UNIVERSIDADE FEDERAL DO RIO GRANDE
PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

OS PRIMEIROS ESTÁGIOS DE VIDA DA SAVELHA (*Brevoortia pectinata*) NO ESTUÁRIO DA LAGOA DOS PATOS

EVANDRO MALANSKI

Dissertação apresentada ao Programa de Pós-graduação em Oceanografia Biológica da Universidade Federal do Rio Grande, como requisito à obtenção do título de MESTRE

Orientador: José Henrique Muelbert

RIO GRANDE
Julho 2011

“*Estou aqui de passagem, mas não vim a passeio...*”

(Stab – Planet Hemp)

AGRADECIMENTOS

À Cecília, pelo companheirismo, carinho e compreensão;

Aos novos amigos, conquistados durante o curso, pelas diversas conversas, brincadeiras, e aprendizado. Em algum momento, cada um destes foi fundamental, por me dar apoio e crescermos juntos com as idéias discutidas, tanto do meio social como acadêmico. Destaque para alguns: Haig They, Martina, Priscila, Pryscilla, Rafael, Renatinha, Samantha, *et al.*;

À família, sempre longe, mas que procuram entender a minha busca pelo conhecimento;

Ao Laboratório de Ecologia do Ictioplâncton e seus integrantes, pela infraestrutura cedida, por me fornecer o material utilizado para esta dissertação, e pela acolhida na chegada a Rio Grande;

À diversos professores da FURG, que quando procurados estavam dispostos a ensinar e discutir, e à própria Universidade Federal do Rio Grande – FURG, por me proporcionar esta vivência;

Ao curso de graduação em Oceanografia na Universidade do Vale do Itajaí – UNIVALI, por me ensinar a base de conhecimento necessária para os estudos que tenho desenvolvido;

À Mônica, amiga e conselheira.

ÍNDICE

LISTA DE TABELAS

LISTA DE FIGURAS

RESUMO

Os padrões associados às condições ambientais e a distribuição espaço-temporal dos estágios iniciais da savelha, *B. pectinata*, e de seu desenvolvimento no estuário da Lagoa dos Patos foram investigados através de dados históricos. Dois outros experimentos complementares foram realizados: um para avaliar as técnicas de medição (tradicional e por imagem) em larvas de peixes; e, outro, para investigar o efeito dos principais conservantes (álcool 70 % e formaldeído 3,6 %) sobre as larvas de savelha. Diferença significativa na medição de larvas de peixes entre ambas as técnicas não foi observada, e os coeficientes de variação foram similares, o que sugere que as técnicas produzem o mesmo resultado médio, apesar do ganho de precisão pela técnica de análise por imagem. Um encolhimento significativo foi observado para ambos os conservantes, e os fatores de correção para as larvas de *B. pectinata* em formaldeído foi $CP_{vivo} = 1{,}0799 \times CP_{pós\text{-}conservação}$, e em álcool foi $CP_{vivo} = 1{,}1415 \times CP_{pós\text{-}conservação}$. No período de 1975 a 2009, um total de 10479 ovos e 14066 larvas e juvenis foram coletados através de amostragens planctônicas, e também amostrados parâmetros da água (salinidade e temperatura). As larvas foram medidas e classificadas de acordo com seu estágio de desenvolvimento. Cada estágio de desenvolvimento foi caracterizado pelo seu intervalo de tamanho, onde a menor larva em estágio vitelínico mediu 2,16 mm, e o maior juvenil teve 43,25 mm. As análises dos padrões de distribuição indicam desova fora da Lagoa dos Patos, em salinidade mais elevada, sendo importantes períodos sazonais para os ovos o inverno, primavera e início de verão, e posteriormente ocorre um transporte para dentro da região estuarina nos estágios mais iniciais de desenvolvimento, associados a valores menores de salinidade e temperatura. A distribuição dos juvenis indicou um retorno para a região costeira, e neste estágio o outono foi um período muito importante. Estes resultados são importantes para o conhecimento desta espécie, e serão úteis para o planejamento no gerenciamento deste recurso.

Palavras-chave: Clupeidae, ambiente costeiro, condições ambientais, padrão ecológico.

ABSTRACT

Patterns associated to environmental conditions and to the spatial and temporal distributions of the early stages of Argentine menhaden, *B. pectinata*, and of its development in the Patos Lagoon estuary were investigated using historical records. Two other complementary experiments were performed: one to evaluate the measurement techniques (traditional and image) in larval fishes; and, other, to investigate the effect of main conservatives (alcohol 70 % and formaldehyde 3.6 %) over Argentine menhaden larvae. Significant difference in the measurement of fish larvae between techniques was not observed, and the coefficients of variation were similar, suggesting that these two techniques produce the same average result, despite the gain in precision by image analysis technique. A significant shrinkage was observed for both preservatives, and the correction factors for *B. pectinata* larvae in formaldehyde was $SL_{live} = 1.0799 \times SL_{post\text{-}conservation}$, and in alcohol was $SL_{live} = 1.1415 \times SL_{post\text{-}conservation}$. From 1975 to 2009 a total of 10479 eggs and 14066 fish larvae and juveniles were collected at plankton surveys, and sampled water parameters (salinity and temperature), too. Larvae were measured and classified according to their developmental stage. Each development stage was characterized by its size range, which the smallest yolk-sac larvae measured 2.16 mm, and the largest juveniles had 43.25 mm. Analysis of the distributional pattern indicate spawning outside the Patos Lagoon, in high salinities, being important seasonal period for the eggs the winter, spring and early summer, and after occur a transport to the inner estuarine region in the earlier developmental stages, associated to lesser temperature and salinity values. Distribution of juveniles indicated a return to the coastal region, and in this stage the autumn was a very important period. These results are important for knowledge this specie, and will be useful for planning the management of this resource.

Keywords: Clupeidae, coastal environment, environmental conditions, ecological pattern.

1. CONTEXTUALIZAÇÃO

As relações existentes nos biossistemas, como no ambiente marinho e seus organismos, geram diversos estudos para compreensão dos fenômenos e padrões existentes na natureza. Por definição, estes estudos ecológicos se preocupam de forma ampla com os níveis de sistema, além daqueles do organismo (Odum & Barrett, 2008).

Diversos componentes da natureza são estudados em vários níveis, tais como os fenômenos ambientais, que podem ser eventos em escalas temporais tanto curtas (*e.g.* ondas e marés) como longas (*e.g.* ENSO), e ocorrem para manutenção do equilíbrio existente no nosso planeta através de processos de retroalimentação (Odum & Barrett, 2008). Como fenômenos biológicos, existem os eventos de crescimento, de reprodução e de desenvolvimento, que também variam sua escala temporal entre cada espécie, mas são fundamentais para a manutenção de suas populações.

Segundo a hipótese Gaia (Lovelock, 1979), a interação entre estes fenômenos gera a modificação dos padrões existentes na natureza, contudo com a manutenção do equilíbrio (homeorese) (Odum & Barrett, 2008). Pelo método científico, estes padrões são verificados através de dados que possam ser medidos e/ou descritos. Neste sentido, com a descrição e compreensão dos padrões existentes, e as suas respectivas inter-relações, podem ser projetados modelos que relacionam os ganhos e perdas decorrentes de cada fenômeno (Odum & Barrett, *op. cit.*).

Com a análise dos ovos e larvas de peixes, é possível a determinação de padrões reprodutivos encontrados para cada espécie, como áreas e épocas de desova, tempo requerido para o desenvolvimento larval, características ecológicas de suas populações, entre outros. O estudo deste estágio de vida é importante por ser caracterizada por um

período crítico, por ser determinante para a manutenção das gerações futuras de determinada população (Alvarez-Cadena *et al.*, 1984), e por estar suscetível a variação dos fenômenos ambientais, que podem ser estressantes aos organismos.

As grandes variações nos parâmetros ambientais são características dos estuários (Kennish, 1986; Seeliger & Odebrecht, 2010). Contudo, o estresse provocado por essa condição contrasta com a alta produtividade biológica, o que o torna favorável como área de berçário e criação para larvas e juvenis de muitas espécies de peixes, caso encontrado no estuário da Lagoa dos Patos (Muelbert & Weiss, 1991; Sinque & Muelbert, 1998; Muelbert *et al.*, 2010).

Os ciclos hidrológicos dominam a dinâmica do estuário da Lagoa dos Patos, através de processos oceanográficos e meteorológicos. O vento e a chuva são identificados como os principais fatores forçantes da circulação, distribuição da salinidade e nível de água deste sistema lagunar (Möller *et al.*, 2009; Möller & Fernandes, 2010), e que também influenciam a alta variabilidade da clorofila-*a* (Odebrecht & Abreu, 1998; Abreu *et al.*, 2010; Odebrecht *et al.*, 2010).

Desta forma, os fenômenos atuantes na Lagoa dos Patos também influenciam os estágios iniciais dos peixes, tanto diretamente, através das condições dos parâmetros da coluna d'água, como indiretamente, por prover condições para desenvolvimento de alimento para as larvas de peixes. Para as espécies que usufruem deste ambiente, estes fenômenos, capazes de modificar as condições do meio, podem torná-lo tanto favorável como desfavorável aos estágios iniciais de vida das espécies.

Os estudos que envolveram ovos e larvas de peixes no estuário da Lagoa dos Patos identificaram que são 3 as espécies mais abundantes e dominantes nesta área: *Brevoortia pectinata*, *Lycengraulis grossidens* e *Micropogonias furnieri*, que somados

correspondem a 88% da abundância dos ovos e 66% das larvas amostrados (Weiss, 1981; Muelbert, 1986; Muelbert & Weiss, 1991; Sinque & Muelbert, 1998). As duas primeiras espécies pertencem a um grupo de peixes reconhecidos por serem forrageiros, podendo ou não servir de alimento para o homem, e seu estudo é importante pois são o alimento para vários outros níveis tróficos, dentre estes alguns que nós exploramos como recurso pesqueiro (Pauly, 2007). Neste sentido, os padrões encontrados para estas espécies forrageiras podem refletir indiretamente nos recursos que são consumidos pelos seres humanos. A busca por esses padrões é necessária para o conhecimento das intra e inter-relações existentes no ambiente natural.

A *Brevoortia pectinata* (Jenyns, 1842) é espécie de savelha encontrada na região, que além de ser forrageira, está entre os peixes mais importantes e benéficos para consumo humano pelos seus elevados níveis de gordura corporal (Visentainer *et al.*, 2007; Simopoulos, 2004, *apud* Visentainer *et al.*, 2007). Os padrões reprodutivos descritos para esta espécie são de desova e cria no estuário da Lagoa dos Patos, onde seus ovos são encontrados em águas mais salinas e suas larvas e juvenis entram no estuário para completar seu desenvolvimento (Weiss & Krug, 1977). Ainda no Hemisfério Sul, a espécie de mesmo gênero *B. aurea* desova e se desenvolve no ambiente estuarino (Acha & Macchi, 2000). Outras espécies congêneres no Hemisfério Norte, tais como *B. patronus* e *B. tyrannus* , caracterizam-se por desovar no ambiente costeiro, e seus ovos serem transportados para dentro do estuário, ambiente que serve como área de berçário/criação dos seus estágios iniciais, até um posterior retorno ao ambiente marinho (Nelson *et al.*, 1977; Deegan, 1990; Stegmann *et al.*, 1999). A busca por padrões de distribuição dos primeiros estágios de vida de peixes, descritos através

de parâmetros mensuráveis da coluna d'água, são fundamentais para essas explicações, obtenção de intra e inter-relações, e monitoramento de estoques reprodutivos.

Os padrões observados na natureza nem sempre são tão claros. Contudo, tendências podem ser melhor identificadas com o aumento do número de observações dos fenômenos investigados, o que melhora a qualidade da informação utilizada e possibilita a representação dos padrões pelas observações mais prováveis ocorridas no estudo. Neste sentido, este trabalho tem como objetivo avaliar e investigar os padrões e relações encontrados com os estágios iniciais de desenvolvimento da espécie de savelha *Brevoortia pectinata* do estuário da Lagoa dos Patos a partir de uma coleção histórica.

2. ANÁLISES

Visto a necessidade da análise de muitos dados para a verificação adequada dos padrões existentes na natureza, foram estudadas quais seriam os melhores métodos para otimizar sua obtenção. Neste sentido, este trabalho avaliou, desenvolveu e inovou:

ι. as técnicas de medição em larvas de peixes;

ιι. os problemas relacionados às medidas das larvas de peixes pós-conservação; e

ιιι. os padrões encontrados na história de vida dos estágios iniciais da savelha, *Brevoortia pectinata*, no estuário da Lagoa dos Patos.

Os resultados destas análises foram estruturados na forma de artigos científicos, seguindo uma ordem lógica e cronológica da sua obtenção, e que estão nos apêndices dessa dissertação. Os detalhes técnico-científicos estão descritos nestes apêndices, o que

torna necessária sua leitura para melhor compreensão dos pontos discutidos ao longo do texto da dissertação.

3. RESULTADOS E DISCUSSÃO

3.1. Técnicas de medição de larvas de peixes

Para a medição das estruturas das larvas de peixes, é tradicionalmente utilizado um microscópio com uma das oculares apresentando um retículo micrométrico pré-calibrado. Neste método, a manipulação do organismo é corriqueira, pois é necessário deixá-lo na posição correta para a medição através do retículo. A manipulação demasiada pode danificar o organismo e causar uma medição imprecisa. Em outro sentido, a manipulação do organismo ainda vivo é uma das causas do seu estresse, e que pode favorecer sua morte (Martínez-Palacios *et al.*, 2002).

Como um método alternativo a técnica tradicional de medição de estruturas em larvas de peixes, foi proposto medições com o auxílio de fotografia digital (Fig. 1). Essa técnica tem como objetivo melhorar a precisão da medição das estruturas, e também possibilitar um processamento de forma mais rápida e ágil.

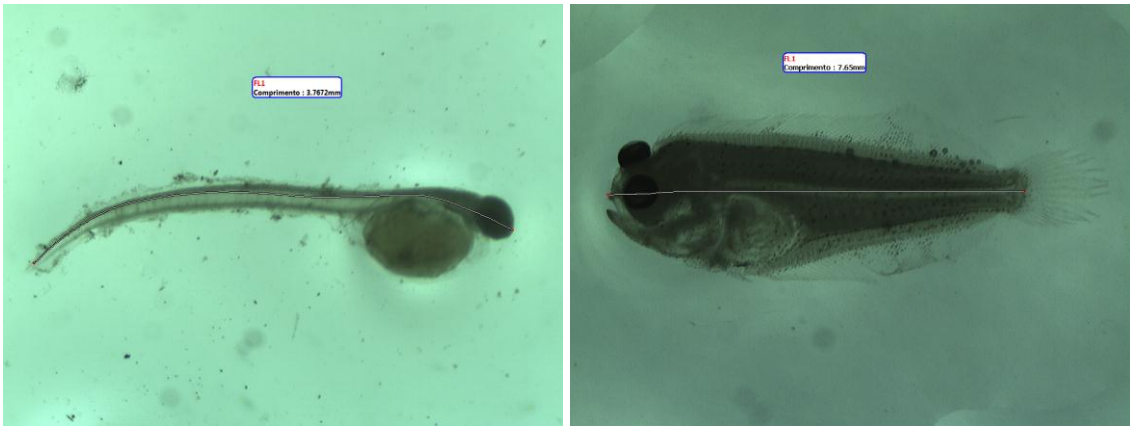

Fig. 1: Fotografia e medição (indicado pela linha ao longo do corpo) de larvas de peixes. Clupeiforme e Pleuronectiforme, respectivamente, nas imagens acima.

Foi descrito cada uma das técnicas de medição, como segue no artigo do **Apêndice 1** (Malanski & Muelbert, *Submt.* a) desta dissertação. Noventa larvas foram medidas por 3 leitores com diferentes graus de experiência utilizando duas técnicas de medição (tradicional e por imagem). Os resultados demonstraram que a medição do tamanho das larvas de peixes com ambas as técnicas não apresentaram diferenças significativas, portanto devem produzir o mesmo resultado médio. Sendo assim, estudos que utilizem medições pelas duas técnicas são válidos. Também, neste sentido, são válidos os estudos que corrigem o tamanho das larvas de peixes a partir de resultados obtidos com a outra técnica de medição (*e.g.* medição pela técnica de análise por imagem, com correção por fórmula obtida com medição pela técnica tradicional, e vice-versa).

A técnica de análise por imagem, comparativamente com a técnica tradicional para medição de organismos com o auxílio do microscópio, foi mais precisa, com o ganho na escala de uma unidade decimal em precisão. Contudo, este fato gerou um

aumento na variação dos resultados, mesmo que não significativo, e com isso ocorre diminuição da acurácia na comparação entre analisadores.

Cuidados sempre devem ser tomados na calibração das técnicas, pois isto será fundamental para a obtenção das medidas. Foi observado que na técnica de análise por imagem ocorre diferença do tamanho do pixel nos eixos vertical e horizontal, por isso é necessário atenção nessa calibração, para evitar a medição incorreta.

Vantagens e desvantagens de cada técnica para os métodos de medição de larvas de peixes estão descritos no **Apêndice 1** desta dissertação, bem como perspectivas para o futuro.

3.2. Modificação de tamanho em larvas da savelha *B. pectinata* por ação do conservante

Diversas áreas de estudo, como a aqüicultura (Martínez-Palacios *et al.*, 2002), a biologia pesqueira (Warlen *et al.*, 2002), a ecologia (Deegan, 1990) e modelagem do transporte de organismos (Baumann *et al.*, 2003), utilizam o tamanho de larvas de peixes. Normalmente não é obtido o tamanho larval em vida, por problemas logísticos, e as larvas são mantidas em conservante para posterior análise.

Uma das principais causas que afeta a acurácia no conhecimento do tamanho dos peixes é o encolhimento de suas estruturas corporais com o uso dos conservantes (Theilacker, 1980; Hjörleifsson *et al.*, 1992), o que pode gerar erros e/ou inconsistências nos estudos que utilizam esse dado. Por isso, diversos estudos são feitos para a obtenção do tamanho da larva em vida através do tamanho da larva pós-conservação (Pepin *et al.*, 1998; Fey *et al.*, 2005; Santos *et al.*, 2009).

Como verificado no artigo do **Apêndice 2** (Malanski & Muelbert, *Submt*. b) desta dissertação, foi obtido a modificação de tamanho em larvas da savelha *Brevoortia pectinata*, e descrita a equação para correção do efeito dos principais conservantes utilizados em estudos ictioplanctônicos (álcool 70 % e formaldeído 3,6 %). Estes resultados foram obtidos a partir de coletas de larvas de *B. pectinata* no ambiente natural, medição dessas larvas previamente a sua conservação, e repetição da medição ao longo de 90 dias de conservação.

Ambos os conservantes geraram modificações de tamanho com tendência ao encolhimento, e essa modificação foi significativa até 15 dias após sua conservação com o uso do álcool, e 30 dias com o uso de formaldeído.

As menores larvas de savelha tiveram as menores modificações de tamanho quando comparadas com as larvas maiores (proporcionalmente ao comprimento padrão), diferindo de outros estudos (Fey *et al*., 2005; Santos *et al*., 2009).

Através das regressões lineares entre os tamanhos das larvas da savelha *B. pectinata* em vida e pós-conservação para cada conservante, foram obtidas as fórmulas para correção do tamanho (Fig. 2). Com a validação dessas regressões, foi observado que somente os valores associados a inclinação da reta seriam suficientes de serem usados para o retrocálculo do tamanho da larva em vida, como segue nas fórmulas abaixo para cada tipo de conservante:

[Álcool] $CP_{vida} = 1{,}1415 \times CP_{pós\text{-}conservação}$

[Formaldeído] $CP_{vida} = 1{,}0799 \times CP_{pós\text{-}conservação}$

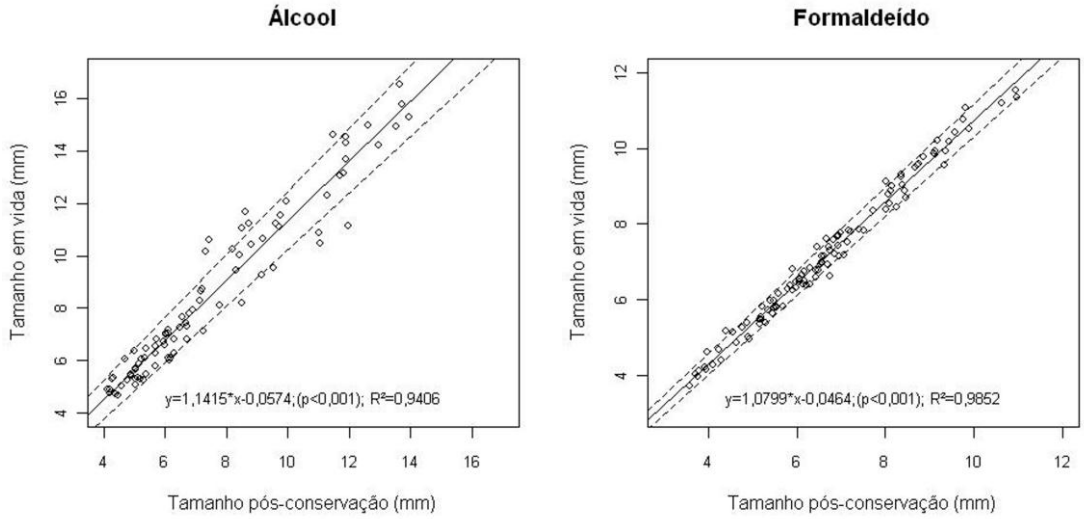

Fig. 2: Regressão linear com o intervalo de confiança de 95% para os parâmetros da regressão, entre o tamanho das larvas de *B. pectinata* em vida (mm) e após modificação do comprimento larval (mm), nos experimentos com álcool 70 % e formaldeído 3,6 % como conservantes.

3.3. Padrões encontrados na história de vida dos estágios iniciais da savelha *B. pectinata*, no estuário da Lagoa dos Patos

No mundo todo, os Clupeidae, compreendidos pelos arenques, sardinhas e savelhas, são considerados peixes de elevada importância ecológica e econômica, apesar do tamanho pequeno e curto período de vida (Whitehead, 1985; Pauly, 2007). Os Clupeiformes (Clupeidae e Engraulidae) são os peixes com maiores capturas mundiais (FAO, 2009), tanto utilizado para consumo humano, como também para isca em outras pescarias (Fischer *et al.*, 2004; Santos & Rodrigues-Ribeiro, 2000). Algumas espécies de clupeídeos não são utilizados diretamente para consumo humano, mas possuem importância pelos seus lipídeos (para produzir óleo de peixe), especialmente devido a alta concentração do ácido graxo ômega-3 nesta espécie fitoplanctívora (*e.g.* gênero *Brevoortia*) (Visentainer *et al.*, 2007). Essa importância incentivou diversos estudos

econômicos e ecológicos direcionados a pesca de suas espécies, as áreas de distribuição de seus cardumes, aos seus processos reprodutivos e de recrutamento, às suas relações tróficas, e por fim ao gerenciamento pesqueiro.

Na foz da Lagoa dos Patos, um ambiente interessante é criado no encontro das águas continentais e oceânicas, formando um estuário com cerca de 1000 km² de área (Seeliger & Odebrecht, 2010). O estuário da Lagoa dos Patos (ELP) é dominado pelos ciclos hidrológicos da bacia de drenagem, sofrendo pressão de processos oceanográficos e meteorológicos. O vento e a chuva são identificados como os principais fatores forçantes da circulação, da distribuição de salinidade e dos níveis de água no sistema da Lagoa dos Patos (Möller *et al.*, 2009; Möller & Fernandes, 2010). É verificado ainda uma alta variabilidade diária ou semanal da clorofila-*a*, e também oscilações anuais e interanuais, principalmente influenciado pelo vento (Odebrecht & Abreu, 1998; Abreu *et al.*, 2010; Odebrecht *et al.*, 2010).

Para o zooplâncton, a maior diferença dos padrões sazonais parece estar relacionado à temperatura da água, que varia de 9 a 15 °C no inverno, e de 25 a 28 °C no verão, mas a variação espacial mostra relação com a salinidade e o vento (Montú *et al.*, 1998). O ELP ainda foi caracterizado como importante para os estágios iniciais de vida de diversas espécies de peixes, devido sua variabilidade de habitats, suprimento abundante de alimento, proteção contra predadores, e a natureza da dinâmica do estuário, que contribui para a presença esporádica de muitas espécies oceânicas no estuário (Sinque & Muelbert, 1998; Muelbert *et al.*, 2010).

Estudos pretéritos do ictioplâncton no estuário da Lagoa dos Patos (Weiss & Krug, 1977; Weiss, 1981; Muelbert, 1986; Muelbert & Weiss, 1991) mostram que a savelha *B. pectinata* está entre as espécies de peixes mais abundantes encontradas na

região, e somam com outras duas espécies (*M. furnieri* e *L. grossidens*) 88% dos ovos e 66% das larvas amostradas no ELP (Sinque & Muelbert, 1998). Loebmann *et al.* (2001) identificaram que *B. pectinata* é a espécie de Clupeidae mais abundante da ictiofauna no ELP. Fischer *et al.* (2004) destacaram que esta espécie pelágica é encontrada em cardumes próximos à costa em águas superficiais, e seus juvenis são comumente encontrados em estuários. Também ocorre presença de outros clupeídeos na fase adulta no ELP, tais como *H. clupeola*, *P. harroweri*, *P. platana* e *R. arcuata* (Fischer *et al.*, *op cit.*).

A semelhança entre espécies de clupeídeos tem causado debate na literatura. Cousseau & Díaz de Astarloa (1993) estudaram o gênero *Brevoortia* na costa Sul-Americana do Atlântico e concluíram que as 2 espécies que coexistem entre o Rio Grande do Sul (Brasil) e Bahia Blanca (Argentina), *B. aurea* e *B. pectinata*, são a mesma espécie. Garcia *et al.* (2008), utilizando a genética na filogeografia da savelha (gênero *Brevoortia*), concluiu que somente *B. aurea* ocorre na região costeira do Uruguai, afirmando que só existe essa espécie no Atlântico Sudoeste. Cousseau & Perrota (2009) comentam sobre a semelhança dos estágios iniciais da *B. aurea*, sinônima da *B. pectinata*, com *R. arcuata*. Esta semelhança pode gerar dúvidas quanto a identificação, contudo será mantida a denominação *B. pectinata* para os ovos e larvas coletados no ELP até que seja esclarecida a denominação final da espécie que ocorre na região.

O habitat onde estes pequenos peixes se desenvolvem é fundamental para a manutenção das espécies. Nelson *et al.* (1977) destacaram a importância do transporte dos ovos e larvas de savelha para áreas estuarinas na costa leste dos EUA. Deegan (1990) discutiu a utilização de estuários como área de berçário/criação dos estágios

iniciais do ciclo de vida da savelha do Golfo (*Brevoortia patronus*) na Louisiana, e já Stegmann *et al*. (1999) definiram potenciais áreas de desova para a savelha do Atlântico (*B. tyrannus*), porém agora tratando este peixe como estuarino-dependente, ou seja, pelo menos uma fase de sua vida se passa no estuário.

Como mostrado no artigo do **Apêndice 3** (Malanski & Muelbert, *Submt*. c) desta dissertação, foram analisados dados históricos de coletas planctônicas de uma vasta área, compreendida pelo estuário da Lagoa dos Patos e adjacências próximas (Fig. 3), com revisão da identificação de ovos e larvas da savelha *Brevoortia pectinata*, para a compreensão dos padrões encontrados nas fases iniciais do ciclo de vida deste clupeídeo. Os resultados obtidos provavelmente refletem as condições mais próximas das ideais para o seu desenvolvimento, uma vez que foram analisados 10479 ovos e 3621 larvas.

O padrão observado nos estágio larvais da savelha *B. pectinata*, foi que a menor larva recém eclodida possui 2,16 mm, mas essas larvas com a presença do vitelo podem possuir até 5,91 mm. Contudo, a menor larva medida sem a presença do vitelo (em estágio de pré-flexão) possuiu 3,58 mm (Fig. 4).

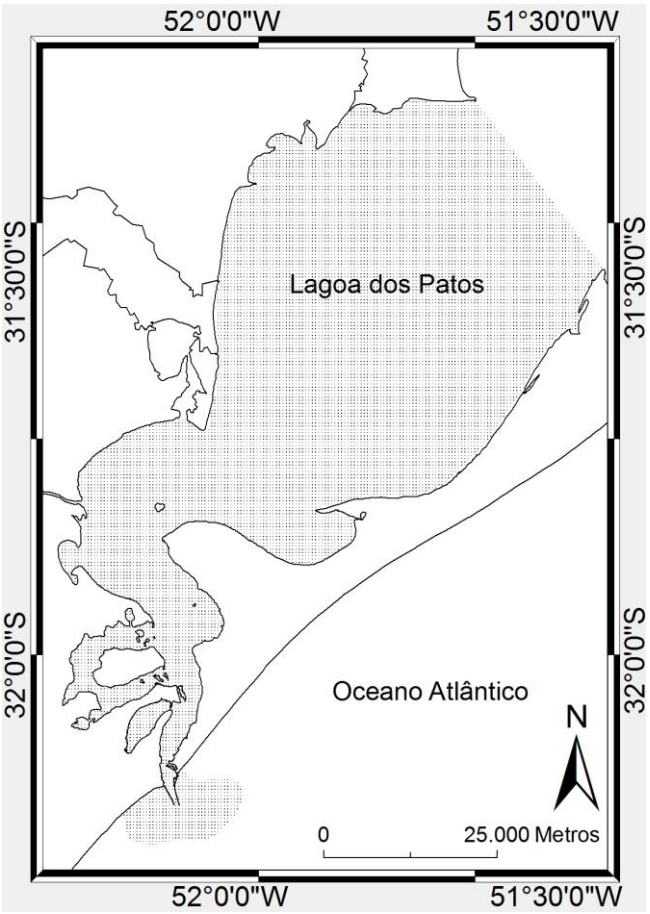

Fig. 3: Localização da área de estudo, com a delimitação pela área rachurada dos limites de coleta de plâncton.

Para as larvas em pós-flexão, que já possuem a formação dos raios da nadadeira caudal, e consequentemente um maior poder de locomoção, o menor tamanho medido neste estágio foi de 9,39 mm, mas não foram maiores que 22,27 mm (a maior larva deste estágio). Após esse tamanho, ou foram classificadas em estágio de metamorfose, ou já como juvenis. O menor juvenil medido possuiu 20,11 mm (Fig. 4). Os resultados foram semelhantes àqueles já descritos por Weiss & Krug (1977). Houveram diferenças com o estudo da *B. aurea*, no estuário do Rio de La Plata (Cássia & de La Rosa, 1993),

as quais ainda mantém as incertezas sobre a existência de uma única espécie de *Brevoortia* no Atlântico Sul.

A salinidade se mostrou como um fator altamente associado da ocorrência dos estágios iniciais da savelha no ELP. Tal fator, se observado em conjunto com os dados de distância da barra de Rio Grande, mostram que a desova ocorre na região externa da Lagoa dos Patos, com a entrada destes estágios iniciais para dentro da lagoa ao longo do seu desenvolvimento, e posterior retorno ao ambiente marinho já no estágio juvenil (Fig. 5). Para *B. aurea*, a desova ocorre dentro do estuário, que também diferiu do presente estudo (Acha e Macchi, 2000).

Já quando observado o padrão de ocorrência em relação a temperatura, foi descrito que o principal período de desova para a savelha ocorre no inverno, com o desenvolvimento das larvas dentro da lagoa em temperaturas menores, até retorno para o ambiente marinho em condições de temperatura da água mais quentes (Fig. 5).

Com isso, podemos caracterizar o estuário da Lagoa dos Patos como área muito importante para os estágios iniciais de vida da savelha *B. pectinata*. Modificações ocorridas neste ambiente, tanto pela ação humana como por eventos ambientais, irão alterar os padrões atuais desta espécie, e deverão afetar o recrutamento deste clupeídeo.

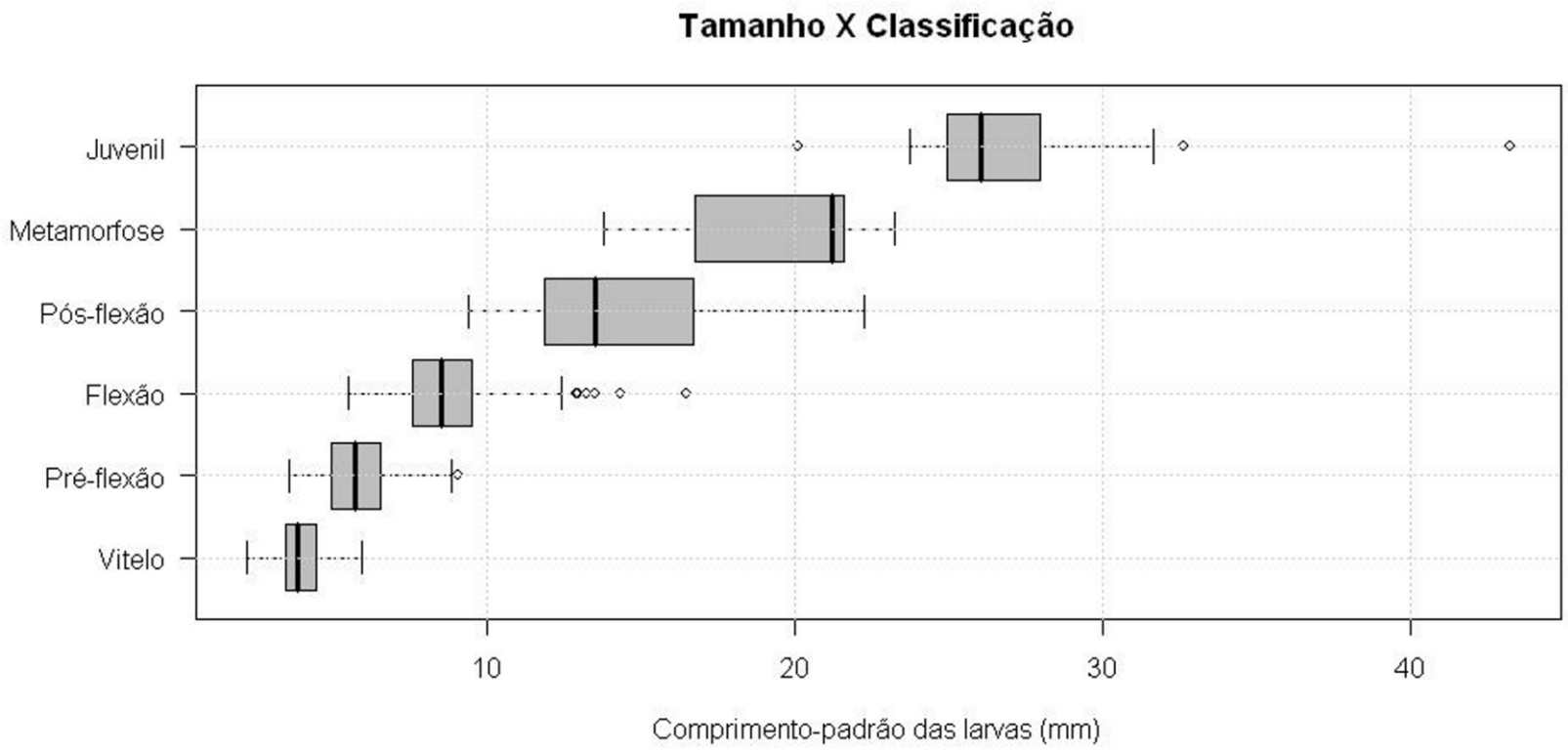

Fig. 4: Intervalos de comprimento padrão (mm) dos estágios iniciais da savelha (*B. pectinata*).

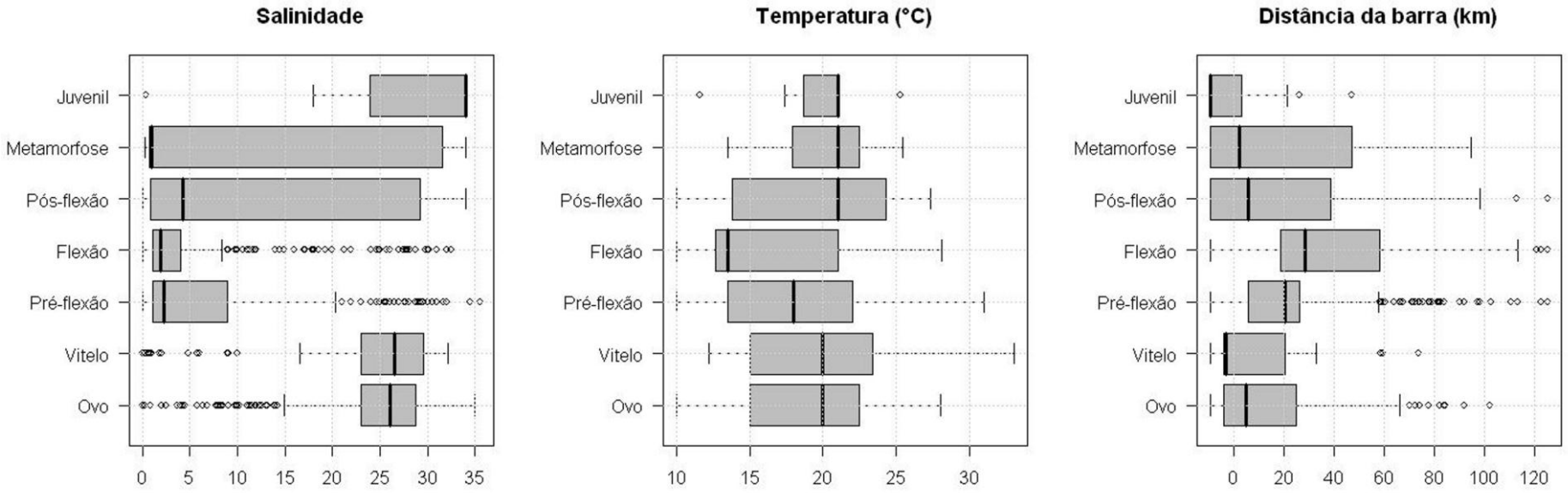

Fig. 5: Intervalos de salinidade, temperatura da água (°C), e distância da barra de Rio Grande (km), que foram encontrados nas capturas dos estágios iniciais da savelha (*B. pectinata*). Valores positivos de distância da barra indicam coletas de larvas para dentro do estuário, enquanto valores negativos indicam para fora da barra (ambiente marinho).

4. CONSIDERAÇÕES FINAIS E RECOMENDAÇÕES

Este estudo gerou importantes resultados para o conhecimento dos estágios iniciais de desenvolvimento da savelha *Brevoortia pectinata* no estuário da Lagoa dos Patos.

Obras de engenharia são comuns neste ambiente estuarino, como a construção dos portos marítimos na cidade de Rio Grande (entre os mais importantes portos do continente americano em produtividade atualmente), e os molhes (estruturas fixadoras da barra, que mantém aberto o canal de acesso a lagoa e direcionam o fluxo de corrente de vazão). Estas obras não necessariamente impactam o ambiente, contudo, indiretamente, aumentam a pressão das atividades humanas sobre o ambiente em questão, e consequentemente o risco de incidentes que podem afetar o recrutamento de diversas espécies de organismos.

Desta forma, este trabalho recomenda a realização de estudos futuros que envolvam modelos de dispersão de ovos e larvas de peixes; estudos de inter e intra-relações para determinadas espécies; e, recrutamento e mortalidade para os estágios iniciais de vida dos peixes. Estes estudos deverão gerar um banco de dados importante para a região, que serão importantes no planejamento do gerenciamento de estoques pesqueiros, e para dar suporte para a avaliação de impactos ambientais sobre as populações e comunidades de peixes, visto a realização de diversas obras de engenharia neste ambiente estuarino.

5. REFERÊNCIAS BIBLIOGRÁFICAS

Abreu, P. C.; M. Bergesch; L. A. Proença; C. A. E. Garcia & C. Odebrecht. 2010. Short- and Long-Term Chlorophyll *a* Variability in the Shallow Microtidal Patos Lagoon Estuary, Southern Brazil. ***Estuaries and Coasts***, 33: 554–569. DOI: 10.1007/s12237-009-9181-9.

Acha, E. M. & G. J. Macchi. 2000. Spawning of Brazilian menhaden, *Brevoortia aurea*, in the Río de la Plata estuary off Argentina and Uruguay. ***Fishery Bulletin***, 98(2): 227–235.

Álvarez-Cadena, J. N.; M. Aquino-Guzmán; F. Alonzo Rojo; J. G. Millán-García & F. Torres-Salinas. 1984. Composición y Abundancia de las Larvas de Peces en el Sistema Lagunar Huizache-Caimanero. Parte I. Agua Dulce 1978. **Anales del Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México**. 11(1): 163-180.

Baumann, H.; P. Pepin; F. J. M. Davidson; F. Mowbray; D. Schnack & J. F. Dower. 2003. Reconstruction of environmental histories to investigate patterns of larval radiated shanny (*Ulvaria subbifurcata*) growth and selective survival in a large bay of Newfoundland. **ICES Journal of Marine Science**, 60: 243-258. DOI: 10.1016/S1054-3139(03)00019-5.

Cassia, M. C. & de la Rosa, S. B. G. 1993. Características diferenciales del desarrollo larval de *Brevoortia aurea* en el Atlantico Sudoccidental. ***Frente Maritimo***, 14: 63-69.

Cousseau, M. B. & J. M. Díaz de Astarloa. 1993. El Género *Brevoortia* en la Costa Atlántica Sudamericana. Montevideo. ***Frente Marítimo***, 14: 49-57.

Cousseau, M. B. & R. G. Perrota. Atlas de Sensibilidad Ambiental de la Costa y el Mar Argentino: Peces. Disponível em: http://atlas.ambiente.gov.ar/tematicas/mt_02/pdfs/PC_Brevoortia_aurea.pdf Acesso em: 31/07/2009.

Deegan, L. A. 1990. Effects of estuarine environmental conditions on population dynamics of young-of-the-year gulf menhaden. ***Marine Ecology Progress Series***, 68: 195–205.

FAO. 2009. Part 1 – Word Review of Fisheries and Aquaculture, *In*: ***The State of Word Fisheries and Aquaculture (SOFIA)***. Roma, Itália. 176p.

Fey, D. P. & J. A. Hare. 2005. Length correction for larval and early-juvenile Atlantic menhaden (*Brevoortia tyrannus*) after preservation in alcohol. ***Fishery Bulletin***, 103(4): 725–727.

Fischer, L. G.; L. E. D. Pereira & J. P. Vieira. 2004. ***Peixes Estuarinos e Costeiros***. Rio Grande/RS, Brasil. Ecoscientia. 139p.

García, G.; J. Vergara & V. Gutiérrez. 2008. Phylogeography of the Southwestern Atlantic menhaden genus *Brevoortia* (Clupeidae, Alosinae). ***Marine Biology***, 155: 325-336.

Hjörleifsson, E. & G. Klein-MacPhee. 1992. Estimation of live standard length of winter flounder *Pleuronectes americanus* larvae from formalin-preserved, ethanol-preserved and frozen specimens. ***Marine Ecology Progress Series***, 82: 13-19.

Kennish, M. J. 1986. ***Ecology of Estuaries: Physical and Chemical Aspects. Volume I***. Boca Raton/FL, USA. CRC Press. ISBN: 0-8493-5892-2. 245p.

Loebmann, D.; A. M. Garcia; M. B. Raseira; L. A. Ramos & J. P. Vieira. 2001. Distribuição espacial de *Brevoortia pectinata* (Jenyns, 1842) (Pisces: Clupeidae) nas

zonas rasas do estuário da Lagoa dos Patos, RS, Brasil. *In*: ***Annals of XIV EBI***, São Leopoldo.

Lovelock, J. E. 1979. ***Gaia: A New Look at Life on Earth***. New York, USA, Oxford University Press. 157p.

Malanski, E. & J. H. Muelbert. *Submetido* a. Measuring Small Organisms: Evaluation of Traditional and Image Techniques for Larval Fishes.

Malanski, E. & J. H. Muelbert *Submetido* b. Size Changes in Argentine menhaden Larvae *Brevoortia pectinata* Due to Preservation.

Malanski, E. & J. H. Muelbert *Submetido* c. Life History Patterns of Early Stages of Argentine menhaden, *Brevoortia pectinata* (Jenyns, 1842), in a Subtropical Estuary of Southwestern Atlantic Ocean.

Martínez-Palacios, C. A.; E. B. Tovar; J. F. Taylor; G. R. Durán & L. G. Ross. 2002. Effects of temperature on growth and survival of *Chirostoma estor estor*, Jordan 1879, monitored using a simple video technique for remote measurement of length and mass of larval and juvenile fishes. ***Aquaculture***, 209: 369-377.

Möller JR., O. O.; J. P. Castello & A. C. Vaz. 2009. The Effect of River Discharge and Winds on the Interannual Variability of the Pink Shrimp *Farfantepenaeus paulensis* Production in Patos Lagoon. ***Estuaries and Coasts***, 32(4): 787–796.

Möller, O. & E. Fernandes. 2010. Hidrologia e Hidrodinâmica. *In*: Seeliger, U. & C Odebrecht (Eds.). ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. pp. 15–27.

Montú, M.; A. K. Duarte & I. M. Gloeden. 1998. O Ambiente e a Biota do Estuário da Lagoa dos Patos: Zooplâncton. *In*: Seeliger, U.; C. Odebrecht & J. P. Castello (Eds.).

1998. ***Os Ecossistemas Costeiro e Marinho do Extremo Sul do Brasil***. Rio Grande. Ecoscientia. pp. 43-46.

Muelbert, J. H. 1986. Estrutura e dinâmica do ictioplâncton da área de canal no estuário da Lagoa dos Patos, RS, no período de outono/81 a verão/83. Tese de Mestrado, Fundação Universidade do Rio Grande. FURG. 88p.

Muelbert, J. H. & G. Weiss. 1991. Abundance and Distribution of Fish Larvae in the Channel of the Patos Lagoon Estuary, Brasil. *In*: Hoyt, R. D. (Ed.). ***Larval Fish Recruitment and Research in the Americas: Proceedings of the 13th Annual Fish Conference***. NOAA Technical Report NMFS 95. Mérida, México. pp. 43-54.

Muelbert, J. H.; E. Muxagata & S. Kaminski. 2010. As Comunidades Zooplanctônicas. *In*: Seeliger, U. & C Odebrecht (Eds.). ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. pp. 65–75.

Nelson, W. R.; M. C. Inghan & W. F. Schaaf. 1977. Larval transport and year class strength of Atlantic menhaden, *Brevoortia tyranus*. ***Fishery Bulletin***, 75(1): 23-41.

Odebrecht, C. & P. C. Abreu. 1998. O Ambiente e a Biota do Estuário da Lagoa dos Patos: Microalgas. *In*: Seeliger, U.; C. Odebrecht & J. P. Castello (Eds.). 1998. ***Os Ecossistemas Costeiro e Marinho do Extremo Sul do Brasil***. Rio Grande. Ecoscientia. pp. 36-40.

Odebrecht, C.; M. Bergesch; S. Medeanic & P. C. Abreu. 2010. A Comunidade de Microalgas. *In*: Seeliger, U. & C Odebrecht (Eds.). ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. pp. 49–63.

Odum, E. P. & G. W. Barrett. 2008. ***Fundamentos de Ecologia***. São Paulo/SP, Brasil. Cengage Learning. ISBN: 978-85-221-0541-0. 612p.

Pauly, D. 2007. Tales of a Small, But Crucial, Fish. ***Science***, 318(5851): 750-751. doi:10.1126/science.1147800.

Pepin, P.; J. F. Dower & W. C. Leggett. 1998. Changes in the probability density function of larval fish body length following preservation. ***Fishery Bulletin***, 96(3): 633-640.

Santos, J. N. S.; F. G. Araújo & D. S. Silva. 2009. Length correction for early-juvenile Brazilian herring *Sardinella janeiro* (Eigenmann, 1894) after preservation in formalin, ethanol and freezing. ***Neotropical Ichthyology***, 7(1): 87-92.

Santos, R. C. & M. Rodrigues-Ribeiro. 2000. Demanda de isca vivas para a frota atuneira catarinense na safra de 1998/99: CPUE, composição e distribuição das capturas. ***Notas Técnicas FACIMAR***, 4: 97-101.

Seeliger, U. & C. Odebrecht (Eds.). 2010. ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. 180p.

Sinque, C. & J. H. Muelbert. 1998. O Ambiente e a Biota do Estuário da Lagoa dos Patos: Ictioplâncton. *In*: Seeliger, U.; C. Odebrecht & J. P. Castello (Eds.). ***Os Ecossistemas Costeiro e Marinho do Extremo Sul do Brasil***. Rio Grande/RS, Brasil. Ecoscientia. pp. 56-60.

Stegmann, P. M.; J. A. Quinlan; F. E. Werner; B. O. Blanton & P. Berrien. 1999. Atlantic menhaden recruitment to a southern estuary: defining potential spawning regions. ***Fishery Oceanography***, 8(2):111-123.

Theilacker, G. H. 1980. Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. ***Fishery Bulletin***, 78: 685-692.

Visentainer, J. V.; M. D. Noffs; P. O. Carvalho; V. V. Almeida; C. C. Oliveira & N. E. Souza. 2007. Lipid Content and Fatty Acid Composition of 15 Marine Fish Species from the Southeast Coast of Brazil. ***Journal of the American Oil Chemists' Society***, 84: 543–547. DOI: 10.1007/s11746-007-1070-4.

Warlen, S. M.; K. W. Able & E. H. Laban. 2002. Recruitment of larval Atlantic menhaden (*Brevoortia tyrannus*) to North Carolina and New Jersey estuaries: evidence for larval transport northward along the east coast of the United States. ***Fishery Bulletin***, 100: 609-623.

Weiss, G & L. C. Krug. 1977. Características do desenvolvimento e metamorphose de *Lycengraulis olidus* (Engraulidae) e *Brevoortia pectinata* (Clupeidae) no Estuário da Lagoa dos Patos, Brasil. Rio Grande. Atlântica, 2(1): 83-117.

Weiss, G. 1981. Ictioplancton del Estuario de Lagoa dos Patos, Brasil. PhD. Thesis, Faculdad de Ciencias Naturales y Museo, Universidad Nacional de la Plata, 164p.

Whitehead, P. J. P. 1985. FAO species catalogue: Volume 7 – Clupeoid Fishes of the World (suborder Clupeioidei). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings. Part 1 – Chirocentridae, Clupeidae and Pristigasteridae. ***FAO Fisheries Synopsis***, 125(7/1): 1-303.

6. APÊNDICES

6.1. Apêndice 1 – MALANSKI, E & MUELBERT. *Submetido* a. Measuring Small Organisms: Evaluation of Traditional and Image Techniques for Larval Fishes. 23 pp. (*manuscrito*)

Obs.: Artigo científico submetido para *Limnology and Oceanography: Methods.*

MEASURING SMALL ORGANISMS: EVALUATION OF TRADITIONAL AND IMAGE TECHNIQUES FOR LARVAL FISHES

Malanski, E.[1]; Muelbert, J. H.

Programa de Pós-Graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG

[1] (e-mail: evandromalanski@furg.br)

ABSTRACT

This study describes and evaluates the traditional and image techniques to measure larval fishes. For this, it was used 90 fish larvae of different taxa to compare the results obtained by each technique. Three analyzers measured the larvae in each technique. There were no significant differences in measurement between each technique (ANOVA, p=0.8357), and their coefficient of variation were similar, suggesting that both techniques produce the same average result. However, calibrations of both techniques indicate gain of precision in one decimal unit with the use of image analysis technique (*e.g.* 8x magnification – 0.0104 mm/pixel in "X"-axis) instead of the traditional technique (*e.g.* 8x magnification – 0.1250 mm/division). A difference observed between horizontal and vertical pixel size may lead to false/incorrect measurements, and reveals that care is necessary in the calibration of the image analysis technique. Advantages and disadvantages for each technique are discussed in this study.

RESUMO

Este estudo descreve e avalia as técnicas tradicional e por imagem para medição de larvas de peixes. Para isto, foram usadas 90 larvas de peixes de diferentes taxa para comparar os resultados obtidos com cada técnica. Três analisadores mediram as larvas em cada técnica. Não foram observadas diferenças significativas nas medições entre cada técnica (ANOVA, p=0,8357), e os coeficientes de variação foram similares, sugerindo que estas técnicas produzem o mesmo resultado médio. Entretanto, a calibração das técnicas indica um ganho de precisão em uma unidade decimal com o uso da técnica de análise por imagem em vez da técnica tradicional. Uma diferença observada entre o tamanho do pixel nos eixos horizontal e vertical da imagem pode conduzir a medição falsa/incorreta, e revelam que cuidados são necessários na calibração da técnica de análise por imagem. Vantagens e desvantagens para cada técnica são discutidas neste estudo.

Keywords: digital picture, fish larvae, image analysis.

INTRODUCTION

Knowledge of larval fish size is used to study fish growth (Martínez-Palacios *et al*., 2002), to identify larval fish and its larval stages (Smith *et al*. 1977; Moser, 1996; Richards, 2006), to understand the effect of the preservative in larval fish length (Fox, 1996; Pepin *et al*. 1998; Santos *et al*., 2009), and other for ecological and transport studies of fish larvae (Deegan, 1990; Baumann *et al*. 2003; Santos *et al*., 2005). In natural environment, larval fish has fast growth to reduce mortality (Hunter, 1984;

Pepin, 1991), despite other factors discussed by Houde (2008). In fish culture, the larval fish growth indicates a higher yield of feed used (Rønnestad *et al*., 1999).

A microscope with one ocular containing a pre-calibrated micrometer reticle is traditionally used to measure the structures of fish larvae. In this technique, manipulation of the body of larval fish is common, it is necessary to lay the larvae in a correct position for measurement with the reticle. Excess handling can damage the body of the organism, and cause inaccurate measurements. Increase in manipulation of living organisms is one cause of stress, which may lead to their death.

Capturing images is an alternative tool that reduces handling of organisms. There are many uses in the study of microscopic organisms that can capture images, both for measuring structures (Martínez-Palacios *et al*., 2002; Almesjö *et al*., 2007), and to recognize patterns in images (Tang *et al*. 1998; Staciarini, 2004). In digital imaging, a sensor converts light into a function with coordinate "X" and "Y", and stores this information in the form of a matrix (Gonzalez *et al*., 1993). Each of the values of this matrix corresponds to a picture element (or pixel), and the set of pixels form a digital image (Gonzalez *et al*., *op cit*.).

Measurements with the aid of digital image have been proposed as an alternative to the traditional technique for measuring structures in fish larvae. The goal of this technique is to improve the precision in the measurement of structures, and also to enable a faster and more agile processing. The aim of this paper is to describe the methodologies step by step, to highlight the results obtained by this study, and to discuss source of errors and future prospects for the use of the measurement method.

MATERIALS AND METHODS

A total of 90 fish larvae, with representatives of Clupeiforms, Perciforms and Pleuronectiforms, from the collection of the Laboratório de Ecologia do Ictioplâncton (LEI/IO-FURG) were separated in 3 lots of 30 larvae, according to their size. The size separation allowed the use of the same lot of larvae at 3 different stereomicroscope magnifications: 8x, 10x and 20x. Fishes larvae were observed at the same magnification in both techniques, the image field captured by the digital camera, and the ocular with the micrometer reticle. This allowed both techniques are compared. The measurement experiments and equipments used were described separately, below.

Image technique

A Motic digital camera, model Moticam2300 with 3.0 MP resolution was mounted in the Opton stereomicroscope. The camera was connected to a computer via USB port, and the capture and image processing was possible with the Motic Images Plus 2.0^{ML} software. Larvae were placed in the microscope and their images were captured by the camera, and its picture and magnification were annotated.

Digital images for each 90 larvae were captured in an uncompressed format, with high color definition (TIFF – Tagged Image File Format) with a resolution of 1024x768 pixels (upper limit of the camera used). These images were projected at a HetchBoard panel, model HB80, with an interactive area of 79", connected to the computer via USB port, and with the aid of the stylus and the eBeam Interact software version 1.3.1, the larva were measured. Larval measurements were made from a line drawn at the larval bodies, where the larval fish standard length would be measured

(from the snout, passing by the intermediate position of the notochord, until the end of the notochord, with no deviations). These lines are formed by pixels, and will correspond the size that wants to do. Calibration of this technique will define the size of each pixel, consequently, the larval fish length.

The interactive panel was used for its ease in getting larval fish length, its ability to reproduce images in big size through the projector, its agility and precision with the measurements.

Once the image was taken, the larva was kept aligned in the same magnification and measured through the ocular (traditional technique).

Traditional technique

In the traditional technique, a stereoscopic microscope Opton, model TNE-10TN, and one ocular with a micrometer reticle were used. This technique involves placement of a fish larvae in a rectilinear position at the microscope, to be capable to measure its length correctly, and count how much divisions of the micrometer reticle cover the larval fish length. Number of divisions and magnification used for each measurement were written down to obtain the appropriate larval size.

Calibration of the ocular in the microscope will be define the size of each reticle division for each magnification used.

Calibration techniques

Both techniques required a calibration to obtain the larval fish length.

A histological calibration slide, with a cross representing 1 mm in each axis subdivided into 100 divisions (Fig. 1), was used to calibrate the techniques. Its digital image was

captured for each magnification used in the experiment. These images were projected on the interactive panel, and with the image processing software, a line was drawn at the ends of the cross, and checked for the number of pixels equivalent in the horizontal ("X") and vertical ("Y") axis. It was possible with this procedure to know the precision of this technique, in mm/pixel (the minimum size capable to be detected by the equipment used), by each magnification.

The same histological calibration slide was used to calibrate the ocular micrometer reticle for each magnification by counting the number of divisions in the reticle corresponding to 1 mm of the cross of the calibration slide. With a simple calculation, it was possible to know the size of each division of the micrometer reticle presenting at the ocular of the microscope, that will correspond by the precision of this technique, in mm/division.

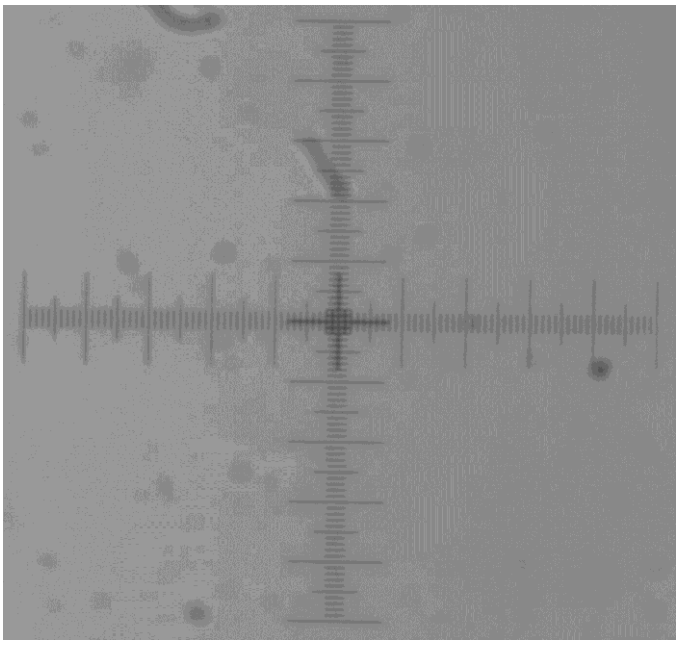

Fig. 1: Picture of the cross 1x1 mm represented in the calibration histological slide at 50x magnification of the stereoscopic microscope.

Analysis and comparisons

To evaluate differences between techniques, the same measurements of larval fishes (standard length) were performed by three different analyzers, being: an analyzer with experience in ichthyoplankton studies and fish larvae measurement; an analyzer with experience just in ichthyoplankton studies; and, an analyzer with experience in measuring planktonic organisms.

The methodology of measurement for each technique were discussed, standardized and followed by all 3 analyzers. To avoid cross-influence of larval fish length reading, each analyzer noted on a separate worksheet his measurements. *A posteriori*, measurements were compared using Analysis of Variance (ANOVA) to investigate whether there were significant differences: 1 - between techniques; and, 2 - between analyzers for different techniques. Statistical routines adapted were conducted with the use of *R* software (*R* Development Core Team, 2006).

Concepts of accuracy involve systematic and random effects, and precision involves only random effect (Monico *et al.*, 2009). In this case, precision of the equipment used for measurement is a random effect, and the difference between analyzers during the measurement is a systematic and random effects. In this sense, the accuracy of the techniques were obtained with the comparison of the *p*-value of analyzers by the ANOVA of each technique. *P*-value equal to 1 (one) means lack of significant differences, and the same average result. On the other hand, *p*-value iqual to 0 (zero) means the data are completely different. So, with the comparison of the *p*-values of analyzers for each technique, it is possible to conclude what technique is more accurate.

RESULTS

Calibration of each technique

Calibration of the digital image capture technique obtained higher degree of precision when compared to the traditional technique (Tab. 1). These results shown the gain of precision in one decimal unit (*i.e.* 8x magnification – image technique=0.01 mm/pixel, and traditional technique=0.1 mm/division). Despite higher precision, there was observed a difference in the pixel size for the image analysis technique, when compared the "X" and "Y" pixel size in the images. This difference found for the three magnifications used in this work is around 6% higher in the "Y"-axis.

Fish larvae measurements

When using the traditional technique, the 3 analyzers found an average of 5.60 mm/larvae SL (SD=1.80 mm) for the 90 fish larvae measured in the study. The smallest fish larvae measured had 3.52 mm, and the largest had 11.87 mm (Tab. 1). When analyzed by magnification, at 8x larvae measured between 5.87 and 11.87 mm (SL); at 10x, larval length varied between 3.60 and 7.20 mm (SL); and, at 20x, larval length ranged between 3.52 and 5.05 mm (SL). There were no significant differences between analyzers, and this technique presented great accuracy (ANOVA, p=0.9826) (Tab. 2, Fig. 2, Fig. 3).

For the image analysis technique, the 3 analyzers found an average of 5.62 mm/larvae SL (SD=1.85 mm) for the 90 fish larvae used in this study. The smallest fish larvae measured had 3.23 mm, and the largest had 12.24 mm (Tab. 1). When analyzed by

magnification, at 8x larvae measured between 5.91 and 12.24 mm (SL); at 10x, larval length varied between 3.60 and 7.29 mm (SL); and, at 20x, larval length ranged from 3.24 and 4.95 mm (SL). There were no significant differences between the analyzers, and the accuracy of this technique was less than the traditional technique (ANOVA, p=0.5335) (Tab. 3, Fig. 2, Fig. 3).

When analyzing the differences between the techniques (Fig. 4), the measurements obtained at 8x and 10x magnification by the image analysis technique were larger when compared to the tradicional technique. At 8x, the average difference was 0.08 mm (SD=0.19 mm), and at 10x, the average difference was 0.06 mm (SD=0.10 mm). At 20x magnification, the fish larvae measurements obtained by traditional technique were on average 0.10 mm (SD=0.09 mm) larger when compared to measurements obtained by image analysis technique. The result of ANOVA indicated that there were no significant difference between the two techniques used for three magnifications and among the 3 analyzers (p=0.8357) (Tab. 4, Fig. 3). The coefficient of variation remained similar between the two techniques at different magnifications (Tab. 1).

Tab. 1: Results obtained for the calibration of each technique (precision) and with the measurements of fish larvae. There is indicated the technique, the magnification, the precision obtained on the "X" and "Y"-axis (mm/pixel) and on the micrometer reticle (mm/division), the smallest and largest fish measured (mm), the average and standard deviation of larval fishes length (mm/larvae) measured, and the coefficient of variation (%).

		Technique precision							
Technique	**Magnification**	**"X" -axis (mm/pixel)**	**"Y"-axis (mm/pixel)**	**Micrometer reticle (mm/division)**	**No. of larval fishes measured**	**Length of the smallest larval fish (mm)**	**Length of the largest larval fish (mm)**	**Average and standard deviation (mm/larva)**	**Coefficient of variation (%)**
IMAGE	**-**	-	-	-	90	3,23	12,24	5,62 ± 1,85	32,83
TRADITIONAL	**-**	-	-	-	90	3,52	11,87	5,60 ± 1,80	31,93
IMAGE	**8x**	0,0104	0,0111	-	30	5,91	12,24	7,78 ± 1,41	18,03
TRADITIONAL	**8x**	-	-	0,1250	30	5,87	11,87	7,70 ± 1,37	17,68
IMAGE	**10x**	0,0081	0,0086	-	30	3,60	7,29	4,97 ± 0,85	16,99
TRADITIONAL	**10x**	-	-	0,1000	30	3,60	7,20	4,90 ± 0,88	17,78
IMAGE	**20x**	0,0040	0,0042	-	30	3,24	4,95	4,11 ± 0,39	9,46
TRADITIONAL	**20x**	-	-	0,0513	30	3,52	5,05	4,21 ± 0,38	8,97

Tab. 2: Results of Analysis of Variance (ANOVA), for traditional technique. (Df – Degrees of freedom; SS – Sum of squares; MS – Mean of squares).

Error: Larvae					
	Df	SS	MS	F value	*p*-value
Residuals	29	89.18	3.07		
Error: Within					
	Df	SS	MS	F value	*p*-value
Analyzer	2	0.02	0.01	0.02	0.9826
Magnification	2	616.12	308.06	457.52	0.0000
Analyzer:Magnification	4	0.04	0.01	0.01	0.9996
Residuals	232	156.21	0.67		

Tab. 3: Results of Analysis of Variance (ANOVA), for image analysis technique. (Df – Degrees of freedom; SS – Sum of squares; MS – Mean of squares).

Error: Larvae					
	Df	SS	MS	F value	*p*-value
Residuals	29	94.62	3.26		
Error: Within					
	Df	SS	MS	F value	*p*-value
Analyzer	2	0.85	0.42	0.63	0.5335
Magnification	2	663.67	331.84	492.41	0.0000
Analyzer:Magnification	4	0.19	0.05	0.07	0.9909
Residuals	232	156.35	0.67		

Tab. 4: Results of Analysis of Variance (ANOVA), for both techniques. (Df – Degrees of freedom; SS – Sum of squares; MS – Mean of squares).

Error: Larvae					
	Df	SS	MS	F value	*p*-value
Residuals	29	183.23	6.32		
Error: Within					
	Df	SS	MS	F value	*p*-value
Analyzer	2	0.43	0.21	0.34	0.7139
Magnification	2	1278.94	639.47	1006.77	0.0000
Technique	1	0.03	0.03	0.04	0.8357
Analyzer:Magnification	4	0.13	0.03	0.05	0.9954
Analyzer:Technique	2	0.44	0.22	0.35	0.7051
Magnification:Technique	2	0.86	0.43	0.68	0.5087
Analyzer:Magnification:Technique	4	0.10	0.03	0.04	0.9969
Residuals	493	313.14	0.64		

This study did not record the time spent to acquire measurements for each technique. However, one approach to estimate the time taken by each technique is possible based on the experience gained from this experiment. To obtain a single measurement of a fish larvae with image analysis technique, there was necessary to capture its digital image, which consisted in placing the fish larvae under the microscope (approximately 10 s dispended), check whether it was in the capture field of image and repositioning it if necessary (t≅10 s), and, capture its digital image (t≅10 s). The measurement consists of the projection of the digital image on the interactive panel (t≅10 s), and, to trace on the image the line corresponding to the larval fish length (t≅10 s). Thus, the image analysis technique required about 1 minute from the beginning to the end of the procedure.

When irregularities were observed in the picture of fish larvae (*i.e.* fish larvae misplaced during the capture of its image), there was possible to obtain its length in the digital image with a trace corresponding to the measure that wanted to be obtained (Fig. 5). This pass did not demand extra time to obtain the measurement.

In order to obtain the measurement of a single larval in the traditional technique, there was necessary to place the organism under the microscope (t≅10 s), and, place it under the field of the micrometer reticle in a straight position (time varies, between 30 and 180 s). Thus, this technique required 50 to 200 s between the beginning and the end of the procedure. The time required in the traditional technique was more variable because of the type of fish larvae and its degree of conservation had high influence to positioning it in the slide, because it is necessary the larvae to be straight and laying in on of its side.

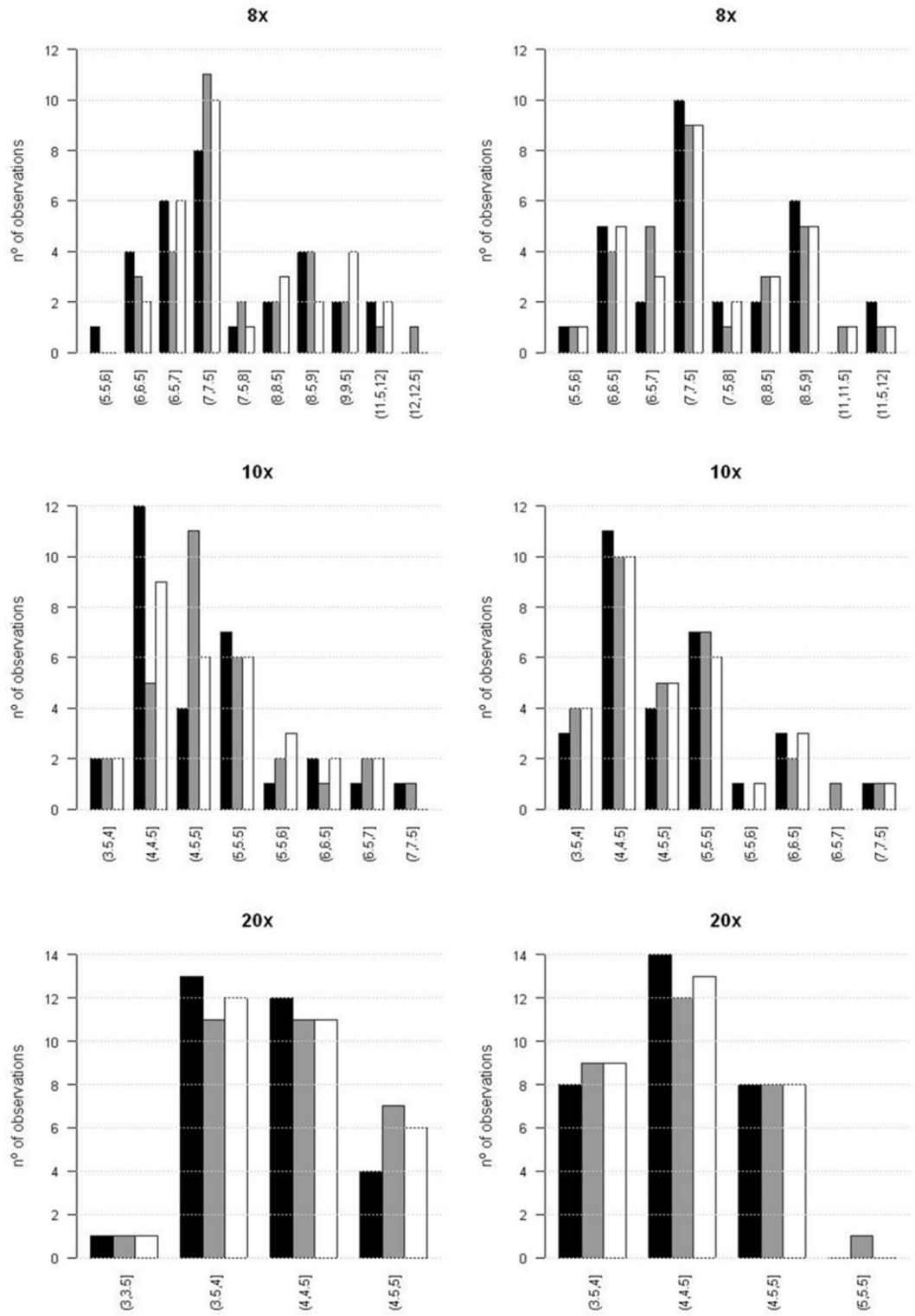

Fig. 2: Number of observations for larval fish length intervals (mm) for each analyzer (1 – black bars, 2 – gray bars, and 3 – white bars). Charts on the left refer to image analysis technique, while the right side refer to traditional technique.

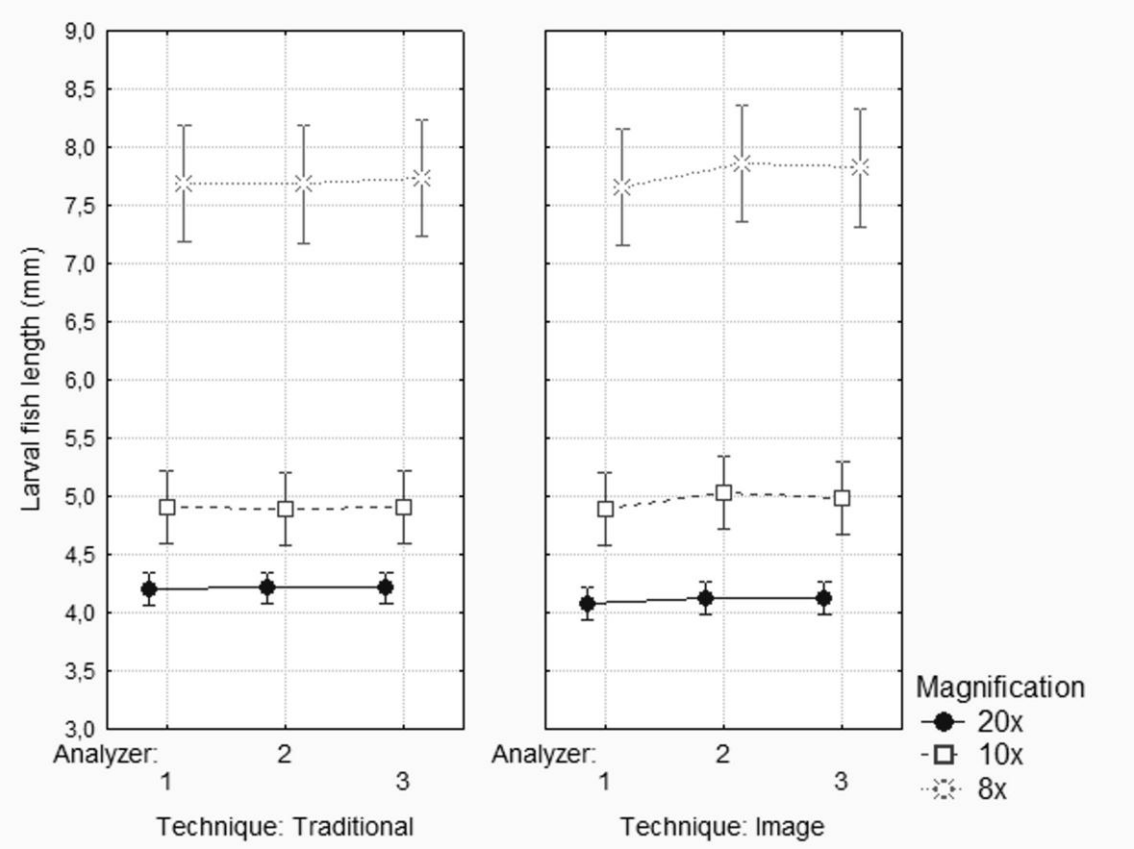

Fig. 3: Average measurement of larval fishes standard length (mm), with the 95 % confidence interval, obtained for each analyzers in each magnification (8x, 10x and 20x) between each technique (traditional and image) used in this study.

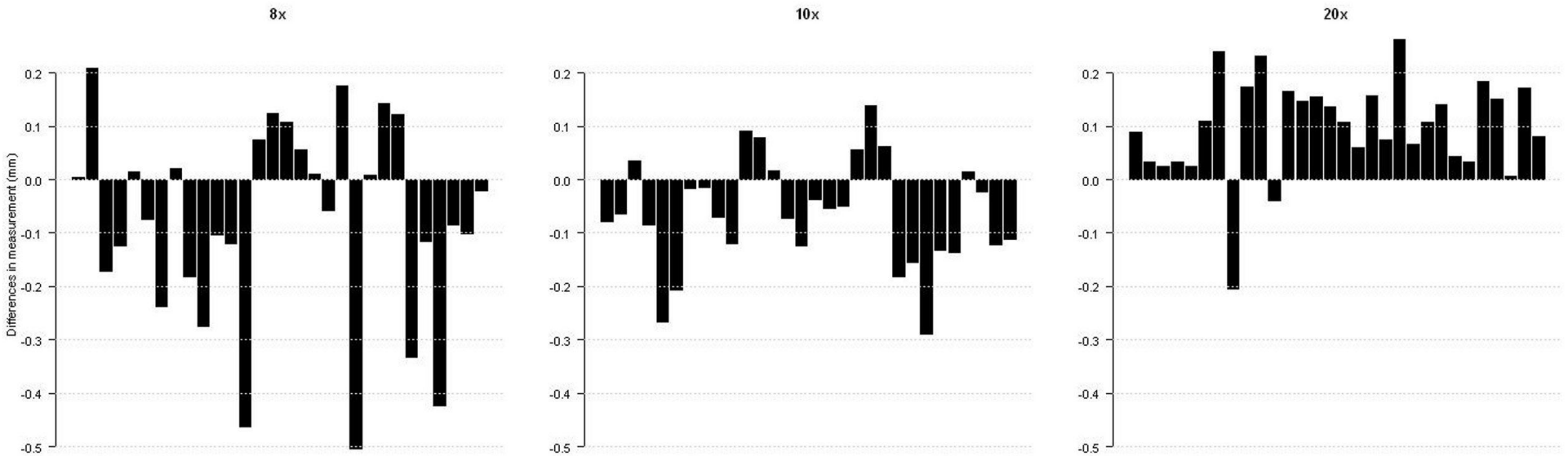

Fig. 4: Differences in measurement between traditional and image analysis technique for all 90 larvae. Positive values indicate the larger length of fish larvae when measured by traditional technique, while the negative value indicates larger length of the fish larvae when measured by image analysis technique.

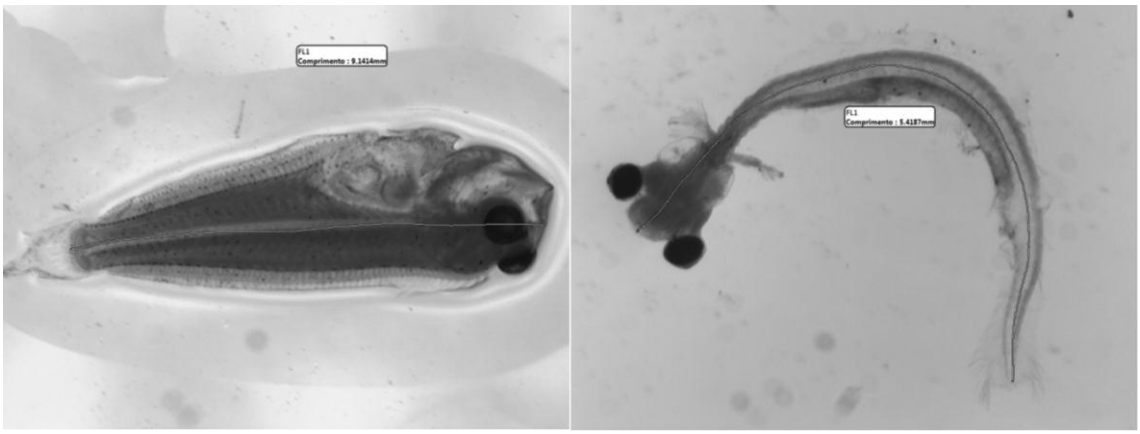

Fig. 5: Digital images for larval fishes (Pleuronectiform and Clupeiform) taken during this study. The lines along the larvae give their length, and the positioning of the larvae did not compromise their measurements in these cases.

DISCUSSION

There are no significant differences between measurements taken by traditional and image analysis technique. The coefficient of variation suggests a similar dispersion of data between techniques, which strengthens the existence of a single average result for the measurements. However, results reveal that the image analysis technique allows for greater precision for measuring larval fish size when compared to the traditional technique.

The small differences observed in the probability of significant differences for analyzers in the ANOVA between the techniques reflect an alteration in the accuracy of each technique. Requirement for greater precision can lead to an increase of variability in results, which although not significant, can generate a lower accuracy when the

techniques are compared. It is assumed that this occurred in the study with the 90 organisms measured.

Care should be taken at the time of calibration to produce valid measurements. As seen in the image analysis technique, a difference between the vertical and horizontal pixel size can lead to false/incorrect measurements.

There are advantages and disadvantages in the use images to obtain measurements in organisms. Main advantages are the precision of the technique, the storage of photographic records that occupied less space than samples, less manipulation of organisms, the agility that influences directly in the time needed to obtain data, in ergonomics for providing from the researchers less exposure to the microscope and the possibility of merging with less stressful environment of the interactive panel. The disadvantages of this method are related to the need for extra equipments to capture and process images, which raise the cost of the experiment, and, the 2D view plan of the image (Fig. 5). A 2D image serves only as a record of the organism's position, which does not allow for the manipulation of the organism in the picture. This is important for moving or rotating the organism, and adjusting the focus and direction of light incidence in some parts of the organism.

Major advantages of the traditional technique are the possibility of manipulation/rotation of the organism, control of brightness, light incidence, magnification and focus that can be adjusted during the measurement. Also, there are no extra financial expenditure on equipment acquisition for processing and analyzing images. Its disadvantages are highlighted by the lost of agility, the excessive handling that may cause damage to the organism, and the time spent with a microscope and with poor posture (ergonomics).

Some researchers emphasize the agility and facilities provided by image methods. Tang *et al.* (1998) comment that the Video Plankton Recorder (VPR) technique facilitates the research, because with videos is possible to obtain increased information in larger-scale sampling, without increasing the amount of sampling storage space during a research cruise, since space be a limiting factor. Martínez-Palacios *et al.* (2002) highlights the ease of working with video to avoid manipulation and consequently high mortality in studies involving fish growth. The facility to store the organism's samples in digital images (records), and the possibility to work with a larger amount of information in future studies make the image method very attractive.

Improvement of the methodologies used to measure organisms structures, making them faster and more precise than traditional methods can encourage some study areas to use these results. For example, modeling studies of transport of eggs and fish larvae (Baumann *et al.*, 2003; Santos *et al.*, 2005), could readily incorporate measurements of fish larvae, and gain an important data to verify, calibrate and confirm model results.

Interesting studies can be performed to improve the time/precision to get results for ichthyoplankton studies. Among them, the studies related to the patterns recognition in images of eggs and fish larvae by processing image software, and with the use of a scanner to obtain image of several eggs and fish larvae for post-processing, which can maximize the agility to get results faster. Similar studies are conducted with phytoplankton and zooplankton, using specific equipments, such as FlowCAM (Sieracki *et al.*, 1998; Ide *et al.*, 2008) and Zooscan (Gorsky *et al.*, 2003; Grosjean *et al.*, 2004).

CONCLUSIONS

This work leads us to conclude that there are no differences between traditional and image analysis technique when used to measure fish larvae.

The use of digital images coupled with the interactive panel can facilitate the research that requires agility and/or precision.

The major advantage of the traditional technique is the lower financial costs and the possibility to manipulate fish larvae and the microscope while getting results.

ACKNOWLEDGMENTS

Thanks: Vanessa Schmitt and NG Haig They helped in carrying out this work; the director of the Núcleo de Tecnologia da Informação (NTI-FURG) loaned the equipment needed; EM benefited from a scholarship from the Coordenação de Aperfeiçoamento de Pessoas de Nível Superior (CAPES), and JHM from a CNPq grant (Proc. 309181/2009-7).

LITERATURE CITED

Almesjö, L. & C. Rolff. 2007. Automated measurements of filamentous cyanobacteria by digital image analysis. ***Limnology and Oceanography: Methods***, 5: 217-224.

Baumann, H.; P. Pepin; F. J. M. Davidson; F. Mowbray; D. Schnack & J. F. Dower. 2003. Reconstruction of environmental histories to investigate patterns of larval

radiated shanny (*Ulvaria subbifurcata*) growth and selective survival in a large bay of Newfoundland. ***ICES Journal of Marine Science***, 60: 243-258. doi:10.1016/S1054-3139(03)00019-5.

Deegan, L. A. 1990. Effects of estuarine environmental conditions on population dynamics of young-of-the-year gulf menhaden. ***Marine Ecology-Progress Series***, 68: 195-205.

Fox, C. J. 1996. Length changes in herring *(Clupea harengus)* larvae: effects of capture and storage in formaldehyde and alcohol. ***Journal of Plankton Research***, 18(4): 483-493.

Gonzalez, R. C. & R. E. Woods. 1993. Digital Image Processing. New York. ***Addison-Wesley Publications***. 509p.

Gorsky, G. & P. Grosjean. 2003. Qualitative and quantitative assessment of zooplankton samples. ***GLOBEC International Newsletter***, 9(1): 5.

Grosjean P.; M. Picheral; C. Warembourg & G. Gorsky. 2004. Enumeration, measurement, and identification of net zooplankton samples using the ZOOSCAN digital imaging system. ***ICES Journal of Marine Science***, 61: 518-525. doi:10.1016/j.icesjms.2004.03.012.

Houde, E. 2008. Emerging from Hjort's Shadow. ***Journal of Northwest Atlantic Fishery Science***, 41: 53-70.

Hunter, J. R. 1984. Feeding Ecology and Predation of Marine Fish Larvae, *in*: *Lasker, R. Marine Fish Larvae*. ***Washington Sea Grant***. 131p.

Ide, K.; K. Takahashi; A. Kuwata; M. Nakamachi & H. Saito. 2008. A rapid analysis of copepod feeding using FlowCAM. ***Journal of Plankton Research***, 30(3): 275-281.

Martínez-Palacios, C. A.; E. B. Tovar; J. F. Taylor; G. R. Durán & L. G. Ross. 2002. Effects of temperature on growth and survival of *Chirostoma estor estor*, Jordan 1879, monitored using a simple video technique for remote measurement of length and mass of larval and juvenile fishes. ***Aquaculture***, 209: 369-377.

Monico, J. F. G.; A. P. Dal Póz; M. Galo; M. C. dos Santos & L. C de Oliveira. 2009. Acurácia e precisão: revendo os conceitos de forma acurada. ***Boletim de Ciências Geodésicas***, 15(3): 469-483.

Moser, H. G. (eds.). 1996. The Early Stages of Fishes in the California Current Region. ***CalCOFI Atlas 33***. 1505p.

Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life-history stages of marine fish. ***Canadian Journal of Fisheries and Aquatic Sciences***, 48(3): 503-518.

Pepin, P.; J. F. Dower & W. C. Leggett. 1998. Changes in the probability density function of larval fish body length following preservation. ***Fishery Bulletin***, 96(3): 633-640.

R Development Core Team, 2006. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0.

Richards, WJ. 2006. Early Stages of Atlantic Fishes: an Identification Guide for the Western Central North Atlantic. II Volume Set. Boca Raton. ***CRC Press***. 2640p.

Rønnestad, I.; A. Thorsen & R. N. Finn. 1999. Fish larval nutrition: a review of recent advances in the roles of amino acids. ***Aquaculture***, 177: 201-216.

Santos, A. J. P.; J. Nogueira & H. Martins. 2005. Survival of sardine larvae off the Atlantic Portuguese coast: a preliminary numerical study. ***ICES Journal of Marine Science***, 62: 634-644. doi:10.1016/l.icesjms.2005.02.007.

Santos, J. N. S., F. G. Araújo & D. S. Silva. 2009. Length correction for early-juvenile Brazilian herring *Sardinella janeiro* (Eigenmann, 1894) after preservation in formalin, ethanol and freezing. ***Neotropical Ichthyology***, 7(1): 87-92.

Sieracki, C. K.; M. E. Sieracki & C.S Yentsch. 1998. An imaging-flow system for automated analysis of marine microplankton. ***Marine Ecology-Progress Series***, 168: 285-296.

Smith, P. E. & S. L. Richardson. 1977. Standard techniques for pelagic fish eggs and larva surveys. ***FAO Fisheries Technical Paper***, 175: 1-100.

Staciarini, F. 2004. Processamento de imagem digital aplicado à análise de otólitos do peixe-rei marinho Odontesthes argentinensis (Valenciennes, 1835). ***Graduation monograph (Oceanology)***. Fundação Universidade do Rio Grande (FURG), Rio Grande. 26p.

Tang, X.; W. K. Steward; L. Vincent; H. Huang; M. Marra; S. M. Gallager & C. S. Davis. 1998. Automatic Plankton Image Recognition. ***Artificial Intelligence Review***, 12: 177-199.

6.2. Apêndice 2 – MALANSKI, E & MUELBERT. *Submetido* b. Size Changes in Argentine menhaden Larvae, *Brevoortia pectinata*, Due to Preservation. 14 pp. (*manuscrito*)

Obs.: Artigo científico submetido para *ICES Journal of Marine Science.*

SIZE CHANGES IN ARGENTINE MENHADEN LARVAE, *Brevoortia pectinata*, DUE TO PRESERVATION

Malanski, E.[1]; Muelbert, J. H.

Programa de Pós-Graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG

[1] (e-mail: evandromalanski@furg.br)

ABSTRACT

The goal of this study was to investigate the effect of two principal types of preservatives utilized in larval fish research (3.6 % formaldehyde and 70 % alcohol) in *B. pectinata* larvae. One hundred and ninety four larvae were collected during oceanographic cruises in the Patos Lagoon estuary. Larvae were measured fresh and changes in standard length (SL) followed for 90 days. Larvae preserved with formaldehyde measured from 3.70 to 11.54 mm SL (n=107), while larvae in alcohol ranged from 4.69 to 16.56 mm SL (n=87). A significant shrinkage was observed for both preservatives (ANOVA, $p<0,000$). Significant changes in length occurred until 30 days after preservation with the use formaldehyde, and until 15 days for alcohol. Smaller larvae had smaller size changes than that of larger fish larvae (proportionally to the standard length), differing to other studies. The correction factor calculated for *B. pectinata* larvae in formaldehyde was $SL_{live} = 1.0799 \times SL_{post\text{-}preservation}$, and in alcohol was $SL_{live} = 1.1415 \times SL_{post\text{-}preservation}$.

RESUMO

O objetivo deste estudo foi investigar o efeito dos dois principais tipos de conservantes utilizados em pesquisas com larvas de peixes (formaldeído 3,6 % e álcool 70 %) em larvas de *B. pectinata*. As cento e noventa e quatro larvas utilizadas neste estudo foram capturadas durante cruzeiros oceanográficos realizados no estuário da Lagoa dos Patos. O comprimento padrão (CP) das larvas foi medido logo após a coleta e seguido durante 90 dias. Larvas preservadas em formaldeído mediram de 3,70 a 11,54 mm CP (n=107), enquanto que em álcool variaram de 4,69 a 16,56 mm CP (n=87). Foi observado um encolhimento significativo para ambos os conservantes (ANOVA, $p<0,000$). A modificação de tamanho ocorreu significativamente até 30 dias pós-conservação com o uso de formaldeído, e até 15 dias para o álcool. As larvas de peixes menores tiveram as menores modificações de tamanho quando comparadas com as larvas maiores (proporcionalmente ao comprimento padrão), diferindo de outros estudos. O fator de correção calculado para as larvas de *B. pectinata* em formaldeído foi $CP_{vivo} = 1,0799 \times CP_{pós\text{-}conservação}$, e em álcool foi $CP_{vivo} = 1,1415 \times CP_{pós\text{-}conservação}$.

Keywords: Clupeidae, preservative effects, correction factor.

INTRODUCTION

The length of fish larvae is used in several studies in aquaculture (Martínez-Palacios *et al.*, 2002), fishery biology (Warlen *et al.*, 2002), ecology (Deegan, 1990) and organisms

transport modelling (Baumann *et al.*, 2003). However, due to logistics and sampling constraints, size is normally not obtained when larval fish is alive, and the larvae are kept in preservative for later analysis.

One of the main causes that affect the precision and accuracy in the knowledge of fish length is shrinkage of body structures with the use of preservatives (Theilacker, 1980; Hjörleifsson *et al.*, 1992), which can lead to errors and/or inconsistencies in studies that use this data. Therefore, many studies aim to estimate larval length in life from larval length after preservation (Pepin *et al.*, 1998; Fey *et al.*, 2005; Santos *et al.*, 2009).

This study will investigate the size changes in larval fish Argentine menhaden *Brevoortia pectinata*, an important clupeid found in coastal waters of Southwestern Atlantic Ocean (Muelbert *et al.*, 1991), and thus obtain an equation to correct the effect of preservatives for this species.

MATERIALS AND METHODS

Field collection:

A conical-cylinder plankton net with 500 µm mesh and 60 cm diameter was utilized to collect *Brevoortia pectinata* larvae during oceanographic cruises. Oblique hauls were adopted to collect the highest number possible of *B. pectinata* larvae. Trawl duration were not standardized (t= 7 s ± 3).

Immediately after trawling, fish larvae were screened and separated from the plankton with the use a pipette, to avoid predation and/or damage to body structures. Larvae were

stored in a bottle that was kept cold in an insulated pack with the intention to decrease metabolism and thus the stress of the larvae until the arrival at the laboratory.

Laboratory:

Prior to the preservation of each fish larvae, their images were captured with a digital camera Motic (model Moticam2300) connected to the computer, mounted on a stereoscopic microscope Opton, model TNE-10TN. We adopted this procedure to avoid manipulation of the larvae, and consequent damage to body structures. Fish larvae were only manipulated with a pipette, with which larvae were captured and placed on a histological slide under the microscope. The picture of each whole larvae was captured with the maximum magnification possible in the stereomicroscope to obtain higher precision during measurement. This same procedure was repeated after 5, 10, 15, 30, 60 and 90 days of storage. In order to maintain precision, the magnification was kept the same as used in the first measurement for each larvae throughout the study.

After larvae had their pictures taken, they were stored in numbered bottles with preservative, and recorded the number of bottle, type of preservative used, the day of conservation, and the number of digital photography.

The preservatives used in this experiment were 3.6% formaldehyde in seawater (diluted to 10% of its concentration), buffered with sodium tetraborate, and 70% ethyl alcohol in distilled water (diluted to 76% of its concentration; used 92.8° hydrated ethyl alcohol), not buffered. These are the most common preservatives used in studies with larval fish; the first in more general studies, while the second is used in more specific studies.

The standard length (SL) of each fish larvae was obtained following the procedures of image analysis technique described in Malanski & Muelbert (*Submt*. a). Before

preservation, larvae were measured in seawater, and after preservation, measurements were conducted with the preservative solution. Thus, larvae were categorized by class length in life (*e.g.* 2 mm ≤ x <4 mm), and type of preservative. There was not a prior selection of larval size, but this experiment aimed to cover the largest number of larval length categories for each preservative.

The size change (SC, see equation [1]) was quantified for each larval length category, and its significance evaluated by analysis of variance (ANOVA) for repeated measures and paired *t*-test when necessary (*post hoc*) of each *i*-larvae over the *j*-storage days in each preservative category, and between each *i*-larvae in each preservative, adopting for this the SC_{90} values (time that changes stabilized). Positive values in the formula indicate an increase in larval size, while negative values indicate their shrinkage.

[1] $$SC_j\ (\%) = \frac{SL_{i,j} - SL_{i,0}}{SL_{i,0}} \times 100$$

We performed a linear regression between larval length in life ($SL_{i,0}$) and larval length post-preservation ($SL_{i,90}$). An equation was obtained to estimate the larval length in life from its post-preservation length, and the significance of the parameters of the equation was evaluated.

Analysis were adapted from statistical routines using R software (R Development Core Team, 2006) from several sources available on the Internet.

RESULTS

One hundred and ninety four fish larvae of *B. pectinata* were captured in the Patos Lagoon estuary during this study. Of these, 107 larvae were preserved with formaldehyde and 87 larvae with alcohol. Larvae preserved with formaldehyde ranged from 3.70 to 11.54 mm SL, while larvae preserved in alcohol ranged from 4.69 to 16.56 mm SL (Fig. 1).

Larval size significantly decreased during this study (ANOVA $_{formaldehyde}$: $F= 46.26$; $df= 6$; $p< 0.000$. ANOVA $_{alcohol}$: $F= 54.90$; $df= 6$; $p< 0.000$). Shortly after 5 days of storage a significant difference for both preservatives (*t*-test; $df_{formaldehyde}= 106$; $df_{alcohol}= 86$; $p< 0.05$) was observed. With respect to formaldehyde, the average shrinkage after 5 days of storage was 5.05 % ± 2.90, and remained not significant from 5 to 10, and 10 to 15 days of storage (*t*-test; $p> 0.05$). Significant shrinking occurred from 15 to 30 days of storage, and no significant difference were observed between 30 and 90 days of storage, where in this latter the average shrinkage was 6.66 % ± 3.07. It was not considered the size change after 60 days of storage to get this conclusion, because these data were differentiated enough from the trend occurred in this experiment (Fig. 2).

With respect to alcohol, the average shrinkage after 5 days of storage was 9.31 % ± 4.42, and the size change in larval length remained significant until 15 days of storage (*t*-test; $p< 0.05$). After 15 days no significant difference was observed in size change (*t*-test; $p> 0.05$), and the average shrinkage of 90 days of storage was 11.03 % ± 7.64. The size change after 60 days of storage was not considered to get this conclusion, since data at 60 days presented a different trend than that observed in the remainder of the experiment (Fig. 2).

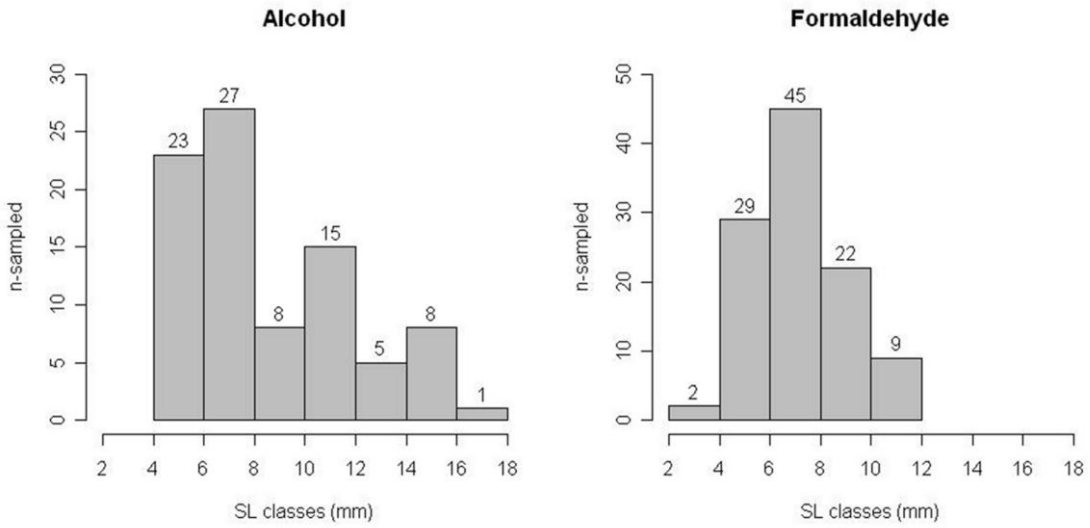

Fig. 1: Distribution of length classes (mm) of *B. pectinata* larvae evaluated for size changes in experiments with 70 % alcohol and 3.6 % formaldehyde as a preservative.

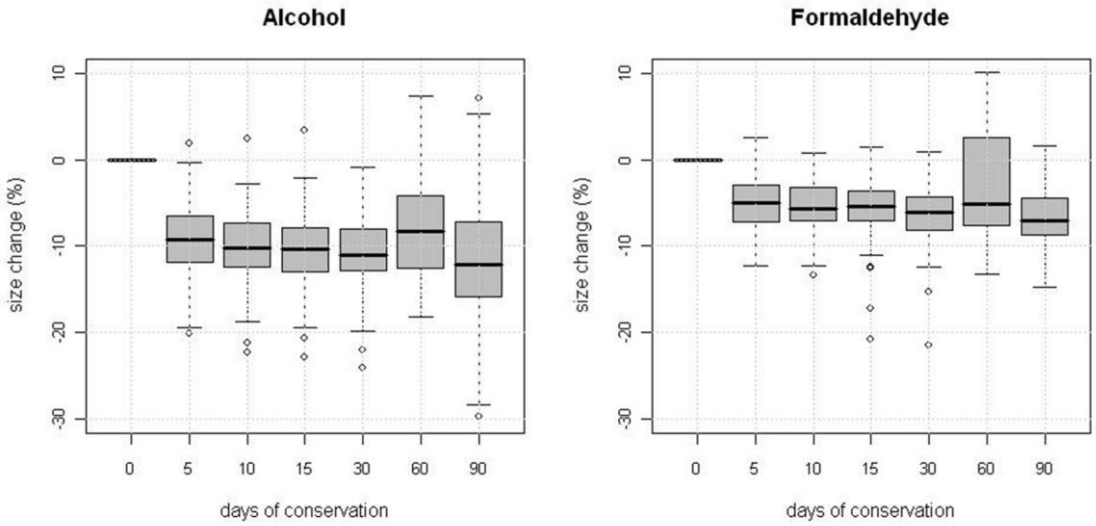

Fig. 2: Size change (%) during the time (days of storage) for *B. pectinata* larvae utilized in experiments with 70 % alcohol and 3.6 % formaldehyde. Box-plots indicate minimum and maximum values (inferior and superior line), median (intermediate black line), and inferior and superior quartiles (gray retangle). Atypical values (outliers) are indicated by "◦".

With the use of formaldehyde, there was no significant difference in size change for the smallest and largest *B. pectinata* larvae (*t*-test for H_0: slope= 0, p= 0.07) (Fig. 3). But, a size effect between larval length in life and in post- preservation was observed (Fig. 4) (regression slope= 1.0799, SE= 0.0129; H_0: slope= 1, *t*-test of regression slope, p< 0.001). However, the *y*-intercept of this regression was not significantly different from zero (regression intercept= –0.0464, SE= 0.80896; H_0: *y*-intercept= 0, *t*-test of regression intercept, p= 0.60). Thus, only the value associated with the slope of the regression can be used as a correction factor of SC for using formaldehyde, according to equation [2].

With the use of alcohol, it is observed that smaller *B. pectinata* larvae shrank proportionally less than that larger (*t*-test for H_0: slope= 0, p< 0.05) (Fig. 3). The size effect between larval length in life and in post-preservation was observed (Fig. 4) (regression slope= 1.1415, SE= 0.0311; H_0: slope=1, *t*-test of regression slope, p< 0.001). However, the *y*-intercept of this regression was not significantly different from zero (regression intercept= –0.0574, SE= 0.2476; H_0: *y*-intercept=0, *t*-test of the regression intercept, p= 0.82). Thus, only the value associated with the slope of the regression can be used as a correction factor of SC for using alcohol, according to equation [3].

[2] $$SL_{live} = 1.0799 \times SL_{post\text{-}preservation}$$

[3] $$SL_{live} = 1.1415 \times SL_{post\text{-}preservation}$$

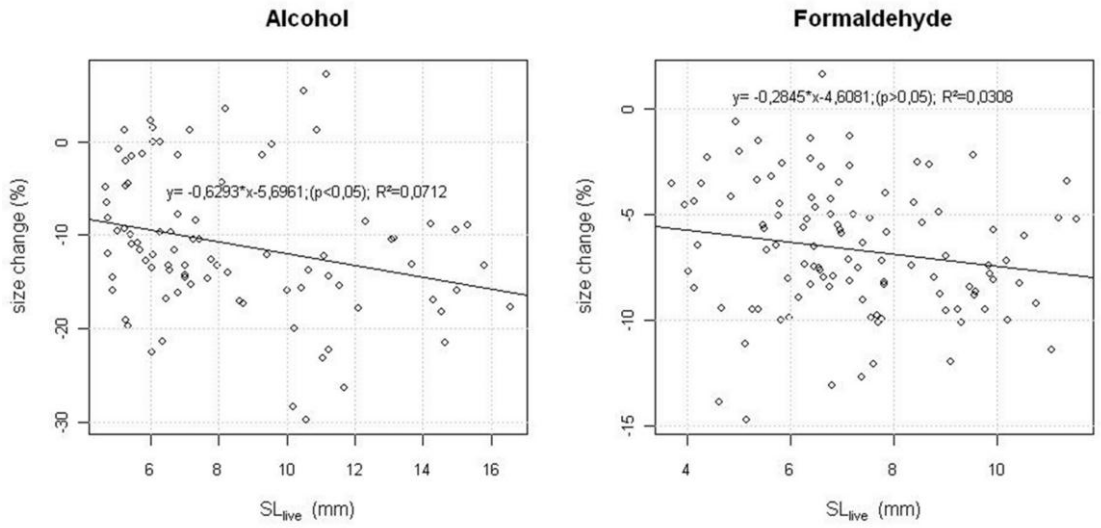

Fig. 3: Size change (%) for each *B. pectinata* larvae in experiments with 70 % alcohol and 3.6 % formaldehyde as a preservative.

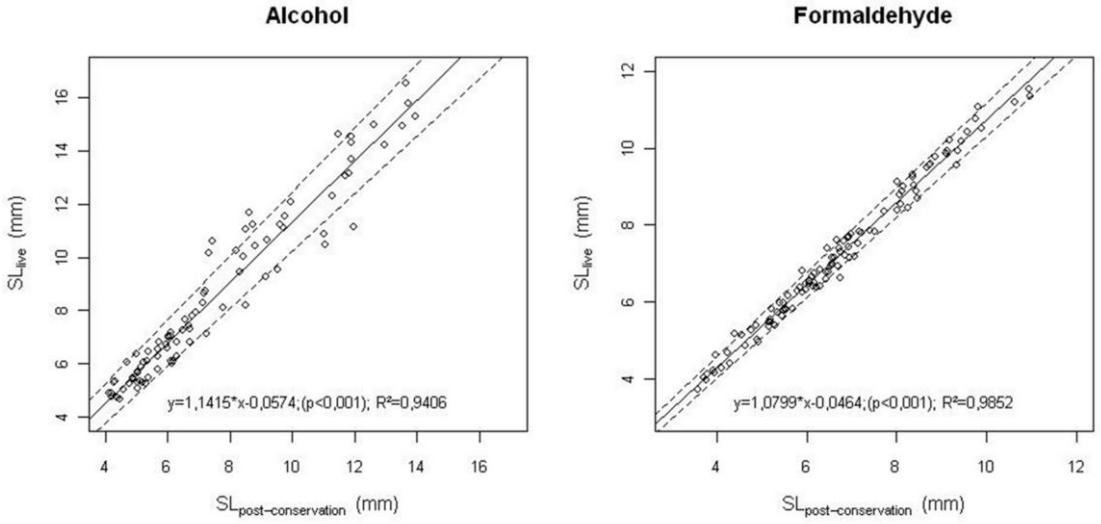

Fig. 4: Linear regression between *B. pectinata* larval length (mm) in life and in post-preservation, with the 95 % confidence interval for the parameters of the regression, in experiments with 70 % alcohol and 3.6 % formaldehyde as a preservative.

DISCUSSION

This work produced important results for studies involving size correction of preserved *Brevoortia pectinata* larvae, an important and frequent clupeid found in the coastal area of the Southwestern Atlantic Ocean. The data indicated a tendency to shrinkage in all size classes of larvae (ranging between 3.70 and 16.56 mm). Shrinkage occurred in less developed larvae (newly hatched) as well as in developed larvae (post-flexion stage), and also independent of the preservative used (70 % alcohol or 3.6 % formaldehyde).

It was found that the size change of larval fish of this experiment occurred significantly until 30 days of storage for formaldehyde, and the shrinkage after 5 days of storage was approximately 76 % of the total observed (90 days), a result that is corroborated by the study of other clupeid (Fox, 1996). On the other hand, shrinkage was significant until 15 days of storage when using alcohol as a preservative, and with 5 days of storage the shrinkage corresponded approximately 84 % of the total observed, result that was not similar when compared with the result obtained by Fox (*op. cit.*) (shrinkage significant until 30 days of storage). Fey *et al.* (2005) observed that with the use of 95 % alcohol, size change was significant between 0 to 3 days of storage, and not significant between 3 to 20 and 20 to 90 days of storage.

Larger larvae presented the greatest size changes, both with use alcohol or formaldehyde as a preservative (Fig. 3). This is the opposite of previous results that showed the greatest size changes occurred in small organisms (Fey *et al.* 2005; Santos *et al.*, 2009). However, these studies assessed organisms ranging from 19.1 to 31.4 mm and from 15.07 to 28.11 mm, respectively. Considering that the largest larvae from the present study had 11.54 mm (formaldehyde) and 16.56 mm (alcohol), it can be inferred

that a maximum size change (asymptote of the SC considering the fish length) occurs close to the post-larvae stage, while smaller or larger sizes of larvae have smaller size changes.

At 60 days of storage, larval length increased instead of shrinking, a result also encountered by Fox (1996). This time interval coincides with mid austral summer, a period when the air temperature in the laboratory was higher than usual. This coincidence suggests that higher temperatures could result in an increase in preserved larval size. However, further studies are needed to confirm this hypothesis. The 60 days storage length data were presented, but were not used for analysis and conclusions presented in the study, a procedure also adopted by Fox (*op. cit.*).

The correction factors of larval length obtained with the linear regressions performed in this study will benefit future studies, particularly those that involve the early stages of Argentine menhaden in South Atlantic, similar to that conducted by Warlen *et al.* (2002) and Malanski (*Submt*. b) who utilized the larval length after storage corrected by this approach.

ACKNOWLEDGMENTS

We are thankfull to the Laboratório de Ecologia do Ictioplâncton (LEI-IO-FURG) and to the Núcleo de Tecnologia da Informação (NTI-FURG) that loaned the equipment needed; EM benefited from a scholarship from the Coordenação de Aperfeiçoamento de Pessoas de Nível Superior (CAPES), and JHM from a CNPq grant (Proc. 309181/2009-7).

LITERATURE CITED

Baumann, H.; P. Pepin; F. J. M. Davidson; F. Mowbray; D. Schnack & J. F. Dower. 2003. Reconstruction of environmental histories to investigate patterns of larval radiated shanny (*Ulvaria subbifurcata*) growth and selective survival in a large bay of Newfoundland. ***ICES Journal of Marine Science***, 60: 243-258. doi:10.1016/S1054-3139(03)00019-5.

Deegan, L. A. 1990. Effects of estuarine environmental conditions on population dynamics of young-of-the-year gulf menhaden. ***Marine Ecology Progress Series***, 68: 195-205.

Fey, D. P. & J. A. Hare. 2005. Length correction for larval and early-juvenile Atlantic menhaden (*Brevoortia tyrannus*) after preservation in alcohol. ***Fishery Bulletin***, 103(4): 725–727.

Fox, C. J. 1996. Length changes in herring (Clupea harengus) larvae: effects of capture and storage in formaldehyde and alcohol. ***Journal of Plankton Research***, 18(4): 483-493.

Hjörleifsson, E. & G. Klein-MacPhee. 1992. Estimation of live standard length of winter flounder *Pleuronectes americanus* larvae from formalin-preserved, ethanol-preserved and frozen specimens. ***Marine Ecology Progress Series***, 82: 13-19.

Malanski, E. & J. H. Muelbert (*Submmited* a). Measuring Small Organisms: Evaluation of Traditional and Image Methods for Larval Fishes. *Limn. Oceanogr. Methods*.

Malanski, E. & J. H. Muelbert (*Submmited* b). Life History Patterns of Early Stages of Argentine menhaden, *Brevoortia pectinata* (Jenyns, 1842), in a Subtropical Estuary of Southwestern Atlantic Ocean. *PLoS ONE*.

Muelbert, J. H. & G. Weiss. 1991. Abundance and distribution of fish larvae in the channel area of the Patos Lagoon estuary, Brazil. Pp. 43-54. In: Hoyt, R. D. (Ed.). Larval fish recruitment in the Americas. Proceedings of the thirteenth annual fish conference. Merida, Mexico, ***NOAA Technical Report NMFS***, 95, 137p.

Martínez-Palacios, C. A.; E. B. Tovar; J. F. Taylor; G. R. Durán & L. G. Ross. 2002. Effects of temperature on growth and survival of *Chirostoma estor estor*, Jordan 1879, monitored using a simple video technique for remote measurement of length and mass of larval and juvenile fishes. ***Aquaculture***, 209: 369-377.

Pepin, P.; J. F. Dower & W. C. Leggett. 1998. Changes in the probability density function of larval fish body length following preservation. ***Fishery Bulletin***, 96(3): 633-640.

R Development Core Team, 2006. ***A Language and Environment for Statistical Computing. R Foundation for Statistical Computing***. Vienna, Austria. ISBN 3-900051-07-0.

Santos, J. N. S.; F. G. Araújo & D. S. Silva. 2009. Length correction for early-juvenile Brazilian herring *Sardinella janeiro* (Eigenmann, 1894) after preservation in formalin, ethanol and freezing. ***Neotropical Ichthyology***, 7(1): 87-92.

Theilacker, G. H. 1980. Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. ***Fishery Bulletin***, 78: 685-692.

Warlen, S. M.; K. W. Able & E. H. Laban. 2002. Recruitment of larval Atlantic menhaden (*Brevoortia tyrannus*) to North Carolina and New Jersey estuaries: evidence for larval transport northward along the east coast of the United States. ***Fishery Bulletin***, 100: 609-623.

6.3. Apêndice 3 – MALANSKI, E & MUELBERT. *Submetido* c. Life History Patterns of Early Stages of Argentine menhaden *Brevoortia pectinata* (Jenyns 1842) in a Subtropical Estuary of Southwestern Atlantic Ocean. 27 pp. (*manuscrito*)

Obs.: Artigo científico submetido para *PLoS ONE*.

LIFE HISTORY PATTERNS OF EARLY STAGES OF ARGENTINE MENHADEN, *Brevoortia pectinata* (JENYNS, 1842), IN A SUBTROPICAL ESTUARY OF SOUTHWESTERN ATLANTIC OCEAN

Malanski, E.[1]; Muelbert, J. H.

Programa de Pós-Graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG

[1] (e-mail: evandromalanski@furg.br)

ABSTRACT

Patterns associated to environmental conditions and to the spatial and temporal distributions of the early stages of Argentine menhaden, *B. pectinata*, and of its development in the Patos Lagoon estuary were investigated using historical records. From 1975 to 2009 a total of 10479 eggs and 14066 fish larvae and juveniles were collected at plankton surveys. Larvae were measured and classified according to their developmental stage. Each development stage was characterized by its size range, which the smallest yolk-sac larvae measured 2.16 mm, and the largest juveniles had 43.25 mm. Analysis of the distributional pattern indicated spawning outside the Patos Lagoon, where the most abundant seasons for eggs were winter, spring and early summer, and transport to the inner estuarine region in the earlier developmental stages. Distribution of juveniles indicated a return to the coastal region, and in this stage the fall was a very important period. These results are important for the knowledge for this species, and to be useful for manage this resource.

RESUMO

Os padrões associados às condições ambientais e a distribuição espaço-temporal dos estágios iniciais da savelha, *B. pectinata*, e de seu desenvolvimento no estuário da Lagoa dos Patos foram investigados através de dados históricos. No período de 1975 a 2009, um total de 10479 ovos e 14066 larvas e juvenis foram coletados através de amostragens planctônicas. As larvas foram medidas e classificadas de acordo com seu estágio de desenvolvimento. Cada estágio de desenvolvimento foi caracterizado pelo seu intervalo de tamanho, onde a menor larva em estágio vitelínico mediu 2,16 mm, e o maior juvenil teve 43,25 mm. As análises dos padrões de distribuição indicou desova fora da Lagoa dos Patos, onde os mais abundantes períodos sazonais para os ovos foram no inverno, primavera e início de verão, com um transporte para dentro da região estuarina nos estágios mais iniciais de desenvolvimento. A distribuição dos juvenis indicou um retorno para a região costeira, e neste estágio o outono foi um período muito importante. Estes resultados são importantes para o conhecimento desta espécie, e serão úteis para o gerenciamento deste recurso.

Keywords: *Brevoortia*, estuary, environmental conditions, Patos Lagoon.

INTRODUCTION

Clupeidae, represented by herrings, sardines and menhadens, are considered fishes of high ecological and economic importance despite the small size and short life period

(Whitehead, 1985; Pauly, 2007). Clupeiforms (Clupeidae and Engraulidae) account for the largest catches worldwide (FAO, 2009), and it is used both for human consumption as bait for other fisheries (Fischer *et al.*, 2004; Santos & Rodrigues-Ribeiro, 2000). Some species of clupeids are not directly used for human consumption, but there are an interest of their lipids (to produce fish oil), specially because the high omega-3 fatty acids in this phytoplanktivorous species (*e.g. Brevoortia* genus) (Visentainer *et al.*, 2007) All this importances encouraged many economic and ecological studies directed for their fishing (FAO, *op. cit.*), distribution school areas, reproductive processes and recruitment, trophic relationships, and finally their fishery management.

Previous studies of ichthyoplankton in the Patos Lagoon estuary – PLE (Weiss & Krug, 1977; Weiss, 1981; Muelbert & Weiss, 1991) showed that a clupeid, Argentine menhaden (*B. pectinata*), is among the most abundant larval fish species found in this region, and with two other species (*M. furnieri* and *L. grossidens*) account for 88 % of egg abundance and 66 % of fish larvae sampled of the PLE (Sinque & Muelbert, 1998). Loebmann *et al.* (2001) found that *B. pectinata* is the most abundant species of Clupeidae in the PLE ichthyofauna. Fischer *et al.* (2004) pointed out that this pelagic species are found in shoals close to shore in surface waters, and their juveniles are commonly found in estuaries. Other adult clupeids, such as *H. clupeola*, *P. harroweri*, *P. platana* and *R. arcuata,* are also presented in the PLE (Fischer *et al.*, *op. cit.*).

Brevoortia is known to use estuaries as a nursery/breeding ground, so this habitat is essential to maintain of their populations. Nelson *et al.* (1977) emphasized the importance of transport of eggs and fish larvae of Atlantic menhaden to estuaries on the U.S. East coast. Deegan (1990) discussed the use of estuaries as nursery/creation area of the early stages of life of Gulf menhaden (*B. patronus*) in Louisiana, and after

Stegmann *et al.* (1999) defined potential spawning areas for Atlantic menhaden (*B. tyrannus*), but now treating this fish as estuarine-dependent (*i.e.* at least one stage of your life cycle is going into the estuary). In the South Atlantic, *B. aurea* eggs were found mainly in shallow estuarine waters (depths <10 m), and with salinity values ranging from 10 to 20 (Acha & Macchi, 2000), indicating estuarine spawning that differs from other species of the same genus.

The geographical distribution of *B. pectinata* is apparently between south Brazil (Rio Grande do Sul) and northwestern Argentina (Bahia Blanca), whereas *B. aurea* inhabit northestern Brazil (Bahia) to the mouth of Rio de la Plata estuary (Uruguay/Argentina) (Whitehead, 1985; Garcia *et al.* 2008).

This coexistence area, and the similarity between different species of clupeids has caused debate in the literature. Cousseau & Díaz de Astarloa (1993) studied the genus *Brevoortia* in the Southwest Atlantic coast and concluded these two species that coexist between Rio Grande do Sul (Brazil) and Bahia Blanca (Argentina) are the same species. Genetic studies of the genus *Brevoortia* revealed that only *B. aurea* occurs in the Uruguayan coast, Southwest Atlantic Ocean (Garcia *et al.* 2008). Cousseau & Perrotta (2009) commented the similarity of the early stages of *B. aurea*, synonym of *B. pectinata*, with *R. arcuata*.

The mouth of the Patos Lagoon is characterized by an estuary with approximately 1000 km² (Odebrecht & Seeliger, 2010). The estuary is as important environment for the early life stages of several fish species because it provides abundant supply of food, protection from predators (Sinque & Muelbert, 1998), their variability of habitats, and the nature of its environment. It forms an interesting and dynamic estuary, created by the encounter of continental and oceanic waters which contribute to the sporadic

presence of freshwater and many marine species (Sinque & Muelbert, 1998; Odebrecht & Seeliger, *op. cit.*).

The Patos Lagoon estuary (PLE) is dominated by hydrological cycles of its drainage basin, and influenced by oceanographic and meteorological processes. Wind and rain are identified as the major factors forcing the circulation, distribution of salinity and water levels in the system of the lagoon (Möller *et al.*, 2009, Möller & Fernandes, 2010). High variability of daily or weekly chlorophyll-*a*, and also annual and interannual variations, are mainly influenced by wind (Odebrecht & Abreu, 1998; Abreu *et al.*, 2009). Major difference in seasonal patterns of zooplankton appear to be related by water temperature, which varies from 9 to 15 °C in winter, and 25 to 28 °C in summer, but the spatial changes showed relationship with salinity and wind (Montu *et al.*, 1998).

Through analysis of historical data, this study reviewed the patterns of environmental conditions, and spatial and temporal distributions, associated to captures of each early developmental stages of Argentine menhaden *Brevoortia pectinata*, an important and frequent clupeid in the Patos Lagoon estuary.

MATERIALS AND METHODS

Similarity between the species of *Brevoortia* may cause doubts about their identification. However, this study will retain the name *B. pectinata* for eggs and larvae collected in the Patos Lagoon estuary until clarification and definition of the final designation of the species occurs in the region.

Eggs and larvae of *Brevoortia pectinata*, collected from 1975 to 2009 were used in the study. The material is part of the collection of the Laboratório de Ecologia do Ictioplâncton from the Instituto de Oceanografia at Universidade Federal do Rio Grande, and was kept preserved and stored in formaldehyde 3.6 %. Samples are the result of several plankton surveys that employed nets to collect the ichthyoplankton.

All these plankton samples investigated a wide research area (Fig. 1) and sampling details can be found in serveral references (Muelbert & Weiss, 1991; Bruno & Muelbert, 2009). Additionally, eggs and larvae collected with plankton samples taken at 7 beach stations between 2000 and 2009 were also used in the analysis. At each sampling point, salinity, temperature and the volume of water filtered by the plankton net were measured.

All samples with presence of clupeids were reviewed and larvae identification was confirmed as *B. pectinata* using the literature (Weiss & Krug, 1977; Richards, 2006; Fahay, 2007). Whenever possible, each larvae identified was measured using one of the methods described in Malanski & Muelbert (*Submt.* a). When possible, eggs and larvae were classified according to developmental stage by the following criteria, modified of Richards (*op. cit.*):

1- Planktonic phase:

   a. **Egg** stage – prior to hatching the body of the organism is surrounded by the chorion, and it is considered an embryonic stage.
   b. **Yolk-sac** stage (vitelinic larvae) – the newly hatched larvae still has some vestige of the yolk-sac, and it does not have some of larval characteristics.
   c. Larval stage – it is divided in 3 other sub-stages:

i. **Pre-flexion** larvae: stage where the larvae has no more trace of the yolk-sac, and still does not have any trace of hippuric bones at the end of the notochord.

ii. **Flexion** larvae: stage when the formation of hippuric bone starts at the end of the notochord, but its formation is not complete, without the formation of caudal fin rays.

iii. **Post-flexion** larvae: the larva has completed the formation of hippuric bones, with the presence of caudal fin rays, but the growth in body height has not yet begun.

d. Transformation stage (**Metamorphosis**): characterized by the loss of larval fish characteristics, and acquisition of adult features. The organism has the complete formation of the caudal fin rays, and an increase in body height begins to reveal the adult trace, but the body is still translucent, characteristic of fish larvae (without scales, which makes the opaque body).

2- Nectonic phase:

a. **Juvenile** stage – stage where the organism has all adult features, but it is not fertile yet.

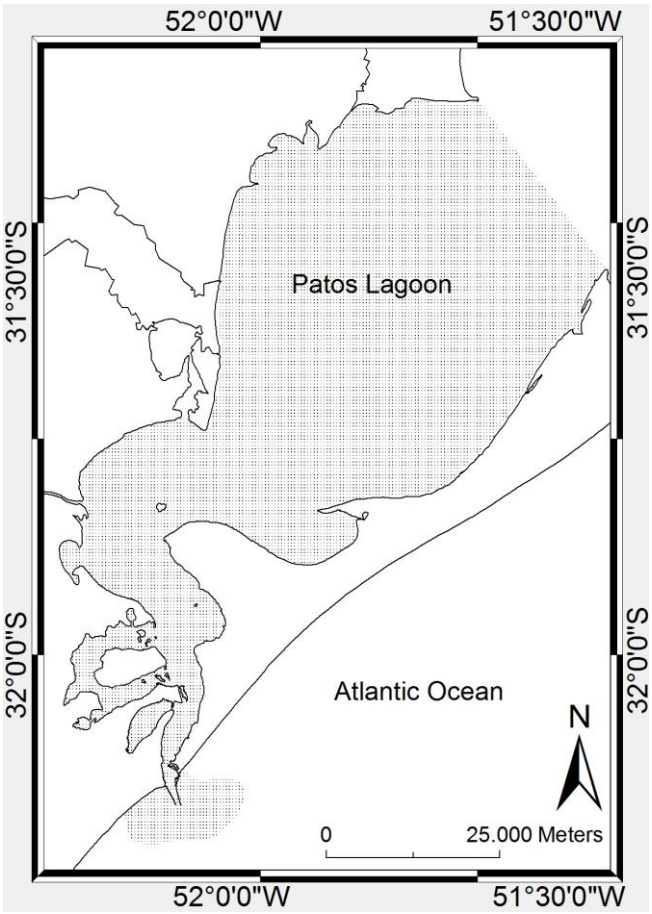

Fig. 1: Study area, where the plankton sample stations were carried out into the shaded limits during 1975 to 2009.

Measurements of standard length (SL) of the early stages of *B. pectinata* kept preserved in the laboratory were adjusted to obtain the SL in life, according to the formula proposed by Malanski & Muelbert (*Submt*. b). Thus, it was possible to obtain the ranges of SL corresponding to each classification of early life stages of this species.

In order to evaluate the spatial distribution of Argentine menhaden, distances from all stations were estimated from a point located exactly at the mouth of the Patos Lagoon estuary. Separating distances were estimated by drawing a single line from the point of

reference to the stations following the existing channels in the study region. In this way, distances should reflect the main drainage route of the Patos Lagoon.

In the samples with positive catches of Argentine menhaden, the larval density (larvae/100 m^3) expressed its CPUE's. For each stage, larval CPUE was summed for each months to show their temporal distributions. Monthly relative frequency (%) was calculated from $CPUE_{total}$ of each larval stage to verify the importance of each seasonal period.

RESULTS

This study identified a total of 10479 eggs and 14066 larvae and juveniles of *B. pectinata*. From these, 9192 larvae and juveniles had their standard length measured, and of which, 3621 were had their developmental stage determined (Fig. 2).

Early stages of this species were registered in all months of the year (Tab. 1), which resulted in a wide range of environmental conditions during their capture (salinity between 0 and 35, and water temperature between 10 and 33.8 °C) (Fig. 3). The maximum distance from coastal marine zone where representatives of this species were found was 125 km into the Patos Lagoon (Fig. 3). Sampling effort was conducted up to 144.5km away from the coastal zone.

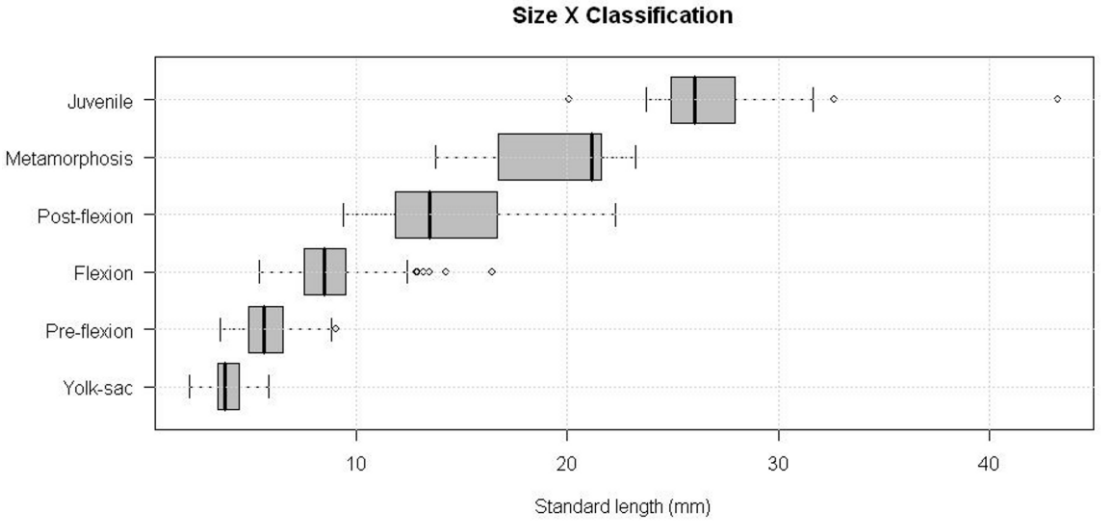

Fig. 2: Standard length intervals (mm) for each early developmental stages of Argentine menhaden (*B. pectinata*). Box-plots indicate minimum and maximum values (inferior and superior line), median (intermediate black line), and inferior and superior quartiles (gray retangle). Atypical values (outliers) are indicated by "◦".

Tab. 1: Relative frequencies (%) for CPUE of early stages of Argentine menhaden (*B. pectinata*) during months of the year. The frequency was standardized by CPUE $_{total}$ of each stage. Dark to light gray indicates the importance of the months for the CPUE's.

Seasons	SUMMER			AUTUMN			WINTER			SPRING		
Months	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Egg	16.73	14.17	0.78	1.30	0.53	0.97	19.08	2.11	16.98	21.34	2.66	3.37
Yolk-sac	93.12	0.05	0.04	0.06	1.18	0.47	3.17	0.92	0.12	0.35	0.22	0.30
Pre-flexion	4.72	7.65	16.37	2.72	0.32	1.97	10.99	1.54	0.61	3.65	44.13	5.34
Flexion	26.60	10.77	6.72	0.37	1.65	8.50	17.87	1.44	0.53	3.67	11.44	10.43
Post-flexion	0.84	7.00	1.71	24.82	3.62	0.44	26.22	5.12	26.74	0.03	1.51	1.95
Metamorphosis	*	1.20	4.99	61.40	29.47	*	1.80	0.33	0.26	0.26	*	0.30
Juvenile	*	0.05	*	41.94	14.96	*	26.74	16.31	*	*	*	*

* No larvae collected.

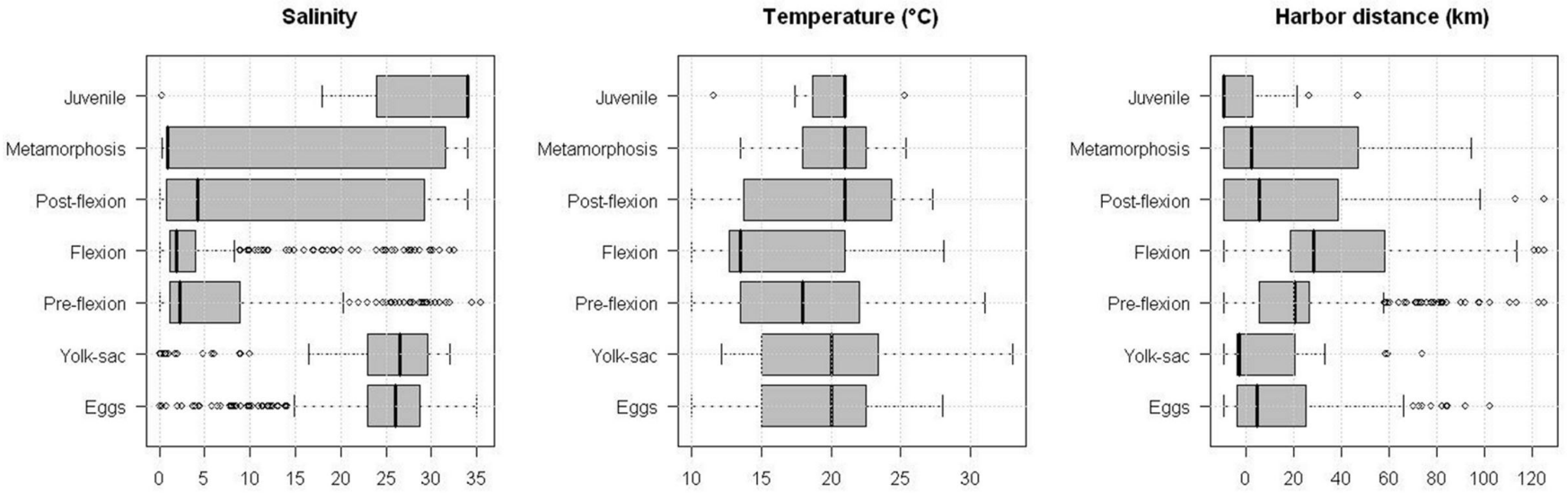

Fig. 3: Salinity, water temperature (°C) and distance of Rio Grande harbor (km) intervals that were found in the captures of early stages of Argentine menhaden (*B. pectinata*). For distance zero indicates an exactly point at the mouth of Patos Lagoon estuary, and positive and negative values indicate distances into the estuary, and distances off the harbor (to adjacent coastal zone), respectively. Box-plots indicate minimum and maximum values (inferior and superior line), median (intermediate black line), and inferior and superior quartiles (gray retangle). Atypical values (outliers) are indicated by "◦".

Egg Stage:

Argentine menhaden eggs were found in the study area during all months. July, September, October, January and February accounted for 88.3 % of its CPUE $_{total}$ (Tab. 1).

Eggs were found related to medium-high salinities (~ 23 and 28.8), and water temperature between 15 and 22.5 °C, data that represent 50 % of the observations (Fig. 3). Fifty percent of the eggs were captured between 9.5 km outside the estuary (in the coastal zone) to 4.6 km into the estuary, and 75 % of the cases were found within 25 km into the estuary (Fig. 3).

Yolk-sac Stage (vitelinic larvae):

Four hundred and thirty-one larvae were identified in this developmental stage, with the smallest and largest larvae possessing 2.16 and 5.91 mm, respectively (Fig. 4). However, the more common standard length (SL) for the vitelinic larvae (lower range of SL, which represents 50 % of the data) ranged from 3.46 to 4.46 mm.

Argentine menhaden vitelinic larvae were found during all months of the year in the study area, but December itself accounted for 93.1 % of its CPUE $_{total}$ (Tab. 1).

The environmental conditions during the presence of this stage were better related to medium-high salinities (~ 23 and 29.6), and water temperature between 15 and 23.4 °C, data that represent 50 % of the observations (Fig. 3). More than 50 % of the larvae were in the coastal zone, and 75 % of the cases were contained within 20.5 km into the estuary (Fig. 3).

Pre-flexion Larvae:

Pre-flexion larvae were represented by 1473 fishes, with the smallest and largest larvae possessing 3.58 and 9.07 mm, respectively (Fig. 4). However, the most common standard length (SL) for pre-flexion larvae ranged from 4.94 to 6.54 mm.

Pre-flexion larvae were found during all months in the study area, of which March, July and November accounted for 71.5 % of its $CPUE_{total}$ (Tab. 1).

Environmental conditions for this stage were better related to low salinities (~ 0 and 2.2), and water temperature between 13.5 and 22 °C, data that represent 50 % of the observations (Fig. 3). Fifty percent of the pre-flexion larvae occurred from the coastal zone to 20.5 km into the estuary, and 75 % of the cases were related up to 26.3 km into the estuary (Fig. 3).

Flexion Larvae:

This developmental stage was represented by 1346 fishes, with the smallest and largest larvae possessing 5.46 and 16.47 mm, respectively (Fig. 4). However, the most common standard length (SL) for flexion larvae ranged from 7.56 to 9.50 mm.

Argentine menhaden flexion larvae were found in the study area all year around, and July, November, December, January and February represented 77.1 % of its $CPUE_{total}$ (Tab. 1).

Flexion larvae were found in low salinities conditions (~ 0 and 1.9), and water temperature between 10 and 13.5 °C, data that represent 50 % of the observations (Fig. 3). Fifty percent of flexion larvae were from the coastal zone to 28.5 km into the estuary, and 75 % of the cases were related up to 58 km into the estuary (Fig. 3).

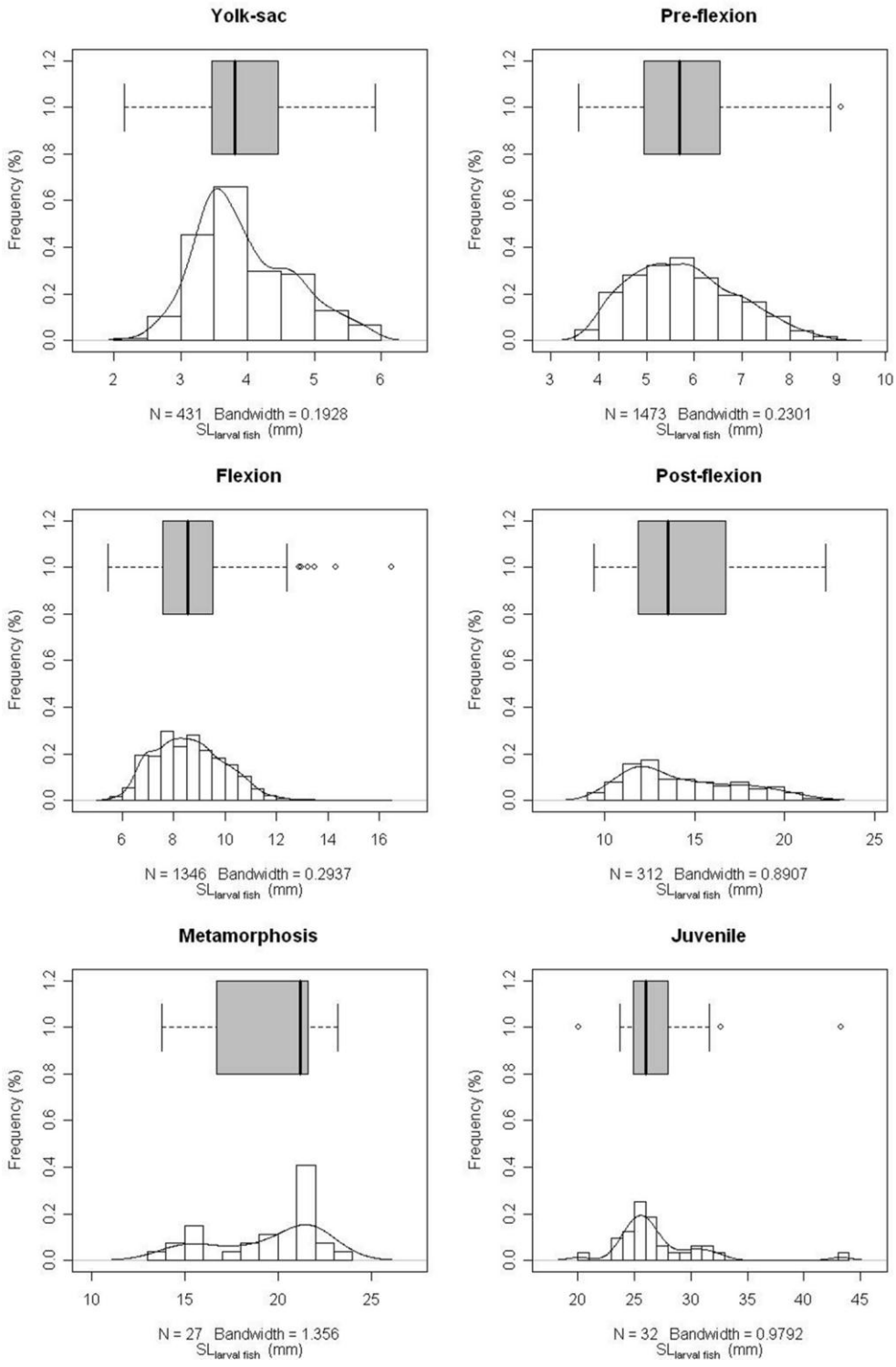

Fig. 4: Relative frequencies (%) related for standard length distributions (mm) of early developmental stages of Argentine menhaden (B. pectinata). N-sampled fishes and bandwidth for each stage are shown. Bandwidth indicates the deviation of the normal curve from original data.

Post-flexion Larvae:

Three hundred and twelve post-flexion larvae were identified, with the smallest and largest larvae possessing 9.39 and 22.27 mm, respectively (Fig. 4). However, the most common standard length (SL) for the post-flexion larvae ranged from 11.88 to 16.67 mm.

Argentine menhaden post-flexion larvae were found during all months in the study area, of which July, September and April accounted for 77.8 % of its CPUE $_{total}$ (Tab. 1).

Environmental conditions to post-flexion larvae were better related to low salinities (~ 0 and 4.2), and water temperature between 13.8 and 24.3 °C, data that represent 50 % of the observations (Fig. 3). Most of the pos-flexion larvae were found from the coastal zone to 5.5 km into the estuary, and 75 % of the cases were related up to 38.5 km into the estuary (Fig. 3).

Transformation Stage (Metamorphosis):

Only 27 fishes were identified in this developmental stage, with the smallest and largest larvae possessing 13.77 and 23.22 mm, respectively (Fig. 4). However, the most common standard length (SL) for the metamorphosis larvae ranged from 16.74 to 21.60 mm.

In January, June and November this stage was not captured in the study area, and in April and May its CPUE represented 90.8 % of the total (Tab. 1).

Environmental conditions to metamorphosis larvae were better related to low salinities (~ 0.3 and 0.9), and water temperature between 17.95 and 22.5 °C, data that represent 50 % of the observations (Fig. 3). Most of them were found from the coastal zone to

2.2 km into the estuary, and 75 % of the cases were related up to 47 km into the estuary (Fig. 3).

Juvenile Stage:

Thirty-two fishes were identified in this developmental stage, with the smallest and largest larvae measuring 20.11 and 43.25 mm, respectively (Fig. 4). However, the most common standard length (SL) for the juveniles ranged from 25 to 27.92 mm.

Juveniles were not captured during January, March, June and from September to December in the study area, and in April its CPUE represented 41,9 % of the total (Tab. 1).

Juvenile distribution was better related to high salinities (~ 34), and water temperature approximately 21 °C, data that represent 50 % of the observations (Fig. 3). Seventy-five percent of the juveniles were found in the coastal zone (Fig. 3).

DISCUSSION

The present study showed the life history patterns of early stages of Argentine menhaden (*Brevoortia pectinata*) in the Patos Lagoon estuary area and nearby surroundings. These results reflect the ideal conditions for the early life cycle development of this species.

Newly-hatched larvae can have 2.16 mm, but they were typically in the range of 3.46 to 4.46 mm SL, similar to the size described by Weiss & Krug (1977), where the length of post-hatching was approximately 3.2 mm. The smallest larva measured without the

presence of the yolk-sac (in pre-flexion) had 3.58 mm, but normally they ranged from 4.94 to 6.54 mm, result very similar to the 5.00 mm found by Weiss & Krug (*op cit.*).

The post-flexion larvae normally had SL ranged from 11.88 to 16.67 mm, but can reach up to 22.27 mm. These larvae already have some capacity for locomotion, because them caudal fin rays are formed. However, they still would not be considered a metamorphosis larvae because was not seen a gain in height size, which case identified in only 27 larval fishes, and these normally had a ranging SL between 16.74 and 21.60 mm. The smallest juvenile of Argentine menhaden measured had 20.11 mm. All these results are very similar to those obtained by Weiss & Krug (1977).

When the results obtained in this study are compared to that obtained by Cassia & de la Rosa (1993), the development of *B. aurea* was different of *B. pectinata*. For example, in the same length which *B. aurea* had started the flexion of urostyle (11 to 12 mm SL), *B. pectinata* larvae are in post-flexion stage and the caudal fin rays are formed. Cassia & de la Rosa (1993) suggested a latitudinal developmental variation in *Brevoortia* genus in the Southwestern Atlantic Ocean, but their sampling site was restricted to the Rio de La Plata estuary. In order to determine that this two species are just one, a study comprising the full distributional range of each of the species is necessary. Garcia *et al.* (2008) also investigated the *Brevoortia* genus using genetic differentiation technique, and concluded that there is just one species in Uruguayan coastal region. In this last study, the latitudinal gradient of the sampling sites was also restricted and the genetic differentiation was performed just by one DNA sequence (mitochondrial *cytochrome-b*). These limitations still leave doubts regarding the presence of one or more species of Brevoortia in the South Atlantic.

The salinity gradient at which the early stages of Argentine menhaden were encountered provide a clue to their life cycle in the Patos Lagoon estuary, which responded to narrow ranges of salinity for each stage along the PLE. Eggs and yolk-sac larvae were found in high salinity waters and associated to the coastal zone (Fig. 3). This result indicates that adults do not enter in the estuary to spawn, and that the spawning area are comprised by the region off the Rio Grande harbor, at the coastal zone.

Pre-flexion, flexion and post-flexion stages were more closely related to lower salinities and greater distances from Rio Grande harbor toward into the Patos Lagoon (Fig. 3). This suggests that eggs and newly hatched larvae are carried into the estuary by currents (Martins *et al.*, 2007; Franzen *et al.*, submitted). Larval stages still belong to the planktonic phase and were also brought by marine currents and/or tide into the estuary. If transport by marine currents were not the case, their occurrence would be very similar to the region near the spawning area of this specie.

Metamorphosis and juvenile stages have the greater locomotion ability than the other stages, and thus can swim and avoid the sampling gear. Despite the low number of individuals of these stages, probably caused by deficiency to capture them by the sampling methodology used (planktonic nets), both were found in conditions of higher salinity, and also a smaller distance from the harbor. These results suggest a return to the marine environment (Fig. 3).

Möller & Fernandes (2010) described that the salinization of the Patos Lagoon peaks in July due to a combination of river discharge and wind effect, altering the waters level into the lagoon. July was the period when most of the developmental stages of Argentine menhaden occurred in the PLE (Tab. 1). Thus, we can say that in winter there is an important spawning for this species, and with the predominance of

south/southwest winds in autumn and winter months, entrance and impoundment of marine waters into the Patos Lagoon is facilitated (Möller & Fernandes, *op cit.*), and can control the transport of fish eggs between the lagoon and marine environment (Martins *et al.*, 2007; Franzen *et al.*, submitted). This will take these early stages to the estuary, where the necessary conditions to development the estuarine-dependent organisms occur (Weiss & Muelbert, 1991; Muelbert *et al.*, 2010). But, during the spring and summer periods were also important for the earliest stages of life of Argentine menhaden (eggs, and vitelinic, pre-flexion and flexion larvae), because the relative frequencies of their CPUE were also higher in this months.

For later larval stages (post-flexion and metamorphosis) and juvenile of Argentine menhaden, the autumn period had importance, suggesting the occurrence of at least one other important spawning period in summer, and that should affect its recruitment, even with the continuous spawning pattern for this species described in the study area (Muelbert *et al.*, 2010).

These results differ in some parts of the pattern described for the Brazilian menhaden (*B. aurea*) in the Rio de la Plata estuary (Acha & Macchi, 2000), where the eggs were found in the inner part of the estuary whith medium salinity values (from 10 to 20). In the present study, at least 75 % of the eggs data were found in medium to high salinity conditions (>23), probably with spawning in the region outside the Patos Lagoon estuary. Continuous spawning was observed for both species, but the importance of eggs during the autumn and winter do not coincide. In the Patos Lagoon estuary, egg are not important during autumn; while in the Rio de La Plata there is another peak in egg abundance during this season. The opposite occurs during winter, when egg abundace revealed an important winter spawning in the Patos Lagoon estuary.

It is known that larger water bodies suffer less influence of temperature changes from the atmosphere. In this sense, during the winter period the marine coastal waters have higher temperature than the shallow waters inside the Patos Lagoon, and vice-versa during summer. So, during winter, earlier stages found outside the estuary and in saltier waters were distributed in higher temperature waters than stages that are further developed and inside of the estuary. The presence of earlier stages of Argentine menhaden in higher low temperature waters outside the Patos Lagoon, strengthen the existence of spawning pattern during the winter period. With further temperature decrease during winter, older stages (pre-flexion and flexion larvae) are transported and captured in the interior of the lagoon in the lowest temperatures.

The pattern described in this study considered only the effects of abiotic factors during the early stages of Argentine menhaden *B. pectinata*. However, life history of marine fishes and the success of their recruitment cannot be described only by few parameters, but rather by a combination of many abiotic and biotic parameters (Sætre *et al.*, 2002), that should produce a positive or negative recruitment (van der Lingen & Huggett, 2003; Huse *et al.*, 2008). In the other hand, environmental impacts on the population of Argentine menhaden in south Brazil should not just be closely related to the spatial and temporal variability of abiotic and biotic water column condition, but rather by their modification by environmental events and/or human activities that affect the entry and/or impoundment of seawater in rivers and coastal lagoons, like episodes of ENSO (*El Niño* South Ocilation), which are capable to modifying the structure of some community, as phytoplankton (Odebrecht *et al.*, 2010), zooplankton (Muelbert *et al.*, 2010), and fishes (Vieira *et al.*, 2010), and human interventions (*i.e.* engineering

works), that not necessary impact the environment directly, but normally increase pressure over this environment (indirect impact).

CONCLUSIONS

The results of this study demonstrate that the Argentine menhaden *Brevoortia pectinata* uses the Patos Lagoon estuary during the early stages of its life cycle. Spawning occurs in the region outside the estuary, with the transport inside the lagoon in favorable areas for their development. Later stages (juvenile) return to the marine environment. The retention process within the estuary is probably the most important event for this species.

Further studies that involve the dispersal models of their eggs and fish larvae, with specific samplings to verify the occurrence of its early stages of life cycle, may prove that the spawning in periods with condition very close to ideal should maximize the survival of these organisms, and consequently maximize its recruitment to adult stock. It is important also to obtain information on other parameters, such as feeding and predation, for the early stages of this species.

Information obtained from this study will be useful in evaluation of environmental impacts on the recruitment of this fish population, especially that live near Rio Grande, south Brazil, if there were known the parameters of the water column.

ACKNOWLEDGMENTS

We thank the Laboratório de Ecologia do Ictioplâncton (LEI-IO-FURG) for providing the infrastructure to develop this study. EM benefited from a scholarship from the Coordenação de Aperfeiçoamento de Pessoas de Nível Superior (CAPES), and JHM from a CNPq grant (Proc. 309181/2009-7).

LITERATURE CITED

Abreu, P. C.; M. Bergesch; L. A. Proença; C. A. E. Garcia & C. Odebrecht. 2010. Short- and Long-Term Chlorophyll *a* Variability in the Shallow Microtidal Patos Lagoon Estuary, Southern Brazil. ***Estuaries and Coasts***, 33: 554–569. DOI: 10.1007/s12237-009-9181-9.

Acha, E. M. & G. J. Macchi 2000. Spawning of Brazilian menhaden, *Brevoortia aurea*, in the Río de la Plata estuary off Argentina and Uruguay. ***Fishery Bulletin***, 98(2): 227–235.

Bruno, M. A. & J. H. Muelbert. 2009. Distribuição Espacial e Variações Temporais da Abundância de Ovos e Larvas de *Micropogonias furnieri*, no Estuário da Lagoa dos Patos: Registros Históricos e Forçantes Ambientais. ***Atlântica***, 31(1): 51-68. doi: 10.5088/atl. 2009.31.1.51.

Cassia, M. C. & de la Rosa, S. B. G. 1993. Características diferenciales del desarrollo larval de *Brevoortia aurea* en el Atlantico Sudoccidental. ***Frente Maritimo***, 14: 63-69.

Cousseau, M. B. & J. M. Díaz de Astarloa. 1993. El Género *Brevoortia* en la Costa Atlántica Sudamericana. Montevideo. ***Frente Marítimo***, 14: 49-57.

Cousseau, M. B. & R. G. Perrota. Atlas de Sensibilidad Ambiental de la Costa y el Mar Argentino: Peces. Disponível em: http://atlas.ambiente.gov.ar/tematicas/mt_02/pdfs/PC_Brevoortia_aurea.pdf accessed in: 31/07/2009.

Deegan, L. A. 1990. Effects of estuarine environmental conditions on population dynamics of young-of-the-year gulf menhaden. ***Marine Ecology Progress Series***, 68: 195–205.

Fahay, M. P. 2007. ***Early Stages of Fishes in the Western North Atlantic Ocean (Davis Strait, Southern Greenland and Flemish Cap to Cape Hatteras)***. Volume I. ISBN: 0-9689167-4-x. Dartmouth/NS, Canada. Northwest Atlantic Fisheries Organization – NAFO. 1696p.

FAO. 2009. Part 1 – Word Review of Fisheries and Aquaculture, *In*: ***The State of Word Fisheries and Aquaculture (SOFIA)***. Roma, Itália. 176p.

Fischer, L. G.; L. E. D. Pereira & J. P. Vieira. 2004. ***Peixes Estuarinos e Costeiros***. Rio Grande/RS, Brasil. *Ecoscientia*. 139p.

Franzen, M. O.; J. H. Muelbert & E. H. L. Fernandes. *Submitted*. Wind events and larval fish recruitment to the Patos Lagoon estuary. ***Journal of Marine Systems***. 36pp.

García, G.; J. Vergara & V. Gutiérrez. 2008. Phylogeography of the Southwestern Atlantic menhaden genus *Brevoortia* (Clupeidae, Alosinae). ***Marine Biology***, 155: 325-336.

Huse, G; A. Salthaug & M. D. Skogen. 2008. Indications of a negative impact of herring on recruitment of Norway pout. ***ICES Journal of Marine Science***, 65: 906–911.

Ibagy, A. S. 1997. Composição, Distribuição e Variação Nictimeral do Ictioplâncton do Estuário da Lagoa dos Patos, RS, Brasil. ***Dissertação de mestrado***. Universidade Federal do Rio Grande – FURG, 127p.

Loebmann, D.; A. M. Garcia; M. B. Raseira; L. A. Ramos & J. P. Vieira. 2001. Distribuição espacial de *Brevoortia pectinata* (Jenyns, 1842) (Pisces: Clupeidae) nas zonas rasas do estuário da Lagoa dos Patos, RS, Brasil. *In*: ***Annals of XIV EBI***, São Leopoldo.

Malanski, E. & J. H. Muelbert. *Submitted* a. Measuring Small Organisms: Evaluation of Traditional and Image Techniques for Larval Fishes. ***Limnology and Oceanography Methods***.

Malanski, E. & J. H. Muelbert. *Submitted* b. Size Changes in Argentine menhaden Larvae, *Brevoortia pectinata*, Due to Preservation. ***ICES Journal of Marine Science***.

Martins, I. M.; J. M. Dias; E. H. Fernandes & J. H. Muelbert. 2007. Numerical modelling of fish eggs dispersion at the Patos Lagoon estuary – Brazil. ***Journal of Marine Systems***, 68: 537–555.

Möller JR., O. O.; J. P. Castello & A. C. Vaz. 2009. The Effect of River Discharge and Winds on the Interannual Variability of the Pink Shrimp *Farfantepenaeus paulensis* Production in Patos Lagoon. ***Estuaries and Coasts***, 32(4): 787–796.

Möller, O. & E. Fernandes. 2010. Hidrologia e Hidrodinâmica. *In*: Seeliger, U. & C Odebrecht (Eds.). ***O Estuário da Lagoa dos Patos: Um Século de Transformações***.

Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. pp. 15–27.

Montú, M.; A. K. Duarte & I. M. Gloeden. 1998. O Ambiente e a Biota do Estuário da Lagoa dos Patos: Zooplâncton. *In*: Seeliger, U.; C. Odebrecht & J. P. Castello (Eds.). 1998. ***Os Ecossistemas Costeiro e Marinho do Extremo Sul do Brasil***. Rio Grande. Ecoscientia. pp. 43-46.

Muelbert, J. H. & G. Weiss. 1991. Abundance and Distribution of Fish Larvae in the Channel of the Patos Lagoon Estuary, Brasil. *In*: Hoyt, R. D. (Ed.). ***Larval Fish Recruitment and Research in the Americas: Proceedings of the 13th Annual Fish Conference***. NOAA Technical Report NMFS 95. Mérida, México. pp. 43-54.

Muelbert, J. H.; E. Muxagata & S. Kaminski. 2010. As Comunidades Zooplanctônicas. *In*: Seeliger, U. & C Odebrecht (Eds.). ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. pp. 65–75.

Nelson, W. R.; M. C. Inghan & W. F. Schaaf. 1977. Larval transport and year class strength of Atlantic menhaden, *Brevoortia tyranus*. ***Fishery Bulletin***, 75(1): 23-41.

Odebrecht, C. & P. C. Abreu. 1998. O Ambiente e a Biota do Estuário da Lagoa dos Patos: Microalgas. *In*: Seeliger, U.; C. Odebrecht & J. P. Castello (Eds.). 1998. ***Os Ecossistemas Costeiro e Marinho do Extremo Sul do Brasil***. Rio Grande. Ecoscientia. pp. 36-40.

Odebrecht, C.; M. Bergesch; S. Medeanic & P. C. Abreu. 2010. A Comunidade de Microalgas. *In*: Seeliger, U. & C Odebrecht (Eds.). ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. pp. 49–63.

Pauly, D. 2007. Tales of a Small, But Crucial, Fish. ***Science***, 318(5851): 750-751. DOI:10.1126/science.1147800.

Richards, W. J. 2006. ***Early Stages of Atlantic Fishes: an Identification Guide for the Western Central North Atlantic. Volume I***. ISBN: 0-8493-1916-1. Boca Raton/FL, USA. CRC Press. 2640p.

Santos, R. C. & M. Rodrigues-Ribeiro. 2000. Demanda de isca vivas para a frota atuneira catarinense na safra de 1998/99: CPUE, composição e distribuição das capturas. ***Notas Técnicas FACIMAR***, 4: 97-101.

Seeliger, U. & C. Odebrecht (Eds.). 2010. ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. 180p.

Sinque, C. & J. H. Muelbert. 1998. O Ambiente e a Biota do Estuário da Lagoa dos Patos: Ictioplâncton. *In*: Seeliger, U.; C. Odebrecht & J. P. Castello (Eds.). ***Os Ecossistemas Costeiro e Marinho do Extremo Sul do Brasil***. Rio Grande/RS, Brasil. Ecoscientia. pp. 56-60.

Stegmann, P. M.; J. A. Quinlan; F. E. Werner; B. O. Blanton & P. Berrien. 1999. Atlantic menhaden recruitment to a southern estuary: defining potential spawning regions. ***Fishery Oceanography***, 8(2):111-123.

Sætre, R.; R. Toresen & T. Anker-Nilssen. 2002. Factors affecting the recruitment variability of the Norwegian spring-spawning herring (*Clupea harengus* L.). ***ICES Journal of Marine Science***, 59: 725–736.

van der Lingen, C. D. & J. A. Huggett. 2003. The role of ichthyoplankton surveys in recruitment research and management of South African anchovy and sardine, *In*: Browman, H. I. & A. B. Skiftesvik (Eds.). ***The Big Fish Bang. Proceedings of the***

26th Annual Larval Fish Conference. Institute of Marine Research. Bergen, Norway. ISBN: 82-7461-059-8. pp. 303–343.

Vieira, J. P.; A. Garcia & L. Moraes. 2010. A Assembléia de Peixes. *In*: Seeliger, U. & C Odebrecht (Eds.). ***O Estuário da Lagoa dos Patos: Um Século de Transformações***. Universidade Federal do Rio Grande – FURG. Rio Grande/RS, Brasil. ISBN: 978-85-7566-144-4. pp. 77–88.

Visentainer, J. V.; M. D. Noffs; P. O. Carvalho; V. V. Almeida; C. C. Oliveira & N. E. Souza. 2007. Lipid Content and Fatty Acid Composition of 15 Marine Fish Species from the Southeast Coast of Brazil. ***Journal of the American Oil Chemists' Society***, 84: 543–547. DOI: 10.1007/s11746-007-1070-4.

Weiss, G & L. C. Krug. 1977. Características do desenvolvimento e metamorphose de *Lycengraulis olidus* (Engraulidae) e *Brevoortia pectinata* (Clupeidae) no Estuário da Lagoa dos Patos, Brasil. Rio Grande. ***Atlântica***, 2(1): 83-117.

Weiss, G. 1981. Ictioplancton del Estuario de Lagoa dos Patos, Brasil. PhD. Thesis, Faculdad de Ciencias Naturales y Museo, Universidad Nacional de la Plata, 164p.

Whitehead, P. J. P. 1985. FAO species catalogue: Volume 7 – Clupeoid Fishes of the World (suborder Clupeioidei). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings. Part 1 – Chirocentridae, Clupeidae and Pristigasteridae. ***FAO Fisheries Synopsis***, 125(7/1): 1-303.