UNIVERSIDADE FEDERAL DO RIO GRANDE
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BIONOMIA DA CORVINA *Micropogonias furnieri* NO EXTREMO SUL DE SUA ÁREA DE OCORRÊNCIA, ATRAVÉS DA ANÁLISE QUÍMICA DE OTÓLITOS

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ÍNDICE

Resumo

O objetivo desta Tese foi estudar o ciclo de vida da corvina *Micropogonias furnieri* em relação a utilização de estuários, mudanças de habitat e interações entre estoques presentes na região costeira do Atlântico sudoeste, através da análise microquímica de otólitos. Entre 2004 e 2005 foram coletados otólitos de peixes adultos (idades entre 3 e 40 anos) no Brasil, Uruguai e Argentina e juvenis (0^+) no Brasil. Com os juvenis foi realizado um cultivo com duração de 3 meses para avaliar a influência de salinidade e temperatura na incorporação de Sr, Ba, Mg e Mn em otólitos *sagittae*. Otólitos de adultos e juvenis foram incluidos em resina, seccionados em serra metalográfica e analizados em “laser ablation inductively plasma mass spectromer”. Os resultados sugerem que temperatura influencia positivamente a incorporação de Mg e negativamente a de Sr. Os resultados apontam para o fato de que a concentração absoluta do elemento na água não é decisivo para sua incorporação nos otólitos, mas sim a relação do elemento com o Ca disponivel na água. A comparação de corvinas de água doce e marinha, demonstrou que as concentrações de Sr e Ba são bons indicadores de hábitat. O Sr aumenta com a salinidade e o bario diminui. As corvinas coletadas na Lagoa Mirim funcionam como padrões naturais para animais de água doce. A corvina foi caracterizada como espécie marinha estuarino dependente, que utiliza estuários nas fases iniciais de vida, podendo prolongar sua estadia nas proximidades destes locais por até 15 anos. Três padrões de mudança de hábitat de longo prazo foram observados. O padrão “A” inclui animais que tendem a ocupar hábitats cada vez mais marinhos ao longo da vida. O padrão “B” representa animais que, após saírem dos estuários, estabilizam sua movimentação em mais águas mais salgadas; e o padrão “C” considera indivíduos que passam toda a vida nas proximidades de zonas estuarinas. Foi constatada a existência de 3 estoques de corvina no Atlântico Sudoeste: entre Santos e Rio Grande; entre Rio Grande e Partido de la Costa, e nas proximidades de El Rincón. Estes estoques ocupam áreas definidas, porém com sobreposição, o que confere à espécie uma movimentação latitudinal com baixo grau de filopatria.

Abstract

The aim of this study was to evaluate the life cycle of *Micropogonias furnieri* regarding estuarine use, habitat change and stock interaction along south-west Atlantic, using otolith microchemistry. Adult fish were sampled between 2004 and 2005 in Brazilian, Argentinean and Uruguayan coastal waters. Juveniles were sampled at Patos Lagoon estuary, in order to evaluate the influence of salinity and temperature on Sr, Ba, Mg, and Mn incorporation in saggital otoliths. For adult and juvenile, otoliths were extracted, included in resin, cut with low speed saw and analyzed in laser ablation inductively coupled plasma mass spectrometer. Results showed Sr negatively and Mg positively influenced by temperature respectively. Salinity presented a complex influence. Apparently elemental incorporation in otoliths is preferably influenced by elemental concentration in the water relative to Ca (Me:Ca), and not by the absolute concentration of elements in the water. Sr and Ba were accurate indicators of habitat when otoliths from fresh and marine water fish were compared. When Sr increased towards higher salinities and barium decreased. *M. furnieri* from Mirim Lagoon lived for the entire life in that environment suggesting its use as natural standards for freshwater. The results revealed whitemouth croaker to be estuarine-dependent, using estuaries mainly during the early life. Some fish, however, stayed in the proximity of estuaries until 15 yr. Three patterns of long-term habitat change where found. Pattern “A” includes individuals that search for more marine habitats along the life. Pattern “B” indicates individuals that tend to stabilize the long-term migration in high salinity waters. Pattern “C” includes animals that spend the entire life in areas close to estuaries. There were no preferences of patterns between sample sites. It is suggested the existence of three *M. furnieri* stocks along south-west Atlantic Ocean: around Santos; between Uruguay and Prtido de la Costa; and, around Bahía Blanca. These stocks are reasonably overlapped, which suggests some degree of interdependency between the three countries. These results suggest *M. furnieri* to have some degree of phylopatry with latitudinal migration.

Prefácio

Esta Tese foi estruturada conforme o modelo alternativo proposto pelo Programa de Pós-Graduação em Oceanografia Biológica. Portanto, na primeira parte do trabalho, redigida em português, foram apresentados aspectos gerais da introdução, metodologia, resultados, discussão e conclusões. A segunda parte foi redigida em inglês e inclui artigos independentes e com objetivos específicos estruturados conforme as normas de cada revista onde o respectivo artigo foi ou será submetido. Cada anexo pode ser lido de maneira independente do restante da tese.

1. Introdução

1.1 – Espécie em estudo

A corvina *Micropogonias furnieri* (Desmarest, 1823) é uma espécie demersal pertencente à Família Sciaenidae. Ocorre desde as Antilhas até o golfo de San Matías, na Argentina (Chao, 1978), habitando águas doces (Anexo II), estuarinas (Castello, 1986) e costeiras até 100 metros de profundidade (Haimovici *et al.*, 1996). Tem preferência por substratos arenosos e lodosos e não realiza migrações verticais (Vazzoler, 1975). Quando juvenil, a corvina alimenta-se durante todo o dia, intensificando o forrageamento durante o período diurno e quando as condições de transparência da água são melhores, o que sugere um comportamento alimentar também associado a visão (Figueiredo e Vieira, 2005). Entre os itens alimentares mais frequentes da dieta de juvenis, estão os macro-invertebrados bentônicos, particularmente poliquetos e tanaidáceos (Gonçalves, 1997; Figueiredo e Vieira, 2005). Quando adulta, tende a se alimentar também de peixes e pequenos caranguejos (Campos, 1998).

O ciclo de vida da corvina é razoavelmente bem descrito. A desova é do tipo parcelada, sendo possível uma única fêmea de porte medio desovar entre três e sete milhões de ovos ao longo de uma estação reprodutiva (Macchi *et al*., 2003), que ocorre preferencialmente nos meses quentes e nas proximidades de estuários (Vazzoler, 1991), ou mesmo no interior destes (Macchi e Christiansen, 1996). Os ovos, que são planctônicos, eclodem em aproximadamente 20 a 24 horas e as larvas recém eclodidas medem em torno de 1,8 mm (Albuquerque, 2003). Ovos e larvas são transportados para zonas mais internas dos estuários, constituindo um dos taxa mais abundantes encontrados no ictioplâncton do estuário da Lagoa dos Patos (Muelbert e Weiss, 1991). No Rio Grande do Sul, o tranporte de ovos e larvas de corvina para dentro do estuário é favorecido pela ocorrência de ventos do quadrante sul que promovem a entrada de água salgada no estuário da Lagoa dos Patos (Möller et al., 1991). Quando ganham maior capacidade de natação, os pequenos juvenis procuram áreas de baixio, onde encontram melhores condições de segurança e alimentação (Gonçalves, 1997). À medida que crescem, ainda dentro dos estuários, vão buscando águas mais profundas e, em torno de 18 a 35 cm de comprimernto total (dependendo da latitude onde se encontra) atinge a maturação sexual, preferencialmente fora dos estuários (veja Vazzoler, 1991 para detalhes). Porém, alguns indivíduos permanecem dentro de estuários e tendem a amadurecer sexualmente mais cedo (Castello, 1986).

A corvina representa um dos recursos pesqueiros mais importantes do Oceano Atlântico Sudoeste (Haimovici e Umpierre, 1996). Apesar de sua ampla distribuição geográfica, se torna economicamente importante ao sul dos 23º de latitude sul, constituindo um dos principais recursos demersais do sul do Brasil (Vasconcelos e Haimovici, 2006), o principal do Uruguai (Norbis, 1995) e um dos principais da Argentina (Lasta e Acha, 1996). No sul do Brasil, as estimativas de biomassa desta espécie diminuiram de 200.000 t em 1996 para 70.000 em 2002 (Haimovici e Ignácio, 2005). A captura por unidade de esforço (CPUE) desta espécie ainda para o Sul do Brasil tem diminuido sistematicamente, caindo de aproximadamente uma tonelada por dia de pesca em 1980 para 0,2 tonelada em 2002 (Vasconcelos e Haimovici, 2006). Na zona comum de pesca entre Argentina e Uruguai, particularmente situada no estuário do Rio da Prata, tem sido observada uma diminuição das capturas anuais, de 60.000 t em 1995 para 30.000 t em 2003 (Carozza et al., 2004). Desta forma, atualmente é considerada um recurso sob forte sobrepesca (Vasconcellos e Haimovici, 2006).

Ao longo de sua distribuição na costa leste da América do Sul esta espécie apresenta variações em padrões de crescimento, reprodução e características morfométricas e merísticas (Vazzoler, 1991; Haimovivi e Umpierre, 1996; Norbis e Verocai, 2005), que levam a diferentes conclusões sobre quantos e quais são os estoques de corvina presentes nesta área. Contudo, de acordo com estudos genéticos, não existe separação entre as populações que ocorrem desde os 23º até os 40º de latitude sul (Maggioni *et al*., 1994; Levy *et al*., 1998; Puchnick-Legat e Levy, 2006;), mas ocorre uma separação significativa entre esses animais e a população existente no norte do Brasil (Puchnick-Legat e Levy, 2006).

Como se trata de uma espécie de elevada tolerância à salinidade e temperatura, o que caracteriza um animal eurióico, é razoável pensar que a corvina pode se movimentar livremente ao longo da costa sudeste da América do Sul, o que poderia ocasionar a elevada homogeneidade genética citada anteriormente. A possibilidade da livre movimentação ao longo da costa promoveu o interesse em se buscar uma ferramenta que não levasse em consideração características de crescimento, merísticas, morfométricas ou genéticas, mas que considerasse o espaço utilizado pelos animais ao longo de suas vidas, ou seja, a influência direta ambiente sobre os indivíduos.

Juntamente com esta dúvida sobre distribuição e identificação de estoques, surgiu o interesse em avaliar o tipo de relação ecológica que as corvinas apresentam com os estuários e que tipo de habitat os adultos buscam uma vez que deixam estes ambientes. A ferramenta aqui sugerida para se estudar estas questões é baseada na análise da composição química de otólitos, que será abordada a seguir.

1.2 Otólitos: Conceitos e Aplicações

Todos os animais vertebrados apresentam estruturas similares em seus ouvidos, particularmente no que se refere aos canais semi-circulares, cuja função mais básica consiste em perceber a posição da cabeça relativamente à força da gravidade (Manley, *et al*. 2004). O ouvido interno da maioria dos vertebrados não mamíferos contém três órgãos otolíticos, também conhecidos como as câmaras sacculo, lagena e utrículo, que apresentam em seus interiores cristais de carbonato de cálcio (Popper e Lu, 2000). Para a maior parte dos vertebrados esses cristais formam uma massa, contudo, em peixes teleósteos esses cristais são solidificados formando uma estrurura cristalina e densa denominada otólito (Popper *et al*., 2005).

Os otólitos crescem através da deposição concêntrica de camadas de carbonato de cálcio e de uma proteína de alto peso molecular chamada otolina (Campana, 1999). Esse crescimento em forma de camadas induz à formação de anéis de crescimento diários, comumentes observados em otólitos de larvas e juvenis (Pannela, 1971). Ao longo do ano, fatores como flutuação da temperatura, reprodução e/ou migração podem induzir à formação de camadas hialinas e opacas (Reibish, 1899), que para *M. furnieri* estão associadas aproximadamente às estações quentes e frias, respectivamente (Schwingel e Castello, 1990). A função fisiológica destas estruturas está fortemente ligada à audição e ao equilíbrio do animal na coluna d’água. De acordo com a espécie, os peixes podem ouvir sons em frequências desde 50 até 180.000 Hz (Mann *et al.*, 2001). Aparentemente o tamanho do otólito interfere nas características auditivas dos peixes. Grandes otólitos estão relacionados a melhor percepção de baixas frequências enquanto menores otólitos possibilitam a audição de frequências mais altas (Fay, 1988). A corvina também é conhecida como “roncador”. Ela emite sons de baixa frequência, que muito provavelmente são percebidos pelos outros indivíduos de mesma espécie, tendo em vista o grande tamanho de seus otólitos *sagittae*.

Além de ser uma estrutura que marca o tempo de vida dos peixes teleósteos, os otólitos também apresentam outras duas características que possibilitaram os mais recentes avanços a cerca do estudo da história de vida dos peixes. A primeira é que, a medida que o animal cresce, além de carbonato de cálcio e otolina, são depositados outros elementos em pequenas concentrações, que guardam uma relação com sua disponibilidade na água em que os peixes se desenvolvem (Fowler *et al.*, 1995). A segunda seria que, após serem depositados o carbonato de cálcio, a otolina e estes elementos menores, os otólitos não sofrem transformações ou modificações químicas, o que significa dizer que são quimicamente inertes (Campana e Neilson, 1985). Portanto, se marcam o tempo de vida e registram informações químicas relativas ao estado do meio ambiente, os otólitos guardam a “data” na qual estas informações foram adquiridas. Como não modificam estas informações ao longo do tempo, é possível determinar que condições ambientais os peixes experimentaram ao longo de suas vidas.

Com base nestas características, estudos com otólitos passaram a avaliar também migração, identificação de estoques, determinação de locais de nascimento e reconstruções de temperatura e salinidade do ambiente (veja Campana, 1999 para revisão). Para se extrair estas informações químicas dos otólitos, algumas técnicas desenvolvidas pela química analítica têm sido sugeridas e serão mencionadas a seguir.

1.3. Extraindo informações químicas de otólitos

A química analítica disponibiliza hoje de várias técnicas para analise de materiais carbonáticos, sejam de origem biológica ou mineral. Particularmente para a análise de otólitos, duas abordagens são principalmente utilizadas:

(1) Análises de dissolução do otólito inteiro (ex. Volpedo e Cirelli, 2006): esta modalidade analisa amostras em estado líquido, sendo portanto necessária a digestão ácida dos otólitos. Embora frequentemente utilizada, esta abordagem descarta toda a informação temporal existente nos otólitos e disponibiliza ao pesquisador um valor médio de concentração para cada elemento analisado. É principalmente usada para comparação de estoques e/ou populações, uma vez que se espera que animais vivendo em locais distintos, apresentem diferentes assinaturas químicas em seus otólitos. As ferramentas mais utilizadas para o desenvolvimento desta abordagem são espectrometria de absorção atômica (AAS), espectroscopia de emissão atômica através de plasma indutivamente acoplado (ICP-AES) e espectrometria de massas através de plasma indutivamente acoplado (ICPMS).

(2) Análises de seções de otólitos: Para esta modalidade os otólitos são cortados em seções, sendo mantida a integridade das informações temporais. São utilizados equipamentos específicos para extrair o material dos otólitos, que são acoplados aos equipamentos que medem as concentrações dos elementos que se deseja analisar. Entre os equipamentos mais utilizados estão o indutor de partículas por emissão de raios-X (PIXE), que permite a análise de porções de otólitos com diâmetros entre 3 e 20 µm (veja Secor e Rooker, 2000 para detalhes), e espectrometria de massas através de plasma indutivamente acoplado com amostras extraídas a laser (LA-ICPMS), que permite análise em áreas com diâmetros superiores a 5 µm (veja Belloto e Miekeley, 2000 para detalhes)

LA-ICPMS foi a técnica usada neste trabalho. É utilizada como método para análises multi-elementares em amostras sólidas biológicas e geológicas. Esta técnica é fragmentada em dois processos acoplados e independentes. O primeiro é a extração da amostra, que acontece através da ablação a laser (LA). Durante o processo de ablação, parte do material exposto ao pulso de laser é vaporizada, parte é liquefeita e parte é arrancada da amostra em fase sólida, formando uma cratera de tamanho previsível (Yilbas *et al.*, 2008). Imagens da cratera formada pela ablação a laser em otólitos podem ser vistos no anexo II do presente estudo. Após extraído da superfície dos otólitos, o material é transportado para o ICPMS juntamente com um fluxo de argônio de aproximadamente 15 L s^{-1}, através de tubos de teflon.

Explicando de maneira resumida, ao entrar no ICPMS o material é bombeado pelo centro de uma tocha de argônio indutivamente acoplado, onde atinge uma temperatura que varia de 5.000 a 10.000 ^{o}C. Nesta temperatura, a amostra passa para estado de plasma (gás em altíssimas temperaturas) ou matéria atomizada, ou seja, as moléculas são dissociadas em íons. Estes íons são direcionados para dentro do ICPMS, passando por duas aberturas consecutivas, sendo dirigidos a uma câmara com 4 cilíndros paralelos (quadrupolo) aos quais é aplicado um potencial elétrico, que funciona como imã, atraindo os íons que não interessam à análise. Dessa forma, apenas os elementos de interesse são quantificados, atingindo uma sensibilidade da ordem de partes por milhão, bilhão, ou até trilhão, dependendo do elemento analisado e do equipamento utilizado (veja Eggins, 1997 para mais detalhes).

Diversos elementos têm sido quantificados com objetivos diversos. Entre eles, o mais estudado é o estrôncio. Esse metal existe em maiores concentrações em águas salgadas e tem sido usado como ferramenta para o estudo da migração de várias espécies (Secor e Rooker, 2000). Desta forma, um animal que vive em águas mais salgadas deve apresentar em seus otólitos concentrações mais altas de estrôncio que outro animal da mesma espécie que vive em águas mais doces (vide anexo II). Outro elemento bastante utilizado como forte indicador de águas doces é o bário (Vries *et al*., 2005). Este elemento é proveniente do sedimento erodido dos continentes e transportado pelos rios (Nozaki *et al*., 2001). Portanto, animais que vivem em áreas de água doce tendem a apresentar maiores concentrações de bário em seus otólitos (vide anexo II). Contudo, foi evidenciado que as concentrações desses elementos nos otólitos se devem não somente à sua concentração absoluta na água, mas principalmente à concentração relativa ao cálcio (Elsdon and Gillanders, 2005; anexo I). Outros elementos como magnésio, manganês, zinco, dentre outros, têm sido utilizados principalmente para a geração de assinaturas químicas mais complexas e, portanto, mais específicas para determinado local.

2. Objetivo e Hipóteses

O objetivo geral desta tese é avaliar a ocupação espacial da corvina *M. furnieri* durante sua história de vida entre o sudeste do Brasil e o norte da Argentina, a partir da análise da constituição química de otólitos.

Objetivos específicos:

A) Avaliar a possibilidade de se adquirir informações bio-ecológicas a partir da análise química de otólitos de corvina (Anexos I e II).

Hipótese 1: A concentração de elementos químicos em otólitos de corvina não é influenciada por salinidade e temperatura.

B) Estudar o uso de estuários e mudança de hábitat da corvina, ao longo do seu ciclo de vida (Anexo III).

Hipótese 2: A concentração de estrôncio em otólitos de corvina durante primeiro e segundo anos de vida é semelhante à concentração esperada para otólitos de animais que utilizaram estuários nesta fase de vida.

Corolário: Se esta hipótese for verdadeira, conclui-se que os animais amostrados passaram a fase inicial de vida (1 e 2 anos) em áreas estuarinas.

Hipótese 3: Uma vez que as concentrações médias anuais de estrôncio em otólitos ultrapassam os valores esperados para áreas estuárinas, seus valores permanecem estáveis, não havendo variações para o resto da vida.

Corolário: Se esta hipótese for verdadeira, após deixar os estuários, a corvina tende a manter-se em um habitat definido (com relação a salinidade) para o resto da vida.

Hipótese 4: Os parâmetros K e Sr_{∞} provenientes das curvas de mudança de habitat são semelhantes entre todos indivíduos amostrados.

Corolário: Se esta hipótese for verdadeira, a corvina apresenta apresenta uma única estratégia de mudança de hábitat ao longo do extremo sul de sua distribuição.

C) Estudar a interação entre grupos populacionais de corvina entre Brasil, Uruguai e Argentina (Anexo IV):

Hipótese 5: Não existem diferenças significativas entre as composições químicas de otólitos provenientes do Brasil, Uruguai e Argentina.

Corolário: Se esta hipótese for verdadeira, existe um único estoque de corvinas ao longo do extremo sul de sua distribuição.

O detalhamento de cada objetivo é apresentado nos artigos (anexos) na seguinte ordem:

Objetivo A – Artigos (anexos I e II): “The effect of temperature and salinity on the uptake of elemental constituents in otoliths of a sciaenid fish” e “A *Micropogonias furnieri* metapopulation trapped in a freshwater coasta1 lagoon as a natural standard for otolith microchemistry”.

Objetivo B – Artigo (anexo III): “Long-term habitat change in a long-lived sciaenidae fish: an approach based on otolith microchemistry”.

Objetivo C – Artigo (anexo 4): “Evidence for *Micropogonias furnieri* stock segregation at Southwester Atlantic Ocean, as revealed by otolith microchemistry”.

3. Material e Métodos – linhas gerais

3.1. Coleta de material

As corvinas coletadas para este trabalho são, em sua maioria, provenientes da pesca industrial realizada no Brasil, no Uruguai e na Argentina (vide mapa do anexo IV). As amostragens foram realizadas entre 2004 e 2005 através de visitas a empresas de pesca dos três países. No Brasil, as amostragens foram realizadas em Santos e no Rio Grande do Sul (Rio Grande). Amostras adicionais foram coletadas na Lagoa Mirim (interior do Rio Grande do Sul e divisa com Uruguai) e no estuário da Lagoa dos Patos, onde foram coletados os juvenis utilizados no experimento referente ao anexo I. No Uruguai, as amostras foram coletadas no porto de Montevidéo e foram provenientes da costa uruguaia do Rio da Prata. Na Argentina, as coletas foram realizadas em Mar del

Plata e foram principalmente provenientes da área de pesca situada nas proximidades das coordenadas 35º S e 56º W, área denominada “Partido de la Costa”. Uma pequena amostra foi tomada em El-Rincón, Bahia Blanca.

Exceto os juvenis coletados para o experimento (anexo I), todos os indivíduos tiveram os otólitos *sagittae* removidos, limpos e acondiconados em envelopes de papel. Foram registrados dados referentes a comprimento total e sexo de cada exemplar. Em laboratório, os otólitos foram emblocados em resina e cortados em serra metalográfica, tão proximo ao núcleo quanto possível. As seções, de aproximadamente 0,5 mm foram fixadas em lâminas histológicas com cola de secagem rápida e guardadas para posterior análise química.

3.2 – Experimento salinidade x temperatura

Os juvenis coletados na Lagoa dos Patos foram transferidos à Estação Marinha de Aquicultura, onde foram aclimatados e receberam, todos juntos, um banho de 24 horas em solução salina de alizarina a 150 ppm, para marcar o início do experimento com o objetivo de avaliar a influência de salinidade e temperatura na deposição de Sr, Ba, Mg e Mn. O experimento foi realizado em duas mesas com temperatura (14 e 24 ^{o}C) controlada. Em cada mesa foram colocados nove tanques de 15 litros cada, sendo 3 tanques submetidos a cada salinidade (6, 16 e 32). Cada tanque recebeu 6 juvenis de corvina. Os animais foram mantidos por 3 meses sob mesma alimentação, fotoperíodo (12 x 12 h) e regime de troca de água (90% ao dia). Ao término deste experimento, os juvenis foram anestesiados através de banhos em solução de benzocaína a 50 ppm e congelados para posterior análise. De cada juvenil foram extraídos os otólitos *sagittae*, que foram processados da mesma forma que os otólitos dos adultos.

3.3. Análises químicas

A quantificação das concentrações de Sr, Ba, Mg e Mn foi realizada na Pontifícia Universidade Católica do Rio de Janeiro, através de uma colaboração realizada com o Laboratório de Espectrometria de Massas do Departamento de Química. Foram realizadas 3 visitas, totalizando aproximadamente um mês de trabalho analítico.

Os equipamentos utilizados foram um sistema de ablação a laser Nd-YAG (CETAC, LSX 100) operando a 266 nm de comprimento de onda e potência de 0,4 – 0,6 mJ pulso^{-1}, e dois ICPMS (ELAN 5000 e 6000) operando em potências de respectivamente 1300 e 1500 watts. Os aparelhos foram calibrados através de padrões de carbonato de cálcio contendo quantidades conhecidas de cada elemento. Essa calibragem possibilitou a obtenção de limites de detecção de Sr = 4,9 ppm; Ba = 0,78 ppm; Mg = 0,73 ppm; e Mn = 0,23 ppm. O ajuste dos equipamentos permitiu a realização de perfis com largura entre 15 e 30 µm, que levam a uma resolução temporal de aproximadamente 3 a 4 meses de vida do peixe analisado.

Para os animais adultos, os perfís foram realizados do núcleo até a borda de cada otólito. Para os juvenis, foram realizados perfis paralelos à borda, logo após a marca gerada pela alizarina, para garantir que somente o material depositado durante o período de cultivo fosse considerado. Uma varredura adicional foi realizada nestes otólitos com a finalidade de determinar as concentrações de estrôncio características do estuário da Lagoa dos Patos.

3.4. Análise estatística

A análise estatística foi empregada de acordo com o objetivo específico de cada estudo. Para o experimento (anexo I) foi aplicada análise de variância fatorial

(ANOVA) e teste de Tukey, para comparação entre as concentrações de cada elemento entre os tratamentos de salinidade e temperatura. Esta mesma ferramenta foi aplicada para a determinação de assinaturas químicas em otólitos para os diferentes ambientes (anexo II). Para a avaliação de uso de habitat (anexo III) foram aplicadas funções logísticas e exponenciais com a finalidade de modelar e caracterizar respectivamente o tempo de escape da influência estuarina (TEEI) e a mudança de hábitat de longa duração. Análise de variância foi utilizada para comparar os parâmetros das funções exponenciais entre locais; teste do Qui-quadrado, para avaliar diferenças quanto à direção da migração observada no inicio da vida dos adultos; e, escalonamento multidimensional para avaliar se as funções obtidas para mudança de habitat de longa duração formaram grupos significativamente distintos. Para a avaliação de conexão entre os estoques amostrados (anexo IV), foi aplicado escalonamento multidimensional e análise de similaridade para testar sobreposição entre os estoques em função dos quatro elementos analisados. As concentrações médias de cada elemento químico também foram comparadas entre locais através de análise de variância.

4. Síntese dos resultados

Nas condições experimentais foi observado que a deposição de Sr, Ba, Mg e Mn nos otólitos de corvina são influenciados por temperatura e salinidade de forma bastante complexa (ver figura 2 do anexo I). Aparentemente, a salinidade, por si só, tem pouca influência na incorporação destes elementos, sendo mais importante a concentração na água de cada metal relativa à concentração de cálcio (razão Me:Ca). O único elemento que apresentou uma tendência clara foi o bário, com maiores concentrações observadas em salinidades mais baixas. Porém, a razão Ba:Ca também cresceu com a diminuição da salinidade. A temperatura influenciou negativamente a incorporação de Sr e positivamente a incorporação de Mg.

Em condições ambientais (anexo II), verificou-se que Sr e Ba apresentam tendências antagônicas. Estrôncio apresentou maiores concentrações para otólitos de animais provenientes de águas salobras e salgadas e bário para águas mais doces. Também observou-se que não há influência ontogenética na incorporação de Sr. Bário apresentou um forte aumento de incorporação ao longo do primeiro ano de vida, podendo se alongar até 5 anos para animais vivendo em água doce (vide figuras 4 e 5 do anexo II). As concetrações de Sr e Ba determinadas para água doce, estuarina e marinha podem ser vistas na figura 6 do anexo II.

Analisando os perfís de Sr ao longo da vida das corvinas (anexo III), foi constatado que enquanto juvenil, a corvina realiza uma busca ativa por salinidades mais baixas. Foi observado que o tempo necessário para que as concentrações de Sr nos otólitos apresentassem valores superiores aos valores calculados para influência estuarina (TEEI) variou de 4 (para Rio Grande) a mais de 7 anos (Uruguai). Quatro padrões de mudança de habitat foram observados, de acordo com as tendências de crescimento das concentrações de Sr em função da idade (vide figura 4 no anexo III) . O padrão “A” caracteriza os animais que apresentam uma concentração de Sr nos otólitos crescente ao longo de toda a vida. O padrão “B” inclui animais cuja concentração de Sr atinge um patamar elevado, mas estabiliza depois de certa idade. O padrão “C” apresenta concentrações de Sr baixas e muito próximas àquelas esperadas para áreas de influência estuarina. O padrão “D” descreve animais que viveram sempre em água doce. Estes animais entraram na Lagoa Mirim quando jovens e ficaram aprisionados após a construção da eclusa, no canal de São Gonçalo, que liga a Lagoa Mirim à Lagoa dos Patos. Não houve predominância significativa de nenhum padrão para os diferentes locais amostrados ao longo da costa do Atlântico sudoeste. Isso sugere que não há organização latitudinal de comportamentos de mudança de habitat, ou seja, esta espécie apresenta uma estratégia de dispersão para todos os locais em que possa sobreviver.

As análises de escalonamento multidimensional e de similaridades para as concentrações de Sr, Ba, Mg e Mn (anexo IV), indicou alto grau de separação para o primeiro ano de vida entre animais da Lagoa Mirim e das zonas costeiras (Argentina, Uruguai e Brasil). Analisando apenas animais da zona costeira, para o primeiro ano de vida, observou-se uma boa separação entre Santos e Argentina, Santos e Uruguai e Santos e Bahía Blanca. Uruguai e Mar del Plata foram estatisticamente similares, mas apresentaram razoável separação dos animais de Bahía Blanca. Rio Grande não apresentou um padrão estatisticamente diferente dos demais locais. Ao analizar-se o último ano de vida, Rio Grande apresentou boa separação do estoque Platense (Uruguai + Partido de la Costa) e de Bahía Blanca, mas mostrou-se similar ao estoque de Santos.

5. Avaliação das hipóteses e discussão geral dos resultados

5.1. Informaçõe ambientais em otólitos de corvina

A hipótese 1 aborda a possibilidade de que mudanças de ambiente sejam registradas através da incorporação de elementos químicos em otólitos de corvina. Esta hipótese foi recusada, uma vez que a deposição de elementos químicos em otólitos de corvina foi influenciada por salinidade e temperatura (anexos I e II).

Embora a salinidade sozinha não aparente influenciar significativamente a deposição de Sr, Ba, Mg e Mn nos otólitos, a temperatura foi significativamente uma influência positiva para Mg e negativa para Sr (Anexo I). Uma clara diferença ficou evidenciada entre as concentrações de Sr e Ba em otólitos de animais que viveram em ambientes diferentes (Anexo II).

Estes resultados em princípio parecem contraditórios, pois a salinidade não influenciou a deposição de Sr em condições experimentais, mas influenciou no ambiente. Com base nessa aparente contradição, surge a questão do que seria mais importante para a incorporação de Sr e Ba nos otólitos: A concentração absoluta destes elementos na água ou sua composição relativa ao Ca? Estudos recentes mostram que a composição relativa é o principal agente influenciador da incorporação de elementos em otólitos (Elsdon and Gillanders, 2005). Isso significa que se a salinidade variar e as razões Me:Ca na água não, a concentração desses elementos nos otólitos tende a se manter constante para diferentes niveis de salinidade. Contudo, na natureza, as razões mudam entre os ambientes dulcícolas, estuarinos e marinhos (Nozaki *et al*., 2001; Elsdon e Gillanders, 2005) e esta mudança acaba refletida nos otólitos. Isso significa que é possível verificar se um animal passou por água doce, estuarina ou marinha, embora para este trabalho, não seja possível, ainda, determinar numericamente as salinidades experimentadas pelos indivíduos ao longo de suas vidas. Portanto, estudos futuros deverão levar em consideração a composição da água de lagoas, estuários e zonas costeiras onde se pretenda trabalhar.

5.2. Sobre o uso de estuários e mudanças de hábitat

Estas hipóteses abordam o tipo de uso dos estuários e à estratégia de vida observada após a fase de adultos desta espécie. A hipótese 2 foi aceita e as hipóteses 3 e 4 foram recusadas, ou seja, a corvina utiliza estuários e águas salobras no início de sua vida (hipótese 2); após sairem dos estuários, os indivíduos, em sua maioria, continuam buscando águas mais salgadas (hipótese 3); e, foram observados quatro padrões de mudança de hábitat ao longo da vida (hipótese 4).

A aceitação destas três hipóteses implica na descrição de um comportamento complexo de utilização estuarina em fases iniciais ou mesmo em fases mais adiantadas do ciclo de vida da corvina. De acordo com a hipótese 2, sugere-se que a corvina seja classificada como uma espécie marinha estuarino-dependente, ou seja, uma espécie que necessita de estuários para completar seu ciclo de vida. A ausência de indivíduos que não apresentam em seus otólitos sinais químicos da utilização de estuários (figura 2, anexo III), permite sugerir que indivíduos que não tiveram contato com estes ambientes, não representem uma parcela significativa dos estoques adultos. Pelo menos não em número suficiente para que sejam detectados em uma amostra como a realizada pelo presente trabalho. Sendo assim, o conceito de estuarino dependência proposto por Darnell e Soniat (1979): “espécies que usualmente requerem estuários para algum momento do seu ciclo de vida” parece se adequar bem à espécie aqui estudada. Uma teoria complementar a esta sugere que sejam consideradas estuarino dependentes aquelas espécies cuja perda do estuário poderia afetar adversamente suas populações (Whitefield *et al.*, 1994). Portanto, as evidências aqui apresentadas estão de acordo com ambas as teorias.

Com relação ao complexo modo pelo qual juvenis ou pre-adultos de *M. furnieri* migram de hábitat estuarino para marinho e mesmo dentro de habitats marinhos (hipótese 3 e 4), foi observado que as idades determinadas para “fuga” da influência estuarina são bastante variáveis entre locais. Foi observado um maior tempo de permanencia em áreas de influência estuarina para animais coletados no Uruguai, onde ocorre justamente uma maior influência das águas do Rio da Prata (Plata Plume Water – PPW, Piola *et al.*, in press.). Os quatro padrões de mudança de hábitat de longa duração (ver Fig. 4, anexo III) parecem representar uma estratégia espécie-específica que objetiva distribuir indivíduos, o que minimizaria a competição intra-específica que pode acontecer em zonas de alta densidade populacional. Haimovici *et al.* (1996) observou que maiores corvinas (e provavelmente mais velhas) são capturadas preferencialmente em zonas mais profundas, o que corrobora os resultados deste trabalho, pois maiores concentrações de estrôncio foram encontradas para as idades mais avançadas, indicando uma tendência de movimentos para áreas mais salinas a medida em que os animais envelhecem. Contudo, essa maior concentração de estrôncio poderia significar também que animais mais velhos tendem a passar menos tempo em áreas de influência estuarina durante a reprodução. Desta forma fica claro que existe uma mudança para habitats mais marinhos, entretanto não se pode afirmar se essa mudança de habitat é função de movimentos para águas mas salgadas ou é função de menor tempo de permanência em águas mais salobras. Apesar disso, mudanças de habitat para áreas de maior salinidade podem estar associadas ao fato da disponibilidade de alimento aumentar no sentido água doce – salgada para animais adultos. Como foi observado para a costa do Brasil, Uruguai e Argentina, que a diversidade de crustáceos parece aumentar com profundidade e salinidade (Bremec e Gilberto, 2006; Capítoli e Bemvenuti, 2004; Gilberto *et al.*, 2004; Pires, 1992), sugere-se que este seja um dos possíveis fatores que motivem a mudança de habitat da corvina para áreas mais salgadas e profundas.

5.3. Interação entre estoques no Atlântico sudoeste

A hipótese 5 considera a existência de um estoque comum e contínuo que abrange o Atlântico sudoeste. Esta hipótese foi recusada em função da existência de estoques aproximadamente bem definidos ao longo da área de estudo ao se considerar a análise de assinaturas químicas em otólitos de corvina. Três estoques foram definidos, com razoável sobreposição: Entre Santos e Rio Grande, Entre Rio Grande e Partido de la Costa e em Bahía Blanca (vide anexo IV). Informações baseadas em microquímica de otólitos tendem a representar o espaço que os peixes utilizam ao longo da vida. Neste caso, deve se entender por espaço o conjunto de fatores físicos e químicos que ocorrem em determinado local. Portanto, estes resultados não contradizem as informações existentes na literatura, que sugerem uma distribuição contínua (estudos genéticos) ou uma distribuição particionada (caracteres merísticos, parâmetros populacionais), mas sim complementa estas informações. Os resultados deste trabalho evidenciam a existência de três grandes estoques, que apresentam alguma sobreposição entre si. Eles são representados por animais situados entre Santos e Rio Grande (estoque brasileiro), por animais situados entre Rio Grande e Mar del Plata (estoque Platense) e por animais que se concentram nas proximidades de Bahía Blanca (≈ 40º S). Estes três estoques apresentam um certo grau de sobreposição (Tab. 3, Anexo IV), que aparenta ser mais forte em Rio Grande. Uma vez que foram encontrados padrões de assinaturas químicas para diferentes locais, sugere-se que a corvina apresente alguma fidelidade regional. A ausência destes padrões para Rio Grande no primeiro ano de vida e consequente presença de um padrão para o último ano de vida, sugere que os animais coletados em Rio Grande sejam principalmente provenientes de outros locais, mas que mantiveram-se na costa de Rio Grande por tempo suficiente para que fosse gerado um padrão químico para este local. Contudo, Rio Grande é considerada uma das princiais áreas de desova da corvina na América do Sul, o que contradiz a possibilidade de que animais coletados em Rio Grande sejam principalmente provenientes de outros locais. É, portanto, possível que a forte variabilidade oceanográfica presente no estuário da Lagoa dos Patos e zona costeira adjacente contribuam para uma maior variabilidade na microquímica de otólitos, impedindo a formação de um padrão específico para animais provenientes desta área. Por outro lado, os animais coletados apresetaram diferentes idades. Sabendo que o Estuário da Lagoa dos Patos é um ambiente de forte variabilidade interanual, é esperado que estes animais tenham experimentaram diferentes condições ambientais durante sua fase juvenil. Isto também pode ter ocasionado a variabilidade observada na constituição química dos otólitos de animais provenientes de Rio Grande.

Esta sobreposição de estoques, ou grupos populacionais, implica na existência de fluxos de indivíduos entre Brasil, Uruguai e Argentina, corroborando resultados encontrados para estudos com parâmetros populacionais e caracteres merísticos (Vazzoler, 1991; Haimovici e Umpierre, 1996) e estudos genéticos (Levy *et al.*, 1998; Puchnick-Legat e Levy, 2006; Maggioni *et al.*, 1994). Portanto, parece haver uma migração latitudinal entre os três países, o que poderia ajudar a explicar as mudanças de hábitat de longa duração propostas no Anexo III.

6. Conclusão geral

Otólitos de corvina são capazes de acumular informações ambientais relativas a temperatura (Anexo I) e salinidade (Anexo II), que são expressas em diferenças nas concetrações de Sr, Ba, Mg e Mn nessas estruturas. Contudo, existe uma influência da ontogenia na deposição de Ba, Mn e Mg, aumentando as concentrações destes elementos nos estágios iniciais de vida. Estrôncio é particularmente indicado para avaliar mudanças de salinidade, pois não apresenta variação ontogenética, nem é influenciado por taxas de crescimento (Anexo II).

A corvina exibe um padrão complexo de mudança de hábitats ao longo do seu ciclo de vida (Anexo 3), requerendo estuários e águas salobras em fases iniciais e buscando águas mais salinas após a maturação sexual. Porém, esta busca por hábitats cada vez mais marinhos não é expressa por todos os indivíduos da população, implicando na existência de três padrões de mudança de hábitat, que ocorrem igualmente entre Brasil, Uruguai e Argentina. Estes padrões parecem constituir uma estratégia específica para disperção destes animais ao longo da plataforma, desde zonas mais costeiras, com forte influência de água doce (padrão 1) até áreas mais profundas e distantes do desague continental (padrão 3).

Foram identificados três grandes estoques de *M. furnieri* no Atlântico sudoeste, que apresentam diferentes graus de sobreposição. O primeiro estoque habita as proximidades de Santos, o segundo é situado entre Uruguai e Partido de la Costa (Argentina), e o terceiro habita as proximidades de Bahía Blanca (Argentina). Os animais capturados em Rio Grande não apresentaram assinaturas químicas que possibilitassem sua separação com relação aos outros estoques para o primeiro ano de vida. Para o último ano, foi observada uma razoável separação dentre animais de Rio Grande e dos demais locais, sugerindo um estoque fortemente sobreposto com os demais.

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ANEXO I

The effect of temperature and salinity on the uptake of elemental constituents in otoliths of a sciaenid fish

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The effect of temperature and salinity on the uptake of elemental constituents in otoliths of a sciaenid fish

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Abstract

The effect of salinity and temperature on the incorporation of strontium, barium, magnesium and manganese in otolith of juvenile *Micropogonias furnieri* was tested under controlled conditions in laboratory. Field captured juveniles were reared for three months under two temperatures (14 and 24 °C) and three salinity treatments (6, 16, 32). Otolith slices were analyzed using LA-ICPMS. Elemental analysis showed that absolute concentrations of Sr, Mg and Mn have increased with water salinity, but Me:Ca ratios of these elements remained approximately constant. Absolute concentration of Ba and Ba:Ca ratios in the water decreased with salinity. Results suggested negative effect of temperature on strontium and positive on magnesium incorporation in otoliths. Strontium was not affected by salinity during period of experiment. Barium was negatively affect by salinity, however, Ba:Ca ratios in the water decreased with salinities, which did not allow to evaluate the specific effect of salinity on its deposition. Magnesium did not show a clear pattern of incorporation among treatments. A strong interactive effect of salinity and temperature was observed for manganese, with a slight trend to decrease towards higher salinities. These results show that the incorporation of different chemical elements in otoliths responds differently to temperature and salinity and emphasize that otolith microchemistry studies should be developed as a species-specific issue.

1. Introduction

In recent years, microchemistry of carbonate-like structures in marine organisms has increasingly been used as a tool to understand their life history. Environmental history reconstruction has been developed from foraminifera (Rosenthal, et al., 1997), corals (Lea et al., 1989), mussel shells (Gillikin, 2005) and fish otoliths (Elsdon and Gillanders, 2003). Otoliths are solid structures composed mostly by calcium carbonate and little amount of non-collagenous organic matrix (Campana, 1999). They are located inside the inner ear of teleost fishes, and their major function is related to fish balance and hearing. Besides calcium carbonate and organic matrix, other elements have been found to compose otoliths in lower concentrations, and there is a general acceptance that their concentration in the otoliths reflects their availability in the surrounding waters (Campana, 1999). Once deposited, the constituents will not be reabsorbed or re-worked, characterizing otoliths as chemically inert structures (Campana and Neilson, 1985). Otolith analysis are also applied to age determination studies in annual (Reisbish, 1899) and daily basis (Pannela, 1971). Therefore, by associating their time-keeping property to the water chemical composition, otoliths are expected to store temporal environmental information.

In the past two decades many studies have been developed to determine the role of environmental factors on otolith elemental uptake (e.g. Elsdon and Gillanders, 2004; Bath and Wunschel, 2006), but controversy on the effect of salinity and temperature still remains. Compiled evidence suggests that otolith uptake of different trace elements respond differently to salinity and temperature (See Campana, 1999 for a review). For example, temperature effect on strontium incorporation in otoliths was found to be positive for *Leiostomus xamthurus* (Martin et al. 2004), negative for *Gadus morhua* (Townsend et al., 1995) and neutral for *Girella elevata* (Gallahar and Kingsford, 1996). Although salinity presents a generally positive effect on strontium deposition for *Acanthopagrus butcheri* (e.g. Elsdon and Gillanders 2002), a neutral effect has also been reported for *Sciaenops ocellatus* (Rooker et al. 2004). The opposite results shown in these studies reinforce that otolith uptake of different constituents responds differently to salinity and temperature and the role of their influence seems to be specie-specific (Geffen et al., 1998; Swearer et al., 2003). Considering the goal of otolith microchemistry studies as natural tools to infer the past environmental situations experienced by a fish, it becomes essential to understand how temperature and salinity influence elemental uptake in different species.

Whitemouth croaker (*Micropogonias furnieri* Desmatest) is an eurihaline sciaenid widely distributed along the eastern coast of the Atlantic Ocean, occurring from the Gulf of Mexico to the Gulf of San Matías (Argentina). It is a long-life and multiple spawner species (Haimovici, 1976; Macchi and Christiansen, 1992). Spawning has been registered in coastal and estuarine waters (Macchi et al., 1996; Acha et al., 1999; Jaureguizar et al., 2008), and in Southern Brazil it occurs mainly during spring and summer (Ibagy and Sinque, 1995; Haimovici and Ignácio, 2005). Juveniles are found inside Patos Lagoon estuary (South Brazil – 32°S and 52°W) during the entire year (Castello, 1986) and adults occur in coastal and estuarine areas during the spawning season. Whitemouth croaker supports important fisheries in the south-west Atlantic shelf (Lasta and Acha, 1996; Vasconcellos and Haimovici, 2006). Although several methods have been applied to determine stock differentiation between Argentina, Uruguay and Brazil, there is not a general agreement about stock dynamics in the region (see Volpeto et al., 2007 for a review). Additionally, the migratory behavior of *M. furnieri* between coastal and estuarine areas is not objectively understood.

Otolith elemental analysis represents an important tool that would be able to explain *M. furnieri* habitat change from estuarine to shelf environments and connectivity between stocks of Brazil, Uruguay and Argentina. In order to do so, the first step is to understand the role of salinity and temperature on otolith composition. Therefore, the aim of this study is to test the influence of salinity and temperature on the incorporation of strontium, barium, magnesium and manganese in otoliths of *M. furnieri*.

2. Materials and Methods

2.1. Experimental design

A three months experiment was setup to examine the influence of water salinity and temperature on trace element incorporation in otolith of juvenile whitemouth croaker. During winter of 2004, one hundred and thirty young of the year *M. furnieri,* of approximately 130 mm in total length, were collected in the Patos Lagoon estuary using a beach seine. At laboratory, all fish were gently transferred to a 200 L tank for acclimatization in 18^{o}C seawater. The experiment was projected to test the influence of two temperatures (14 and 24^{o}C) and three salinities (6, 15, and 32) on the incorporation of Sr, Ba, Mn and Mg to otoliths of *M. furnieri*. A total of 18 tanks were used, with three replicates for each combination of salinity and temperature (6 treatments). Temperature was controlled with 2 hot-cold regulated water tables set for 14 ^{o}C and 24 ^{o}C. Each water table received nine 15 L tanks with the 3 salinity treatments. Because of the large volume of water to be renewed daily (> 200 L) water salinity was controlled through direct dilution of seawater using tap water aired for 24 h.

After acclimatization, fish were put in a bath of alizarin complexone solution (24 h, 150 ppm), in order to mark new experimentally-produced otolith material. One hundred and eight fish were randomly selected and transferred to the experimental treatments, with density of 6 fish per 15 L tank. Fish that died during the first week were replaced in order to maintain the initial number. After the second and third week, dead fish were removed from the study. Fish that diet after one month of experiment were individually labeled and frozen for posterior analysis.

Photoperiod was set to 12:12 h and a commercial diet was offered once a day. During the entire experiment, 90% of the water and accumulated detritus were siphoned daily, and replaced with freshly prepared water. Three 300 L stock-tanks with water under controlled salinity were completed daily in order to guarantee the next day water change. At the end of the experimental period all fish were anesthetized with benzocain 200 ppm, and frozen in individual plastic bags for posterior analysis.

2.2. Otolith processing and chemical analysis

Saggital otoliths were extracted, washed, labeled and stored dry. Only left otoliths were embebbed in epoxy resin, sectioned through the core using low speed saw and separated in slices of 0.3 to 0.4 mm. Sections were mounted onto glass slides with crazy glue. Their surfaces were polished with silicon carbide paper (n^o 8000); washed with deionized (DI) water (Milli-Q, Millipore, Bedford, USA); ultrasonically cleaned for 3 minutes; and, rinsed three times with DI water. Slides were dried in horizontal flow cabinet before analysis.

Analytical measurements were performed with a Nd:YAG CETAC LSX 100 Laser Ablation system operating at 266 nm, coupled to an ELAN 6000 (PerkinElmer – SCIEX) inductively coupled plasma-mass spectrometer (ICP-MS). The laser was configured for Q-switched mode operation, defocused, a pulse frequency of 20 Hz, scan speed of 40 μm/s, and power of 0.4 to 0.6 mJ per pulse. Under these conditions the ablated crater in the otoliths presented diameter about 15 μm. The ablated material was conducted through a Teflon coated tube into the ICP-MS using Argon as carrying gas (0.85 dm^3 min^{-1}). The ICP was operated at 1500 W, with outer gas flow and intermediate gas flow of 15 and 1.1 dm^3 min^{-1}, respectively. The determined elements (masses) were ^{43}Ca, ^{86}Sr, ^{138}Ba, ^{24}Mg and ^{55}Mn.

The quantification was performed using the external calibration mode. Standards constituted by pressed powder $CaCO_3$ discs with known analyte concentration, and suprapur $CaCO_3$ discs were used as blanks. These standards were proposed and produced by Bellotto and Miekeley (2000; 2006). The measured signal (counts per second – cps) for each element was normalized with reference to ^{43}Ca for correction of the bias induced by differences on the amount of ablated material. The limit of detection (LOD) was obtained by scanning blank discs 10 times. Each mean profile was used as a single analyte concentration and the standard deviation (σ) from the 10 values was considered. The LODs were calculated as 3σ divided by the sensitivity (inclination) of the respective calibration curve and they were 0.47 $\mu g\ g^{-1}$ for barium, 4.9 $\mu g\ g^{-1}$ for strontium, 0.91 µg g^{-1} for magnesium and 0.39 µg g^{-1} for manganese.

2.3. Water analysis

Temperature and salinity were measured once a day for all 18 tanks. Three water samples were taken from the water stock-tanks per month for each salinity. Acid-washed vials were used to transport the water samples to be analyzed by an Optima 4300 DV inductively coupled plasma mass spectrometer (PerkinElmer). The equipment was set for external calibration mode using Titrisol® (Merck KGaA, Darmstadt, Germany) reference material as standards. Detection limits were 0.010 mg L^{-1} for calcium, 0.0002 mg L^{-1} for strontium, 0.0002 mg L^{-1} for barium, 0.0001 mg L^{-1} for manganese and 0.002 for magnesium.

2.4. Data analysis

Nominal experimental temperatures (14° and 24° C) and salinities (6, 16 and 32) will be used for data presentation and discussion. Partition coefficients (D_{Me}) between metals (Me) in otolith and in water were calculated for each treatment by dividing the mean Me:Ca ratio in otoliths by the mean Me:Ca ratio in the respective experimental water (from the stock-tanks). Differences on absolute elemental concentrations in otoliths and partition coefficients among treatments were tested for each element using Factorial Analysis of Variance (Factorial ANOVA). In order to satisfy ANOVA assumptions, data from both magnesium and barium were Ln(x + 1) transformed, but for visual purposes all elements are presented as absolute concentrations and partition coefficients. When significant effect of treatments were detected (Factorial ANOVA, $P<0.05$), means were compared using Tukey test. Differences on D_{Me} among treatments were assessed using factorial ANOVA as well. Significance was attributed when *F* and Tukey tests presented $P<0.05$.

3. Results

Eleven fish died within one month of experiment, and 20 did not produce enough otolith material after alizarin mark and could not be analyzed. Seventy-seven fish were analyzed to produce the results presented in this study, 40 from 14°C and 37 from the 24°C.

3.1. Water chemistry

Absolute elemental concentrations in water were significantly different among all salinity treatments, except for manganese. Concentrations increased with salinity for Sr, Mg and Mn and decreased for Ba. Mn:Ca ratios were lower only at salinity 16. The variability of Me:Ca ratios were higher for Mn than for Sr, Mg and Ba (Fig. 1). Slightly but significantly lower Sr:Ca and Mg:Ca ratios were found for salinity 6, compared to salinities 16 and 32. Ba:Ca ratio was inverse, decreasing towards higher salinities (Fig. 1). Manganese presented the lowest experimental concentrations with the highest standard errors for all absolute concentrations. No significant differences were observed between salinities for both absolute and relative manganese concentrations.

3.2. Absolute elemental concentration in otoliths

Elemental concentration in otoliths did not vary within tanks from the same treatment (Table 2). Different effects of salinity and temperature on absolute concentrations were observed for all analyzed elements.

Strontium presented lower concentration for 24°C (Fig. 2), and its mean concentration ranged from 1440 to 1600 μg g4^{-1} for 14 °C and from 1320 to 1390 μg g^{-1} in 24°C.

Barium mean concentration decreased towards higher salinities, but temperature and salinity effects and their interaction were significant (Table 2). The highest barium mean concentration in otoliths was observed for treatment 6-24 °C (5.3 μg g^{-1}) and it was significantly different from the other treatments. Otolith barium concentrations did not differ between the salinities 16 and 32 at both temperatures.

Magnesium mean concentrations ranged from 6.4 μg g^{-1} to 32.5 μg g^{-1}. Temperature presented a significant effect on magnesium deposition for salinities 6 and 32 (Fig. 2; Table 2). Within the 14 °C treatment there were not significant differences among salinities. For 24 °C magnesium mean concentrations were significantly lower for salinities 6 and 16 than for salinity 32.

Manganese concentration was significantly affected by temperature and salinity with a strong interactive effect at salinity 16 (Table 2; Fig. 2). The general pattern shows two groups of significantly distinct manganese concentrations. The first one shows mean concentrations about 10 μg g^{-1} for salinities 6 and 16 at 14°C, and salinity 6 at 24 °C. The second presents mean concentrations about 4 μg g^{-1} and is composed by otoliths from salinities 16 and 32 from 24 °C and salinity 32 from 14 °C.

3.3. Partition coefficients

The results show great variability for all elements in otoliths of *Micropogonias furnieri* (Fig. 3). The lowest coefficients were observed for magnesium, with values ranging from 0.00001 to 0.00004 indicating strong elemental discrimination. Strontium partition coefficients ranged from 0.35 to 0.69. The effect of salinity on D_{Sr} was significant

(Table 3), with significantly lower D_{Sr} observed for salinities 16 and 32 than for salinity 6. Temperature affected D_{Sr} significantly for salinity 32, with the colder water inducing higher D_{Sr} values. For barium, partition coefficients ranged from 0.06 to 0.66. Salinity presented a positive effect on D_{Ba} (Table 3). The general effect of temperature on D_{Ba} was positive (Table 3) however, no significant differences were observed between temperatures. For magnesium, partition coefficients ranged from 0.05×10^{-4} to 0.35×10^{-4}. Temperature presented a positive effect on D_{Mg} and there was an interactive effect of salinity and temperature (Tab. 3). Therefore, for temperature 14 ^{o}C there were no differences of D_{Mg} between salinities. For temperature 24 ^{o}C, significant differences were observed between salinities 6 and 16. Manganese partition coefficients ranged from 0.03 to 0.9. The effect of interaction between salinity and temperature on D_{Mn} was observed for salinity 16 (Fig. 3), following the same pattern observed for its absolute concentrations.

4. Discussion

Results observed throughout this study show that the deposition of Sr, Ba, Mg and Mn in otoliths of *M. furnieri* is not a simple result of absolute or relative (Me:Ca) concentrations of these elements in the water. However, it is product of complex interactions between elemental availability in the water, temperature and salinity. Since a single element can show the same concentration on otoliths from fish reared under two different sets of experimental conditions (as observed for Mn; Fig. 2 and 3), general patterns of elemental deposition have to be drawn with care. Furthermore, since salinity and temperature influenced differently each element, the results observed in this study will be analyzed and discussed individually.

4.1. Strontium

There is a general acceptance that in nature strontium concentrations in otoliths are positively affected by salinity. Therefore, higher strontium concentrations should be encountered on otolith from high salinity waters (See Campana, 1999 for a review). That characteristic turned strontium in a very suitable element to study diadromy based on otolith microchemistry. However, different species have shown differences on the response of strontium deposition in otolith of fish reared under the same conditions (Rooker et al., 2004; Swearer et al., 2003) supporting the idea of a species-specific pattern of deposition. For *M. furnieri* the absolute strontium concentrations in otoliths was not influenced directly by salinity (Fig. 2), as observed for *Sciaenops ocellatus* (Rooker et al. 2004).

Some studies have addressed the variations of strontium deposition in otoliths to the Sr:Ca ratios in the water, and not directly to changes on salinity (Gallahar and Kingsford, 1996; Bath et al. 2000; Elsdon and Gillanders 2005). When analyzing partition coefficients (Fig. 3), significantly higher mean D_{Sr} was observed for salinity 6 and temperature of 14 ^{o}C (Table 3) suggesting a lower discrimination of strontium (higher D_{Sr}) at low salinities. This pattern was also observed for *Acantopagrus butcheri* reared under controlled conditions at brackish water (Vries et al., 2005). The range of partition coefficients for strontium observed for *M. funieri* was higher (0.35 to 0.69) than the observed for other studies. For *Lutjanus griseus*, Martin and Wuenschel (2006) have observed partition coefficients ranging from 0.21 to 0.38, despite their experimental range (salinity from 5 to 45 and temperature from 18 to 33 ^{o}C) being larger than ours (Table 4). For *A. butcheri*, Elsdon and Gillanders (2005) have presented D_{Sr} varying approximately from 0.2 to 0.5.

The existence of significant discrimination of strontium incorporation to otoliths, and the absence of significant higher strontium concentration for higher salinities may be the result of different factors. The variability of strontium discrimination has been addressed to physiological regulation of the amount of strontium transported to the endolinph (Bath et al., 2000). Other possible source of this variability would be the phase of ontogeny where fish were at the beginning of the experiment. *Micropogonias furnieri* at juvenile stanza has been showed to actively search oligohaline waters (Albuquerque et al. *in prep*). Therefore juveniles would not be physiologically ready to be exposed to high salinity waters, which could cause additional stress to the fish and maybe retard the response of strontium concentration in otoliths or cause the higher variability observed for salinity 32. This large variability could mask the final absolute concentration in otoliths on this experiment.

In the current study, temperature was slightly inversely correlated to strontium absolute concentrations and partition coefficients**,** with significant effect for salinity 32. Temperature is expected to be an important variable affecting strontium incorporation in otoliths, albeit it does not show a single pattern of influence for different species (see Campana, 1999). One explanation for the inverse effect of temperature is that physiological process regulating ion transport from the ambient water into the saccular endolinph have been suggested to be relaxed at low temperatures, intensifying strontium incorporation on otoliths (Townsend et al., 1992). However, neutral and positive effects of temperature have been recorded as well (see citations on introduction). According to Kalish (1989) the effect of temperature on strontium deposition may be indirect, with a primary effect on growth rates and reproduction. As expected, *M. furnieri* juveniles held in our study grew faster in warmer water, therefore, strontium incorporation was inversely correlated to fish growth rate, as observed for *Haemulon plumieri* (Sadovy and Severin, 1992). The strontium variability observed in otoliths of *M. furnieri* difficulted the evaluation of the effect of salinity on its deposition since small differences (but significant for salinity 6) on Sr:Ca ratios among treatments were found. Therefore, in this experiment it was not possible to reach a conclusive result about effect of water salinity on strontium incorporation in otoliths. On the other hand, strontium was negatively affected by temperature.

4.2. Barium

Barium concentration in otoliths as an environmental indicator has received more attention in the last 10 years. The variability of Ba:Ca ratios in otoliths has been addressed to the Ba:Ca availability in the surrounding water were fish developed (Bath et al., 2000; Elsdon and Gillanders, 2004; Vries et al., 2005). Most part of estuarine barium comes from barium-rich river-suspended sediment particles (Nozaki et al., 2001). The maxima values appear when freshwater encounters saltwater and after the maxima presents a linear decrease towards more salty waters (Coffey et al., 1997). Consequently it is expected a negative correlation between salinity and barium concentration in otoliths. As expected, in this study *M. furnieri* presented a negative relationship between otolith barium concentrations and salinity and a positive relationship with Ba:Ca in the water. Since Ba:Ca ratios in the experimental water were not similar between salinities (see Fig. 1), the influence of solely salinity on otolith could not be evaluated. Therefore, analyzing partition coefficients helped to verify that barium was more discriminated (lower D_{Ba}) at lower salinities. The D_{Ba} observed for *M. furnieri* varied from 0.06 to 0.66 which agreed with the ranges observed in other species (see table 4). Albeit absolute barium concentrations in otoliths of *M. furnieri* followed the trend presented by Ba:Ca ratios in water, the analysis of D_{Ba} revealed that proportionally more barium was deposited in *M. furnieri* otoliths at higher salinities. In the same way Vries et al. (2005) found increased barium discrimination at lower salinities (with high Ba:Ca in the water) for otolith of *A. butcheri*. These authors argued that otolith barium uptake was facilitated by increased strontium concentrations in water, if Ba:Ca levels in the water were higher than 20 µmol mol^{-1}.

In the present study, not significant difference was found for barium deposition in otoliths of *M. furnieri* between temperatures, except for a significant positive effect observed for salinity 6 (see Fig. 2). Since no effect of temperature were observed for D_{Ba} as well, our data corroborates that temperature does not affect barium deposition in fish otoliths as reported for *L. griseus* (Martin and Wuenschel, 2006) and *Leiostomus xanthurus* (Bath et al. 2000; Martin and Thorrold, 2005;). However, temperature effect has been presented for otoliths of *A. butcheri* (Elsdon and Gillanders, 2002). The last authors argue that the absence of a temperature effect on barium deposition could be related to the insufficient temperature range experimented in other studies. The temperature range tested here was that commonly experienced by *M. furnieri* in south Brazil, therefore this experiment seems to be coherently designed and it supports the neutral effect of temperature on barium deposition in otoliths.

4.3 Magnesium

Magnesium has been suggested to be a temperature indicator in organisms that contain internal or external carbonatic structures and it has been used as a palaeothermometer, been positively affected by temperature in the calcite of coralline algae (Kamenos et al. 2008), foraminifer shells (Lea et al. 1989) and bivalve shells (Klein et al., 1996). The present study revealed that temperature affected positively magnesium deposition in otoliths of *M. furnieri*, except for salinity 16, where the interactive effect with temperature was detectable. A negative but not significant effect of temperature on magnesium deposition and a positive correlation with somatic growth rates were observed for *L. xanthurus* (Martin and Thorrold, 2005), which suggests a physiologically determined elemental uptake. As expected, *M. furnieri* reared at 24^{o}C grew faster than in 14^{o}C, and the difference of the absolute magnesium concentrations and D_{Mg} could be due to differentiated growth rates and not due to a direct effect of temperature.

A general pattern of magnesium deposition in otoliths and salinity was not observed. Although the experimental water presented different absolute magnesium concentrations (correlated to salinity, since it was diluted from sea water), the Mg:Ca ratios were very close among treatments, with a slight but significant lower concentration for salinity 6. Magnesium absolute concentrations of ~ 5 to 40 μg g^{-1} found in our study were in average higher than those from adults of *Gadus morhua* (~17 – 21 μg g^{-1}; Jónsdóttir et al., 2006) and *Sebastes* spp. (~7 – 12 μg g^{-1}; Campana et al. 2007) studied in nature. The larger concentrations observed here when compared to other studies could be due to differences on stage of life, since high magnesium concentrations has been showed to occur before one year of life for *M. furnieri* (Albuquerque unpubl. data) and in the otolith core of different species (Ruttemberg et al., 2005).

Significant lower discrimination of magnesium found for warmer treatment (higher D_{Mg}) reinforces the positive effect of temperature on magnesium deposition, as observed for absolute concentrations. The analysis of partition coefficients, when compared to other elements allows emphasizing the strong discrimination of magnesium for fish otoliths at all salinities. Magnesium was over thousand times more discriminated than the other elements, which agreed with results found for otoliths of other species (see table 4). Therefore, magnesium deposition in otoliths seems to be highly physiologically controlled, as suggested by Martin and Thorrold (2005). Adding this regulation to the absence of patterns between salinities observed in this study, implies that magnesium might not be useful for evaluating changes on environmental characteristics related to salinity.

4.4. Manganese

Temperature and salinity had a strong interactive effect on manganese deposition in otoliths of *M. furnieri*. That interaction does not allow designing general patterns for the experimental treatments. A large variability on absolute manganese concentrations in the water was found for each salinity treatment, however it was not reflected on the water Mn:Ca ratios. When otoliths from treatments with salinities 6 and 32 are examined (Fig. 1), absolute manganese concentrations and partition coefficients presented similar patterns with no significant differences between temperatures, but they were contrary to the absolute manganese concentration in the water. Inverse trends between otolith magnesium concentration and water availability have already been reported in fish (Elsdon and Gillanders, 2003). On the other hand, temperature presented a significant negative effect only at salinity 16. These results are opposite to the findings of Fowler et al. (1995) that observed higher manganese concentrations at higher salinities for otolith of *Micropogonias undulatus*. Since we studied a co-generic species and Fowler et al. (1995) did not measured elemental concentrations nor Mn:Ca ratios in the experimental waters it is hard to explain the nature of this difference.

Absolute concentrations of manganese found in otolith of *M. furnieri* varied from ~1 to 10 $\mu g\ g^{-1}$. These values are higher than concentrations of other fish species in nature. Campana et al. (2007) found manganese concentrations from ~ 0.1 to 0.3 μg g^{-1} on otoliths of adult *Sebastes* spp from different locations in Canadian waters. Manganese concentrations from ~ 0.7 to 1.2 μg g^{-1} were determined for otoliths of in *G. morhua* (Jónsdóttir et al., 2006). Nevertheless, according to the summary of publications presented by Campana (1999), manganese mean concentration amongst different species would be about 10 ppm, that means approximately 10 μg g^{-1}.

The elevated variability of measurements of manganese in otoliths between replicate tanks (Elsdon and Gillanders, 2002) and in the water between tanks (Martin and Thorrold, 2005) had made it difficult to interpret the influence of salinity and temperature on manganese uptake. However, this problem should be minimized with the use of partition coefficients, as applied by Martin and Thorrold (2005), who's found positive effect of temperature at salinity 15 for D_{Mg} in juveniles of *L. xanthurus*. Interestingly, for *M. furnieri* we found exactly the opposite, with a strong negative effect of temperature for salinity 16. Manganese has been suggested to be mostly discriminated ($D_{Mn,}$<1) for experimental water with Mn:Ca above ambient levels, and incorporated (D_{Mn}>1) for experimental Mn:Ca below ambient levels (Elsdon and Gillanders, 2003), which seems to reflect the physiological activity in order to maintain homeostasis. If the response of manganese to shifts on temperature and salinity is already contradictory, we highlight that additional care should be taken at laboratory experiments since manganese has been suggested to be an indirect indicator of hypoxia in aquatic environments (Thorrold and Shuttleworth, 2000). Therefore, oxygen availability would be an extra source of manganese variability on fish otoliths. Based on the large variability of concentrations and the absence of a clear pattern of the influence of temperature and salinity on manganese deposition in otoliths of *M. furnieri*, we suggest manganese should not be used as an environmental indicator.

5. Conclusion

The current study presents the first information regarding the influence of environmental factors on elemental incorporation in otoliths of *M. furnieri*. Our data show the complexity which strontium, barium, magnesium and manganese are incorporated in otoliths. Manganese and magnesium were disregarded as useful indicators of salinity. We show that strontium concentration in otoliths can not be directly related to salinity if the Sr:Ca in the water does not increase. Another experiment stabilizing Sr:Ca ratios in the water will be mandatory in order to determining clearly the salinity effect on strontium deposition in otoliths of *M. furnieri*. Barium presented a good correspondence with their relative concentration in the water and it would be used as an indirect salinity indicator if there is a previous knowledge of its natural dilution between fresh and saltwater. The effect of salinity and temperature on elemental uptake in fish otolith remains controversial, particularly to elements like magnesium and manganese which just few studies have tried to describe. Since many different conclusions have been shown for all studied elements among many fish species, we emphasize the importance of studying elemental uptake as a species-specific process and not as a general characteristic explained for fish otoliths.

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Table 1- Summary of nominal salinity (NS), effective mean salinity (ES, ± SD), and mean experimental temperature (ET ± SD) for water from each tank during the experiment for the two water tables (WT).

WT	ET (^oC)	Tank	NS	ES
1	13.6 ± 0.9	T2	6	6.4 ± 1.5
		T8	6	6.2 ± 2.2
		T10	6	6.8 ± 1.7
		T1	16	15.8 ± 1.5
		T5	16	16.3 ± 2.1
		T7	16	16.2 ± 1.8
		T3	32	32.3 ± 2.4
		T4	32	32.7 ± 2.9
		T9	32	32.4 ± 3.0
2	23.8 ± 1.7	T12	6	6.6 ± 1.4
		T16	6	6.3 ± 1.4
		T19	6	6.4 ± 3.2
		T13	16	16.7 ± 1.4
		T17	16	15.7 ± 1.1
		T20	16	15.9 ± 1.3
		T14	32	32.2 ± 2.4
		T15	32	31.8 ± 2.5
		T18	32	31.8 ± 2.6

Table 2 – Result for 2-way ANOVA for testing the effect of salinity (S), temperature (T) and replicate tanks (Tk) on the mean absolute concentrations of strontium (Sr), barium (Ba), magnesium (Mg) and manganese (Mn) on otoliths of *M. furnieri* reared in laboratory.

		Sr			Ba		
Effect	df	MS	*F*	*P*	MS	*F*	*P*
T	1	389012	7.75	*0.007	22.80	11.49	*0.001
S	2	76786	1.53	0.225	70.56	35.57	*0.000
Tk	2	45463	0.90	0.409	0.129	0.06	0.937
T x S	2	31259	0.62	0.539	17.16	8.65	*0.000
T x Tk	2	10639	0.21	0.809	1.498	0.75	0.474
S x Tk	4	104974	2.09	0.093	0.156	0.07	0.988
T x S x Tk	4	116977	2.33	0.066	0.166	0.83	0.506

		Mg			Mn		
Effect	df	MS	*F*	*P*	MS	*F*	*P*
T	1	14.97	48.53	*0.000	100.53	8.62	*0.005
S	2	1.76	5.70	*0.005	159.30	13.66	*0.000
Tk	2	0.08	0.26	0.773	5.27	0.45	0.638
T x S	2	0.83	2.68	0.077	81.18	6.96	*0.002
T x Tk	2	0.09	0.31	0.736	0.30	0.02	0.974
S x Tk	4	0.18	0.60	0.661	13.18	1.13	0.351
T x S x Tk	4	0.35	1.16	0.337	17.37	1.48	0.217

Table 3 – Result for 2-way ANOVA for testing the effect of salinity (S), temperature (T) and replicate tanks (Tk) on partition coefficiens between water and *M. furnieri* otoliths for strontium (D_{Sr}), barium (D_{Ba}), magnesium (D_{Mg}) and manganese (D_{Mn}).

		D_{Sr}			D_{Ba}		
Effect	df	MS	*F*	*P*	MS	*F*	*P*
T	1	0.04	7.92	*0.000	< 0.01	14.76	*0.000
S	2	0.07	14.81	*0.005	0.16	464.51	*0.000
T x S	2	0.01	2.36	0.099	< 0.01	0.017	0.983

		D_{Mg}			D_{Mn}		
Effect	df	MS	*F*	*P*	MS	*F*	*P*
T°	1	< 0.01	106.50	*0.000	0.07	70.39	*0.000
S	2	< 0.01	1.34	*0.259	0.06	64.48	*0.000
T° x S	2	< 0.01	17.80	*0.000	0.03	32.00	*0.000

Table 4 – Synthesis of partition coefficients (D) of Sr, Mg, Mn and Ba, found on the literature, regarding temperature and salinities ranges evaluated in each study.

Species	Temp. range (^{o}C)	Salinity range	D_{Sr}	D_{Mg}	D_{Mn}	D_{Ba}	Author
L. griseus	18–33	5–45	0.21 – 0.38	7.6×10^{-6} – 8.9×10^{-5}	0.018 – 1.02	0.043 – 0.45	A
A. butcheri	12–24	5–30	0.42 – 0.6	0.0004 – 0.0005	0.2 – 0.55	0.26 – 0.79	B
L. xanthurus	17–26	15, 25	0.2 – 0.43	0.00014 – 0.001	0.055 – 0.92	0.11 – 1.123	C
A. butcheri	~ 21	5, 32	~ 0.22 – 0.55	–	–	~ 0.03 – 0.25	D
M. furnieri	12, 24	6–32	0.35 – 0.69	6×10^{-6} – 3.4×10^{-5}	0.03 – 0.9	0.06 – 0.66	E

A – Bath Martin and Wuenschel (2006)
B – Data from Elsdon and Gillanders (2002), as estimated by Bath Martin and Wuenschel (2006)
C – Martin et al. (2004) and Martin and Thorrold (2005), withdrew from Bath Martin and Wuenschel (2006)
D – Vries et al. (2005)
E – Present study

Figure Captions

Figure 1 – Mean concentrations and Me:Ca ratios of strontium (Sr), barium (Ba), magnesium (Mg) and manganese (Mn) in experimental water at salinities 6, 16 and 32. Bars (left) denote absolute concentrations and lines (right) indicate the Me:Ca ratios. For Mn, ratios were multiplied for 10 to improve graph visualization. Error bars = standard error.

Figure 2 - Absolute mean concentrations of strontium (Sr), barium (Ba), magnesium (Mg) and manganese (Mn) in otoliths of *M. furnieri* reared in laboratory under different salinities and temperatures. Black and white squares represent treatments of 14^{o}C and 24^{o}C respectively. Error bars = standard error.

Figure 3 – Partition coefficients calculated between otolith Me:Ca and water Me:Ca for strontium (Sr), barium (Ba), magnesium (Mg) and manganese (Mn). Circles and triangles represent treatments of 14^{o}C and 24^{o}C respectively.

Figure 1

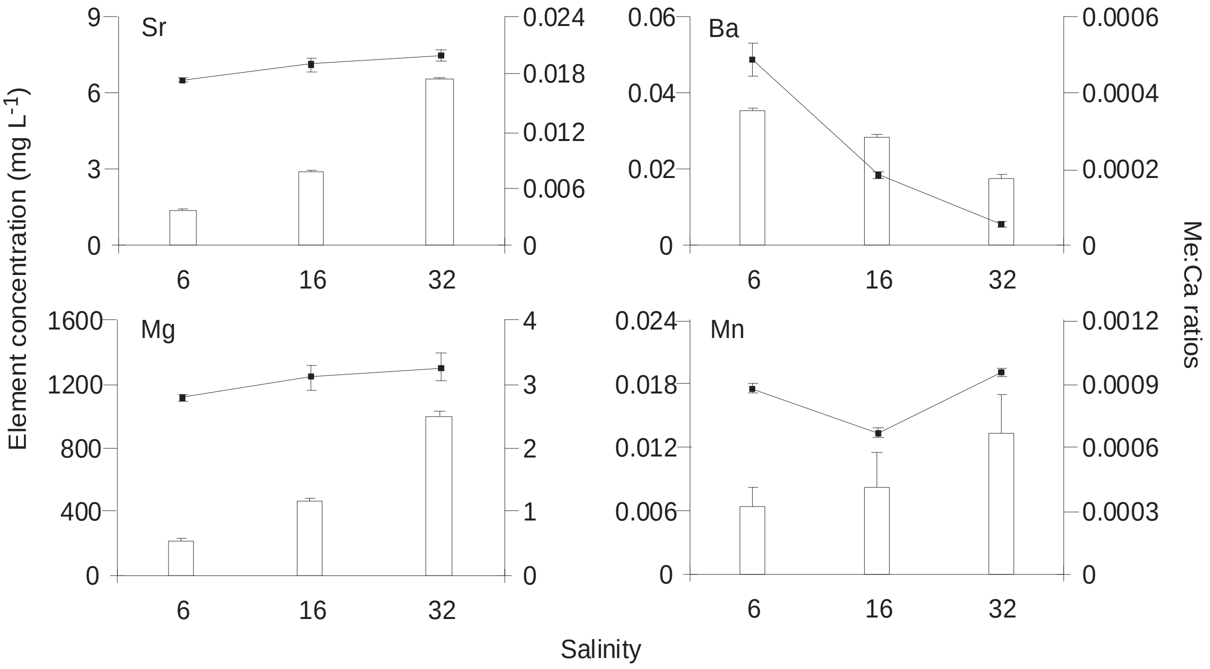

Sr
Ba
Mg
Mn
Element concentration (mg L-1)
Me:Ca ratios
Salinity
0
3
6
9
0
0.006
0.012
0.018
0.024
0
0.02
0.04
0.06
0
0.0002
0.0004
0.0006
0
400
800
1200
1600
0
1
2
3
4
0
0.006
0.012
0.018
0.024
0
0.0003
0.0006
0.0009
0.0012
6
16
32

Figure 2

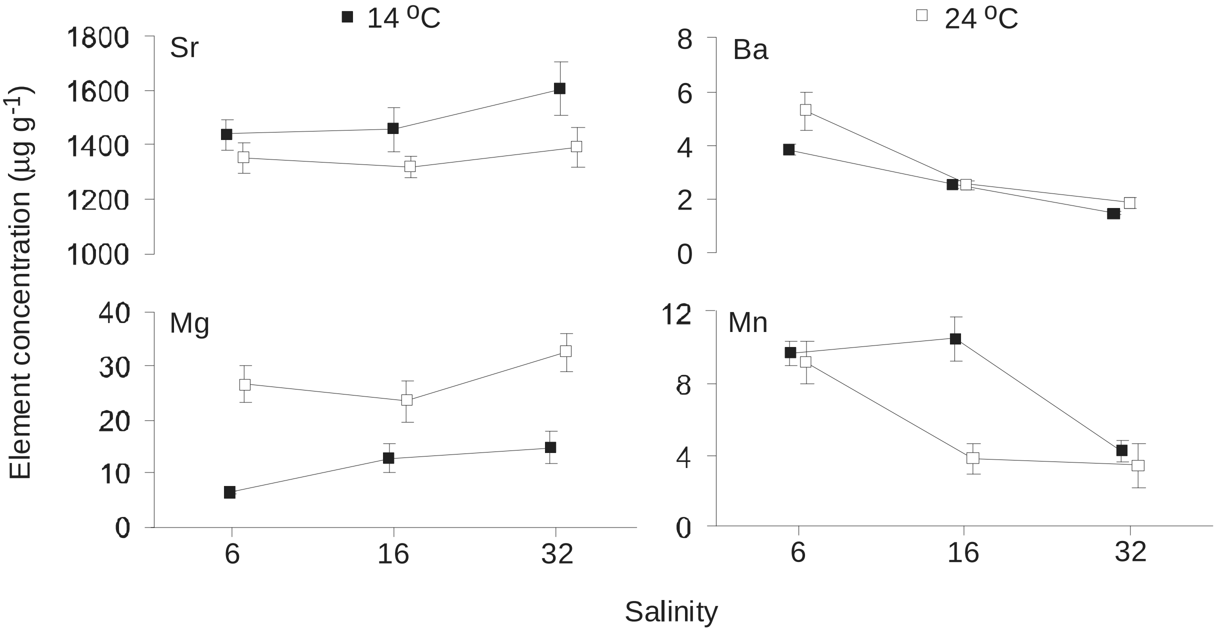
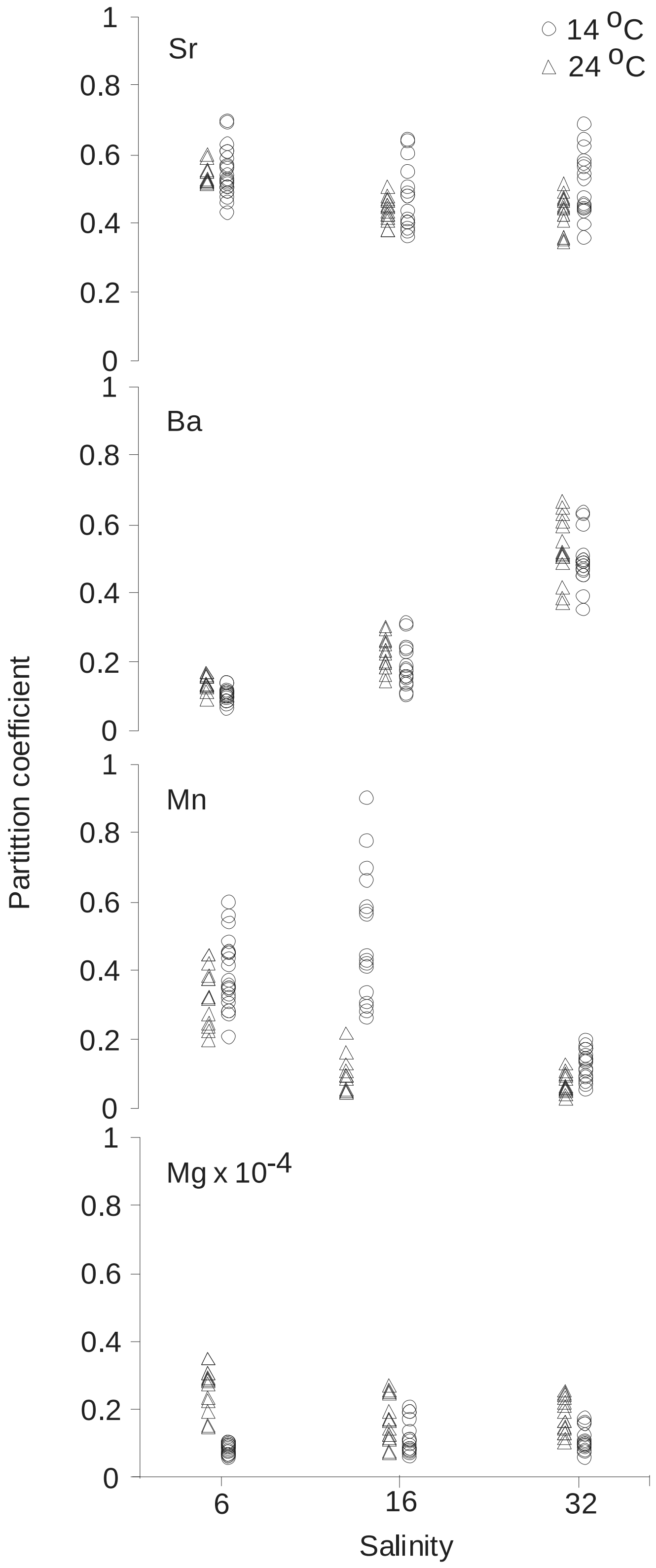

■ 14 °C
□ 24 °C
Sr
Ba
Mg
Mn
Element concentration (µg g-1)
1800
1600
1400
1200
1000
8
6
4
2
0
40
30
20
10
0
12
8
4
0
6
16
32
6
16
32
Salinity

Figure 3


○ 14 °C
△ 24 °C
Sr
Ba
Mn
Mg x 10-4
Partition coefficient
0
0.2
0.4
0.6
0.8
1
6
16
32
Salinity

ANEXO II

Micropogonias furnieri trapped in a freshwater coastal lagoon as a natural standard for otolith microchemistry

Albuquerque, C.Q., Miekeley, N., Muelbert, J.H.

(Submetido à Marine and Freshwater Research)

Micropogonias furnieri trapped in a freshwater coastal lagoon as a natural standard for otolith microchemistry

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Abstract. We analyzed strontium and barium concentrations in otoliths of *M. furnieri* collected from freshwater and marine water habitats at South Brazil. Otoliths were analyzed using LA-ICPMS with the objective of establishing elemental signatures for each habitat and to examine the applicability of otolith chemistry as a tool for study habitat use by fish. The results suggest a positive effect of salinity on strontium accumulation and a negative one on barium deposition. Strontium and barium mean signatures in otoliths of *M. furnieri* were respectively 710 and 111.6 µg g^{-1} for freshwater, 2069 and 16.7 µg g^{-1} for estuarine, and 2990 and 2.7 µg g^{-1} for marine water. Barium concentrations in otoliths from freshwater specimens presented significantly higher concentrations than the observed in other species and ontogenetic processes were observed to increase barium deposition at the initial fish life. For marine fish, mean strontium concentration increased from about 2000 to 2900 µg g^{-1} along the fish's life, suggesting a change from estuarine to marine habitat. Strontium in otoliths from freshwater fish decreased to a low and uniform level of about 710 µg g^{-1} for the rest of the life, characterizing a singular type of sea-to-freshwater migration for *M. furnieri*. Strontium and barium are suitable for studying habitat use by *M. furnieri*.

Additional keywords: Strontium, barium, laser ablation, ICPMS, whitemouth croaker

Running head: Strontium and Barium as fish habitat indicators

Introduction

Otoliths, or ear stones, are calcified structures composed of aragonite and otolin that develop inside the inner ears of teleost fish. They have been extensively used in fisheries and fish ecology studies, since their properties as age and growth indicators were described (Reisbish 1899; Pannela 1971). In the last two decades, a new and powerful approach for otolith studies has emerged: the association of time-preserving properties of otoliths to the environmental conditions experienced by fish throughout their life (Radtke 1984). Otoliths are metabolically inert structures and the elements incorporated to it are related to their concentration in the surrounding waters as fish grow. Therefore, otolith chemistry is expected to reflect physical and chemical characteristics of the environment (Campana and Neilson 1985; Fowler *et al.* 1995a). Due to these attributes, the lifetime of fish could be recorded as chemical information in the otoliths. Strontium, barium, and other trace metals (e.g. Zn, Pb) can be deposited into the otolith matrix, reflecting spatial and temporal changes of the environment where fish grew. This characteristics have been used to study fish migration between salinity gradients and for the determination of nursery areas (Thorrold *et al.* 1998; Volk *et al*. 2000; Tzeng *et al.* 2002; Zlokovitz *et al*. 2003). Some studies have used this approach to identify fish stocks (Campana *et al*. 1999) and to determine fish age (Kalish 1993).

In general, Sr and Ba in otoliths increase according to their availability in the water (Bath *et al.* 2000; Elsdon and Gillanders 2002). Since strontium concentrations trend to increase towards saltwater and barium towards freshwater (Surge and Lohmann 2002; Jacquet *et al.* 2005), it is expect that salinity affects positively Sr and negatively Ba deposition in otoliths. The validation of this approach has mostly been conducted under artificial conditions that can hardly reproduce the natural environment. Almost no attempts have been made to confirm laboratory results with those obtained from wild conditions (but see Elsdon and Gillanders 2005a). In the present study we mimic laboratory conditions in a natural environment. We investigated strontium and barium concentrations in otoliths of the whitemouth croaker *Micropogonias. furnieri* collected from two different habitats: a freshwater coastal lagoon (Mirim Lagoon) and a marine/brackish water environment (Rio Grande) in Southern Brazil (Fig. 1).

The Patos Lagoon complex (Fig. 1) is the world’s largest chocked coastal lagoon system (Kjerve, 1986). It is an oligohaline-freshwater ecosystem and its southern area presents the common high variability on salinity of estuarine waters (Seeliger 2001). The communication to the ocean occurs through a single 740 m width entrance channel, and the hydrology is mainly influenced by wind and freshwater discharge (Kjerve 1986; Möller *et al*. 2001). During summer, the estuary commonly receives large inputs of saltwater, which are enhanced when there is an association to low pluviometric levels and predominance of SE wind (Castello and Möller 1978). These saltwater intrusions reach the São Gonçalo channel, which is the unique communication between Patos and Mirim lagoons (Fig. 1). To avoid damages to the rice farms in this region, Brazilian and Uruguayan governments have constructed a floodgate on São Gonçalo channel in 1977 (Fig. 1-A) to control saltwater intrusion. Since then, the Mirim Lagoon has been characterized as a freshwater environment.

Whitemouth croaker is a eurihaline sciaenid widely distributed along the eastern coast of the Atlantic Ocean, occurring from the Gulf of Mexico to the Gulf of San Matías (Argentina). It has a relatively long life-span with observed ages of about 37 years (Haimovici and Umpierre 1996). Together with other sciaenids, *M. furnieri* supports over 50% of the total fisheries production in Southern Brazil (Vasconcellos and Haimovici 2006) and it is a biomass-dominant species, which is also an important fisherys in Argentina and Uruguay (Lasta and Acha 1996). Spawning has been registered in coastal and estuarine waters (Macchi *et al*. 1996; Acha *et al.* 1999; Jaureguizar *et al. in press*), and in Southern Brazil it occurs mainly during summer (Ibagy and Sinque 1995). Juveniles are found inside Patos Lagoon estuary during the entire year (Castello 1986) and adults can be captured mainly during spawning season. This species is also caught in the Mirim Lagoon, despite 30 years of isolation from estuarine waters (Burns *et al*. 2006).

Therefore, this situation presents ideal conditions for a natural fresh/brackish/marine water experiment. *M. furnieri* in the Mirim Lagoon inhabits an environment with no salinity variability for the last 30 years, while its counterparts in the estuary and adjacent marine coast are influenced by both, salinity and temperature. We use this setup to test the hypothesis of strontium and barium incorporation in otoliths as a result of salinity variability and ontogeny using the freshwater individuals as natural standards for otolith microchemistry. Furthermore, results of this study will provide basal concentrations for strontium and barium in the otoliths of *M. furnieri* that may allow better understanding of its life cycle (e.g. migration), habitat use, and the long-term variability of elemental fluctuations in fish otoliths.

Materials and Methods

Sampling and sample preparation

Ten adults of *M. furnieri* were sampled in December 2005 in the north area of the Mirim Lagoon (Fig. 1) with a 300-m long gillnet (50 and 70-mm mesh size). Fish specimens were randomly sub-sampled from the total capture. Other ten adults were obtained from commercial fisheries in the coastal Rio Grande area (Fig. 1) at the same time. All sampled specimens were sexed, measured in total length, and saggital otoliths were extracted, cleaned and stored dry. The left otoliths were then embedded in crystal polyester resin and transverse sections were cut with a low-speed diamond blade saw as close to the core as possible. Thin sections (0.4 mm) were mounted onto glass slides with glue. Prior to chemical analysis, otolith surfaces were polished with silicon carbide paper (n^o 8000), washed with deionized (DI) water (Milli-Q, Millipore, Bedford, USA), sonicated for 3 minutes, and rinsed three times with DI water. The slides were dried in a horizontal flow cabinet before analysis.

Elemental determinations by LA-ICPMS

Analytical measurements were performed with a Nd:YAG CETAC LSX 100 Laser Ablation system operating at 266 nm, coupled to an ELAN 5000 (PerkinElmer – SCIEX) inductively coupled plasma-mass spectrometer. The laser was configured for Q-switched mode operation and operated at a pulse frequency of 20 Hz, a scan speed of 40 µm/s, and a laser beam energy of 0.6 – 0.8 mJ. This low energy was used to avoid perforation of the thin sections; however, it obviously compromised the achieved detection limits. Under these experimental conditions, the ablated crater diameter on otoliths was about 30 µm (Fig. 2). The sampling time allowed two average measurements per second, and each measurement was the result of 10 laser shots that integrated an area of approximately 1300 μm^2 (50 µm of length). Since most part of annuli presented a width between 200 and 300 μm and each measurement is based on a 50-μm long scan, the temporal resolution corresponded between two and tree months of life time. More information on the operational characteristics of the LA-ICPMS system used is summarized in Table 1.

The determined elements (masses) were ^{43}Ca, ^{86}Sr, ^{138}Ba, ^{24}Mg and ^{55}Mn, but only results for ^{86}Sr and ^{138}Ba are presented in this study. Analytical quantification was performed in the external calibration mode using a series of matrix matched in-house standards. These standards were constituted by pressed powder $CaCO_3$ discs with known analyte concentrations, and similar discs of suprapur $CaCO_3$ were used as a blank. These standards were proposed and produced by Bellotto and Miekeley (2000; 2007), and further details on their preparation, validation and application were described in these publications. The measured signals (counts per second – cps) for ^{86}Sr and ^{138}Ba were normalized with reference to ^{43}Ca for correction of the bias induced by differences on the amount of ablated material. Limits of detection (LOD, 3σ), determined from the blank discs and the sensibility (inclination) of the respective calibration curve, were 0.46 μg g^{-1} for barium and 5.31 μg g^{-1} for strontium. All concentrations were expressed as micrograms of Ba or Sr per gram of otolith material, considering otolith material as a virtually pure CaCO3 matrix.

Due to the difficult visualization of the otolith core in the CCD camera picture, three parallel scans were conducted perpendicularly to the growth increments (Fig. 2), from the core to the edge of each otolith section. Only the scan yielded over or closest to the core was considered. In order to improve the accuracy of the measurements, the otoliths were randomly analyzed and a blank scan without laser was conducted after each otolith scan. The laser chamber was purged for 60 s prior to each new otolith analysis to clean the chamber of residual aerosol from the previous ablation.

After chemical analysis, otoliths were photographed under a microscope. Otolith images were measured using the UTHSCSA ImageTool programme (University of Texas Health Science Center at San Antonio, Texas, ftp://maxrad6.uthscsa.edu) in order to assign ages to specific locations of the scanned profile. Annual growth increments have been validated by Shwingel and Castello (1990). Since the profiles have a very similar pattern, and in order to avoid too many graphs, we will show only four representative profiles from each environment.

Model assumptions and statistical analysis

Whitemouth croaker from the coastal area spend their initial life at estuarine environments and move to the marine adjacent waters, where they live as adults, reproducing close to the estuaries (Vazzoler 1975; Vazzoler *et al*. 1999). Because of that presumed behavior, a tendency of increased strontium concentration is expected along their life span, due to the general higher concentrations of this element in sea water (Surge and Lohmann 2002; Rosenthal 1981). By the other side, fish that entered the Mirim Lagoon as juveniles were trapped in a freshwater environment, and strontium is therefore not expected to increase with fish age. Otherwise, we expect to find higher barium concentrations in otoliths from the Mirim Lagoon freshwater environment since this element presents commonly higher concentrations in fresh than in saltwater.

Based on these assumptions, statistical comparisons were made regarding integrated concentrations for the first year of life (FYL), for the last year of life (LYL) and for the whole fish life (WFL, complete profile). Differences on average concentrations of strontium and barium were analyzed between sampled areas and time of life using one-way ANOVA and Tukey HSD *post hoc* test (Sokal and Rohlf 1969). In order to satisfy the assumptions of normality and homogeneity of variances, both variables were log-transformed according to $\log_{10}(x+1)$. Significance was attributed at $P < 0.05$. Trends on strontium increase through the life were assessed using linear correlation (Sokal and Rohlf 1969) and significant increase was admitted for the correlation that reached both $p < 0.05$ and $r > 0.6$.

A semi-quantitative model was established to infer life history patterns of *M. furnieri* and to attribute fresh, estuarine and marine water signatures to otolith composition. Based on literature data (Muelbert and Weiss 1991; Castello 1986), we assume *M. furnieri* had its larval and initial juvenile phase in the estuary of Patos Lagoon. Therefore, the estuarine chemical signature was inferred from strontium and barium concentrations measured for the FYL region for otoliths from fish collected in the ocean. The marine and the freshwater signals were determined for the LYL assuming that fish spent the last year around the site where they were collected. Ontogenetic effects were analyzed based on the assumption that if an elemental pattern is ontogeny-mediated, it should be observable for both freshwater and estuarine/marine environments.

Results

Despite being older, fish obtained from the Mirim Lagoon were significantly smaller (ANOVA, $P < 0.05$) than fish collected from the coast of Rio Grande (Table 2). Mean barium concentration was significantly higher in otoliths from freshwater fish (ANOVA, $P < 0.01$) and strontium was higher in coastal fish (ANOVA, $P < 0.01$, Table 2).

Strontium

Strontium mean concentration presented different trends and values between the sampled areas. Considering the concentration for whole otolith surface averaged for all individuals, saltwater fish presented a mean value of 2496 ± 366 μg g^{-1} (Table 2), which was significantly higher than the value observed for freshwater fish (728 ± 64 μg g^{-1}, ANOVA, $P < 0.05$). Analyzing the otoliths surface by periods of life, significant differences were observed among all combinations of FYL and LYL (Table 3).

The general pattern shows higher strontium concentrations in otoliths from saltwater fish (Fig. 3). Among them, average strontium concentrations for FYL ranged from 1845 to 2300 μg g^{-1} and they were significantly lower than mean concentrations for LYL, which ranged from 2175 to 4180 μg g^{-1} (Table 3, Fig. 3). For otoliths from freshwater fish, the inverse occurred, with mean concentrations for FYL (ranging from 632 to 967 μg g^{-1}) slightly higher than for LYL (642 to 851μg g^{-1}). Strontium concentrations measured along the scan axis of otoliths from freshwater fish presented a stable and flat pattern almost through the complete otolith surface, but with an increase of concentration observed at the FYL region (Fig. 4). Except for otolith number 5, all otoliths from freshwater fish presented significant higher strontium concentrations at FYL than at LYL region (Tukey *HSD* test, $P < 0.05$, Fig. 3). The uniform patterns observed in the strontium curves for fish from the Mirim Lagoon were not observed in fish from Rio Grande, where the concentrations increased significantly with fish age ($r > 0.6$ and $P < 0.05$, Fig. 4). Strontium begun to increase between one to five years, for most part of the individuals. Mean ± sd strontium signatures were determined as 2990 ± 635 μg g^{-1} for marine, 2069 ± 238 μg g^{-1} for estuarine and 710 and 111.6 μg g^{-1} for freshwater habitats (Fig. 6).

Barium

Barium patterns showed an inverse behavior from that found for strontium, with higher concentrations measured close to the core. Higher mean values were observed for otoliths from freshwater than from salt water (ANOVA, $P < 0.05$, Table 2). FYL presented higher barium concentrations than LYL for fresh and saltwater fish (Table 3). For otoliths from the Mirim Lagoon specimens, barium ranged from 83 to 211 $\mu g\ g^{-1}$ for FYL and from 83 to 167 $\mu g\ g^{-1}$ for LYL. Otoliths from coastal Rio Grande presented mean concentrations from 0.6 to 42 $\mu g\ g^{-1}$ for FYL and <0.46 to 11.8 $\mu g\ g^{-1}$ for LYL.

For most freshwater individuals, the otolith core presented barium concentrations above 100 $\mu g\ g^{-1}$ (Fig. 5). After the otolith core, towards the edge, barium concentrations were followed by a peak around the first year for freshwater and before one year for saltwater fish (Fig. 5). In those individuals barium maxima reached almost 300 $\mu g\ g^{-1}$, declined to about 100 $\mu g\ g^{-1}$ after 5 years, and remained at approximately this concentration for the rest of fish's life.

For fish from marine habitat, maximum barium concentration reached about 40 $\mu g\ g^{-1}$ and presented a fast decline before the first year to levels sometimes below 0.46 $\mu g\ g^{-1}$ (Fig. 5). In general, barium profiles presented a similar pattern within habitats, with minor differences due to the peak intensity, and the absence of a peak for one otolith.

Mean ± sd barium signature for freshwater fish (Fig. 6) was characterized by concentrations of about 111.6 ± 30 $\mu g\ g^{-1}$, which contrasted with the much lower signal of otoliths from the marine habitat (2.7 ± 3.3 $\mu g\ g^{-1}$). Barium mean signature for estuarine fish was estimated as 16.7 ± 1.6 $\mu g\ g^{-1}$.

Discussion

Strontium and barium profiles

Strontium and barium variability in fish otoliths has been increasingly applied to evaluate migration between fresh and saltwater environments. Most studies point out that strontium deposition is an indicator of water salinity (e.g. Secor and Rooker 2000; Elsdon and Gillanders 2002; Rooker *et al.* 2002; Arai *et al.* 2007), and that barium varies inversely to salinity (Vries *et al*. 2005; Bath and Wuenschel 2006; Hamer *et al*. 2006). In marine waters, concentration of barium is lower than estuarine or freshwater and controlled by barite ($BaSO_4$) precipitation (Jacquet *et al.* 2005). Due to this fact and the higher solubility of strontium in sulfate-rich environments (e.g. ocean water), the partitioning of strontium between water and otolith (and also other carbonate-like structures: e.g. corals, mussel shells) is more favorable. Our results corroborate this assumption. Otoliths from freshwater specimens presented strontium concentrations of about 2 to 4 times lower than otoliths from estuarine and marine fish. Barium concentrations in otoliths from freshwater fish were up to 100 times higher than that from marine habitat.

For ocean-collected *M. furnieri*, strontium concentrations increased as fish grows, with high concentrations towards the edge of otoliths. After strontium began to increase, its concentration did not return to the same previous level. If we consider the increase of strontium concentrations in otoliths as an indicator of salinity (e.g. Campana 1999), that pattern could indicate a move towards a marine habitat. Strontium increase towards otolith edge was observed for chum salmon *Oncorhynchus keta*, as an indicative of fish migration as well (Arai *et al.* 2007). However, a magnification of strontium concentration due to slow growth rates at old ages has been observed for *Epinephelus guttatus* (Sadovy and Severin 1994), suggesting that strontium could not be used to make inferences about fish migration. According to Elsdon and Gillanders (2002), ontogeny does not affect the incorporation of strontium in otoliths of *Acanthopagrus butcheri*. The influence of salinity, temperature and growth rates on elemental composition of otoliths have already been studied in *Micropogonias undulatus* and variability on strontium deposition have been attributed to otolith size, as promoted by differences on experimental temperatures, and ontogeny (Fowler *et al.* 1995 a,b). However, there is not a general agreement about the influence of growth rates and ontogeny on otolith microchemistry. The growth rate effect on elemental incorporation was also not established for otoliths of *Leiostomus xanthurus* (Bath *et al.* 2000). Since *M. furnieri* from the Mirim Lagoon lived constantly in freshwater and there was no strontium increase toward older ages, which presumably presents lower growth rates, this indicates that strontium is not influenced by fish ontogeny nor growth rates. Therefore, otolith strontium concentration can be used as a good indicator of water salinity where fish grew. We believe that the temporal resolution of 2 to 3 months obtained from the LA-ICPMS equipment used here would be detailed enough to reveal a seasonal temperature effect on strontium deposition. However, the absence of a visual seasonal pattern suggests that strontium deposition is not importantly influenced by temperature.

The barium profiles showed temporally well-defined peaks during the first year of life. After those peaks towards the otolith edge, barium concentrations in otoliths decreased to very low levels for fish from coastal Rio Grande and maintained about 100 $\mu g\ g^{-1}$ for fish from Mirim Lagoon. Despite the difference in magnitude, with higher barium concentration measured for otoliths from Mirim Lagoon fish, the occurrence of the peaks was consistent between environments. This consistency and the lack of an equivalent pattern in the strontium profiles suggest that barium deposition in otoliths of *M. furnieri* is regulated by an ontogeny-mediated process during its initial life. According to Ruttenberg *et al.* (2005), there is a trace metal enrichment (including barium) in the core region for a variety of fish species worldwide and it could be inherited by the mother. In a recent work about transgenerational mark of embryonic otoliths, Thorrold *et al.* (2006) have injected radioactive barium ($^{137}BaCl_2$) into females of *Amphiprion melanopus* and *Centropristis striata* and observed clear barium signatures in otoliths from the progeny. This finding suggests that the concentrations of barium in the otolith core could reflect the mother's environment. However, the peaks observed for Mirim Lagoon specimens got extended to almost five years of life, and that extra deposition of barium could not be inherited by the mother for such long time. The effect of ontogeny in otolith microchemistry seems to be specific to for different elements and species. Despite there is a certain negative relationship between environmental salinity and barium concentration on otoliths of *M. furnieri*, that relationship is masked during the beginning of the life. Therefore, barium concentration must be interpreted carefully during the fist years of life, under the risk of obtaining incorrect conclusions about fish movement and environmental conditions.

When comparing strontium and barium profiles for freshwater fish, it becomes evident that strontium represents a better indicator for salinity because it delineates a more constant pattern along of the fish's life than that observed for barium. Assuming similar temperature fluctuations for both sampled areas and considering temperature as the other main variable acting on the freshwater environment, our results show that temperature could not mask the change in strontium concentration in otoliths as a result of salinity variations during fish's life. In other words, the effect of other environmental variability (e.g. temperature) on strontium deposition is weaker than changes in salinity. This indicates that strontium concentration in otoliths can be adequately used to study *M. furnieri* habitat change between marine, brackish and freshwater. Differently, the oscillations observed for barium concentrations in otoliths from freshwater croakers were sometimes higher than 60 μg g^{-1} (excluding the values observed during the five initial years). That oscillation is higher than the total barium range observed for some marine individuals. It could be expected that barium concentration in otoliths from a stable freshwater environment should be therefore similarly stable, but that was not true. Relatively strong variability was observed for barium concentrations along the growth axis of *M. furnieri* otoliths. For estuaries, Elsdon and Gillanders (2005b) found significant variability of barium and other elements in the water over periods of hours, days, weeks and seasons. Most part of estuarine barium comes from barium-rich river-suspended sediment particles (Nozaki *et al.* 2001). Removal and release of trace elements in water are processes mainly controlled by precipitation and sorption (including ion-exchange) onto particles (Coffey *et al.* 1997). Since the Mirim Lagoon receives water from a large drainage basin (together with the Patos Lagoon, the drainage basin is about 263,876 km^2 – Seeliger *et al.* 1996), it is therefore suitable that barium fluctuations in otoliths from Mirim Lagoon fish are related to other environmental factors than local salinity or temperature.

Strontium and barium reference concentrations

Considering the *M. furnieri* group in the Mirim Lagoon as an indicator for freshwater signature, the concentrations of strontium and barium measured in their otoliths may be used as a natural and reliable reference for freshwater environments (about 700 μg g^{-1} for Sr and 100 μg g^{-1} for Ba, see Fig. 6). By the other side, the elemental signatures in otolith from saltwater specimens (about 2900 μg g^{-1} for Sr and 2 μg g^{-1} for Ba, Fig. 6) may also be considered as a reference for marine environments.

Based on the life cycle of *M. furnieri*, which allows us affirming that this species spends its initial life in estuarine waters (Vazzoler 1975; Macchi and Christiansen 1996; Sinque and Muelbert 1997; Acha *et al.* 1999; Vazzoler 1999; Jaureguizar *et al.* 2003), a typical signature may also be inferred for this environment. This signature can be estimated from the mean concentration of strontium and barium for the first year of life for fish collected at Rio Grande coast (about 2000 μg g^{-1} and 16 μg g^{-1} for Sr and Ba respectively –

Fig. 6). Complementarily, we used the freshwater and saltwater signals as reference values. to confirm the coherence of the estimated estuarine concentrations.

Campana (1999) has summarized trace element concentrations in otolith from the tree major aquatic habitats and reported strontium values (mean ± standard error) of 2137 ± 127 µg g^{-1}, 1937± 70 µg g^{-1}, and 698 ± 111 µg g^{-1} for marine, estuarine and freshwater species, respectively. He also presented barium mean concentrations of 3.7 ± 0.6 µg g^{-1} for marine, 8.2 ± 2.7 µg g^{-1} for estuarine and 11 ± 2.8 µg g^{-1} for otolith from freshwater fish. In our study, we observed very similar strontium concentrations for freshwater and estuarine environments, but higher and more variable concentrations for the marine habitat (2990 ± 635 µg g^{-1}), corroborating the mean concentrations of about 2800 µg g^{-1} reported for strontium in otoliths of *M. furnieri* from the San Blás Bay, Argentine (Volpedo and Cirelli 2006). For barium, the concentrations determined here were about ten times higher for the Mirim Laggon than the mean concentration published for freshwater species (Campana 1999).

In theory, the concentrations of metals in otolith should preserve a distinct relationship to their concentrations in the water (Hamer *et al.* 2006; Elsdon and Gillanders 2004; Bath *et al.* 2000), but sometimes this may not occur due to specie-specific characteristics (Swearer *et al.* 2003). The noticeably higher barium values determined in otoliths of *M. furnieri* from freshwater (Mirim Lagoom) could be promoted by abnormal high barium concentrations in its water. Unfortunately, water data are not yet available, neither comparative data on Ba concentrations for otoliths of this species from other freshwater environments. This information would be necessary in order to define the barium concentration levels found here as a species-specific attribute or as an environmental-induced result.

Implications for M. furnieri life cycle

Strontium and barium results from our study suggest interesting information on the life cycle of *M. furnieri*. Fish collected in the Mirim Lagoon were 35 years old on average. Strontium values were high close to the core, suggesting those fish entered the lagoon before one year of life. Lower and relatively constant strontium values observed thereafter suggested that *M. furnieri* lived inside the Mirim Lagoon for the rest of their life. Since no adults younger than 30 years were captured, it can be inferred that no more entrance of this species has occurred in the Mirim Laggon. About 30 years ago, a floodgate was built in the São Gonçalo channel, the only communication between the estuary of Patos Lagoon and Mirim Lagoon. Our results demonstrate that this closure was effective on preventing significant fish migration between the coast of Rio Grande and Mirim Lagoon, and it is the most plausible explanation for the absence of young fish inside the Lagoon. If *M. furnieri* could reproduce in the Mirim Lagoon, the absence of higher strontium concentration at the otolith core would be common. It is known that *M. furnieri* spawning in the Río de la Plata estuary is coincident with the bottom salinity front (Macchi and Christiansen 1996; Acha *et al.* 1999). In the Patos Lagoon Estuary, spawning occurs in coastal waters (Ibagy and Sinque 1995) and larvae occurrence is associated to salt water intrusions (Sinque and Muelbert 1997). Therefore, these evidences indicate that *M. furnieri* requires salt water for its reproduction and therefore it would not be able to hatch inside the Mirim Lagoon.

For this reason, we characterize the Mirim Lagoon *M. furnieri* as a land- locked group and not as a population, since reproductive activity is one of the attributes defining the biological concept of population. Moreover, that group will probably be extinct in the near future because of the fishery activity in that Lagoon, and the absence of adequate stock renewal.

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Table 1. Operating conditions for the ICPMS instrument and the Nd: YAG laser (266 nm) used in this study.

ICP MASS SPECTROMETER	ELAN 5000A (Perkin-Elmer/Sciex)
Sweeps/reading	3
Replicates	200 – 360 (depending on the otolith size)
Scanning mode	“Peak-hop”
Dwell time	40 ms
Forward power	1300 W
Carrier gas flow (Ar)	0.850 dm^3 / min
Outer gas flow (Ar)	15.0 dm^3 / min
Intermediate gas flow (Ar)	1.00 dm^3 / min
Isotopes measured (*m/z*)	^{43}Ca, ^{86}Sr, ^{138}Ba, ^{24}Mg and ^{55}Mn
LASER ABLATION SYSTEM	LSX-100 (CETAC)
Mode	Q-switched
Laser beam energy	0.6 - 0.8 mJ
Beam divergence	0.8 mRad
Beam size diameter	1.0 mm
Defocus	10 µm
Crater diameter in otoliths	about 30 µm
Pulse width	< 8-12 ns
Repetition rate	20 Hz
Rastering rate	40 µm/s
Distance between lines	80 µm
Pre-ablation time	6 s
Total acquisition time	100 - 200 s (depending on the otolith size)

Table 2. Mean age, total length (TL), and mean concentrations of Sr and Ba in otoliths of *M. furnieri* sampled at the coastal Rio Grande (marine habitat) and the Mirim Lagoon (freshwater habitat). SD = Standard deviation.

Region	*n*	Age (years) mean ± sd	TL (cm) mean ± sd	Sr (μg g^{-1})* mean ± sd	Ba (μg g^{-1})* mean ± sd
Rio Grande	10	14.1 ± 6.7	60.7 ± 7.2	2496 ± 366	6.9 ± 5.5
Mirim Lagoon	10	34.3 ± 1.1	41.7 ± 3.2	728 ± 64	113 ± 22

*Concentrations averaged for all otoliths using the entire scanned profile from each otolith.

Table 3. Comparison of Sr and Ba concentrations between periods of life and sampled areas for otoliths of *M. furnieri*. Mean concentrations and standard deviation ($n = 10$ individuals per sampled area) for the first year of life (FYL) of fish from the Mirim Lagoon and Rio Grande are expressed as M_{FYL} and R_{FYL}, and for the last year of life (LYL) as M_{LYL} and R_{LYL}, respectively. The P values (resultant from Tukey's tests) were e expressed as <0.01 to avoid large decimal numbers in the table.

		Sr (mean µg g^{-1} ± sd)				Ba (mean µg g^{-1} ± sd)			
		M_{FYL}	R_{FYL}	M_{LYL}	R_{LYL}	M_{FYL}	R_{FYL}	M_{LYL}	R_{LYL}
		834	2069	710	2990	138	16.7	111.6	2.7
		± 194	± 238	± 76	± 635	± 57	± 20.4	± 30	± 3.3
	M_{FYL}	-	<0.01	<0.01	<0.01	-	<0.01	0.45	<0.01
P values	R_{FYL}		-	<0.01	<0.01		-	<0.01	<0.01
	M_{LYL}			-	<0.01			-	<0.01

Figure Legends

Figure 1 – Sampling area at South Brazil. A = Floodgate of São Gonçalo Channel.

Figure 2 – Photomicrograph of a left otolith section of a 23-years old *M. furnieri* from Rio Grande showing the laser ablation rims. The images were taken using incident light (principal image) and transmitted light (zoom appendix). The otolith core is indicated by the letter C and the locations of annuli are indicated by numbered arrows. Scales represent 1 mm (white bar) and 100 µm (black bar).

Figure 3 – Strontium and barium average concentrations for the first (FYL) and last (LYL) year of life for *M. furnieri* otoliths from the Mirim Lagoon (white bars) and coastal Rio Grande (grey bars). Error bars indicate standard deviation.

Figure 4 – Strontium concentrations for whole analyzed scans for otoliths of *M. furnieri*. The arrows indicate the location of the 1, 5, 20 and 35th annuli for specimens of the Mirim Lagoon, and 1, 5 10 and 20th annuli for the Rio Grande individuals.

Figure 5 – Barium concentrations for whole analyzed scans for otolith of *M. furnieri*. The arrows indicate 1, 5, 20 and 35th annuli for specimens of the Mirim Lagoon, and 1, 5 10 and 20th annuli for the Rio Grande individuals.

Figure 6 – A semi-quantitative conceptual model of strontium and barium signatures in otoliths of *M. furnieri* for marine, estuarine and freshwater habitats at South Brazil.

Figure 1

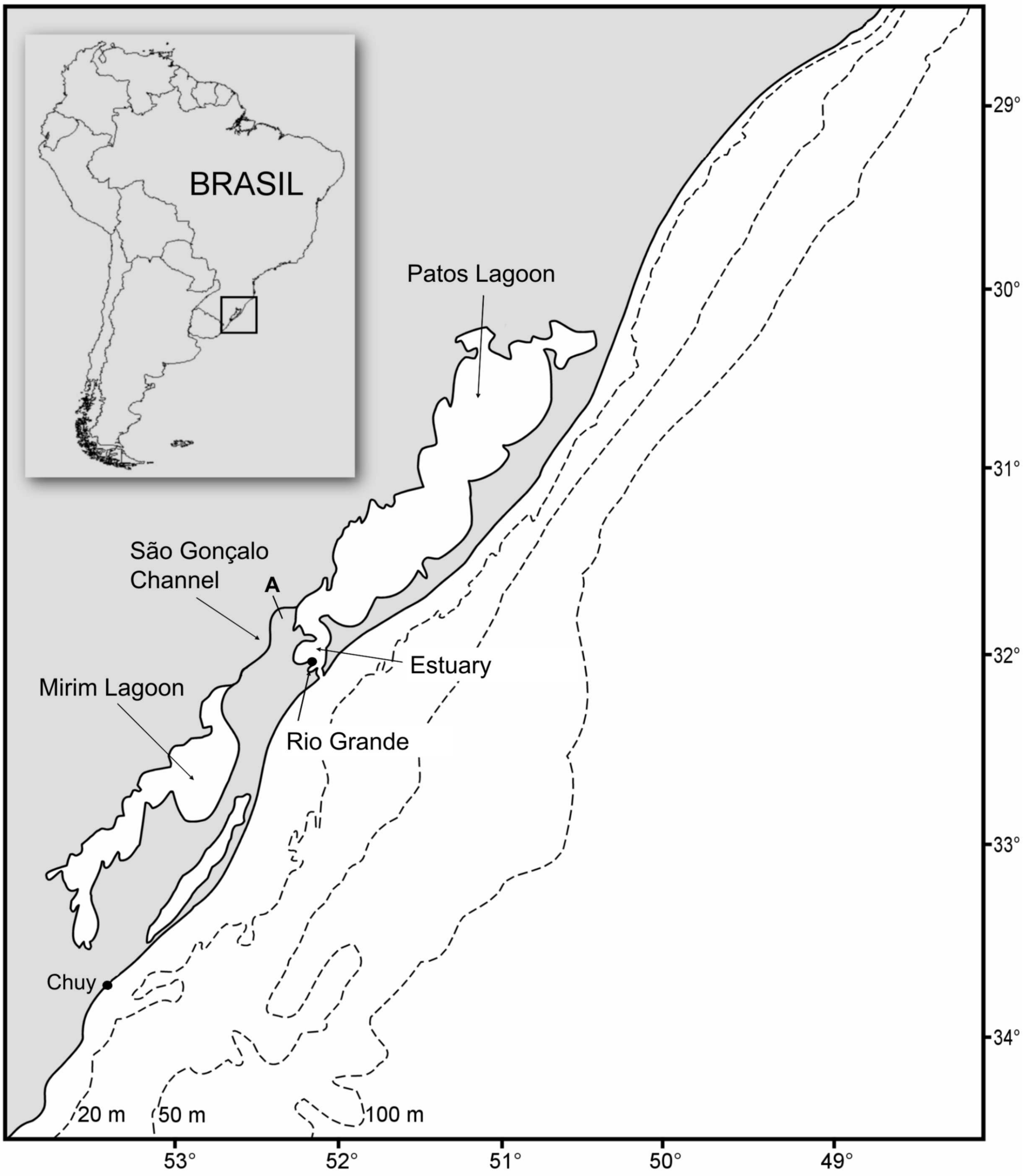

BRASIL
Patos Lagoon
São Gonçalo
Channel
A
Estuary
Mirim Lagoon
Rio Grande
Chuy
20 m
50 m
100 m
29°
30°
31°
32°
33°
34°
53°
52°
51°
50°
49°

Figure 2

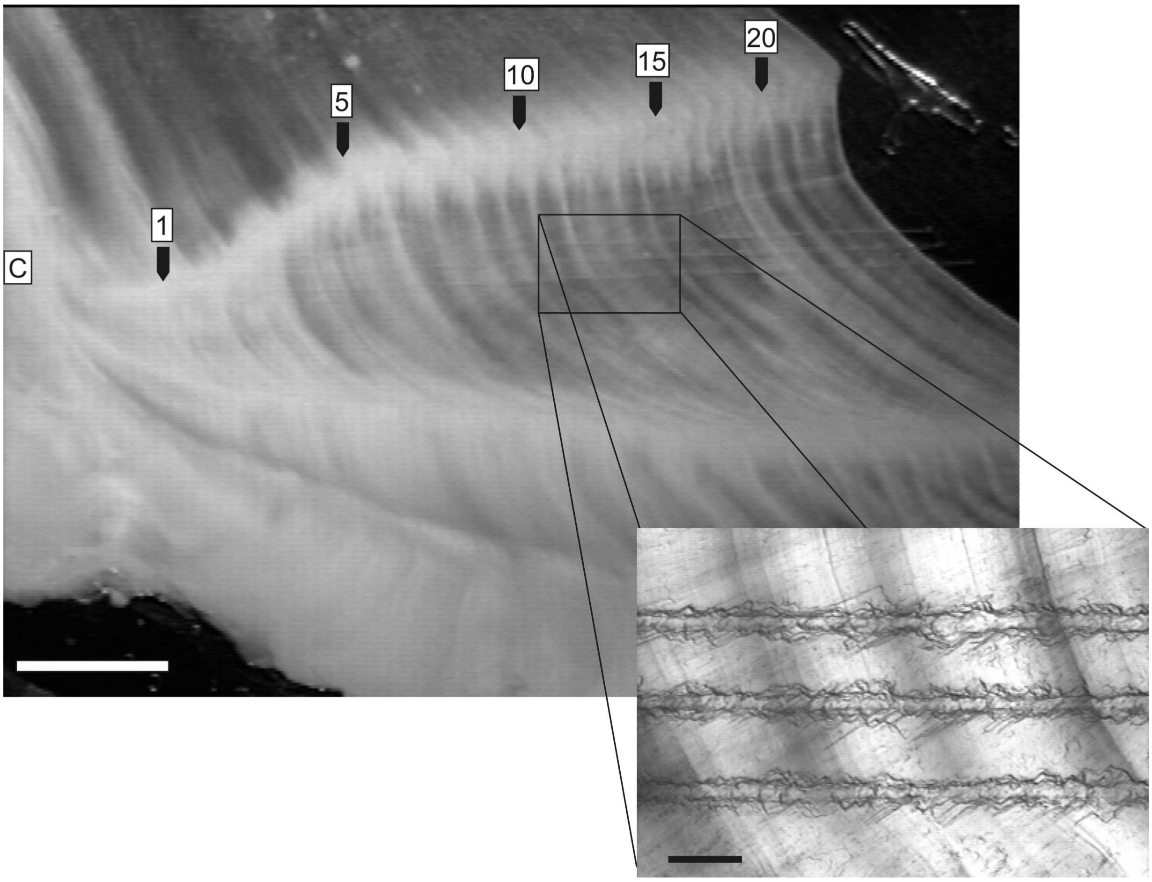


Figure 3

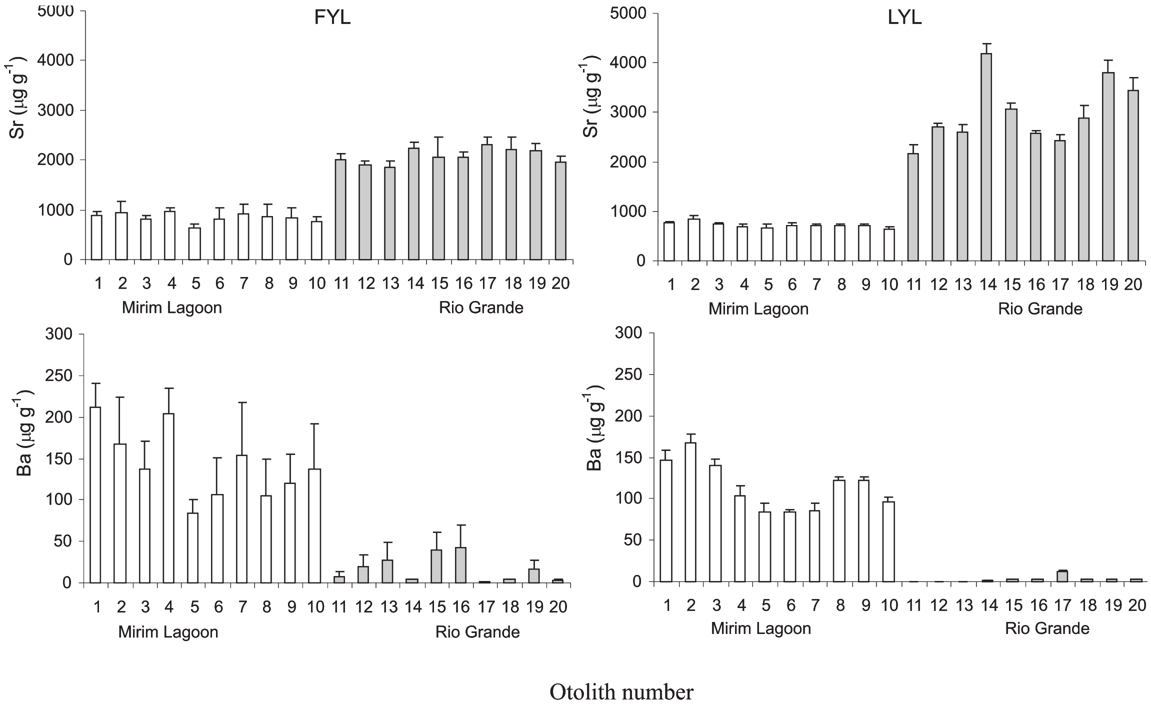

FYL
LYL
Sr (µg g^{-1})
Ba (µg g^{-1})
0
1000
2000
3000
4000
5000
50
100
150
200
250
300
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Mirim Lagoon
Rio Grande
Otolith number

Figure 4

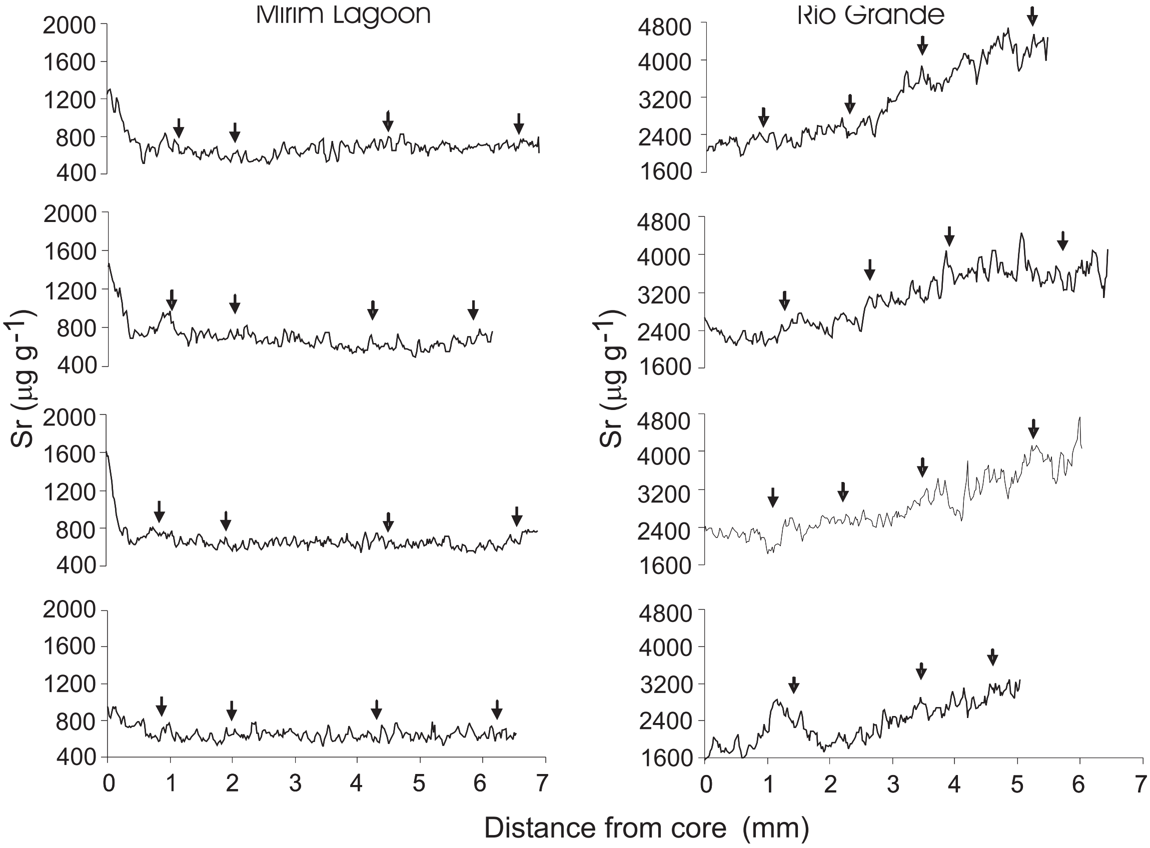

Mirim Lagoon
Rio Grande
Sr (µg g^{-1})
2000
1600
1200
800
400
4800
4000
3200
2400
1600
0
1
2
3
4
5
6
7
Distance from core (mm)

Figure 5

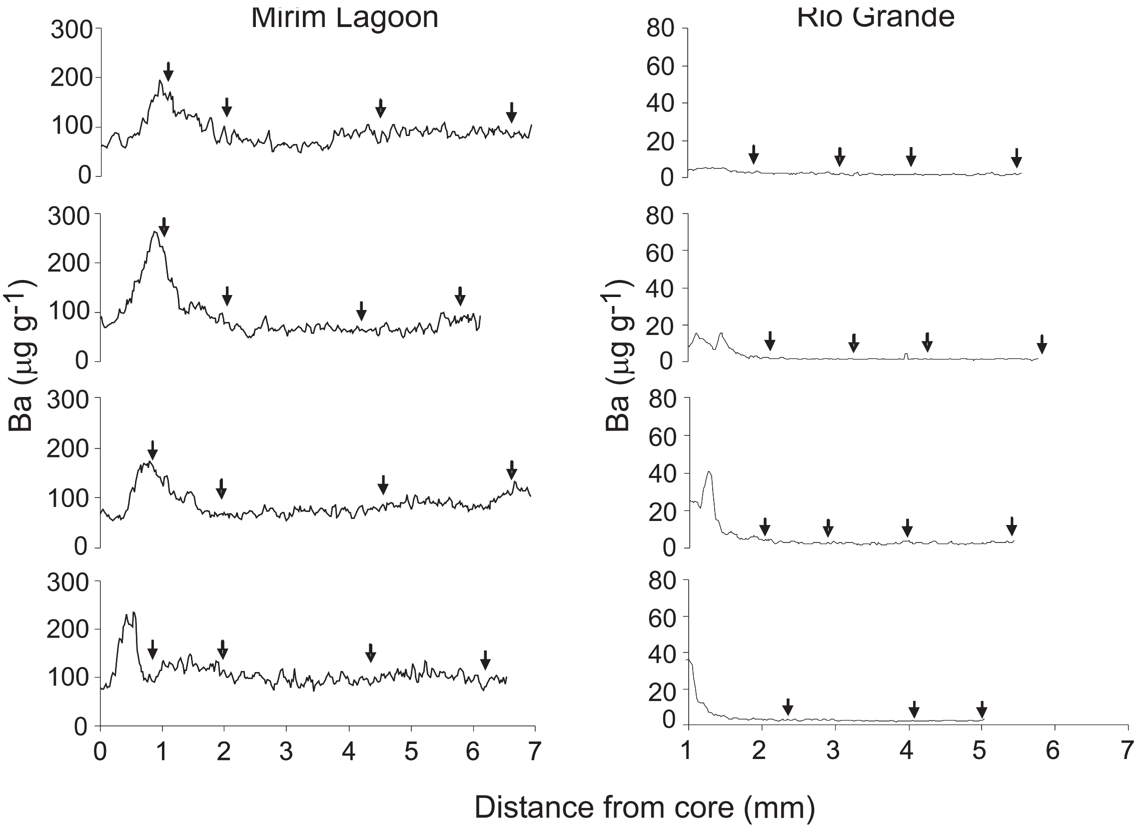
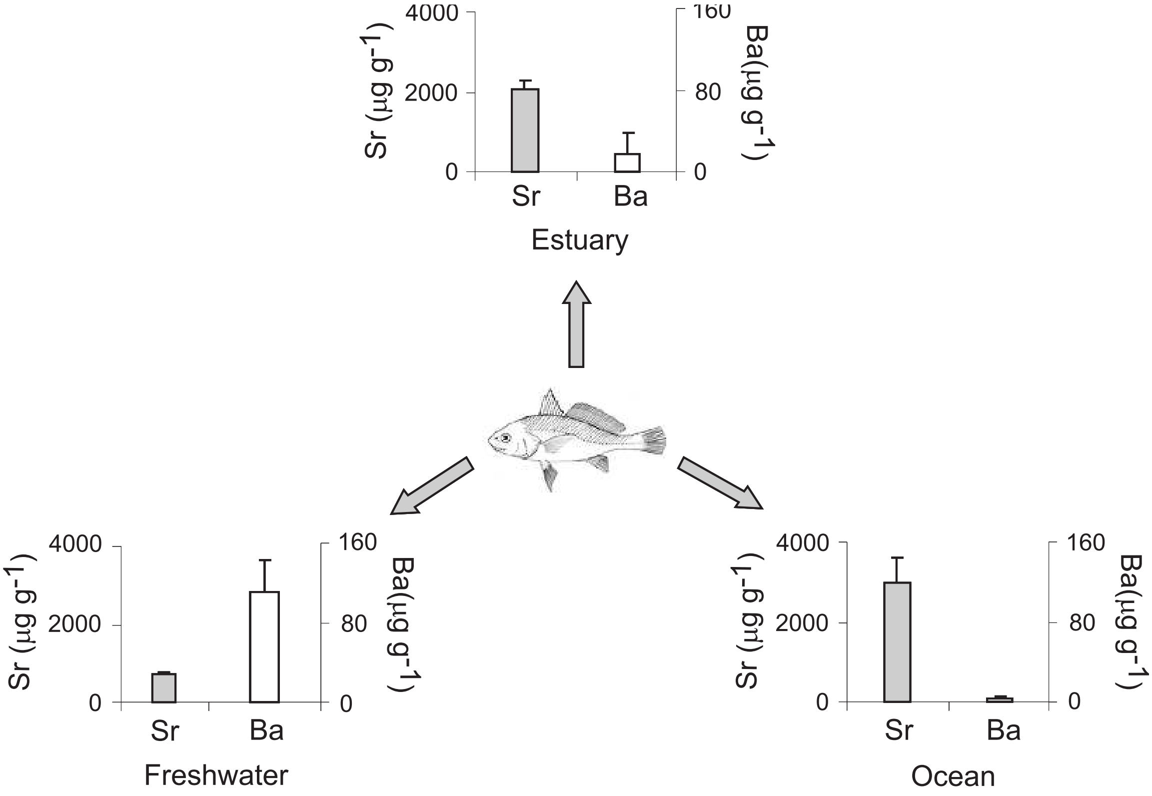

Mirim Lagoon
Rio Grande
Ba (µg g^{-1})
300
200
100
0
80
60
40
20
0
1
2
3
4
5
6
7
Distance from core (mm)

Figure 6


4000
2000
0
Sr (μg g^{-1})
160
80
0
Ba(μg g^{-1})
Sr
Ba
Estuary
4000
2000
0
Sr (μg g^{-1})
160
80
0
Ba(μg g^{-1})
Sr
Ba
Freshwater
4000
2000
0
Sr (μg g^{-1})
160
80
0
Ba(μg g^{-1})
Sr
Ba
Ocean

ANEXO III

Ontogenetic habitat change in a long-lived sciaenidae fish: an approach based on otolith microchemistry

Albuquerque, C.Q., Miekeley, N., Muelbert, J.H.

Ontogenetic habitat change in a long-lived sciaenidae fish: an LA-ICPMS study applied on otolith microchemistry

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Abstract

Strontium concentrations on otoliths of whitemouth croaker *Micropogonias furnieri* from Brazilian (São Paulo and Rio Grande), Uruguayan and Argentinean coastal waters were examined in order to determine patterns of estuarine use and fish migration towards coastal habitats in the South-West Atlantic continental shelf. Additionally, samples from a Brazilian freshwater coastal Lagoon (Mirim Lagoon) and an estuary (estuary of Patos Lagoon) were taken to provide elemental reference concentrations for freshwater and estuarine habitats. A LA-ICPMS calibrated with $CaCO_3$ standards was used to measure absolute strontium concentrations along profiles from the core to the edge of the otoliths. The strontium profiles were individually averaged by fish age and analyzed for the interpretation of estuarine use through evaluation of initial strontium concentrations (ages 0 and 1). Habitat change was assessed interpreting the time of escape from estuarine influence (TEEI), measured as the time at which strontium concentration in otoliths have reached values about 2000 μg g^{-1}. Long-term habitat change was interpreted by adjusting an exponential function to the strontium profiles along fish life time. The results show strontium concentrations in otoliths to be about 800 μg g^{-1} for freshwater, 1800 for estuarine and generally over 2000 μg g^{-1} for coastal marine habitats. Whitemouth croaker presented consistent signals of estuarine habitats for the ages 0 and/or 1 for all regions. The average TEEI ranged from 4 yr for south Brazil to 7.5 yr for Uruguay. This result suggests estuarine dependence as a species-specific attribute characteristic of *M. furnieri*. Three long-term habitat change patterns were observed: (1) Pattern A represents fish that do not stabilize the long-term habitat change and search more saline waters for the entire life; (2) Pattern B comprises fish that stabilizes long-term habitat change at high salinity waters; and, (3) Pattern C represents fish that spend the entire life close to estuarine influenced areas. Except for the absence of pattern A for Argentinean coast, the lack of latitudinal organization of these migratory patterns suggests a species-specific strategy of fish displacement along the South-West Atlantic continental shelf.

Keywords: *Micropogonias furnieri*, otolith microchemistry, strontium, estuarine use, habitat change, fish migration.

1. Introduction

Many theories have been proposed and many concepts have been elaborated in order to explain and understand the existing movements performed by fish among habitats for different taxonomic groups [1]. Understanding the migratory patterns of animals in nature represents key information on ecology, particularly when regarding to their conservation. In this study we will approach the study of movements of fish from estuarine-brackish towards coastal-marine habitats, not in seasonal, but in life-time basis. Therefore, the term “migration” would not be appropriate here. Recent studies raised two classifications approaching the use of estuaries by fish [2], regarding fish that move but do not complete the fresh-marine water migration: “Marine stranglers” which contains species that spawn at sea and typically enter estuaries only in low numbers; and, “Marine migrants” which refers to species that spawn at sea and often enter estuaries in substantial numbers.

The whitemouth croaker, *Micropogonias furnieri*, is an eurihaline long-lived (>40 years) sciaenid species widely distributed along the eastern coast of the Atlantic Ocean, occurring from the Gulf of Mexico to the Gulf of San Matías (Argentina). Together with other sciaenids, *M. furnieri* supports over 50% of the total fisheries production in Southern Brazil [3] and it is a biomass-dominant species, which also represent important fisheries in Argentina and Uruguay [4]. It is one of the most studied fish species in South America and ecological aspects related to reproduction, growth, diet, nursery areas and spatial distribution are already known in Argentina and Uruguay [5, 6, 7] and Brazil [8, 9, 10, 11]. In Argentina and Uruguay, *M. furnieri* spawns preferably in the inner part of the Río de la Plata estuary [12] and in Brazil it spawns mostly in the outer part of Lagoa dos Patos estuary [10]and Cananeia Lagoon [13] Eggs and larvae are transported to estuaries [9] and the juveniles inhabit estuarine shallow zones [7]. Despite the seasonal reproductive movements towards estuarine regions, the movements carried out by *M. furnieri* during its life is not well understood. It is suggested that during the first sexual maturation young adults emigrate to the coastal zones where they spawn, and occupy the continental shelf being fished mostly under 50 m of depth (8, 14). Therefore, at a first approach *Micropogonias furnieri* could be considered a “marine migrant” fish species, as defined by previous studies [2]. However, there is not a conclusion concerning the strategies of estuarine utilization for this species along the life. Estuarine dependency [15] and estuarine opportunism [16] have been described as strategies of habitat use by *M. furnieri*. These evidences demonstrated that whitemouth croaker movement patterns and life cycle may be more complex than suggested so far.

Many techniques have been developed in order to follow fish movement over time. Some examples are mark-recapture methods [17], telemetry [18], passive integrated transponder [19] and natural [20] and artificial [21] chemical tags on otoliths. Otolith microchemistry has been increasingly used to access fish migration since otoliths were described as metabolically inert structures that grow continuously throughout fish life and store information of the surrounding environment [22]. Therefore, otoliths record information about the environment were fish grew, particularly regarding salinity [23], temperature [24] and water chemistry [25]. [26] pointed out the advantages of studying fish migration from otolith chemistry based on the fact that each single fish is naturally tagged (otolith approach) and returns an entire life of information, while tag methods only provide information at two moments (time of tagging and recapture). Strontium and barium availability in otoliths have already been reported as good indicators of habitat use by *M. furnieri*, with high barium concentrations observed for otoliths from freshwater fish and high strontium concentrations found for marine ones [27]. Although migration regards diverse temporal scale, from days to years, most part of studies on fish movements have focused the attention on the seasonal based migratory behavior (e.g. reproduction). There is almost no attempts regarding a large temporal scale movement or eventual changes on habitat change trends along the life of long-lived fish species (but see [28]).

In this study, movements of *M. furnieri* based on the interpretation of strontium profiles in otolith from fish collected at Brazilian, Argentinean and Uruguayan waters is investigated over large time scales. More specifically, we will evaluate the estuarine use by *M. furnieri* and test the hypothesis of *M. furnieri* as presenting a single habitat change strategy in the South-West Atlantic Ocean.

2. Material and methods

2.1. Sampling and sample preparation

The study was developed in the coastal region of the South-west Atlantic Ocean, and includes the Argentinean, Uruguayan and Brazilian coasts (Fig. 1). Between 2004 and 2005, 147 adults of whitemouth croaker (*M. furnieri*) were collected from commercial fisheries at north coast of Argentina (*n* = 34), Uruguay (n = 32), Rio Grande/Brazil (*n* = 31), São Paulo/Brazil (*n* = 30) and Mirim Lagoon/Brazil (*n* = 20). Ten juveniles were collected in the estuary of Patos Lagoon.

All sampled specimens were measured in total length, sexed and sagittal otoliths were extracted, cleaned and stored dry. The left otoliths were then embedded in crystal polyester resin and transverse sections were cut through the core using a low-speed diamond blade saw. Thin sections (0.4 mm) were mounted onto glass slides with crazy glue. Prior to LA-ICPMS analysis, otolith surfaces were polished with silicon carbide paper (n^o 8000), washed with deionized (DI) water (Milli-Q, Millipore, Bedford, USA), sonicated for 3 minutes, and rinsed three times with DI water. The slides were dried in a horizontal flow cabinet before analysis.

2.2. Elemental determinations

Analytical measurements were performed with a Nd:YAG CETAC LSX 100 Laser Ablation system operating at 266 nm, coupled to an ELAN 6000 (PerkinElmer – SCIEX) inductively coupled plasma-mass spectrometer (ICP-MS). The laser was configured for Q-switched mode operation, defocused, a pulse frequency of 20 Hz, scan speed of 40 μm/s, and power of 0.4 – 0.6 mJ per pulse. Under these conditions the beam diameter was about 15 μm. The ablated material was conducted through a Teflon coated tube into the ICP-MS using Argon as carrying gas (0.85 dm^3 min^{-1}). The ICP was operated at 1500 W with outer and intermediate gas flow of 15 and 1.1 dm^3 min^{-1} respectively. The determined elements (masses) were ^{43}Ca, ^{86}Sr, ^{138}Ba, ^{24}Mg and ^{55}Mn. However, only ^{86}Sr concentrations were evaluated for this work. The quantification was performed using the external calibration mode. The standards were constituted by pressed powder $CaCO_3$ discs with known analyte concentration, and suprapur $CaCO_3$ discs were used as a blank. These standards were proposed and produced by [29,30]. The measured signal (counts per second – cps) for each element was normalized with reference to ^{43}Ca for correction of the bias induced by differences on the amount of ablated material. To obtain the limits of detection (LOD), the blank discs were scanned 10 times. Each mean profile was used as a single analyte concentration and the standard deviation (σ) from the 10 values was considered. The LOD was calculated as 3σ divided by the sensitivity (inclination) of the respective calibration curve and it was 4.9 μg g^{-1}. The limit of quantification (10σ / inclination) was determined as 49 μg g^{-1}.

2.3. Data analysis and model description

After chemical analysis, otoliths were photographed under a microscope. Otolith images were measured using the UTHSCSA ImageTool programme (University of Texas Health Science Center at San Antonio, Texas, ftp://maxrad6.uthscsa.edu) in order to assign ages to specific locations on the scanned profile. The strontium profiles were analyzed individually by fish and were averaged by year and plotted against age for each sex. Therefore, seasonal signals were not considered in this study. The otolith strontium concentration relative to the range of estuarine influence was calculated as the average concentration and standard deviation of strontium in otoliths, measured between ages 0 and 1.

Tree approaches have been considered in order to characterize the long-term habitat change patterns. The first one studied the existence of a juvenile pattern of movement and it is related to the estuarine use by *M. furnieri*. Juvenile movement was evaluated thorough the strontium concentrations observed for the ages 0 and 1, using the "juvenile movement index" (JMI) to evaluate the direction of juvenile movements. JMI was calculated for all individuals by subtracting strontium mean concentration of age 0 from age 1, except for fish from Mirim Lagoon. Therefore, neutral, positive and negative values of JMI indicate respectively juvenile residency, movement toward higher salinities and movement towards lower salinities, between ages 0 and 1. To be considered negative or positive, the absolute value of JMI have to be greater than 9.9 $\mu g\ g^{-1}$ (or lower than -9.9 $\mu g\ g^{-1}$) which represents the limit of quantification of the LA-ICPMS analysis performed here.

The second approach investigates the moment in which *M. furnieri* escapes from the estuarine influence (Time of Escape from Estuarine Influence – TEEI). This attribute was calculated as the cumulative frequency of individuals out from the estuarine influence by age for the four coastal regions. These frequencies were fitted (by least squares) to a logistic function (1) in order to determinate the theoretical age where 50% of the individuals are out of estuarine influence (F_{50}). The logistic model is defined by the equation:

$$F_i = \frac{1}{1+e^{(a-T_i)}} \qquad (1)$$

Where F_i is the relative frequency of individuals out of estuarine influence at age i, a is the model parameter that represents the TEEI and T_i is the age i.

The third approach described the overall long-term habitat change pattern. Since we arbitrarily expect that individuals older than 10 years have already started to express their pattern of long-term habitat change, a exponential function (2) was fitted to each strontium profiles for fish older than 10 years (AR, n = 8; UR, n = 21; RG, n = 21 ; SP, n = 12) using least squares estimation. The long-term movement curve (LTMC) is defined by the function:

$$Sr_i = Sr_\infty\{1-e^{[-K(T_i-T_o)]}\} \qquad (2)$$

where Sr_i is the mean strontium concentration at age i; Sr_∞ is the level of strontium concentrations in which the fish have stabilized its long-term movement; and, K represents how fast the fish reaches the long-term stability. This procedure was not performed for Mirim Lagoon fish because LTMC was not suitable to describe strontium accumulation patterns for that region. Data from the fist year (age 0) where removed from this analysis to improve the curve fitting.

2.4. Statistical analysis

Differences on strontium concentration and LTMC parameters among regions were tested using one way ANOVA, after the assumptions of normality, homogeneity of variances and mean-variance relationship were verified. The LTMC parameter T_0 was disregarded from the analysis because of the lack of ecological meaning. Differences on the frequencies of patterns of JMI and LTMC among sites were tested using x^2 test. Additionally, LTMC parameters were analyzed by multidimensional scaling (MDS) in order to verify the existence of similarities. The significance of these similarities was tested trough Analysis of Similarity (ANOSIM). Both MDS and Analysis of Similarity were carried out trough the Primer 5.0 computer package (Plymouth Marine Laboratory).

3. Results

Adult whitemouth croaker presented mean total length ranging from 39.2 to 61.2 cm and mean ages ranging from 7.2 to 35.3 years (Tab. 1). The number of females (78) examined was slightly higher than the number of males (69). Females were predominant from São Paulo, Rio Grande and Argentina and males were predominant in Uruguay and Mirim Lagoon.

3.1. Strontium signatures

Initial strontium mean concentrations in otoliths (age 0 and 1) ranged from 645 $\mu g\ g^{-1}$ at Mirim Lagoon (Fig. 2–A) to 2500 $\mu g\ g^{-1}$ at São Paulo (Fig. 2–B). Significant differences on strontium concentrations of initial ages were observed between Mirim Lagoon (freshwater) and the other areas (Fig. 2; Tab. 1, $P < 0.05$). However, no significant differences were observed amongst the coastal sampled areas. Average strontium concentration in otoliths of juvenile *M. furnieri* was not significantly different from the initial concentration observed for adult fish (Tab. 1, $P > 0.05$). Higher mean concentration was observed for older ages for all sampled areas except for Mirim Lagoon (Fig. 2) and reached 4400 $\mu g\ g^{-1}$ in fish from Rio Grande (Fig. 2–C). There were no significant differences on strontium concentrations between sexes for age 0 and 1 (ANOVA, $P > 0.05$) and no differences on visual patterns were observed on long-term strontium concentration between sexes.

Negative values of JMI were significantly more abundant, particularly for Argentina, Uruguay and São Paulo (Tab 2). The largest proportion of negative JMI were observed respectively for Uruguay and Argentina. For Rio Grande, the three types of JMI were equally abundant. Positive JMI values were the second most abundant JMI type and its higher proportion was observed for Rio Grande. Neutral JMI occurred in significant smaller proportion on individuals from Uruguay and Argentina.

The logistic function showed that the time of escape from estuarine influence (TEEI) was variable according to the sampled area (Fig. 3). São Paulo, Argentina and Rio Grande presented close values of TEEI when compared to Uruguayan ones, which presented the highest TEEI (Fig. 3). After age 14 no more otoliths were observed with strontium concentration inside the range determined for estuarine influence.

LTMC parameters were summarized at table 3 and no significant differences were observed among areas (ANOVA, $P > 0.05$). Three theoretical habitat change patterns were raised according to the shape of LTMC (Fig. 4). A fourth pattern (D) was designed in order to represent the individuals from freshwater, and there are no values of Sr_∞ and K for this category. The patterns A, B, and C, were significantly different among themselves (ANOSIM, $P < 0.05$, Fig. 5). Therefore, long-term habitat change strategies were described as follows: Pattern A, presents very high values of Sr_∞ (> 4000) and very low K values ($K < 0.1$); Pattern Pattern B presents high Sr_∞ ($4000 > Sr_\infty > 3000$) and intermediate K values ($0.2 > K > 0.1$); and, for Pattern C it is observed low values of Sr_∞ (< 3000) and high values of K ($K > 0.2$). The co-occurrence of these strategies did not show any significant predominance between sites (Tab. 2). The patterns B and C were observed for all sampled areas while pattern A was not observed for Argentina. Three representative examples of individual strontium profiles show the fitting between theoretical and observed curves from each strategy of long-term migration (Fig. 6).

4. Discussion

4.1. Assumptions and concepts

The analysis of strontium in otoliths has been largely used to evaluate fish migration throughout a gradient of salinities [23; 20; 31]. A positive relationship between strontium concentration in otoliths and salinity has been refered in many studies (see [31] for a review). In sciaenids, positive relationship was observed for *Micropogonias undulatus* [32], *Pogonias cromis* [33] and *Micropogonias furnieri* [27]. Therefore, differences in the concentration of strontium along the otolith profile reflect changes in the salinity of the environment that fish experienced throughout its life time. Existing evidence supports the assumption that observed changes in strontium concentration is the result of movements between a salinity gradient. The extent of that gradient was not determined and for that reason we will not refer to absolute salinity concentrations but to general patterns of habitat change.

Recent studies demonstrated that whitemouth croaker that lived over 31 yr in freshwater (Mirim Lagoon) lack ontogenetic induced variation of strontium concentration along the otolith profiles [27]. The otolith strontium concentration found for those fish represent a confident strontium signature for freshwater fish.

Another important evidence is the capability to assign strontium signatures to estuarine areas. Since *M. furnieri* reproduction occurs inside and close to estuaries, and juveniles are expected to growth mainly inside estuaries (see introduction for references), it is assumed that strontium signature for the range of estuarine influence is represented by the group of strontium concentrations measured in otoliths, between 0 and 1 yr. Consequently, the range of estuarine strontium signal is expanded over that previously estimated [27] according to the values presented on table 1. Otoliths analyzed from juvenile fish assure these strontium concentrations as confident signatures for the estuary of Patos Lagoon. Since a close pattern of initial strontium concentrations in otoliths has been observed for all sampled areas (except freshwater), it is assumed that initial levels of strontium as true signatures for estuaries and estuarine influence areas.

Within many other definitions, estuaries can be described in a broad sense as "transitional water bodies" [2]. That approach has been recently used because there is not a single definition able to cover all the types of estuaries around the world. Here, estuaries are taken as dynamic systems able to present salinities ranging from < 1 to 34 PSU [34]. Therefore, under appropriate meteorological conditions there will be an estuarine condition in the coastal adjacent region outside from the physical boundaries of the estuaries. Particularly, between Rio Grande and Uruguay, a widespread influence of the continental discharge affects the salinity of coastal waters [35]. That estuarine influence (salinities lower than 33) reaches 50 meters of depth during winter and could potentially affect *M. furnieri* stocks particularly during years with high freshwater discharges (e.g. El Niño years). To that estuarine condition along the shelf, we have attributed the term "estuarine influence". This understanding is important because a fish collected in a coastal area can present a strontium signature in otoliths reasonably close to the signature expected for a true estuarine-collected fish.

4.2. Estuary utilization by whitemouth croaker

The role of estuarine use fish has been studied in many ways and two concepts seem to be fundamental to describe the fish species that uses estuaries at least in part of their life cycles: (*i*) Estuarine-dependency and (*ii*) Estuarine-opportunism. These concepts are variable according to author and context of study [36] and leads to a conceptual confusion that would be easily solved by previously defining the terms to be adopted in any scientific work. Here, estuarine-dependent will refer to fish as "species which usually require the estuarine habitats for some stage of the life"[37]. Following the same author, estuarine-opportunist can be described as "coastal marine species that inhabit estuaries with some regularity but which do not require these habitats". According to conceptual approaches, a fish species should be considered estuarine dependent only if the loss of estuarine habitats affects adversely its population [38]. The strategy of estuarine use by whitemouth croaker has been discussed and there is still not definitive conclusions about this issue. It has been suggested that *M. furnieri* would be characterized as estuarine-opportunist based on the fact that eggs and larvae are passively transported towards estuarine shallow areas and on the existence of juvenile fish growing in the coastal area of Rio Grande [16]. On the other hand, the use of estuarine areas by *M. furnieri* has been suggested to be obligatory for larvae and juvenile fish [15]. Nevertheless, there is not a study evaluating specifically this issue. The main question generating different points of view is the presence of juveniles growing far from estuaries. However, there are no studies assessing the time extent in which those fish were really growing outside from estuaries. The juvenile presence away from estuaries would be related to some period of estuary extent to the shelf as promoted by NE winds [39]. Since *M. furnieri* is an eurioic species, there would be no physiological problems regarding the survival of these individuals. Many authors have showed the strong presence of *M. furnieri* along estuarine environments. Salinity and temperature were suggested do not influence juvenile spatial distribution at estuary of Patos Lagoon [8]. This finding was corroborated by other authors [7] that have indicated turbidity as the main influence factor for the youngest age-classes in the Río de la Plata estuary, however salinity would be more important for old juvenile and adults. Our results show that the majority (about 71%) of the juvenile *M. furnieri* actively search for low-salinity waters during the first and second years of life (negative JMI, table 2). This trend was based on the absolute decrease of strontium between ages 0 and 1 (negative values observed for JMI). The 34% remaining fish presented both emigrational trends (search for more saline waters – 25%) and residency strategies (4%). However, not a single fish presented strontium concentration over the limit expected for estuarine influence for both age 0 and 1. This active search for low-salinity waters and the absolute predominance of estuarine strontium signatures at the otolith zones corresponding to ages 0 and 1 represent a direct evidence of brackish water search by fish. Therefore the association to estuarine waters seems to be an obligatory characteristic for the *M. furnieri* ecological success; indicating that *M. furnieri* should be considered an estuarine-dependent species. The importance of estuarine waters for sciaenid fish has been recognized [40] and species as *Leiostomus xanthurus*, *Micropogonias undulatus* and *Pogonias cromis* has already been proposed to present the estuarine-dependency strategy of estuary utilization [36]. Looking at *M. furnieri* profiles (Fig. 4), when the range of estuarine influence over the coastal areas is considered, this estuarine-dependence seems to enclose more than the juvenile time. The values of TEEI (Fig. 3) over the sexual maturation time (1 – 3 years, [8; 41; 42] reinforce the idea that many adult fish still inhabit the vicinities of estuaries after reproduction. TEEI over 10 yr among the three sampled regions were observed, suggesting these areas are still being used for several old individuals. However, as the fish grow there is a decreasing trend of estuarine use what characterizes the long-term habitat change that will be discussed next.

4.3. Long-term habitat change

The examination of otolith strontium of *M. furnieri* suggests the existence of different strategies of long-term habitat change for the Southwest Atlantic Ocean. The strontium profiles presented three reasonably distinct patterns based on the shape and on the parameters of the LTMC. Pattern “A” can be defined as the behavior in which there is not a stabilization of the strontium increase along fish’s life and the asymptotic concentration is not reached (mean Sr_{∞} = 5000 μg g^{-1} and mean K = 0.04). Therefore, individuals included on strategy A present a constant search for more saline waters along its life. That characteristic may implicate in two hypotheses: (1) the fish spend increasingly more time in marine habitats. Since reproduction occurs at mesohaline low Sr^{++} waters, close to estuaries [10] or into the estuaries [12], that pattern could indicate that the reproductive period is getting shorter or less frequent as the fish is getting old, what promotes higher mean annual strontium concentrations in their otoliths. (2) Fish search increasingly more salty waters along the life. Therefore, whitemouth croaker would move to deeper waters as it is getting old, avoiding estuarine influenced areas and getting higher strontium concentrations on its otoliths. Larger *M. furnieri* are preferably captured at deeper waters [14; 43] what supports the second hypothesis. However, there is not evidence for either reject or support the hypothesis one.

Pattern B includes fish that stabilize the long-term habitat change with high but reachable asymptotic strontium concentration (mean Sr_{∞} = 3378 μg g^{-1} and mean K = 0.13). Consequently fish from strategy B emigrate to marine waters, and at some time it stabilizes the time spent between marine or estuarine influenced waters. Therefore it seems to have reached the equilibrium between the time spent at reproduction (low salinity waters) and the rest of the year (high salinity waters). If considered in total number for all sample sites, Pattern B represented the most abundant habitat change pattern.

For the pattern C, the long-term habitat change towards marine waters seems not occur, and the individuals tend to maintain the proximity of estuarine waters as evidenced by the low strontium concentrations during the entire life (mean Sr_∞ = 2630 μg g^{-1} and mean K = 0.38). These individuals would in broad sense be called “short migrants” and could represent an important part of the population mainly caught by the artisanal fisheries on the shallow waters of the Southwest American continental shelf.

Migration would be motivated mainly by feeding (search of food), climatic changes (search for more suitable climactic conditions) and reproduction [1]. For instance, size-specific dispersion has been initially suggested [44] to explain sciaenid movements towards higher salinities. According to them, the here called long-term habitat change would represent a trade-off between increased predation risk and food availability since both are positively related to salinity. Similar long-term habitat change trends were for striped bass and were suggested to occur because striped bass larger preys tend to inhabit more saline waters [28]. At the coastal area of Rio Grande it has been showed an increment of macrobenthic diversity towards deep waters, particularly from 11 to 45 m of depth [45]. In Argentinean and Uruguayan waters it was observed an increased trophic diversity of Polychaete towards higher salinities [48], and a positive effect of salinity and depth on macrobenthic diversity [49]. In the same way, higher biomass and density of benthic megafauna were observed between 50 to 100 m than between 10 to 50 m of depth for São Paulo coast [46]. Since the benthic infauna and epifauna constitute the most important *M. furnieri* prey groups [47; 50], it would be therefore suggested feeding as one of the possible causes for the long-term habitat change exhibited by *M. furnieri*. However, additional studies should be developed in order to evaluate feeding habits for older age classes. Besides food availability and risk of predation, intra-specific competition would be a plausible factor for inducing habitat change. As suggested by Haimovici & Ignácio (2005), *M. furnieri* growth has been suggested to be strongly dense-dependent [42], therefore, these differential movements along the life would mean ecological strategies to avoid intra-specific competition.

Interestingly, for all sample areas there was a mix of displacement patterns and an absence of latitudinal organization of the respective patterns which suggests the existence of patterns of movement more complex than proposed earlier. Therefore, the hypothesis of *M. furnieri* to present a single migratory behavior in the Southwest Atlantic Ocean is rejected. When habitat change strategies A, B and C are examined, it is possible to observe fish occupying very different waters as they were “trying” to avoid common areas where population density would be high and therefore the problems inherent to these densities would be avoided as well. Another interesting issue to think about would be the possible effect of a huge commercial capture that could act as an evolutionary pressure negatively selecting fish with more aggregative behavior, and could potentially increase the numbers of fish presenting dispersive behavior. In future studies, this issue should be investigated by examining otoliths from old collections and comparing the frequencies of long-term habitat change types with that observed in the present study.

5. Conclusion

This study represents the fist evaluation of long-term habitat change in fish with the use of otolith microchemistry. The results draw the attention to the existence of different behavioral patterns for fish after they become adults. These patterns of habitat use should be considered by life history studies. The analysis of strontium profiles on otoliths demonstrated the dependence of *M. furnieri* to estuarine and brackish waters, and showed different patterns of coastal-oceanic fish displacement along the continental shelf. Future studies should focus on feeding and reproductive strategies among different ages of adult fish, to help to understand the reason for the long-term habitat change observed in the present study.

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Table 1 – Description of *M. furnieri* sampled at South America. Total length is indicated by TL and the Estuarine Influence Range (EIR) was based on otolith strontium concentration for ages 0 and 1.

Site (**Adults**)	Male	Female	Age (years) mean ±SD	TL (cm) mean ±SD	EIR mean Sr (μg g^{-1}) ±SD
SP / Brazil	11	19	9.9 ± 4.4	46.2 ± 3.8	1869 ± 228
RG/ Brazil	15	16	14.8 ± 9.8	61.2 ± 5.9	1794 ± 213
Mirim Lagoon / Brazil	11	9	35.3 ± 0.7	42.9 ± 4.1	820 ± 55
Uruguay	19	13	10.7 ± 5.8	51.7 ± 9.7	1698 ± 195
Argentina	13	21	7.2 ± 6.8	39.2 ± 9.5	1755 ± 213
Site (**Juveniles**)					
RG/Brazil (n = 10)	NA	NA	< 1 year	7.8 ± 0.9	1751 ±101

* Sr concentration for Mirim Lagoon does not represent EIR.

Table 2 – Absolute and relative frequencies of Juvenile Migratory Index types (JMI) and Long-term habitat change patterns among sites. Significant differences were tested by x^2 test.

	JMI				Habitat change pattern			
Site	Positive (23%)	Negative (66%)	Neutral (11%)		A (33%)	B (40%)	C (27%)	
	o	*o*	*o*	Tot	*o*	*o*	*o*	Tot
São Paulo	9	*20	1	30	2	4	6	12
Rio Grande	13	15	3	31	8	8	5	21
Uruguay	5	**27	*0	32	7	7	7	21
Argentina	5	**28	*1	34	0	6	2	8
Total	32	**90	**5	127	17	25	20	62

** $\alpha = 0.01$
* $\alpha = 0.05$

Table 3 – Mean parameters of long-term migration curve calculated by site and ANOVA results for the comparison for each parameter between sites.

	SP (n = 12)	RG (n = 21)	UR (n = 21)	AR (n = 8)	ANOVA	
Von Bertalanffy Parameters	mean (±se)	mean (±se)	mean (±se)	mean (±se)	*F*	*P*
Sr_∞	3635 (±316)	3929 (±239)	3501 (±239)	3219 (±387)	0.98	>0.4
K	0.26 (±0.05)	0.15 (±0.04)	0.18 (±0.04)	0.17 (±0.06)	0.92	>0.4
T_0	-3.8 (±1.4)	-6.4 (±1.1)	-7.2 (±1.1)	-3.3 (±1.7)	2.06	>0.1

Figure 1– Study area in the South-West Atlantic Ocean. Black circles indicate approximately sites were *M. furnieri* samples were collected at São Paulo (SP), Rio Grande (RG), Mirim Lagoon (ML), Uruguay (UR), and Argentina (AR). Juveniles (J) where collected in the Patos Lagoon estuary.

Figure 2 – Strontium profiles averaged by age for *M. furnieri* sampled at Mirim Lagoon (A), São Paulo (B), Rio Grande (C), Uruguay (D) and Argentina (E). White and black circles indicate respectively males and females. Each line represents a single individual. Shaded area indicates the strontium concentrations representative for the estuarine influence range (EIR), as showed in Table 1.

Figure 3 – Proportion of *M. furnieri* individuals out from the estuarine influence range by age, according to a logistic function for each sample site. T_{50} represents the age in which 50% of the individuals presented strontium concentrations in otoliths higher than the estuarine influence range.

Figure 4 – Conceptual model showing the four types of habitat change strategies for *M. furnieri*. Types A, B and C were modeled according to the long-term migration curve adjusted for otolith strontium profiles along fish's life. Type D was based in the *M. furnieri* group confined in Mirim Lagoon.

Figure 5 – Multidimensional scaling based on long-term migration parameters (K and Sr_{∞}) for strategies A, B, and C. Circles indicates significant different groups (ANOSIN, $P<0.05$).

Figure 6 – Examples strontium profiles in otolith of *M. furnieri*, averaged by age and adjusted to the strategies A (Rio Grande), B (Argentina), and C (Uruguay).

Figure 1

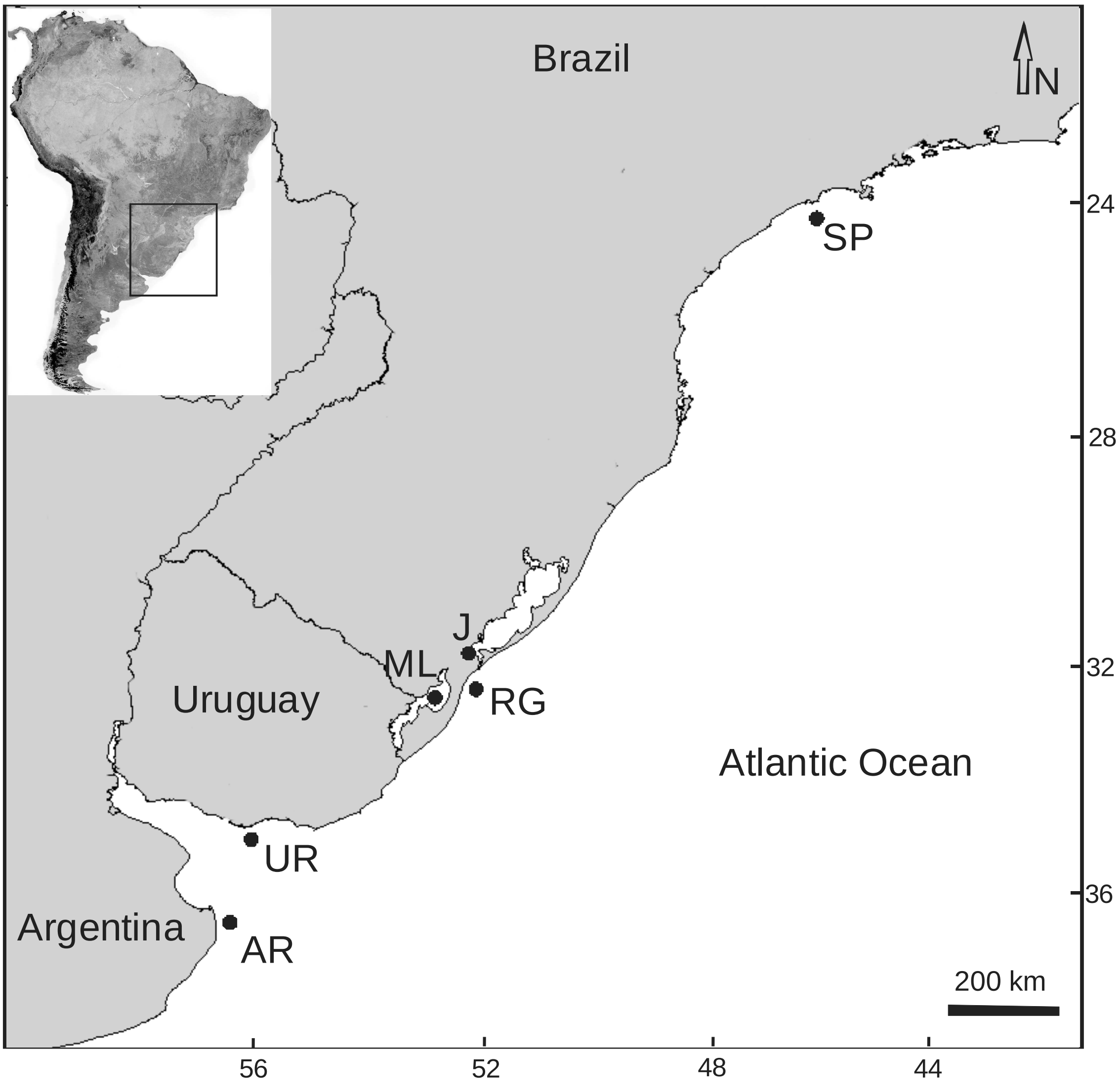

Brazil
N
24
SP
28
J
ML
32
RG
Uruguay
Atlantic Ocean
UR
36
Argentina
AR
200 km
56
52
48
44

Figure 2a

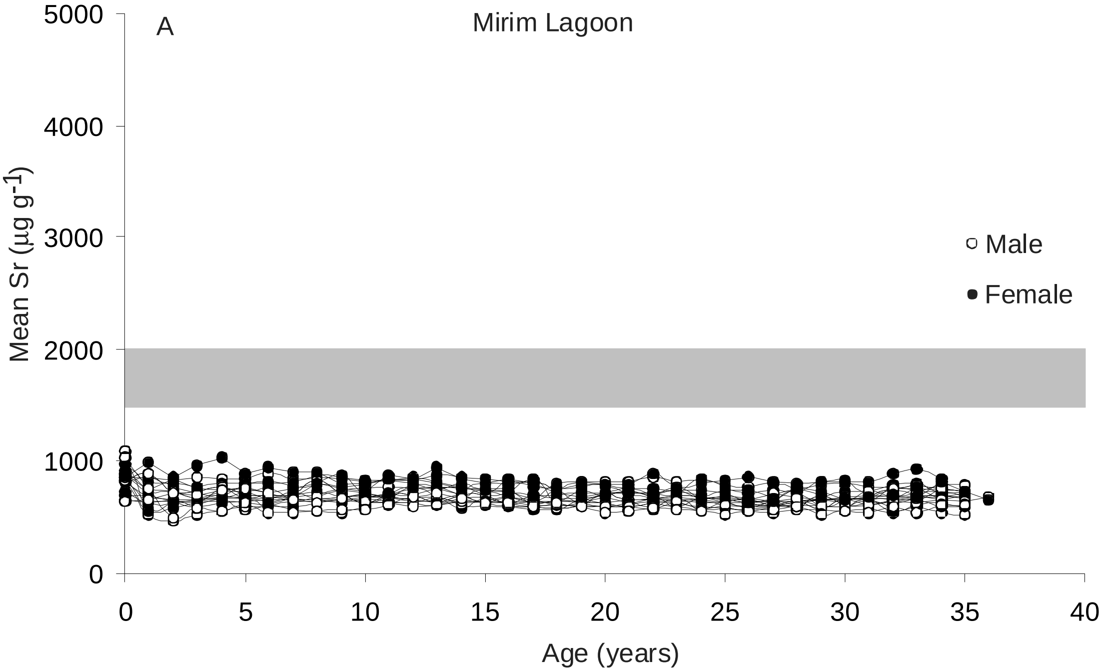

A
Mirim Lagoon
Mean Sr (µg g-1)
5000
4000
3000
2000
1000
0
Male
Female
0
5
10
15
20
25
30
35
40
Age (years)

Figure 2b

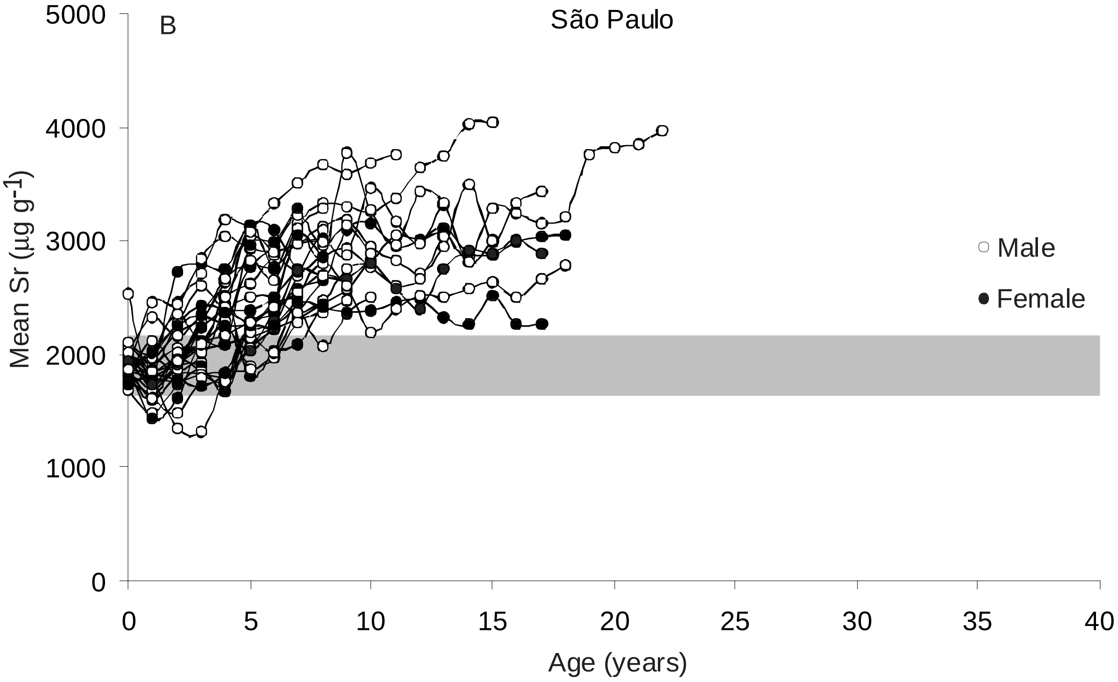

B
São Paulo
Mean Sr (µg g-1)
5000
4000
3000
2000
1000
0
Age (years)
0
5
10
15
20
25
30
35
40
○ Male
● Female

Figure 2c

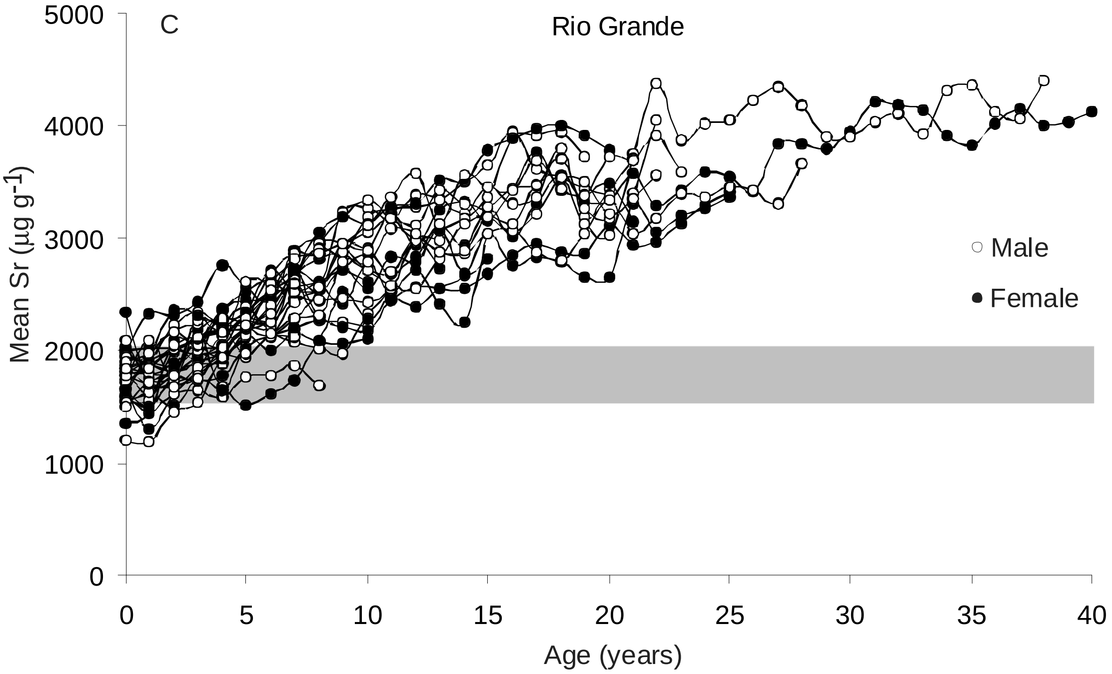

C
Rio Grande
Mean Sr (µg g^{-1})
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
40
Age (years)
○ Male
● Female

Figure 2d

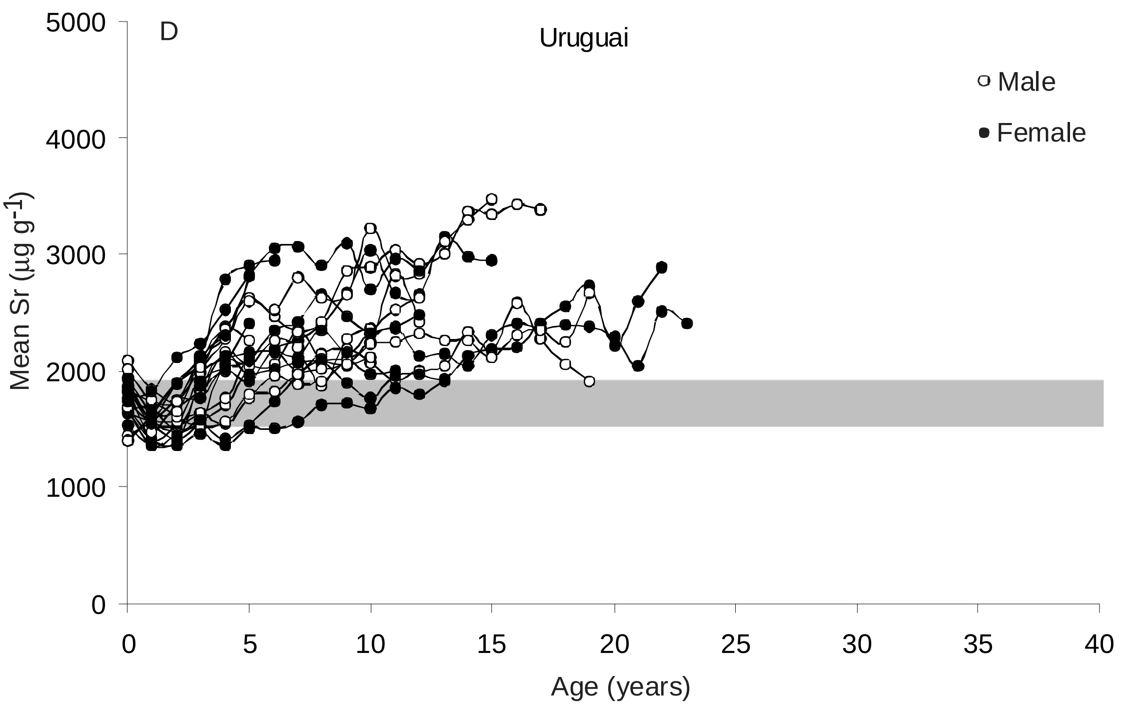

D
Uruguai
Male
Female
Mean Sr (µg g-1)
Age (years)
0
1000
2000
3000
4000
5000
0
5
10
15
20
25
30
35
40

Figure 2e

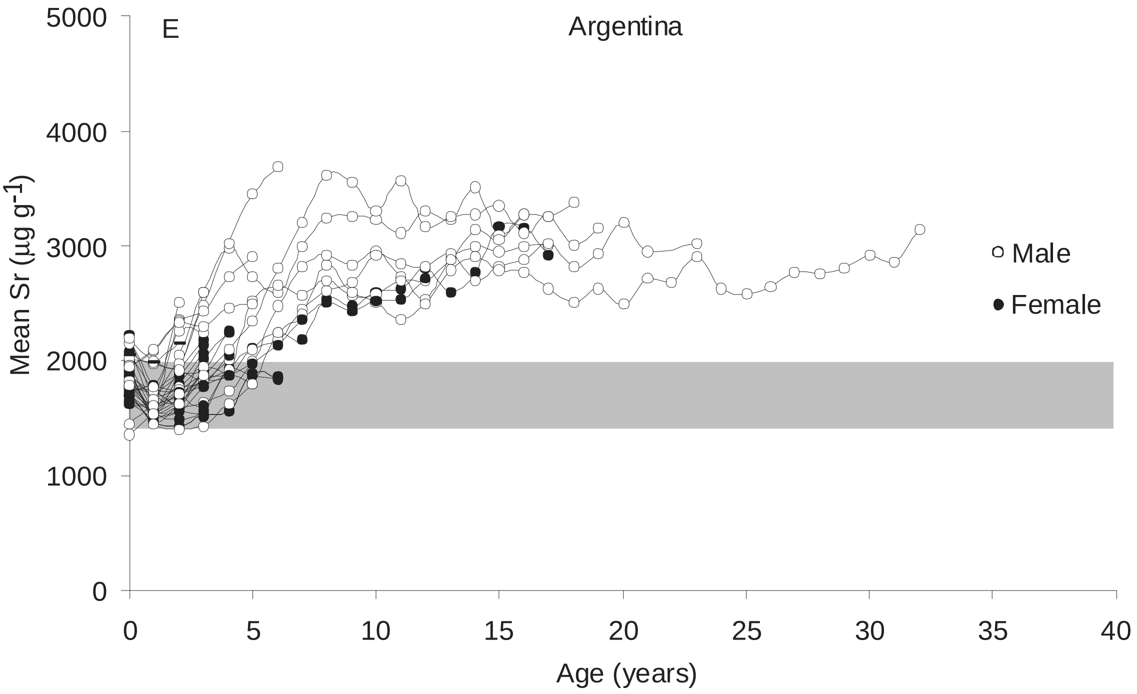

E
Argentina
Mean Sr ($\mu g\ g^{-1}$)
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
40
Age (years)
○ Male
● Female

Figure 3

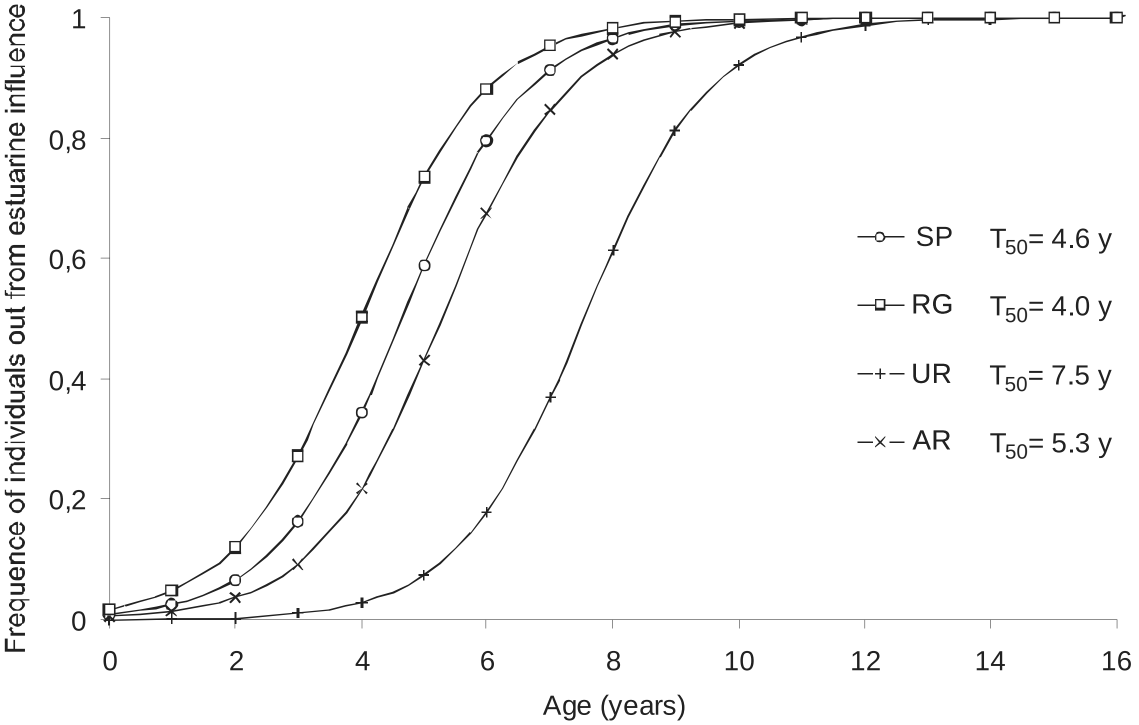

Frequence of individuals out from estuarine influence
1
0,8
0,6
0,4
0,2
0
0
2
4
6
8
10
12
14
16
Age (years)
SP T_{50}= 4.6 y
RG T_{50}= 4.0 y
UR T_{50}= 7.5 y
AR T_{50}= 5.3 y

Figure 4

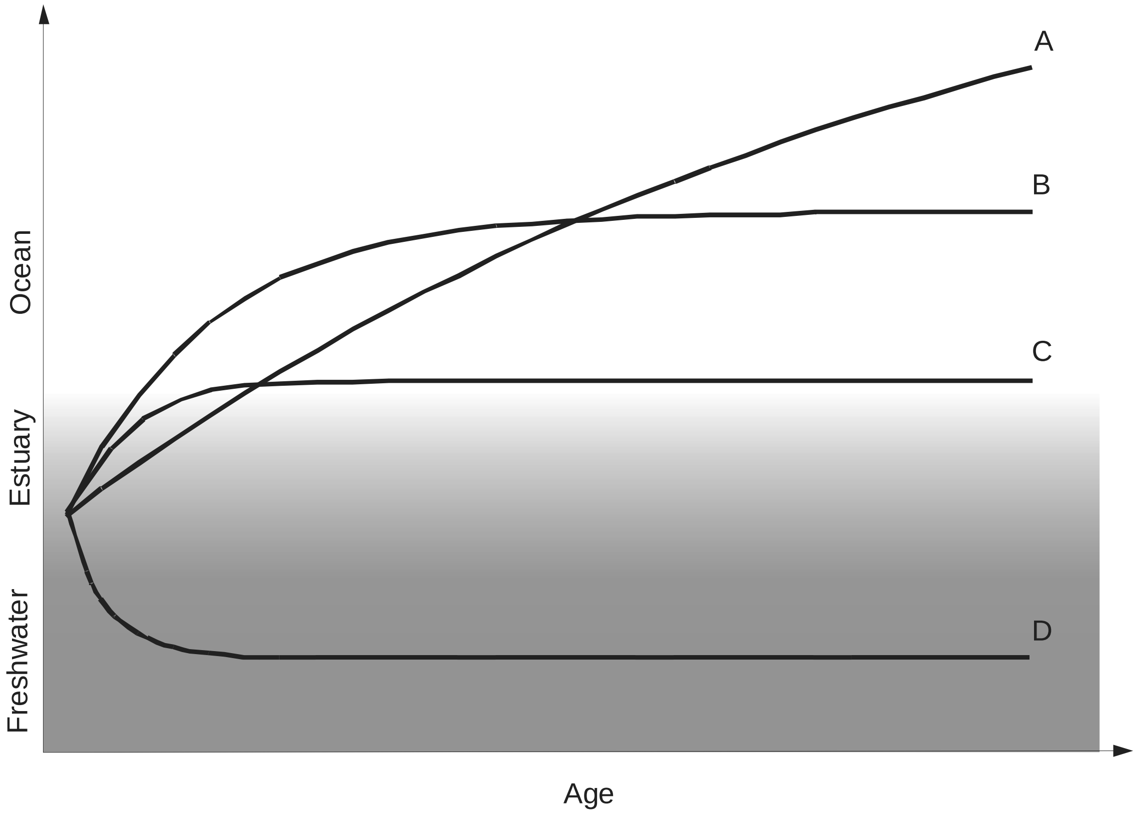


Figure 5

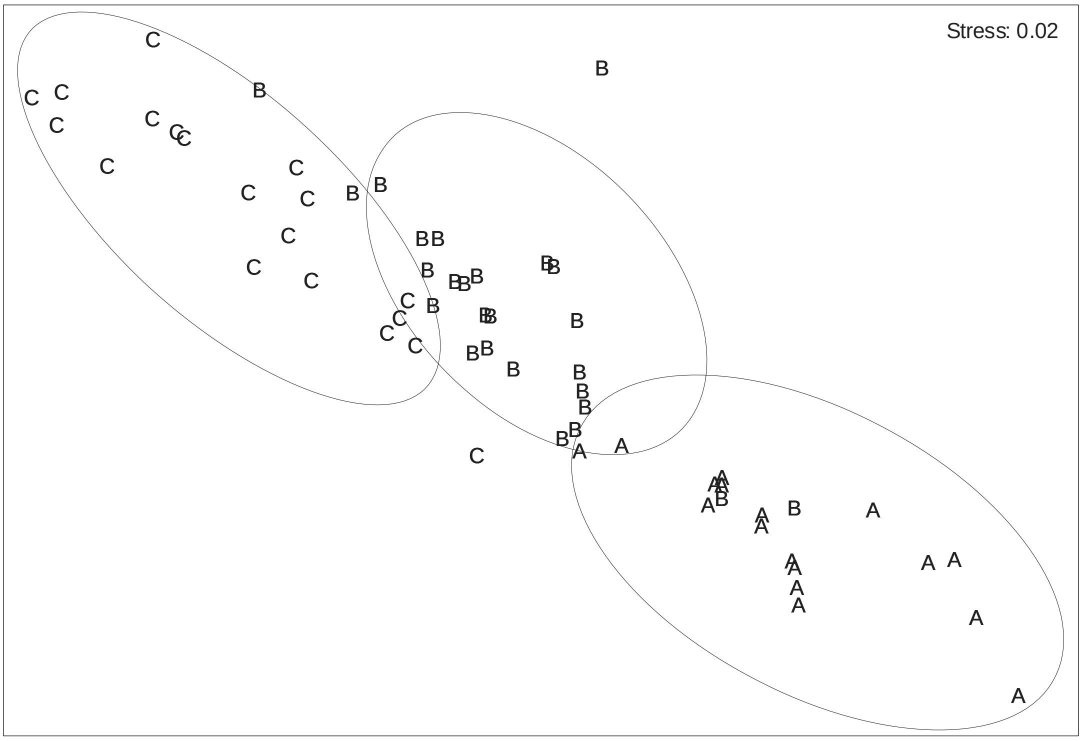
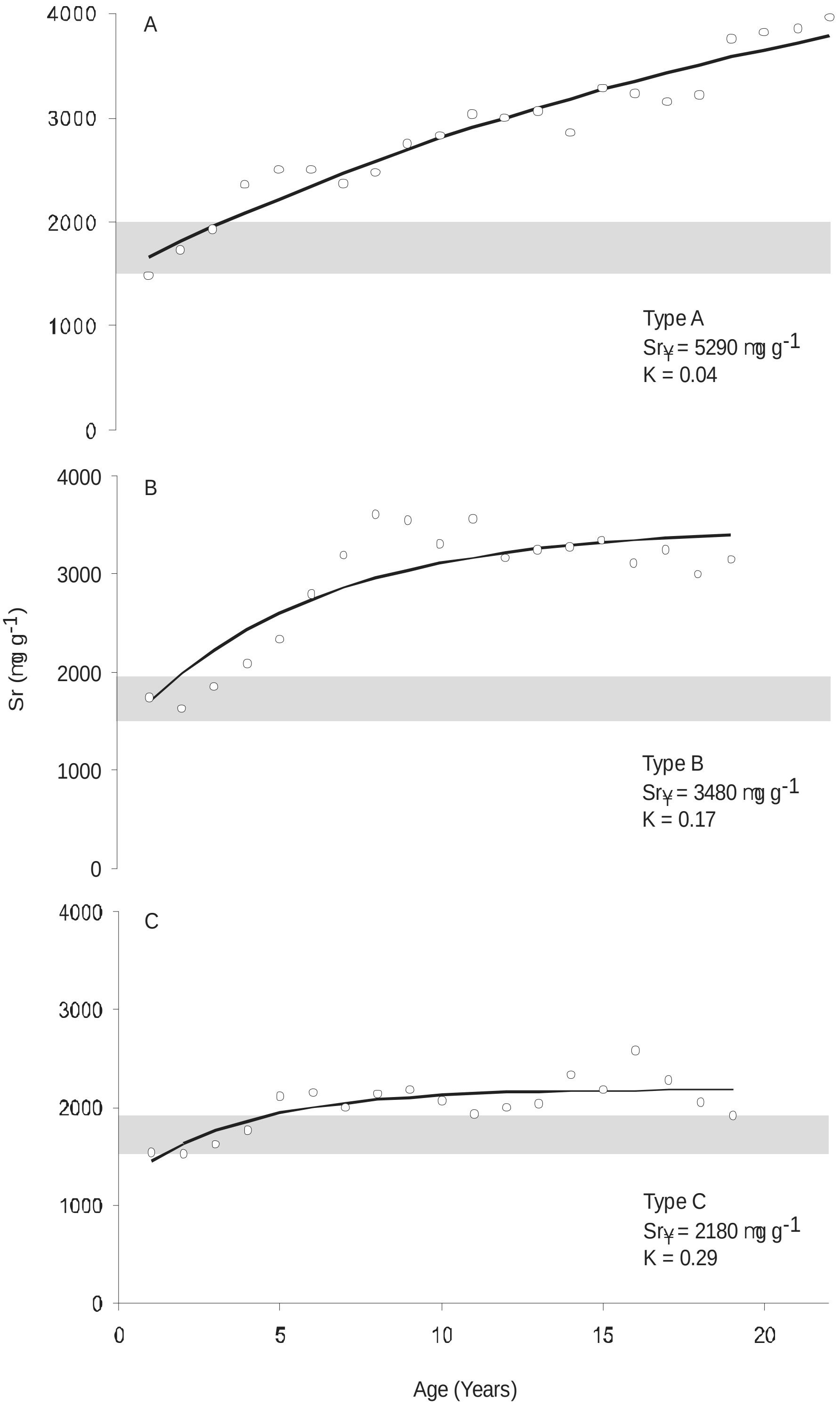


Figure 6


A
4000
3000
2000
1000
0
Type A
Sr¥ = 5290 mg g-1
K = 0.04
B
4000
3000
2000
1000
0
Type B
Sr¥ = 3480 mg g-1
K = 0.17
C
4000
3000
2000
1000
0
Type C
Sr¥ = 2180 mg g-1
K = 0.29
Sr (mg g-1)
0
5
10
15
20
Age (Years)

ANEXO IV

Evidence for *Micropogonias furnieri* stock segregation at Southwester Atlantic Ocean, as revealed by otolith microchemistry

Cristiano Q. de Albuquerque, Norbert Miekeley, Andrés J. Jaureguizar, José H. Muelbert

(Periódico a determinar)

Evidence for *Micropogonias furnieri* stock segregation at Southwest Atlantic Ocean, as revealed by otolith microchemistry

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Abstract

Elemental composition of *Micropogonias furnieri* otoliths from southern Brazil, Uruguay and northern Argentina was examined by LA-ICPMS in order to evaluate stock mixture or separation along this region. Analyses were performed approaching the otolith portion related to the first year and last years of fish's life. The results suggest significant differences on the concentration of Sr, Ba, Mg and Mn between otoliths from freshwater and coastal water fish. Along the coastal areas, for first year, stocks were partially overlapped suggesting a degree of connection between Bahía Blanca and Partido de La Costa-Uruguay (Platensis) stock. These stocks presented reasonable separation from Santos (Brazil) stock. Rio Grande stock could not be well separated from Santos and presented a strong overlap with the other stocks. For last year of life a

degree of separation was observed between Rio Grande and Platensis, and Rio Grande and Bahía Blanca stocks. These results constitute an evidence for partial separation of *M. furnieri* stocks from Southwest Atlantic Ocean.

1. Introduction

The whitemouth croaker *Micropogonias furnieri* comprises one of the most abundant fishery resources of the Southwest Atlantic ocean (Haimovici and Umpierre, 1996). Although it is distributed from Antilhas to Gulf of San Mathias (Argentina, Chao, 1978), the major part of the catch is obtained from southern Brazilian, Uruguayan and northern Artgentinean coasts (Lasta and Acha 1996; Vasconcellos and Haimovici 2006). Total landings reached about 130,000 t in 1995, 70,000 t for southern Brazil (Haimovici and Ignácio, 2005), and 60000 t for Argentine-Uruguayan Common Fishing Zone (Carozza and Hernandez, in press). Catch per unit or effort (CPUE, t day^{-1}) was observed to continuously decrease from about 1 t day^{-1} in 1980 to 0.2 t day^{-1} in 2002 for southern Brazil (Vasconcellos and Haimovici, 2006), and total biomass was estimated to decrease from 200,000 t in 1996 to 70,000 t in 2002 for southern Brazil,(Haimovici and Ignácio, 2005) and from 280,000 t in 1991 to 220,000 t in 2008 Argentine-Uruguayan Common Fishing Zone (Carozza and Hernandez, in press) (Carozza and Hernandez, in press). The steady decrease in biomass of the whitemouth croaker emphasizes the actual status of heavily overfished fishery resource (Vasconcellos and Haimovici, 2006). Since *M. furnieri* is captured under a wide latitudinal range, evaluating the interdependency among the existing stocks becomes fundamental to support production models that assess the exploitation status of fish populations (e.g. Vasconcelos and Haimovici, 2006) and to support management strategies.

Many studies based on differences of *M. furnieri* growth, reproduction and meristic characters have assessed stock identification in Southwest Atlantic Ocean. Results from these studies have postulated the presence of two stocks at southern Brazil (23 – 29^{o} S and 29 – 33^{o} S; Vazzoler, 1971); two size groups reproducing at Río de la Plata estuary (35 – 36 ^{o} S; Norbis and Verocai, 2005); and, another stock around Bahía Blanca coast (≈40 ^{o}S; Volpedo and Cirelli, 2006). On the other side, genetic studies have indicated existence of a continuous distribution between 23^{o} and 40^{o} S (Levy et al., 1998; Maggione et al., 1994: Puchnick-Legat and Levy, 2006). Therefore, stock differentiation of whitemouth croaker in the southwest Atlantic Ocean remains unclear.

The trace elements analysis of otolith represents one of the most recent techniques used to discriminate fish stocks. The basic premise supporting the applicability of this technique is that otoliths are chemically inert structures (Campana and Neilson, 1985) and as rings accrete, trace elements are deposited into successive layers in some relation to environment (Campana 1999). Thus, provided that there are spatial gradients in environment conditions (e.g. trace element concentration), otoliths can carry a permanent record, or fingerprint, that allows researchers to retroactively track fish through time and space (e.g. Fowler et al., 1995, Gillanders 2002, Frodie and Levin 2008). The trace elements of otoliths (e.g. Sr, Ba, Mn, Mg) have been successfully employed in stock evaluation (Edmonds et al., 1999; Campana, et al., 2000; Jónsdóttir, et al., 2006). Based on this approach, two different stocks of *M. furnieri* were identified at northern Argentina (Volpedo and Cirelli, 2006) which raised the possibility that different stocks could occur at other areas of important fishery grounds (Uruguay and southern Brazil). Here, the concept of stock is understood as an economically important group of fish inhabiting a common area.

The aim of this study is to determine if there is major changes in the spatial distribution of trace elements (Sr, Ba, Mg and Mn) in otolith of *M. furnieri* from Brazilian to Argentinean coasts in order to characterize the existing stocks along this area.

2. Material and Methods

2.1. Sampling and sample preparation

The study was developed in the coastal region of the South-West Atlantic Ocean, and includes northern Argentinean, Uruguayan and southern Brazilian coasts (Fig. 1). Between 2004 and 2005, 173 adults of whitemouth croaker (*M. furnieri*) were collected from commercial fisheries (Tab. 1) from Argentina (Partido de la Costa, Bahía Blanca), Uruguay, and Brazil (Rio Grande, Mirim Lagoon, and Santos). Eight juveniles (JUV) were collected in the Patos Lagoon estuary using a beach seine net.

All sampled specimens were measured in total length and sagittal otoliths were extracted, cleaned and stored dry. The left otoliths were then embedded in crystal polyester resin and transverse sections were cut through the core using a low-speed diamond blade saw. Thin sections (0.4 mm) were mounted onto glass slides with crazy glue. Prior to LA-ICPMS analysis, otolith surfaces were polished with silicon carbide paper (n^o 8000), washed with deionized (DI) water (Milli-Q, Millipore, Bedford, USA), sonicated for 3 minutes, and rinsed three times with DI water. The slides were dried in a horizontal flow cabinet before analysis.

2.2. Elemental determinations

Analytical measurements were performed with a Nd:YAG CETAC LSX 100 Laser Ablation system operating at 266 nm, coupled to an ELAN 6000 (PerkinElmer – SCIEX) inductively coupled plasma-mass spectrometer (ICP-MS). The laser was configured for Q-switched mode operation, defocused, a pulse frequency of 20 Hz, scan speed of 40 μm/s, and power of 0.4 – 0.6 mJ per pulse. Under these conditions the beam diameter was about 15 μm. The ablated material was conducted through a Teflon coated tube into the ICP-MS using Argon as carrying gas (0.85 $dm^3\ min^{-1}$). The ICP was operated at 1500 W with outer and intermediate gas flow of 15 and 1.1 $dm^3\ min^{-1}$ respectively. The determined elements (masses) were ^{43}Ca, ^{86}Sr, ^{138}Ba, ^{24}Mg and ^{55}Mn. The quantification was performed using the external calibration mode. The standards were constituted by pressed powder $CaCO_3$ discs with known analyte concentration, and suprapur $CaCO_3$ discs were used as a blank. These standards were proposed and produced by Bellotto and Miekeley (2000; 2007). The measured signal (counts per second – cps) for each element was normalized with reference to ^{43}Ca for correction of the bias induced by differences on the amount of ablated material. To obtain the limits of detection (LOD), the blank discs were scanned 10 times. Each mean profile was used as a single analyte concentration and the standard deviation (σ) from the 10 values was considered. The LOD was calculated as 3σ divided by the sensitivity (inclination) of the respective calibration curve and it was 4.9 $\mu g\ g^{-1}$ for Sr; 0,78 for Ba; 0.73 for Mg; and 0.23 $\mu g\ g^{-1}$for Mn.

2.3. Statistical analysis

Concentrations of Sr, Ba, Mn and Mg were averaged by age, based on the analysis of annual growth increments in otoliths (Schwingel and Castello, 1990), and results relative to first (FYL) last year of life (LYL) from each fish were considered in the analysis. The elements were individually compared among themselves using analysis of variance (ANOVA, $P<0.05$). The spatial distribution of trace elements (similarity of otolith elemental composition among sites) was assessed through Multidimensional Scaling (MDS) and the significance of the similarities was tested by Analysis of Similarity (ANOSIM). Both analyses were carried out trough the Primer 5.0 computer package (Plymouth Marine Laboratory). Similarity matrices were produced using Bray-Curtis distance and the elemental concentrations were log(x+1) transformed in order to decrease the importance of zero values on the analysis. The ANOSIM results were interpreted in accordance to the PRIMER manual (Clarke & Gorley, 2001), according to the values of *R* (strength of spatial separation). However, a different scale of R-values is proposed as follows: R-values >0.75 indicates strongly separated groups, R>0.5 indicates overlapping, but clearly different groups, R > 0.4 is overlapped and reasonably different groups, R>0.2 means weak separation and R< 2 indicates no possible separation of the analyzed groups.

In order to improve MDS visualization of the differences among sites, three situations were approached and ANOSIN was applied separately for each one. Situation

A evaluates if there are differences in elemental composition of otolith between coastal (SP, JUV, RG, UR, MAR, EL) and freshwater (MI); Situation B analyses the similarity between all sample sites except for freshwater fish; and Situation C groups fish otoliths from Partido de la Costa and Uruguay. This last group was called Platensis stock (PLA). The three situations were tested for both FYL and LYL.

3. Results

Whitemouth croaker presented variable age and sizes (Tab. 1), being largest at Rio Grande and oldest at Mirim Laggon. Elemental concentrations in otolith were significantly different among sites for FYL and LYL (Fig. 2; Tab. 2), except for manganese, that presented no differences among sites for LYL ($P>0.05$). Fish from Mirim Lagoon presented otolith strontium concentrations (≈ 700 – 900 $\mu g\ g^{-1}$) drastically lower and barium concentrations (≈ 110 – 160 $\mu g\ g^{-1}$) over 10 times higher than concentrations observed for otolith from coastal fish (Sr ≈ 170 $\mu g\ g^{-1}$; Ba ≈ 1 – 30 $\mu g\ g^{-1}$).

ANOSIM analysis showed significant differences between different pairs of sample sites (Tab. 3). When all individuals for FYL and LYL are compared, MDS shows two well defined groups with no overlapping (Global R > 0.97; Fig. 3a). These groups comprise otolith elemental composition of fish from coastal and freshwater environments.

The second MDS situation (Fig. 3b) presented a global R of 0.31 for FYL and 0.26 for LYL, indicating a weaker separation than the observed between fresh and coastal water fish. For FYL (Fig. 3b), the main differences were found when comparing between pair of sites SP-UR, SP-MAR and UR-EL (see Tab. 3 for R-values). On the other side, FYL of fish from Rio Grande presented a widespread distribution over all groups (Fig. 3b; Tab. 3). Fish from Bahía Blanca presented otolith composition overlapped but reasonably separated from Uruguay and Partido de la Costa (Tab. 3). Fish from Partido de la Costa and Uruguay presented otoliths with approximately similar elemental composition.

When the general division between groups was analyzed, LYL data presented weaker separation (Global R = 26) than FYL (Global R = 0.31; Fig. 3b;4b). However, otoliths of fish from Rio Grande presented a more defined pattern for FYL than for LYL, presenting weak to reasonable differences from Bahía Blanca, Partido de la Costa and Uruguay and no separation from Santos. No differences were observed among the pairs of sites RG-SP, UR-MAR and MAR-EL (see Tab. 3 for R values).

When considering the Platensis stock (Situation C; Fig. 3c; 4c), the global R was 0.38 for FYL and 0.24 for LYL. Otolith composition of fish from Platensis stock was well separated from SP (strongest difference) and JUV, and reasonably different from RG and EL (Tab. 3). Fish from Bahía Blanca were similar to SP, RG and JUV fish. For LYL (Fig. 3c), weak differences were observed between SP-RG and PLA-EL (Tab. 3). Dissimilarities were also present between PLA-SP, PLA-RG, EL-SP and EL-RG. The spatial arrangement of the sites was improved for situation C, and allowed for a better and simpler understanding of the general stock organization of *M. furnieri*.

4. Discussion

4.1 – Assumptions and limitations

Differences on the elemental composition of *M. furnieri* otoliths presented in this study suggest the occurrence of spatially distinct fish groups, here called stocks. Here we understand stock as fish groups inhabiting a specific habitat and presenting a recognizable chemical signature on the otoliths of their individuals.

Fish from Mirim Lagoon have spent their entire life in a freshwater environment (Albuquerque et al. in prep a). They were analyzed to show that chemical composition of *M. furnieri* otoliths can discriminate between two diverse environments (see Fig. 3a;4a). However, interpretation of chemical differences as results of fish spatial distribution requires prudence. The basic assumption of chemical temporal stability of otolith composition and the environmental influence on the rate of elemental deposition have been show for Sr, Ba, Mn, Fe, and Pb (Campana, 1999). However, recent results have indicated that for *M. furnieri* barium may change during development with a higher deposition during the first year of life (Albuquerque et al. in prep a). Furthermore, this species is expected to move from estuarine to coastal habitats between juvenile and adult stanza, since lower strontium concentrations were measured close to the otolith cores of marine whitemouth croakers (Albuquerque et al. in prep b). Consequently, comparison between early (FYL) and late stages (LYL) of otolith elemental composition for *M. furnieri* has to be interpreted with care. Therefore, only the patterns within FYL and within LYL should be considered to infer spatial distribution of *M. furnieri* stocks.

Once determined that otolith chemistry is influenced by the environment where fish grew, it is assumed that if fish are randomly distributed, an identifiable sub-regional pattern of otolith chemistry will not be present. On the other hand, if a recognizable pattern on otolith elemental composition exists, and the sub-regions present different chemical and-or physical water characteristics, it is coherent to suppose differences on fish utilization of the main region. Following the same logic, if a group of fish presents any clear pattern of chemical composition, some degree of philopatry will be credited to it. Since the chemical profiles analyzed here correspond to a temporal scale of one year of life, it is difficult to infer that a fish collected in one region spent one entire year (for

LYL) or the entire life (for FYL) around the same coastal region. Nevertheless, if many fish caught in this region present a pattern of otolith elemental composition, some degree of philopatry may be attributed to it. When analyzing the data presented here, patterns were observed between fish from fresh and coastal waters, and within coastal waters, suggesting the existence of chemical signatures among the groups and some degree of spatial organization of these fish.

4.2. Spatial organization of M. furnieri stocks

This study has shown that whitemouth croakers are non-randomly distributed along the south-west Atlantic Ocean. There are consistent differences in the elemental composition of otoliths among sample sites, suggesting some degree of spatial separation of these stocks. It is suggested the presence of three significantly defined stocks of *M. furnieri*: Santos (around 28^{o} S), Platensis (between 32^{o} and 38 ^{o} S) and Bahía Blanca (38 ^{o} S southwards). A latitudinal increase or decrease of individual elemental concentrations in otoliths was not observed, but some elements show a modal trend. Barium, a strong indicator of freshwater in otoliths of *M. furnieri* (Albuquerque et al. in prep-a), and strontium, tracer for marine waters (CITA), concentration presented the maximum and minimum values respectively around the Uruguay and Rio Grande. This pattern is related to coastal environment under freshwater influence, as Rio de la Plata (Uruguay) and Lagoa dos Patos (Rio Grande). The Río de la Plata freshwater input drains the second largest watershed in South America, discharging an average of 24.000 m^3s^{-1} (Jaime et al., 2002), generating an estuarine system with an area of roughly 35.000 km^2 (Guerrero et al. 1997; Framiñan et al., 1999, Piola et al. 2000). The Patos Lagoon drainage basin (201,626 km2) is one of the largest in Latin America, and the freshwater mean annual discharge is ca. 2,000 m^3 s^{-1}, although large year-to-year variation can occur (700–3,000 m^3 s^{-1})(Moller et al. 2001).

When considering coastal *M. furnieri*, the major differences were observed between otolith composition of fish from Santos and from the Platensis stock. These differences were consistent for FYL and LYL, and indicated that the groups have spent considerable part of their life, if not the entire life, in different regions. These two regions are influenced by two very distinct oceanographic characteristics. The Santos stock is under the influence of the warm and salty tropical waters (T^o>25°C; S >36; Campos et al., 1995) from the Brazil Current (BC), the intrusion of South Atlantic Central Water ($T^o \approx 17.1^oC$, S ≈ 35.8; Piola et al. *in press*) and weak freshwater input. The whitemouth croaker inhabits Partido de la Costa/Uruguay region are subject to direct influence of strong freshwater input from the Plata Plume Water (PPW, 10 – 11 °C , 30<S<31; Lucas et al. 2005, Piola et al. *in press*). Fish from this region also experience influence from Sub-Antartic Shelf Waters (SASW, 7 – 11 °C; 33.4<S<34; Lucas et al. 2005, Piola 2000; Piola et al. *in press*). These distinct oceanographic conditions are suggested to explain part of the differences observed between Santos and Platensis stocks.

Clear similarities were found between fish from Partido de la Costa and Uruguay (Fig. 3, Tab. 3), which allowed to cluster these fish in a single stock here called Platensis Stock (Fig. 3c). When comparing Platensis stock with fish from Santos, as observed earlier, a remarkable dissimilarity is observed suggesting that mixture can occur, but in low extent. On the other hand, a significant dissimilarity was observed between Partido de la Costa and Bahía Blanca, and Uruguay and Bahía Blanca. These dissimilarities were stronger than the one observed when comparing Platensis and Bahía Blanca fish, what suggests that grouping Uruguay and Partido de la Costa into Platensis stock was weakly informative for evaluating the differences between the pairs Partido de la Costa and Bahía Blanca, and Uruguay and Bahía Blanca. Bahía Blanca estuary is a particular environment where the evaporation encompasses the freshwater supply and salinity can reach 40 during warm seasons (Hoffmeyer, 2004). Differences on otolith composition between Bahía Blanca and Partido de la Costa were previously suggested to be significant (Volpedo and Cirelli, 2006) and support the results presented here.

Otolith signatures for fish from Rio Grande overlapped with the other groups, particularly for FYL. The absence of chemical patterns at the early life is suggested to be due to the strong interanual hydrochemical variability in the estuary of Patos Lagoon (Niencheski and Baumgarten, 1998). Since the collected fish presented different ages, they have born in different years. Therefore, these fish experienced diverse environmental conditions at the early life what probably have promoted the absence of a clear elemental fingerprint for fish from Rio Grande. Interestingly, otolith composition for LYL showed a pattern significantly different from Argentina, Uruguay and Bahía Blanca. The existence of differences or elemental signatures between LYL of otoliths from Rio Grande and the other regions suggests that fish collected in Rio Grande have remained there time enough to incorporate a local elemental signature, specific to the last year of life.

The overlapping of *M. furnieri* stocks in large or small extent observed in this study, supported by otolith microchemistry, agreed with the homogeneity presented by genetic evidences (Maggione, et a., 1994; Levy et al., 1998; Puchnick-Legat and Levy, 2006), since genetic studies are expected to indicate population homogeneity even though small amount of stock overlapping occurs (Edmonds, et al., 1989).

The results of this work support the existence of tree stocks of *M. furnieri* in the South-west Atlantic Ocean. These stocks are partially overlapped, but significant differences on otolith elemental composition suggest these stocks use preferably one of the three distinct environments (regions of Santos, Uruguay/Partido de la Costa, and south of Bahía Blanca. The stock from Rio Grande could not be characterized. Otolith microchemistry techniques have the advantage of present a direct evidence of spatial distribution of fish, while genetic or population studies infer fish distribution based upon species-specific biological data. Therefore the diverse information on *M. furnieri* stock distribution generated by otolith microchemistry, genetics, population parameters and meristic characters are complementary. This indicates that all methods should be considered to support management policies. For species as *M. furnieri*, with homogeneous genetic distribution over a wide region, if population parameters differ, it will be more effective for management purposes to divide this region in sub-regions and manage them separately. Otolith composition studies can bring the adequate information to execute this separation.

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Table 1 – Summary of *Micropogonias furnieri* collected at south-western Atlantic Ocean.

Site	Abbreviation	Location	*n*	Age (years) mean ± SD	TL (cm) mean ± SD
Santos / BRA	SP	24º S	25	9.6 ± 5.3	46.2 ± 3.8
Rio Grande / BRA	RG	32º S	31	15.5 ± 9.2	60.4 ± 7.6
Mirim Lagoon / BRA	ML	33º S	20	35.3 ± 0.7	42.9 ± 4.1
Juveniles - ELP / BRA	JUV	32º S	8	< 1 year	7.8 ± 0.9
Uruguay / UY	UR	35º S	50	12.5 ± 7.2	50.1 ± 8.5
Partido de la Costa / ARG	MAR	38º S	38	7.4 ± 5.2	39.2 ± 9.5
Bahía Blanca / ARG	EL	39º S	9	3.3 ± 1.6	35.4 ± 1.7

Table 2 – Analysis of variance performed separately for each analyzed element between regions for first (FYL) and last (LYL) years of life in otoliths of *Micropogonias furnieri*.

	FYL			LYL		
Effect	MS	*F*	*P*	MS	*F*	*P*
Mg	832	7.9	< 0.01	194	1.69	0.14
Mn	82.9	6.2	< 0.01	7.8	5.70	< 0.01
Sr	29×10^5	72.6	< 0.01	14×10^5	54.5	< 0.01
Ba	49×10^2	133.6	< 0.01	47×10^3	588.9	< 0.01

Table 3 – ANOSIM analysis comparing dissimilarities of otolith elemental composition between pairs of sample regions (Rio Grande = RG, Santos = SP, Junveniles = JUV, Uruguay = UR, Partido de la Costa = PC, Bahía Blanca = BB) for first year of life (FYL) and last year of lyfe (LYL). For situation B the comparisons were performed between all individual regions. For situation C data of Partido de la Costa and Uruguay were grouped into Platensis stock. R values indicate the degree of differentiation of each pair of regions according to the legend below.

		Situation B						Situation C			
FYL	Site	RG	JUV	UR	PC	BB	Site	RG	JUV	PLA	BB
	SP	0.23	0.24	**0.65**	**0.55**	0.31	SP	0.23	0.24	**0.58**	0.16
	RG		0.11	0.24	0.19	0.01	RG		0.11	0.29	0.01
	JUV			**0.51**	**0.59**	0.03	JUV			0.48	0.02
	UR				0.13	**0.51**	PLA				0.274
	PC					0.41					
LYL	Site	RG	UR	PC	BB		Site	RG	PLA	BB	
	SP	0.04	0.33	0.45	0.41		SP	0.05	0.34	0.41	
	RG		0.31	0.35	0.43		RG		0.29	0.43	
	UR			0.03	0.21		PLA			0.15	
	PC				0.05						

R-Values

< 0.2	>0.2	>0.4	**>0.5**

Figure captions

Figure 1 – South-western Atlantic Ocean, showing the sample sites. Santos = SP, Rio Grande = RG; Mirim Lagoon = ML; Patos Lagoon (juvenile) = J; Uruguay = UR; Partido de la Costa = PC; Bahía Blanca = BB.

Figure 2 – Elemental mean concentration (±SD) averaged for first year of life (FYL) and last year of life (LYL) measured for otoliths of *M. furnieri*. Sample sites abbreviations follow Fig. 1.

Figure 3 – Multidimensional scaling for otoliths of *M. furnieri*, regarding the concentrations of Sr, Ba, Mg and Mn for fisrt year of life. The data was grouped according to habitat (A), and according to the sample site (B). For graph C, data from Partido de la Costa and Uruguay were grouped in Platensis stock.

Figure 4. Multidimensional scaling for otoliths of *M. furnieri*, regarding the concentrations of Sr, Ba, Mg and Mn for Last year of life. Graphs A, B and C follow captions of Fig. 3.

Figure 1

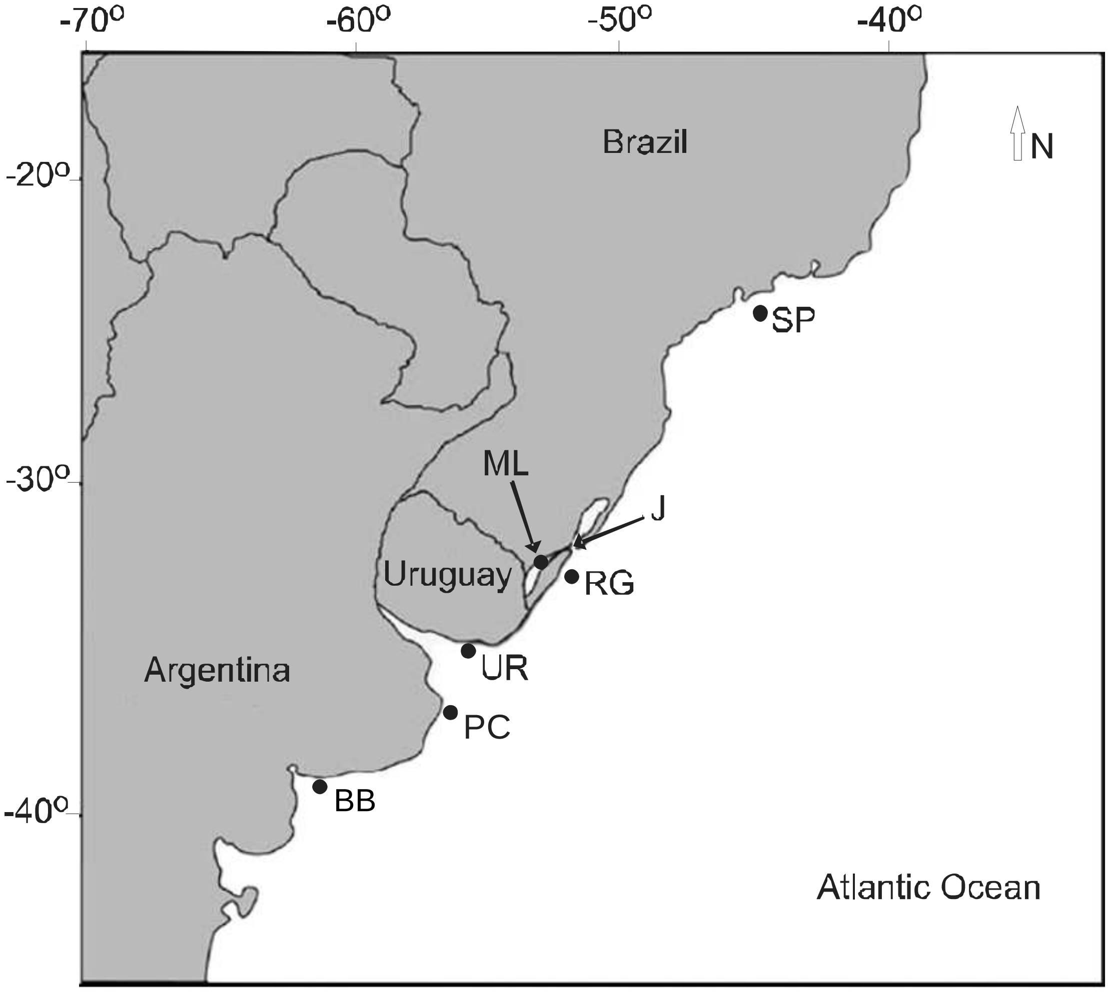

-70º
-60º
-50º
-40º
-20º
-30º
-40º
Brazil
N
SP
ML
J
Uruguay
RG
UR
Argentina
PC
BB
Atlantic Ocean

Figure 2

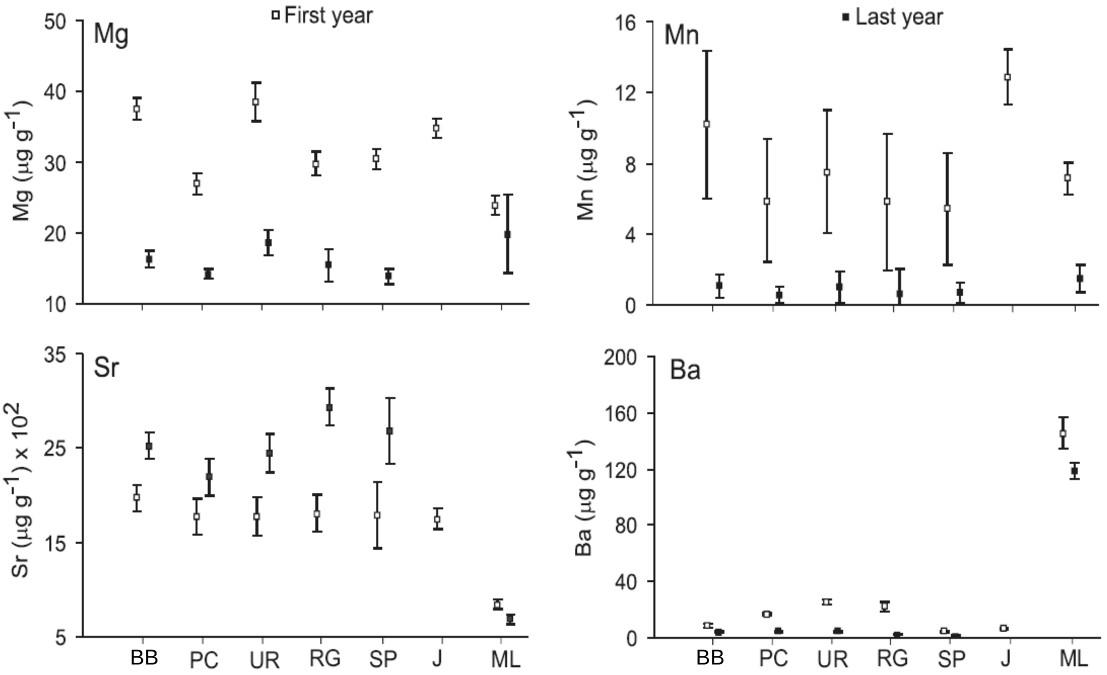

Mg
First year
Mg (µg g⁻¹)
50
40
30
20
10
Mn
Last year
Mn (µg g⁻¹)
16
12
8
4
0
Sr
Sr (µg g⁻¹) x 10²
35
25
15
5
Ba
Ba (µg g⁻¹)
200
160
120
80
40
0
BB
PC
UR
RG
SP
J
ML

Figure 3

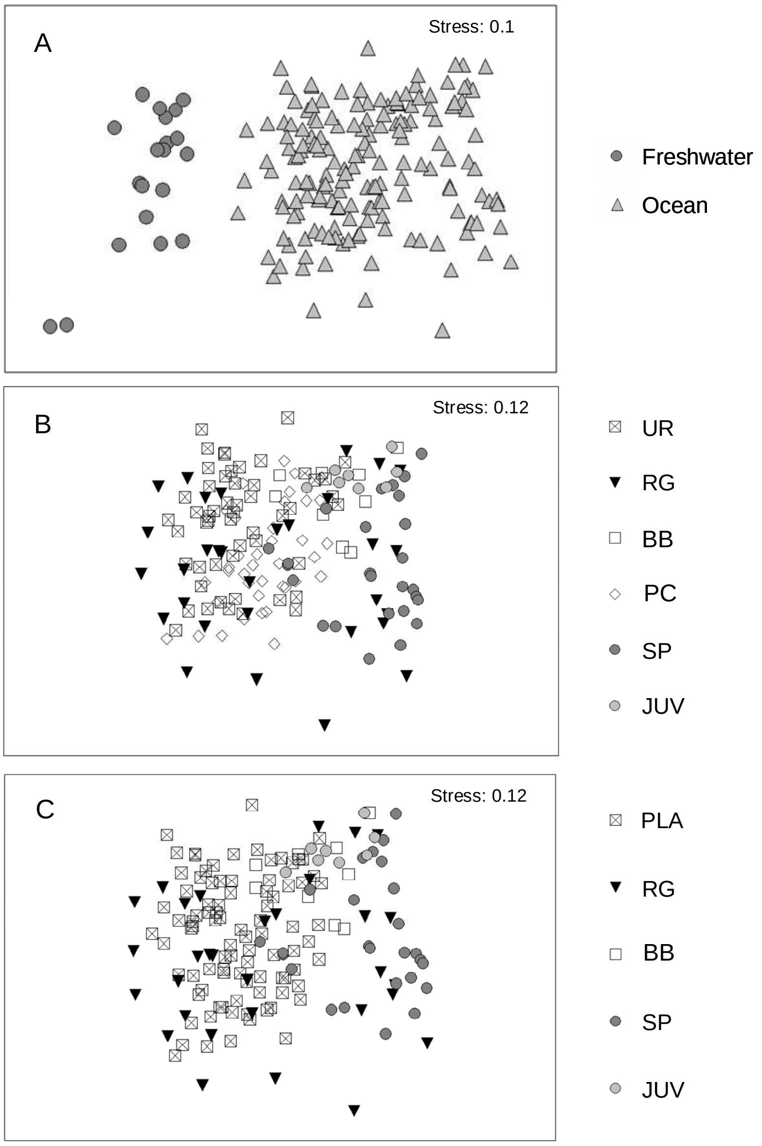
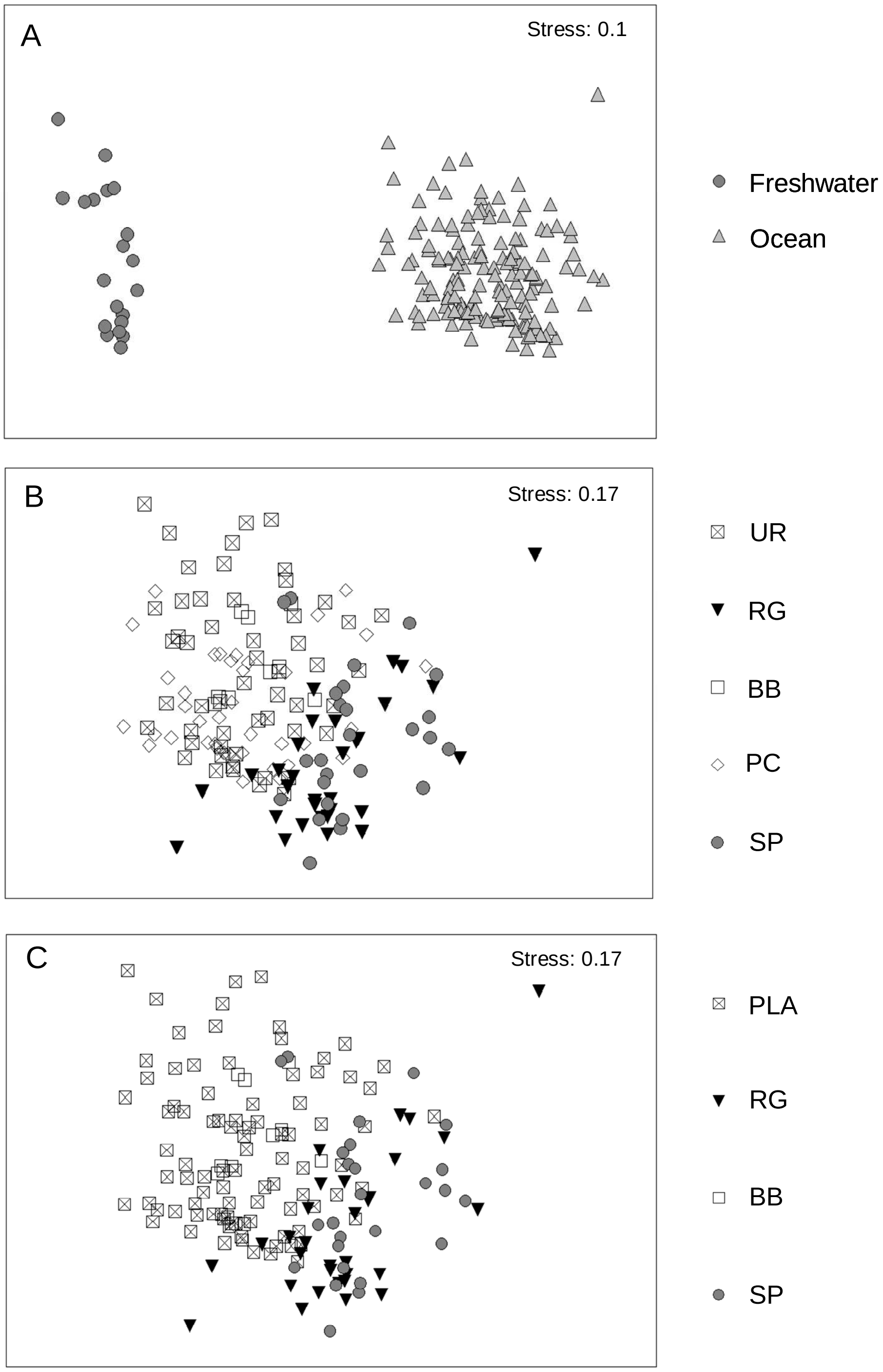

A
Stress: 0.1
Freshwater
Ocean
B
Stress: 0.12
UR
RG
BB
PC
SP
JUV
C
Stress: 0.12
PLA
RG
BB
SP
JUV

Figure 4


A
Stress: 0.1
● Freshwater
▲ Ocean
B
Stress: 0.17
☒ UR
▼ RG
☐ BB
◇ PC
● SP
C
Stress: 0.17
☒ PLA
▼ RG
☐ BB
● SP