated, multi-host life cycle, the development of fish nematodes is successful in non-disrupted ecosystems (Molnár *et al.* 2006).

Adult Aspidogastridae and larval Nematoda exhibited several distributions on *M. furnieri* and used the host in several ways. Interspecific interactions among parasites are likely to occur in the former type of communities because their members exploit similar resources in the same general habitat but are unlikely in the latter type of communities, which consist of species that are often encysted and unlikely to compete with one another for host resources (Poulin & Valtonen 2001). Furthermore, low pathogen parasites can occur in high abundance in the host without negatively influencing its Kn (Moreira *et al.* 2010).

There was no difference between host indices and parasitism abundance. The relationship between parasitism abundance and total length (Lt), HSR, GSR and Kn of each parasite group was verified, but there was no significant correlation observed. Similar results were seen in several different environments by Tavares-Dias *et al.* (2000), Lizama *et al.* (2006), Guidelli *et al.* (2009), Tavernari *et al.* (2009) and Velloso and Pereira Jr. (2010). Poulin (2007) reported that low parasite pathogenicity makes correlation impossible because it did not influence the Kn/parasitism ratio.

Mouritsen *et al.* (2010) reported anisakid nematodes in Atlantic cod (*Gadus morhua* Linnaeus, 1758) and Greenland cod (*Gadus ogac* Kröyer, 1847) and compared levels of parasites with the condition factor of the hosts., but the both species of cod do not appear to suffer any reduction in condition factor with increasing infection intensity. Relative Condition Factor (Kn) can serve as a quantitative indicator of fish welfare and is an important tool for studying host-parasite interactions (Lizama *et al.* 2006). The occurrence of each parasite group was considered independently of the others, and no difference was observed when the mean relative condition factor (Kn) values of each fish group and of the non-parasitized group were compared to the standard Kn=1.0. Velloso & Pereira Jr. (2010) found the same results for ectoparasites and *M. furnieri*, and Dias *et al.* (2004), also found the same results in the *Perodoras granulosus* nematodes. These results indicated the balance between hosts and parasites that is expected for wild fish. Fish that live in natural ecosystems are rarely free from infections and, frequently, the most affected hosts are not able to survive (Barber *et al.* 2000). In artificial aquaculture environments, fish are at risk from acute outbreaks of parasite infection, resulting from the maintenance of high stock density (Barber *et al.* 2000).

Although nematodes are important pathogens, their direct pathogenic effect is much less important than their role as causative agents of zoonoses (Molnár *et al.* 2006). Anisakiasis is a serious zoonotic disease, and in the last two decades, there has been a dramatic increase in its reported prevalence throughout the world (Chai *et al.* 2005; Lima dos Santos & Howgate 2011). Furthermore, consumer attitudes regarding the presence of nematodes in food could also have a great impact on the market value of fish products and stresses the need for strict monitoring and diagnosis of nematode infections in marine fish stocks (Molnár *et al.* 2006).

Lima dos Santos & Howgate (2011) indicate that an association between aquaculture and human diseases caused by fish-borne parasites is now well documented, and research is needed in geographical areas where the diseases are endemic. The present work contributes to the body of knowledge concerning *M. furnieri* endoparasitosis tolerance levels using data collected on fish welfare. Understanding the tolerance of *M. furnieri* to endoparasites will help the aquaculture and fishery industries be aware of warning signs that signal the presence of zoonotic nematodes.

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Table 1: Prevalence values (P%), mean intensity (MI) and mean abundance (Ax) of the endoparasitic fauna of *Micropogonias furnieri* (Desmarest, 1823) collected at the two sites (pre-limnic and estuarine) of Patos Lagoon Estuary, RS, Brazil, and the values of both sites added together (All Estuary). *Significant values $p \leq 0.05$.

Endoparasites	Pre-limnic			Estuarine			All Estuary		
	P%	MI	MA	P%	MI	MA	P%	MI	MA
Nematoda	94.68	19.17*	18.15*	96.55	6.83*	6.60*	95.03	13.19	12.53
Aspidogastridae	44.68*	3.50	1.56	67.82*	1.75	1.75	55.80	2.96	1.65

CAPÍTULO III

Parasitism, fish welfare and aquaculture: can this interaction be sustainable?

Artigo formatado pelas normas da revista Aquaculture.

Situação: ainda não submetido.

Parasitism, fish welfare and aquaculture: can this interaction be sustainable?

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ABSTRACT

In this review the effects of parasites on fish are related to fish welfare and aquaculture, considering the latest available information. Here we adopt a function-based definition, centre on an animal’s ability to adapt to its present environment. Parasitic organisms naturally exist in an unstable equilibrium with their hosts and can affect their host's health in many ways. Tools that utilize organ indicators and relative fish condition factors affecting parasitism levels are important in understanding the ecological relationship between parasites and hosts. However, the development of aquaculture can affect this equilibrium. Under culture conditions a variety of parasites can occur in high numbers, can lead to economic loss. Health management is an important tool for the prevention of disease in aquaculture and vital to sustain aquaculture production. Multiple factors interact and influence the outcome of pathogen transmission and disease development. The severity of disease depends on the parasite capacity of infection and environmental quality in which fishes are kept. A better understanding of what pathogens currently exist in aquatic ecosystems can help to determine natural distributions of pathogens and hosts and identify if, and how, new diseases or parasites are introduced. The development of Best Management Practices and codes of conduct can improve biosecurity and reduce the risk of disease transmission. The contribution of fish to food and nutrition security will become increasingly important. All forward projections anticipate a need for increased supply of fish protein and aquaculture now accounts for almost 50 percent of fish consumed by humans. The development of sustainable aquaculture involves species selection, production systems, animal genetics, good health management, understanding of animal welfare and optimized feed and feeding. Looking forward, research should incorporate these concepts into production, whereas the modern aquaculture is based on three pillars: profitable production, social development and preservation of the environment. Diseases are part of our nature, and when understanding like this, it is easier get along with their occurrence. Prophylaxis and surveillance should be the guiding of the work that consider modern aquaculture; treatment should only be used when strictly necessary, preventing introduction of drugs into the environment. So we can say that it is possible - and very important in the current scenario - to sustain the interaction between parasites, welfare and aquaculture.

KEYWORDS: Relative Condition Factor (Kn); Biosecurity; Health; Farmed Fish; Wild fish;

1. Introduction

Parasites have been recognized as an important component of global biodiversity (Poulin and Morand, 2004). Limited research has been undertaken on diseases of wild fish at the population level, due to the tremendous difficulties associated with the study of diseases in wild fish populations: infected fish often die and disappear before they can be detected (Bergh, 2007).

In this review the effects of parasites on fish are restricted to macroparasites, an artificial group of metazoan parasites (May and Anderson, 1979), on teleost fish hosts. Parasites are expected to exert considerable selection pressure on host organisms, and are likely to have played a significant role in the evolution of many aspects of fish behavior and ecology (Barber, 2007).

The high-density living conditions in aquaculture facilities can lead to outbreaks of diseases that likely occur at low levels in natural populations. Under mariculture conditions, a variety of both monoxenous and heteroxenous parasites can occur in high numbers (Rückert et al., 2009). Such infestations/infections can lead to enormous economic loss due to resulting mass mortalities (Tucker et al., 2002). The case of salmonids in Norway is one of the best-studied examples of interactions between wild and cultured fish, which *Gyrodactylus salaris* epizootic has proved a serious threat to native host populations (Bakke et al., 2007).

Health and welfare are among other factors increasingly influencing consumption decisions (FAO, 2010). Health management is an important tool for the prevention of disease in aquaculture and vital to sustain aquaculture production (Harikrishnan et al., 2010). Therefore, consumers demand guarantees that their food has been produced, handled and sold in a way that is not dangerous to their health, respects the environment and addresses various other ethical and social concerns (FAO, 2010).

Huntingford et al. (2006) summarize the concept of welfare in three broad categories: 1) Feelings-based definitions, are set in terms of subjective mental states; 2) Function-based definitions, centre on an animal's ability to adapt to its present environment; 3) Nature-based definitions, arise from the view that each species of animal has an inherent biological nature that it must express.

The development of a better array of welfare indicators is necessary to facilitate understanding when laboratory tests are not possible (Huntingford et al., 2006). Accordingly, the examination of the relationship between parasite abundance and welfare host indices, like relative condition factor (Kn), hepatosomatic relation (RHS) and gonadosomatic relation (RGS) can contribute to the knowledge regarding parasitosis tolerance levels (Velloso and Pereira Jr., 2010; 2011). In this way, the aims of this study are review and analyze the latest information about the influence of parasitism on the welfare of fish and its relation to aquaculture.

2. Fish Parasitism

Parasitic organisms naturally exist in an unstable equilibrium with their hosts, but could be no 'ideal' natural state for the relationship between host and pathogen, as the somewhat misleading terms 'equilibrium' or 'natural balance' indicate (Bergh, 2007). Parasites can affect their host's health in many ways, and it is not possible to draw a general conclusion about their effect on host health; however, the presence of parasites is not exclusively negative (Sures, 2008). Fish parasites may be useful indicators of fish and aquatic health (Silva-Souza et al., 2006; Lafferty, 2008).

Tools that utilize organ indicators and relative fish condition factors affecting parasitism levels are important in understanding the ecological relationship between parasites and hosts (Lizama et al., 2006). Unlike high-virulence parasites, the low parasite pathogenicity makes correlation impossible because it did not influence the Kn/parasitism ratio (Poulin, 2007). However, environmental changes and anthropogenic activities, such as for instance the development of aquaculture can affect this equilibrium (Reno, 1998).

In the same way, is probably unrealistic to expect that fish kept in captivity under natural or semi-natural conditions to be maintained in a parasite-free condition, and in general, low level infections with co-evolved parasites within the intensity range normally encountered for the population of origin are unlikely to raise significant welfare concern (Barber, 2007; Tonguthai, 1997). The aim of aquaculture should not be a mythical disease-free cultured animal, but rather an animal for which the impact of diseases and general level of welfare is acceptable, at least when compared to wild individuals of the same species (Bergh, 2007).

However, if the levels of infection developed by fish in captivity, if the particular strains or species of parasites to which fish are exposed are changed by the husbandry process, or if husbandry practices alter a fish's capacity to tolerate normal levels of infection, then parasites may become a welfare issue for captive housed fish (Barber, 2007).

3. Fish Welfare

The concept of animal welfare is complex and the word is used in a number of different ways (Dawkins, 1998; Appleby, 1999). In this article, we adopt a function-based definition (Huntingford et al., 2006) centre on an animal's ability to adapt to its present environment. Here good welfare requires that the animal be in good health with its biological systems functioning appropriately and not being forced to respond beyond their capacity (Huntingford et al., 2006).

Browman and Skiftesvik (2011) warning that while concerns about the welfare of aquatic organisms are valid, research on this topic should be grounded in the scientific method, strictly respect the science boundary. There is no single measure of welfare and although a wide range of physiological, biochemical and behavioral measures are used to assess welfare, none of these are considered reliable in isolation and multiple measures need to be taken (Broom, 1997).

Physical health is the most universally accepted measure of welfare and is undoubtedly a necessary requirement for good welfare (Ashley, 2007). The relative condition factor (Kn) corresponds to the ratio between observed and theoretically expected weight for a given length (Ranzani-Paiva and Silva-Souza, 2004) and is a quantitative indicator of fish welfare (Vazzoler, 1996). Different species have differing biological and environmental requirements and show differing responses to aquaculture conditions (Ashley, 2007). In addition to the hepatosomatic relation (HSR) and gonadosomatic relation (GSR) factors, the relative condition factor (Kn) is an important tool for studying host–parasite relationships (Tavares-Dias et al., 2000; Lizama et al., 2006; Ranzani-Paiva and Silva-Souza, 2004; Yamada et al., 2008; Moreira et al., 2010;

Velloso and Pereira Jr., 2010; 2011). Adequate welfare standards are required to minimize stress and reduce the incidence of disease and its consequent impacts on production and profits (Hall et al., 2011).

4. Diseases in the context of aquaculture

Today, diseases are generally viewed by science as an integral part of the existence of all animals and plants, including both cultured and wild populations (Bergh, 2007). Multiple factors interact and influence the outcome of pathogen transmission and disease development (Rückert et al., 2009; Krkošek, 2010; Johansen et al., 2011). Improvements in the understanding of fish diseases have been made in recent years with greater focus now on fish welfare (Bostock et al., 2010).

Aquaculture activities may alter the geographic distribution of diseases, or alter the host–parasite balance (Vincent and Font, 2003; Bergh, 2007). The severity of disease depends on the parasite capacity of infection and environmental quality in which fishes are kept (Krkošek, 2010; Martins et al., 2010). Once a disease outbreak ensues, effective health management requires three basic steps: problem identification, diagnosis, and corrective management—all of which must be performed in a timely manner to avoid further losses (Wise et al., 2004). Domestication of organisms unavoidably also involves 'domestication' of their pathogens (Bergh, 2007).

The public and scientific debate often oversimplifies the concept of welfare in aquaculture by neglecting the obvious reference to the state and condition of wild fish (Huntingford et al., 2006; Bergh, 2007). It is important to remember that poor health can be both a cause and a result of poor welfare (Ashley, 2007). A counter-intuitive negative association between parasitism and the 'health' of a fish stock suggests that parasites might be sensitive indicators of the status of a fishery (Lafferty, 2008). It can be hypothesised however, that increased population density enhances disease proliferation unless improved prophylactic measures are applied as an integrated part of the development of the aquaculture industry (Johansen et al., 2011).

The ultimate goal of aquaculture could not, and thus should not, be a mythical disease-free cultured animal, but rather an animal for which the impact of diseases and general level of welfare is acceptable, at least when compared to wild individuals of the same species (Bergh, 2007). In this way, biosecurity can be understood as the management of biological risks in a comprehensive and systematic manner to protect the health and welfare of animals, plants and people, and to maintain the functions and services of ecosystems (FAO, 2010).

5. Pharmacological treatment is really necessary?

A better understanding of what pathogens currently exist in aquatic ecosystems can help to determine natural distributions of pathogens and hosts and identify if, and how, new diseases or parasites are introduced. To promote health and minimize stress, fish should be provided with their basic needs: sufficient space, good water quality, a nutritionally complete diet, limited physical disturbance, protection from predators, and prudent handling (Wise et al. 2004). Thus, the development of Best Management Practices and codes of conduct can improve biosecurity and reduce the risk of disease transmission (FAO, 1995; NACE, 2000).

The application of risk analysis offers an effective management tool whereby, despite limited information, pragmatic decisions can be made that provide a balance between competing environmental and socio-economic interests (FAO, 2010). The side effects and risks cannot be completely eliminated; instead we should actively choose the level of impact on animal welfare and impact on the environment that we are willing to accept (Bergh, 2007).

Whenever multiple factors contribute to the disease process, it makes the diagnosis more difficult and often complicates corrective management (Wise et al., 2004). Environmental standards have been developed for many of the compounds used as medicines by aquaculture and food safety standards, designed to protect consumers from exposure to potentially harmful medicinal and other chemical residues, are driving more responsible use (Hall et al., 2011). Development and refinement of methods to minimize the side effects of prophylaxis and environmental impacts of disease outbreaks and treatment will be increasingly important tasks (Bergh, 2007). However, this tool needs research, databases and other vital sources of information and knowledge so that it can effectively support biosecurity assessments, surveillance, diagnostics, early warning, emergency preparedness and contingency planning (FAO, 2010).

Although technologies and measures for aquatic animal disease prevention, control and treatment have improved significantly in recent years, abuse of antimicrobials and other veterinary drugs and associated environmental and human health risks remain a major concern (Hall et al., 2011). The treatment of infected fish should be performed only if strictly necessary, since any type of prophylactic or therapeutic intervention can cause stress, which can further aggravate the state of health of these animals, which can often be quite debilitated by the action of parasites (Schalch et al., 2009).

6. Importance of aquaculture in the current scenario

All forward projections anticipate a need for increased supply of fish protein to meet the health needs and general aspirations of societies (Bostock et al., 2010). Fish representing about 10% of total exports of agricultural products by value, seafood exports from wild fisheries and aquaculture in 2008 had a combined value of US$102 billion an 83% increase from 2000 (FAO, 2010). Aquaculture now accounts for almost 50 percent of fish consumed by humans, and this share is expected to increase further to meet future demand (FAO, 2010).

The development of aquaculture has caused many problems to environment, however, there are signs that such problems are commonly confined to the early stages of intensification and can be overcome as the sector matures (Asche, 2008). The interactions of aquaculture with the environment needs to be evaluated in a rational way that allows the benefit of environmental services to be used but not over-exploited and impacted on (Bostock et al., 2010). There's no doubt that something will be lost in the transition to mass aquaculture, as fish — the last true wild food — are domesticated to support human beings, in much the same way we tamed cattle, pigs and chickens thousands of years ago (Walsh, 2011).

But if we're all going to survive and thrive in a crowded world, we'll need to cultivate the seas just as we do the land and aquaculture can be one more step toward saving ourselves (Walsh, 2011). Underlying development of sustainable aquaculture involves species selection, production systems, animal genetics, good health management, understanding of animal welfare and optimized feed and feeding (Bostock et al., 2010).

Aquaculture production systems offer unique opportunities for prevention and control of diseases that are not available to capture fisheries from the wild (Lima dos Santos and Howgate, 2011). Looking forward, there is strong focus on improving the efficiency of resource utilization through management and integration or more technological solutions available through advances in engineering and bioscience (Bostock et al., 2010).

7. Conclusions

Aquaculture may influence diseases of wild fish populations either by providing vectors for transmission of pathogens into new geographic areas, or by altering the balance in host–parasite dynamics by increasing the number of available hosts (Bergh, 2007). We also need to know more about diseases in fish, about the links between stress, immune function and disease states and therefore about the relationship between health and welfare (Huntingford et al., 2006). Modern industrial aquaculture with state-of-the-art prophylaxis probably represents a major improvement in controlling fish diseases, thus increasing fish welfare (Bergh, 2007).

However, the importance of aquaculture cannot be denied. In a world where 925 million people are still estimated to be undernourished in 2010 (FAO, 2010b), every initiative for food production should be welcomed. The contribution of fish to food and nutrition security will become increasingly important particularly in the African and Asian countries where there is growing domestic and regional demand (Hall et al., 2011).

Therefore aquaculture is indeed to optimize processes that already occur in nature. The quality of the food produced to be prioritized, but also the welfare of this "food" as a living organism under cultivation. Thus, we should consider parasites as part of this process, monitoring them, but without the claim of exclude them. So we can say that it is possible - and very important in the current scenario - to sustain the interaction between parasites, welfare and aquaculture.

Looking forward, research should incorporate these concepts into production, whereas the modern aquaculture is based on three pillars: profitable production, social development and preservation of the environment. Diseases are part of our nature, and when understanding like this, it is easier get along with their occurrence. Prophylaxis and surveillance should be the guiding of the work that consider modern aquaculture; treatment should only be used when strictly necessary, preventing introduction of drugs into the environment.

The knowledge of diseases in the natural environment - wild organisms - is fundamental to establish standards parameters for comparison if they occur in the culture environment. Prophylaxis studies and also the development of tools that can assist the producer in deciding the need and choice of treatment should be prioritized.

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DISCUSSÃO GERAL

A aquicultura pode influenciar na ocorrência de doenças em populações naturais de peixes, tanto pela introdução de patógenos em novas áreas geográficas como pela alteração da dinâmica hospedeiro-parasita (Bergh 2007). Nesse sentido, o conhecimento do parasitismo no ambiente natural é fundamental para estabelecer parâmetros para comparação, caso a doença venha a ocorrer no ambiente de cultivo.

No primeiro e no segundo capítulo, a relação entre a abundância do parasitismo, e os índices somáticos (IHS, IGS e Kn) foi verificada para cada espécie/grupo parasita, mas não houve correlação. Resultados semelhantes foram observados por Tavares-Dias *et al.* (2000), Lizama *et al.* (2006), Guidelli *et al.* (2009) e Tavernari *et al.* (2009). Parasitos com baixa patogenicidade podem ocorrer em alta abundância no hospedeiro sem influenciar negativamente seu fator de condição relativo (Kn) (Poulin 2007; Moreira *et al.* 2010). Estes resultados sugerem o equilíbrio entre hospedeiros e parasitos, o que é esperado para peixes em ambiente natural. Peixes que vivem em ecossistemas naturais raramente são livres de infecções e, frequentemente, os espécimes mais afetados não sobrevivem (Barber *et al.* 2000). Em ambientes artificiais como os cultivos, os peixes estão em risco de surtos agudos de infecções parasitárias, resultantes das facilidades oferecidas pelas altas densidades em que se encontram muitas vezes os hospedeiros (Barber *et al.* 2000). O fator de condição relativo é, assim, uma importante ferramenta para o estudo das interações parasita-hospedeiro (Lizama *et al*. 2006) e pode ser um indicador quantitativo do bem estar dos peixes (Vazzoller 1996). Portanto, pode-se sugerir que os índices parasitológicos, dentro dos limites encontrados em *Micropogonias furnieri* neste estudo são toleráveis, não influenciando o bem-estar do hospedeiro.

A comparação das amostras por ambientes do estuário, pré-limnico e estuarino, mostrou algumas diferenças na diversidade e intensidade de espécies ectoparasitas encontradas. Os resultados sugerem que a hirudinea *Myzobdella uruguayensis* Mañé Garzón e Montero, 1977 e o copepoda *Gauchergasilus euripedesi* (Montú, 1980) preferem a área pré-límnica, onde a água menos salgada é a condição dominante. Este é um resultado importante para o cultivo de *M. furnieri*, pois sugere que o controle destes parasitos pode ser realizado por regulação dos níveis de salinidade.

Entre os endoparasitos, houve uma diferença significativa nos valores de prevalência de Aspidogastridae na área pré-límnica em comparação com a área estuarina. Esta diferença pode ser explicada pelo ciclo de vida de Aspidogastridae spp., onde tanto moluscos como teleósteos marinhos são necessários para completar o ciclo de vida (Rohde 2001). O impacto da salinidade sobre interações parasito-hospedeiro é um regulador potencial dos processos de transmissão do parasito em áreas intertidais em que trematódeos são parasitas comuns, tanto de invertebrados como de vertebrados (Lei & Poulin 2011).

A maioria dos nematodas encontrados neste estudo estavam em estágio larval e pode-se verificar a presença de Anisakideos, parasitas comuns da corvina (Pereira Jr. & Costa 1996; Alves & Luque 2001; Pereira Jr. *et al*. 2004a; Pereira Jr. *et al.* 2004b). A maioria dos nematóides atinge a maturidade sexual por meio de um complexo ciclo de desenvolvimento envolvendo um hospedeiro intermediário ou possivelmente paratênico (Molnár *et al.* 2006). Mouritsen *et al.* (2010) relataram anisakideos no bacalhau do Atlântico (*Gadus morhua* Linnaeus, 1758) e no bacalhau da Gronelândia (*Gadus ogac* Kröyer, 1847) e compararam os níveis da parasitose com o fator de condição dos hospedeiros, verificando que não ocorre alteração no bem-estar dos hospedeiros, corroborando com os dados encontrados neste estudo. Apesar dos nematóides muitas vezes serem importantes patógenos, seu efeito patogênico direto é muito menos importante do que seu papel como agentes causadores de zoonoses (Molnár *et al.* 2006). As atitudes dos consumidores em relação à presença de nematóides em alimentos, especialmente pela estética do produto parasitado, pode ter um grande impacto sobre o valor de mercado do pescado, salientando a necessidade de rigoroso monitoramento (Molnár *et al.* 2006).

Parasitos têm sido reconhecidos como um importante componente da biodiversidade (Poulin & Morand 2004) e podem ser indicadores importantes da saúde de peixes e ambientes aquáticos, pois podem ser sensíveis a pesca intensiva e poluição, mostrando diminuição da abundância ao longo do tempo (Lafferty 2008). Parasitos podem afetar a saúde dos hospedeiros de muitas maneiras, porém, sua presença não é exclusivamente negativa (Sures 2008). Doenças são parte da natureza, e quando entendidas desta forma, torna-se mais fácil lidar com a sua ocorrência. Profilaxia e vigilância devem ser os princípios orientadores na aquicultura moderna; o tratamento deve ser usado apenas quando estritamente necessário, evitando a introdução de drogas no meio ambiente.

A aquicultura sustentável está baseada em três pilares: a produção lucrativa, desenvolvimento social e preservação do meio ambiente (Valenti 2002). Nesse sentido, são necessários mais estudos sobre doenças em peixes, sobre as relações entre o estresse, a função imune e estados de doença e, portanto, sobre a relação entre a saúde e o bem-estar (Huntingford *et al.* 2006). Estudos de profilaxia e também o desenvolvimento de ferramentas que possam ajudar o produtor a decidir a necessidade e escolha do tratamento devem ser priorizados.

Aquicultura deve ser a otimização de processos que já ocorrem na natureza. A qualidade dos alimentos produzidos deve ser priorizada, mas também o bem-estar deste "alimento" enquanto organismo vivo do cultivo. Dessa forma, os parasitos devem ser considerados como parte deste processo; devem ser monitorados, mas sem a pretensão de excluí-los. Assim, pode-se dizer que é possível - e muito importante no cenário atual - sustentar a interação entre os parasitos, bem-estar de peixes e aquicultura.

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CONCLUSÃO E RECOMENDAÇÕES

- Considerando o volume de informações já disponíveis sobre *Micropogonias furnieri* e os resultados encontrados neste trabalho, pode-se dizer que a corvina mostrou-se um modelo biológico apropriado para o estudo das interações entre parasitismo, bem-estar de peixes e aquicultura.
- O conhecimento das doenças no ambiente natural é fundamental para estabelecer parâmetros para comparações, caso venham a ocorrer no ambiente de cultivo.
- Os índices parasitológicos encontrados em *M. furnieri* na natureza, neste estudo, são toleráveis e não influenciam o bem-estar do hospedeiro.
- A comparação das amostras por ambientes do estuário, pré-limnico e estuarino, mostrou algumas diferenças na riqueza e intensidade de parasitos encontrados, como *Myzobdella uruguayensis*, *Gauchergasilus euripedesi* e Aspidogastridae.
- A salinidade parece ser um regulador potencial dos processos de transmissão do parasito, sendo uma informação importante para o cultivo de *M. furnieri*, pois sugere que o controle destes parasitos pode ser realizado por regulação dos níveis de salinidade.
- Profilaxia e vigilância devem ser os princípios orientadores na aquicultura moderna; o tratamento deve ser usado apenas quando estritamente necessário, evitando a introdução de drogas no meio ambiente.
- Estudos sobre profilaxia e também sobre o desenvolvimento de ferramentas que possam ajudar o produtor a decidir a necessidade e escolha do tratamento devem ser priorizados.

ANEXOS

ECTOPARASITAS

Região Pré-límnica - teste t single sample (Ho: Kn = 1,0)

Test of means against reference constant (value)

variables	mean	std dv	n	std err	-95%	95%	reference constant	t value	df	p
machos	0,970417	0,150339	24	0,030688	0,906934	1,033900	1,000000	-0,96400	23	0,345072
femeas	1,003223	0,119902	70	0,014331	0,974633	1,031812	1,000000	0,22486	69	0,822751
nao parasitado	1,007065	0,169457	33	0,029499	0,946978	1,067152	1,000000	0,23950	32	0,812248
parasitado	0,988237	0,100380	61	0,012852	0,962528	1,013945	1,000000	-0,91526	60	0,363717
sem hirudinea	1,000207	0,167622	37	0,027557	0,944319	1,056095	1,000000	0,00751	36	0,994049
com hirudinea	0,991367	0,096159	57	0,012737	0,965853	1,016881	1,000000	-0,67781	56	0,500685
sem copepode	0,990656	0,133231	80	0,014896	0,961007	1,020305	1,000000	-0,62727	79	0,532290
com copepode	1,018790	0,095815	14	0,025608	0,963468	1,074113	1,000000	0,73378	13	0,476108
sem monogenoidea	1,001215	0,130072	75	0,015019	0,971288	1,031142	1,000000	0,08088	74	0,935757
com monogenoidea	0,969709	0,121112	19	0,027785	0,911335	1,028083	1,000000	-1,09019	18	0,290009
1	0,996090	0,090288	32	0,015961	0,963538	1,028642	1,000000	-0,24498	31	0,808089
2	0,998072	0,125323	2	0,088617	-0,127912	2,124056	1,000000	-0,02176	1	0,986152
3	0,730002		1				1,000000			
1-2	1,006040	0,099431	7	0,037581	0,914082	1,097998	1,000000	0,16072	6	0,877588
1-3	0,958178	0,103080	14	0,027549	0,898661	1,017695	1,000000	-1,51807	13	0,152934
2-3	1,048372		1				1,000000			
1-2-3	1,044067	0,113865	4	0,056933	0,862883	1,225252	1,000000	0,77403	3	0,495316

Região Estuarina - teste t single sample (Ho: Kn = 1,0)

Test of means against reference constant (value)

variables	mean	std dv	n	std err	-95%	95%	reference constant	t value	df	p
machos	0,978965	0,099450	31	0,017862	0,942487	1,015444	1,000000	-1,17764	30	0,248199
femeas	1,007652	0,161365	56	0,021563	0,964439	1,050866	1,000000	0,35489	55	0,724032
nao parasitado	0,995138	0,108000	47	0,015753	0,963428	1,026848	1,000000	-0,30865	46	0,758979
parasitado	1,000125	0,176007	40	0,027829	0,943835	1,056415	1,000000	0,00449	39	0,996441
sem hirudinea	0,998658	0,151197	68	0,018335	0,962061	1,035255	1,000000	-0,07320	67	0,941866
com hirudinea	0,993038	0,108814	19	0,024964	0,940592	1,045485	1,000000	-0,27887	18	0,783527
sem n. argentinensis	0,992548	0,105405	66	0,012974	0,966637	1,018460	1,000000	-0,57433	65	0,567726
com n. argentinensis	1,012775	0,225451	21	0,049197	0,910151	1,115399	1,000000	0,25967	20	0,797774
sem n. avaginata	0,995524	0,127974	74	0,014877	0,965874	1,025173	1,000000	-0,30091	73	0,764342
com n. avaginata	1,008287	0,213678	13	0,059263	0,879163	1,137411	1,000000	0,13983	12	0,891115
1	0,983525	0,103520	18	0,024400	0,932046	1,035005	1,000000	-0,67519	17	0,508633
2	1,003692	0,252194	8	0,089164	0,792853	1,214532	1,000000	0,04141	7	0,968124
3	1,033266		1				1,000000			
1-2	1,164275		1				1,000000			
1-3	-	-	-	-	-	-	-	-	-	-
2-3	1,006205	0,223041	12	0,064386	0,864492	1,147919	1,000000	0,09637	11	0,924957
1-2-3	-	-	-	-	-	-	-	-	-	-

Estuário Total - teste t single sample (Ho: Kn = 1,0)

Test of means against reference constant (value)

variables	mean	std dv	n	std err	-95%	95%	reference constant	t value	df	p
machos	0,973855	0,122258	55	0,016485	0,940804	1,006906	1,000000	-1,58597	54	0,118585
femeas	1,004727	0,138351	126	0,012325	0,980334	1,029120	1,000000	0,38353	125	0,701979
nao parasitado	0,999075	0,136231	80	0,015231	0,968759	1,029392	1,000000	-0,06071	79	0,951745
parasitado	0,992392	0,132971	101	0,013231	0,966142	1,018642	1,000000	-0,57500	100	0,566582
sem copepoda	0,992504	0,136697	167	0,010578	0,971619	1,013388	1,000000	-0,70869	166	0,479513
com copepoda	1,029252	0,094846	14	0,025349	0,974490	1,084015	1,000000	1,15400	13	0,269276
sem hirudinea	0,994532	0,155178	105	0,015144	0,964501	1,024562	1,000000	-0,36110	104	0,718760
com hirudinea	0,996471	0,098776	76	0,011330	0,973900	1,019043	1,000000	-0,31145	75	0,756325
sem n. argentinensis	0,997914	0,119525	141	0,010066	0,978014	1,017815	1,000000	-0,20722	140	0,836143
com n.argentinensis	0,986293	0,177735	40	0,028102	0,929451	1,043136	1,000000	-0,48775	39	0,628459
sem n. avaginata	0,996898	0,127923	166	0,009929	0,977295	1,016502	1,000000	-0,31238	165	0,755147
com n.avaginata	0,978166	0,194812	15	0,050300	0,870282	1,086049	1,000000	-0,43408	14	0,670845
sem monogenoidea	0,998104	0,120334	139	0,010207	0,977923	1,018286	1,000000	-0,18574	138	0,852924
com monogenoidea	0,986218	0,173447	42	0,026764	0,932168	1,040268	1,000000	-0,51497	41	0,609338
1	1,009495	0,120540	2	0,085235	-0,073513	2,092504	1,000000	0,11140	1	0,929369
2	0,993781	0,096418	50	0,013636	0,966379	1,021183	1,000000	-0,45607	49	0,650356
3	0,957573	0,244032	9	0,081344	0,769993	1,145153	1,000000	-0,52158	8	0,616094
4	1,011554		1				1,000000			
5	0,973457	0,218967	22	0,046684	0,876373	1,070542	1,000000	-0,56856	21	0,575684
1-2	1,014630	0,099067	7	0,037444	0,923008	1,106252	1,000000	0,39072	6	0,709498
1-3	-	-	-	-	-	-	-	-	-	-
1-4	-	-	-	-	-	-	-	-	-	-
1-5	1,064739		1				1,000000			
2-3	0,987874	0,113502	13	0,031480	0,919286	1,056463	1,000000	-0,38519	12	0,706838
2-4	0,957858		1				1,000000			
2-5	0,981130	0,106863	15	0,027592	0,921951	1,040308	1,000000	-0,68391	14	0,505197
3-4	0,982196	0,218638	12	0,063115	0,843280	1,121111	1,000000	-0,28209	11	0,783111
1-2-3	1,055848	0,111300	4	0,055650	0,878744	1,232951	1,000000	1,003547	3	0,389538
1-2-4	-	-	-	-	-	-	-	-	-	-
1-3-4	-	-	-	-	-	-	-	-	-	-
2-3-4	0,916724		1				1,000000			
1-2-3-4	-	-	-	-	-	-	-	-	-	-

ENDOPARASITAS

Região Pré-límnica - teste t single sample (Ho: Kn = 1,0)

Test of means against reference constant (value)

variables	mean	std dv	n	std err	-95%	95%	reference constant	t value	df	p
NAO_PARA	0,967245	0,047777	3	0,027584	0,848560	1,085930	1,000000	-1,18747	2	0,356957
PARASIT	0,995757	0,130134	91	0,013642	0,968655	1,022858	1,000000	-0,31106	90	0,756471
Com Nema	0,994029	0,129266	89	0,013702	0,966799	1,021259	1,000000	-0,43575	88	0,664083
Sem Nema	1,009395	0,121959	5	0,054542	0,857963	1,160827	1,000000	0,17225	4	0,871602
Com Aspido	0,995209	0,108824	42	0,016792	0,961297	1,029121	1,000000	-0,28534	41	0,776824
Sem Aspido	0,994554	0,143178	52	0,019855	0,954693	1,034415	1,000000	-0,27428	51	0,784978
Machos	0,970417	0,150339	24	0,030688	0,906934	1,033900	1,000000	-0,96400	23	0,345072
Femeas	1,003223	0,119902	70	0,014331	0,974633	1,031812	1,000000	0,22486	69	0,822751

Região Estuarina - teste t single sample (Ho: Kn = 1,0)

Test of means against reference constant (value)

variables	mean	std dv	n	std err	-95%	95%	reference constant	t value	df	p
NAO_PARA	1,176418	-	1	-	-	-	1,000000	-	-	-
PARASIT	0,995349	0,141954	86	0,015307	0,964914	1,025784	1,000000	-0,30381	85	0,762012
Com Nema	0,995618	0,144278	83	0,015837	0,964114	1,027123	1,000000	-0,27667	82	0,782729
Sem Nema	1,035034	0,103903	4	0,051951	0,869701	1,200366	1,000000	0,67435	3	0,548401
Com Aspido	1,009730	0,102920	52	0,014272	0,981077	1,038383	1,000000	0,68172	51	0,498499
Sem Aspido	0,979158	0,186726	35	0,031563	0,915015	1,043300	1,000000	-0,66035	34	0,513476
Machos	0,978965	0,099450	31	0,017862	0,942487	1,015444	1,000000	-1,17764	30	0,248199
Femeas	1,007652	0,161365	56	0,021563	0,964439	1,050866	1,000000	0,35489	55	0,724032

Estuário Total - teste t single sample (Ho: Kn = 1,0)

Test of means against reference constant (value)

variables	mean	std dv	n	std err	-95%	95%	reference constant	t value	df	p
NAO_PARA	1,027900	0,093159	4	0,046580	0,879663	1,176137	1,000000	0,59898	3	0,591399
PARASIT	0,994610	0,134964	177	0,010145	0,974590	1,014631	1,000000	-0,53129	176	0,595889
Com nema	0,993973	0,135538	172	0,010335	0,973573	1,014373	1,000000	-0,58316	171	0,560552
Sem Nema	1,021582	0,105100	9	0,035033	0,940796	1,102369	1,000000	0,61605	8	0,554978
Com Aspido	1,001425	0,101193	94	0,010437	0,980699	1,022152	1,000000	0,13656	93	0,891670
Sem Aspido	0,988778	0,162707	87	0,017444	0,954100	1,023455	1,000000	-0,64334	86	0,521714
Machos	0,973855	0,122258	55	0,016485	0,940804	1,006906	1,000000	-1,58597	54	0,118585
Femeas	1,004727	0,138351	126	0,012325	0,980334	1,029120	1,000000	0,38353	125	0,701979