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ICTIOPLÂNCTON E A CONSERVAÇÃO DE REGIÕES COSTEIRAS: O CASO DO ESTUÁRIO DA LAGOA DOS PATOS

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“A minha família que desde o início da minha vida
acadêmica me deu todo o incentivo e
suporte necessário! Em especial ao meus
pais, Cyro e Rosangela, por todo o
carinho. Mesmo tão longe, sempre tão
presentes!”

"For most of history, man has had to fight nature to survive; in this century he is beginning to realize that, in order to survive, he must protect it" Jacques-Yves Cousteau

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RESUMO

A relevância ecológica dos estuários para diversas espécies (e.g. *Micropogonias furnieri*), associada a importância econômica fazem com que os interesses sócio-econômicos colidam com os de conservação nestes ambientes. Devido a estreita relação entre a sobrevivência de ovos e larvas de peixes e o sucesso no recrutamento, o conhecimento sobre o ictioplâncton torna-se relevante para a conservação estuarina. Estudos sobre ictioplâncton estuarino geralmente abordam aspectos taxonômicos e ecológicos das espécies, e existe uma lacuna de informações sobre a identificação de áreas prioritárias para a conservação, em especial, utilizando dados sobre estágios iniciais de peixes. Em virtude do pouco conhecimento a respeito de áreas essenciais para a conservação e da relação entre a distribuição da abundância de larvas e de juvenis de peixes em estuários, é essencial estudar a incorporararação de informações sobre as primeiras fases do ciclo de vida dos peixes no planejamento para a conservação. Na presente tese, o conceito de "*habitat essencial (essential fish habitat)*" foi aplicado para a corvina *M. furnieri*, enquanto que o planejamento sistemático para a conservação de estuários foi baseado em informações de distribuição espacial e composição do ictioplâncton, vegetação aquática submersa, sedimento e batimetria do estuário da Lagoa dos Patos (ELP). Nesse contexto, as hipóteses de trabalho foram: a) existe uma correlação entre os diferentes estágios de vida da corvina e os habitats no estuário da Lagoa dos Patos; b) cada estágio de desenvolvimento (e.g. ovo, larva e juvenil) da corvina possui um habitat essencial específico e está correlacionado com diferentes variáveis ambientais; c) a inclusão de dados de ictioplâncton gera diferenças na prioridade de planejamento espacial para a conservação; e d) a assembleia ictioplanctônica das áreas rasas do ELP apresenta variabilidade temporal e espacial na abundância e composição das espécies que influenciam as ações de conservação. Os resultados da tese possibilitaram correlacionar cada estágio de vida da corvina com um habitat estuarino e com diferentes variáveis ambientais. Além disso, considerando as variações temporais na ocorrência da espécie no ELP foi possível identificar os *habitats essenciais* para cada estágio de desenvolvimento, mostrando que principalmente as enseadas rasas e os canais profundos são essenciais para a manutenção do recrutamento da corvina no ELP. Considerando a proteção do seu *habitat essencial,* foi observado que a adição de zonas em um plano de conservação não altera as prioiridades espaciais. A inclusão de dados do ictioplâncton contribui de maneira significativa para a priorização espacial para a conservação no ELP. Os resultados mostraram que para alcançar os objetivos de conservação, algumas enseadas rasas do estuário devem ser consideradas como áreas de proteção, ressaltando ainda mais a importância desses habitats no ELP. Além disso, ovos e larvas de peixes nas enseadas rasas do ELP possuem uma alta variabilidade entre meses, anos e pontos amostrais, a qual pode ser principalmente associada com a intrusão de água salgada no estuário. Como consequência, foi encontrada uma alta variabilidade nos padrões de distribuição espacial dos ovos e larvas de peixes, com espécies distintas ocupando as duas zonas propostas pela ação de manejo aplicada atualmente ao ELP. Estudos de longa duração funcionam como uma boa estratégia para auxiliar a tomada de decisão para a conservação, uma vez que possibilitam compreender as variações nos padrões espaciais de distribuição das espécies ao longo do tempo. Em conclusão, no caso do estuário da Lagoa dos Patos, um plano de conservação que combine ações de manejo previstas em políticas públicas (e.g. enquadramento das águas) e informações sobre a dinâmica da comunidade biológica (e.g. dados sobre estágios iniciais de peixes) é extremamente necessário para a proteção do ecossistema e de sua biodiversidade.

Palavras-chave: ictioplâncton, estuário da Lagos dos Patos, *Micropogonias furnieri*, habitat essencial, conservação de estuários, planejamento sistemático para a conservação

ABSTRACT

The ecological importance of estuaries for many species (e.g. *Micropogonias furnieri*) associated with the economic importance of these regions results in conflict between conservation and social-economic interests. Due to the close relation between survival of fish early stages and recruitment processes knowledge about ichthyoplankton becomes extremely relevant to estuarine conservation. Icthyoplankton studies on estuaries are restricted to taxonomic and ecological understanding and there is a lack of information on the identification of priority areas for conservation, mainly on how to incorporate early life stages in the design process. Since there is little knowledge about essential areas for conservation and the relation among larvae and juvenile abundance and composition at estuaries, it is crucial to study the incorporation of information on early life stages of fish on systematic planning. In the present study, the essential fish habitat concept was applied to the whitemouth croaker *M. furnieri*, and the approach of systematic conservation planning was based on information on the spatial distribution and composition of fish eggs and larvae, submerged aquatic vegetation, sediment type, and bathymetry in the Patos Lagoon estuary (PLE). In this sense, the hypotheses were: a) different life stages of *M. furnieri* are correlated with distinct estuarine habitats at PLE; b) each ontogenetic stage (e.g. eggs, larvae and juveniles) of *M. furnieri* has a specific essential habitat and it is correlated to different environmental variables; c) incorporation of information on the spatial distribution and composition of ichthyoplankton into systematic conservation planning changes spatial priorities for conservation; and, d) ichthyoplankton from shallow embayments exhibits temporal and spatial variability on abundance and composition that can facilitate conservation actions on estuaries. Results show that each life stages of the whitemouth croaker is correlated to an estuarine habitat and to different environmental variables. Moreover, it was possible to identify the essential habitats for each life stage of the species by considering the temporal variation on the occurrence pattern. Shallow embayments and channels were identified as the main habitats to guarantee the recruitment process at PLE. Considering its essential habitat protection, it was observed that adding zones in a conservation plan do no change the spatial priorities for conservation. Incorporating fish eggs and larvae data significantly contributes to spatial conservation prioritization at PLE, and reveals that shallow embayments must be considered to protection, highlighting the importance of these habitats at PLE. Fish eggs and larvae in shallow embayments at PLE have high variability within months, years and sampling stations, which can mainly be associated with the entrance of salt water into the estuary. Also, it was found high variability on spatial distribution patterns of fish eggs and larvae, and that distinct species inhabit both zones currently proposed for conservation management at PLE. Long-term studies can be a helpful strategy to assist conservation decision making, since they provide information to understand the variability on spatial distribution of species throughout time. In conclusion, in PLE, a management plan that combines the Framework for Water Classification to information of the dynamic nature of biological communities is extremely necessary to achieve the protection of the ecosystem and its biodiversity.

Keywords: ichthyoplankton, Patos Lagoon estuary, *Micropogonias furnieri*, essential fish habitat, estuarine conservation, systematic conservation planning

1. CONTEXTUALIZAÇÃO

1.1 O estuário da Lagoa dos Patos

Os estuários são ecossistemas de elevada produtividade biológica, que aliada aos gradientes ambientais, favorecem o recrutamento e o desenvolvimento inicial de várias espécies de peixes, incluindo aquelas de interesse econômico (Mann & Lazier, 2006). Do ponto de vista das características da coluna d'água, um estuário pode ser dividido em três setores: a) com influência direta do oceano adjacente (baixo estuário); b) com marcada mistura de água salina e doce (estuário médio); e, c) dominado pela água doce (estuário superior) (McLusky & Elliott, 2006). Nesse contexto, destaca-se a Lagoa dos Patos (RS), que apresenta em seu limite sul um ecossistema estuarino de aproximadamente 1.000 Km², representando 10% da área total da laguna (Seeliger, 2001).

A Lagoa dos Patos (32°S, Brasil) é uma laguna subtropical quente do tipo estrangulada que possui uma relação significativa entre a precipitação total na bacia hidrográfica e a descarga anual de água de doce (Odebrecht et al., 2010). Cerca de 85% da água presente na bacia de drenagem do sistema Patos-Mirim é proveniente dos rios Guaíba, Camaquã e do Canal do São Gonzalo (Garcia, 1998). O deságue de água doce varia consideravelmente durante as estações do ano, principalmente em anos com a influência do El Ñino. De maneira geral, anos de El Ñino estão associados com alta pluviosidade e, consequentemente, baixa salinidade. Em anos com a influência da La Ñina o padrão encontrado é o inverso: baixa pluviosidade e alta salinidade (Odebrecht et al., 2010). A troca de água com o Oceano Atlântico adjacente ocorre através de um canal de 0,5 a 3 Km de largura, e é controlada principalmente pelo vento e a descarga de água doce, resultando numa variação do limite geográfico do estuário (Odebrecht et al., 2010; Seeliger, 2001; Garcia, 1998). A circulação, distribuição da salinidade e o nível de água no estuário da Lagoa dos Patos são controlados basicamente pelo vento, sendo predominate o regime de ventos NE-SW (Garcia, 1998).

O ecossistema estuarino da Lagoa dos Patos é composto por diferentes habitats: coluna d’água, planícies entre-marés não vegetadas (300 km^2), marismas (40 km^2), bancos de fanerógamas (120 km^2), e substratos artificiais (Seeliger, 2001). Essas características ambientais do estuário da Lagoa dos Patos (ELP) propiciam diversos habitats para uma ampla variedade de espécies (Seeliger, 2001), incluindo as de interesse econômico como a corvina *Micropogonias furnieri*, a tainha *Mugil Liza* e o camarão-rosa *Farfantepenaeus paulensis*, que dependem desse ecossistema para o sucesso no recrutamento e manutenção das populações adultas que sustentam a pesca artesanal local (Vieira & Scalabrin, 1991; Vieira, 1991; Abreu & Castello, 1998). Esta atividade entrou em declínio em 1982, e desde então é realizada em nível de subsistência (Reis & D’Incao, 2000). Além da sobrepesca, as mudanças ambientais causadas pelos eventos ENSO podem atuar de maneira sinérgica, influenciando na produção dos estoques pesqueiros locais (Möller et al., 2009; Odebrecht et al., 2010; Schroeder & Castello, 2010).

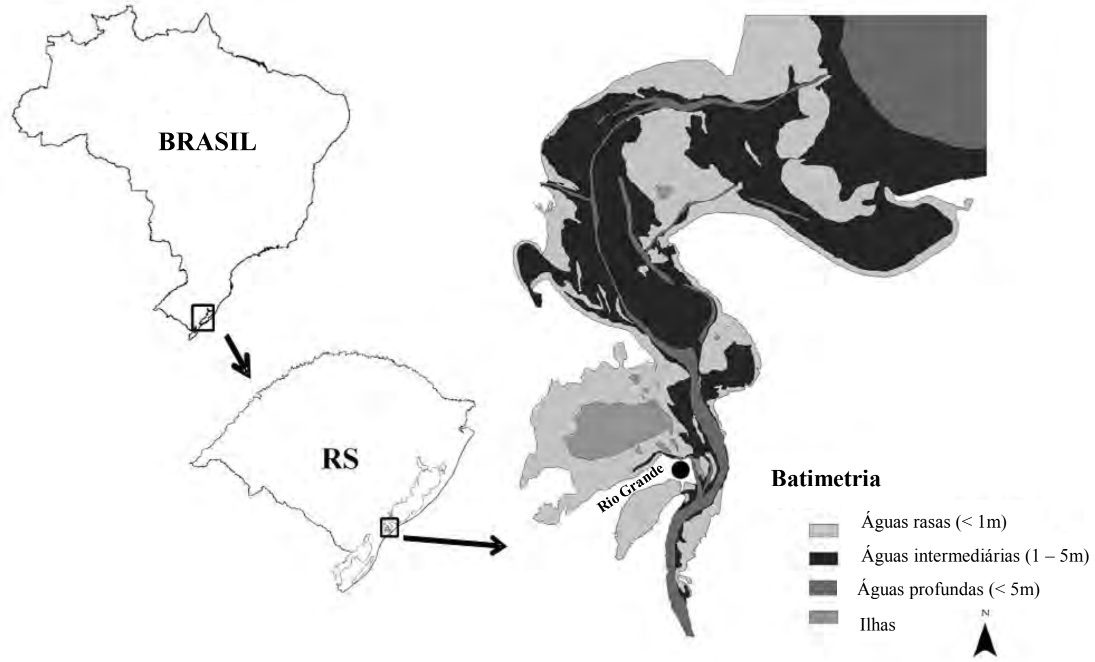


Figura 1: Localização do estuário da Lagoa dos Patos no estado do Rio Grande do Sul (Brasil), e detalhe de sua batimetria. Fonte: Adaptado de Costa et al. (2014).

Desde os trabalhos pioneiros relacionados ao ictioplâncton, ficou clara a relação existente entre a sobrevivência das larvas de peixes, o sucesso no recrutamento e a manutenção da população adulta (Houde, 2008). A variabilidade do recrutamento pode ser resultado de inúmeros processos que operam em diferentes escalas de tempo e espaço, e representa um processo integrado durante os estágios de ovo e larva (Leggett & Deblois, 1994; Houde, 2008). Associado a isso, a degradação dos habitats também afeta diretamente as diferentes fases do ciclo de vida de um peixe, e pode tomar várias formas, desde ações que impedem o uso de uma área por uma população a ações que podem alterar características importantes dos habitats, como reduzindo sua função de área de alimentação e/ou desova (Rosenberg et al., 2000). Desta forma, o conhecimento sobre o ictioplâncton se torna relevante para a conservação de ambientes estuarinos. No ELP, ovos e larvas de peixes são transportados para o interior do estuário pelas correntes de fundo, e os processos de retenção e sobrevivência desses organismos na região estuarina são dependentes da troca de água com o oceano adjacente e dos ventos predominantes (Martins et al., 2007; Muelbert & Weiss, 1991; Odebrecht et al., 2010; Sinque & Muelbert, 1997).

Os estudos sobre ictioplâncton no ELP datam da década de 1970, com o objetivo de estudar a influência da hidrodinâmica na distribuição espaço-temporal e no ciclo de vida das espécies (Muelbert et al. 2010), e incluem os trabalhos de Weiss (1981), Mafalda Jr. (1989), Muelbert & Weiss (1991), Ibagy (1997), Bruno & Muelbert (2009), Filgueras (2009) e Schmitt (2010). Apesar de estudos sobre a distribuição e a influência dos fatores físicos no ictioplâncton serem desenvolvidos em vários sistemas estuarinos no Brasil, ainda são escassos os trabalhos que relacionam a distribuição dos ovos e larvas no plâncton com os diferentes habitats estuarinos e com a abundância de juvenis das diferentes espécies, assim como sua importância para a conservação.

1.2 Conservação estuarina

As áreas marinhas protegidas (AMP) surgem no cenário atual como potencial ferramenta na conservação da biodiversidade e manejo pesqueiro, pois podem prevenir o colapso de estoques, permitir o aumento da produção larval (Johnson et al., 1999; Botsford et al., 2003), e proteger regiões críticas como áreas de berçário, de desova e de alta diversidade de espécies (Allison et al., 1998). Com base nos trabalhos realizados por Sumaila (1998), Bohnsack (1999), e Aburto-Oropeza et al. (2011), pode-se citar como os potenciais benefícios da AMP: 1) aumento, proteção e exportação da biomassa, ou seja, como a produção aumenta, ocorre a dispersão de ovos e larvas de maiores classes de tamanho, sendo fonte de fornecimento de recrutamento para a área adjacente; 2) ajuda no restabelecimento do estoque pesqueiro em áreas de pescaria através de emigração de juvenis e adultos; 3) manutenção da estrutura etária da população natural; 4) segurança contra o fracasso do manejo em áreas de pescaria; e, 5) proteção da qualidade genética. Desta maneira, áreas que abrigam estágios de vida vulneráveis (e.g. ictioplâncton) devem ser consideradas como prioritárias para a conservação. Trabalhos que apresentam os potenciais benefícios de uma área estuarina protegida são escassos, e incluem os trabalhos desenvolvidos na "Kennedy Space Center Reserve" para a ictiofauna (Johnson et al., 1999) e para o ictioplâncton (Reyier et al., 2008).

Além das AMP, a aplicação do conceito de *Essential Fish Habitat* (EFH) dentro do contexto do manejo ecossistêmico, surge como uma solução para minimizar a perda de habitat devido a crescente atividade antrópica em regiões costeiras e estuarinas (Rosenberg et al., 2000). O conceito de EFH tem como base a relação entre a qualidade do habitat e a produtividade pesqueira, sendo definido como "*águas e substratos necessários para uma determinada espécie de peixe para desova, criação, alimentação e/ou crescimento até a maturidade*", e desde 1996 faz parte do "*Sustainable Fisheries Act*" do governo norte-americano (Koenig et al., 2000; Rosenberg et al., 2000; Levin & Stunz, 2005). Assim, a identificação dos principais habitats utilizados por uma determinada espécie durante seu ciclo de vida, e a influência das variáveis ambientais na conectividade entre esses habitats é essencial para a aplicação de medidas de manejo, visando a conservação dos ecossistemas e estoques pesqueiros (Barletta et al., 2010).

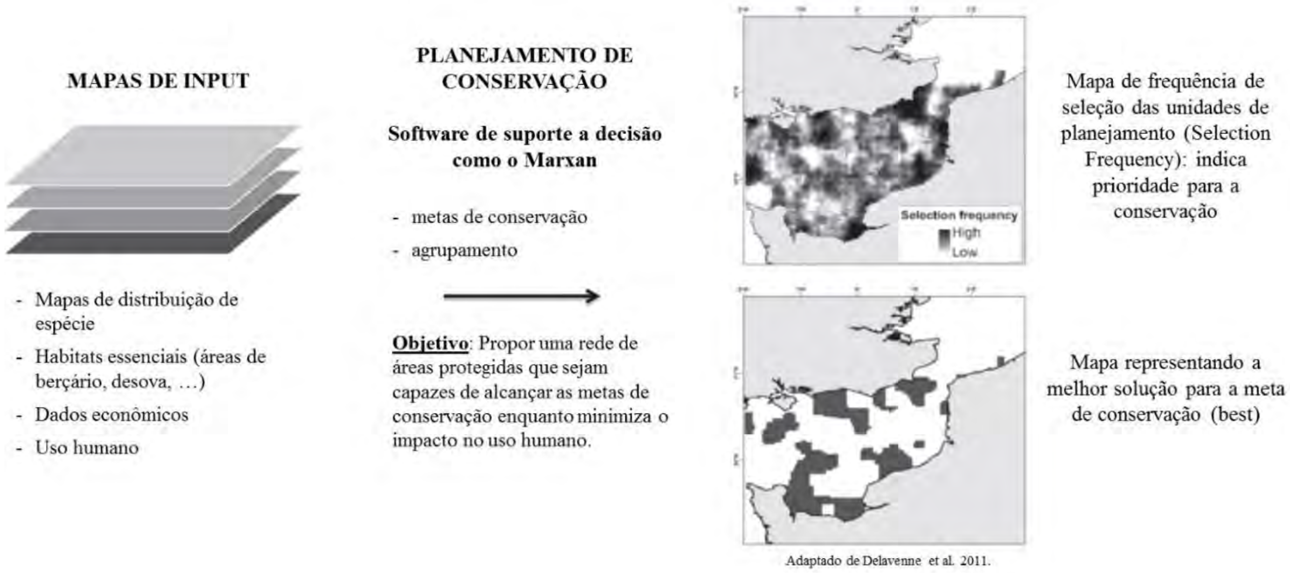
Nesse contexto, *habitats essenciais* podem ser identificados através dos padrões de utilização de diferentes áreas pela espécie, considerando sua variabilidade na abundância dentre os diferentes estágios de desenvolvimento. Para o ELP, a corvina *M. furnieri*, devido a quantidade de informações já publicadas, e por ser uma espécie que utiliza este estuário de maneira sistemática durante toda sua ontogenia (Vieira et al., 1998), foi escolhida como modelo para a identificação de *habitats essenciais* no ELP.

No Brasil, como em grande parte do mundo, as áreas protegidas são estabelecidas de maneira que, por vezes, os locais escolhidos nem sempre são os de maior interesse para a conservação da biodiversidade e/ou para o incremento de pescarias (Margules & Pressey, 2000; Roberts, 2000; WWF, 2011). Nesse cenário, surge o planejamento sistemático para a conservação (PSC, Figura 2), o qual é uma disciplina que envolve aspectos biológicos, matemáticos e sociais (Possingham et al., 2006), que visa identificar diferentes configurações de áreas complementares para alcançar os objetivos de conservação (Pressey et al., 2007). Os principais objetivos do PSC são: 1) representação adequada de todos os componentes da biodiversidade na área estudada; 2) assegurar a persistência da biodiversidade no futuro; e, 3) alcançar esses objetivos com a maior economia de recursos possível (Sakar & Illoldi-Rangel, 2010). Uma vez que informações sobre a biodiversidade total de uma área geralmente são incompletas, os objetos de conservação utilizados no PSC agem como representantes para a biodiversidade local (Possingham et al., 2006; Shokri & Gladstone, 2009). Considerando as informações disponíveis para o ELP, a presente tese utilizou como indicativo da biodiversidade o ictioplâncton. Com este enfoque, o objetivo foi garantir a conservação dos habitats estuarinos utilizados pelos estágios iniciais de peixes no ELP. Nesta tese, estágios inciais de peixes referem-se aos ovos, larvas e juvenis de peixes, sendo que dependendo do objetivo analisado estes estágios podem contemplar somente o ictioplâncton.

No ELP, como em diversos ecossistemas estuarinos no mundo, os múltiplos interesses associados a diversas atividades humanas tornam a zona costeira mais vulnerável aos impactos antrópicos (Niencheski et al., 2006; Tagliani et al., 2007), fazendo necessária a implantação de medidas de recuperação e conservação da produtividade e integridade dos recursos (Tagliani et al., 2003). Estudos pretéritos na região (Asmus et al., 1988; Asmus et al., 1989) classificaram, através de mapeamentos temáticos, as diferentes áreas de utilização no ELP em três categorias: zonas de preservação ambiental (sem uso), zonas de conservação (uso limitado e controlado) e zonas de desenvolvimento, sendo possível identificar as zonas de conflito de uso. Apesar de o Plano Ambiental do município de Rio Grande contemplar o Zoneamento Ecológico-Econômico Municipal (ZEEM) baseado nessas três categorias, e apresentar o enquadramento das águas incorporado no ZEEM (Tagliani & Asmus, 2011), a área da Lagoa dos Patos ainda carece de informações no sentido da identificação de áreas prioritárias para a conservação, e que considerem a dinâmica do ecossistema no planejamento. Em especial, informações sobre o uso deste ambiente pelos primeiros estágios de vida de peixes.

Considerando que métodos quantitativos para a identificação de áreas priotitárias para a conservação já terem sido amplamente aplicadas para os ambientes terrestres e marinhos (Moilanen et al., 2009; Pittman et al., 2011), a priorização espacial em estuários está em sua infância quando comparada com estes ecossistemas. Poucos estudos aplicaram o planejamento sistemático para a conservação em estuários (Neely & Zajac, 2008; Geselbracht et al., 2009; Shokri & Gladstone, 2009; Shokri et al., 2009; Shokri & Glastone, 2013a; Shokri & Gladstone, 2013b), sendo que a maioria destes teve por objetivo avaliar o “proxy” da biodiversidade estuarina para a seleção de áreas protegidas em estuários (Shokri et al., 2009; Shokri &

Gladstone, 2009; Shokri & Gladstone, 2013a). Dentre eles, somente dois estudos utilizaram uma ferramenta de suporte a decisão para a conservação (Neely & Zajac, 2008; Geselbracht et al., 2009). Um conceito importante é que processos ecológicos em estuários podem interagir através da interface oceano-continente, criando um padrão espacial complexo e dinâmico para muitos fatores (e.g. biológicos, físicos, químicos, e sócio-econômicos), e em diferentes escalas de tempo e espaço (Pittman et al., 2011). A alta heterogeneidade espacial e variabilidade que pode ser encontrada nos estuários significa que estratégias de manejo devem incorporar a dinâmica ecológica numa abordagem espacial (Pittman et al., 2011). Além da heterogeneidade espacial, as espécies que habitam os estuários e ambientes costeiros também podem ser afetados pela própria sazonalidade e por eventos climáticos, como o *El Niño* e *La Niña*. No caso do ELP, sabe-se que estes eventos tem um grande influência na ocorrência e distribuição de diversas espécies, uma vez que estes alteram a precipitação, e consequentemente, os gradientes de salinidade do estuário. Entretanto, a priorização espacial é tratada como um problema estático, ignorando a dinâmica dos fatores (Moilanen et al., 2009). Para ecossistemas dinâmicos, como os estuários, a análise temporal dos dados é um importante componente que deve ser integrado no planejamento sistemático para a conservação, e vital para a implementação de estratégias de manejo.



- Mapas de distribuição de espécie
- Habitats essenciais (áreas de berçário, desova, ...)
- Dados econômicos
- Uso humano

Software de suporte a decisão como o Marxan

- metas de conservação
- agrupamento

Objetivo: Propor uma rede de áreas protegidas que sejam capazes de alcançar as metas de conservação enquanto minimiza o impacto no uso humano.

Mapa de frequência de seleção das unidades de planejamento (Selection Frequency): indica prioridade para a conservação

Mapa representando a melhor solução para a meta de conservação (best)

Adaptado de Delavenne et al. 2011.

Adaptado de Le Pape et al., 2014.

Figura 2: Procedimento geral utilizado para analisar cenários utilizando ferramentas de suporte a decisão para o planejamento da conservação. Neste exemplo, os resultados foram produzidos no software Marxan. Fonte: Adaptado de Le Pape et al., 2014.

1.3 Perguntas e Hipóteses

Em virtude do pouco conhecimento a respeito de áreas essenciais para a conservação em estuários e da relação entre a distribuição da abundância de larvas e de juvenis de peixes, é essencial testar a incorporararação de informações sobre as primeiras fases do ciclo de vida dos peixes no planejamento para a conservação. No presente estudo, o conceito de "*habitat essencial (essential fish habitat)*" foi aplicado para a corvina *M. furnieri*, visando a identificação de padrões de ocupação do habitat pela espécie por diferentes estágios do seu ciclo de vida, e a relação destes estágios de vida com as variáveis ambientais. Além disso, técnicas de priorização espacial para a conservação baseada em informações de distribuição espacial de ovos e larvas das espécies de peixes, vegetação aquática submersa, sedimento e batimetria, foram utilizadas visando a identificação das áreas prioritárias para a conservação no ELP. Devido a importância dos primeiros estágios de vida na regulação das populações de peixes, o trabalho parte da hipótese de que a inclusão de dados de ictioplâncton gera diferenças nas prioridades espaciais para a conservação.

Nesse contexto, as hipóteses do trabalho foram:

1) existe uma correlação entre os diferentes estágios de vida da corvina e os habitats no estuário da Lagoa dos Patos;
2) cada estágio de desenvolvimento (e.g. ovo, larva e juvenil) da corvina *M. furnieri* possui um habitat essencial específico e está correlacionado com diferentes variáveis ambientais;
3) a inclusão de dados de ictioplâncton gera diferenças na prioridade de planejamento espacial para a conservação;
4) a assembleia ictioplanctônica das áreas rasas do ELP apresenta variabilidade temporal e espacial na abundância e composição das espécies, as quais influenciam as ações de conservação.

1.4 Objetivos

A tese teve como objetivo principal testar a importância da incorporação de informações sobre os primeiros estágios de vida dos peixes no planejamento para a conservação em estuários.

Com isso, os objetivos específicos foram:

a) analisar a relação entre a abundância de ovos, larvas e juvenis de *Micropogonias furnieri* no ELP;
b) identificar os *habitats essenciais* para cada estágio de desenvolvimento da corvina *M. furnieri* no ELP;
c) testar se um plano de conservação baseado em zonas altera as prioridades espaciais em relação a um plano de conservação simples (sem zonas) para estuários;

d) definir áreas prioritárias para a conservação de peixes no ELP com base nos primeiros estágios de vida;

e) analisar a longo prazo a abundância e composição da assembleia ictioplanctônica nas áreas rasas do ELP, e se estes resultados podem auxiliar nos planos de manejo.

2. ANÁLISE

Os resultados deste trabalho foram estruturados na forma de sequência de artigos científicos, os quais se encontram disponíveis como apêndice nesta tese. A apresentação principais resultados, das peculiaridades metodológicas e discussão foi dividida em três capítulos principais. Um resumo do banco de dados utilizados e metodologia de coleta empregada em cada artigo científico encontra-se na Tabela 1. Cada capítulo apresenta também o elo entre os resultados e o contexto em que se encontram na tese. Visando facilitar o entendimento do trabalho, a descrição dos detalhes técnicos sobre a metodologia utilizada ficará restrita ao texto de cada artigo incluído no apêndice. Portanto, para a compreensão da tese é fundamental a leitura completa dos apêndices.

Tabela 1: Resumo dos dados utilizados em cada artigo científico produzido na presente tese. (*): arrastos de fundo foram realizados em áreas profundas (profundidade de 4 a 14 metros); arrastos de meia-água foram realizados em áreas com mais de 8 metros de profundidade, e arrastos de praia foram realizados em estações localizadas em águas rasas (profundidade média de 1,5 m).

Capítulo 1: ***A corvina Micropogonias furnieri e seu habitat essencial no estuário da Lagoa dos Patos***

Artigo 1: Estuarine early life stage occupancy patterns of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) from the Patos Lagoon, Brazil.
Artigo 2: Dealing with temporal variation and different life stages of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) in species distribution modeling to improve estuarine essential fish habitat identification
Artigo 3: Ensuring the protection of an estuarine essential fish habitat by spatial conservation prioritization analysis with different management zones

Dados	**Ictioplâncton**		**Juvenis e adultos**	
Período não-contínuo de 11 anos	Período 1: Dezembro de 1975 até Fevereiro de 1978 Período 2: Abril de 1981 até Fevereiro de 1983		Período 1: Dezembro de 1975 até Fevereiro de 1978 Período 2: Janeiro de 1979 até Outubro de 1974	
Coleta: periodicidade, número de estações por cruzeiro, e profundidade	Período 1: quinzenal e/ou mensal; 20 a 30; e arrastos de superfície	Período 2: quinzenal; 5; e arrastos estratificados	Período 1: quinzenal e/ou mensal; 20 a 30; limitada pelo calado da embarcação	Período 2: mensal; variável (cruzeiro durava mais que um dia); dependente do do tipo do arrasto(*)

Capítulo 2: ***Planejamento espacial eficiente para a conservação do estuário da Lagoa dos Patos***

Artigo 4: Incorporating early life stages of fishes into estuarine spatial conservation planning

Dados	**Ictioplâncton**
	Dezembro de 1975 até Fevereiro de 1978
Coleta: periodicidade, número de estações por cruzeiro, e profundidade	quinzenal e/ou mensal; 20 a 30; e arrastos de superfície

Capítulo 3: *Influência da variabilidade estuarina nas ações de conservação*

Artigo 6: Long-term assessment of temporal variability in spatial patterns of early life stages of fishes to facilitate estuarine conservation actions

Dados	**Ictioplâncton**
	Janeiro de 2000 até Dezembro de 2012
Coleta: periodicidade, número de estações por cruzeiro, e profundidade	mensal; 5; e, arrastos de superfície

2.1 *Capítulo 1: A corvina Micropogonias furnieri e seu habitat essencial no estuário da Lagoa dos Patos*

Este capítulo é composto pelos seguintes artigos:

- Costa, M.D.P., Muelbert, J.H., Moraes, L.E., Vieira, J.P. & Castello, J.P. 2013. Estuarine early life stage occupancy patterns of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) from the Patos Lagoon, Brazil. Fisheries Research, 160, 77-84 **(Apêndice 1)**
- Costa, M.D.P., Muelbert, J.H., Vieira, J.P. & Castello, J.P. Dealing with temporal variation and different life stages of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) in species distribution modeling to improve estuarine essential fish habitat identification. Submetido para a Hydrobiologia **(Apêndice 2)**
- Costa, M.D.P., Possingham, H.P., Muelbert, J.H., Vieira, J.P., Castello, J.P., Copertino, M.S. & Tagliani, P.R.A. Ensuring the protection of an estuarine essential fish habitat by spatial conservation prioritization analysis with different management zones **(Apêndice 3)**

A corvina *Micropogonias furnieri* é uma das principais espécie-alvo da atividade pesqueira na costa do Brasil. Ao longo do seu ciclo de vida, a corvina habita águas marinha, doce e salobra, e pode utilizar diferentes habitats estuarinos para completar seu desenvolvimento. Os padrões de distribuição espacial e temporal da abundância da corvina durante os diferentes estágios de vida já foram descritos para diversos ecossistemas (Costa & Araújo, 2003; Giannini & Paiva Filho, 1990; González-Sansón et al., 1996), incluindo a Lagoa dos Patos (Castello, 1986; Vieira, 2006). Neste ecossistema estuarino, a espécie ocorre durante todo o ano, podendo ser registrado todos os seus estágios de desenvolvimento. Apesar das diversas informações existentes sobre a corvina no estuário da Lagos dos Patos ou em outros ambientes, ainda existe uma lacuna de estudos que combinem as informações de ovos, larvas e juvenis da espécie. Com isso, o estudo apresentado no **apêndice 1** teve como principal objetivo investigar o padrão de distribuição espacial de diferentes estágios de desenvolvimento da corvina no estuário da Lagoa dos Patos, sendo o primeiro estudo a analisar o padrão de ocupação desde os ovos até indivíduos maiores que 160 mm da espécie em um estuário.

Os resultados demostraram que cada estágio de desenvolvimento está correlacionado com um habitat estuarino, e associado com diferentes variáveis ambientais, as quais podem influenciar sua ocorrência no estuário. De maneira geral, os ovos foram associados com o ambiente mais costeiro, enquanto que as larvas foram abundantes em todo o canal estuarino. Juvenis de diversos tamanhos exibiram um padrão espacial bem definido: indivíduos menores que 30 mm foram associados com as áreas de canal, indivíduos entre 30 – 160 mm foram associados principalmente com águas rasas e profundas, enquanto que indivíduos maiores que 160 mm foram relacionados com as águas profundas. Uma abordagem integrada como a que foi utilizada no apêndice 1 é importante para melhor compreender a ecologia da corvina e sobre os diferentes habitats que são requeridos pela espécie ao longo de seu desenvolvimento, sendo um primeiro passo para a identificação do habitat essencial da espécie. Esses resultados mostraram uma ocupação complexa do habitat estuarino pela corvina durante cada estágio de vida, o que tem implicações para a ampliação do conhecimento sobre a espécie, assim como para sua conservação e manejo.

A compreensão completa do uso do habitat por uma espécie durante todo o seu ciclo de vida é essencial para se aprimorar as medidas de manejo e conservação. Atualmente, existem diversos métodos que podem ser utilizados para a identificação do *habitat essencial* de uma espécie, incluindo a análise de padrões de densidade em cada habitat, índices e modelagem da distribuição das espécies (Benaka, 1999; Valavanis, 2008). Os resultados obtidos a partir dessas metodologias podem ser utilizados para gerar mapas de adequação de habitat, os quais refletem a relação entre os descritores do ambiente e a distribuição espacial da espécie (Le Pape et al., 2014). Atualmente, a modelagem da distribuição das espécies é uma das principais ferramentas utilizadas para a identificação de habitats essenciais (Valavanis, 2008). Independente da abordagem estatística, a modelagem de distribuição da espécie tem como objetivo predizer a distribuição de uma espécie numa determinada região e estimar a relação entre o registro da espécie e as variáveis preditores (Guisan & Zimmermann, 2000; Hirzel & Le Lay, 2008; Elith & Leathwick, 2009), assumindo que a distribuição observada de uma espécie reflete seus requerimentos ecológicos (Hirzel & Le Lay, 2008).

No sentido de ampliar o conhecimento para a conservação dos diferentes habitats requeridos pela corvina para seu desenvolvimento, o estudo apresentado no **apêndice 2** teve como principal objetivo testar a hipótese de que a preferência de habitat e a variabilidade na ocorrência durante o ciclo de vida influencia os resultados dos modelos em estudos de distribuição das espécies. Se correta, a inclusão dessas informações na modelagem irá aprimorar a identificação dos habitats essenciais e o planejamento da conservação em estuários. Nossa pergunta é: a incorporação de diferentes estágios do ciclo de vida pode afetar os resultados finais da identificação dos habitats essenciais? Na maioria das vezes, os *habitats essenciais* para uma espécie são identificados somente com informações sobre juvenis ou adultos, sem considerar informações sobre ovos e larvas, e consequentemente, sem uma visão integrada dos diferentes habitats necessários para a espécie completar seu ciclo de vida. Isso é particularmente importante para espécies como a corvina, a qual muda de uma vida basicamente planctônica durante seus estágios iniciais para uma vida demersal ao longo do seu desenvolvimento. Neste caso, foi utilizada a abordagem de modelagem de distribuição da espécie para avaliar a influência da variação temporal na ocorrência e na preferência de habitat durante diferentes estágios do ciclo de vida da corvina no ELP. Além disso, analisar se a adição dos diferentes estágios do ciclo de vida podem influenciar nos resultados espaciais do habitat essencial. Nesse sentido, foram testados dois cenários: 1) considerando todos os dados de presença da espécies, independente do estágio de vida e da variação temporal; e 2) considerando a ocorrência de cada estágio de vida separadamente para cada estação do ano. Nesse último caso, foi testado um modelo considerando juvenis e adultos independente da sua classe de tamanho, e outro considerando cada classe de tamanho separadamente. Em todos os cenários foi possível analisar a influência das variáveis ambientais na distribuição da corvina no ELP, além de considerar a natureza dinâmica do ecossistema do ELP através da modelagem da distribuição para cada estágio de desenvolvimento em cada estação do ano. A performance do modelo foi analisada através da metodologia “True Skill Statistic” (TSS, Hanssen & Kuipers, 1965; Allouche et al., 2006). Os valores do TSS podem ser classificados em: 0 – 0,4: pobre; 0,4 – 0.5: razoável; 0,5 – 0,7: bom; 0,7 – 0,85: muito bom; 0.85 – 0,9: excelente; e de 0,9 – 1,0: perfeito. Em geral, a performance preditiva dos modelos foram maior do que 0,4, indicando que predição variou de razoável (0,4 – 0,5) a muito boa (0,7 – 0,85). O cenário 1, considerando todos os dados de presença da corvina no ELP, independente do estágio de desenvolvimento, apresentou uma performance preditiva boa (0,56). Entretanto, quando consideramos o efeito da variabilidade temporal para cada estágio de desenvolvimento (cenário 2), podemos observar que 64% dos modelos alcançaram uma melhor performance preditiva. Nesse caso, houveram somente 3 exceções onde o TSS foi menor que 0,4. Nesse sentido, assumiu-se que os modelos considerando o impacto da variabilidade temporal na presença e distribuição dos diferentes estágios de vida podem melhorar os resultados dos modelos. Consequentemente, os resultados apresentados nesse estudo são baseados nos modelos do cenário 2. Esses resultados mostraram que considerar diferentes estágios de vida e estações do ano separadamente nos modelos auxiliam a considerar as variações espaciais e temporais na distribuição de cada estágio de desenvolvimento da espécie. Além disso, foi possível observar que diferentes variáveis ambientais podem influenciar a ocorrência de cada estágio de vida no estuário da Lagoa dos Patos. Independente do estágio de desenvolvimento, a temperatura e a salinidade foram as variáveis que mais contribuíram para os modelos.

Apesar das diferentes metodologias e ferramentas para a identificação dos *habitats essenciais*, nenhuma delas permite identificar as prioridades espaciais para a conservação e que integre diferentes fatores. A priorização espacial para a conservação é importante principalmente para aqueles ambientes com usuários múltiplos, como os estuários, e para espécies com ampla distribuição no ambiente, como a corvina. A priorização considera o custo de uma ação para a conservação. Este custo não é uniformemente distribuído no espaço, e existem diferentes tipos de custos relacionados com ações para a conservação (Wilson et al., 2009). Dentre eles, o custo de oportunidade representa o benefício perdido quando uma ação impossibilita uma atividade rentável que poderia ocorrer num determinado local, por exemplo, quando uma determinada área que é utilizada para a pesca é selecionada como área de proteção (Wilson et al., 2009). Em ambientes marinhos e costeiros, o custo de oportunidade geralmente é representado pela renda da atividade pesqueira que será perdida caso a área seja declarada como área protegida. A aplicação dessa abordagem em análises de planejamento para a conservação tem sido testada em diversos estudos, os quais mostram que a incorporação dessa informação auxilia a alcançar resultados mais custo-efetivos do que quando o custo é negligenciado na análise (Naidoo et al., 2006; Klein et al., 2008; Carwardine et al., 2008; Ban & Klein, 2009; Mazor et al., 2014). Nesse contexto, o principal objetivo do estudo apresentado no **apêndice 3** foi estabelecer um plano de conservação para o ambiente estuarino da Lagoa dos Patos, assegurando a proteção do habitat essencial da corvina. Nesse caso, nossa principal pergunta era se a adição de mais zonas no plano de conservação altera as prioridades espaciais de conservação. Este foi o primeiro estudo a incorporar dados de diferentes estágios do ciclo de vida de uma espécie em uma ferramenta de suporte de decisão para a conservação para priorizar espacialmente a proteção do habitat essencial de uma espécie. Neste sentido, foram testados três planos de conservação baseados nas categorias de zoneamento da IUCN para áreas marinhas protegidas (Dudley, 2008): 1) plano I: sem zoneamento; 2) plano II: incluindo três zonas: zona de snatuário, zona de proteção do habitat, e zona de múltiplo uso; e, 3) plano III: com quatro zonas, incluindo uma zona de santuário, zona de proteção do habitat I, zona de proteção do habitat II, e zona de múltiplo uso. Para cada plano foram testados diferentes cenários baseados na meta de conservação (15% e 30%) e custo de oportunidade. Nesse caso, o custo de oportunidade foi estimado baseado na pesca artesanal de peixes e crustáceos do ELP. A análise dos resultados de cada plano de conservação foi realizada atráves da frequência de seleção de cada área do ELP para uma determinaza zona, e para comparar as soluções testadas para cada zona foi realizada uma análise de cluster. De maneira geral, nossos resultados não foram sensíveis aos custos de oportunidade testados. Os resultados mostraram que independente do custo de oportunidade e da meta de conservação, a incorporação de mais zonas em um plano de conservação não altera as prioridades espaciais para a conservação. Com isso, sugerimos que um sistema de zoneamento pode ser inserido futuramente em planos de conservação.Além disso, também mostramos que um plano de conservação pode assegurar a proteção de um *habitat essencial* através da incorporação de diferentes estágios de desenvolvimento de uma mesma espécie. Metas de conservação distintas produziram diferentes soluções para a zona de santuário, entretanto, não influenciaram as soluções para as demais zonas. No caso da corvina no estuário da Lagoa dos Patos, as águas rasas são as principais áreas para a proteção do habitat essencial da corvina. Além da corvina, outras espécies como o camarão-rosa (*Farfantepenaus paulensis*) utilizam essas áreas rasas para seu desenvolvimento e recrutamento, reforçando a necessidade de que essas áreas sejam consideradas em futuras ações de manejo no ELP visando a manutenção do recrutamento e da população adulta dessas espécies.

2.2 Capítulo 2: Planejamento espacial eficiente para a conservação do estuário da Lagoa dos Patos

Este capítulo é composto pelos seguintes artigos:

- Costa, M.D.P., Possingham, H.P. & Muelbert, J.H. Incorporating early life stages of fishes into estuarine spatial conservation planning. Submetido para o Aquatic Conservation: Marine and Freshwater Ecosystems **(Apêndice 4)**

- Costa, M.D.P. & Muelbert, J.H. Efficient estuarine conservation planning: taking into account the dynamic nature of the ecosystem. Submetido para o Brazilian Journal of Nature Conservation **(Apêndice 5)**

Muitas espécies alteram os habitats necessários para completar seu desenvolvimento ao longo do seu ciclo de vida, resultando em distribuições espaciais distintas para cada estágio de desenvolvimento. Essa alta conectividade e variação temporal pode ter grande influência na eficiência de uma rede de áreas protegidas em alcançar seus objetivos de conservação. Desta maneira, para um eficiente planejamento de conservação em estuários é essencial considerar sua natureza dinâmica e a alteração de habitats ao longo dos diferentes estágios de desenvolvimento da espécie e de sua variabilidade temporal. Neste sentido, sugerimos que os padrões de distribuição e composição de ovos e larvas de peixes podem ser um bom “proxy” para considerar a dinâmica na conservação estuarina, uma vez que diferentes assembleias de ictioplâncton caracterizam diferentes regimes hidrográficos (Muelbert & Weiss, 1991).

A maioria das espécies de peixes que dependem dos estuários para completar seu desenvolvimento apresentam estágios iniciais de vida planctônicos enquanto que juvenis e adultos da mesma espécie geralmente dependem de habitats diferentes, e.g. demersal. Portanto, a proteção integral da espécie depende da proteção dos diferentes estágios de vida, e isto é essencial para garantir o sucesso no processo de recrutamento e a manutenção da população adulta. Nesse contexto, o objetivo do estudo apresentado no **apêndice 4** foi avaliar a importância de se incorporar dados de ovos e larvas de peixes numa abordagem de planejamento sistemático para a conservação do estuário da Lagoa dos Patos. Nesse sentido, nossa principal pergunta era se a inclusão de dados de ovos e larvas de peixes afeta os resultados do planejamento para a conservação, e como estes são afetados pela incorporação do custo de oportunidade.

Nesse estudo foram testados duas classificações de conservação: uma onde todas as unidades de planejamento no estuário estão disponíveis para a conservação, e outra onde unidades de planejamento classificadas como Classe C segundo a resolução CONAMA não estão disponíveis para a conservação. Essa segunda classificação representa que águas utilizadas para usos multiplos, principalmente navegação, não estariam disponíveis para comporem as áreas protegidas no estuário. Além disso, também foram testadas duas metas de conservação, 30% e 50% para a criação de áreas protegidas com e sem as informações da distribuição espacial de ovos e larvas de peixes. Para este fim, foi utilizado o software de suporte a decisão Marxan, o qual tem por objetivo alcançar as metas de conservação ao menor custo (Ball et al., 2009). Os resultados formaram 2 grupos separando os cenários com e sem dados de ictioplâncton indicando que incorporar essas informações gera um grande impacto nas prioridades espaciais para a conservação no estuário da Lagoa dos Patos. Além disso, foi possível observar que independentemente do custo de oportunidade as prioridades espaciais foram significativamente afetadas pela inclusão dos dados de ovos e larvas de peixes. As águas rasas foram selecionadas como as áreas mais importantes para a conservação, independente de como foi calculado o custo de oportunidade. A abordagem utilizada nesse trabalho foi a primeira a combinar estimativas de dados espaciais da pesca artesanal com um plano de conservação que incorpora dados sobre os estágios iniciais de peixes. No caso do estuário da

Lagoa dos Patos, as águas rasas foram as mais importantes para se atingir as metas de conservação.

A abordagem de manejo baseado no ecossistema e planejamento espacial marinho emergiu como uma estratégia para a gestão dos usos múltiplos do ambiente de maneira sustentável, tornando-se uma realidade em muitos países. Os estuários estão entre os ecossistemas costeiros mais produtivos, e devido a sua alta importância social e econômica, também está entre os ecossistemas costeiros mais impactados por atividades antrópicas. Uma das características mais importantes deste sistema é que os processos podem interagir ao longo da interface continente – oceano, criando um padrão dinâmico e complexo para muitos componentes em diferentes escalas de tempo e espaço. Neste contexto, o trabalho apresentado do **apêndice 5** traz uma revisão sobre como lidar com a natureza dinâmica dos estuários no planejamento para a conservação. A depleção dos recursos estuarinos e costeiros, e a alteração da estrutura, funções, e processos ecológicos através das atividades humanas se tornaram bem mais aparentes. Como resultado disso, aumentou-se a demanda por dados espaciais que representassem os atributos do ecossistema, incluindo características ecológicas e sociais, para a produção de resultados eficientes e custo-efetivos de priorização espacial. Um dos maiores desafios para o planejamento espacial para regiões costeiras é a incorporação de atributos que reflitam a dinâmica do ecossistema. Neste trabalho, sugerimos que a inclusão da variação temporal pode ser uma boa alternativa para resolver o problema da priorização espacial estática. Neste sentido, os monitoramentos de longo-prazo são uma boa estratégia para aumentar a disponibilidade de dados nas escalas temporais e espaciais adequadas. Essa informação irá permitir que pesquisadores e tomadores de decisão tenham um conhecimento maior sobre as causas de variações, identificar áreas de interesse especial, predizer a distribuição e os efeitos de alterações ambientais na biodiversidade, e estabelecer níveis de proteção apropriados, permitindo um aumento na eficiência do planejamento para a conservação. Estudos futuros sobre priorização espacial em estuários devem testar diferentes atributos como “proxy” para representar a alta variabilidade destes ecossistemas, e considerar as diferentes condições do habitat e a conectividade entre os habitats.

2.3 Capítulo 3: Influência da variabilidade estuarina nas ações de conservação

Este capítulo é representado pelo artigo:

Costa, M.D.P. & Muelbert, J.H. Long-term assessment of temporal variability in spatial patterns of early life stages of fishes to facilitate estuarine conservation actions **(Apêndice 6)**

Os resultados anteriores destacam a importância da inclusão de informações sobre ovos e larvas de peixes para a conservação estuarina, e a importância das águas rasas do estuário da Lagoa dos Patos para os estágios iniciais de peixes, e que estas devem estar incluídas dentro dos planos de manejo da região. Nesse sentido, o trabalho apresentado no **apêndice 6** tem como principal objetivo analisar se alterações de longo prazo sobre a composição e abundância de ovos e larvas de peixes em águas rasas do estuário da Lagoa dos Patos influenciam as ações de conservação em ecossistemas estuarinos. Neste sentido, a hipótese foi de que a variabilidade temporal do ictioplâncton resulta em padrões espaciais que afetam as ações para a conservação em estuários. Para isso, comparamos a distribuição de ovos e larvas de peixes em cinco pontos amostrais com a classificação do enquadramento das águas proposta pelo CONAMA (2005), sendo essa classificação a única ação de conservação implementada para a região estuarina no Plano Ambiental de Rio Grande (Tagliani & Asmus, 2011). A análise de agrupamento foi utilizada para analisar os padrões espaciais de distribuição de ovos e larvas durante o período do estudo, e identificar as espécies indicadoras dos grupos formados. Além disso, foi utilizado um modelo linear generalizado para analisar a variabilidade da abundância de ovos e larvas de peixes em relação com as variáveis ambientais. Os resultados mostraram que ovos e larvas de peixes no estuário da Lagoa dos Patos exibiram uma alta variabilidade entre meses, anos, e pontos amostrais, os quais podem ser associados principalmente com o gradiente de salinidade e com o local do ponto amostral no estuário. Além disso, também foi possível observar uma alta variabilidade nos padrões de distribuição espacial dos ovos e larvas de peixes, e que espécies distintas ocupam as duas diferentes zonas propostas dentro do enquadramento das águas. Devido a natureza dinâmica destes ecossistemas, os processos ecológicos podem criar um padrão espacial complexo para vários componentes biológicos, físicos e químicos, os quais podem influenciar na distribuição e ocorrência de muitas espécies. A alta variabilidade associada aos estuários, tanto em escala temporal quanto espacial, torna necessários que planos de manejo incorporem essa dinâmica em suas estratégias de conservação. Nesse sentido, estudos de longa duração são uma boa estratégia para auxiliar as tomadas de decisão para a conservação, uma vez que possibilitam o entendimento das variações nos padrões espaciais de distribuição das espécies ao longo do tempo. No caso do estuário da Lagoa dos Patos, um plano de manejo que combine o enquadramento das águas e informações da comunidade biológica (como dados sobre estágios iniciais de peixes) é extremamente necessário para a proteção do ecossistema e de sua biodiversidade.

3. CONSIDERAÇÕES FINAIS

Variabilidade ontogenética, dinânmica e conservação

Como todo sistema estuarino, o estuário da Lagoa dos Patos (ELP), é composto por um complexo mosaico de habitats, os quais estão sujeitos a diferentes interações físicas, químicas e biológicas (Pihl et al., 2002). A seleção de um determinado habitat estuarino por uma espécie pode estar relacionada a diferentes fatores, como a disponibilidade e complexidade estrutural, abundância de presas e predadores, processos de transporte e condições ambientais locais (Blaber & Blaber, 1980; França et al., 2009). A identificação dos habitats utilizados pelos peixes durante seu ciclo de vida, além das variáveis ambientais que influenciam sua ocorrência e distribuição, é extremamente importante para o estudo da dinâmica de populações, coonservação e manejo, além de auxiliar a identificar *habitats essenciais* e a também no desing de áreas protegidas marinhas (Benaka, 1999). Nesse sentido, a conservação dos habitats estuarinos torna-se relevante para a manutenção do recrutamento e da biodiversidade.

No caso da corvina *M. furnieri* no ELP, os padrões temporais e espaciais da espécie estão basicamente relacionados com os requerimentos ecológicos de cada estágio de desenvolvimento. O estudo de caso para a identificação do *habitat essencial* da corvina mostrou uma ocupação complexa do habitat pela espécie durante seu ciclo de vida, o que tem profundas implicações para o manejo e conservação da espécie. Desta forma, ressalta-se a importância de estudos que integrem mais de uma fase de desenvolvimento e de longa duração no estabelecimento de ações para a conservação. As enseadas rasas do ELP são um habitat essencial para diversos estágios de desenvolvimento da corvina. A importância desses habitats já foi ressaltada por Castello (1985) por servirem como ambiente propício para a produção primária por algas bentônicas e fanerógamas marinhas, e ao desenvolvimento, alimentação e proteção para muitos peixes e crustáceos. Espécies como o camarão-rosa (*Farfantepenaus paulensis*) utilizam essas mesmas áreas rasas para seu desenvolvimento e recrutamento, reforçando a necessidade de que essas áreas sejam consideradas em futuras ações de manejo no ELP visando a manutenção do recrutamento e da população adulta dessas espécies. Desta forma, podemos concluir que considerar tais áreas como zonas de conservação em um plano de zoneamento para o ELP é extremamente necessário para a conservação dos *habitats essenciais* da espécie. Neste caso, foram confirmadas as hipóteses de que existe uma correlação entre os diferentes estágios de vida da corvina e os habitats no estuário da Lagoa dos Patos (hipótese 1), e de que cada estágio de desenvolvimento (e.g. ovo, larva, juvenil e adulto) da corvina *M. furnieri* possui um habitat essencial específico no estuário da Lagoa dos Patos e este é relacionado com diferentes variáveis ambientais (hipótese 2).

Além da corvina, muitas outras espécies dependem do ELP para completarem seu desenvolvimento, sendo que a maioria destas possui um estágio inicial de vida planctônico, o que significa muitas vezes que adultos da mesma espécie vivem num habitat completamente diferente. Nesse sentido, proteger diferentes estágios de vida torna-se essencial para o sucesso no recrutamento e também para a manutenção da população adulta. Além disso, estuários são ambientes extremamente dinâmicos o que significa que planos para a conservação devem tentar incorporar essa variabilidade para se alcançar um resultado eficiente. A abordagem de planejamento sistemático para a conservação ainda está em sua infância no que se refere a ecossistemas estuarinos. Os resultados com relação a priorização espacial apresentados na tese contribuem para o avanço nessa área, mostrando dois resultados principais: 1) um plano de zoneamento pode ser desenho futuramente num plano de conservação (Apêndice 3); e 2) a incorporação de ovos e larvas de peixes em análises de planejamento sistemático para a conservação em estuários altera significativamente as prioridades espaciais para conservação (Apêndice 4). Estes são os primeiros trabalhos a combinar estimativas de dados espaciais para a pesca artesanal com um plano de conservação que inclue estágios iniciais de peixes (Apêndice 4), e o primeiro a incorporar dados de diferentes estágios de vida de uma espécie em uma ferramenta de suporte a decisão para priorizar espacialmente a proteção do habitat essencial de uma espécie (Apêndice 3). Estes resultados confirmam a hipótese de que a inclusão de dados de ictioplâncton altera gera diferenças na prioridade de planejamento espacial para a conservação (hipótese 3).

Durante os últimos doze anos, as áreas rasas do ELP apresentaram alta variabilidade na composição e abundância dos ovos e larvas de peixes, associada principalmente com a distribuição da salinidade no estuário, mês de coleta e posição do ponto amostral. Além disso, essas áreas apresentam uma alta variabilidade nos padrões de distribuição espacial dos ovos e larvas de peixes ao longo dos anos, com diferentes espécies indicando os diferentes grupos de assembleias encontrados. Estes resultados confirmam a hipótese de que a assembleia ictioplanctônica das áreas rasas do ELP apresenta variabilidade temporal e espacial na abundância e na composição das espécies, os quais influenciam as ações de conservação. Comparando esses resultados com as classes do enquadramento das águas, a qual é única ação de conservação implementada no ELP através do Plano Ambiental de Rio Grande (Tagliani & Asmus, 2011), é possível concluir que esta medida sozinha não é suficiente para proteger o ecossistema estuarino da Lagoa dos Patos. Um plano ambiental contemplando o enquadramento das águas juntamente com informações que reflitam a dinâmica dos estuários é de extrema importância. Devido a relação entre a sobrevivência dos estágios iniciais de peixes, o processo de recrutamento e a manutenção da população adulta, o uso de dados de ovos e larvas de peixes é uma boa estratégia para se auxiliar no planejamento para a conservação estuarina.

4. RECOMENDAÇÕES PARA ESTUDOS FUTUROS

Nesta última seção, gostaria de compartilhar algumas recomendações para futuros estudos:

1) O uso de dados históricos e longas séries temporais: A disponibilidade de dados históricos ainda não é uma realidade no Brasil. Entretanto, o estuário da Lagoa dos Patos conta com um banco de dados para as mais diversas áreas desde os anos de 1970, o que possibilitou muitas das análises aqui realizadas. Entretanto, o mesmo não acontece para a zona costeira adjacente, sendo este é o habitat dos adultos de muitas espécies que dependem do ELP para seu desenvolvimento, como por exemplo a corvina. Nesse sentido, visando uma aprimoração no planejamento para a conservação do ELP e de suas espécies, seria

interessante ter a zona costeira adjacente incluida no planejamento. Isso não foi possível nessa tese pois a maior parte dos dados utilizados foram dos anos 1970 e 1980 os quais representavam uma maior coberta espacial, porém restrita ao estuário. Geralmente, estudos baseados em longas séries temporais não contínuas estão associados a algumas dificuldades. Considerando a presente tese posso citar: 1) amostragens realizadas com diferentes metodologias; 2) existência de lacunas dentro da série temporal; 3) e diferenças entre as séries amostrais do ictioplâncton e de juvenis. Entretanto, apesar dessas dificuldades, foi possível obter um padrão bem claro para a ocupação da corvina no ELP;

2) Cobertura espacial do estuário dos dados recentes: Um fator que restringiu o uso dos dados recentes (2000 – 2012) de ictioplâncton para o capítulo 3 foi a sua pequena cobertura espacial da área do estuário, representando somente cinco pontos amostrais em áreas rasas. As análises utilizadas nos capítulos 1 e 2 demandavam uma grande cobertura espacial da área de estudo, fazendo com que, novamente, somente os dados históricos fossem utilizados nesses capítulos. É claro que um alto grau de incerteza pode ser associado a planos de conservação baseados em informações não recentes, porém, algumas estratégias foram tomadas para se diminuir essa incerteza, como a entrada de informações do ictioplâncton no Marxan separadas por mês e por ano, garantindo que a variabilidade temporal fosse incorporada na análise. Além disso, o objetivo principal da tese foi o teste da aplicação da metodologia com os dados. No caso de uma real implementação do plano para a conservação sugere-se a re-análise com dados mais recentes;

3) Estimativa dos custos de oportunidade para a pesca artesanal do ELP: Outro fator associado a incerteza dos resultados dos apêndices 3 e 5 são relacionados ao cálculo das estimativas do custo de oportunidade para a pesca artesanal. Esse cálculo foi baseado na única informação disponível de estatística pesqueira para a atividade artesanal para a região, na qual não é possível separar o que é proveniente do estuário e o que é da zona costeira

adjacente. Desta forma, tais estimativas podem estar sobre-estimando o valor das áreas de pesca do estuário. Infelizmente, a estatística pesqueira confiável para a atividade artesanal ainda não é uma realidade para muitos portos brasileiros.

4) Inserir a condição do habitat no planejamento para a conservação em estuários: Usualmente, a priorização espacial para a conservação assegura que habitats e espécies sejam representados a um menor custo econômico. Entretanto, ela ignora os impactos de outras atividades humanas no ambiente. A incorporação da condição do habitat inserida em análises de priorização espacial para a conservação ainda é escasso, e inexistente para ecossistemas estuarinos.

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6. APÊNDICES

Capítulo 1: A corvina *Micropogonias furnieri* e seu habitat essencial no estuário da Lagoa dos Patos

6.1 Apêndice 1 – Costa, M.D.P., Muelbert, J.H., Moraes, L.E., Vieira, J.P. & Castello, J.P. 2014. Estuarine early life stage occupancy patterns of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) from the Patos Lagoon, Brazil. Fisheries Research, 160, 77-84.

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Estuarine early life stage habitat occupancy patterns of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) from the Patos Lagoon, Brazil

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ABSTRACT

The whitemouth croaker *Micropogonias furnieri*, inhabits marine and brackish waters, and is one of the main fishery targets along the Brazilian coast. The species uses different estuarine habitats as a nursery during its early life cycle. The main objective of this study was to investigate the estuarine spatial distribution pattern of *M. furnieri* in the Patos Lagoon Estuary (PLE) during different development stages. A non-continuous 11-year interval was used to understand development stage spatial variability and the influence of environmental variables in different sized abundance. Results demonstrated that each developmental stage is correlated with an estuarine habitat and is associated with different environmental variables that influence their occurrence. Eggs were associated with coastal environments, whereas larvae were abundant in the estuarine channel area. Juveniles of different sizes exhibited a different spatial pattern: post-settlement individuals (<30 mm TL) were associated with channel areas, individuals between 30 and 160 mm were related to shallow and deep environments; and, individuals larger than 160 mm were mainly associated with deep waters. These results revealed complex estuarine habitat occupancy by *M. furnieri* during its early life stages, with implications to the knowledge of its biology, conservation and management actions, such as the identification of the essential fish habitat.

1. Introduction

The whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) is a Sciaenidae which inhabits marine, brackish and freshwater environments. This species uses estuaries as a nursery ground and adults occur in shallow waters, usually around continental shelf areas, over muddy and sandy substrata (Nelson, 2006). The species can be found in the western Atlantic from the Gulf of Mexico (20 N) to Argentina (41 S) (Vazzoler, 1991). *M. furnieri* occurs year-around in the Patos Lagoon estuary (PLE), from its early stages (Muelbert and Weiss, 1991) to a size range of 10 to >250 mm TL (Vieira, 2006). The species ranks first in number in bottom trawl samples and also among the four dominant species in beach seine samples (Vieira, 2006), and depends on the PLE ecosystem to successfully recruit and maintain the adult population that supports local traditional fisheries (Abreu and Castello, 1997; Odebrecht et al., 2010). In Brazil, it is one of the main target species in fisheries (Haimovici and Ignácio, 2005) with annual catches over 40.000 tons (MPA, 2010).

In a recent review (Haimovici and Ignácio, 2005) the whitemouth croaker fishery was identified as in an overfishing status since 1960. Additionally to overexploitation, environmental changes caused by ENSO events can act in a synergic manner and influence local production (Moraes, 2011; Möller et al., 2009; Odebrecht et al., 2010; Schroeder and Castello, 2010). ENSO events have a direct effect in this ecosystem, influencing the salinity distribution in the estuary, with low salinity (associated with high river discharge and an increase with rainfall) during El Niño, and the highest values of salinity in La Niña years (Odebrecht et al., 2010). These conditions have a great influence on the distribution of early life stages of whitemouth croaker, therefore regulating the recruitment success of the species (Moraes, 2011; Muelbert et al., 2010; Vieira et al., 2010). This scenario reinforces the need to understand

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the importance of estuarine habitat for the different development stages during early life.

The Patos Lagoon (32°S, Brazil) is a warm subtropical river-dominated chocked lagoon with a significant relationship between total rainfall at the hydrographic basin and annual river discharge (Odebrecht et al., 2010). The estuarine region comprises about 1000 km^2, and represents 10% of the lagoon total area. Water exchange with the coastal ocean is controlled by wind forcing and freshwater discharge, resulting in variable geographic estuarine limits as this ecosystem is conditioned by climatic factors (Odebrecht et al., 2010; Seeliger, 2001). This ecosystem is predominantly shallow and composed of different estuarine habitats. Shallow waters (<1.5 m), intermediate waters (1.5–5.0 m), deep waters (>6.0 m), unvegetated subtidal flats (300 km^2), seagrass beds (120 km^2), marginal salt marshes (40 km^2) and artificial hard substrates represent the main habitat for marine invertebrates and fish species (Seeliger, 2001). Fish eggs, larvae and juveniles are transported into the PLE by deep channel currents; and retention and survival of these organisms in the estuarine region are dependent on the water exchange and prevailing winds (Martins et al., 2007; Muelbert and Weiss, 1991; Odebrecht et al., 2010; Sinque and Muelbert, 1997).

In determining the importance of estuaries for marine animals it is noteworthy that these ecosystems consist of a complex mixture of many habitat types and that these habitats are subjected to physical, chemical and biological interactions (Pihl et al., 2002). Habitat selection by fish species in an estuary may be associated to availability and structural complexity, prey and predator abundance, physical transport process and local environmental conditions (Blaber and Blaber, 1980; França et al., 2009). In this sense, the degradation and losses of these estuarine habitats can potentially affect fishes and fisheries that depend on them to survive. Knowledge of estuarine habitat usage during life cycle can serve as a conservation basis for species as the concept of Essential Fish Habitat, which encloses "those waters and substrate necessary for a fish for spawning, feeding or growth to maturity" (Minello, 1999).

The estuarine spatial and temporal patterns and variations of distribution of *M. furnieri* abundance during the juvenile stage is recorded for a variety of ecosystems (Costa and Araújo, 2003; Giannini and Paiva Filho, 1990; González-Sausón et al., 1996), including Patos Lagoon estuary (Castello, 1986; Vieira, 2006). Indeed, these spatial variation reflect ecological requirements of each development stage, and associated environmental features (Figueiredo and Vieira, 1998; Gonçalves et al., 1999). Despite the existing information about *M. furnieri* in the PLE and other environments as the Rio de La Plata estuary (Acha et al., 2012; Braverman et al., 2009; Giberto et al., 2007; Jaureguízar et al., 2003, 2008; Militelli et al., 2013), an integrated study that combines information on eggs, larvae and juveniles is still lacking. This is the first study that analyzes the occupational pattern of the species in an estuarine ecosystem covering from eggs to individuals larger than 160 mm. The integrated approach is important to better comprehend the ecology and to determine the pattern of use of the PLE by whitemouth croaker during its early life cycle, and can be considered as a first step to the identification of essential fish habitats for the species. So, the main objective of this study was to comprehend the estuarine habitat occupancy patterns of whitemouth croker *M. furnieri* in the PLE during different development stages (e.g. eggs, larvae and juveniles), and understand stage variability and the influence of environmental variables in stage abundance.

2. Materials and methods

2.1. Sampling methods

The analysis of the estuarine habitat occupancy pattern of the whitemouth croaker was based on abundance data of eggs, larvae and juveniles sampled in PLE (Fig. 1) during a non-continuous 11-year interval (1975–1984), composed by two sampling periods (1975–1978, and 1979–1984). Table 1 summarizes information for both ichthyoplankton sampling periods. Samples were taken with a conical plankton net (500 μm, 60 cm mouth diameter) equipped with a flow-meter, which was towed for about 3 min in surface water during the first sampling period. During the second sampling period, three depth strata (surface, mid-water and bottom depths) were sampled using the same net without a closing system. All samples were preserved in a 4% formalin solution. Water for salinity and temperature measurements were collected at each sampling station and depth strata with a Niskin bottle. Salinity was registered using an American Optical refractometer. Temperature was recorded in degrees Centigrade and was obtained with a thermometer. Since the aim of the present study is not the vertical variability of ichthyoplankton, mean egg and larval abundance, temperature and salinity data was used for each sampling station.

Data for juvenile abundance also covered two periods. The first coincided with the first ichthyoplankton sampling period (from

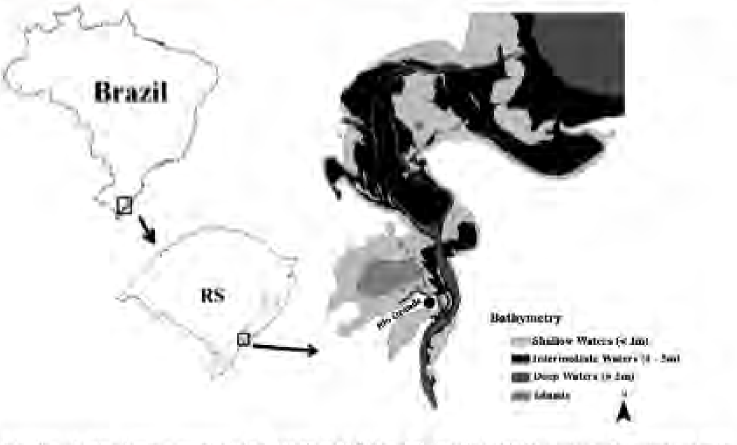

Fig. 1. Location of Patos Lagoon estuary in Rio Grande do Sul State (RS, Brazil) and bathymetry in the estuarine area.

Source: C. Taglioni.

Table 1

Summary of ichthyoplankton sampling procedures in Patos Lagoon Estuary (Brazil) for data used in the present study. Period I corresponds to the Project "Lagoa dos Patos" and Period II to the Project "Lagoa Estratificada" conducted by the Universidade Federal do Rio Grande.

	Dates	Periodicity	Number of stations per cruise	Depth
Period I	December 1975 to February 1978	Fortnightly and/or monthly	20–30[a]	Surface
Period II	April 1981 to February 1983	Fortnightly	5	Stratified[b]

[a] Cruise duration longer than a day.

[b] Stations 1–3 were sampled at surface, mid-water and bottom; stations 4 and 5 were sampled only at the surface and bottom.

December, 1975 to November, 1978) and the second covered six years of sampling (January, 1979 to October, 1984) which partially overlaps with the second ichthyoplankton period. In the first period a bottom trawl (9 m long, with an effective opening of 6.1 m, a variable mouth height of 0.5–1.0 m, 10 mm mesh near the codend) was towed for 5–20 min at 2 knots, and covered an area of approximately 1900 m^2. The catch per unit of effort (CPUE) was estimated using the number of individuals captured during a standardized 20 min trawl. During the second period, three different kinds of nets were used: (a) a beach seine (9 m long, 13 mm bar mesh in the wings and 5 mm in the center 3 m section) towed for 20 m covering an area of approximately 60 m^2; (b) a bottom trawl (8 m head rope shrimp trawl, 11 m ground rope, 2 m legs on each side, 1.3 cm bar mesh wings and body with a 0.5 cm bar mesh cod end liner, and a pair of weighted otter doors) towed for 5 min, covering an area of 1600 m^2; and (c) mid-water trawl (rectangular trawl, 7.1 m headline and ground rope, 5.8 m wing ropes, 30 m sweep line, 1.3 cm bar mesh, 0.5 cm bar mesh cod-end liner, using the same otter doors as the bottom trawl) towed under the surface at a depth of 8–12 m (or about 3–8 m from the bottom) for 5–30 min at 90–110 m/min, covering an area of approximately 1600 m^2 (Chao et al., 1985). Table 2 summarizes the sampling periods for juveniles. Sampling stations were classified according to depth following this scheme: period I was divided in shallow (<1.5 m), intermediate (1.5–5 m) and deep water (>6 m) according to Seeliger (2001); and the period II was divided according the sampling method, which reflects the depth at the sampling station: shallow (beach seine net) and deep water (bottom trawl net and mid-water trawl). Sampling with the beach seine was more continuous over time than those with bottom trawl and mid-water trawl (Supplementary Material 1). Temperature (°C) and salinity were recorded for each sampling station during both sampling periods.

2.2. *Data analysis*

Fish eggs and larvae of *M. furnieri* were sorted and identified under a binocular stereo-microscope following Weiss (1981). Standard length of larvae from Period II was measured. Eggs and larvae abundance were estimated as the number of individuals per 100 m^3. Juveniles (beach seine, bottom and mid-water trawl) were identified, sorted and the total biomass determined; when the catch was large, samples were divided in sub-samples, and all individuals from each sub-sample were measured (total length, TL) to the nearest millimeter. Monthly, depending on the period and sampling gear, juvenile abundance was estimated as catch per unit of effort (CPUE). CPUE by size class (CPUE-TL) was obtained by multiplying the ratio of the total number of individuals caught to the total number of individuals measured by the number of individuals measured for each 10 mm size class for period 1 and for each 5 mm size class for period 2 (Garcia et al., 2001; Vieira, 1991, 2006). As the total number of whitemouth croaker obtained in each haul was not recorded for the first period, the CPUE was estimated based on length–weight relationship proposed by Castello (1986) using the same dataset from Period I. The first step to estimate the total number of withemouth croaker was to calculate the average weight for each size class using the length–weight relationship proposed by Castello (1986). From this relationship, it was possible to estimate the weight for each sample by simply multiplying the number of individuals collected by the average weight for each size class. The next step was to calculate the contribution of each size class to the total weight considering the number of individuals in the subsample, this means that we used the value found on step two above and divided by the total weight for each sample. Following this, we estimated the proportional weight of each size class in relation of the weight in the subsample and then in relation to the total weight captured. The last step was to multiply the value found on step five by the number of individuals captured and then divided by the value found on step four. To evaluate our estimates we used the same procedure for Period II data and the estimates were very close to the measured values.

Based on juvenile occurrence and abundance juvenile length classes were classified in four Size Ecological Taxa (SET, further information in Vieira (2006)): SET I (<30 mm), SET II (30–90 mm), SET III (90–160 mm) and SET IV (> 160 mm). All abundance data were $\log(x+1)$-transformed and analyzed.

A PERMANOVA analysis based on Bray–Curtis similarity resemblance matrix was used to compare the abundance in CPUE of each development stage (eggs, larvae and juvenile) of *M. furnieri* between the two periods, using as factors period (fixed, 2 levels) and year (random, nested in period, 6 levels). Juvenile data was separated into shallow and deep waters, and this analysis was done separately. The results of this analysis (Table 3) showed that abundances were not significantly different ($p > 0.05$) between periods, which allowed the combination of both temporal series in the cross-correlation analysis. The relationship between abundance of fish eggs, larvae and juvenile (SET I, SET II, SET III and SET IV) were evaluated by cross-correlation analyses, using PAST software, in order to identify the lags between developmental stages. For this analysis,

Table 2

Summary of juvenile sampling procedures in Patos Lagoon Estuary (Brazil) for data used in the present study. Period I corresponds to the Project "Lagoa dos Patos" and Period II to the Project "BELAP" conducted by the Universidade Federal do Rio Grande.

	Dates	Periodicity	Number of stations per cruise	Depth
Period I	December 1975 to February 1978	Fortnightly and/or monthly	20–30[a]	Limited by the vessel haul (0.9 m)
Period II	January 1979 to October 1984	Monthly	Variable[a]	Dependent on the net type[b]

[a] Cruise duration longer than a day or two.

[b] Bottom trawl was performed at stations in deep areas (depth: 4–14 m) in the PLE, mid-water trawl in areas with more than 8 m depth, and beach seine net was performed in stations located at shallow areas (mean depth < 1.5 m) in PLE.

Table 3
Values of pseudo-*F* and (*p*-value) from PERMANOVA for *M. furnieri* abundance in Patos Lagoon estuary.

Abundance (CPUE)	Period	Year (Period)
M. furnieri eggs	0.44 (0.58)	0.64 (0.72)
M. furnieri larvae	2.76 (0.13)	1.34 (0.24)
Shallow waters juveniles	4.33 (0.08)	1.04 (0.40)
Deep waters juveniles	2.46 (0.11)	1.66 (0.08)

the juvenile data used was selected in order to have the same temporal series as ichthyoplankton data ("Projeto Lagoa", December 1975 to February 1978; and data from beach seine and bottom trawl of Project BELAP, April 1981 to December 1982). Only the CPUE data from shallow and deep areas were used due the lack of sampling in intermediate areas during the second period of the study.

The association of eggs and larvae abundance of *M. furnieri* to temperature, salinity, freshwater outflow and period was identified using a multiple regression performed by STATISTICA software. For SETs of *M. furnieri*, the relationship with environmental variables (temperature, salinity, freshwater outflow, period and depth) was identified based on data from Period I and II using canonical correspondence analysis (CCA) performed by CANOCO 4.5 software. Juveniles in SET I (>30 mm TL) were excluded from this analysis because they represented only 2% of CPUE total and exhibited a high difference between Periods I and II. The intent of this was to avoid that ecological patterns were masked by the CPUE temporal variation of SET I. The sum of the monthly river discharge of three rivers (Jacuí, Taquari and Camaquã) located north of Patos Lagoon were used as a proxy for the freshwater outflow of the drainage basin of the Patos-Mirim Lagoon System (Vaz et al., 2006). Monte-Carlo permutation test (5000 permutations) for the first canonical axis and for all canonical axes was conducted to test the significance of the relations.

3. Results

3.1. *M. furnieri* eggs and larvae

A total of 380,085 eggs and 8384 larvae were collected throughout the study period; Period I accounted for 124,594 (32.8%) eggs and Period II for 255,491 (67.2%). For larvae, Period I accounted for 1957 (23.3%) larvae Period II for 6427 (76.7%). Egg abundance varied from zero to 858.5 eggs/100 m^3 during Period I and between zero and 8951 eggs/100 m^3 in Period II. Larval abundance varied from zero to 13.9 larvae/100 m^3 during Period I, and between zero and 1009 larvae/100 m^3 in Period II. Highest egg and larval abundance occurred during the spring and summer months of both periods.

3.2. *M. furnieri* juveniles

Total estimated catches of *M. furnieri* juveniles was 92,703 individuals for Period I, and 96,488 fishes for Period II. CPUE based on bottom trawl samples varied from zero to 975.5 inds/month in shallow waters, between 101.2 and 782.5 inds/month in intermediate waters, and between zero and 1530.4 inds/month in deep waters during Period I. During period II, CPUE varied from zero to 22.7 inds/month in shallow waters with beach seine; from 2.6 to 1306.5 inds/month in deep waters with bottom trawl; and between 1 and 293 inds/month with mid-water trawl. The CPUE was high all of throughout almost Period I in all depths. During Period II, highest CPUE values were associated with warmer months and mainly with deep waters.

3.3. *Stage distribution and spatial and temporal analysis of size ecological taxa*

Seasonal abundance anomalies of *M. furnieri* juveniles reveal an abundance pattern that varied seasonally in habitat type in both

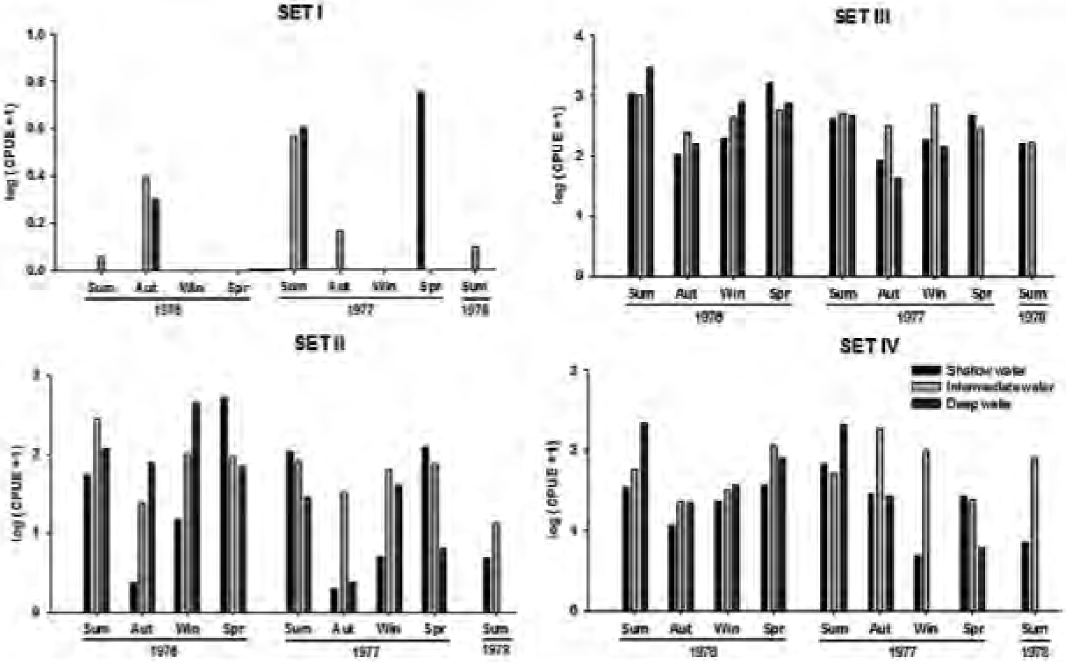

Fig. 2. Seasonal abundance (CPUE log(x + 1)-transformed) for each size ecological taxa (SET) of *M. furnieri* during Period I in Patos Lagoon estuary.

periods (Supplementary Materials 2–4). During the first period it was possible to observe that *M. furnieri* occurred in all estuarine habitats, but mainly at intermediate and deep waters (Supplementary Material 2). In the second period larvae was observed entering in the estuary during the reproductive season (spring, summer and august), and juveniles occupying both habitats, mainly deep waters (Supplementary Materials 3 and 4).

SET I (<30 mm TL) was mainly associated with intermediate and deep waters and with summer and autumn during Period I (Fig. 2), whereas SET II (30–90 mm TL) occurs throughout the year and can be related to all estuarine habitats, but mainly intermediate and deep waters. SET III (90–160 mm TL) and SET IV (>160 mm TL) occurred through all estuarine habitats and year round (Fig. 2).

For period II (Fig. 3), CPUE showed a different spatial and temporal pattern for each SET. SET I had peaks of abundance that ranged between years, but mostly associated with deep waters. SET II can also occur at shallow waters, occupying all estuarine habitat during all year. SET III is also present in shallow waters, but in low abundance. SET IV almost not occurs in shallow waters, and is mainly related with deep waters during the year (Fig. 3).

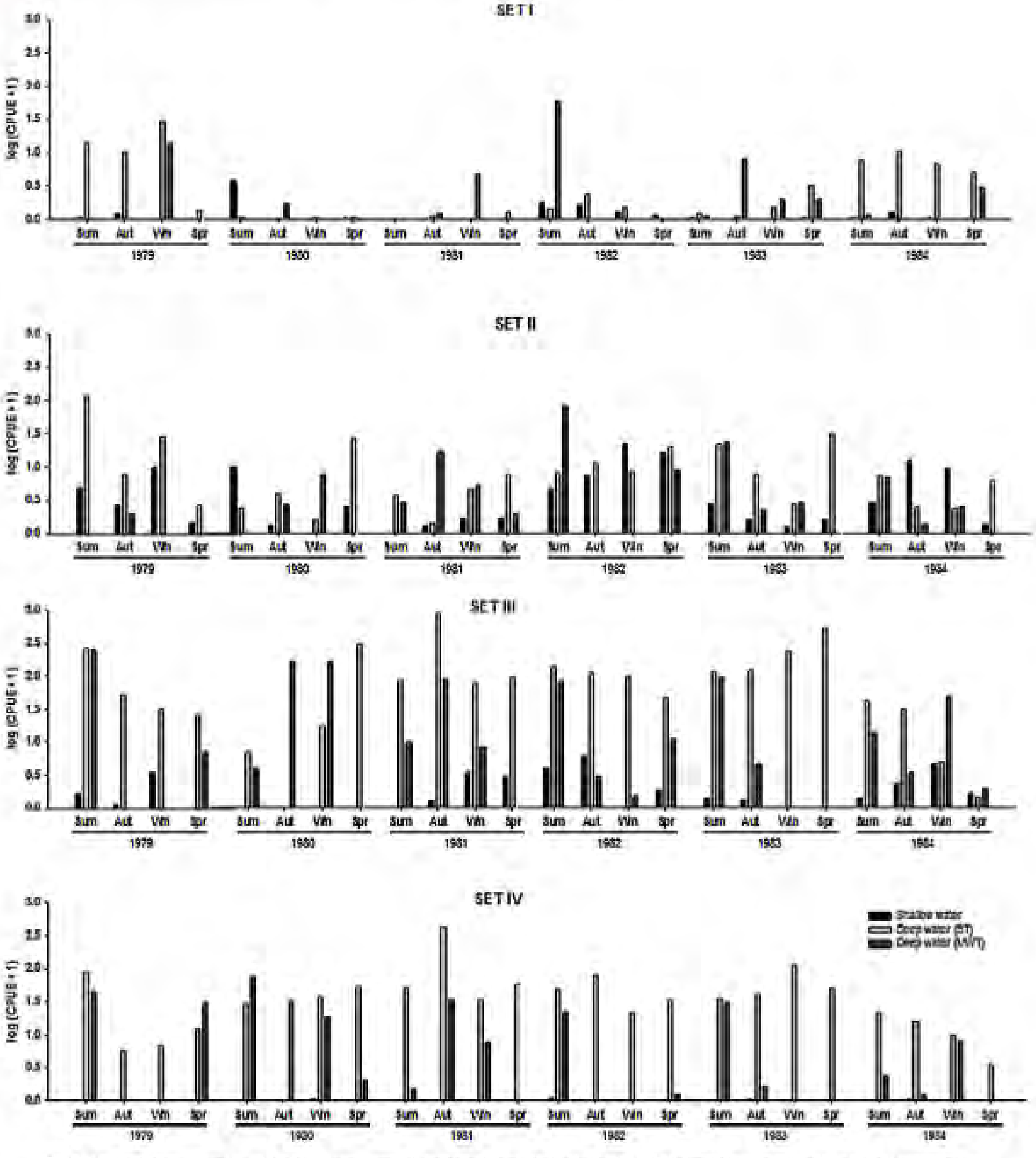

Fig. 3. Seasonal abundance (CPUE log(x + 1)-transformed) for each size ecological taxa (SET) of *M. furnieri* during Period II in Patos Lagoon estuary.

Table 4
Multiple regression results for eggs and larvae abundance of *M. furnieri* (log (x + 1)-transformed) and environmental variables (temperature, salinity, freshwater outflow and period).

Environmental variables	Eggs		Larvae	
	Adjusted R^2 = 0.45		Adjusted R^2 = 0.53	
	Beta	*P*	*Beta*	*P*
Temperature	0.55	0.00	0.55	0.00
Salinity	0.57	0.00	0.40	0.01
Freshwater outflow	0.20	0.22	−0.08	0.57
Period	−0.02	0.88	0.15	0.33

3.4. *M. furnieri* ontogenetic stages and abiotic variables

The multiple regressions for *M. furnieri* eggs and larvae and environmental variables demonstrated that both development stages were positively associated with temperature and salinity (Table 4). The adjusted R^2 registered was 0.45 for eggs and 0.53 for larvae.

The first CCA ordination axis accounted for 71.1% of the total explained variance. Among the significant variables, depth was closely correlated to the first axis, whereas period and salinity were correlated to the second axis. The ordination diagram shows two gradients: the first gradient corresponds to variations in depth, contrasting chiefly SET II and IV; and the second gradient corresponds to period and salinity variations. In this case, contrasting SET II and IV with SET III (Fig. 4, Table 5).

3.5. Estuarine occupational pattern of *M. furnieri* in Patos Lagoon estuary

The cross-correlation analysis revealed the different habitat use patterns for whitemouth croaker in Patos Lagoon estuary (Supplementary Materials 5 and 6). During Period I (Supplementary Material 5), this analysis showed that the species abundance in some development stages were out phase. For instance, a 1 month delay was found in time elapsed between eggs and larvae ($r = 0.50$, $p = 0.00$) and between SET II and SET III at Deep waters (D) ($r = 0.49$, $p = 0.00$). The full table of cross-correlation results (Supplementary Material 5) showed that changes in the abundance of other development stages were in phase or that the observed lags were not significant. The correlations were lower mainly during first juveniles stages (SET I and II).

During Period II, significantly higher correlations between development stages of *M. furnieri* were observed with 0 and 3 months delay (Supplementary Material 6), suggesting greater synchrony in the occupation of the different estuarine habitats by the species into Patos Lagoon estuary. Again, the correlations were lower mainly during earlier juvenile stages (SET I and II).

Table 5
Results of the partial canonical correspondence analysis (partial CCA) performed for *M. furnieri* size ecological taxa (CPUE log (x + 1)-transformed) in Patos Lagoon estuary.

	CCA axes	
	1	2
Summary statistics for ordination axis		
Eigenvalues	0.012	0.005
Species–environment correlations	0.403	0.373
Cumulative percentage variance		
Of species data	11.0	15.5
Of species–environment relation	71.1	100

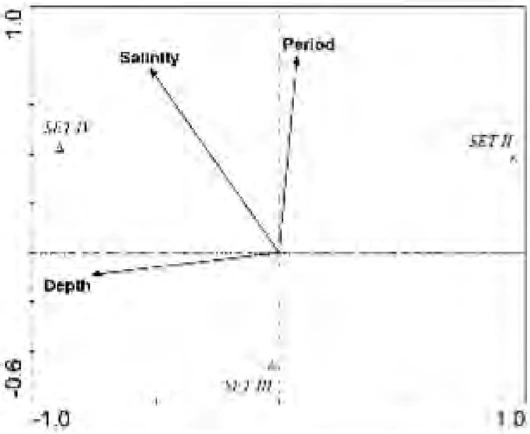

Fig. 4. Canonical correspondence analysis (CCA) ordination diagram for *M. furnieri* juveniles based on CPUE log (x + 1)-transformed for each of the size ecological taxa (SET) in Patos Lagoon estuary.

4. Discussion

M. furnieri is classified as an estuarine dependent species (Albuquerque et al., 2012; Vieira and Castello, 1997) and in this paper we describe the movements and usage of different size ecological taxa of *M. furnieri* in the estuarine habitats of Patos Lagoon Estuary (PLE). The species inhabits the adjacent marine surf zone of Patos Lagoon estuary (Busoli and Muelbert, 1999, 2003; Lima and Vieira, 2009; Rodrigues and Vieira, 2012), the upper limnetic part of the Patos Lagoon (Milani and Fontoura, 2007; Vieira et al., 2010), the shelf, and also invading freshwater environments (Albuquerque et al., 2010, 2012). Whitemouth croaker systematically uses this lagunar environment along its ontogeny (Vieira, 2006; Vieira and Castello, 1997). Eggs and larvae are common throughout much of the year, although they have highest densities in spring and summer, which is the main reproductive and recruitment period of the species to PLE (Bruno and Muelbert, 2009; Muelbert and Weiss, 1991). Eggs and larvae of this species are most abundant in the mouth of PLE (Ibagy and Sinque, 1995), and enter the estuary associated with the intrusion of salt water (Muelbert and Weiss, 1991; Sinque and Muelbert, 1997).

Temperature and salinity positively influenced the abundance of *M. furnieri* eggs and larvae in PLE. This pattern was also registered by other studies in this ecosystem (Bruno and Muelbert, 2009; Muelbert and Weiss, 1991). However, other variables could be interacting and affecting the structure of this life stages in PLE estuary. Wind influences the transport of fish eggs and larvae of *M. furnieri* in the PLE (Martins et al., 2007). Moreover, the relationship between juveniles and environmental variables confirmed that each size ecological taxa could be structured by different variables. Variations in depth contrasted SET II (30–90 mm) with SET IV (>160 mm), suggesting that this reflects the habitat preferences of each SET. Larger juveniles (SET IV) were chiefly associated with deep environments, meanwhile SET II and III could use both habitats (shallow and deep waters). Turbidity is another important abiotic factor that can structure juvenile estuarine fish assemblage since it is related to reduction of predation and, generally, is coincidental with an increased food supply in shallow waters (Blaber and Blaber, 1980; Jaureguizar et al., 2003).

Vegetated shallow areas can also be an important factor in structuring fish abundance and diversity in estuaries (Gillanders, 2006). Large areas of shallow waters are colonized by *Ruppia maritima* in

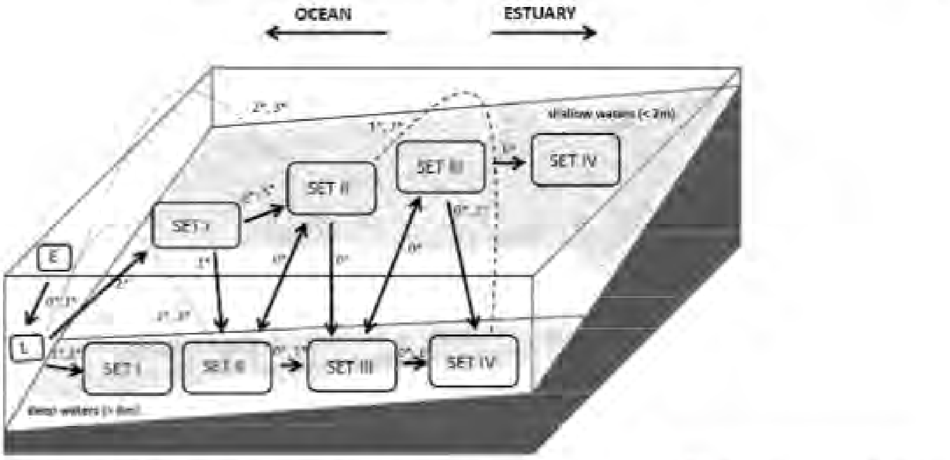

Fig. 5. Schematic diagram of the habitat use by early life stages of whitemouth croaker *Micropogonias furnieri* in Patos Lagoon estuary, Brazil (numbers represent the time-lag result in months of the cross-correlation analysis between each development stage; * represent significant time-lag result; E: eggs; L: larvae; SET I: size ecological taxa I, <30 mm TL; SET II: size ecological taxa II, 30–90 mm TL; SET III: size ecological taxa III, 90–160 mm TL; and SET IV: size ecological taxa IV, >160 mm TL).

PLE, and develop during spring and summer, providing an essential habitat for feeding, development and protection for early life stages of fishes and invertebrates (Castello, 1986; Seeliger, 2001). Juveniles of *M. furnieri* use both, vegetated and unvegetated areas at PLE, but mainly SET I use *R. maritima* shallow areas during day and night (Garcia and Vieira, 1997). As the total area of *Ruppia* beds tend to be variable within years the contribution and the function as nursery area of these habitats for some species, as whitemouth croaker *M. furnieri*, can also be variable between years (Seeliger, 2001).

M. furnieri shows a defined temporal and spatial pattern of habitat use in the PLE according to each life stage. Fig. 5 shows a conceptual model of the habitat use by *M. furnieri* at Patos Lagoon estuary. The changes in the spatial distribution can be related to phases of the life cycle (Costa and Araújo, 2003) and studied period. In the PLE, juveniles in SET I (<30 mm TL) can concentrated both in shallow (vegetated and unvegetated areas) and deep waters, although they were scarce in shallow waters during Period I. Juveniles in SET II (30–90 mm TL) and SET III (90–160 mm TL) can be found throughout the estuary, in shallow and deep estuarine habitats. Juveniles larger than 160 mm TL (SET IV) were registered in estuarine beaches during the first period, but apparently are more related to the deep waters (Vieira, 2006), indicating the initial migration to continental shelf to spawn (Fig. 5). Castello (1986) demonstrated that this migration to continental shelf can be associated with the first sexual maturity, which is around 180–205 mm TL. This pattern is further emphasized by differences in feeding behavior and diet preferences between deep and shallow areas of PLE (Fgiueiredo and Vieira, 1998). In the La Plata estuary, a similar pattern for different age-classes habitat preferences within the estuary was found for this species (Jaureguizar et al., 2003). Furthermore, these authors suggested that the habitat selection can be driven by environmental variables.

Although the study did not focus on an outer–inner estuarine habitat use perspective, it is known that *M. furnieri* tend to move by active swimming toward low-salinities regions in the PLE during the first and second year of life (Albuquerque et al., 2012). Strong co-occurrence between *M. furnieri* juveniles and freshwater-vagrant species were registered in the PLE, suggesting close association between whitemouth croaker and periods of low salinity (Moraes, 2011). The occurrence of this species in estuarine beaches of PLE can be explained by an active transverse displacement from deep waters to shallow regions (Moraes, 2011). Other studies show similar patterns in the Río de la Plata estuarine region, where the oldest individuals are associated with the outer area (more saline) toward the mouth of the estuary, while the youngest individuals inhabit the inner areas (brackish-freshwater) (Jaureguizar et al., 2003). In Sepetiba Bay (Rio de Janeiro, Brazil), recruits of *M. furnieri* tended to be concentrated in beaches of the inner zone of the bay (Costa and Araújo, 2003), a response associated with diet and the fact that shallow areas generally create favorable conditions for recruitment and growth. Studies that integrate ichthyoplankton and juvenile data are important to better comprehend the biology and ecology of the species and associated with the understanding of the estuarine habitat using patterns during the life history which are useful tools to management actions, such as the essential fish habitat identification.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2013.10.025.

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6.2 Apêndice 2 – Costa, M.D.P., Muelbert, J.H., Vieira, J.P. & Castello, J.P. Dealing with temporal variation and different life stages of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) in species distribution modeling to improve estuarine essential fish habitat identification. Submetido para a Hydrobiologia.

Hydrobiologia

Dealing with different life stages of whitemouth croaker Micropogonias furnieri (Desmarest, 1830) in estuarine essential fish habitat identification

--Manuscript Draft--

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Abstract:	Understanding of the habitat usage of a species is essential for assessing impacts from human activities, conservation, and management issues. All of the life stages of Micropogonias furnieri, an important species of the artisanal fishery, inhabit the estuarine area of Patos Lagoon year-around. A model of the seasonal distribution of each life stage of M. furnieri in Patos Lagoon Estuary was conceived to understand the seasonal distribution pattern of the different stages of the species in the estuarine area as a step for essential fish habitat identification. Our results showed that models can account for different spatial and seasonal variability that are influenced by different environmental variables. Variability in abundance and habitat preferences among different developmental stages must be considered when identifying the essential fish habitat of species with complex life cycles, such as M. furnieri, which could improve conservation and management outcomes.
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Dealing with temporal variation and different life stages of whitemouth croaker *Micropogonias furnieri* (Desmarest, 1830) in species distribution modeling to improve estuarine essential fish habitat identification

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Short Title: Essential fish habitat identification based on different life stages

ABSTRACT

Understanding the habitat usage of a species is essential for assessing impacts from human activities, conservation, and management issues. *Micropogonias furnieri*, an important species of the artisanal fishery, inhabits different habitats of the Patos Lagoon estuary year-around according to the stage of its life cycle. Our aim was to test the hypothesis that habitat preferences and occurrence variability during the life cycle influences the model outcomes in the study of species distribution. Also, we want to evaluate if the addition of different life stages to the models can affect the final outcome of essential fish habitat identification. Our results showed that models outcome were affected when considering temporal variability and the addition of different life stages. In this sense, we suggest that variability in abundance and habitat preferences at different developmental stages must be considered when identifying essential fish habitat of species with complex life cycles, such as *M. furnieri*. This measure could improve conservation and management outcomes.

Key words: essential fish habitat; estuary; fish eggs and larvae; juveniles; *Micropogonias furnieri*

INTRODUCTION

Essential fish habitat is defined as “*those waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity,*” in regards to the importance of the habitats for the maintenance of the population (Minello, 1999). This is a concept originated in the United States of America, when the Magnuson-Stevens Fisheries Conservation and Management Act recognized the importance of habitat for the maintenance of fisheries stocks. There are several methods to identify essential fish habitat of a species, including analyses of density patterns within each habitat, indices, and species distribution models (Benaka, 1999; Valavanis, 2008). These statistical approaches can be used to build habitat suitability maps, reflecting the link between descriptors of the environment and the spatial distribution of the species (Le Pape et al., 2014). These maps represent those habitats required by the species to complete their development. In this context, the identification of essential fish habitats is of extreme relevance to improve the conservation and management of aquatic resources.

Currently, species distribution modeling is one of the main tools used to build habitat suitability maps to identify essential fish habitat (Valavanis, 2008). Regardless of the statistical approach, species distribution modeling aims to predict the distribution of a species across a region, estimate the relationship between the species records and predictor variables, and provide one powerful way to overcome the sparseness of information that usually exists for a species (Guisan & Zimmermann, 2000; Hirzel & Le Lay, 2008; Elith & Leathwick, 2009). This approach assumes that the ecological requirements of a species are reflected by its observed distribution (Hirzel & Le Lay, 2008). In the case of essential fish habitat, species distribution modeling is generally based on presence records of adults, ignoring early life stages or temporal variability.

Coastal environments, such as estuaries and lagoons, are highly dynamic ecosystems because they are directly influenced by seasonality, and they act as nursery grounds for a wide variety of species, including many marine fish species. These ecosystems are also areas that help sustain marine resources and biodiversity; however, they are easily impacted by human activities. Habitat disturbance, especially habitat loss, is noted as one of the main drivers in biodiversity loss in coastal environments (Barletta et al., 2010). Additionally, decreases in water quality is also a main threat for a wide variety of species that depend on these ecosystems to complete their life cycle (Cattrijsse et al., 2002; Pihl et al., 2002).

Micropogonias furnieri (whitemouth croaker, Desmarest, 1830) is a Scienidae species, which inhabit marine, brackish, and freshwater environments, and they usually use estuaries as a nursery ground (Nelson, 2006). In Brazil, whitemouth croaker represents one of the main target fish species in fisheries, with annual catches over 40.000 tons (MPA, 2010). It has been heavily fished and has been declared as being overexploited (Haimovici and Ignácio, 2005). In Southern Brazil, Patos Lagoon is recognized as an important habitat for this species, because the species occurs in this estuary throughout its entire life cycle.

Whitemout croaker is an estuarine dependent species (Vieira & Castello, 1997; Albuquerque et al., 2012), and depending on the developing stage, it can be found inhabiting the upper limnetic part of the Patos Lagoon to the adjacent marine surf zone and shelf (Costa et al., 2014). Since spawning occurs in the marine environment adjacent to the lagoon, early stages are most abundant at the mouth of the estuary, depending on the salt water intrusion entering the estuarine region to occupy the inner part of the estuary (Muelbert & Weiss, 1991; Ibagy & Sinque, 1995). In this system, in addition to overfishing, ENSO events can also influence regional production in the Patos Lagoon Estuary (PLE), acting in a synergic manner leading to overexploitation (Möller et al., 2009; Odebrecht et al., 2010; Schroeder and Castello, 2010). Additionally, several threats, such as untreated domestic effluent from 7 million inhabitants, industrial production, dredging, and port activities, can be linked to impacts on the PLE (Barletta et al., 2010). In the PLE, each life stage of whitemouth croaker exhibits a well-defined temporal and spatial pattern of habitat use, each stage being correlated with different environmental variables that can influence their occurrence (Costa et al., 2014). The model we present here assesses the relevance of considering different life stages of a species to achieve a complete essential fish habitat identification regarding the seasonal and spatial distribution. In this sense, our aim was to test the hypothesis that habitat preference and occurrence variability during the life cycle influences the model outcomes in the study of species distribution. If this is correct, inclusion of this information in modelling approach will improve estuarine essential fish habitat identification and conservation planning. Our open question is, can the addition of different life stages affect the final outcome of essential fish habitat identification?

MATERIAL AND METHODS

Study area

The Patos Lagoon (32°S) is a subtropical chocked lagoon (Kjerfve, 1986) that is located in Southern Brazil. Most of the lagoon area is freshwater, but an estuarine system of approximately 1,000 km² is formed in its connection with the Atlantic Ocean (Fig.1) (Seeliger, 2001; Odebrecht et al., 2010). This ecosystem is mostly conditioned by climatic factors, such as the water exchange with the ocean, which is mainly controlled by wind forcing and freshwater discharge (Seeliger, 2001; Odebrecht et al., 2010), and results in a dynamic estuarine region with variable geographic limits. These features can also influence the occurrence and distribution patterns of most of the species inhabiting the estuarine area (Seeliger, 2001; Odebrecht et al., 2010).

Patos Lagoon estuary is a highly dynamic and physiographic diverse area. Most of the estuary is shallow (< 1.5 m), with many estuarine habitats such as water column in intermediate waters (1.5 – 5.0 m) and channels (> 5.0 m), unvegetated subtidal soft-bottoms and intertidal flats, seagrass beds, salt marshes, and artificial hard substrates. All of these habitats provide nursery areas for a wide variety of species, including *M. furnieri* (Seeliger, 2001), which depend on this ecosystem to successful develop, feed and grow. This species is one of the main target fish species in the local artisanal fishery in the PLE (Odebrecth et al., 2010), with individuals found year-around inhabiting the lagoon, ranging in age from the early stages to adulthood (Castello, 1986; Muelbert & Weiss, 1991; Vieira, 2006).

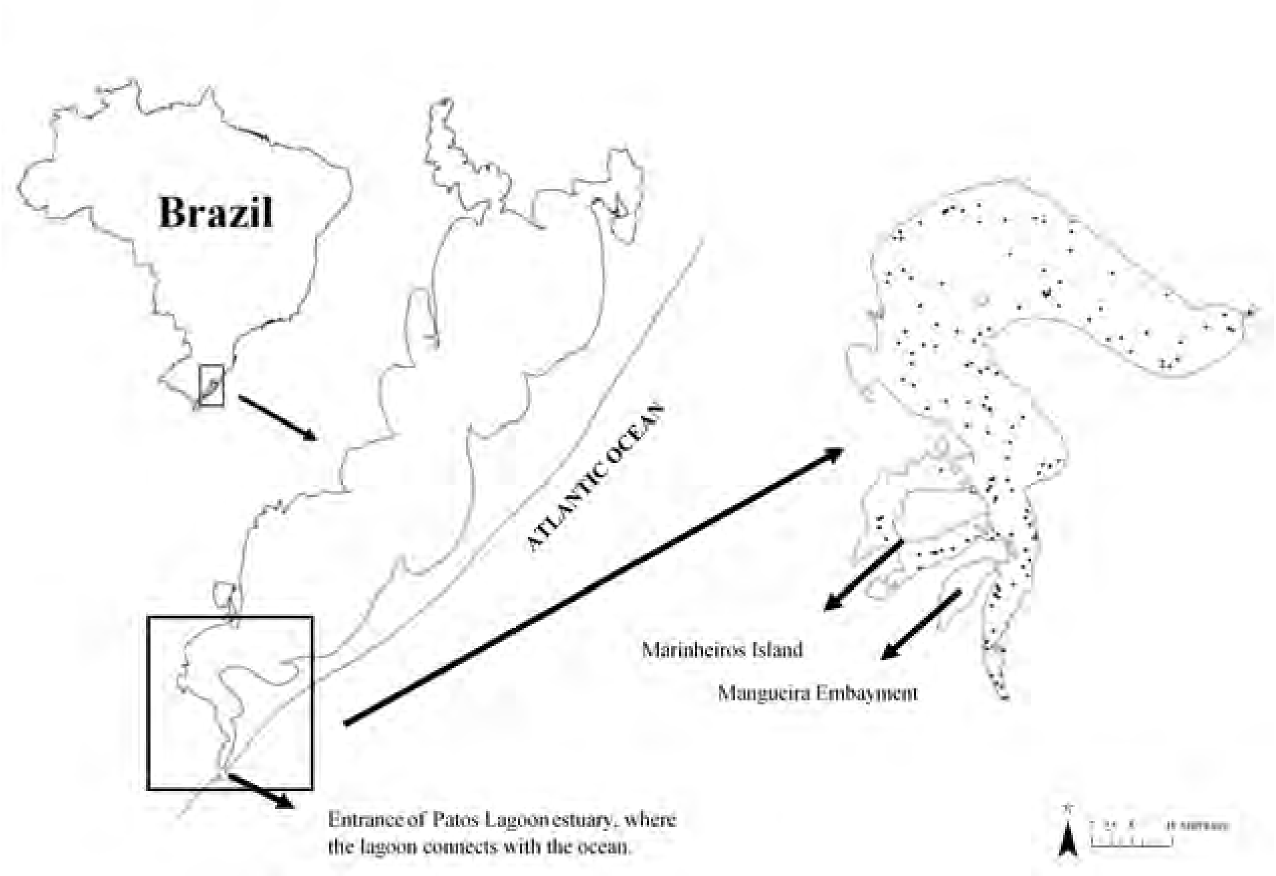

Figure 1: Location of Patos Lagoon in Brazilian coast and sampling stations in the estuarine region.

Essential Fish Habitat identification

Whitemouth croaker habitat suitability maps were built based on records of eggs, larvae, juveniles, and adults in the PLE. We used a long-term dataset to extract information on the occurrence of whitemouth croaker in the PLE to analyze abundance of each life stage during the study period. Ichthyoplankton samples were taken with a conical plankton net (500 µm, 60 cm mouth diameter) equipped with a flow-meter, which was towed for 3 minutes in surface water (Costa et al., 2014). Juveniles and adults were classified into length classes: < 30 mm, 30 - 90 mm, 90 – 160 mm, and > 160 mm, and samples were taken with different methods throughout the study period: 1) a bottom trawl (9 m long, with an effective opening of 6.1 m, a variable mouth height of 0.5 – 1.0 m, 10 mm mesh near the codend) was towed for 5 – to 20 min at 2 knots, and covered an area of approximately 1900 m², the swept area was estimated using the number of individuals captured during a standardized 20 min trawl; 2) a beach seine (9 m long, 13 mm bar mesh in the wings and 5 mm in the center 3 m section) towed for 20 m covering an area of approximately 60 m²; 3) a bottom trawl (8 m head rope shrimp trawl, 11 m ground rope, 2 m legs on each side, 1.3 cm bar mesh wings and body with a 0.5 cm bar mesh cod end liner, and a pair of weighted otter doors) towed for 5 min, covering an area of 1600 m²; and 4) mid-water trawl (rectangular trawl, 7.1 m headline and ground rope, 5.8 m wing ropes, 30 m sweep line, 1.3 cm bar mesh, 0.5 cm bar mesh cod-end liner, using the same otter doors as the bottom trawl) towed under the surface at a depth of 8 – 12 m (or about 3 to 8 m from the bottom) for 5 to 30 min at 90 to 110 m/min, covering an area of approximately 1600 m² (Chao et al., 1985; Costa et al., 2014). In general, our data represented occurrence records from a non-continuous period from December, 1975 to April, 2001 (Fig. 1). The data selected represented the best spatial coverage of sampling stations in the PLE. Eggs and larvae density were estimated as the number of individuals per 100 m^3. Juvenile and adult abundance were estimated as catch per unit effort (CPUE) by length class. CPUE by length class was obtained by multiplying the ratio of the total number of individuals caught (N_t) by the number of individuals measured for each 10 mm size class (N_{lc})(Vieira, 1991, 2006; Garcia et al., 2001):

$$CPUE_{lc} = {}^{1}\!/_{N_t} \times N_{lc}$$

The environmental variables used in the identification of essential fish habitat were temperature, salinity, bathymetry, sediment type, and information about submerged aquatic vegetation, which were analyzed at a fine spatial resolution (< 1 km, cell precision of 0.0016). Temperature and salinity data registered at each sampling station during the cruises were interpolated for the entire area of the PLE using the Inverse Distance Weighted methodology, which estimated the cell values by averaging the values of sample data points in the neighborhood of each cell (Shepard, 1968). Bathymetry was used as a categorical variable that was classified as shallow (< 1.5 m), intermediate (1.5 – 5 m), or deep water (> 5 m). Sediment type was also a categorical variable and was classified according to Calliari (1980) based on sand-silt-clay content in 10 classes: silt, silty sandy, silty clay, sandy silty, mixed sandy+silt+clay, clay silty, sand, sandy clay, clay sandy, and clay. Submerged aquatic vegetation data were based on the percentage of coverage (%) of *Ruppia maritima*, *Zannichellia palustris*, and macroalga and meadow height (B. Gianasi, *unpublished information*). Information on submerged aquatic vegetation was available only for autumn and summer due to its occurrence period in Patos Lagoon estuary. The inner estuarine limit for sampling of vegetation was narrower than the limits of other environmental variables. This means that autumn and summer models will show a different spatial limit. The Mangueira Embayment (Fig. 1) exhibited a low resolution of environmental and biological data, and for this reason it was excluded from the model.

We used MAXENT software version 3.3.3 (Phillips & Dudík, 2008) to assess the influence of temporal variation in occurrence and habitat preferences during the life cycle of whitemouth croaker at Patos Lagoon estuary, and evaluate if the addition of different life stages into the modeling process can impact the final outcome of the species essential fish habitat. In this sense, we tested 2 main scenarios: 1) considering all presence data from the species, regardless of life stage or temporal variation; and 2) considering the occurrence of each life stage separately for each season. In the last case, we also tested a model considering juveniles and adults regardless of their length class, and then ran models to each length class separately. In spite of the high abundance of individuals smaller than 30 mm, this length class was not modeled separately because of the lack of data present at different sampling stations; however, the data were included in the abundance analysis and in the model that considered all length classes of juveniles. We set 25% of the species occurrence data for testing and 75%

for training the models. Data were crossvalidated and all runs were set with a random seed with a convergence threshold of 0.00001 with 5,000 iterations and with 10,000 background points. Predictive performance of the models were analyzed according to True Skill Statistic (TSS, Hanssen & Kuipers, 1965; Allouche et al., 2006). TSS values can range between -1 (no agreement) and 1 (total agreement) and are classified as: 0 – 0.4= poor; 0.4 – 0.5= fair; 0.5 – 0.7= good; 0.7 – 0.85= very good; 0.85 – 0.9= excellent; and, 0.9 – 1.0= perfect. TSS was chosen since it is not affected by the size of the study area or the prevalence of the presence points. Then, the models selected to build the essential fish habitat mapping were re-ran using data randomly subsampled and replicated 15 times. The logistic threshold output was used and a 10 percentile training presence logistic threshold was used for the suitable habitat identification of whitemouth croaker in the PLE.

RESULTS

Assessing models performance

In general, predictive performance of models were higher than 0.4, indicating a prediction ranged between fair (0.4 – 0.5) and very good (0.7 – 0.85) (Table 1). Scenario 1, considering all presence data of whitemouth croaker at Patos Lagoon estuary regardless of temporal variation in the occurrence of each development stage, showed a good predictive performance (0.56). However, when considering the effect of temporal variation in the presence of each life stage (Scenario 2), we can observe that in 64% of the runs it is possible to reach an improved predictive performance for the models (Table 1). In this scenario, we had only three exceptions where TSS values were smaller than 0.4 (Table 1). In this sense, we can assume that models considering the impact of temporal variation in the presence and distribution of different life stages can improve modeling outcomes. Consequently, further results showed in this study are based on models from scenario 2.

Table 1: True Skill Statistic (TSS) values obtained for different scenarios ran with Maxent for distinct development stages (eggs, larvae, all juveniles and SETs) of *Micropogonias furnieri* at Patos Lagoon estuary (Brazil). In bold, poor (smaller than 0.4) predictive performance values.

Scenario 1 (considering all presence data, regardless life stage or temporal variation)
TSS = 0.56

Scenario 2 (considering presence data of different life stages and temporal variation)				
Life Stage	Summer	Autumn	Winter	Spring
Eggs	0.69	0.55	-	0.44
Larvae	0.53	0.73	-	**0.27**
Juveniles (All)	0.59	0.42	0.65	0.59
Length class 30 – 90 mm	**0.39**	0.51	**0.29**	0.57
Length class 90 – 160 mm	0.59	0.65	0.65	0.59
Length class > 160 mm	0.62	0.74	0.62	0.54

-: no presence data in this season.

Relative contribution of predictor variables for each model

The relative contribution of each predictor varied for each developmental stage of whitemouth croaker and also for seasons (Table 2). For eggs and larvae, the main variables were temperature and salinity; however, the submerged aquatic vegetation and bathymetry also contributed to the results. Salinity and temperature were the main predictor variables for models that included juvenile and adult data combined; however, bathymetry was important during autumn and sediment was important during winter. The submerged aquatic vegetation contributed less than 10% for this scenario in each season.

A different contribution pattern for predictor variables was observed when modeling the spatial distribution of juveniles and adults classified into length classes. Temperature and salinity were the main contributing variables to the occurrence of individuals ranging in size from 30 – 90 mm during spring in the Patos Lagoon Estuary. At the same time, bathymetry and sediment also contributed to the model (Table 2). Individuals ranging in size from 90 – 160 mm in the summer, winter, and spring had the same temperature and salinity as the main variables; however, during autumn, the contribution of each variable was more evenly distributed among predictors. In this case, sediment, bathymetry, and *Ruppia maritima* coverage also contributed to the model results. Individuals larger than 160 mm showed similar results; however, sediment contribution increased in winter, and macroalgae coverage became important during autumn (Table 2).

Table 2: Mean percent contribution of the predictor variables to seasonal Maxent models for each development stage of *Micropogonias furnieri* in Patos Lagoon estuary (Brazil).

Life Stage	Temperature	Salinity	Sediment	Bathymetry	Macroalgae coverage	*Zannichelia palustris* coverage	*Ruppia maritima* coverage	Meadow height
Eggs Summer	0.3	66.5	10.8	4.9	6.4	0.3	10.5	0.3
Eggs Autumn	20.6	50.3	5.9	14.2	0.6	1.3	4	3.1
Eggs Spring	15.3	73.6	2.5	8.6	-	-	-	-
Larvae Summer	0.2	78.4	3.3	3.9	*	1.5	3.2	9.5
Larvae Autumn	25.5	18.3	8.5	31.1	6.7	3.6	6.3	*
Juveniles Summer	6.1	80.8	6.6	1.1	*	1.9	2.4	1
Juveniles Autumn	31.2	29.6	6.1	14.5	5.9	0.6	6.7	5.4
Juveniles Winter	25.1	48.6	16.5	9.8	-	-	-	-
Juveniles Spring	37	43.4	5.3	14.3	-	-	-	-
30 – 90 mm Spring	32.9	52.9	3.8	10.4	-	-	-	-
90 – 160 mm Summer	4.5	83.1	6	0.8	*	2.1	2.6	0.8
90 – 160 mm Autumn	30.6	25.6	7.3	17.3	1.5	0.2	17.5	*
90 – 160 mm Winter	27.7	48.7	15.2	8.4	-	-	-	-
90 – 160 mm Spring	35.3	47.9	4.7	12.1	-	-	-	-
> 160 mm Summer	3.7	85.8	3.9	1.2	*	2.2	2.8	0.5
> 160 mm Autumn	30.1	31.2	7	11.6	8.7	0.8	10.6	*
> 160 mm Winter	26	48.4	14.8	10.8	-	-	-	-
> 160 mm Spring	30.5	47.1	5.8	16.7	-	-	-	-

-: predictor variable was not available for this season and was not used in the model.
*: predictor variable did not contribute to the model.

Seasonal distribution patterns and essential habitat mapping

Our results showed a well-defined seasonal distribution pattern of whitemouth croaker in the PLE. Eggs and larvae occurred year-round, except for winter (Fig. 2a). In general, high egg densities were associated with spring and summer time (Fig. 2a). A similar pattern of seasonal variability was observed for larvae, with an increase in density during summer and autumn (Fig. 2a). Juveniles and adults of whitemouth croaker registered year-round at the PLE (Fig. 2b and c). When classified by length class, individuals smaller than 30 mm were most abundant during autumn and summer (Fig. 2b and c). High CPUE values were found during the entire study period for individuals from 30 – 90 mm and from 90 – 160 mm; however, CPUE decreased during 2000 and 2001 for individuals larger than 160 mm and their occurrence was restricted to the spring and summer (Fig. 2b and c).

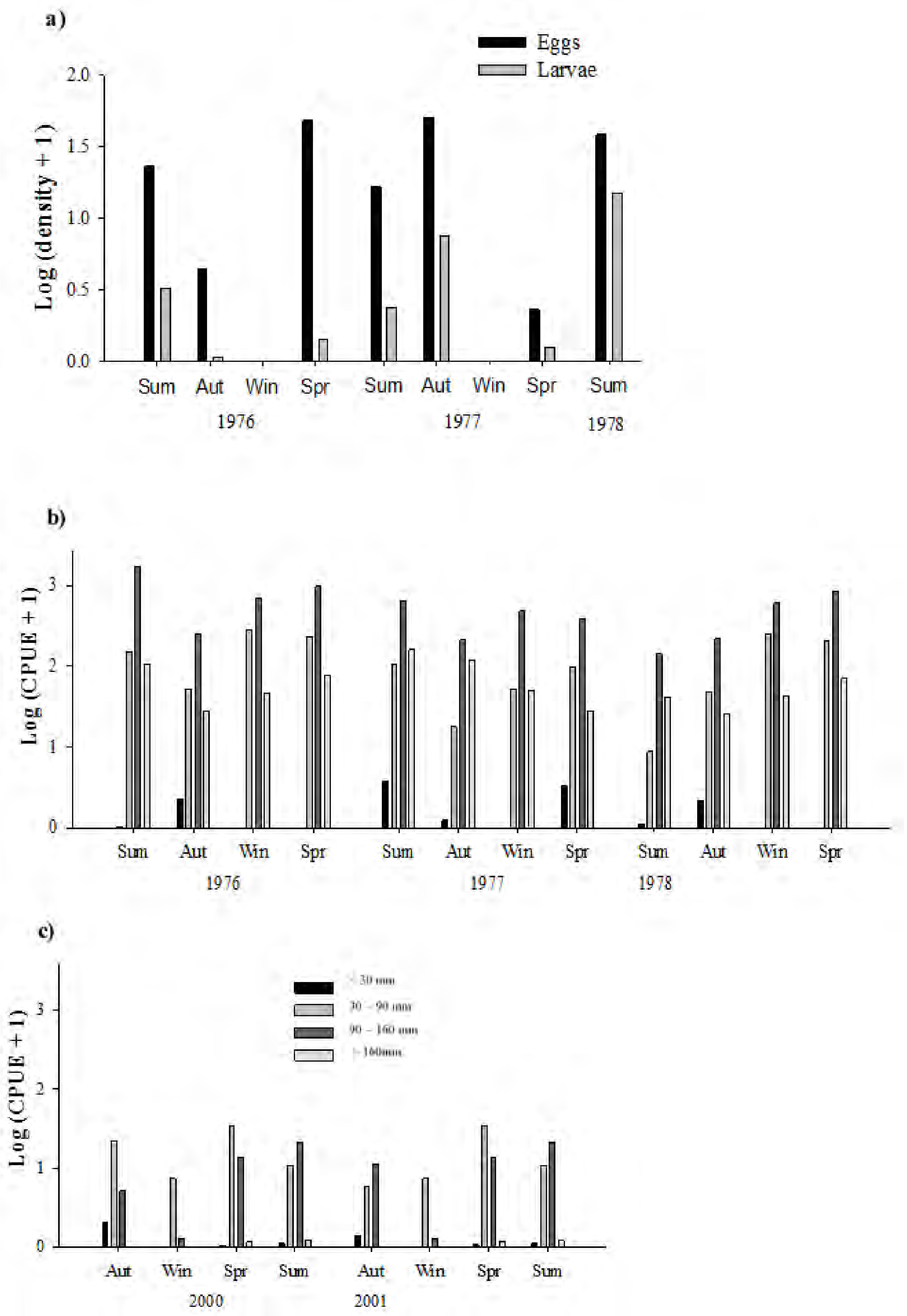

Figure 2: Seasonal mean abundance of eggs and larvae (A) and seasonal mean capture per unit effort (CPUE) of juveniles and adults (B and C) of *Micropogonias furnieri* at Patos Lagoon estuary.

When analyzing seasonal spatial distribution patterns from modeling results we can observe that each ontogenetic stage of whitemouth croaker has distinct habitat requirements during its life cycle (Fig 3 to Fig. 5). In the case of eggs and larvae, it is clear that Patos Lagoon estuary is an important area for the species development and growth during spring (Fig. 3). During this season, eggs and larvae of whitemouth croaker are spread throughout the estuary. During autumn and winter, distribution patterns mostly reflect retention areas due to estuarine circulation patterns (Fig. 3). When examining seasonal distribution patterns for juveniles and adults, we found a distinct habitat usage during the year (Fig. 4 and 5). When analyzing juveniles and adults separated by size class, we observe that individuals from 30 to 90 mm are widely distributed in the estuary, mainly during winter and summer, and occupied both shallow and deep areas of Patos Lagoon estuary (Fig. 4A to 4D). In the opposite, individuals from 90 to 160 mm show a more restrict distribution in the estuary, with a clear temporal variation in the distribution pattern (Fig. 4E to 4H). During autumn (Fig. 4E) and winter (Fig. 4F), individual are more related to shallow sites in Patos Lagoon estuary, however, during spring (Fig. 4G) and summer (Fig. 4H) those individuals are already migrating to deep channels in the estuary. Finally, besides the fact that shallow areas are still suitable for this size class, adults larger than 160 mm (Fig. 5A to 5D) are more associated with deep waters, indicating the spawning migration of these individuals to the adjacent sea. In order to assess the importance of considering different size classes in the modeling process, we ran a scenario considering all data from juveniles and adults, regardless their size (Fig. 5E to 5H). In this case, despite the effect of the temporal variation in the distribution and occurrence, most of the details in the patterns obtained when dealing with each ontogenetic stage separately are lost.

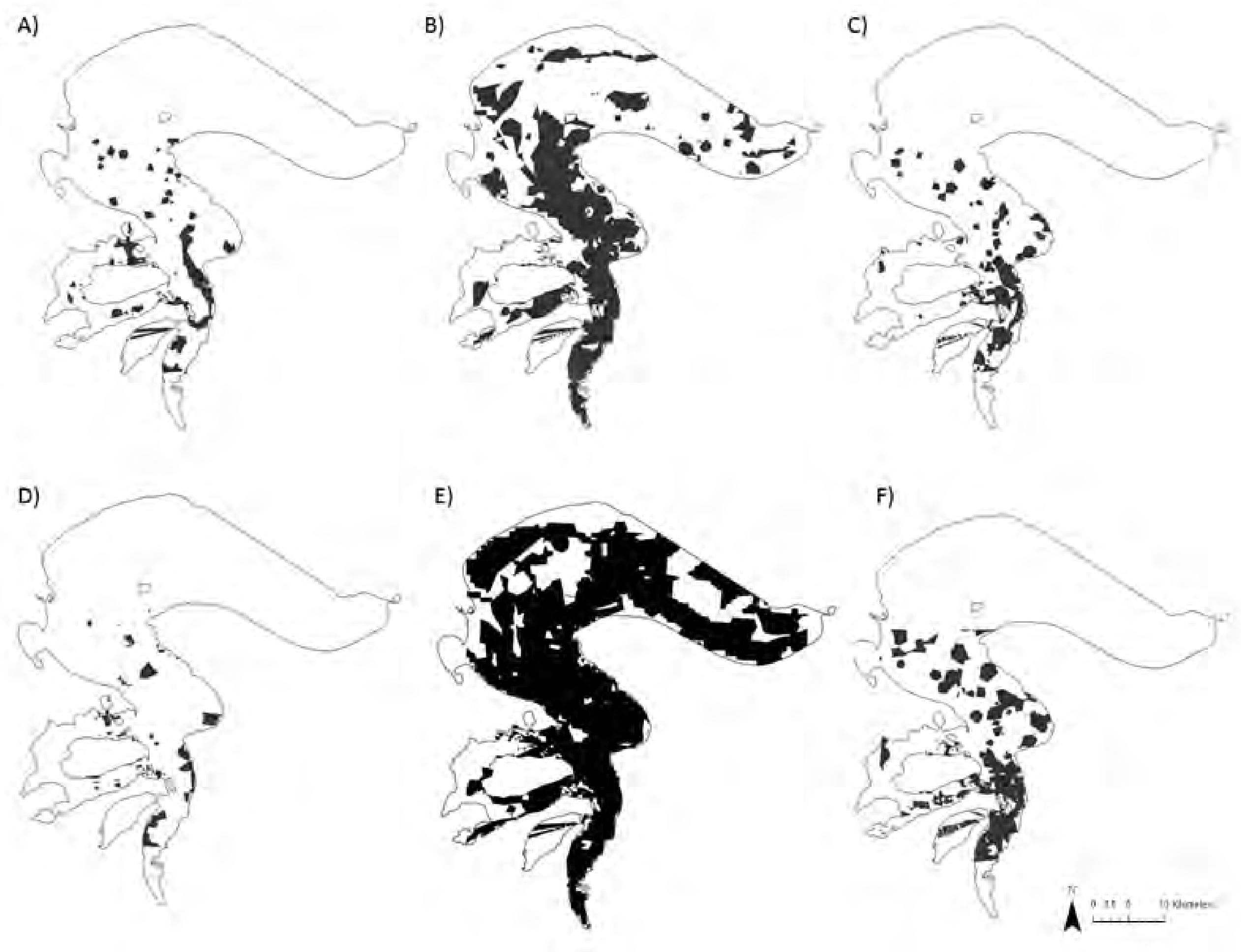

Figure 3: Seasonal distribution modeling of eggs and larvae of *Micropogonias furnieri* at Patos Lagoon estuary based on a 10 percentile training presence logistic threshold: A) habitat suitability mapping of eggs during autumn; B) habitat suitability mapping of eggs during spring; C) habitat suitability mapping of eggs during summer; D) habitat suitability mapping of larvae during autumn; E) habitat suitability mapping of larvae during spring; and, F) habitat suitability mapping of larvae during summer. Spatial limits for autumn and summer are different from winter and spring due to submerged aquatic vegetation information available.

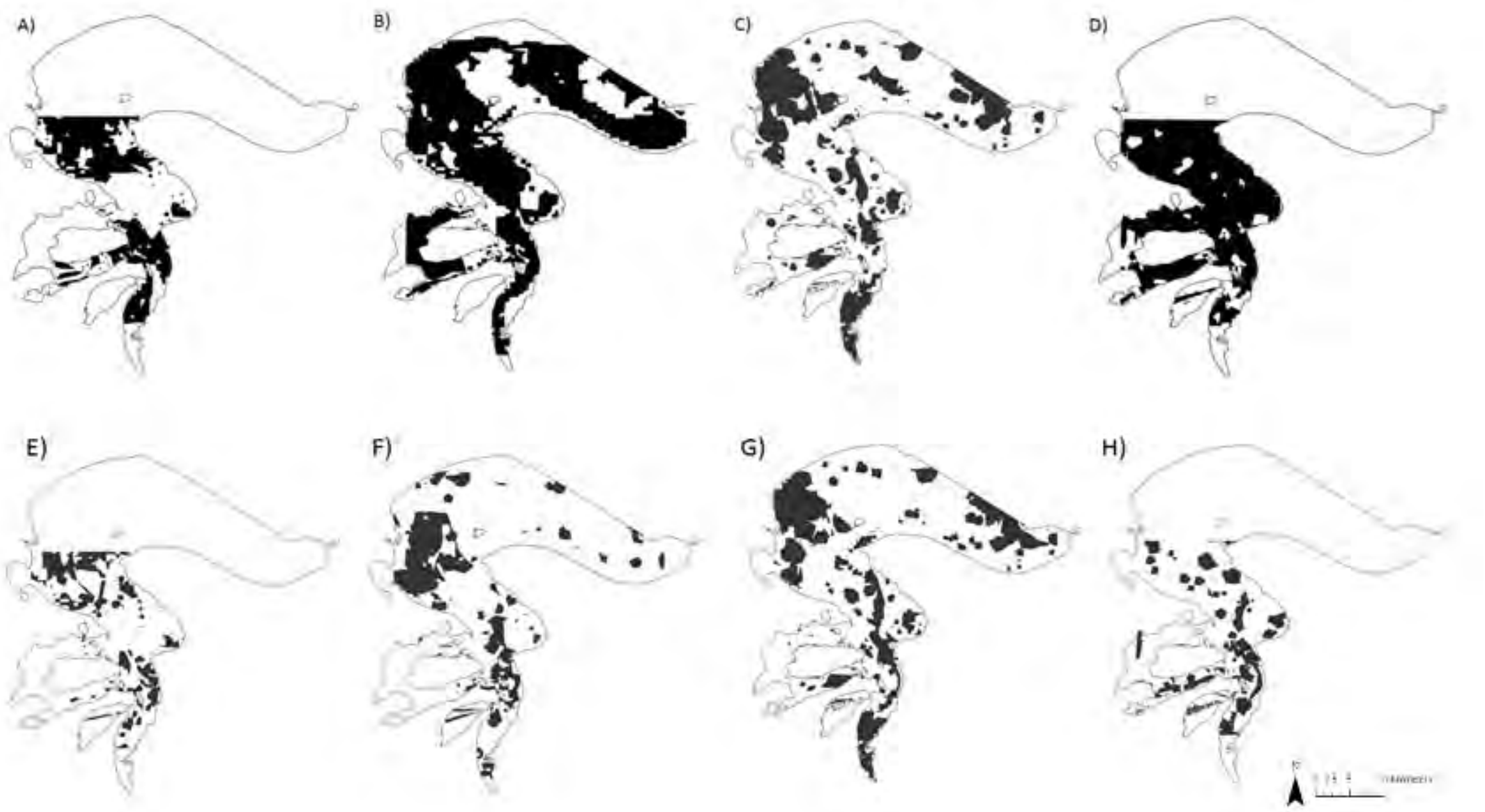

Figure 4: Seasonal distribution modeling of individuals from 30 to 90 mm and from 90 to 160 mm of *Micropogonias furnieri* at Patos Lagoon estuary based on a 10 percentile training presence logistic threshold: A) habitat suitability mapping of individuals from 30 to 90 mm during autumn; B) habitat suitability mapping of individuals from 30 to 90 mm during winter; C) habitat suitability mapping of individuals from 30 to 90 mm during spring; D) habitat suitability mapping of individuals from 30 to 90 mm during summer; E) habitat suitability mapping of individuals from 90 to 160 mm during autumn; and, F) habitat suitability mapping of individuals from 90 to 160 mm during winter; G) habitat suitability mapping of individuals from 90 to 160 mm during spring; and, H) habitat suitability mapping of individuals from 90 to 160 mm during summer. Spatial limits for autumn and summer are different from winter and spring due to submerged aquatic vegetation information available.

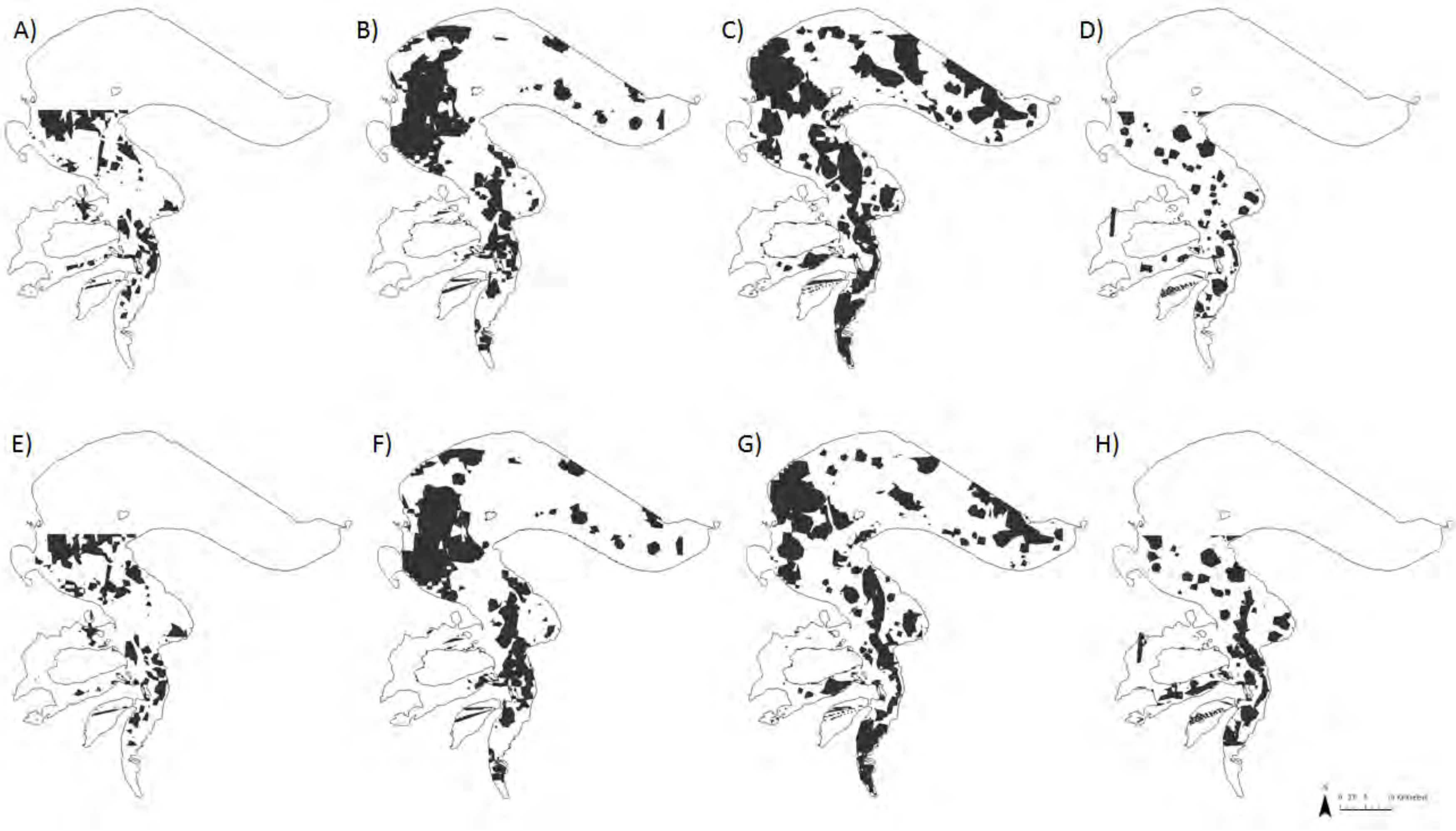

Figure 5: Seasonal distribution modeling of individuals larger than 160 mm and from scenario considering all juveniles and adults of *Micropogonias furnieri* at Patos Lagoon estuary based on a 10 percentile training presence logistic threshold: A) habitat suitability mapping of individuals larger than 160 mm during autumn; B) habitat suitability mapping of individuals larger than 160 mm during winter; C) habitat suitability mapping of individuals larger than 160 mm during spring; D) habitat suitability mapping of individuals larger than 160 mm during summer; E) habitat suitability mapping of all juveniles and adults during autumn; and, F) habitat suitability mapping of all juveniles and adults during winter; G) habitat suitability mapping of all juveniles and adults during spring; and, H) habitat suitability mapping of all juveniles and adults during summer. Spatial limits for autumn and summer are different from winter and spring due to submerged aquatic vegetation information available.

In the attempt to build the essential fish habitat of whitemouth croaker, we combined the different seasonal distribution models into one map representing the essential habitat for each ontogenetic stage (Fig. 6). In this case, deep channels (> 6 m) are suitable habitat for eggs (Fig. 6A), whereas almost the entire area of the PLE is suitable for larvae occurrence (Fig. 6B). In general, mainly intermediate waters and the outermost part of the channel are suitable for individuals from 30 – 90 mm (Fig. 6C); however, shallow waters can also be important for these individuals (Fig. 6C). Individuals from 90 – 160 mm can occur in the entire estuarine area, but deep waters seems to be more important for this developmental stage (Fig. 6D). A similar pattern was found for individuals larger than 160 mm (Fig. 6D) because they are distributed throughout the entire PLE, with deep and intermediate waters representing the main habitat for this size class (Fig. 6E).

DISCUSSION

We showed that considering different life stages and the temporal variation in their occurrence can impact the predictive performance of species distribution models, mainly when using these results for the identification of essential fish habitats. Also, we determined the habitat usage and the influence of environmental variables in the distribution of each development stage of whitemouth croaker in the PLE. In addition, we were able to account for the dynamic nature of this ecosystem by modeling each development stage by season. Estuaries are well known for being dynamic because they have a variable mixture of salt and freshwater, which presents a challenge to the physiology of most of species that inhabit these ecosystems (McLusky & Elliott, 2004). In addition, they are composed of a variety of habitats along and across their main axis, such as saltmarshes, intertidal soft and hard substratum, subtidal soft and hard substratum, subtidal vegetated habitats, and tidal freshwater, which play an important role for shelter and food source for a wide variety of species. In spite of this recognized importance, little attention has been given to conservation of these ecosystems and their habitats. In the case of the PLE, the protection of shallow embayments have been ignored since 1980 (Castello, 1985). These habitats are critically important to many species, including whitemouth croaker, to complete their development. A diversified combination of suitable habitats and environmental conditions are needed in order for whitemouth croaker, an euryhaline species, to successfully colonize in the PL estuary.

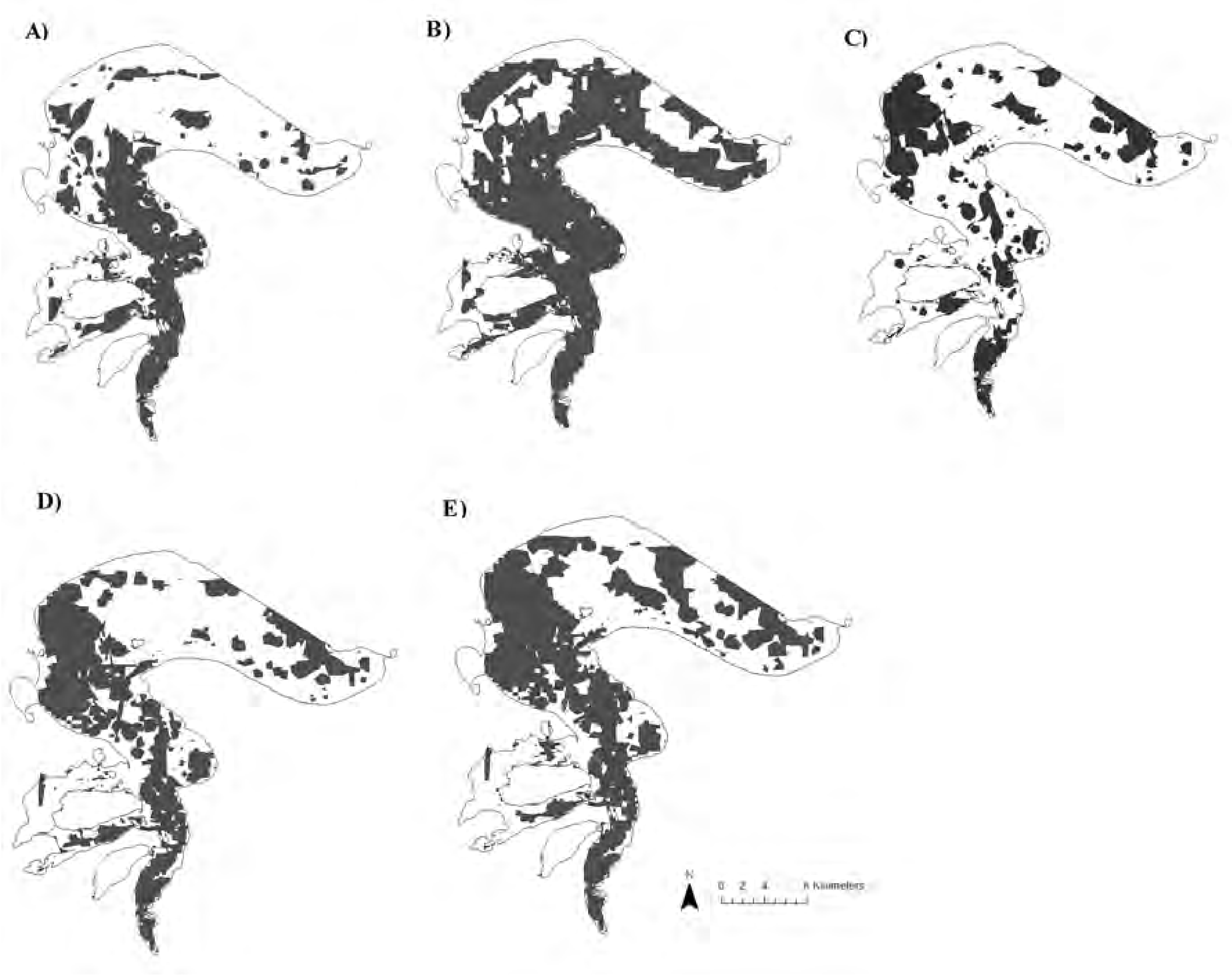

Figure 6: Essential Fish Habitat (EFH) mapping of Micropogonias furnieri at Patos Lagoon estuary, based on the combination of seasonal distribution species modeling for each life stage: A) eggs; B) larvae; C) individuals from 30 to 90 mm; D) individuals from 90 to 160 mm; and, E) individuals larger than 160 mm.

Our results showed that the models can account for different spatial and seasonal variability and they were susceptible to the influence of different environmental variables. Eggs and larvae were common year-round, with highest densities during spring and summer, which were the main reproductive and recruitment period of the species. Studies have already identified salinity and temperature as variables influencing the abundance of whitemouth croaker eggs and larvae in the PLE (Muelbert & Weiss, 1991; Costa et al., 2014). Wind can also influence the entrance of fish eggs and larvae of whitemouth croaker into the PLE (Martins et al., 2007). In addition, our results showed a contribution from submerged aquatic vegetation and bathymetry in their occurrence.

Similarly, temperature and salinity were the main environmental variables influencing the occurrence of juveniles in the PLE; however, in this case, sediment, bathymetry, and submerged aquatic vegetation also contributed to the models. Past studies have already highlighted the importance of vegetated shallow embayments, mainly those covered by *Ruppia maritima.* These estuarine habitats are essential habitats for feeding, development, and protection during the life stages of many invertebrates and fishes (Gillanders, 2006). In Patos Lagoon estuary, *Ruppia maritima* colonize large areas of shallow waters (Seeliger, 2001), and are extremely important to individuals of whitemouth croaker smaller than 30 mm, which use these areas during both the day and night (Garcia & Vieira, 1997). These habitats are also important to pink shrimp *Farfantepenaus paulensis*, which depends on many shallow embayments at PLE to recruit and complete its development (D’Incao, 1991). The distribution of smaller length class (individuals smaller than 30 mm) could not be modeled due to its presence was associated with few sampling points. Despite that, their abundance were extremely high, mainly at stations around “Marinheiros Island”, showing that this shallow area in the PLE could act as an essential habitat for this size group (Fig. 1). Because each length class of whitemouth croaker had been correlated to a different habitat type in this estuarine ecosystem (Costa et al., 2014), these results reinforce that each size class could be ruled by different variables. Meteorological processes that control seawater intrusion into the estuarine system are also responsible for the transport of planktonic organisms into the PLE (Seeliger, 2001). In the case of whitemouth croaker, the intrusion during the early life stages into the PLE is associated with the spawning activity in the adjacent Atlantic Ocean during warmer periods (Ibagy & Sinque, 1995), which coincided with the increased abundance of eggs and larvae (Sinque & Muelbert, 1997). Shallow embayments at Patos Lagoon estuary were suitable habitat for most of ontogenetic stages of whitemouth croaker. Our results showed that to protect whitemouth croaker and to guarantee the success of recruitment process, management plans should consider the protection of channels and most of shallow embayments in the PLE.

Ontogenetic shifts and feeding are cited as important factors that need to be incorporated into marine SDMs (Robinson et al., 2011; Le Pape et al., 2014). This is particularly relevant when dealing with highly dynamic ecosystems, such as estuaries, and with species exhibiting a complex life cycle, such as whitemouth croaker. In our study, we successfully identified the essential fish habitat of whitemouth croaker by accounting for the ontogenetic shift of each developmental stage. Modeling each life stage separately allowed us to identify those areas in the PLE that seemed to be suitable to the species for its development during different times of the year. In practice, these results could also be applied into marine conservation planning. Designing maps of essential fish habitats is usually the first step in conservation, taking into account the habitats required by the species to successfully complete their life cycle (Valavanis et al., 2008; Le Pape et al., 2014); however, to use species distribution modeling in marine conservation planning, it is extremely necessary that the dataset represents the most current information available. Another difficult task is to define the boundaries of an essential fish habitat (whether it should be designated more important 10%, 20% or 30% of the habitat). Despite the inherent uncertainty in modeling results, SDMs are usually used for conservation planning (Wilson et al., 2005; Elith & Leathwick, 2009). In this case, essential fish habitat maps could be used as input information into decision support software, helping to achieve an efficient reserve system (Le Pape et al., 2014). In the particular case of the essential fish habitat, a systematic conservation planning approach could help to spatially prioritize those areas identified as relevant to the maintenance of the population. In addition, essential fish habitat maps for different life stages are useful to fill knowledge gaps about fish distribution and in helping decision makers set better conservation targets and actions for management plans. Our results showed that predictive performances of the models were impacted when considering different life stages and the temporal variation in their occurrence. Also, that each developmental stage of whitemouth croaker can be correlated with an estuarine habitat and influenced by different environmental variables at Patos Lagoon estuary (Costa et al., 2014). In conclusion, we highly suggest that essential fish habitat identification should consider separately different life stages and their temporal fluctuations during the modeling process, accounting for the complexity and different habitat requirements that a species can show throughout their development.

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6.3 Apêndice 3 – Costa, M.D.P., Possingham, H.P., Muelbert, J.H., Vieira, J.P., Castello, J.P., Copertino, M.S. & Tagliani, P.R.A. Ensuring the protection of an estuarine essential fish habitat by spatial conservation prioritization analysis with different management zones. * Submetido na Diversity and Distributions

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Keywords:	essential habitat, estuaries , Marxan with Zones, Micropogonias furnieri, spatial conservation planning, spatial prioritization

Ensuring the protection of an estuarine essential fish habitat by spatial conservation prioritization analysis with different management zones

Short Title: Conservation planning to ensure habitat protection

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ABSTRACT

Aim Essential fish habitat has been identified using a variety of tools and approaches, but, none of them allows us to develop conservation priorities in space that logically integrates the mitigation of different threats. In this context, our aim was to set a conservation plan to ensure the protection of the estuarine essential habitat of *Micropogonias furnieri*. The primary question is, will having more zones in the conservation plan will change the spatial priorities?

Location Patos Lagoon estuary, Brazil.

Methods Using two conservation targets (15% and 30%), we used a decision support tool to compare three conservation plans to evaluate the role of zoning spatial priorities. To reflect the opportunity cost of different zones we used surrogates for the artisanal fishery revenue. We performed a cluster analysis to compare the solutions between the scenarios tested for each zone.

Results We showed that having more zones in a conservation plan does not significantly change spatial priorities. Also, our results showed that a conservation plan should accommodate the protection of an essential habitat by integrating different life stages of a species. In addition, we compared two zoning plans in relation to their ability to reach absolute conservation target and zone target, the sensitivity of our solutions to opportunity cost, and changes in spatial priorities in each zone. Distinct conservation targets produced different locations for sanctuary zone. Using an absolute target of 15% it is possible to achieve a low average cost for most of the scenarios examined.

Main Conclusion We found that regardless of the absolute target and the opportunity cost, having more zones did not significantly change spatial priorities for conservation in Patos Lagoon estuary. Hence, while zoning of protected areas enhances efficiency, the zoning can occur after initial designation of broad areas for protection in this case.

Key words: essential habitat, estuaries, Marxan with Zones, *Micropogonias furnieri*, spatial conservation planning, spatial prioritization

INTRODUCTION

Until recently, fishery management was usually focused on treating each target species separately. This usually involves controls in catch, effort, timing and gear for each fishery. However, with advances in knowledge about interactions between resources and their environment, fishery management has shifted focus towards ecosystem-based management (Benaka, 1999; FAO, 2003). The main goal of the ecosystem approach to fishery (EAF) is to "*ensure that, despite variability, uncertainty and likely natural changes in the ecosystem, the capacity of the aquatic ecosystems to produce fish food, revenues, employment and, more generally, other essential services and livelihood, is maintained indefinitely for the benefit of the present and future generations*" (FAO, 2003). This perspective tries to balance diverse social objectives, taking into account the relationship between biotic, abiotic and human components in the ecosystem and their uncertainties (FAO, 2003).

The concept of Essential Fish Habitat (EFH) was originated in the United States of America when the Magnuson-Stevens Fisheries Conservation and Management Act recognized the importance of habitat for the maintenance of fisheries stocks, and defined the term as "*those waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity*." This perspective puts emphasis on the importance of habitats for the maintenance of aquatic resources (Minello, 1999). In 1996, new amendments to this United States law included conservation measures based on the identification of habitats essential to maintain and support marine resources and sustainable fisheries (Benaka, 1999; Rosenberg *et* *al*., 2000). In EFH, the terms “fish” can be finfish, molluscs, crustaceans, and all other marine animals and plants other than marine mammals and birds; “substrate” refers to the associated biological communities that render these sites suitable for the species; and “waters” including all aquatic areas and their properties (biological, chemical and physical) (NMFS, 1997; Rosenberg *et al*., 2000). In Brazil, as in the rest of the world, most fishery management has been focused on controls of the catch, effort and technical measures for the target species such as mesh size, closed seasons and areas. Besides that, there is an increasing recognition by Brazilian decision makers for the requirement to protect essential fish habitats to stabilise recruitment and stocks (MMA, 2013). Habitat management must involve a close interaction between stakeholders and decision makers, before establishing and applying coastal management policies.

Designation of essential fish habitat occurs through four lines of evidence: 1) presence-absence data of the species; 2) habitat-specific densities of the species; 3) habitat-related measures of growth, reproduction, or survival; and 4) fish production by habitat (Able, 1999; Minello, 1999). Most of the research on essential fish habitat identification is based on data on the species (lines of evidence 1 and 2) because data on population processes and productivity is lacking for most habitats (Able, 1999; Minello, 1999; Valavanis, 2008). Usually, essential fish habitat has been identified by different methods, including analyses of density patterns within habitats, habitat suitability indices and species distribution models (Benaka, 1999; Valavanis, 2008). The result is that all habitats used by a species during all its life history are included in the essential fish habitat designation (Cook & Auster, 2005; Levin & Stunz, 2005). When dealing with species that have complex life cycles and patterns of habitat use, especially in coastal areas where there are a wide variety of human uses, EFH prioritization is a complex and difficult task requiring a lot of data.

While habitat prioritization has already been highlighted in essential fish habitat designation (Able, 1999; Minello, 1999; Cook & Auster, 2005; Levin & Stunz, 2005; Le Pape *et al*., 2014), none of the methods usually used allows us to prioritise protection spatially. To our knowledge, only two studies have tried to prioritize the spatial location of essential fish habitat. Levin & Stunz (2005) developed a three-step approach based on a life cycle model that prioritises life history stage and habitats, while Cook & Auster (2005) used spatial conservation planning software (Marxan) to prioritize essential fish habitat for many species. In this sense, this is the first study to integrate distribution data for eggs, larvae, juveniles and adults larger than 160mm, to systematically and spatially prioritise essential fish habitat for a single species.

The whitemouth croaker *Micropogonias furnieri* (Desmarest 1830) is a Sciaenidae estuarine dependent fish distributed in the western Atlantic from the Gulf of Mexico (20°N) to Argentina (41°S) (Vazzoler, 1991; Vieira & Castello, 1997; Albuquerque *et al*., 2012). In general, the species inhabits marine, brackish and freshwater environments, using estuaries as a nursery ground. The whitemouth croaker is one of the most important commercial fish species in Brazil, reaching annual catches over 40,000 tons (MPA, 2010), but it is already considered overexploited (Haimovici & Ignácio 2005). Patos Lagoon estuary is one of the most important nursery ground for *M. furnieri* in southern Brazil, occurring all year and in every life stage (Castello, 1985, 1986; Muelbert & Weiss, 1991; Vieira, 2006; Costa *et al*., 2014). Patos Lagoon, the largest choked lagoon in the world (Kjerfve, 1986), has an area of approximately 11,000 km^2 (Seeliger, 2001). About 10% of the lagoon is shallow (< 1.5m) estuary, with some intermediate (1.5 – 5.0 m) and deep (> 5 m) channels. The estuarine shallow areas are occupied by submerged aquatic vegetation (up to 120 km^2), bare sediment (300 km^2), fringing salt marshes (40 km^2) and artificial hard substrates (Seeliger, 2001). As with most coastal environments, Patos Lagoon is exposed to a wide variety of threats and pressures, including untreated domestic effluents, rice and cattle farming, industrial pollution, mining, dredging, port activities, and fishing (Barletta *et al*., 2010).

Usually, spatial variation in the location of developmental stages reflects different ecological requirements determined by environmental conditions. In the case of *M. furnieri* in Patos Lagoon, each life stage of the species exhibits a defined temporal and spatial pattern of habitat use driven by feeding preferences (Figueiredo & Vieira, 1998; Gonçalves *et al*., 1999; Costa *et al*., 2014). *Micropogonias furnieri* was chosen as our case study species because of its frequency in experimental bottom trawl, ichthyoplankton and beach seine sampling (Muelbert & Weiss, 1991; Vieira, 2006). Our aim was to establish a conservation plan for an estuarine system ensuring the protection of the essential fish habitat of an estuarine dependent species, *M. furnieri*, integrating data from all life stages (eggs to adults up to 160mm TL). In this case, the primary question is: how will having more kinds of zone available in the conservation planning process change the spatial priorities?

METHODS

Biodiversity Data

The Patos Lagoon estuary (Figure 1) was divided into 2,355 hexagons planning units using QuantumGis software (QGIS Development Team, 2013). Distribution data for forty-one biological and environmental features were used as input data in the analysis, including bathymetry, sediment type, submerged aquatic vegetation coverage, and seasonal distribution of eggs, larvae and juveniles of *M. furnieri*. Submerged aquatic vegetation data comprises both average percentage cover and canopy height of mixed strands of *Ruppia maritima, Zannichellia palustris*, and macroalgae, obtained during the austral summer (February 2011) and autumn (May 2011) (Gianasi, unpublished data). Bathymetry was classified into three classes: shallow (< 1m), intermediate (1 – 5 m) and deep (channels, > 5 m) waters. Sediment type follows the ten classes proposed by Calliari (1980), based on the relative amounts of sand, silt and clay: silt, silty sandy, silty clay, sandy silty, mixed sandy+silt+clay, clay silty, sand, sandy clay, clay sandy, and clay. Distribution data for *M. furnieri* comprises the seasonal spatial distribution of eggs, larvae, juveniles and adults larger than 160mm sampled at Patos Lagoon estuary during a non-continuous period from December, 1975 to April, 2001. Juveniles were classified in four size classes (< 30 mm, 30 – 90 mm, 90 – 160 mm, and > 160 mm). Further information about the sampling methods and seasonal spatial distribution are available in Costa *et al*. (2014).

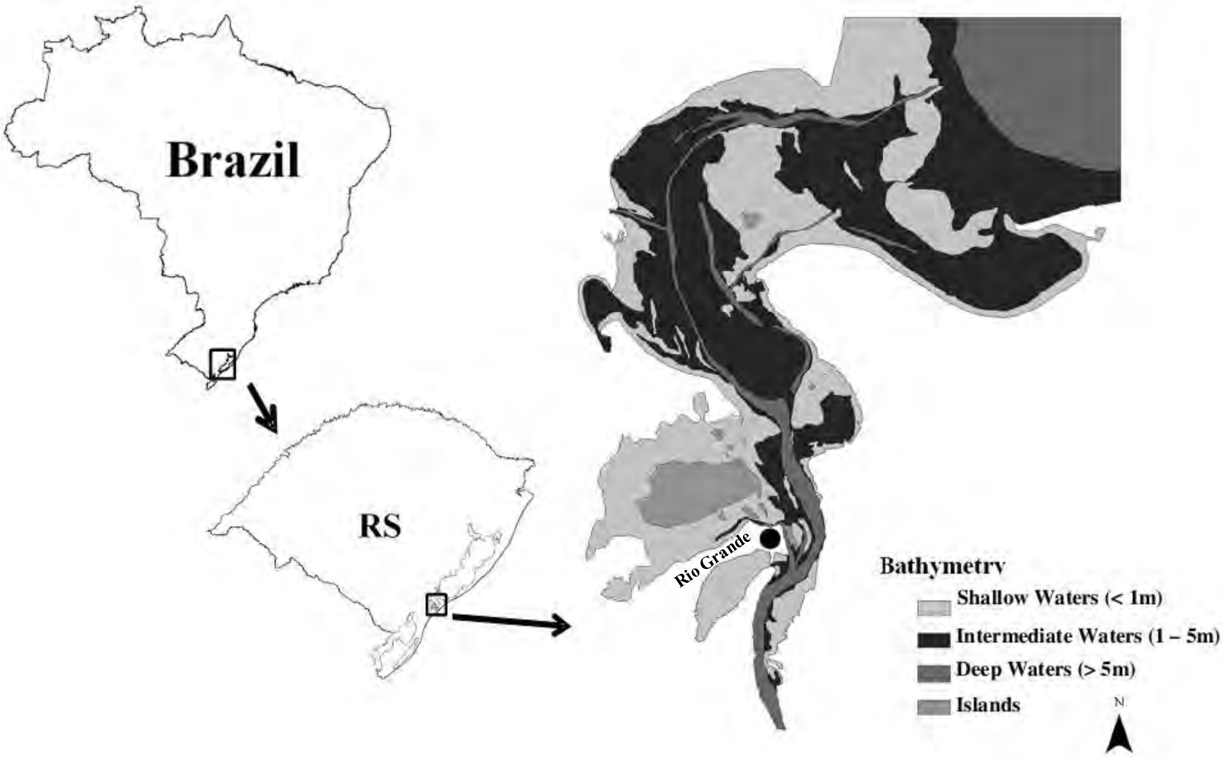


Figure 1: Location of Patos Lagoon estuary in Rio Grande do Sul State (RS, Brazil) and details of the water depth classes in the estuarine area. Modified from Seeliger (2001).

Opportunity cost

Cost-effective conservation planning is important in a multiple use and resource-constrained environment. In conservation planning, there are many types of costs associated with conservation actions (Wilson *et al.*, 2009). Conservation costs are not spatially homogeneous, and among the different kinds of cost, opportunity cost represents the value of a foregone usage (lost benefit when an activity takes place where another used to occur) (Wilson *et al.*, 2009). For example, in aquatic environments, opportunity cost can be the fishery benefit that will be lost if a specific area is declared as a no-take area. Here we estimated the fishing revenue for crustacean and finfish fishery in each fishing site of Patos Lagoon estuary to represent the opportunity cost for local artisanal fishers. The fishing revenue was calculated by adapting the equation for commercial fishing in Mazor *et al.* (2013). To estimate the annual catch of the artisanal fishery (C_i) in each planning unit we assumed that the catch decreases with estuarine depth (D) and to the distance to the nearest landing site (d) weighted exponentially by a constant α and then multiplied by the area (A) of the planning unit (i). We used two different values for α: 0.001 and 0.0001 to reflect uncertainty in how far fishers travel to fish, and these values were used separated in the analysis. Then, we re-ran the analysis incorporating the four layers of opportunity cost (Figure 2). The depth (D) was classified into three classes, being 1 = channels (> 5 m), 2 = intermediate waters (1 – 5 m) and 3 = shallow waters (< 1 m). The catch was normalised by a measure of total effort (R) which is equal to:

$R = \sum_{i=1}^{m} D_i \, e^{-\alpha d} \text{ x } A_i,$

where m is the number of planning units in the estuarine area of Patos Lagoon.

We then multiplied the final value by the total biomass of fish captured (B, ton) in the Patos Lagoon estuary during 2011 (IBAMA/CEPERG, 2012), multiplied by the average price

(C, \$ per ton) of three main target species for the finfish fishery (*M. furnieri*, marine catfish *Genidens barbus*, and mullets *Mugil liza*) and of two crustacean species (shrimp *Farfantepenaeus paulensis* and blue crab *Callinectes sapidus*) in the Patos Lagoon estuary (which were US\$591 and US\$837 per ton, respectively). The final equation used to calculate the opportunity costs at Patos Lagoon estuary is as follows:

$$C_i = \left(\frac{D_i e^{-\alpha d} A_i}{R}\right) \text{ x } B \text{ x } C$$

The four resulting opportunity cost layers for crustacean and finfish fishing in Patos Lagoon estuary are given in Fig. S1.

Systematic Conservation Planning

We used the decision support tool Marxan and Marxan with Zones V. 1.0.1 (Watts *et al.* 2009) to compare three conservation plans for this estuarine system, integrating different life stages (eggs to adults) and ensuring the protection of the essential fish habitat of *M. furnieri*. Marxan uses a simulated annealing algorithm to find alternative good solutions to the minimum set coverage problem (Ball *et al.*, 2009). Marxan with Zones is an extension of Marxan which allows a multiple use planning approach, aiming to find a good solution assigning each planning unit to one zone while meeting the biodiversity targets at a minimum cost (Ball *et al.*, 2009; Watts *et al.*, 2009). In a Marxan analysis, the conservation target is the amount of the conservation feature (component of biodiversity; in our case, forty-one features were used) to be protected, that is, the solution must protect at least this amount of each feature (Ardron *et al*, 2008; Game & Grantham, 2008). In this sense, we aimed to test three conservation plans based on IUCN zoning categories for marine protected areas (Dudley, 2008): 1) plan I: just two zones, protected or otherwise; 2) plan II: allows for a sanctuary zone, habitat protection zone, and multiple use zone; and 3) plan III: allows for a sanctuary zone, habitat protection I zone, habitat protection II zone, and a multiple use zone (Table 1). The four zoning schemes were informed by existing fisheries activities in Patos Lagoon estuary. For all conservation plans, planning units open for navigation (according to the Framework for Water Classification from the Brazilian National Council of the Environment - CONAMA, 2005), were locked out and not available for inclusion in the reserve system or sanctuary and habitat protection zones.

Marxan and Marxan with Zones were run 100 times each with 1,000,000 iterations. Following recommendations in the Marxan manual (Watts *et al*., 2008) the amount of spatial clumping was determined by setting the "boundary length modifier" (BLM) to 10,000, and the "species penalty factor" (spf) was set to 10 for all features. To cluster the different zones we set a different zone boundary cost for each zone. As each run of Marxan produces a different solution, the selection frequency output, which comprises the number of times that a planning unit was included in particular zone across all runs, was chosen to represent the output (Watts *et al*., 2008).

Table 1: Overview of the conservation plans proposed for Patos Lagoon estuary, Brazil.

Conservation Plans	**Sanctuary Zone (IUCN Ia)**	**Habitat Protection Zone (IUCN IV)**		**Multiple Use Zone (IUCN VI)**
Plan I – Simple No zoning scheme: Marxan produces a binary outcome, reserve and non-reserve.	-	-		-
Plan II - Intermediate	X	X		X
		Habitat Protection I	Habitat Protection II	
Plan III - Complex	X	X	X	X
Description (based on IUCN zoning classification - Dudley, 2008)	Areas with high priority to biodiversity conservation. Use and impacts are strictly controlled to ensure protection.	Protect particular species or habitats, needing regular and active interventions to address the conservation of the habitat or species. Habitat Protection I related to the maintenance of pelagic habitat, allowing only crustacean and bottom-related fishing. Habitat Protection II is related to benthic habitat conservation, and allows only pelagic fishing.		Habitat conservation associated with sustainable use of natural resources.

Conservation targets

For each zoning planning scenario, we tested two different biodiversity targets. We set a 15% and 30% target for all features, with a specific contribution fraction for each zone when using Marxan with Zones: 100% for sanctuary zone, 30% for habitat protection (I and II) zone, and 0% for multiple use zone. These reflect how much of a feature is conserved in each zone (Ball *et al.*, 2009). Besides the conservation target in each zone, Marxan with Zones enables you to set a target for each feature in a specific zone (Watts *et al.*, 2008): we set a different target for each life stage (eggs, larvae, and juveniles) in a specific zone: 10% for all life stages at sanctuary zone; 8% for eggs and larvae at habitat protection I and II zone, and 5% for juveniles at habitat protection I and II zone. As our main objective in this study was to design a conservation plan to ensure the protection of the essential fish habitat of *M. furnieri*, we also tested our results using a higher target (20%) for the sanctuary zone. The assumptions were that: 1) fish eggs and larvae are not affected by fishery directly, however, their occurrence, distribution and survival can be influenced by the effects of human activities such as fishing, on the habitat required to complete their development; and 2) *Micropogonias furnieri* has a complex life cycle which includes a habitat change during its growth, from pelagic to benthic habitat. We set the contribution of the multiple use zone as 0% for all life stages of the species.

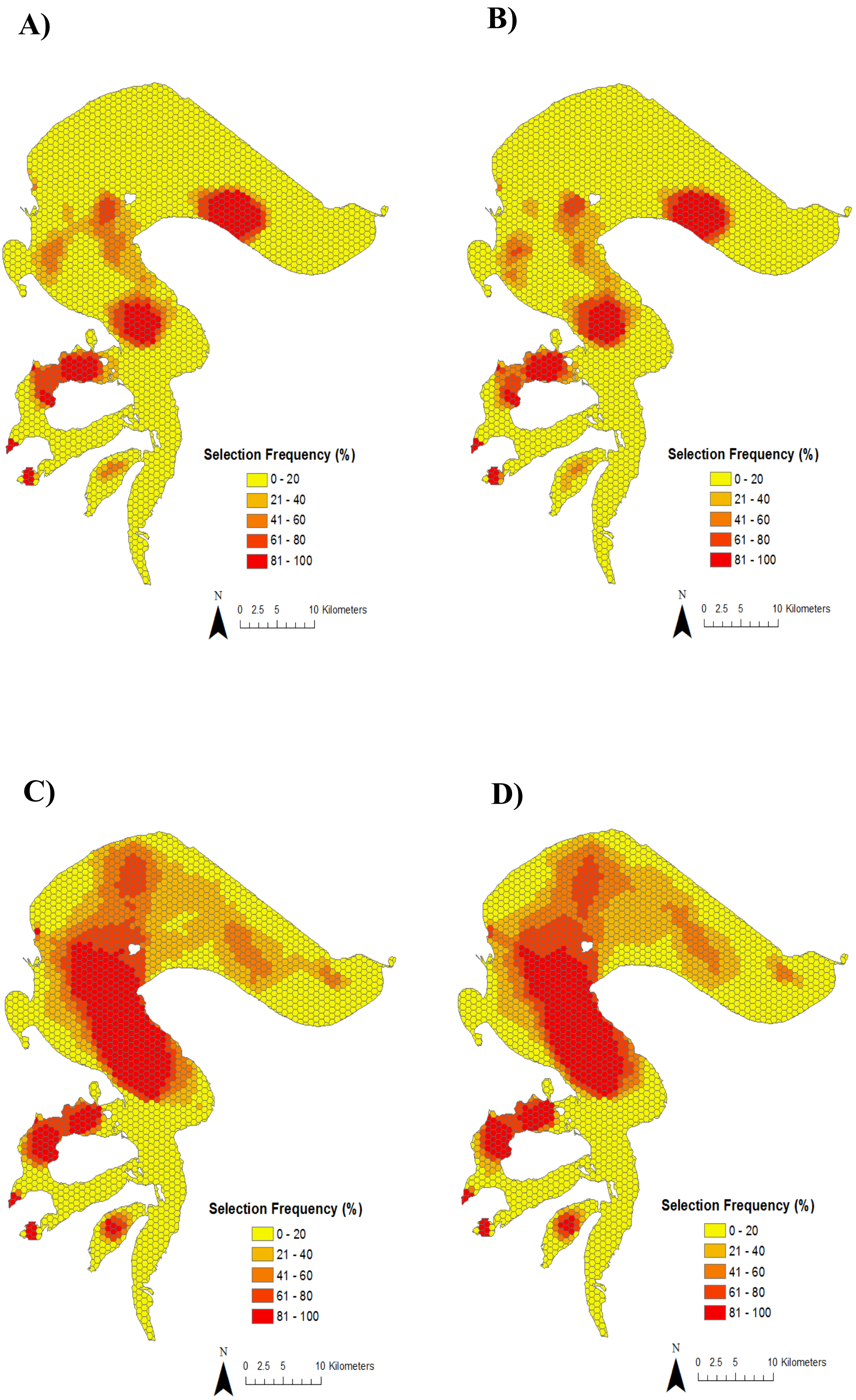
Comparing the solutions and scenarios

To compare the solutions between the scenarios tested for each zone we performed a cluster analysis in R version 2.15.0 (R Development Core Team, 2013) using the Multivariate Analysis of Ecological Communities (vegan package) in R version 2.15.0 (R Development Core Team, 2013). The cluster analysis, when applied to Marxan results, allows the user to group the most similar solutions while achieving conservation targets efficiently (Linke *et al.*, 2011; Harris *et al.*, 2015). We also compared the scenarios based on their average total cost to implement the zoning planning.

RESULTS

We found that having more zones in a conservation plan does not change spatial priorities. In general, sites with high selection frequency for conservation were in the same areas of Patos Lagoon estuary. Our results also showed that a conservation plan can accommodate the protection of estuarine essential fish habitat by integrating different life stages of a species. In addition, we compared our two zoning plans in relation in their ability to reach their absolute conservation targets (15% or 30%) and zone targets, the sensitivity of our scenarios solutions to opportunity cost, and changes in spatial priorities in each zone.

Spatial priorities did not change substantially among all scenarios tested in this study, showing that a zoning scheme can be added later during a conservation plan. Conservation and zone targets were met for all scenarios tested in this study, regardless of how we calculated opportunity cost. Results from the plan I (without zoning) showed a similar spatial priority pattern, regardless of opportunity cost and conservation target (Fig. 2). However, scenarios with a conservation target of 30% prioritised a broader area with high selection frequency in the intermediate estuary (Fig. 2C and 2D). In general, when comparing these results with the conservation plans considering zoning, we observed that shallow waters are the main sites for *M. furnieri* essential fish habitat conservation (Fig. 2, 3, 4, S2 – S7). Planning units selected frequently as sanctuary zone were concentrated in the embayment and shallow waters along the shoreline of the estuary in all scenarios (Fig. 3A, 4A, S2 – S7). Habitat protection zone (I and II) did not show high selection frequencies (< 50%), but site selection was similar among scenarios (Fig. 3B, 4B and 4C). Planning units with high selection frequency selected for the multiple use zone were widely distributed in the estuary, especially in the upper estuary and mouth of the estuary, and reflects the opportunity cost used in the analysis (Fig. 3D and 4D, S1, S2 – S7). In addition, in order to analyse the contribution of each site to the zoning plan, we used a threshold of 50% of selection frequency for each planning unit to visualise highly selected sites (Fig. 5 and 6). In this case, we also observed the similar pattern on spatial priorities for conservation, with a clustered network of sanctuary zone selected mainly at shallow in Patos Lagoon estuary (Fig. 5A and 6A). Habitat protection (I and II) zone showed that small reserves are enough to complete the zoning scenario (Fig. 5B, 6B and 6C), and multiple use zone were set throughout all estuarine region (Fig. 5C, 6D and S1).


A)
Selection Frequency (%)
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
N
0 2.5 5 10 Kilometers
B)
Selection Frequency (%)
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
N
0 2.5 5 10 Kilometers
C)
Selection Frequency (%)
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
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D)
Selection Frequency (%)
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61 - 80
81 - 100
N
0 2.5 5 10 Kilometers

Figure 2: Selection frequency (%) result displayed for each scenario under conservation plan I (no zoning) for the spatial prioritization of the estuarine essential fish habitat of *Micropogonias furnieri* in Patos Lagoon estuary. The cost layer used in this analysis represents the opportunity cost of artisanal fishery (crustacean and finfish fisheries) in Patos Lagoon estuary. A) conservation target of 15% and cost layer with constant $\alpha = 0.001$; B) conservation target of 15% and cost layer with constant $\alpha = 0.0001$; C) conservation target of 30% and cost layer with constant $\alpha = 0.001$; D) conservation target of 30% and cost layer with constant $\alpha = 0.0001$.

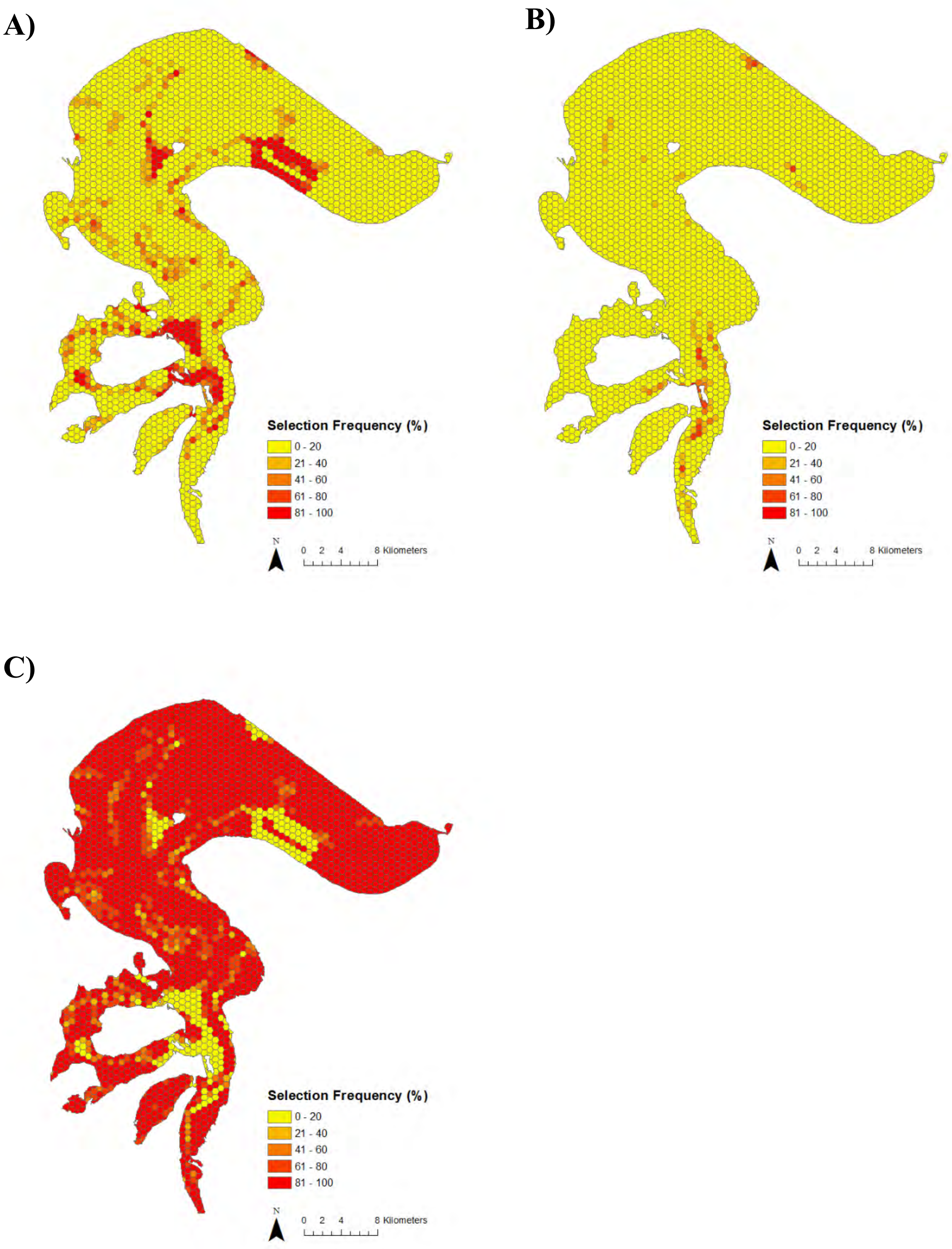

Figure 3: Selection frequency (%) result for the scenario 1 under the conservation plan II, displayed for each zone for the spatial prioritization of the estuarine essential fish habitat of *Micropogonias furnieri* in Patos Lagoon estuary. The cost layer used in this analysis represents the opportunity cost of artisanal fishery (crustacean and finfish fisheries) in Patos Lagoon estuary with constant α = 0.001. A: sanctuary zone; B: habitat protection zone; C: multiple use zone.

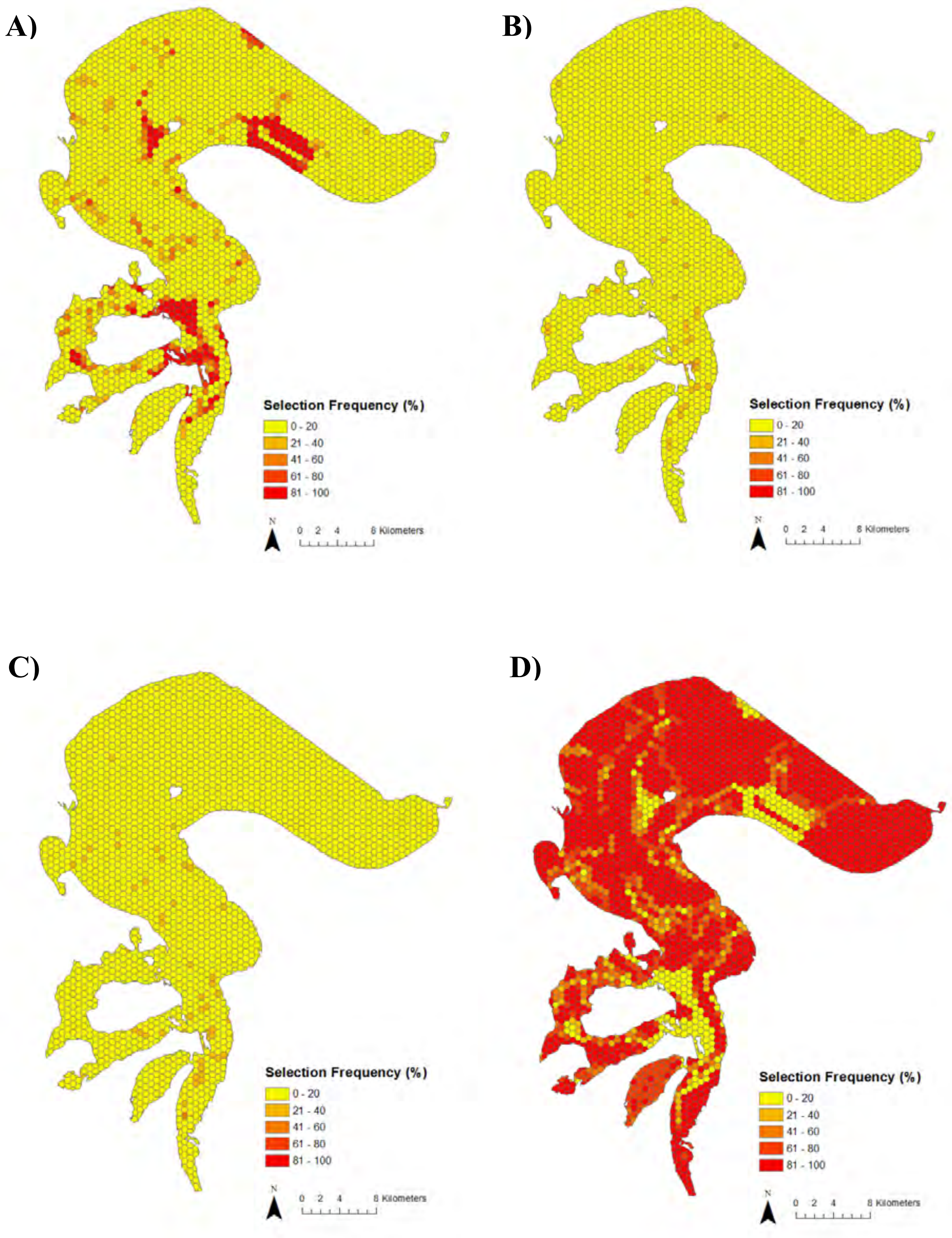

Figure 4: Selection frequency (%) result for the scenario 1 under the conservation plan III, displayed for each zone for the spatial prioritization of the estuarine essential fish habitat of *Micropogonias furnieri* in Patos Lagoon estuary. The cost layer used in this analysis represents the opportunity cost of artisanal fishery (crustacean and finfish fisheries) in Patos Lagoon estuary with constant α = 0.001. A: sanctuary zone; B: habitat protection I zone; C: habitat protection II zone; and D: multiple use zone.

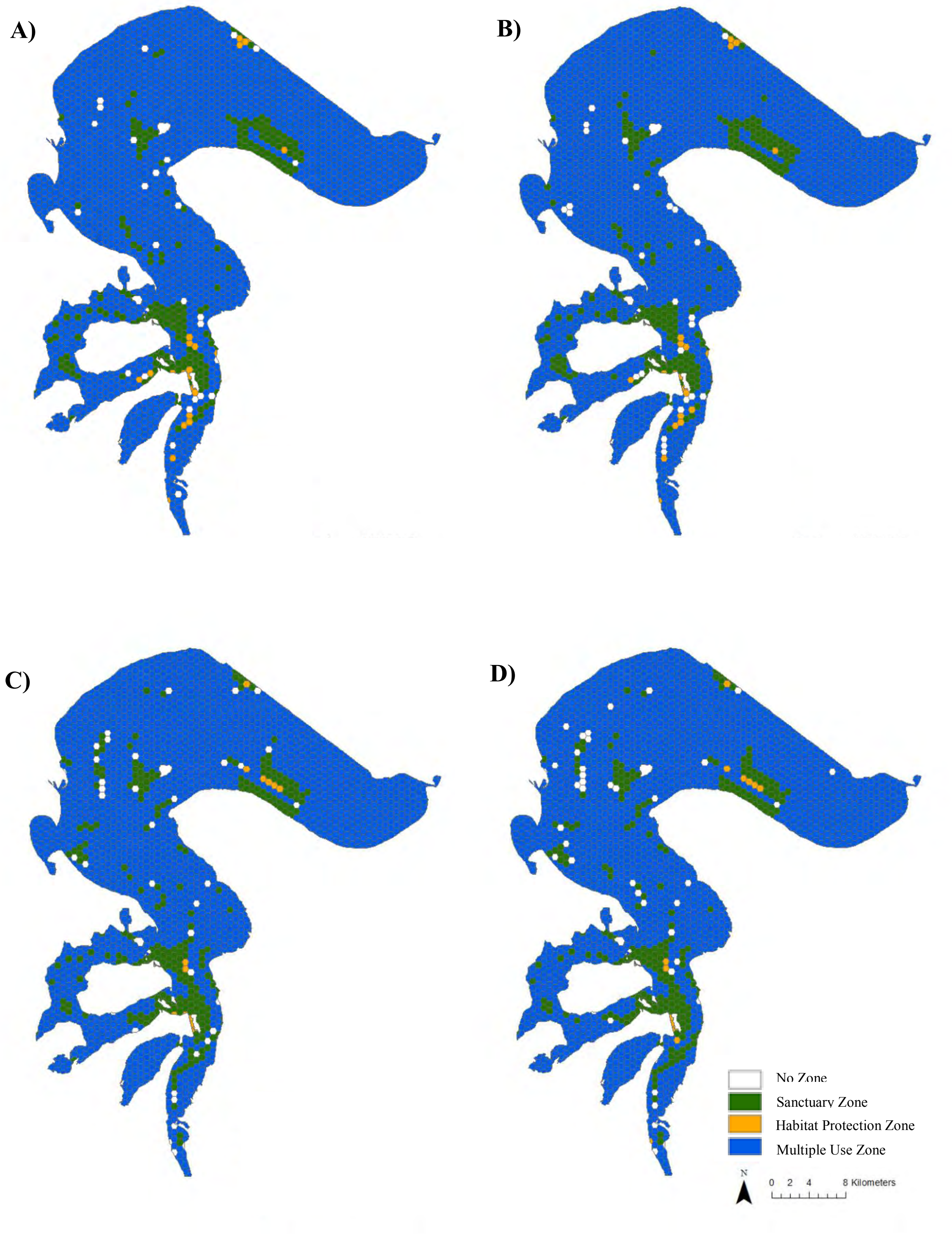

Figure 5: Selection frequency higher than 50% for each zone for the conservation plan II in Patos Lagoon estuary. Some planning units were not selected across all runs and were identified as “no zone”. A) Scenario 1 and cost layer with constant α = 0.001; B) Scenario 2 and cost layer with constant α = 0.0001; C) Scenario 3 and cost layer with constant α = 0.001; and D) Scenario 4 and cost layer with constant α = 0.0001.

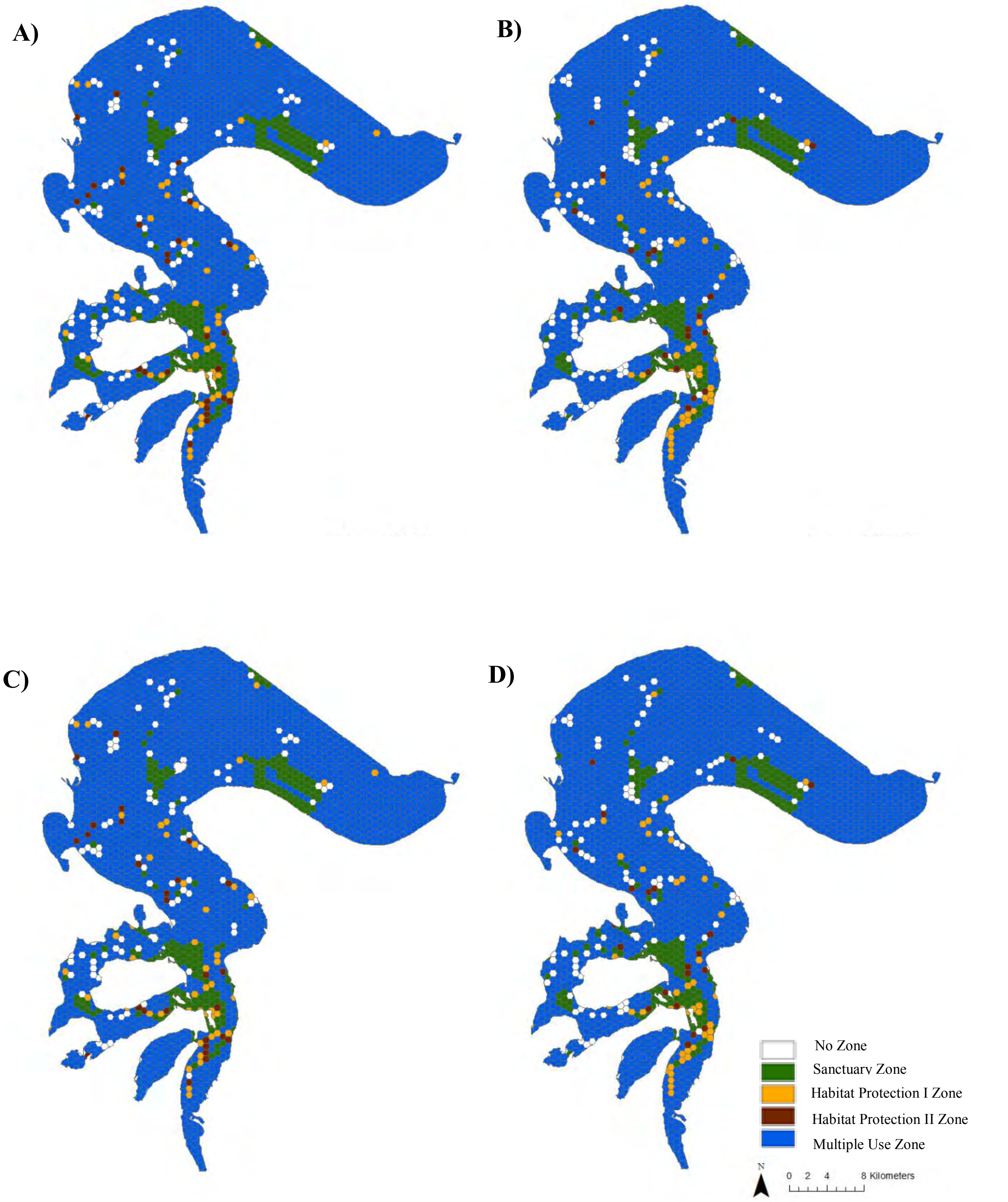

Figure 6: Selection frequency higher than 50% for each zone for the conservation plan III in Patos Lagoon estuary. Some planning units were not selected across all runs and were identified as “no zone”. A) Scenario 1 and cost layer with constant α = 0.001; B) Scenario 2 and cost layer with constant α = 0.0001; C) Scenario 3 and cost layer with constant α = 0.001; and D) Scenario 4 and cost layer with constant α = 0.0001.

A cluster analysis of the Marxan with Zones solutions showed that the absolute target for each feature (15% or 30%) had surprisingly high impact on the location of sanctuary zone for scenarios from conservation plan II (Table 2, Fig. 7, S8 – S13). However, for conservation plan III absolute targets for each feature had very little impact on the location of zones (Table 2, Fig. 8, S8 – S13). One exception was the scenario with a cost layer considering both opportunity costs and a zone target of 20%, which exhibited a high impact of the absolute target (15% or 30%) on the location of the sanctuary zone (Fig. 9). Spatial solutions showed that our results were not sensitive to the different opportunity costs tested (Table 2). However, the average cost among scenarios was variable (Table 2). Using an absolute target of 15% it is possible to achieve a low average cost for most of scenarios tested (Table 2). Hence, we found that regardless of the absolute target and the opportunity cost, having more zones did not substantially change spatial priorities for conservation in Patos Lagoon.

Table 2: Results showing average cost and main cluster results for each scenario ran in Marxan with Zones (cost 1: cost layer with constant α = 0.001; cost 2: cost layer with constant α= 0.0001; and cost 3: cost layer with both constant values, α = 0.001 and 0.0001).

Conservation Plans	Cost (US$ million)	Cluster results
Plan I **Scenario 1:** 15% conservation target	cost 1: 71,2 **cost 2: 71,1**	Not applicable
Scenario 2: 30% conservation target	cost 1: > 100 cost 2: > 100	
Plan II **Scenario 1:** sanctuary zone, habitat protection zone and multiple use zone – 15% conservation target, and 10% of the distribution of all features in sanctuary zone	**cost 1: 78,1** **cost 2: 78,09** cost 3: > 100	Ia x IIb, Ib x IIb: Conservation target significantly produced different solutions for sanctuary zone, regardless zone target and opportunity cost. Figures 7, S8 – S10
Scenario 2: sanctuary zone, habitat protection zone and multiple use zone – 15% conservation target, and 20% of the distribution of all features in sanctuary zone	**cost 1: 90, 392** **cost 2: 90, 650** cost 3: >100	
Scenario 3: sanctuary zone, habitat protection zone and multiple use zone – 30% conservation target, and 10% of the distribution of all features in sanctuary zone	cost 1: >100 cost 2: >100 cost 3: >100	
Scenario 4: sanctuary zone, habitat protection zone and multiple use zone – 30% conservation target, and 20% of the distribution of all features in sanctuary zone	cost 1: >100 cost 2: >100 cost 3:>100	
Plan III **Scenario 1:** sanctuary zone, habitat protection I zone, habitat protection II zone and multiple use zone – 15% conservation target, and 10% of the distribution of all features in sanctuary zone	**cost 1: 86,923** **cost 2: 86,665** cost 3: >100	IIIa x IVa, IIIb x IVb: In general, conservation target, zone target and opportunity cost did not affect spatial priorities for any zone. One exception was IIIb x IVb comparing both scenarios using cost 3, which conservation target significantly produced different solutions for sanctuary zone. Figures 8, 9, S11 – S13
Scenario 2: sanctuary zone, habitat protection I zone, habitat protection II zone and multiple use zone – 15% conservation target, and 20% of the distribution of all features in sanctuary zone	**cost 1: 86,923** **cost 2: 86,665** cost 3: >100	
Scenario 3: sanctuary zone, habitat protection I zone, habitat protection II zone and multiple use zone – 30% conservation target, and 10% of the distribution of all features in sanctuary zone	cost 1: >100 cost 2: >100 cost 3: >100	
Scenario 4: sanctuary zone, habitat protection I zone, habitat protection II zone and multiple use zone – 30% conservation target, and 20% of the distribution of all features in sanctuary zone	cost 1: >100 cost 2: >100 cost 3: >100	

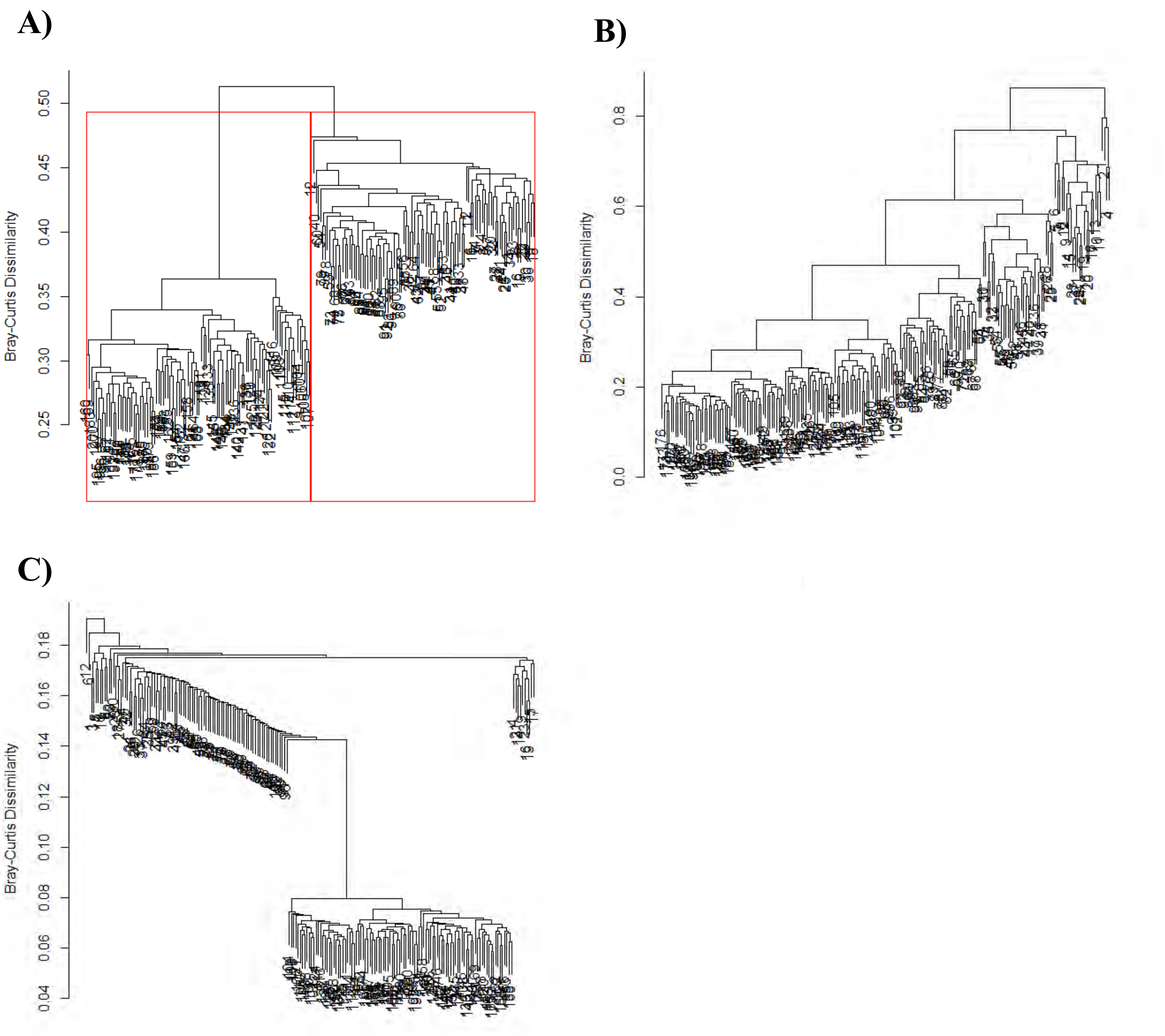

Figure 7: Relationship among 100 solutions for each zone based on conservation target value: 30% (solutions 1 to 100) and 15% (solutions 101 to 200), zone target of 10% for all life stages at sanctuary zone, 8% for eggs and larvae at habitat protection zone, and 5% for juvenile at habitat protection zone, ran in Marxan with Zones. The cost layer used in this analysis represents the opportunity cost of artisanal fishery (crustacean and finfish fisheries) in Patos Lagoon estuary with constant α = 0.001. Data is presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: sanctuary zone; B: habitat protection zone; and C: multiple use zone).

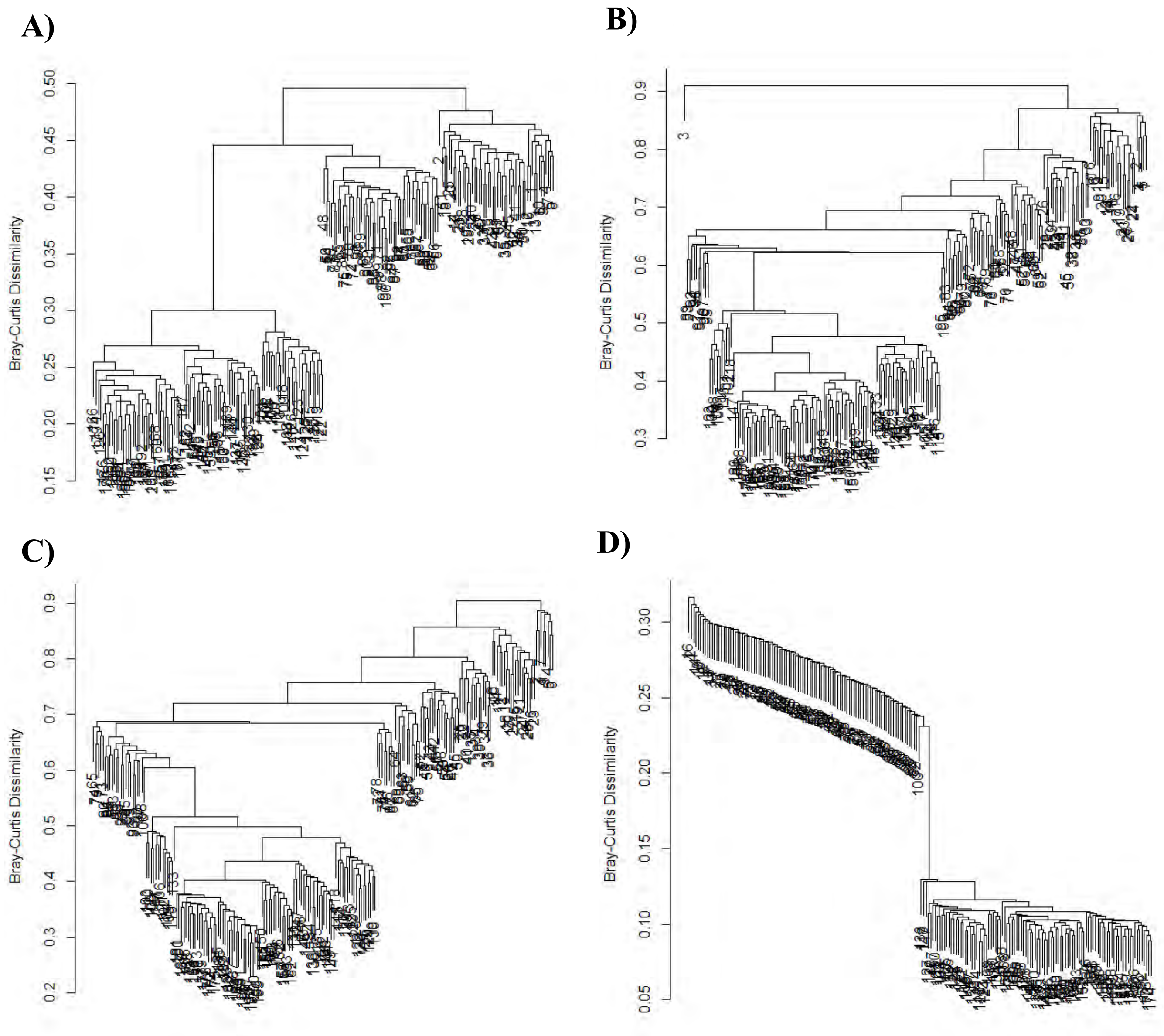

Figure 8: Relationship among 100 solutions for each zone based on conservation target value: 30% (solutions 1 to 100) and 15% (solutions 101 to 200), zone target of 10% for all life stages at sanctuary zone, 8% for eggs and larvae and 5% for juvenile at habitat protection I and II zone, ran in Marxan with Zones. The cost layer used in this analysis represents the opportunity cost of artisanal fishery (crustacean and finfish fisheries) in Patos Lagoon estuary with constant α = 0.001. Data is presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: sanctuary zone; B: habitat protection I zone; and C: habitat protection II zone, D: multiple use zone).

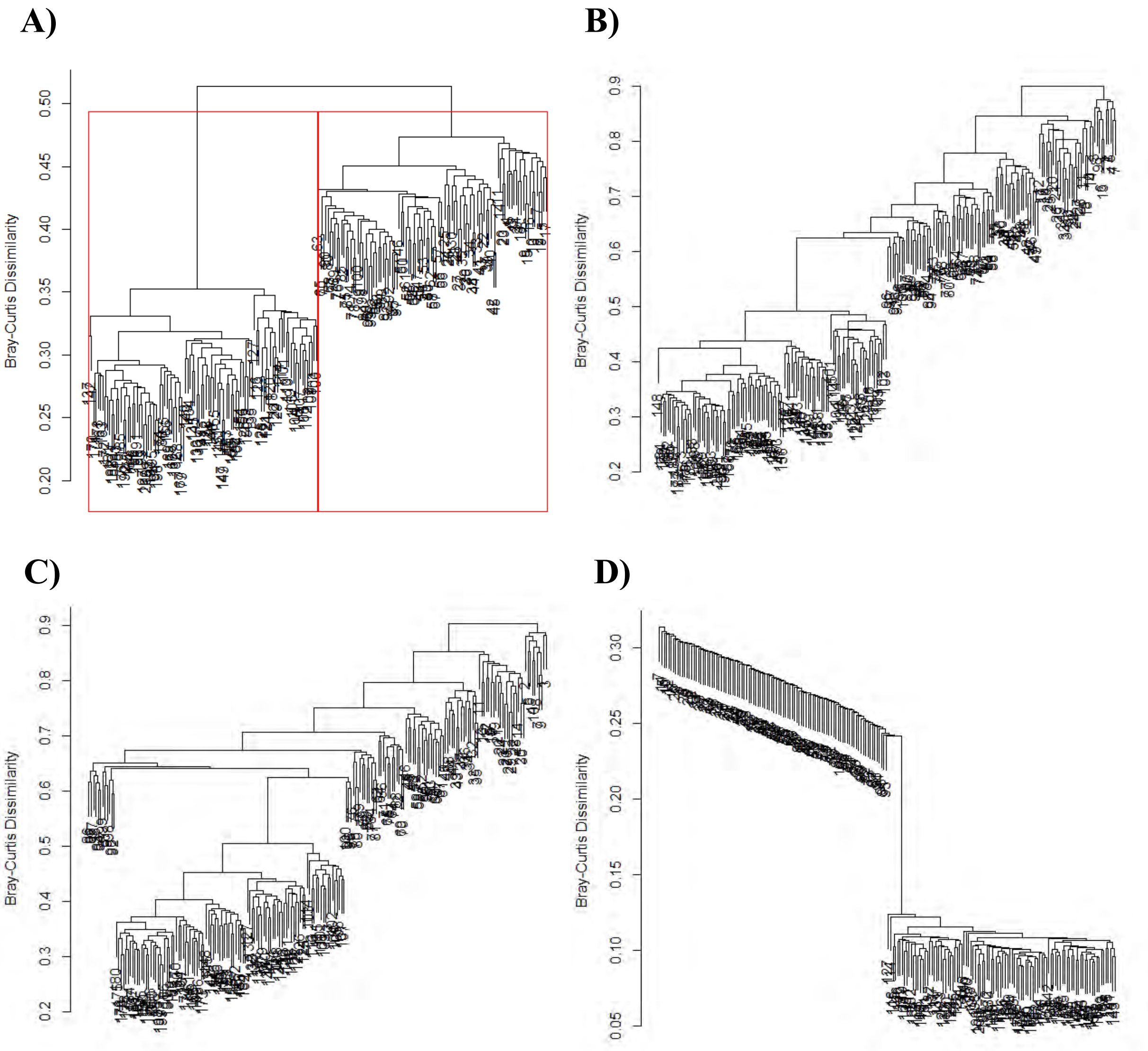

Figure 9: Relationship among 100 solutions for each zone based on conservation target value: 30% (solutions 1 to 100) and 15% (solutions 101 to 200), zone target of 20% for all life stages at sanctuary zone, 8% for eggs and larvae and 5% for juvenile at habitat protection I and II zone, ran in Marxan with Zones. The cost layer used in this analysis represents both opportunity cost of artisanal fishery (crustacean and finfish fisheries) in Patos Lagoon estuary with constant α = 0.001 and 0.0001. Data is presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: sanctuary zone; B: habitat protection I zone; and C: habitat protection II zone, D: multiple use zone).

DISCUSSION

In this study, we showed that regardless of the absolute target and the opportunity cost, having more zones in a conservation plan does not substantially change spatial priorities. Also, a simple conservation plan can accommodate the protection of an estuarine essential fish habitat, fishing and other human activities (e.g. harbour activities). *Micropogonias furnieri* exhibits a complex pattern of occupancy, in which each developmental stage is correlated with an estuarine habitat and influenced by different environmental variables (Costa *et al*., 2014). In this sense, accounting for different life stages of a species is extremely important to achieve the protection of an essential fish habitat. In the case of *M. furnieri* in Patos Lagoon estuary, our results showed that a substantial fraction of shallow areas must be included into more highly protected zones to achieve the protection of the essential fish habitat.

Inspection of the spatial patterns of the solutions showed that our results were not sensitive to the different opportunity costs tested. However, average cost varied among all scenarios. We demonstrated that using an absolute target of 15% it is possible to achieve a low overall opportunity cost for most of the scenarios tested. We also showed that a conservation plan with no zones or considering only 3 zones (conservation plan II) can produce similar and cheaper solutions to protect the essential fish habitat as plans considering 4 zones (conservation plan III), while achieving the same biodiversity targets and guaranteeing human activities. Our results also showed that Marxan with Zones is an efficient tool to account different life stages of a species into zoning plan. A key lever for achieving the conservation of a species with a complex life-cycle in a spatial zoning plan is the zone target which allowed us to set a target for a specific developmental stage in each zone (Ball *et al*., 2009; Watts *et al*., 2008). To this date, the methods used to design the essential fish habitat comprises several options, from analysis of density patterns until more sophisticated species distribution modelling. However, they all consider essential fish habitat as the whole area where the species can occur. In this sense, Marxan with Zones is an efficient tool for spatial prioritize and planning the essential fish habitat protection, especially because it allowed to deal with different levels of protection and incorporating more than one cost layer into a systematic conservation planning (Ball *et al.*, 2009).

Our selection frequency results (Fig. 2 - 6) showed that spatial priorities did not change substantially among scenarios, which means that a zoning scheme can be added further in a conservation planning process. We also observed that shallow waters were among the sites with high selection frequencies in all scenarios tested. The importance of this habitat for fishes has already been highlighted for many estuaries in the world (Minello, 1999; Paterson & Whitfield, 2000; Lazzari & Tupper, 2002), including Patos Lagoon estuary (Vieira *et al.*, 2010). Vegetated shallow waters provides an essential habitat for feeding, development and protection for fishes and invertebrates, especially for their early life stages (Seeliger, 2001; Gillanders, 2006). In Patos Lagoon estuary, juveniles of *M. furnieri* use vegetated and unvegetated areas, but mainly small individuals (> 30mm) occupy shallow waters colonized by *R. maritima* during day and night (Garcia & Vieira, 1997). Vegetated shallow embayments at Patos Lagoon estuary are also important sites to different ontogenetic stages of the pink shrimp *Farfantepenaus paulensis* (Ruas *et al.*, 2014). These habitats were also selected as the main sites for conservation when analysing the importance of incorporating fish eggs and larvae into a spatial conservation planning (M.D.P. Costa, H.P. Possingham & J.H. Muelbert. Incorporanting early life stages of fishes into estuarine spatial conservation planning). Hence, conservation actions in Patos Lagoon estuary should consider incorporating these habitats as priority areas to protect the essential fish habitat of *M. furnieri*.

Our results also showed that using an opportunity cost for crustacean and finfish fishing contributed to achieve an efficient zoning network that enables to meet the conservation targets while minimizing the economic cost lost for artisanal fishers. Artisanal fishing is one of the most important economic activities in Patos Lagoon estuary which in the late 2000 provided support for about 3 thousand fishermen (Kalikoski & Vasconcellos, 2012). Basically, the series of landing data indicates a decreasing rate since 1970 for all main resources (pink shrimp, flatfish, whitemouth croaker, blue crab, catfish, and mullets) primarily due to overfishing (Kalikoski & Vasconcellos, 2012). Besides the fishing pressure, the Patos Lagoon is impacted by human activities such as pollution, dredging, contamination, port activities and the loss of estuarine habitats as salt marshes and seagrass beds (Seeliger & Costa, 1997; Barletta *et al*., 2010). Beyond the importance for conservation in Patos Lagoon estuary, shallow areas are also among the main fishing sites in the estuary (Kalikoski & Vasconcellos, 2012). As it frequently happens during negotiations for management implementation, economic arguments are taken into deep consideration. One of the advantages of incorporating economic costs into spatial conservation prioritization is to successfully deal with usage conflicts, minimizing them while achieving conservation targets (Naidoo et al., 2006; Carwardine *et al*., 2008; Klein *et al*., 2008a, 2008b; Ban & Klein, 2009). By considering zones for fishing activity and for multiple use, and by locking out planning units in the port area from sanctuary zone, our results represent a more realistic scenario that accommodates the main anthropogenic pressures in Patos Lagoon into a conservation planning for *M. furnieri* life stages.

In conclusion, we suggest that when spatial priorities do not change, zones can be established further in a conservation planning process. Also, that different life stages should be incorporated into the conservation plan when dealing with the protection of an essential fish habitat. The protection of the essential habitats associated with spatial prioritization analysis can efficiently deals with a better allocation of management actions and resources. Using this type of information in future management actions should ensure the recruitment and maintenance of the adult population, and consequently, helping to achieve a sustainable fishing. However, we recognize that much needs to be done to devise better methods of spatial planning for dynamic environments such as estuaries, e.g. incorporating the habitat condition into planning process.

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BIOSKETCH

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Author contributions: M.D.P.C., H.P.P. and J.H.M. conceived the ideas; M.D.P.C. collected and analysed the data with advice from H.P.P. and J.H.M.; M.D.P.C. led the writing with the editorial input of other authors.

Additional Supporting Information may be found in the online version of this article:

Figure S1: Cost layers of crustacean and finfish fishery within each fishing area in Patos Lagoon estuary: (a) crustacean fishery cost layer with constant α = 0.001, (b) crustacean fishery cost layer with constant α = 0.0001, (c) finfish fishery cost layer with constant α = 0.001, (d) finfish fishery cost layer with constant α = 0.0001.

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Figure S5: Selection frequency (%) result for the scenario 1 under conservation plan III displayed for each zone for the spatial prioritization of the estuarine essential fish habitat of *Micropogonias furnieri* in Patos Lagoon estuary. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in Patos Lagoon estuary with constant α = 0.0001.

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Additional Supporting Information

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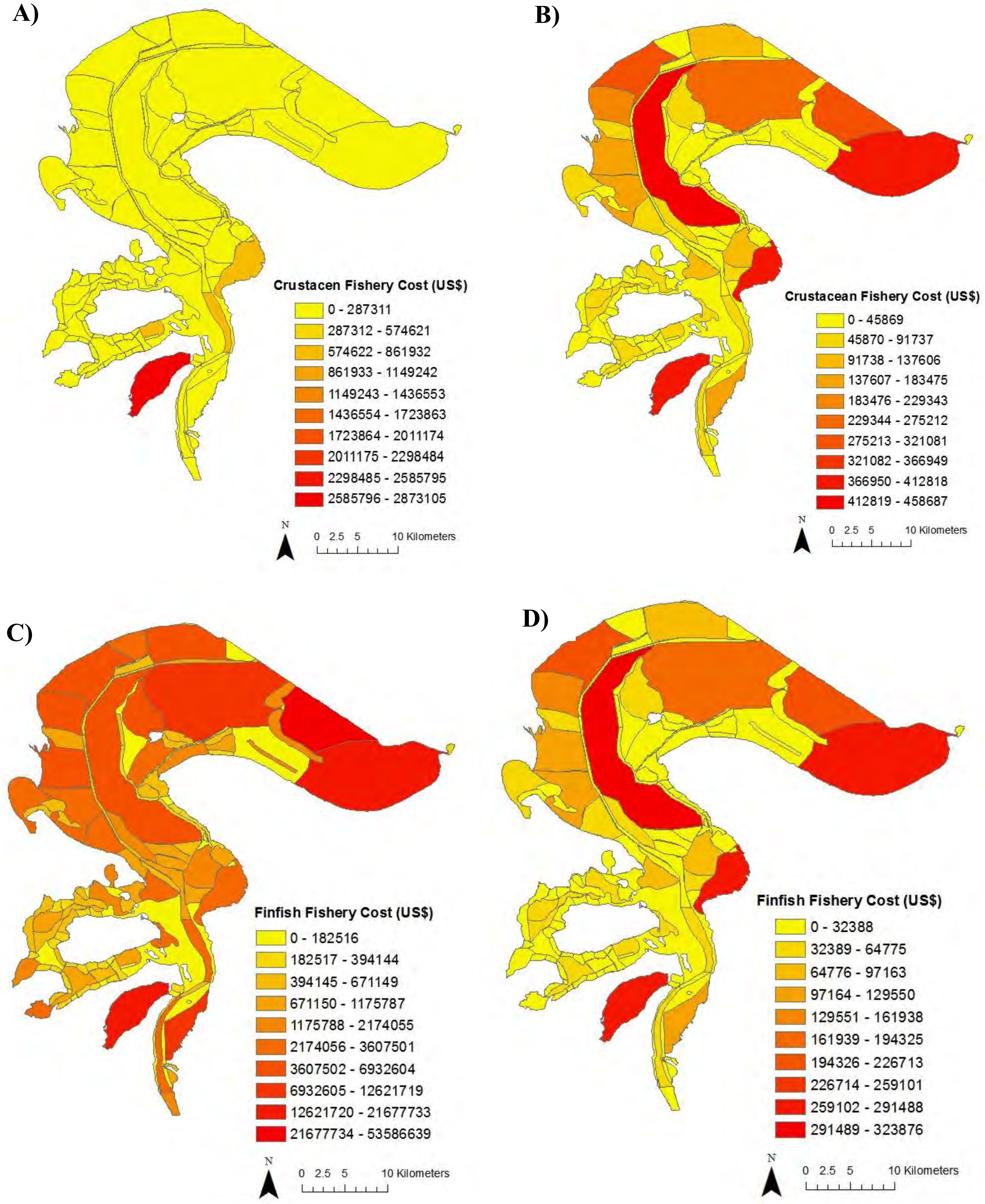

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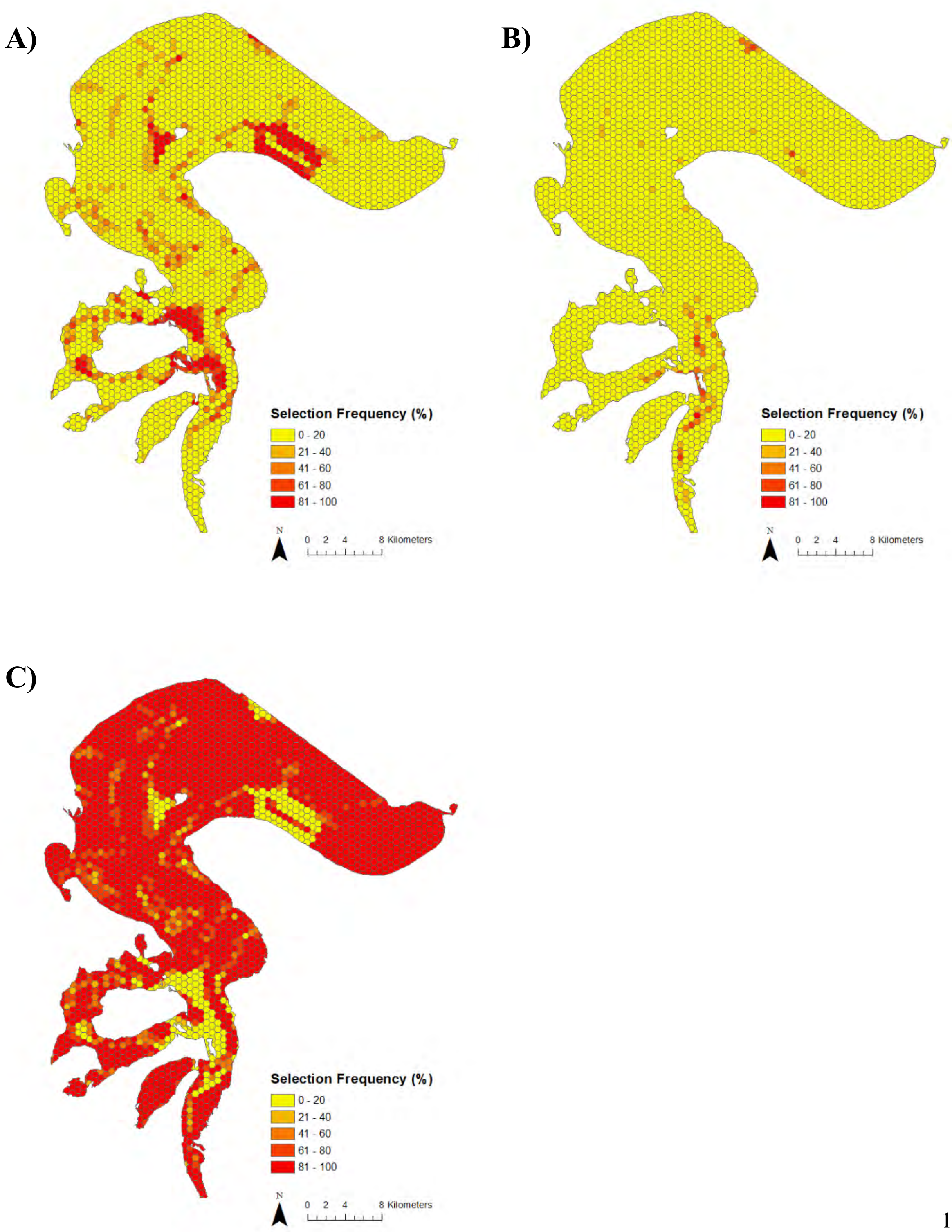

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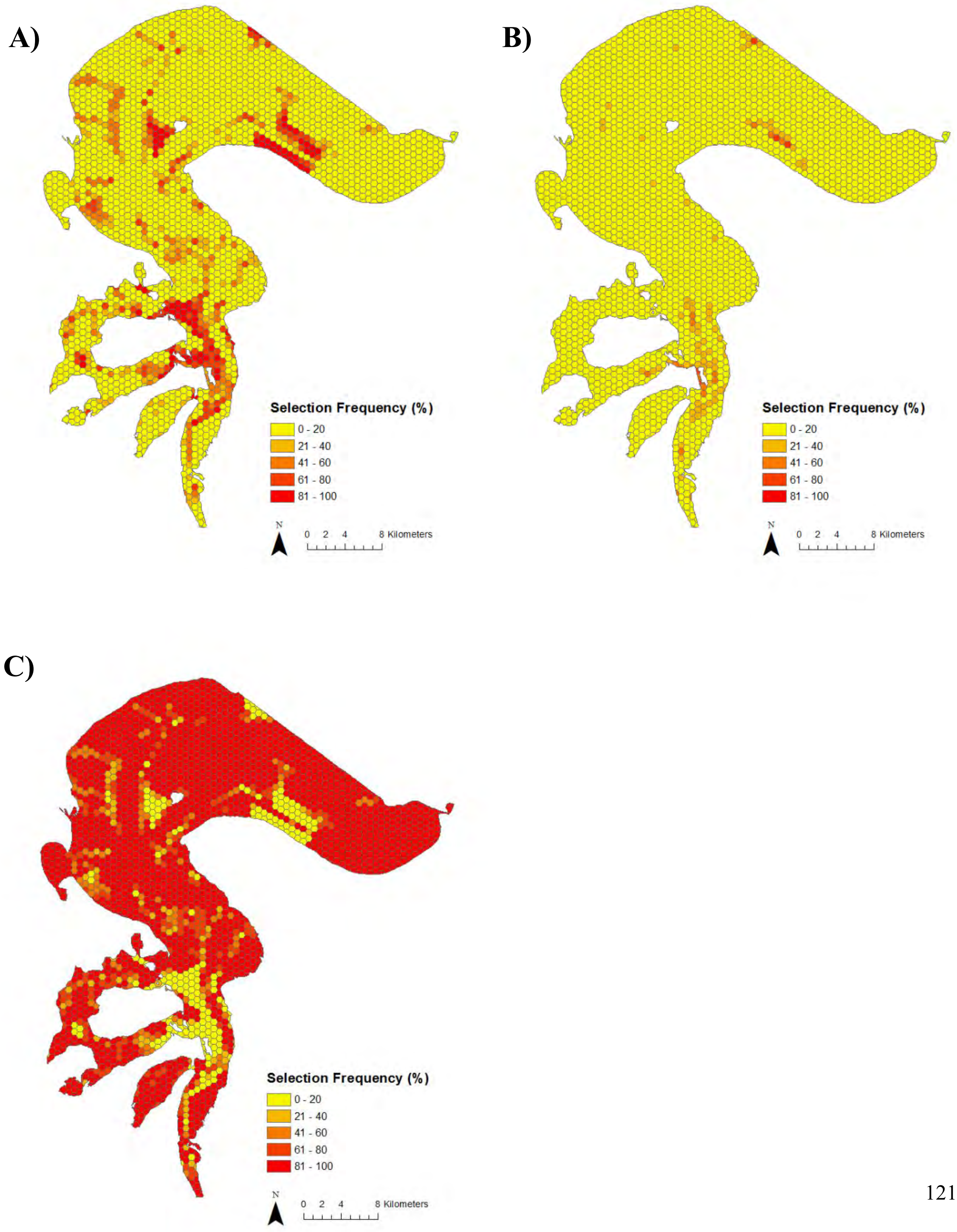

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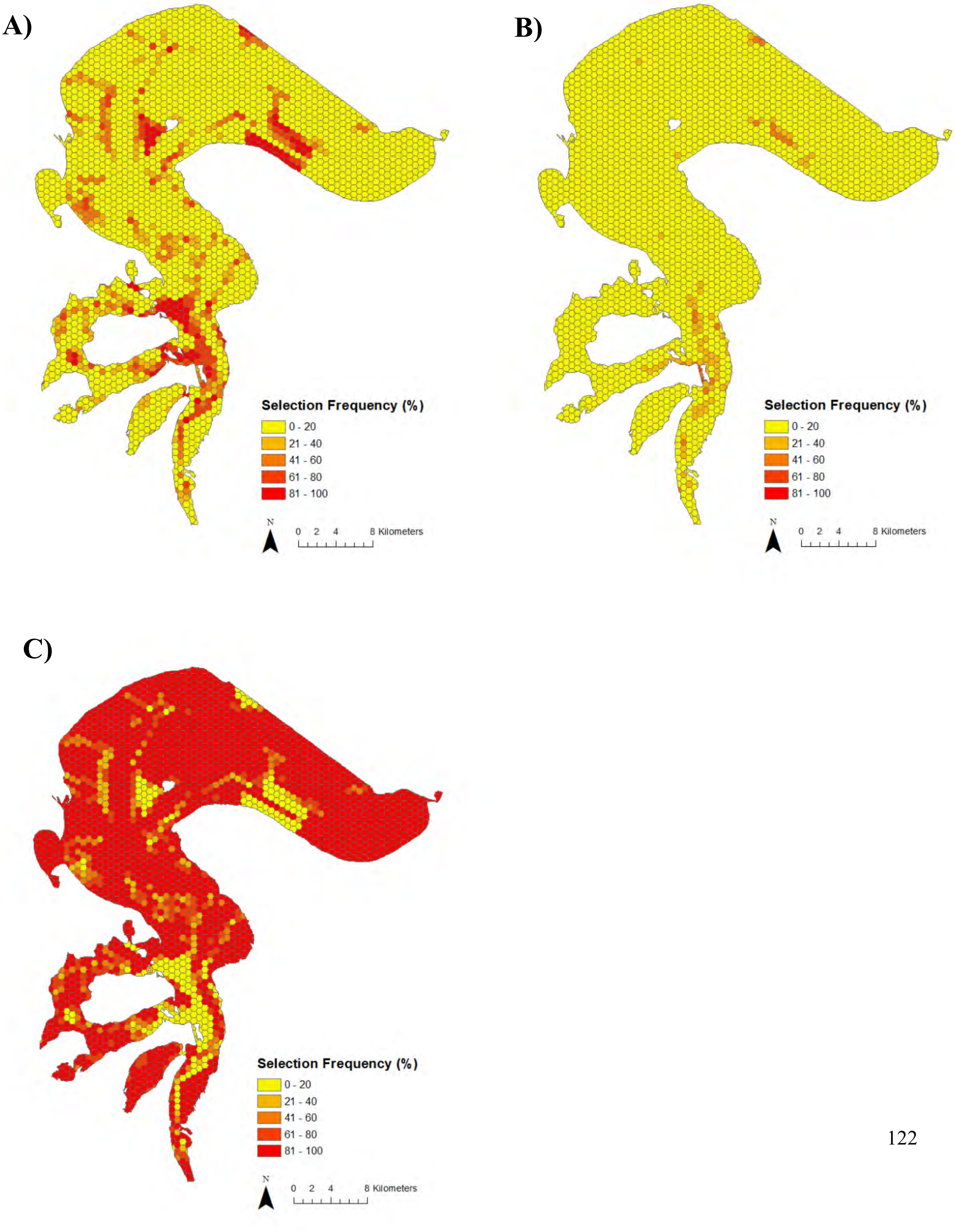

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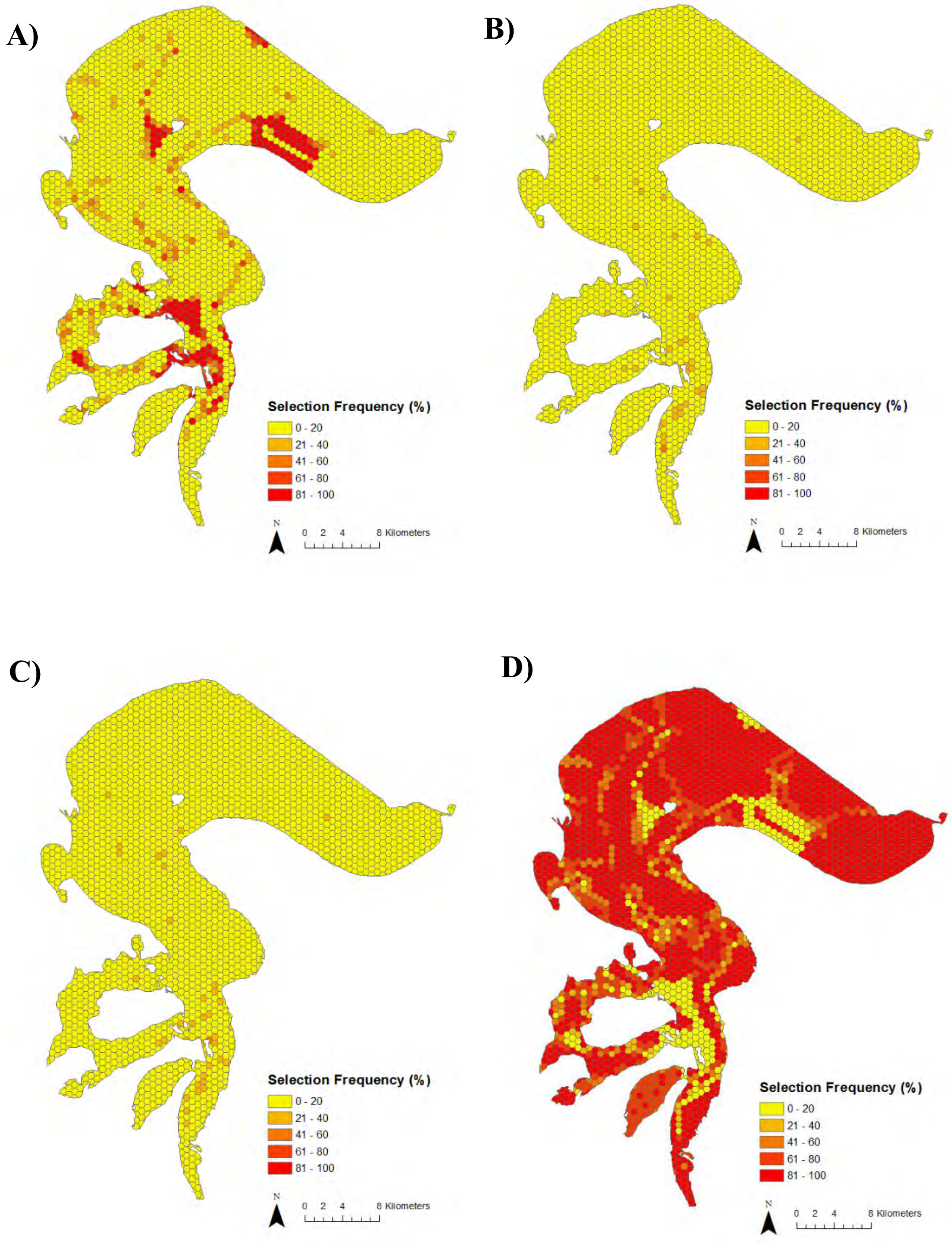

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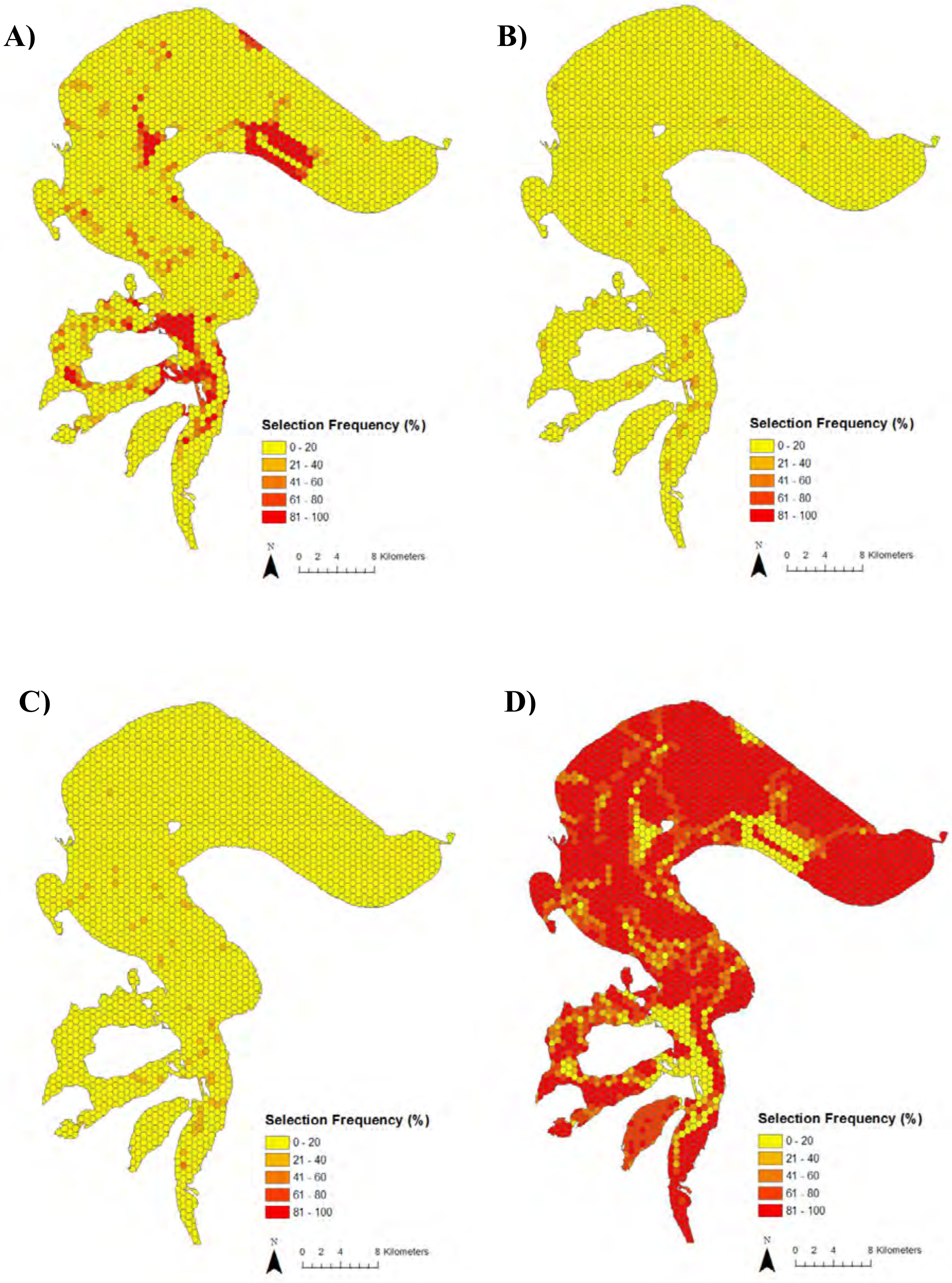

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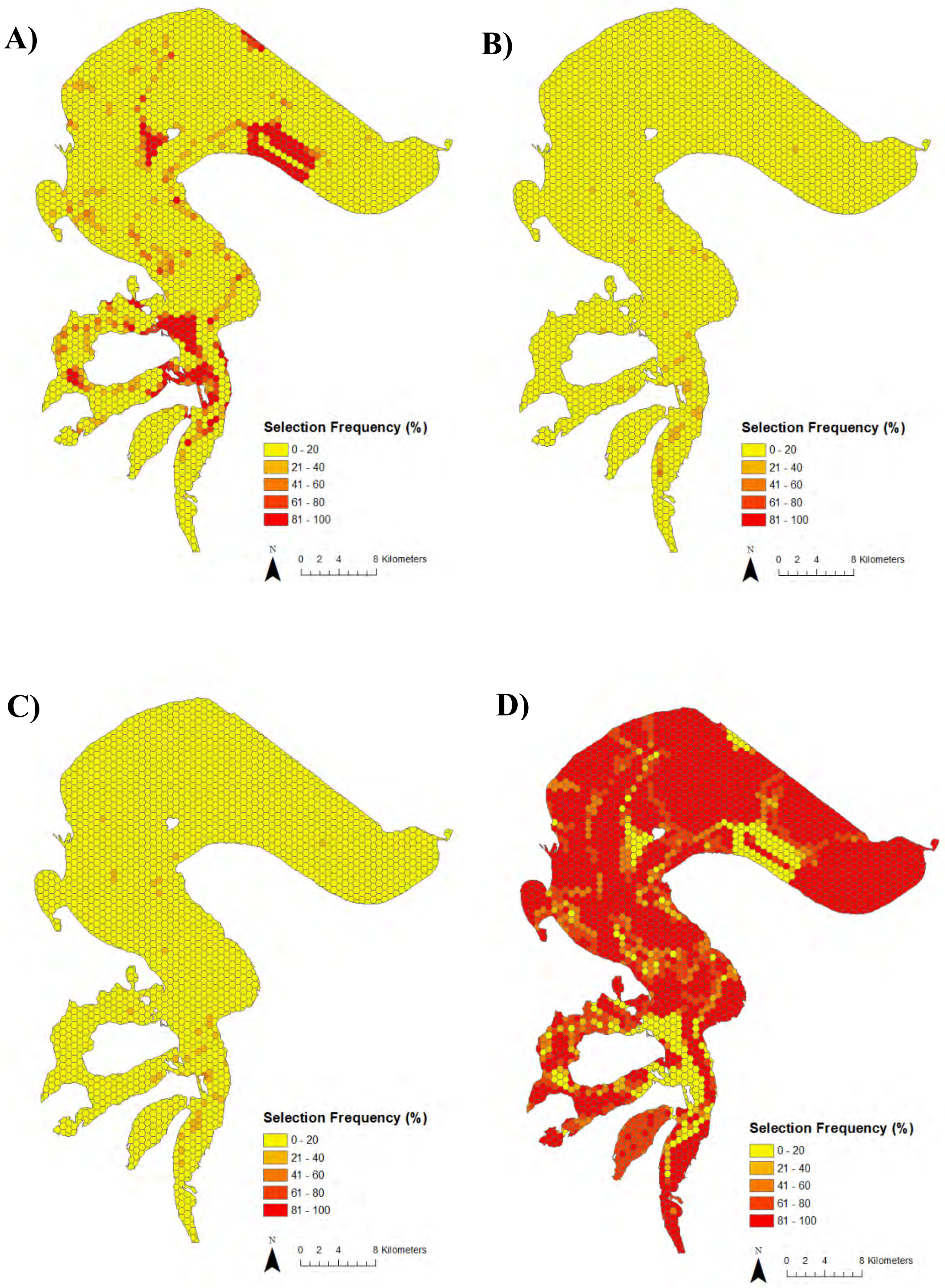

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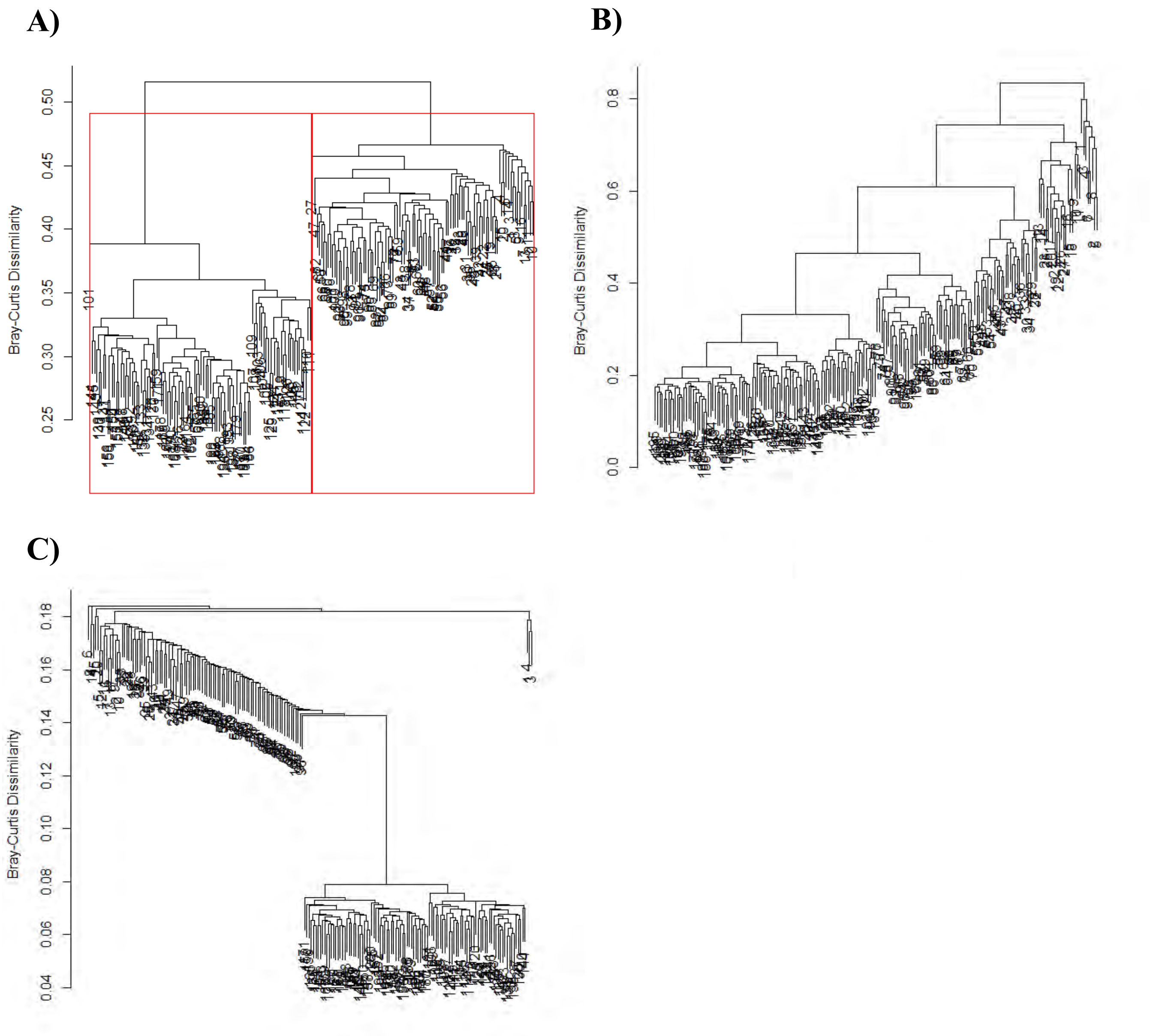

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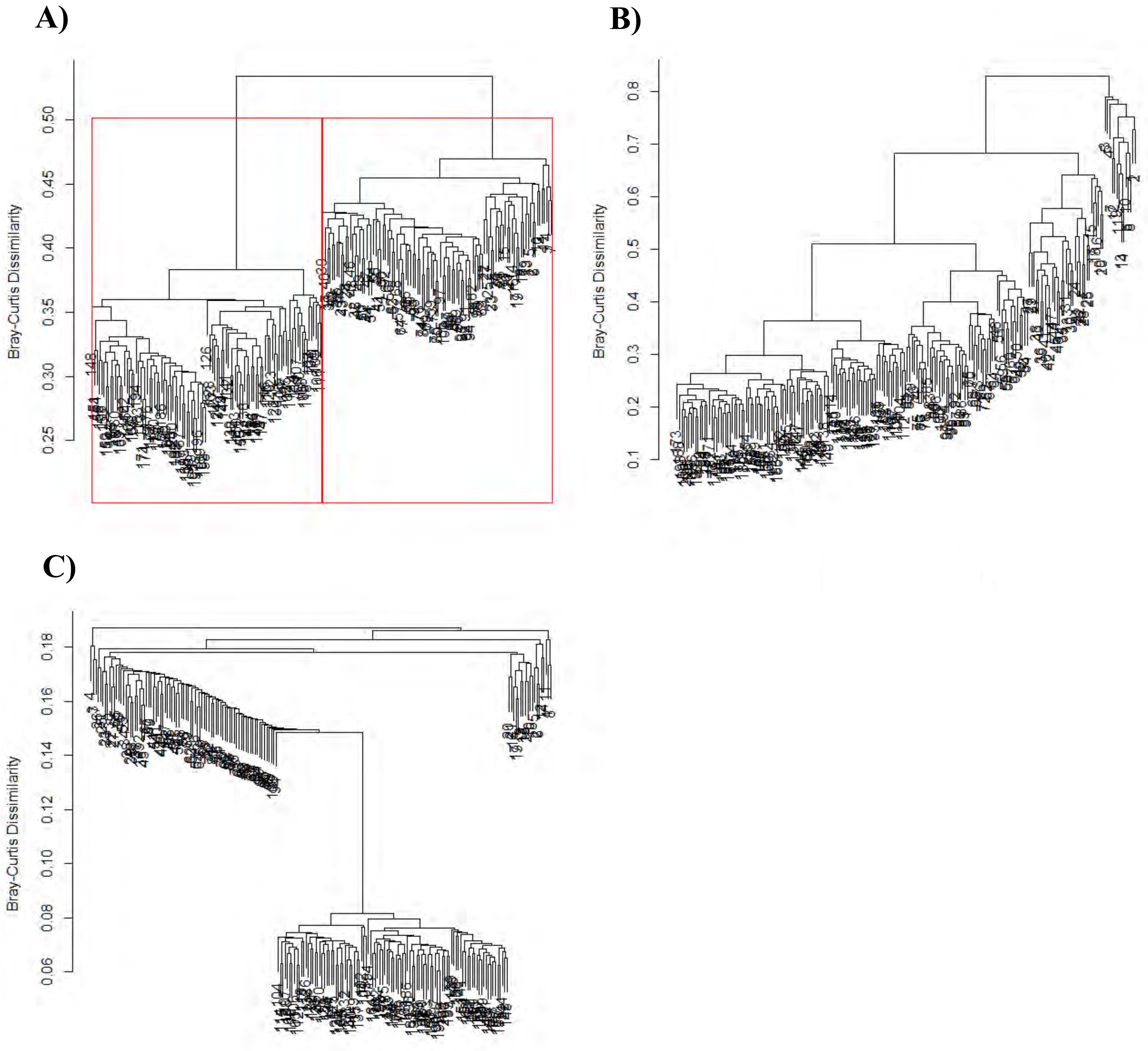

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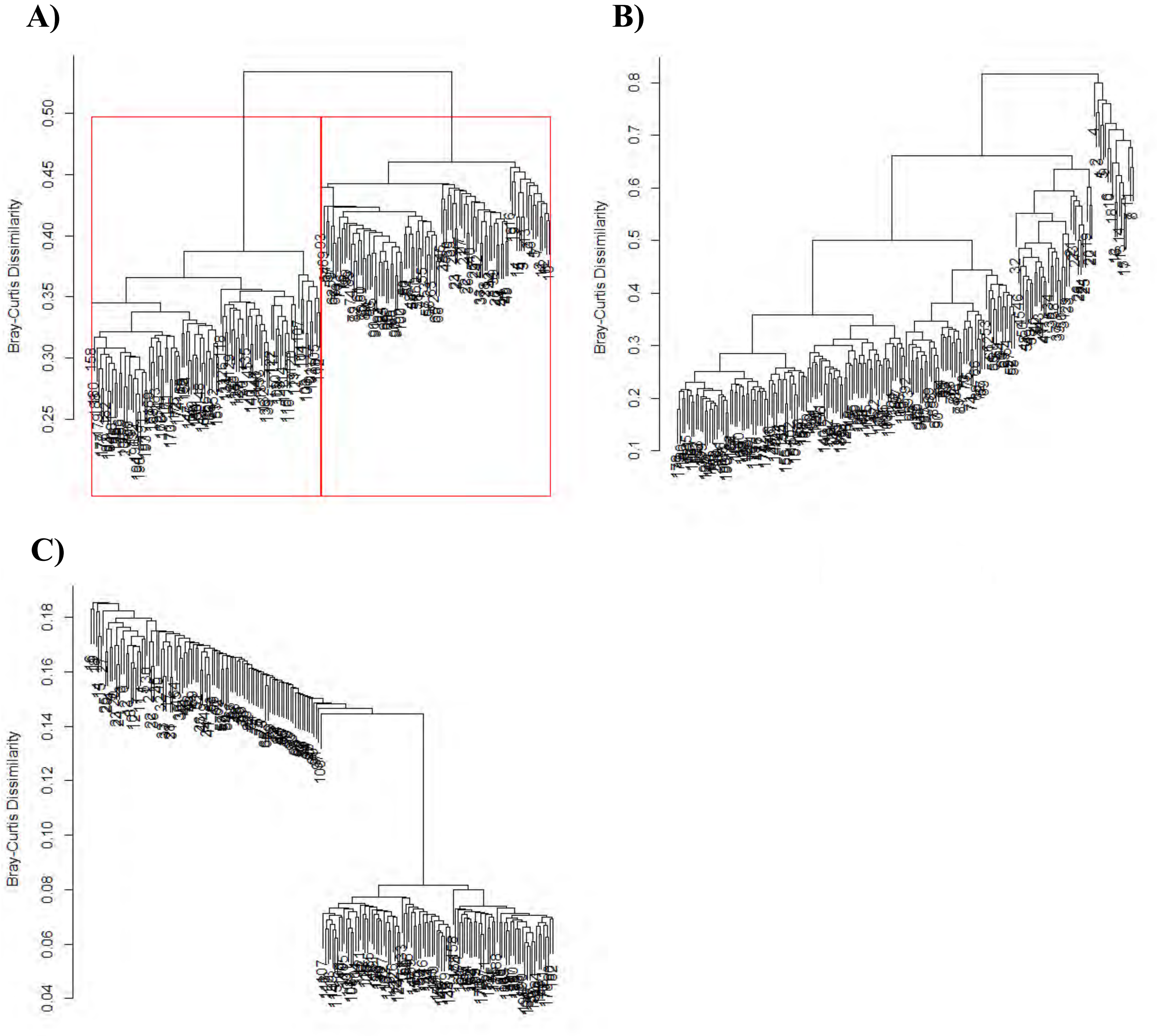

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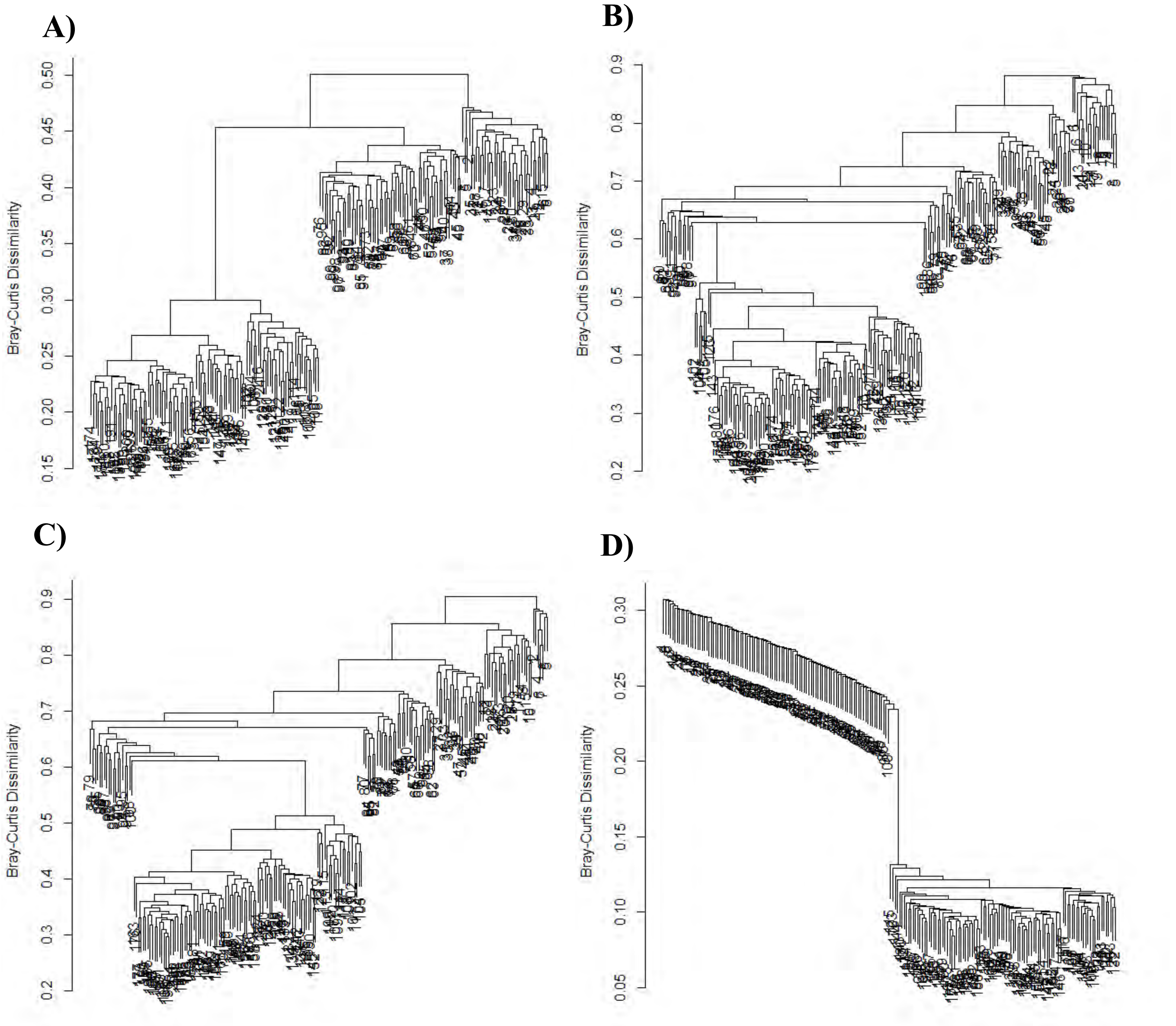

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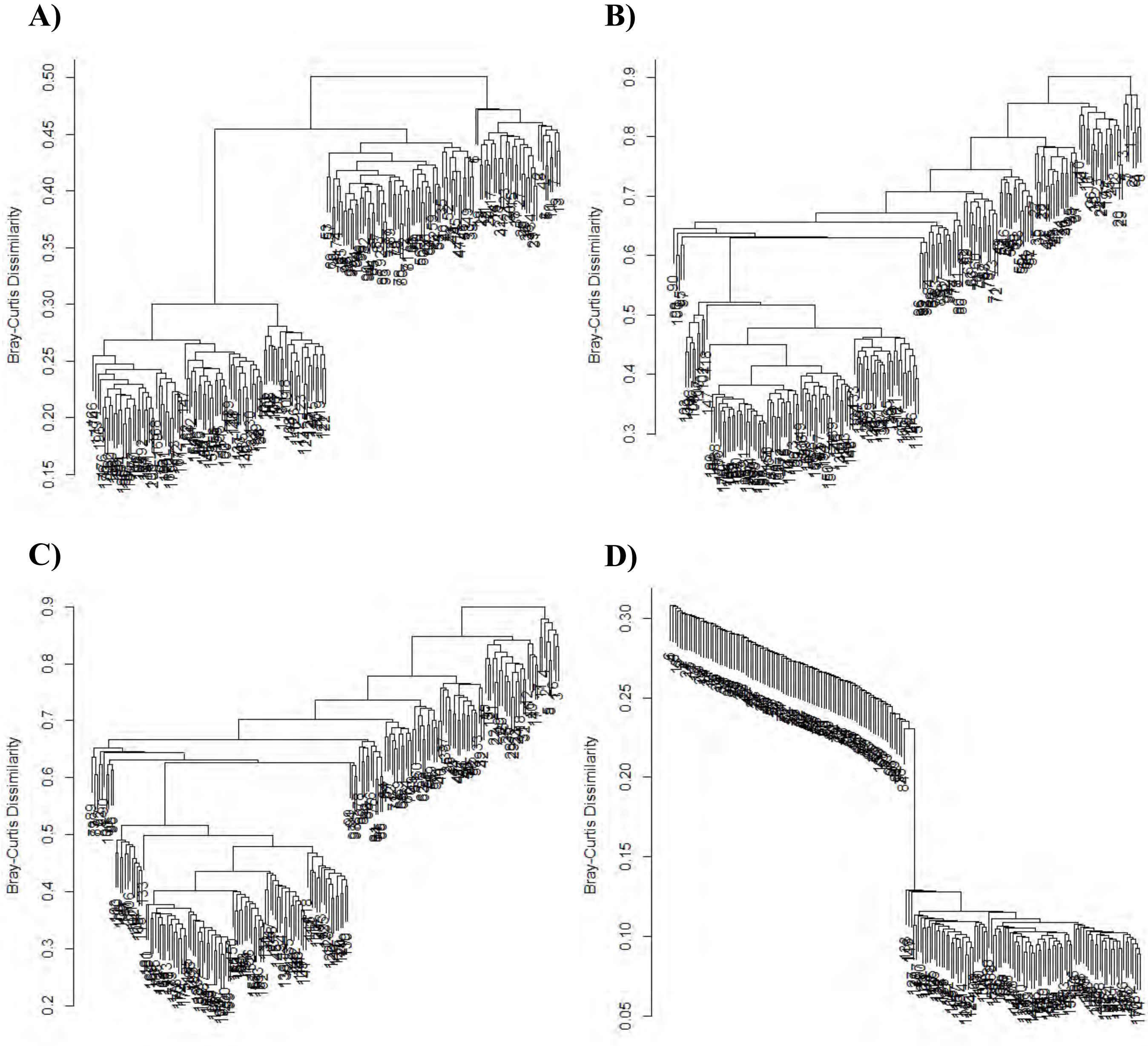

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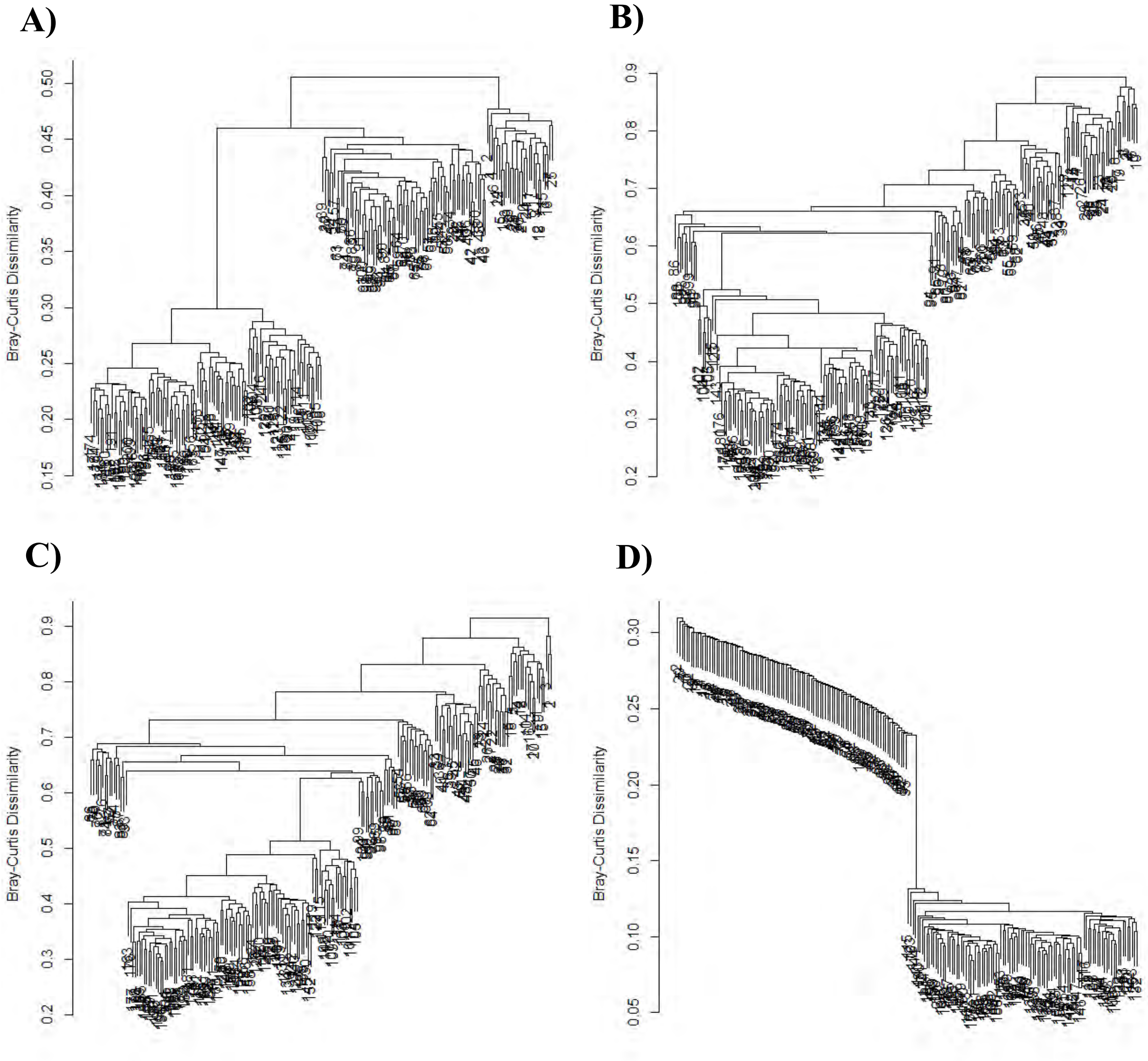

Capítulo 2: Planejamento espacial eficiente para a conservação estuário da Lagoa dos Patos

6.4 Apêndice 4 - Costa, M.D.P., Possingham, H.P. & Muelbert, J.H. Incorporating early life stages of fishes into estuarine spatial conservation planning. Submetido para o Aquatic Conservation: Marine and Freshwater Ecosystems.

AQUATIC CONSERVATION
Marine and Freshwater Ecosystems

Incorporating early life stages of fishes into estuarine spatial conservation planning

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Broad habitat type (mandatory) select 1-2:	estuary < Broad habitat type, lagoon < Broad habitat type, brackish < Broad habitat type
General theme or application (mandatory) select 1-2:	habitat management < General theme or application
Broad taxonomic group or category (mandatory, if relevant to paper) select 1-2:	fish < Broad taxonomic group or category, zooplankton < Broad taxonomic group or category
Impact category (mandatory, if relevant to paper) select 1-2:	

Incorporating early life stages of fishes into estuarine spatial conservation planning

Running Head: Ichthyoplankton and estuarine spatial conservation

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ABSTRACT

1. Many species of fish depend on estuaries to complete their development. Most of them have a planktonic early life-stage, and adults of the same species often live in a different habitat.
2. The aim was to assess the importance of incorporating data on fish eggs and larvae in systematic conservation planning at the Patos Lagoon estuary, Brazil.
3. We ran different scenarios where fish larvae and eggs were or were not included in the systematic conservation planning process.
4. An estimate of artisanal fishing revenue was used as an opportunity cost and compared with a spatially homogeneous cost. Cluster analysis was performed to compare solutions from scenarios that incorporated egg and larvae data with scenarios that did not to assess the impact of cost information on estuarine systematic conservation planning.
5. Regardless of the opportunity cost, the spatial plans fell into two clusters - those with and without fish egg and larvae data. This shows that egg and larvae data have a large impact on spatial conservation priorities.
6. This approach is the first to combine artisanal fishery economic spatial data with a conservation plan that incorporates early life stages.
7. In the case of the Patos Lagoon estuary, shallow areas were particularly important for reaching conservation targets in all scenarios. Considering the dynamic nature of these ecosystems, much work needs to be done to devise better methods of spatial planning in estuaries.

Key words: estuarine conservation; fish eggs; fish larvae; fish conservation; Marxan; systematic conservation planning

INTRODUCTION

Systematic conservation planning addresses the optimal allocation of spatially explicit conservation actions aiming to promote the persistence of biodiversity (Moilanen *et al*., 2009; Watson *et al*., 2011). The main aim of this approach, which has been traditionally used for the design of protected areas in the marine and terrestrial realm, is to achieve biodiversity objectives while minimising economic costs (Smith *et al*., 2006; Watson *et al*., 2011). Few studies have used the systematic conservation planning approach to identify priority areas for conservation in estuaries, and most of them are based on benthic macroinvertebrates and habitat types, which are assumed to be surrogates for all biodiversity (Neely & Zajac 2008; Geselbracht *et al*. 2009; Shokri & Gladstone 2009; Shokri *et al*. 2009; Shokri & Glastone 2013a, b).

The costs of taking conservation actions in different places are not spatially homogeneous, and there are many types of costs related to conservation actions (Wilson *et al*., 2009). Among them, opportunity cost represents the lost benefit when an action precludes a profitable activity that could otherwise occur there, e.g., when a specific site that is used for fishing or navigation is chosen as a no-take marine reserve (Wilson *et al*., 2009). For example, in marine or coastal environments, opportunity cost may be the fishery benefit that will be lost if a specific site is declared as no-take area. The application of opportunity costs in the planning analysis has already been tested in many studies, showing that the incorporation of this information can help to achieve more cost-effective outcomes than when costs are neglected (Naidoo *et al*. 2006; Klein *et al*. 2008; Carwardine *et al*. 2008; Ban & Klein 2009; Mazor *et al*. 2014). In the marine ecosystem, most planning studies have used fisheries information to integrate opportunity cost into the analysis (Ban & Klein 2009). Among the studies that have applied the conservation planning approach in estuaries, only Geselbracht et al. (2009) included opportunity cost in the planning analysis by developing a spatial index of socio-economic factors as opportunity costs. The lack of studies using opportunity cost in estuarine conservation planning exposes a gap in our understanding of the analysis, mainly on the effect of this information on the spatial prioritisation analysis in a highly competitive ecosystem such as estuaries.

Estuaries are among the most dynamic and productive ecosystems in coastal areas, acting as a nursery ground for a wide variety of species. In addition to nursery, breeding and refuge functions, estuaries are also important for ecosystem regulatory functions, disturbance prevention, sediment retention and nutrient regulation (Barbier *et al*., 2011). Unfortunately, estuarine and coastal areas are among the most heavily impacted natural ecosystems, and cumulative impacts from human activities can directly affect the benefits provided by these ecosystems (McLusky & Elliott, 2006). Several fishes, mainly marine species, depend on estuaries to complete their development (Elliott & Hemingway, 2002; McLusky & Elliott, 2006). Most of these species have a planktonic early life stage, which means that adults of the same species sometimes live in different habitats and rely on the dynamic nature of estuaries to complete their life cycle.

The distribution of fish eggs and larvae in an estuary depends on both biological processes (e.g., mortality, spawning and behaviour) (Boehlert & Mundy, 1988; Schultz et al., 2003) and abiotic factors (Govoni, 2005; Babler, 2000). These processes interact synergistically, influencing the occurrence and distribution of ichthyoplankton in estuaries. These early stages are unable to move against currents, being passively transported in the ocean-estuary interface, and they are retained in specific sites in the estuary by physical processes. In addition to fish eggs and larvae, the early life history of fishes that use estuaries includes settled juvenile fishes. This late life stage is already capable of directional swimming. Ontogenetic habitat changes during development results from variation in habitat requirements and can structure the spatial distribution of each developmental stage throughout the life cycle (Le Pape *et al*., 2014). Most estuarine-dependent species complete their development in estuaries and migrate to the ocean as an adult to spawn. Despite the relation between the survival of fish eggs and larvae and recruitment success, no other study has used information about fish eggs and larvae in a systematic conservation plan.

The present study incorporates data on the early life stages of fish (eggs and larvae) into a decision support tool to assess the impact of adding these data into estuarine systematic conservation planning. The open question is, will the inclusion of some of the early life history stages of fishes that use estuaries affect the outcomes of conservation planning, and how is this affected by the incorporation of opportunity costs? While fish eggs and larvae are not directly impacted by fishing, they are affected by other anthropogenic activities such as habitat destruction and pollution (Allison et al., 1998). These factors are of great importance when identifying and designing protected areas, especially marine protected areas created where the main objective is to sustain marine fish population and fisheries (Warner et al., 2000). In cases where recruitment is driven by the retention of eggs and larvae, conservation of retention areas is as important as conservation of adult habitat (Warner et al., 2000). In order to answer the main question of the study, we used a large dataset including physical features (e.g., bathymetry, sediment type, and habitat) of the estuary and the spatial-temporal distribution of six important fisheries species.

METHODS

Study Area

Patos Lagoon (Figure 1) is a warm subtropical, river-dominated choked lagoon located in southern Brazil (32°S, 52°W - Brazil) with a large estuary that is important to several commercial species. This ecosystem is characterised by its ecological (high biological productivity and biodiversity) and socio-economic importance (ports, industries, agriculture, aquaculture, and fisheries) (Odebrecht *et al*., 2010). Ichthyoplankton studies in this estuary were conducted to investigate the distribution and ecology of species and the influence of environmental variables on species distribution and abundance (Muelbert *et al*., 2010). In this system, the retention and survival of early stages of fishes are strongly dependent on water exchange and prevailing winds (Muelbert & Weiss, 1991; Sinque & Muelbert, 1997; Martins *et al*., 2007; Odebrecht *et al*., 2010). Furthermore, the occurrence and distribution of fish eggs and larvae are strongly affected by seasonal variation in salinity and temperature (Muelbert *et al*., 2010). This is the case with many other estuaries around the world (Berasategui *et al*., 2004; Faria *et al*., 2006; Ramos *et al*., 2006; Simionato *et al*., 2008; Primo *et al*., 2011). The Patos Lagoon estuary was chosen as a case study for estuarine spatial conservation prioritisation because of the breadth of existing information and its ecological and economic importance.

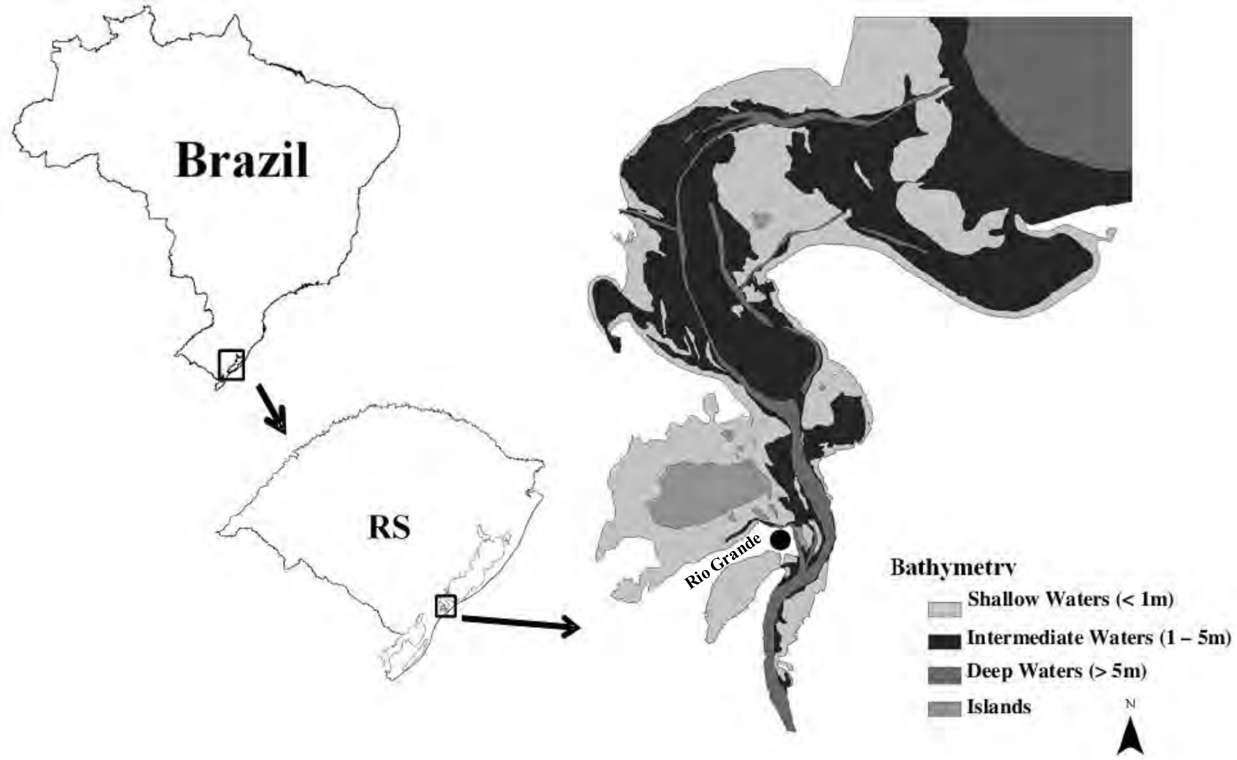

Figure 1: Location of the Patos Lagoon estuary in Rio Grande do Sul State (RS, Brazil) and details of the bathymetry in the estuarine area.

The majority of the lagoon is freshwater (80%), but brackish water creates an estuarine system of approximately 1000 km² in the southern reach. The deep (15 m) and narrow (800 m) inlet of this system acts as a filter attenuating the advance of tidal waves into the estuarine region (Seeliger, 2001, Odebrecht *et al.*, 2010). Wind and freshwater discharge are the main forces controlling water exchange with the open ocean. Hence, the geographic limits of this estuarine ecosystem are conditioned by climatic factors (Odebrecht *et al.*, 2010). The estuarine region of Patos Lagoon (Figure 1) is predominantly shallow (< 1.5 m), although it also has some intermediate waters (1.5 – 5.0 m) and deep waters (> 5.0 m). The estuary is composed of different benthic habitats including unvegetated subtidal flats (300 km²), seagrass beds (120 km²), marginal salt marshes (40 km²) and artificial hard substrates (~ 5 Km-long rocky jetties) (Seeliger, 2001). A wide variety of marine fishes and invertebrates depend on these habitats to complete their development and successfully recruit to the adult population (Seeliger, 2001; Odebrecht *et al.*, 2010).

Conservation features data

The Patos Lagoon estuary was divided into 2,355 hexagonal planning units with sides of 0.5 km using QuantumGIS software (QGIS Development Team, 2013). Distribution data of 64 features were selected as surrogates for biodiversity and included bathymetry, sediment, habitat, and ichthyoplankton (Table 1). Ichthyoplankton data comprise seasonal distribution information over three years for six fish species (*Achirus garmani*, *Brevoortia pectinata*, *Licengraulis grossidens*, *Micropogonias furnieri*, *Parapimelodus valenciennes* and *Thrichiurus lepturus*). These species have the most abundant fish eggs and larvae in the Patos Lagoon estuary (Muelbert & Weiss, 1991; Muelbert *et al.*, 2010) and were chosen to represent the estuarine ecological guilds: marine (*T. lepturus)*, estuarine dependent (*B. pectinata, M. furnieri*), true estuarine (*L. grossidens, A. garmani)*, and freshwater species (*P.*

valenciennes) (McLusky & Elliott, 2006). Because changes in the abundance and occurrence of ichthyoplankton at the Patos Lagoon estuary are determined by fluctuations in salinity ((Muelbert & Weiss, 1991; Muelbert *et al*., 2010), we assumed that the period of interest is a typical period for fish eggs and larvae recruitment. Habitat data were determined from the percent cover of submerged aquatic vegetation of two seagrasses species (*Ruppia maritima* and *Zannichelia palustris*) and macroalgae and the meadow height during summer and autumn (B. Gianasi; Table 1). Bathymetry was divided into three classes following a previous study (Plano Ambiental de Rio Grande, 2007): shallow (< 1 m), intermediate (1 – 5 m) and deep (channels, > 5 m) waters; sediment follows the classification proposed by Calliari (1980) based on sand-silt-clay content in 10 classes: silt, silty sandy, silty clay, sandy silty, mixed sandy+silt+clay, clay silty, sand, sandy clay, clay sandy, and clay. Physical features of the Pathos Lagoon estuary were chosen as surrogates for biodiversity according to their availability and relevance to estuarine diversity. Many species prefer particular water depths (e.g., species that prefer shallow water and those associated with deep channels). The sediment type can be a good surrogate for benthic fauna and other species associated with the estuarine bottom, and submerged aquatic vegetation is an important estuarine habitat, which many species rely on to complete their development (e.g., meadow height can be an indicative measure of complexity of this habitat: the higher the meadow the more developed and complex it is).

Table 1: Description of the features that were used to assess the role of incorporating fish egg and larva data for estuarine conservation in the Patos Lagoon estuary, Brazil (e: eggs; l: larvae).

Feature	Data	Number of layers	Description
Bathymetry	Polygon	3	Shallow, intermediate and deep waters
Sediment	Polygon	7	Classification based on Calliari (1980)
Habitat	Interpolated seasonal survey	16	*Ruppia maritima*, *Zannichelia palustris*, macroalgae, meadow height(*)
Ichthyoplankton species	Output of species distribution modelling** (MAXENT)	38	*Achirus garmani* (e/l), *Brevoortia pectinada* (e/l), *Licengraulis grossidens* (e/l), *Micropogonias furnieri* (e/l), *Parapimelodus valenciennes* (l), *Trichiurus lepturus* (e)

(*) meadow height was classified into 5 classes of height (cm): 0 – 10, 11 – 20, 21 – 40, 41 – 60, and 61 – 150.
(**) only MAXENT outputs with AUC > 0.75 were used.

Opportunity cost

In this study, fishing revenue was chosen as the opportunity cost of closing a planning unit to fishing because artisanal fishery is one of most important economic activities in the Patos Lagoon estuary, and it occurs throughout the estuary (Schafer & Reis, 2008). This cost was added to a flat cost of 1 for all planning units. The flat cost ensures that planning units with no fishing effort are not simply added to the reserve system if they have no conservation benefit. The fishing revenue was calculated using the equation for commercial fishing proposed by Mazor *et al*. (2013). To estimate the annual catch of artisanal fisheries (C_i) in each planning unit, the catch was assumed to be proportional to the estuarine depth (D_{epth}) and to the distance of the nearest landing site (d) weighted exponentially by a constant decay rate α and then multiplied by the area (A_{rea}) of the planning unit (i). In essence, we are assuming that artisanal fishermen tend to concentrate in shallow waters near their villages (Freitas & Tagliani, 2009). Four different values for α were used (0.0001, 0.001, 0.005 and 0.01) to reflect uncertainty in how far fishers travel to fish. The depth was classified as cited above. The catch was normalised by a measure of total effort (R_{effort}) which is equal to:

$$R_{effort} = \sum_{i=1}^{m} D_{epth}\, e^{-\alpha d} \text{ x } A_{rea},$$

where m is the number of planning units in the estuarine area of Patos Lagoon. Then, the final value was multiplied by the total biomass of fish captured ($R_{biomass}$, ton) in the Patos Lagoon estuary during 2011 (IBAMA/CEPERG, 2012) multiplied by the average price ($C_{ost,}$ US$ per ton, IBAMA/CEPERG, 2012) of three main target species (whitemouth croaker *Micropogonias furnieri*, shrimp *Farfantepenaeus paulensis*, and mullets *Mugil* spp.) for artisanal fishery in the Patos Lagoon estuary (which was US$1,000 per ton) which comprises 88.5% of landings in the region (IBAMA/CEPERG, 2012). The final equation used to calculate the opportunity cost for artisanal fishing in each planning unit at the Patos Lagoon estuary is as follows:

$$C_i = \left(\frac{D_{epth} e^{-\alpha d} A_{rea}}{R_{effort}}\right) \text{ x } R_{biomass} \text{ x } C_{ost}$$

The four different opportunity cost layers for artisanal fisheries, each with a different emphasis on the value of nearby fishing grounds, in the Patos Lagoon estuary are given in Figure 2.

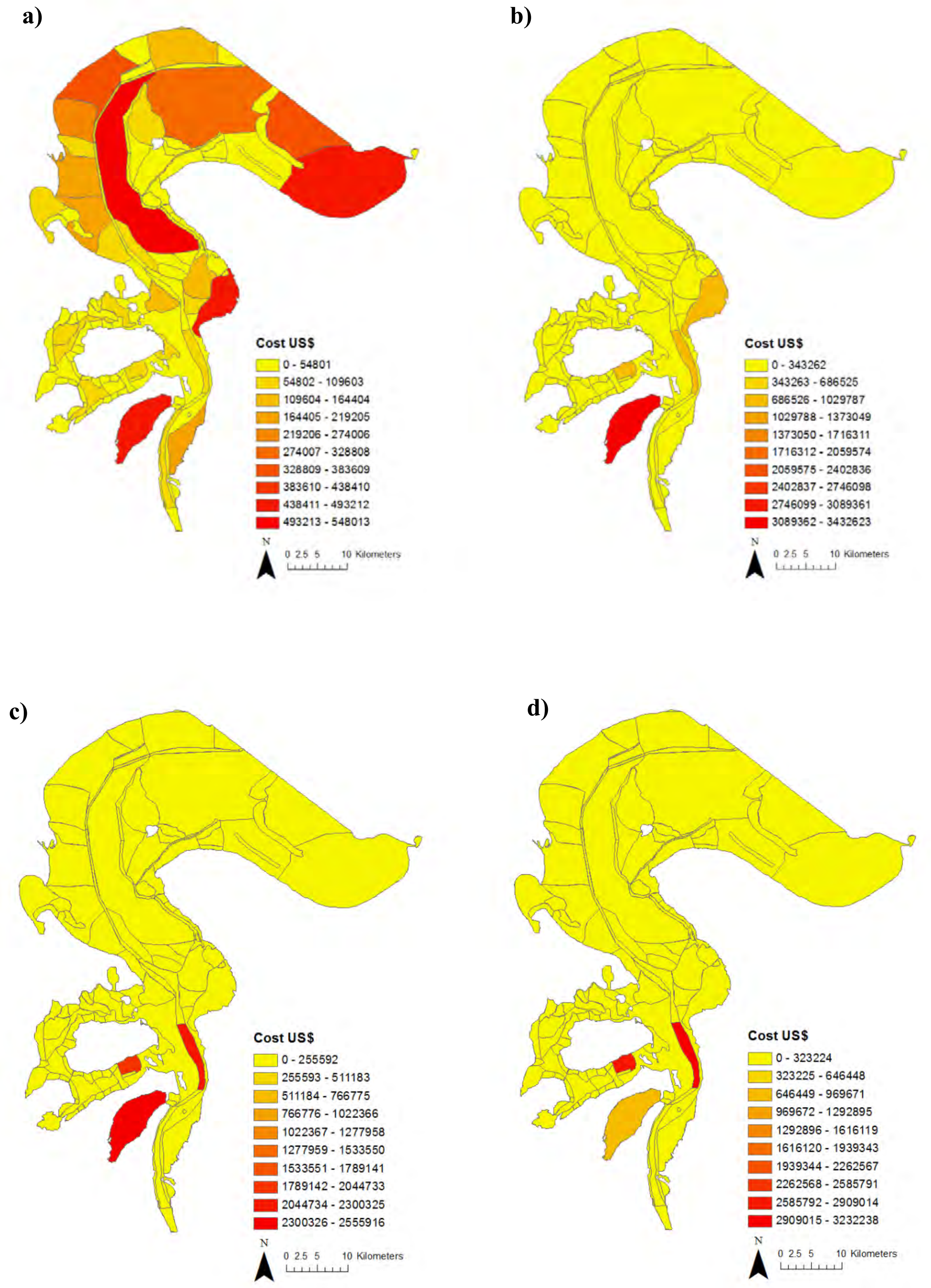

Figure 2: Cost layers of artisanal fishery within each fishing area in the Patos Lagoon estuary: (a) artisanal fishery cost layer with constant α = 0.0001, (b) artisanal fishery cost layer with

constant α = 0.001, (c) artisanal fishery cost layer with constant α = 0.005, and (d) artisanal fishery cost layer with constant α = 0.01.

Conservation problems and scenarios

Two conservation issues were analysed. First, it was assumed that all planning units were available for inclusion in the reserve system. Second, planning units open for navigation, based on the "Framework for Water Classification" (Class 3) from the Brazilian National Council of the Environment (CONAMA, 2005), were locked out and not available for inclusion in the reserve system. This framework classifies marine, freshwater and brackish waters based on their usage and divides brackish water into four classes: Special Class (for which the primary purpose is the preservation of aquatic environments and the maintenance of aquatic communities), Class 1 (waters that can be used for recreational activities, aquaculture, fishing, human consumption after conventional or advanced treatment and irrigation, and the protection of aquatic communities); Class 2 (waters that can be used by fisheries and for recreational activities); and Class 3 (waters allocated for navigation) (CONAMA, 2005). Additionally, two conservation targets were set for each issue: first, to protect 30% of each conservation feature and second, to protect 50% of each habitat. Despite the general use of a 10% target (CBD, 2014) for the protection of marine and coastal environments, the present study followed a precautionary approach and tested higher targets for the features. Different scenarios were run based on costs (opportunity cost and flat cost) and ichthyoplankton data for each combination of conservation issue and target. First, all scenarios were run by including ichthyoplankton data in the analysis. Then, all scenarios were re-run by excluding these data to assess the importance of incorporating the data from different life stages. A cluster analysis was performed using Multivariate Analysis of Ecological Communities (vegan package) in R version 2.15.0 (R Development Core Team, 2013) to compare the solutions from different scenarios. The cluster analysis applied to

Marxan results showed which selection frequency maps (spatial representation of conservation priorities) are most similar (Linke *et al*., 2011).

Systematic conservation planning analysis

Marxan, a decision support tool, was used to assess the importance of incorporating different life stages of fishes into estuarine spatial planning. This tool uses a simulated annealing algorithm to find alternative solutions for conservation planning problem that meets conservation targets at a low socio-economic cost (Ball *et al*., 2009). Marxan was run 100 times each with 1,000,000 iterations. Input parameters were set after calibration, a "boundary length modifier" (BLM) of 10 was adopted to provide reasonable clumping (McDonnell *et al*., 2002), and the "species penalty factor" was set by increasing the value until all targets were met. These input parameters were constant for all scenarios to enable fair comparison. As each run of Marxan produces a different solution, the selection frequency output, which comprises the number of times that a planning unit was selected across all 100 runs, was chosen to represent the conservation priority of a planning unit in each scenario (Ardron *et al*., 2008).

RESULTS

Incorporating fish egg and larvae information into spatial conservation planning has a major effect on spatial priorities. The results of the cluster analysis based on the Marxan solutions showed that most scenarios that used artisanal fishery as an opportunity cost in the Patos Lagoon estuary formed two main groups, splitting the solutions between scenarios that included or excluded ichthyoplankton data, showing that the inclusion of this information produces distinct spatial priorities for conservation. Figure 3 shows the results found for scenarios using a fishing cost decay rate of 0.001. Regardless of the conservation target (30%

or 50%) and whether planning units are locked out of the reserve system, the priorities form two clusters driven by whether we use fish egg and larva data (Figure 3). Cluster analysis results from different scenarios are presented in Figures S1, S2 and S3 under Supporting Information. In general, it was observed that regardless of the opportunity cost used, the priorities are substantially affected by the inclusion of data on fish eggs and larvae.

One exception to this general pattern was found when there is a conservation target of 50% for every habitat, and all planning units are available for inclusion in the reserve system (Figure S2C). It was found that some solutions from this scenario with ichthyoplankton data were grouped with solutions from scenarios without these data. However, when a flat cost was considered instead of an opportunity cost for artisanal fishery, this split patterning disappears, showing that in this case, including fish eggs and larvae data might not be important (Figure 4).

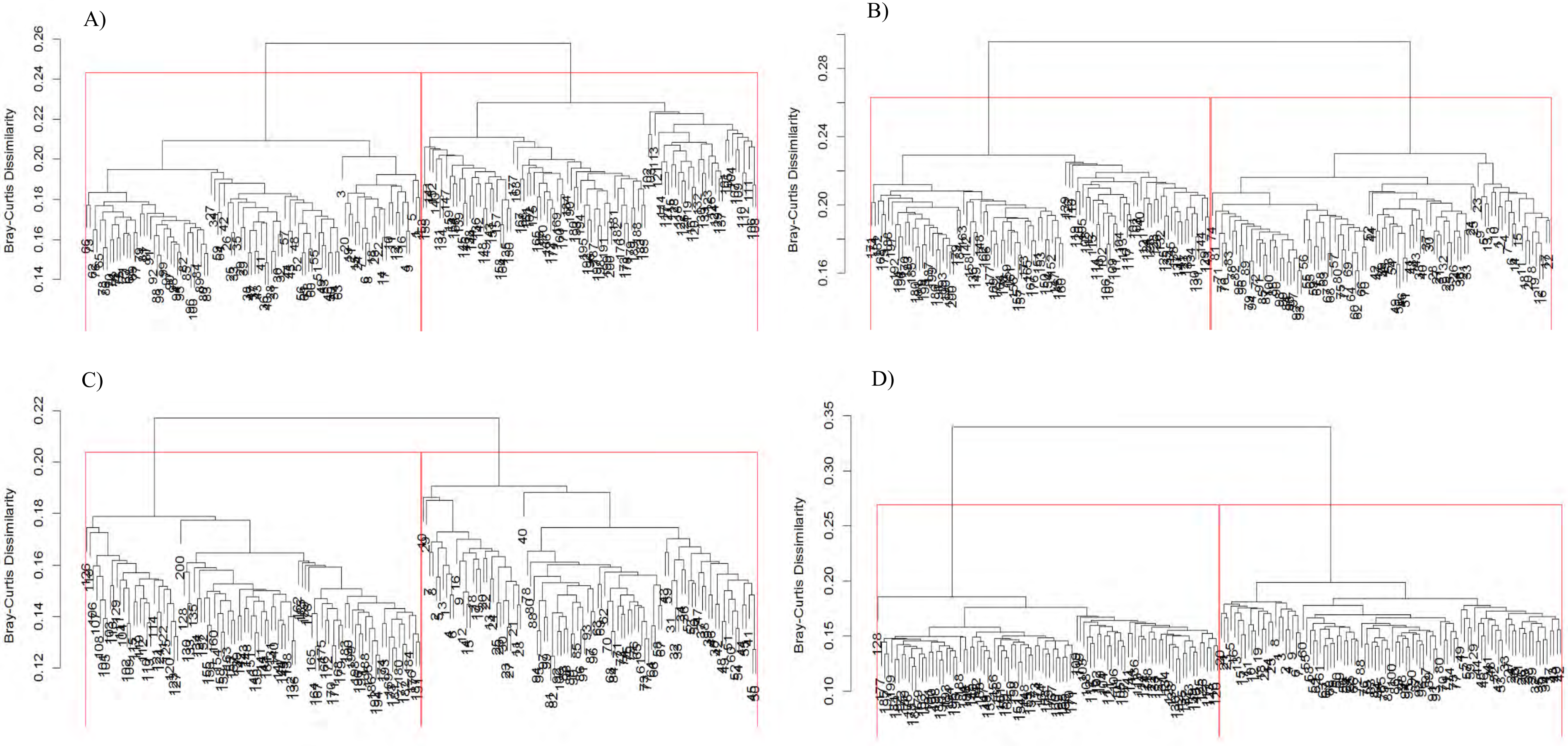

Figure 3: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in Patos Lagoon estuary with constant α = 0.001. Data are presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; C: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

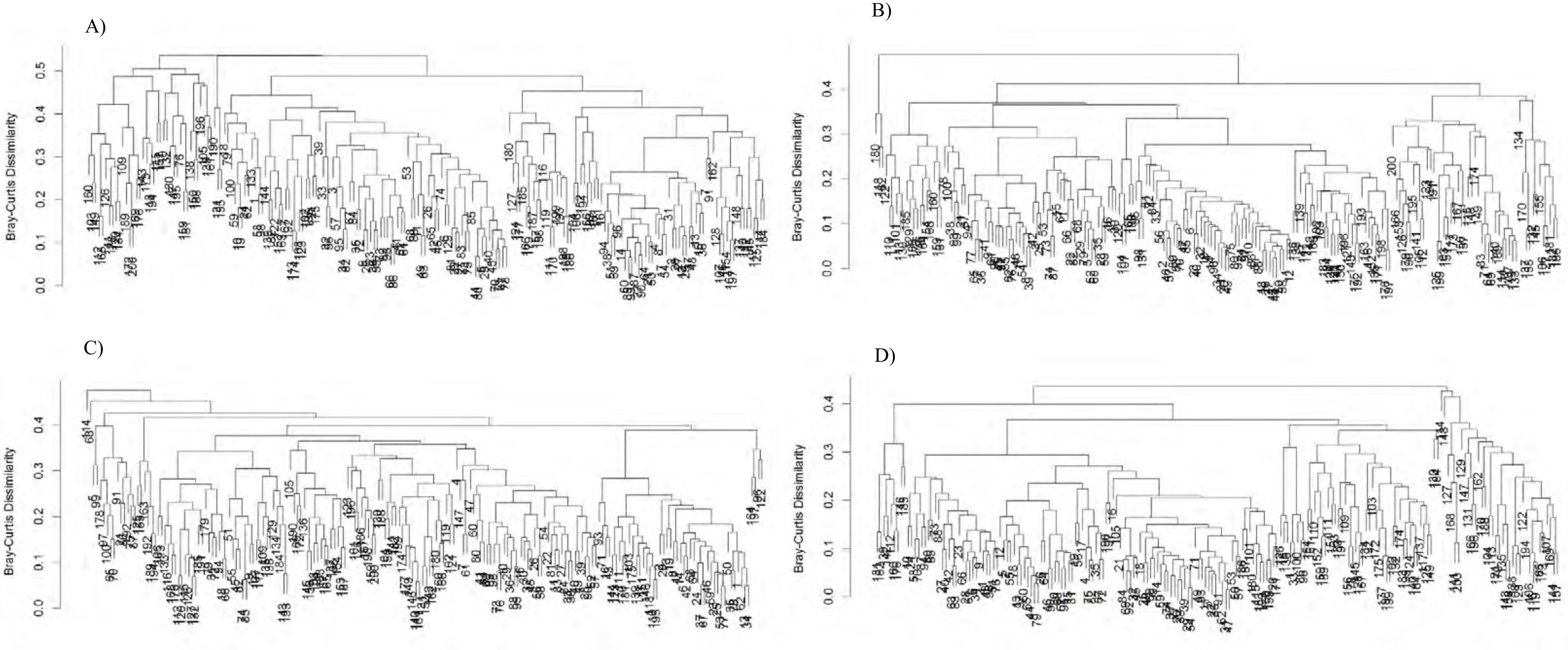

Figure 4: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan. The cost layer used in this analysis represents the flat cost for each planning unit in the Patos Lagoon estuary. Data are presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; C: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

When analysing average cost and the percentage of the target met in each planning design, scenarios considering all planning units available for inclusion in the reserve system were cheaper than those excluding planning units used for navigation from the reserve system (Table 2). Additionally, regardless of cost and conservation target, scenarios where all planning units are available for inclusion in the reserve system exhibited a lower total cost and a higher percentage of targets met when incorporating ichthyoplankton data than when excluding this information. However, planning designs excluding planning units used for navigation showed a different pattern. In this case, scenarios excluding ichthyoplankton showed a lower cost and a lower number of planning units selected in the reserve system than when including these data but with a lower percentage of target met (Table 2). This last pattern is associated with the decrease in sites available for conservation in the estuary, making it more expensive to protect a higher number of features.

The selection frequency of each planning unit or each scenario run with ichthyoplankton data and using a decay rate from fishing site of 0.001 is represented in Figure 5. These results show that high percentages of site selection can be found either clustered or dispersed (Figures 5, S4, S5 and S6). Additionally, it was observed that regardless of the conservation target, shallow areas in the upper part of the estuary and in the middle embayment had among the highest selection frequency (Figure 5). However, in the case of a flat cost, a main and clustered reserve system in the centre of the estuarine area would be enough for conservation purposes (Figure 6). The selection frequency data show that shallow waters are the most important areas for protection in the Patos Lagoon estuary.

Table 2: Results showing average cost, percentage of conservation target met, average of planning units selected, and main cluster results for each scenario run in Marxan (cost 1: cost layer with constant α = 0.0001; cost 2: cost layer with constant α= 0.001; and cost 3: cost layer with constant α = 0.005 and cost 4: cost layer with constant α = 0.01). Cost 5 represents the cost layer with a homogenous value for all planning units (flat cost) and does not have an economic value (in table is represented by (-)).

Planning Scenario	Cost (US$ million)	Targets met (%)	Number of PU selected
Planning Ia – Including Ichthyoplankton 30% conservation target and all planning units available for inclusion in the reserve system	cost 1: 92,455	96.87	686
	cost 2: 92,412	95.31	685
	cost 3: 92,338	93.75	686
	cost 4: 92,307	98.43	686
	cost 5: -	100	957
Planning Ia – Excluding Ichthyoplankton 30% conservation target and all planning units available for inclusion in the reserve system	cost 1: 94,939	92.31	684
	cost 2: 95,008	88.46	684
	cost 3: 94,973	100	684
	cost 4: 94,933	96.15	684
	cost 5: -	96.15	768
Planning Ib – Including Ichthyoplankton 30% conservation target and planning units open for navigation not available for inclusion in the reserve system	cost 1: 116,955	93.75	670
	cost 2: 117,530	89.06	670
	cost 3: 116,930	96.87	670
	cost 4: 116,873	89.06	669
	cost 5: -	96.87	1043
Planning Ib – Excluding Ichthyoplankton 30% conservation target and planning units open for navigation not available for inclusion in the reserve system	cost 1: 112,406	92.30	672
	cost 2: 112,325	88.46	672
	cost 3: 112,407	88.46	672
	cost 4: 112,540	96.30	672
	cost 5: -	92.30	907
Planning IIa – Including Ichthyoplankton 50% conservation target and all planning units available for inclusion in the reserve system	cost 1: 98,372	95.31	705
	cost 2: 98,420	96.87	705
	cost 3: 98,338	93.75	705
	cost 4: 98,229	95.31	705
	cost 5: -	98.43	1000
Planning IIa – Excluding Ichthyoplankton 50% conservation target and all planning units available for inclusion in the reserve system	cost 1: 98,671	96.15	705
	cost 2: 98,595	96.15	705
	cost 3: 98,566	84.61	707
	cost 4: 98,603	88.46	704
	cost 5: -	100	858
Planning IIb – Including Ichthyoplankton 50% conservation target and planning units open for navigation not available for inclusion in the reserve system	cost 1: 128,924	92.18	694
	cost 2: 128,848	93.75	694
	cost 3: 129,005	92.18	693
	cost 4: 129,004	89.06	695
	cost 5: -	98.43	1101
Planning IIb – Excluding Ichthyoplankton 50% conservation target and planning units open for navigation not available for inclusion in the reserve system	cost 1: 98,607	88.46	705
	cost 2: 96,685	92.30	693
	cost 3: 98,457	96.15	704
	cost 4: 98,563	92.30	705
	cost 5: -	100	872

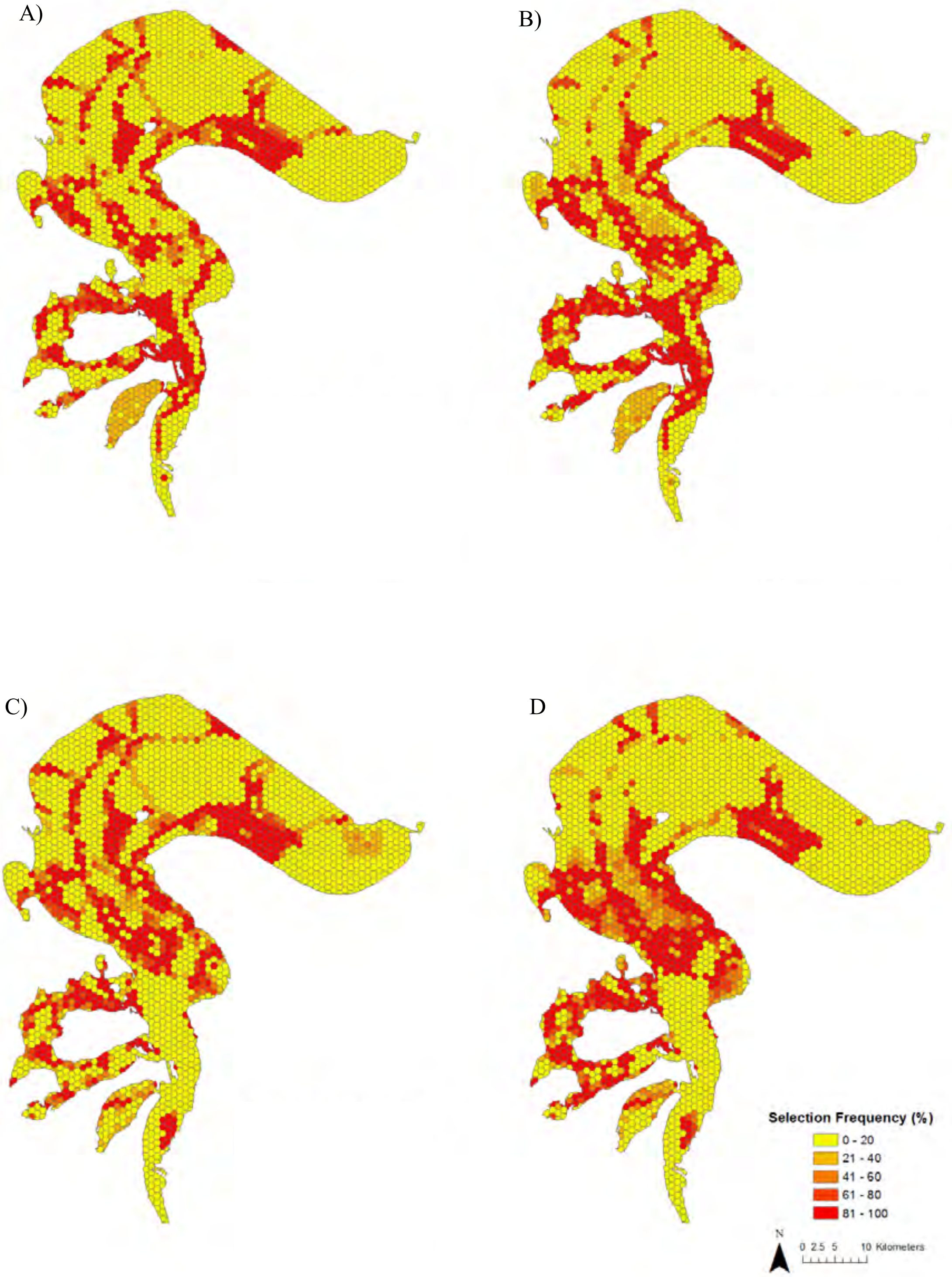

Figure 5: Selection frequency result displayed for each scenario with ichthyoplankton data. The cost layer used in this analysis represents the opportunity cost of artisanal fisheries in the Patos Lagoon estuary with constant α = 0.001. (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; C: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

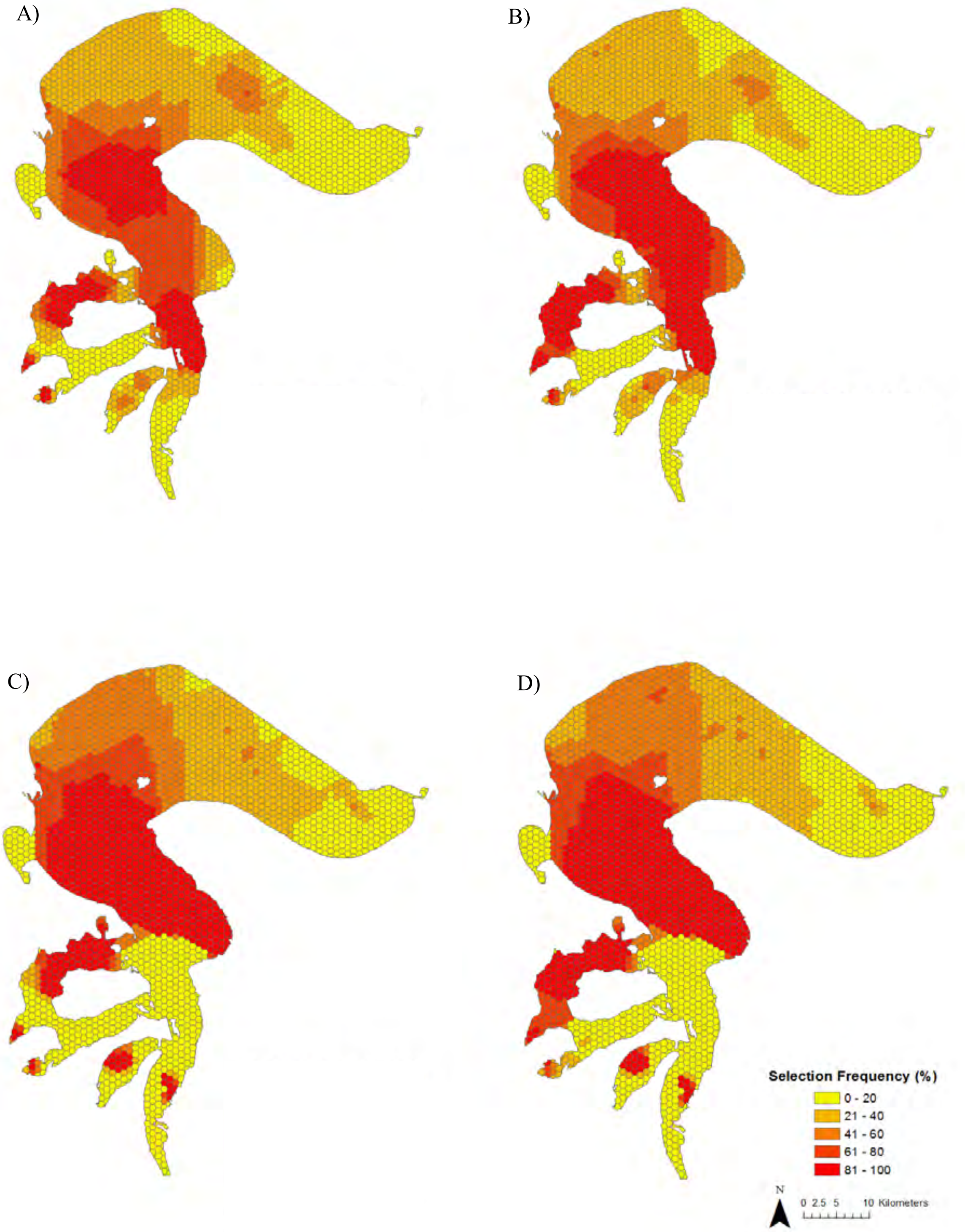

Figure 6: Selection frequency result displayed for each scenario with ichthyoplankton data. The cost layer used in this analysis represents the flat cost for each planning unit in the Patos Lagoon estuary (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; C: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

DISCUSSION

This study shows that information about the early life history stages of fishes and opportunity costs based on artisanal fishery can help to achieve a more cost-effective outcome in the Patos Lagoon estuary. Furthermore, using fish egg and larvae data in conservation planning substantially changes priorities for estuarine conservation. Usually, planktonic stages have a wide distribution and depend on nursery areas, such as estuaries, and spawning sites. In this case, understanding and including the spatial distribution of these life-stages is extremely relevant to increase the effectiveness of reserves (Allison et al., 1998; Warner et al., 2000). Ichthyoplankton represents the most vulnerable stage of the fish life cycle, and mortality rates during this phase can reach almost 99% for most species (Fuiman & Werner, 2002). The mortality of fish eggs and larvae can be associated with various processes, such as predation, nutrition, diseases, unfavourable environmental conditions, anthropogenic causes such as the habitat alteration or destruction and pollution. Protected areas offer a spatially explicit form of protection that enables local control of anthropogenic activities, helping to mitigate numerous pressures such as fishing, pollution threats and habitat disturbance (Allison et al., 1998). Estuaries are among the most important sites for the development of the early life history of fishes. These ecosystems often suffer from anthropogenic impacts that can potentially destroy estuarine habitat (McLusky & Elliot, 2006) and affect nursery functioning for many species (Courrat *et al*. 2009). The protection of the habitat used by early life stages of fish is essential for ensuring success in the recruitment process and to sustain the adult population.

Despite the ecological importance of estuaries, estuarine conservation is in its infancy relative to terrestrial and marine realms. This may be related to the high social and economic values of these areas for humans and also a lower aesthetic appeal compared with other ecosystems (Edgar *et al*., 2000; Neely & Zajac, 2008). Systematic conservation planning studies in estuaries are restricted to a few papers, most of them using macrobenthic invertebrates or habitats as surrogates of biodiversity (Neely & Zajac, 2008; Shokri & Gladstone, 2009, 2013; Shokri *et al*., 2009). Until now, ichthyoplankton or fish data have not been used in estuarine conservation planning. The results showed that incorporating ichthyoplankton data into systematic conservation planning can generate different outcomes than a plan without these data (Figures 3 and 4). Therefore, estuarine conservation plans that solely rely on habitat data for surrogates of biodiversity can produce suboptimal results. In a recent study, Shokri & Gladstone (2013) found that habitat classification schemes that represent variation in estuarine biodiversity are inefficient at effectively designing estuarine protected areas. One of the main aspects of estuaries is the interaction between land and sea processes that creates a complex and dynamic spatial pattern for biological, physical, chemical, and socio-economic components (Pittman *et al*., 2011). This means that an efficient planning scheme must account for ecological dynamics in a spatially explicit framework (Pittman *et al*., 2011). Future studies should include some analysis relating the distribution of planktonic stages, such as fish eggs and larvae, to other dynamic physico-chemical features of the estuary. Furthermore, other socio-economic components with more direct impacts on fish eggs and larvae development, such as water quality, should be examined in future estuarine conservation planning studies.

The Patos Lagoon estuary has been subject to substantial natural and human disturbances. The construction of rocky jetties at the entrance of the estuary may have altered the input and output water flux (Seeliger & Odebrecht, 2010), consequently influencing the transport of fish eggs and larvae into the estuary. Despite the strong seasonal pattern in recruitment of fish eggs and larvae to the Patos Lagoon estuary, long-term studies suggest the existence of decadal variations in climate due to El Niño Southern Oscillation (Muelbert *et al*., 2010). El Niño (high precipitation) and La Niña (low precipitation) events interact with wind patterns in the region and influence the salinity in this ecosystem and consequently control the occurrence and abundance of fish eggs and larvae (Bruno & Muelbert, 2009). Despite these disturbances, the study period showed consistent patterns in salinity and consequently in the recruitment of such early life stages of fishes into the Patos Lagoon estuary (Muelbert *et al.*, 2010). Therefore, the scenarios tested in the present study that incorporated historical ichthyoplankton assessments represent the general pattern of the recruitment process into the study area. In this sense, historical data may be acceptable for inclusion in studies that aim to develop new methods for spatial planning. However, it is necessary to note that historical data were only used because there was no recent information available for the entire estuarine area. Spatial planning for conservation purposes should be based on recent information for the region it intends to preserve where possible.

While the present study highlights the advantage of using ichthyoplankton data for estuarine conservation, it also emphasises the importance of incorporating opportunity cost instead of a spatially homogeneous (flat) cost. The results showed that if a flat cost is used (Figure 6), as is still common in the conservation planning literature, using just abiotic features (e.g., habitat, bathymetry and sediment) is sufficient for achieving conservation targets. In contrast, when a more realistic socio-economic cost was used instead of a flat cost, it was possible to achieve a more effective outcome. Additionally, it was observed that the abiotic features were not adequate surrogates for the biotic elements that were the focus in the planning. This confirms the study by Shokri & Gladstone (2013), which highlighted that habitat alone is an inefficient surrogate for estuarine conservation. Similarly, Stewart & Possingham (2005) outlined the importance of considering opportunity cost in conservation planning and creating representative, efficient, and practical reserve systems that minimise potential economic losses. In the case of the Patos Lagoon estuary, artisanal fisheries and port activity are the main economic uses of the estuarine area; thus, a conservation plan aiming towards a representative and efficient reserve system must take these activities into consideration. In this sense, scenarios using opportunity cost which also excluded planning units used for navigation from the reserve system represents a more realistic solution for the study area as they minimise fishery loss, avoid prioritising reserves in the navigation area in the estuary and achieve conservation targets.

The Patos Lagoon estuary is predominantly a shallow estuarine ecosystem (Seeliger, 2001). The results showed that shallow waters (vegetated or not) are among the most selected sites for conservation in the Patos Lagoon estuary (Figures 1, 5 and 6, Figures S4 – S6). Shallow areas in the upper part of the estuary and in the middle embayment were selected in all scenarios, regardless of the objectives, indicating the importance of these sites for early life fish development. In Patos Lagoon, most shallow waters are colonised by the seagrass *Ruppia maritima*, which develops during warmer seasons (spring and summer) and plays an important role in feeding, development and protection for the early life stages of many fish and invertebrate species (Castello, 1986; Garcia & Vieira, 1997; Seeliger, 2001). In addition, vegetated shallow waters are cited as an important factor structuring fish abundance and composition in estuaries worldwide (Gillanders, 2006). Despite their ecological importance, shallow areas in the Patos Lagoon estuary are also impacted by pollution and contamination from surrounding urban area, dredging, and the loss of seagrass beds and saltmarshes (Seeliger & Costa, 1997; Barletta *et al.*, 2010). Consequently, the efficient management of estuarine shallow waters, vegetated or not, is of paramount importance for conservation and must be accounted for when addressing estuary protection.

In summary, if fish egg and larva data are ignored in the planning process, conservation priorities will be very different. Furthermore, including temporal variability in estuarine biodiversity into systematic conservation planning can be beneficial for achieving an effective outcome because it considers the variations that a species can have in their distribution within different scales of time and space. Considering the present case study, shallow waters are of great importance and must be considered for estuarine conservation and incorporated into estuarine protected areas. Additionally, it is suggested that future studies should incorporate information from adjacent coastal and freshwater ecosystems to create a conservation plan for the entire estuarine ecosystem. The present study also shows that opportunity costs can help achieve an efficient and representative reserve system and highlights the need to incorporate social and economic information into reserve design (Naidoo *et al*., 2006; Carwardine *et al*., 2008; Klein *et al*., 2008a, b; Ban & Klein, 2009). More studies should focus on developing methods for planning conservation in estuarine ecosystems with multiple uses and stressors.

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Additional Supporting Information may be found in the online version of this article:

Figure S1: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan, using cost layer with constant $\alpha = 0.01$.

Figure S2: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan, using cost layer with constant $\alpha = 0.005$.

Figure S3: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan, using cost layer with constant $\alpha = 0.0001$.

Figure S4: Selection frequency result displayed for each scenario with ichthyoplankton data, using cost layer with constant $\alpha = 0.01$.

Figure S5: Selection frequency result displayed for each scenario with ichthyoplankton data, using cost layer with constant $\alpha = 0.005$.

Figure S6: Selection frequency result displayed for each scenario with ichthyoplankton data, using cost layer with constant $\alpha = 0.0001$.

Additional Supporting Information

Figure S1: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in the Patos Lagoon estuary with constant α = 0.01. Data are presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; C: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

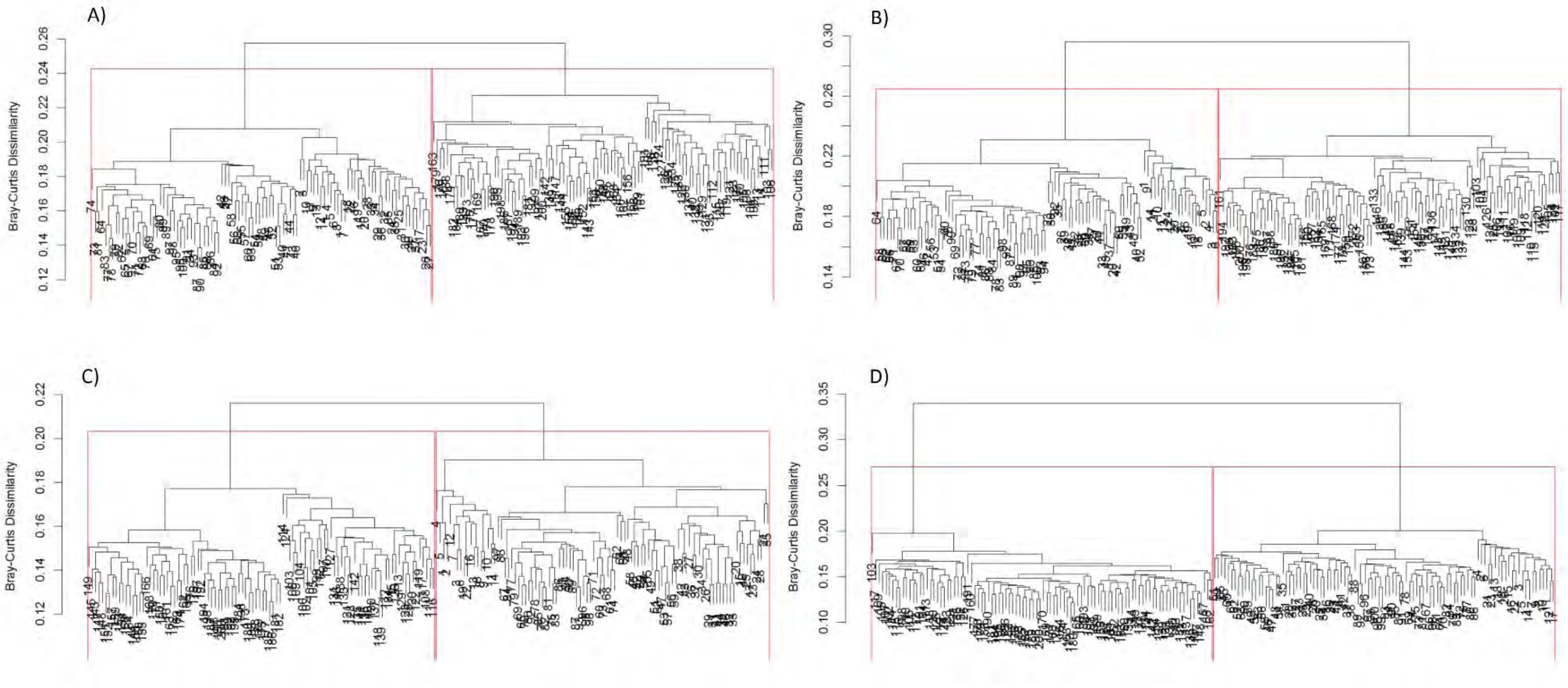


Figure S2: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in the Patos Lagoon estuary with constant α = 0.005. Data are presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; C: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system)

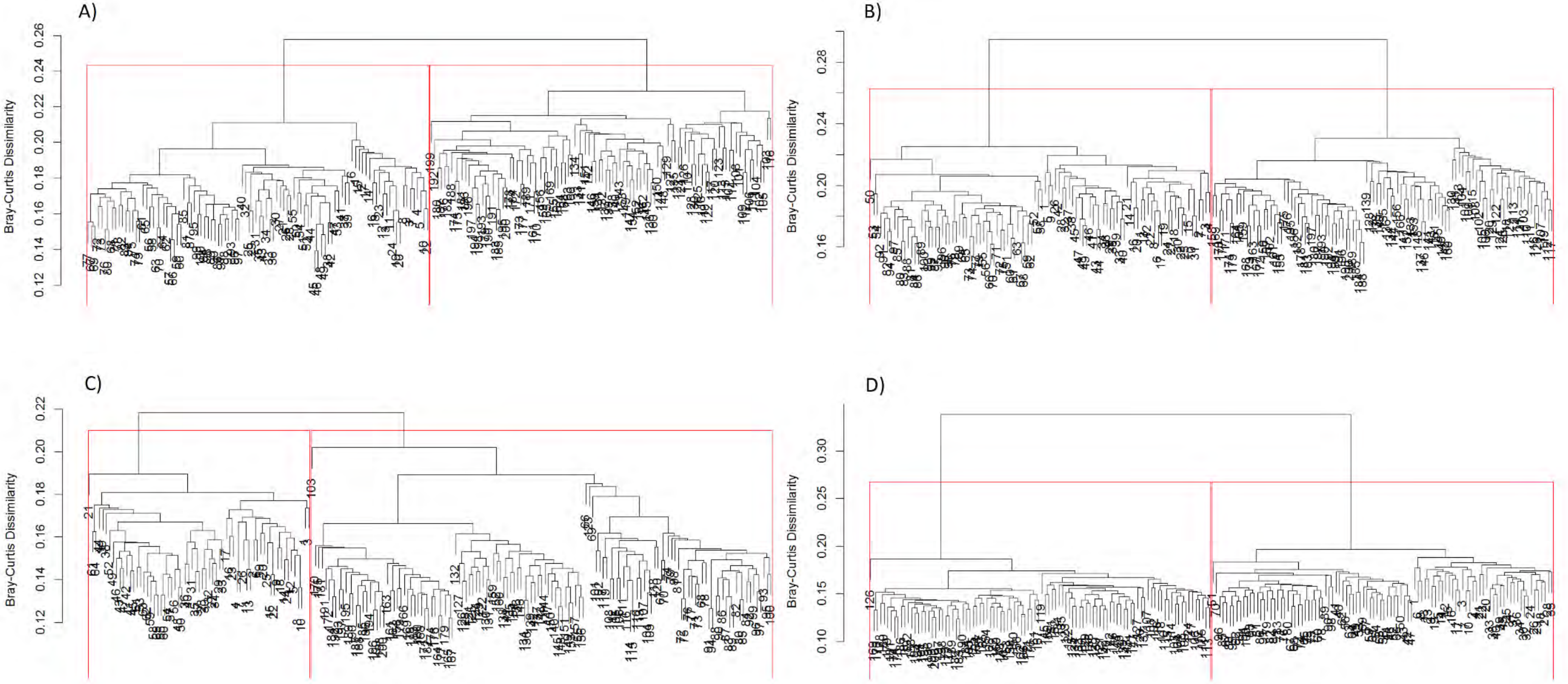


Figure S3: Relationship among 100 solutions for each scenario with (solutions 1 to 100) and without (solutions 101 to 200) ichthyoplankton data run in Marxan. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in the Patos Lagoon estuary with constant α = 0.0001. Data are presented as a dendrogram from a cluster analysis based on Bray-Curtis Dissimilarity (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; C: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

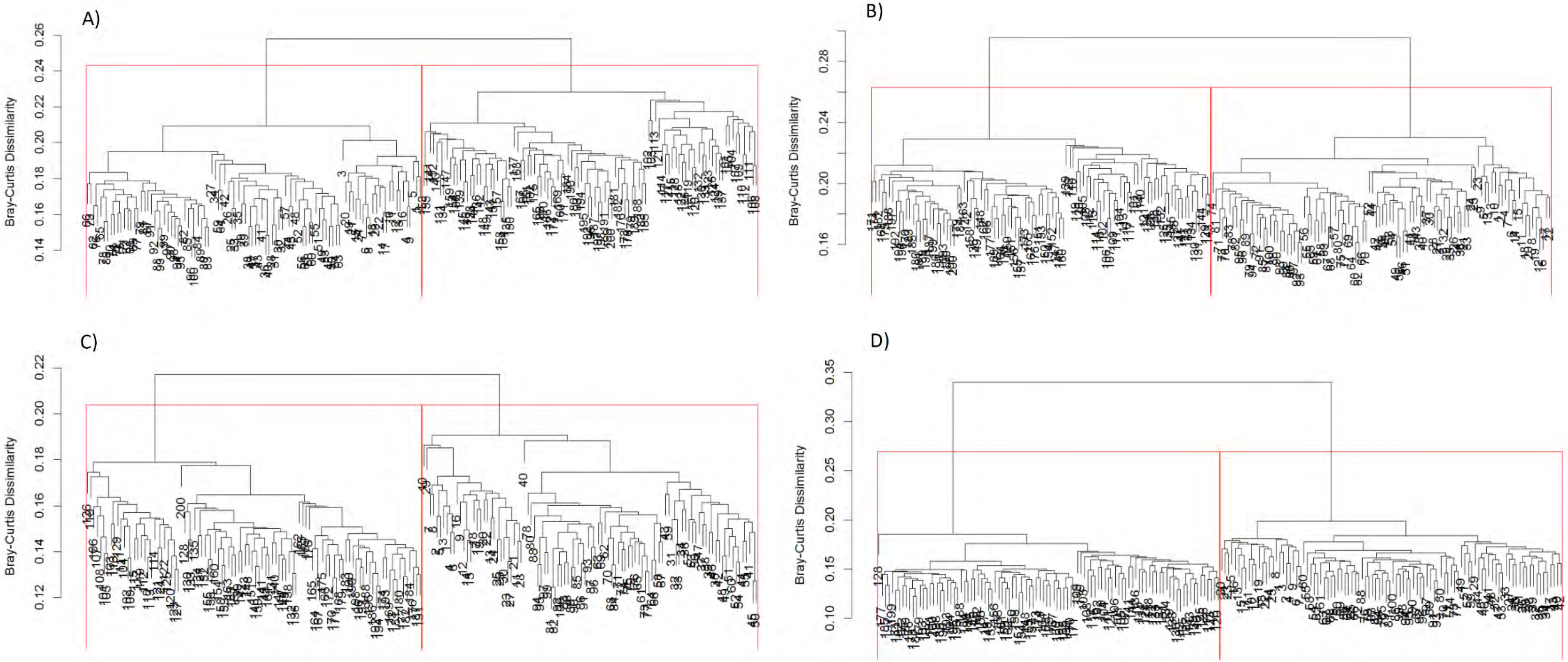


Figure S4: Selection frequency result displayed for each scenario with ichthyoplankton data. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in the Patos Lagoon estuary with constant α = 0.01 (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; C: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

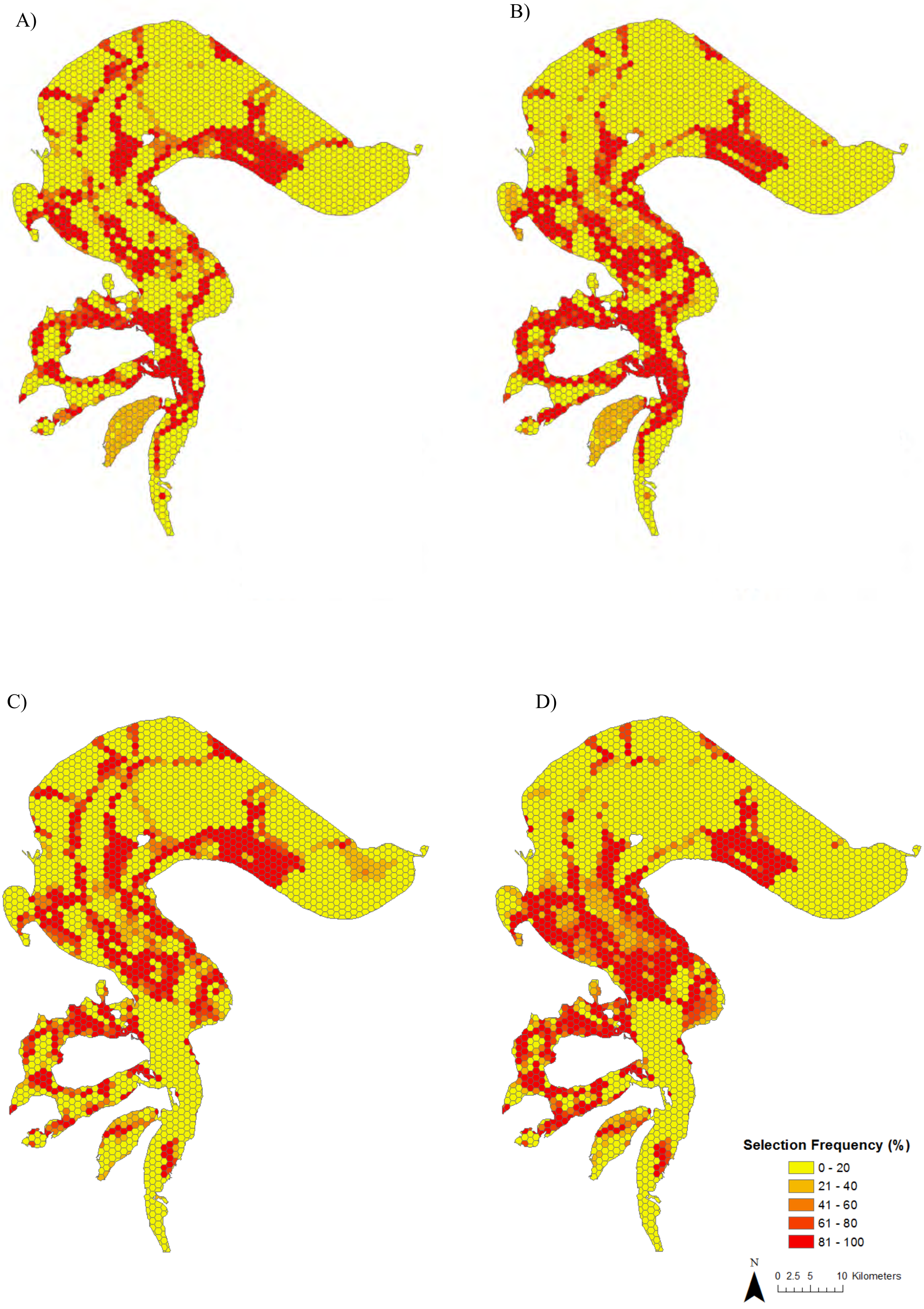

A)
B)
C)
D)
Selection Frequency (%)
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
N
0 2.5 5 10 Kilometers

Figure S5: Selection frequency result displayed for each scenario with ichthyoplankton data. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in the Patos Lagoon estuary with constant α = 0.005 (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; C: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).

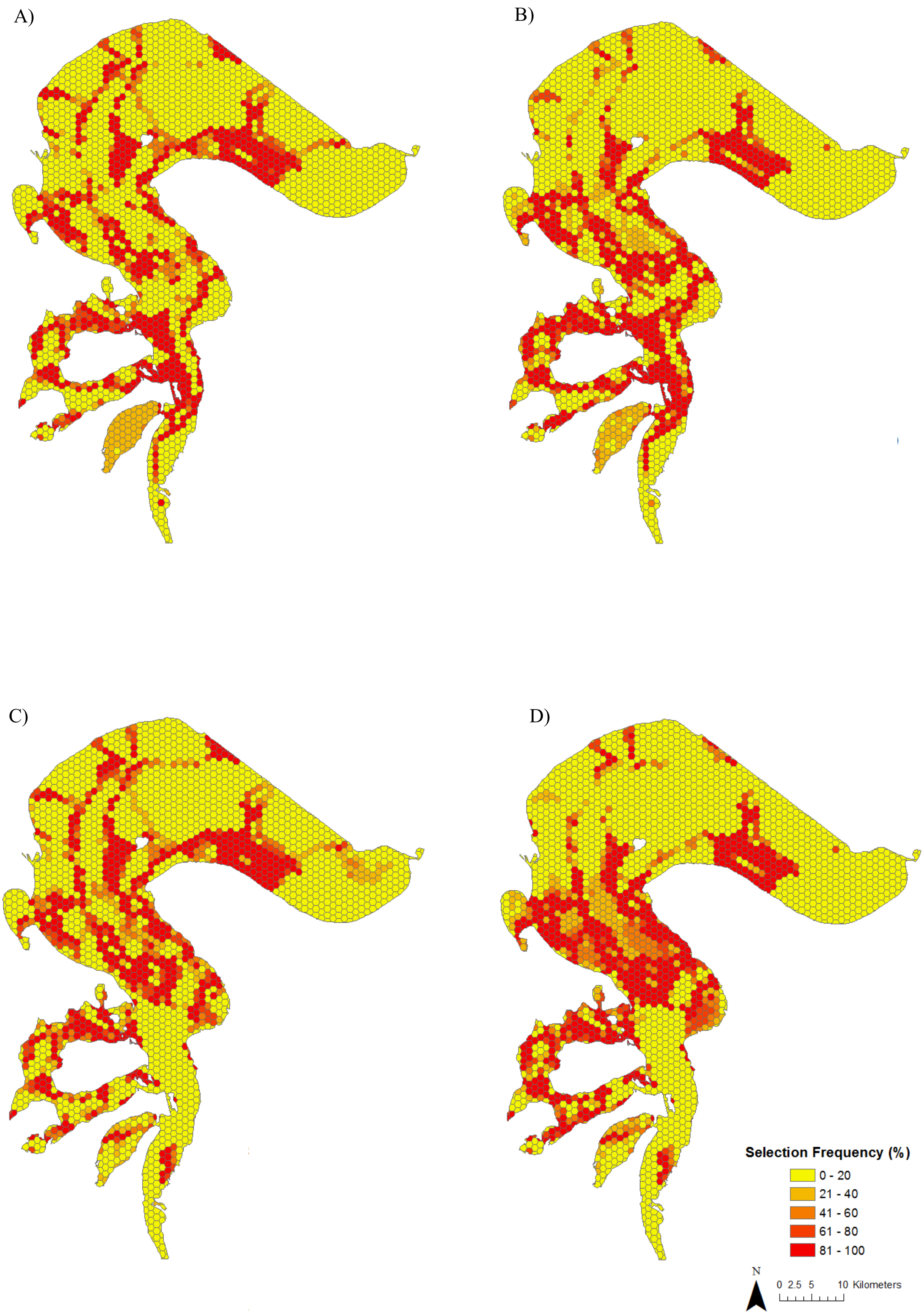
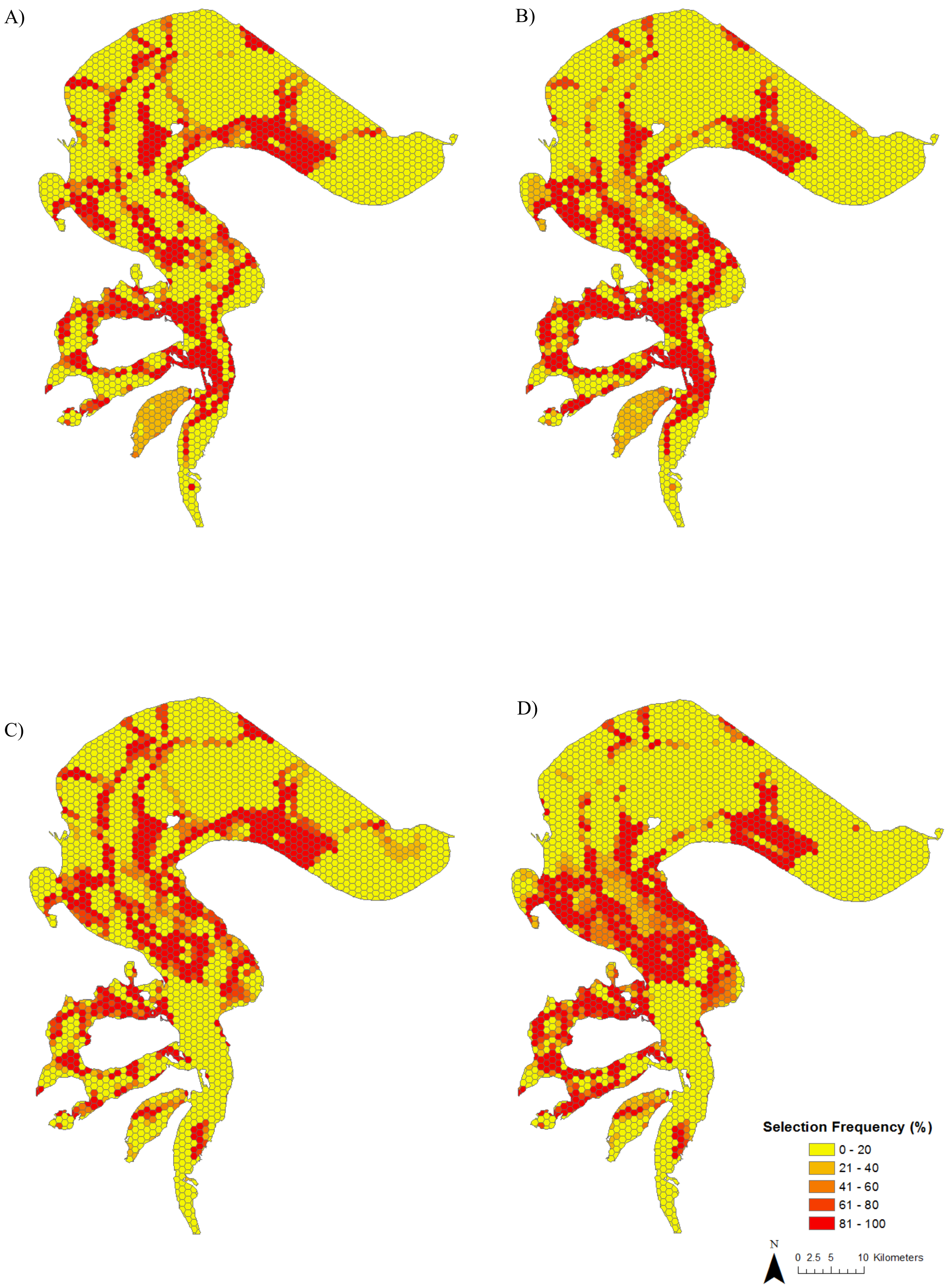

A)
B)
C)
D)
Selection Frequency (%)
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
N
0 2.5 5 10 Kilometers

Figure S6: Selection frequency result displayed for each scenario with ichthyoplankton data. The cost layer used in this analysis represents the opportunity cost of artisanal fishery in the Patos Lagoon estuary with constant α = 0.0001 (A: conservation problem under a conservation target of 30% for all features and all planning units available for inclusion in the reserve system; B: conservation problem under a conservation target of 50% for habitats and all planning units available for inclusion in the reserve system; C: conservation problem under a conservation target of 30% for all features and planning units open for navigation not available for inclusion in the reserve system; and D: conservation problem under a conservation target of 50% and planning units open for navigation not available for inclusion in the reserve system).


A)
B)
C)
D)
Selection Frequency (%)
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
N
0 2.5 5 10 Kilometers

6.5 Apêndice 5 – Costa, M.D.P. & Muelbert, J.H. Efficient estuarine conservation planning: taking into account the dynamic nature of the ecosystem. Submetido para o Brazilian Journal of Nature Conservation (Natureza & Conservação).

Section: Essays and Perspectives

Efficient estuarine conservation planning: taking into account the dynamic nature of the environment

Short title: Estuarine conservation planning

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ABSTRACT

Ecosystem-based management and integrated marine spatial planning have emerged as strategic processes for managing multiple marine uses in a sustainable way, and they have become a priority in many countries. Estuaries are among the most productive coastal ecosystems and are of both economic and social importance. These features also make them one of the most impacted environments in the world, subject to the effects of a range of human activities. Their main peculiarity is that ecological processes can interact across land and sea, creating a complex and dynamic spatial pattern with many components and at different temporal and spatial scales. For such dynamic ecosystems, analysis of temporal data is an important component of effective estuarine conservation planning and is vital to the implementation of successful management strategies. For many species, habitat requirements and occupation can change throughout their life cycle, resulting in different spatial distributions at each ontogenetic stage. High connectivity and temporal variation can influence the ability of protected areas to efficiently reach their conservation objectives. Considering the dynamic nature of estuaries, incorporating variation in spatial and temporal patterns of habitat use among development stages is crucial to creating an effective spatial estuarine plan. With this in mind, we suggest that distribution patterns of different life stages, such as fish eggs and larvae, be included in estuarine conservation planning. In this essay, we aim to discuss recent trends and developments in estuarine conservation that incorporate habitat dynamics into spatial planning.

INTRODUCTION

In the last decades, many definitions and classifications have been proposed for estuaries based on different perspectives (e.g., landform geomorphology, evolutionary origins, and formative processes), purposes (e.g., understanding structure, variability and dynamics, functions and values, and interaction with the adjacent freshwater system and coast), and applications (e.g., categorizing, mapping and management) (Simenstad & Yanagi 2011). However, all of these classifications rely on a basic definition of an estuary based on the following criteria: coastal bodies; border control; tidal action; single or multiple connections with the adjacent shore or coastal saline water body; freshwater input, which produces a statistically significant and circulation-wide dilution of the seawater; and the existence of species that live in the estuary during part of or for their entire life cycle (Perillo 1995). Perillo (1995) has proposed the only definition that includes all of these criteria: *"an estuary is a semi-enclosed coastal body of water that extends to the effective limit of tidal influence, within which seawater entering from one or more free connections with the open sea, or any other saline coastal body of water, is significantly diluted with freshwater derived from land drainage, and can sustain euryhaline biological species from either part or whole of their life cycle"*.

Estuaries are among the most productive ecosystems in the world. They support large populations of a wide variety of species, which makes them of high conservation value (McLusky & Elliott 2006; Ducrotoy & Elliott 2006). In addition to their nursery function, estuaries and their specific habitats are important feeding areas and migration zones for many species (Elliott & Hemingway 2002). Contributions of each estuarine habitat to organisms varies from species to species. Estuarine habitats include tidal freshwater, vegetated and unvegetated habitats, intertidal soft and hard substratum, and subtidal soft and hard substratum. Estuaries are also of economic and social importance to humans. Approximately

41% of the world's population resides in the coastal zone (Martinez *et al*. 2007) and maintains livelihoods that are intrinsically linked to estuaries and coastal ecosystems (Barbier *et al*. 2011). The attractiveness of coastal resources to humans makes estuaries among the most valuable ecosystems on Earth (van den Belt 2011). As a result, ecosystem-based management and integrated marine spatial planning have emerged as priorities for many agencies around world in order to manage multiple marine uses in a sustainable and comprehensive way (Ehler & Douvere 2009; Pittman *et al*. 2011). In this essay, we discuss recent trends and developments in estuarine conservation that include habitat dynamics in spatial planning. We also discuss a case study to suggest that the dynamic nature of estuaries must be accounted for to achieve efficient conservation planning.

Estuarine Ecosystem Services and Threats

Estuaries are located at the land-sea interface. The synergistic relationship between different habitats can enhance the ecosystems services in estuaries, producing cumulative benefits. In general, services provided by estuaries can be more significant than those provided by a single habitat (Barbier *et al*. 2011). Ecosystem services provided by estuaries can be classified into three categories: (a) goods (e.g., products obtained from ecosystems, such as resource harvests and water); (b) services (e.g., social amenities such as tourism and recreation; ecological functions such as water purification and climate regulation; and habitat features such as niches, refugia, and nursery and breeding sites); and (c) cultural significance (e.g., spiritual and religious beliefs and heritage value) (Daily *et al*. 1997; Millennium Ecosystem Assessment 2005; Barbier *et al*. 2011; van den Belt 2011).

Despite the many services provided by estuaries, habitat loss and the cumulative impacts of human activities are intense and increasing in these ecosystems (Millenium Ecosystem Assessment 2005; Barbier *et al*. 2011). Excessive activities and impacts can potentially affect several services provided by coastal ecosystems (Barbier *et al*. 2011). One of the primary causes associated with increased human impacts in estuaries is rapid, uncontrolled population growth and urban development in many coastal cities (Kennish 2002). Of the many effects of human activities on estuaries, pollution inputs (such as nutrient enrichment and chemical contaminants), habitat alteration and loss, overfishing, introduced species, dredging, and freshwater diversions are listed among the most serious threats (Kennish 2002; McLusky & Elliott 2006). In addition, the number of people in coastal populations is estimated to reach six billion by 2025, suggesting that human impacts on estuaries may increase significantly, mainly in developing countries (Weber 1994; Hameedi 1997; Kennish 2002).

For this reason, maintenance and protection of estuaries, coastal ecosystems, and their related services and functions must involve managing entire landscapes, accounting for connectivity among habitats, synergistic effects, and diverse temporal and spatial scales (Barbier *et al*. 2011). Thematic habitat maps of ecosystem services can provide an effective way to incorporate economic impact into management decisions and can be used with a decision support tool to facilitate the decision process (Moilanen *et al*. 2009; Pittman *et al*. 2011). The multiple functions of these coastal ecosystems, along with their ecological importance, make requiring an integrated management of great importance to balance threats, demands and ecological functions of estuaries (Ducrotoy & Elliott 2006).

Estuarine Conservation Planning

The effective allocation of resources to support various ecosystem functions is vital and urgent given the increases in human pressures and the effects of changing global climate on estuaries over the last few decades. The main goal of identifying priority areas for conservation is to mitigate anthropogenic threats and impacts. Therefore, incorporating data on these issues is crucial to effective conservation (Pittman *et al*. 2011). Quantitative methods for identification and spatial prioritization are considered efficient and have been used widely for both terrestrial and marine systems internationally (Moilanen *et al*. 2009; Pittman *et al*. 2011). Spatial prioritization of estuaries is in its infancy when compared with terrestrial and marine ecosystems. The conservation and maintenance of estuaries and their habitats requires knowledge of diversity, species distribution, environmental variables, ecosystem functions, and the relation between species and the environment (Ducrotoy & Elliott 2006). A common practice is the assessment of water quality within estuaries and coastal lagoons based on bioindicators. This approach has become a worldwide practice over the past few years and is especially popular in Europe where the European Water Framework Directive has been implemented (Pérez-Dóminguez *et al*. 2012). Fish are considered a key element to this approach, and several fish-based indices have been proposed (Cabral *et al*. 2012; Pérez-Dóminguez *et al*. 2012). Usually, these indices are based on independent metrics and on assemblage composition or functional attributes of fish species (Pérez-Dóminguez *et al*. 2012).

Despite the need for an integrated management plan, only a few studies have applied a systematic conservation planning approach to estuaries (Neely & Zajac 2008; Geselbracht *et al*. 2009; Shokri & Gladstone 2009; Shokri *et al*. 2009; Shokri & Glastone 2013a; Shokri & Gladstone 2013b). Most of these studies aimed to assess surrogate taxa to select estuarine protected areas (Shokri *et al*. 2009; Shokri & Gladstone 2009; Shokri & Gladstone 2013a), or incorporated the vulnerability of seagrass meadows to anthropogenic disturbances into the selection of priority areas for conservation (Shokri & Gladstone 2013b). Only two studies used decision support software to efficiently achieve estuarine conservation planning outcomes (Neely & Zajac 2008; Geselbracht *et al*. 2009).

The incorporation of economic costs into conservation planning has already been tested in many studies and illustrates that this information can help achieve substantially better outcomes than when costs are ignored (Naidoo *et al.* 2006; Ban & Klein 2009). In general, four economic indices have been considered: uniform cost or area; opportunity costs; multiple socioeconomic costs; and measures of naturalness, or ecological impact of anthropogenic activities (Ban & Klein 2009; Moilanen *et al.* 2009). Among the estuarine conservation planning studies reviewed, only Geselbracht *et al.* (2009) integrated a spatial index of socioeconomic factors as an opportunity cost into the planning design. Most marine conservation planning studies that have incorporated economic data spatially have focused on fisheries information as an opportunity cost (Ban & Klein 2009). In general, conservation planning efforts that integrate economic costs reflecting the conservation action or multiple stakeholder interests are more likely to identify cost-effective outcomes (Naidoo *et al.* 2006; Klein *et al.* 2008; Carwardine *et al.* 2008; Ban & Klein 2009; Mazor *et al.* 2014). This is especially relevant when dealing with a highly dynamic ecosystem with a wide variety of human activities, such as estuaries. Another approach to improving estuarine conservation planning for multiple activities and a diverse array of stakeholder groups is zoning (Ban & Klein 2009). Zoning allows activities to occur in specified areas, provides area-based control, separates conflicting uses, and minimizes negative human impacts on the ecosystem (Day 2002). It can be an efficient way to ensure that multiple objectives (e.g., no-take areas, resources, services and biodiversity conservation) are met (Pittman *et al.* 2011). The use of protected areas in estuaries has produced conflicting results. Initially, areas with "no-fishing" regulations showed greater abundance of fishes, larger fishes and relative abundance of several fish species than sites where fishing was allowed (Johnson *et al.* 1999). However, a few years later, the abundance of sciaenid sport fish larvae across the same estuarine reserve boundary was higher in public areas than in the no-take areas, suggesting that the reserve designation may do little to enhance local recruitment (Reyier *et al*. 2008). These results only reinforce the need to increase the effort to understand how estuarine protected areas can benefit biodiversity and also highlight the importance of applying a systematic conservation planning approach to estuarine protection.

Dealing with estuary dynamics in systematic conservation planning

An important concept underlying the ecosystem management approach is that different areas have different biological production rates. These rates are interlinked with spatial heterogeneity of ecological patterns and processes, including anthropogenic activities (Pittman *et al*. 2011). Ecological processes in estuaries can interact across land and sea, creating complex and dynamic spatial patterns across multiple categories (e.g., biological, physical, chemical, and socioeconomic) (Pittman *et al*. 2011) and spatio-temporal scales. The transfer of material and energy between systems can vary significantly at the land-ocean interface, requiring very specific adaptations for organisms within these ecosystems (Heip *et al*. 2011). Usually, the processes and ecological characteristics of estuaries exhibit high spatial and temporal variability, so that estuaries may act as both a source and sink for materials (Heip *et al*., 2011). High heterogeneity can also be found in spatial distribution patterns of many species that use estuaries as nursery grounds. Differences in spatial patterns can be geographic, periodic, ontogenetic, and cohort-specific (Able 2005; Gillanders *et al*. 2011). Moreover, dispersal abilities in early life stages, habitat quality and habitat quantity can also influence spatial patterns and vary among estuaries, life stages and species (Gillanders *et al*. 2011; Vasconcelos *et al*. 2011).

The high spatial heterogeneity and variability that can be found in estuaries means that management strategies require the incorporation of dynamic ecological features into a spatial framework (Pittman *et al*. 2011). Furthermore, in order to achieve an effective outcome, temporal fluctuations should be considered in conservation planning and management strategies. Unfortunately, dynamic features are usually ignored, and spatial prioritization problems are frequently developed as static issues (Moilanen *et al*. 2009). In these cases, it is assumed that the features in a planning unit stay the same over time, ignoring long-term studies (Moilanen *et al*. 2009). To solve this problem, we can set probabilistic goals or incorporate feature dynamics within dynamic state equations (Possingham *et al*. 2009). Another option for incorporating feature dynamics in spatial prioritization problems is to consider the distribution pattern of a specific feature in different months or seasons as distinct input data (Costa *et al*. (*in review*)). For example, Costa et al. (in review) used data on the monthly spatial distribution of six fish eggs and larvae species over three years as features in a conservation prioritization analysis. This was the first study to consider early life stages of fishes along with bathymetry, habitat and sediment types as surrogates for estuarine biodiversity. Generally, habitat and distribution of benthic macroinvertebrate organisms are used as surrogates for estuarine biodiversity (Shokri *et al*. 2009; Shokri & Gladstone 2009; Shokri & Gladstone 2013a). Habitat classification schemes alone were found to be insufficient for the effective design of an estuarine protected reserve (Shokri & Gladstone 2013a).

For many species inhabiting estuaries, habitat requirements can change during development, which results in different spatial distributions at each ontogenetic stage (Harden 1968; Boltovskoy 1999; Costa *et al*. 2014). For this reason, in addition to spatial and temporal variations, changes in habitat occupation associated with different life stages must also be included in the planning process. Mobility and variability of different life stages can have a great influence on spatial priorities (Costa *et al*. *in review*), on the functioning of protected areas for mobile species (Grüss *et al*. 2011), and on the efficiency of protected areas in reaching appropriate conservation objectives (Grüss *et al*. 2011; Le Pape *et al*. 2014).

With these factors in mind, we suggest that early life stages of species which use estuaries as nursery grounds, such as fish eggs and larvae (Costa *et al. in review*), be included in spatial conservation prioritization.

Case study: Patos Lagoon estuary

Patos Lagoon (32°) is a choked warm temperate coastal lagoon located in southern Brazil (Kjerfve, 1986). There is a significant relationship between the total precipitation in the river basin and the annual freshwater discharge (Odebrecht *et al*. 2010). Freshwater discharge varies during seasons, mainly in El Niño years (Garcia *et al*. 1998). In general, El Niño years are associated with high rainfall and low salinity, while El Niña years are associated with low rainfall and high salinity in the estuary (Odebrecht *et al*. 2010). Water exchange with the Atlantic Ocean is controlled mainly by wind and freshwater discharge. The freshwater discharge occurs via a channel 0.5 – 3.0 Km width, which results in a variable geographic limit for the estuarine region (Odebrecht et al. 2010; Seeliger 2001; Garcia 1998). Wind is the primary force acting in the Patos Lagoon estuary, and NE-SW winds are the most common. These winds control estuarine circulation, salinity distribution, and water levels (Garcia 1998).

Patos Lagoon estuary covers an area approximately 1,000 km^2 (approximately 10% of the lagoon's total area) and is composed of different habitats, including the water column, bare sediment (300 km^2), submerged aquatic vegetation (up to 120 km^2), fringe salt marshes (40 km^2) and artificial hard substrates (Seeliger 2001). These different habitats act as nursery ground for many species, contributing to their survival and development. In addition to its ecological importance, Patos Lagoon estuary is also relevant to many economic and social interests and faces many anthropogenic pressures. Untreated domestic effluents, rice and cattle farming, industrial pollution, mining, dredging, port activities, shipping, and predatory fishing are among the most common pressures in Patos Lagoon.

Micropogonias furnieri (Desmarest, 1830) is a Sciaenidae that inhabits marine and brackish waters and is one of the main targets of fishery activity on the Brazilian coast. The species is present in the Patos Lagoon estuary year round, from eggs to adults larger than 250 mm; and this system is considered one of the species' primary nursery sites in southern Brazil (Vieira 2006). *M. furnieri* exhibits a well-defined spatial pattern of habitat occupation at Patos Lagoon estuary, where each developmental stage is correlated with an estuarine habitat and with different environmental variables influencing their presence (Costa *et al*. 2014). Spawning occurs on the continental shelf, and then eggs and larvae are transported into the Patos Lagoon estuary where they primarily occupy deep waters. Early juveniles (< 30 mm) are also associated with deep waters, though a low abundance reaches shallow waters. Medium size individuals (30– 160 mm) are associated with both estuarine habitats (shallow and deep waters), and larger individuals (> 160 mm) are associated with deep waters, indicating their migration to continental shelf to spawn (Fig. 1) (Castello 1986; Vieira 2006; Costa *et al*. 2014).

This ontogenetic habitat change can also be found in other species that depend on estuaries to complete their development. In the case of Patos Lagoon estuary, another example is the pink shrimp *Farfantepenaeus paulensis*. Spawning occurs in the coastal zone, and larvae are transported into the estuary, which acts as a nursery ground for the larvae (Möller *et al*. 2009). Different size classes are also related to distinct areas in Patos Lagoon estuary (D'Incao 1991). Understanding this complex pattern of habitat occupancy for various estuarine species—here exemplified by the life cycles of *M. furnieri* and *F. paulensis*, where each developmental stage is associated with a specific habitat—is extremely important and should be included in conservation planning. In addition to the variations in habitat occupancy, both species also show variations in spatial and temporal distribution related to environmental variables (Castello 1986; D'Incao 1991; Vieira 2006; Möller *et al*. 2009; Costa *et al*. 2014).

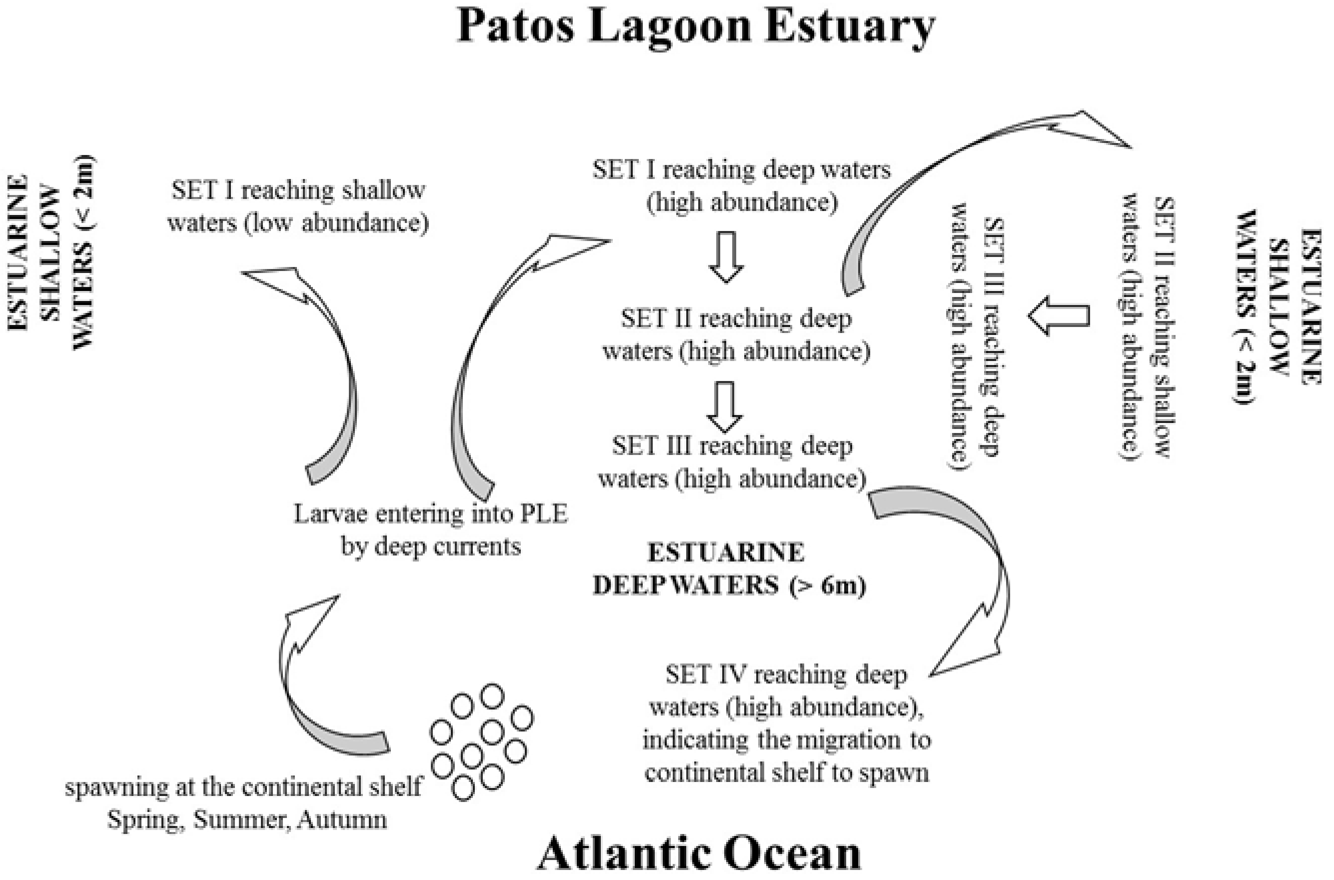

Fig. 1: Schematic diagram of the habitat occupancy pattern by different life stages of *Micropogonias furnieri* in Patos Lagoon estuary (PLE, Brazil) (SET: Size Ecological Taxa, SET I: individuals smaller than 30 mm; SET II: individuals between 30 – 90mm; SET III: individuals between 90 – 160 mm; and SET IV: individuals larger than 160 mm /Further information: Costa *et al*., 2014).

In an attempt to improve conservation and management actions in the Patos Lagoon estuary, two studies were developed to better understand the impact of adding early life stage analyses to estuarine spatial conservation planning (Costa et al., *in review*) and to assess the effect of adding more zones to a conservation plan designed to protect the essential fish habitat of *M. furnieri* (Costa *et al. unpublished*). Results showed that incorporating early life stages of fishes into systematic conservation planning can significantly change the spatial priorities for conservation (Costa *et al. in review*, Fig. 2). Additionally, adding more zones to a conservation plan to protect essential fish habitat does not alter spatial priorities (Costa *et al. unpublished*). In this case, zones can be established further within a reserve system. Considering the Patos Lagoon estuary, both studies showed that protecting shallow embayments was particularly important in the creation of an efficient conservation plan.

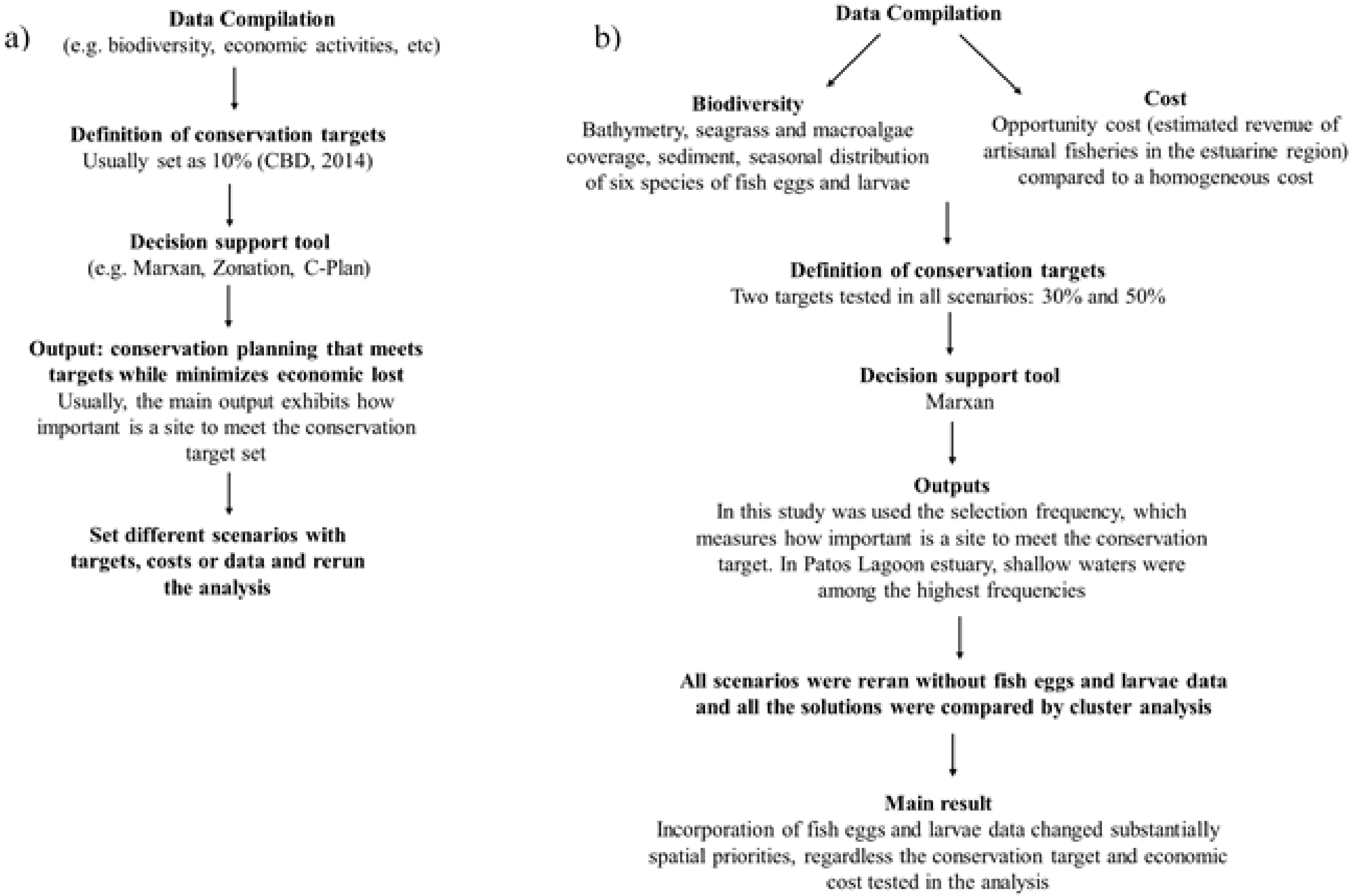

Fig. 2: Flowchart showing the steps followed in usual spatial conservation planning (a) and in the study case at Patos Lagoon estuary (Brazil) to assess the importance of incorporate early life stages of fishes into estuarine spatial planning (b). Further information in Costa *et al*. (*in review*).

Perspectives

In the last decades, the depletion of estuarine and coastal resources, and the alteration of estuarine structures, functions, and ecological processes by human activities have become more apparent. As a result, the demand for spatial data representing ecosystem attributes, including ecological and social characteristics, to support assessment, management and planning has increased. These data are expected to lead to more comprehensive and cost-effective outcomes for conservation planning (Pittman *et al*. 2011). One of the main challenges in coastal conservation planning is to incorporate surrogates that reflect ecosystem dynamics and account for the functional characteristics of the environment. We showed that the incorporation of temporal and spatial data for different life stages (e.g., fish eggs and larvae) into estuarine conservation planning can substantially change spatial priorities for estuarine conservation and serves as a good example for the integration of dynamic processes into planning efforts (Costa et al, *in review*). For this reason, we recommend that different life stages, mainly for those species that occupy a different habitat during each stage of their lifecycle, be included in spatial planning efforts. Additionally, the inclusion of temporal variation in spatial distribution can be a good way to solve the issue of static spatial prioritization.

Long-term monitoring can also be a strategy to improve data availability at appropriate temporal and spatial scales in dynamic ecosystem such as estuaries. This information will allow researchers and planners to better understand the environmental drivers of change, to identify rare and special interest areas, to predict the distribution and effects of environmental changes on biodiversity, and to establish appropriate levels of protection that will increase the efficacy of decision making (Pittman *et al*. 2011; Collins & Childers 2014). From a fisheries point of view, planning should include interactions between fishing and essential fish habitats, both for sustaining marine fish populations and biodiversity conservation, but also to maintain fisheries in a sustainable way (Le Pape *et al*. 2014). Despite past advances, it is well known that estuarine spatial planning is its infancy when compared with terrestrial and marine ecosystems. We recognize that much needs to be performed to devise better methods for comprehensive and cost-effective spatial planning for dynamic ecosystems such as estuaries. Future studies are encouraged to test different surrogates to represent the high variability of these ecosystems and account for distinct habitat conditions and connectivity in spatial prioritization analyses.

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Capítulo 3: Influência da variabilidade estuarina nas ações de conservação

Apêndice 6 – Costa, M.D.P. & Muelbert, J.H. Long-term assessment of temporal variability in spatial patterns of early life stages of fishes to facilitate estuarine conservation actions

Long-term assessment of temporal variability in spatial patterns of early life stages of fishes to facilitate estuarine conservation actions

Costa, MDP & Muelbert, JHM

Highlights

- Ichthyoplankton exhibits high variability among months, years and stations.
- Different taxa inhabits distinct estuarine areas.
- Temperature and salinity influence occurrence and distribution of fish eggs and larvae taxa.
- Temporal variability in spatial patterns of ichthyoplankton distribution facilitate conservation.

Long-term assessment of temporal variability in spatial patterns of early life stages of fishes to facilitate estuarine conservation actions

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ABSTRACT

Estuaries are among the most productive coastal ecosystems, supporting a wide variety of marine fish and their early life stages. Due to its dynamic nature, ecological processes create a complex spatial pattern that influence species distribution and biological production. Besides that, species can have different habitat requirements during their development that result in diverse spatial distribution during their life. Variability in spatial and temporal scales in estuaries implies that management strategies must incorporate these dynamic features in a spatial framework that enables the achievement an efficient management plan. In our study, we incorporate 13-years of data on early life stages of fish (eggs and larvae) from Patos Lagoon estuary (PLE, Brazil) to assess if long-term monitoring can facilitate conservation actions in estuarine ecosystems. We also evaluate the variability in fish eggs and larvae abundance in relation to the Framework for Water Classification (FWC) applied to the study area as a management plan. Our sampling stations were set in two of the three zones proposed by the framework. The open question is, will this data exhibit temporal variability in spatial patterns that can be used to facilitate conservation actions? Results showed that fish eggs and larvae exhibit high variability among months, years and sampling stations in a long-term approach. Occurrence and spatial distribution were mainly associated with salinity and not with space. Also, we found a high variability on spatial distribution patterns of fish eggs and larvae, with distinct species inhabiting both zones. Long-term studies can be a helpful strategy to assist conservation decision making, since it makes possible to understand the variability on spatial distribution of species throughout time. In conclusion, for dynamic estuaries such as PLE, a management plan that combines a fixed strategy, such as the Framework for Water Classification, with long-term information obtained from the variability of the biological community is extremely useful to achieve the protection of the ecosystem and its biodiversity.

Keywords: estuarine conservation, fish eggs, fish larvae, long-term analysis, framework of water classification

1. INTRODUTION

Estuaries are among the most productive coastal environments and support a wide variety of species, specially fish and their early life stages. The main advantages for early life stages that depend on estuaries are related to decreased predation, high food availability for post-larvae and juveniles, and shelter for the eggs and larvae (Blaber, 2000). Another fact that should be emphasized is the importance of the time and place of spawning, since the maintenance of the population depends on the successful recruitment of juveniles to nursery areas and their entry in the adult population (Hinckley et al., 2001).

Due to its dynamic nature, ecological processes in estuaries can interact across continent-ocean interface, and create a complex pattern in biological, physical, chemical or socioeconomic components (Pittman et al., 2011). This complex spatial pattern can influence biological production and, consequently, species distribution. Also, many species that use estuarine ecosystems have different habitat requirements during their development resulting in a diverse spatial distribution for different ontogenetic stages (Costa et al., 2014). The identification of the main characteristics of habitats used by fish throughout their life cycle and environmental influence on the connectivity between habitats is vital to the study of population dynamics, management of fish stocks, definition of key habitats and the design of protected areas (Benaka, 1999). Thus, estuarine habitat conservation becomes extremely important for the maintenance of recruitment process and fish biodiversity and should include the dynamic nature of this environment.

The strategies used by fish eggs and larvae to ingress estuaries are often related to hydrodynamics patterns. These patterns can be altered depending on many abiotic or biotic factors, such as variations in precipitation and changes in salinity gradients, which may affect ichthyoplankton assemblages in most temperate estuaries (Marques et al., 2006; Primo et al., 2011). Distribution patterns of fish eggs and larvae are usually correlated with physical and chemical variables, being salinity and temperature the most relevant parameters influencing ichthyoplankton abundance and occurrence (Marques et al., 2006). Biotic factors, such as food availability, predation and competition can also influence mortality rates and recruitment process in estuaries (Costa et al., 2002). Usually, estuaries also exhibit an extreme temporal and spatial variation in many ecological processes (McLusky and Elliott, 2006). Furthermore, natural variations can interact with human activities, as urban development, overfishing and habitat degradation, and influence the structure of communities that depend on this ecosystem. The heterogeneity and variability in spatial and temporal scales in estuaries and its influence on living resources such as fishes, implies that management strategies must incorporate dynamic features in a spatial framework for conservation (Pittman et al. 2011).

In this sense, it is well known that to understand the ecosystems and the way they respond to anthropic activities requires the ability to describe, interpret and predict temporal and spatial scales of variability (Elliott, 2002). Patos Lagoon, in southern

Brazil, was chosen as a case study because of the existence of a long dataset. Unlike most estuaries in Brazil, Patos Lagoon estuary (PLE) is monitored since 1975 ensuring information in many aspects of its ecology. As in other estuarine systems, environmental variables can interact with human disturbances influencing the recruitment process, maintenance of population, and consequently, the equilibrium of the estuarine ecosystem. And for that, long-term and well-defined monitoring is a necessary strategy to obtain this information. In our study, we incorporate 13-years of continued data on early life stages of fish (eggs and larvae) to assess if long-term information can facilitate conservation actions in estuarine ecosystems. The open question is, can temporal variability in spatial patterns of early life stages of fish be used to facilitate conservation actions?

2. MATERIAL AND METHODS

2.1 Study Area

Patos Lagoon (32°S), the largest choked lagoon in the world (Kjerfve, 1986), is a warm temperate coastal lagoon with an estuarine ecosystem of approximately 1,000 km^2 (around 10% of the lagoon total area) on its southern limit (Seeliger, 2001). Most of the estuary is shallow (< 1.5m), but is also composed by intermediate (1.5 – 5.0 m) and deep (> 5 m) water channels. Estuarine habitats in Patos Lagoon comprehend bare sediment (300 km^2), submerged aquatic vegetation (up to 120 km^2), fringe salt marshes (40 km^2) and artificial hard substrates (Seeliger, 2001). All these habitats contribute to the life cycle of many species of invertebrates and fish to complete their development. Unlike many estuaries, where variability is determined by astronomic tides, PLE is located in a micro-tide region and variability is influenced by meteorological forcing.

Because of that, biological, chemical and physical alterations are determined mainly by winds and precipitation. Low frequency events, such as El Niño Southern Oscillation, also influence the structure of PLE and induce inter-annual fluctuations (Odebrecth et al., 2010). Besides its ecological importance, the estuarine region of Patos Lagoon is of great relevance for many social and economic aspects, being exposed to many anthropic pressures, including untreated domestic effluents, rice and cattle farming, industrial pollution, mining, dredging, port a

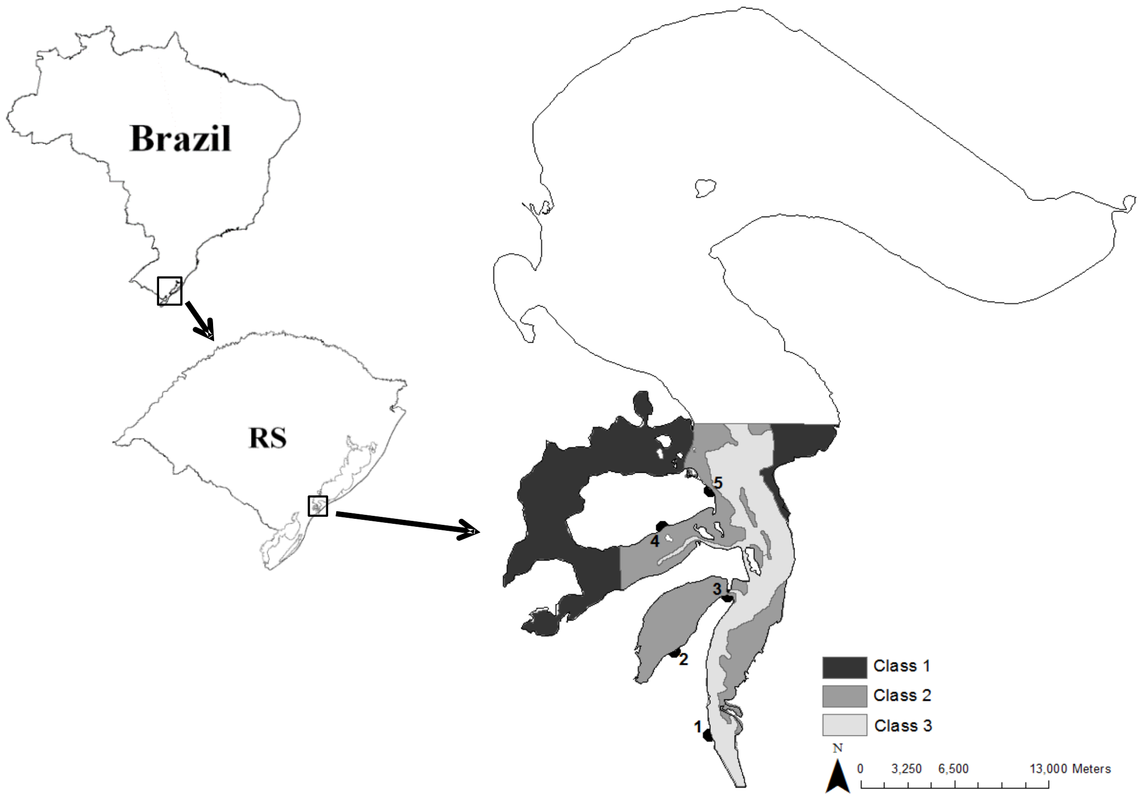


Figure 1: Location of Patos Lagoon estuary (PLE) in the southern Brazilian coast and the water classification based on "Framework of Water Classification" (CONAMA,

2005) and implemented in the Environmental Plan of Rio Grande City (Plano Ambiental de Rio Grande, 2007).

2.2 Ichthyoplankton and conservation analysis

Fish eggs and larvae are transported by deep currents into the estuarine channel of Patos Lagoon, and their retention and survival are dependent on the water exchange and prevailing winds (Muelbert and Weiss, 1991; Martins et al., 2007). In this study, the analysis of the ichthyoplankton assemblage is based on abundance data collected during a continuous 12-year interval (2000 - 2012) in PLE (Figure 1). Samples were taken monthly with a conical plankton net (300 µm, 60 cm mouth diameter) equipped with a flow-meter, towed for about two minutes at surface, at five sampling stations located in shallow sites at the estuary. All samples were preserved in a 4% formalin solution. Temperature (°C) and salinity were recorded for each sampling station during all study period. Fish eggs and larvae were sorted and identified under a binocular stereo-microscope following specialized literature. Eggs and larvae abundance were estimated as the number of individuals per $100m^3$.

Analysis of the distribution patterns of fish eggs and larvae assemblage was conducted by cluster analysis on standardized ichthyoplankton abundance to the number of individuals per 100 m^3 filtered water. A combination between the frequency of occurrence and relative abundance, by multiplying these values, was used to select the most important taxa (Macedo-Soares et al., 2014). Indicator species for each group were calculated as a product of the relative frequency and relative average abundance in each group (Dufrêne and Legendre, 1997). The data matrix was composed by the density of all identified *taxa* transformed by log (x +1), using Bray-Curtis similarity as the distance coefficient and clustering method by averaging their similarity values (UPGMA). Statistical procedures were done R 2.13.1 (R Development Core Team, 2014).

We used a generalized linear model (GLM) in order to analyze the variability on fish eggs and larvae abundance in relation to environmental variables. We defined as the response variable $y_{(i)}$ as the number of fish eggs or larvae in the *ith* plankton tow. At first, we assumed that our response variable $y_{(i)}$ to follow a Poisson distribution. However, as our data showed over-dispersion, we further assumed our response variable $y_{(i)}$ to follow a Negative Binomial distribution with mean μ_i and parameter k. Explanatory variables were temperature, salinity, volume of water filtered by the tow, sampling station, month and year. The last three variables were categorical. The structure of the model was as follow:

$y_{(i)} \sim \mathrm{NB}(\mu_i, k)$

$E(y_i) = \mu_i$ and $\mathrm{var}(y_i) = \mu_i + \mu_i^2 / k$

$\log(\mu_i) = \eta(X_{i1}, \ldots, X_{iq})$

Four alternative models are proposed:

Model 1: η = log (volume) + sampling station + month + year + temperature + salinity

Model 2: η = log (volume) + sampling station + month + year + temperature * salinity

Model 3: η = log (volume) + sampling station * month + year + temperature + salinity

Model 4: η = log (volume) + sampling station + month + temperature + salinity

Selection process to choose the best model for our data was based on Akaike Information Criterion (AIC). All GLM analysis was performed using R 2.15.1 (R

Development Core Team, 2014). Then, we analyzed the abundance variability of the most important taxa in relation to temperature and salinity.

We also analysed the variability of fish eggs and larvae abundance in relation to the “Framework for Water Classification” (FWC) in order to evaluate the contribution of long-term studies on ichthyoplankton to estuarine conservation. The FWC, established by the Brazilian National Council of the Environment (CONAMA, 2005), is the main tool to ensure water quality for multiple uses. This framework classifies marine, freshwater and brackish waters according to their usage as: Special Class, which aims the preservation of aquatic environments preservation and the maintenance of aquatic communities; Class 1, include waters that can be used for recreational activities, aquaculture, fishing, supply for human consumption after conventional or advanced treatment and irrigation, but also aim to protect aquatic communities; Class 2: include waters that can be used by fisheries and recreational activities; and Class 3, include waters allocated for navigation and landscaping (CONAMA, 2005). The classification is based on water quality parameters, such as the amount of organic carbon, dissolved oxygen, pH, and many others inorganic and organic compounds, being specific for each class. The framework has already been used within the environmental plan of Rio Grande City, and it is the only conservation action proposed to the PLE (Plano Ambiental de Rio Grande, 2007). The area of PLE includes waters classified as Class 1, 2 and 3 (Figure 1). However, our sampling stations were set only on Class 2 and 3.

3. RESULTS

The long-term series revealed that fish eggs and larvae exhibit high variability, which can be associated mainly with salinity fluctuations in the estuary. Here, we describe the intra-annual and inter-annual variability in the abundance of fish eggs and larvae, and in the ichthyoplankton assemblage. We also evaluate these results in relation to the zones classified according the Framework for Water Classification at PLE.

3.1 Variability in fish eggs and larvae abundance

Fish eggs and larvae abundance exhibited high variability among sampling stations within years and months (Figures 2 and 3). Among the sampling stations, station 2 was associated with lower abundance of fish eggs (Figure 2), with no recruitment of fish eggs at this site during a few years. Station 4 showed a similar pattern but with higher abundance of eggs (Figure 2). In general, stations 1, 3 and 5 exhibited high abundance of eggs during the study period, but with a clear pattern of variation within years (Figure 2). Fish larvae abundance showed a different pattern (Figure 2). In general, fish larvae were found during all years and stations, with a clear pattern of variation within years.

High variability was also observed when analysing fish eggs and larvae abundance within months (Figure 3). Eggs were more abundant during warmer months (November, December, January and February), and stations 1 and 3 showed recruitment into this sites during the whole year (Figures 3). However, at stations 2, 4 and 5 cold months tended to low eggs abundance (Figures 3). In general, fish larvae recruited to PLE during the whole year, being found at all sampling stations. Despite that, larvae abundance exhibited high variability within months at each sampling station (Figure 3).

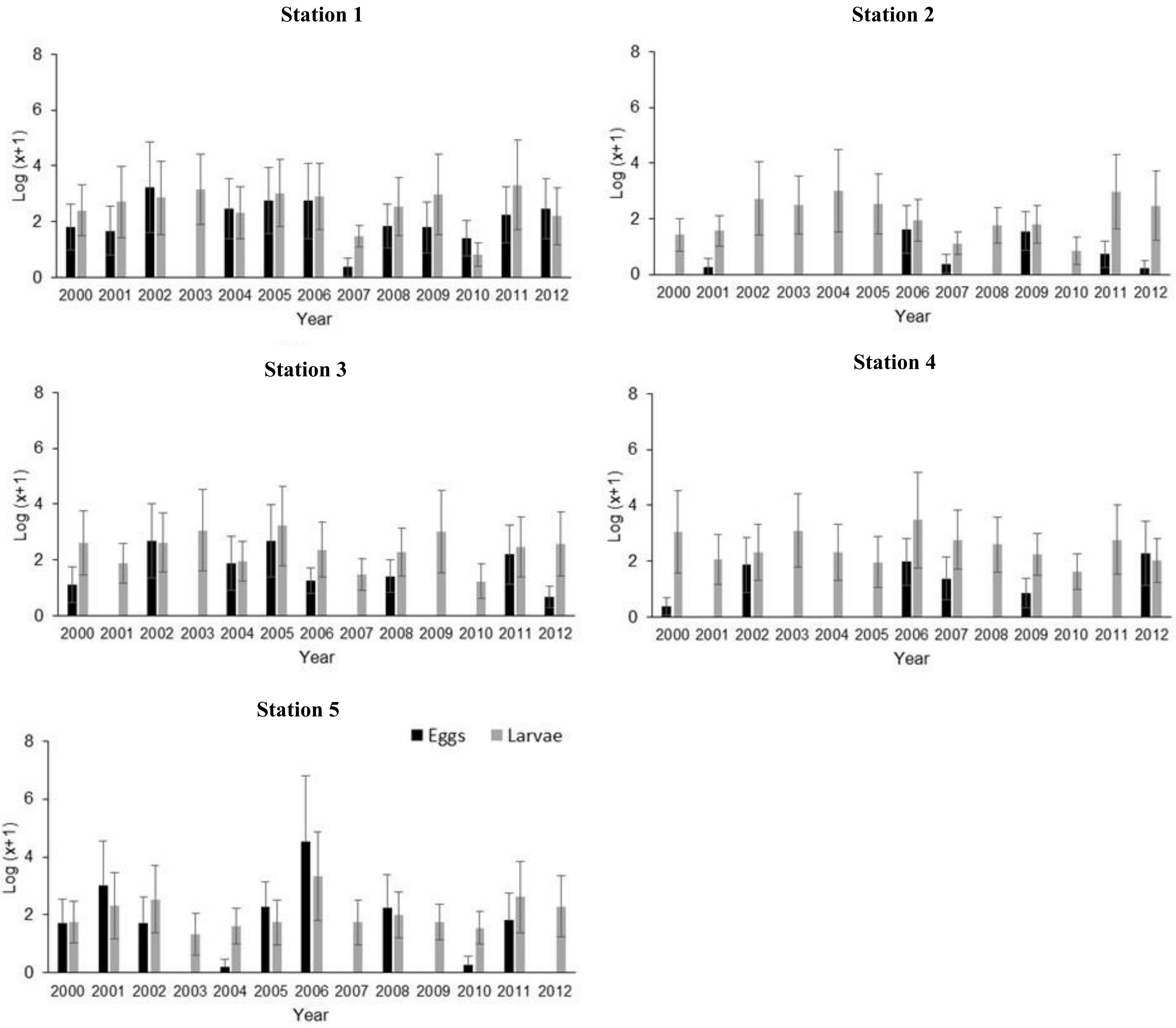
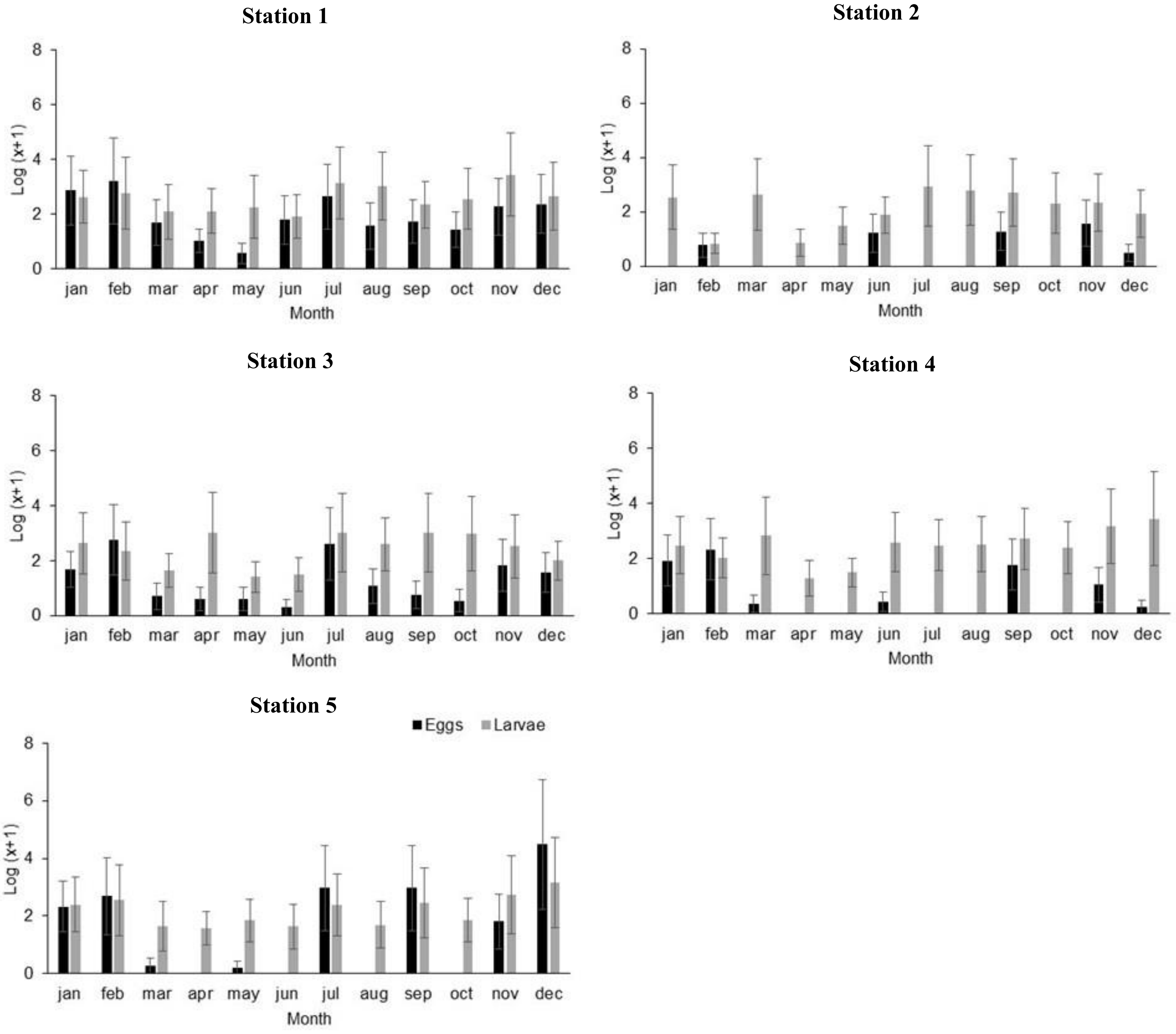


Figure 2: Inter-annual variability in the mean abundance of fish eggs (black bars) and larvae (grey bars) at each sampling station in the Patos Lagoon estuary (Brazil). Bars are ± standard error.



Station 1

Station 2

Station 3

Station 4

Station 5

Figure 3: Monthly variability in the mean abundance of fish eggs (black bars) and larvae (grey bars), from 2000 to 2012, at each sampling station, in the Patos Lagoon estuary (Brazil). Bars are ± standard error.

3.2 Generalized Linear Models

3.2.1 Model selection

Outliers were removed from the analysed data from the GLM modeling after preliminary residual analysis. The values of AIC for all models are summarized in Table I. Two different models were selected as the best model for fish eggs and larvae abundance. Best models based on AIC for fish eggs and larvae abundance were Model 4 and 1, respectively. All analyses that follow refer to these models.

Table I: Akaike information criteria (AIC) for models fitted to fish eggs and larvae abundance data at Patos Lagoon estuary, Brazil.

Model (Distribution for y)	Eggs	Larvae
Model 1	709.68	**1324.70**
Model 2	714.40	1326.60
Model 3	714.25	1341.60
Model 4	**702.84**	1353.70

3.2.2 Parameter estimates

The selected GLMs fitted to abundance data of fish eggs and larvae produced the parameter estimates listed in Table II (columns 2 and 5). The baseline estimate of the logarithm of the expected number of fish eggs $\eta = \log \mu$ at station 1 in November was given by the intercept. This value is adjusted by temperature, salinity, and the volume of water filtered by the tow. Increments for different stations (2 to 5), months (December, January, February and March) were added. In the case of the model selected for fish larvae abundance, the intercept represents the expected number of fish larvae $\eta = \log \mu$ at station 1 in November, 2000. Different months (December, January, February, and

March) and years (2001 to 2012) were also added to the model, and the value was adjusted by temperature, salinity and the volume of water filtered by the tow.

For fish eggs abundance, the months of November, December, and February, and the sampling stations 1, 2 and 4 had statistically significant differences in η. Also, temperature and salinity induced statistically different increments in η. For fish larvae, the monthly effects were different with significant increments in η in November, February and March. Sampling stations 1 to 5, and years 2000, 2002, 2004, 2005, 2007, 2008 and 2009 also exhibited statistically significant increments in η. However, in this case, only salinity induced statistically significant differences in η. For both early life stages the effect of the volume of water filtered by the tow was not significant.

Table II: Estimates, standard errors, and p-values of coefficients for selected GLMs fitted to the number of fish eggs and larvae at Patos Lagoon estuary, Brazil (N/A: not applicable, significant p-values (< 0.05) in bold).

	Fish eggs			Fish larvae		
Parameter	Estimate	Std. Error	P-value	Estimate	Std. Error	P-value
Intercept	7.01	1.89	**0.00**	4.34	1.03	**2.58e-0**
Log(volume)	-0.20	0.15	0.17	0.05	0.07	0.47
Sampling station 2	-2.57	0.77	**0.00**	-1.85	0.32	**1.73e-0**
Sampling station 3	0.63	0.57	0.27	-0.98	0.29	**0.00**
Sampling station 4	-1.51	0.60	**0.01**	-0.94	0.28	**0.00**
Sampling station 5	0.90	0.58	0.12	-0.92	0.30	**0.00**
Month December	1.85	0.68	**0.00**	0.11	0.30	0.71
Month January	1.41	0.73	**0.05**	-0.63	0.34	0.06
Month February	2.76	0.71	**0.00**	-1.18	0.35	**0.00**
Month March	-0.38	0.81	0.63	-1.90	0.38	**7.38e-0**
Year 2001	N/A	N/A	N/A	-0.11	0.48	0.80
Year 2002	N/A	N/A	N/A	1.63	0.47	**0.00**
Year 2003	N/A	N/A	N/A	0.41	0.49	0.40
Year 2004	N/A	N/A	N/A	-1.12	0.50	**0.02**
Year 2005	N/A	N/A	N/A	-1.14	0.49	**0.02**
Year 2006	N/A	N/A	N/A	-0.03	0.48	0.94
Year 2007	N/A	N/A	N/A	-0.98	0.49	**0.04**
Year 2008	N/A	N/A	N/A	-1.04	0.50	**0.03**
Year 2009	N/A	N/A	N/A	-0.99	0.50	**0.04**
Year 2010	N/A	N/A	N/A	-0.79	0.49	0.10
Year 2011	N/A	N/A	N/A	-0.41	0.52	0.43
Year 2012	N/A	N/A	N/A	-0.37	0.50	0.46
Temperature	-0.39	0.08	**5.70e-0**	-0.05	0.04	0.19
Salinity	0.15	0.01	**3.66e-1**	0.02	0.01	**0.04**

3.2.3 Residual analysis

Residual analysis was used to examine the fit of the selected models for fish eggs and larvae abundance. Residuals results of abundance showed that they were within an acceptable range (-3 and 3) for selected models (Figure 4A and C). Also, the quantile-quantile plots (Figure 4B and D) indicated that distribution of residuals were acceptable, since the scatterplots were almost linear. In general, residuals were mostly negative. This is normal, considering the fact that for small values of y residuals are usually negative, and also for y=0 negative residuals are the only possibility.

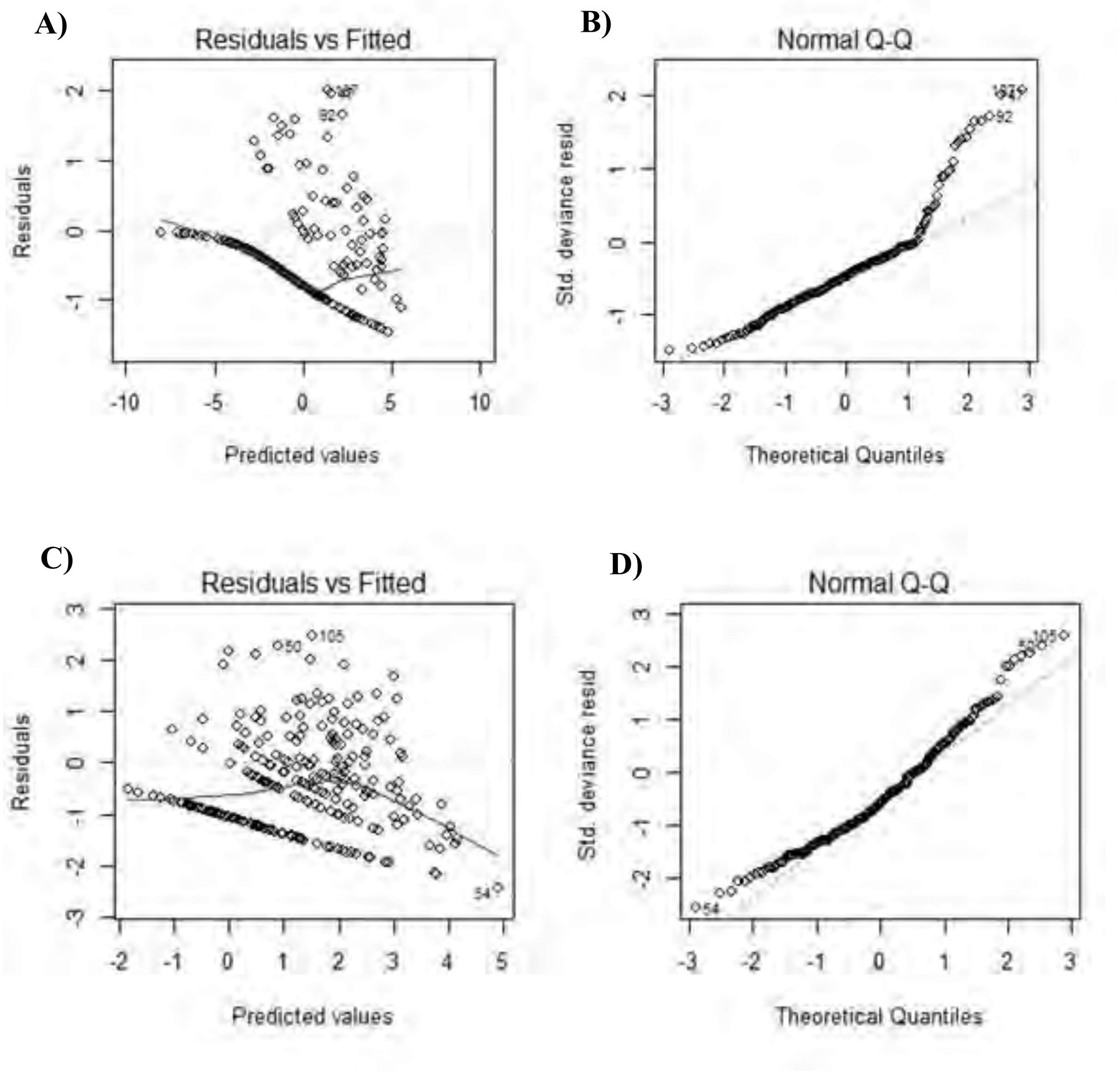


Figure 4: Residuals plots for selected models of fish eggs (A and B) and larvae (C and D) abundance at Patos Lagoon estuary with binomial negative distribution.

3.3 Fish eggs and larvae assemblage

Eggs and larvae identification resulted in 11 and 38 taxa, respectively, for the entire study period (Table III). Identification occurred at species level for 5 and 16 of these taxa for eggs and larvae, respectively. Fish eggs from shallow waters in PLE were also identified at Genus (1), Family (4) and Order (1) level. In the case of fish larvae, identification also resulted in Genus (10), Family (9) and Order (3) level. Ichthyoplankton assemblage was composed by taxa from different guilds: marine species (e.g. *Anchoa marinii* and *Trichiurus lepturus*), estuarine dependent species (e.g. *Micropogonias furnieri*), estuarine residents (e.g. *Achirus garmani*) and freshwater species (e.g. Caracidae and *Poecilia vivipara*). The most important taxa were *Achirus garmani* (eggs), Atherinidae (larvae), *Brevoortia pectinata* (eggs and larvae), Clupeidae (e), Gobiidae (larvae), *Jenynsia* spp. (larvae), *Lycengraulis grossidens* (eggs and larvae), *Menticirrhus americanus* (larvae), *Micropogonias furnieri* (larvae), Mugilidae (larvae), *Odonthestes* spp. (larvae), Sciaenidae (eggs and larvae), Pleuronectiformes (eggs), *Trichiurus lepturus* (eggs). Only 6.2% and 0.4% of eggs and larvae remained unidentified (Table III).

Table III: Abundance of fish eggs and larvae identified at Patos Lagoon estuary during 2000 – 2012. (*) indicates the most abundant taxa that were used to analyse the variability in their abundance in relation to the mean temperature and salinity within year and month at each sampling station.

Taxa	Zone 2	 3	Total Abundance (n°/100m^3)
Eggs			
Engraulidae		x	754.90
Anchoa marinii		x	15.95
Lycengraulis grossidens	x	x	3,320.21
Clupeidae	x	x	8,559.07
*Brevoortia pectinata**	x	x	52,952.77
Atherinidae	x		8.92
Sciaenidae*	x	x	388,817.91
Trichiurus lepturus	x	x	144.07
Pleuronectiformes	x	x	1,618.89
Paralichthys sp.		x	13.87
Achirus garmani	x	x	2,021.66
NI	x	x	38,947.70
Larvae			
Clupeiformes	x		53.19
Engraulidae	x		74.28
Anchoa marinii		x	19.60
Engraulis anchoita	x		29.03
Lycengraulis grossidens	x	x	18,966.33
Clupeidae	x	x	1,264.65
Brevoortia pectinata	x	x	58,564.92
Ramnogaster arcuata	x		29.21
Characiformes	x		52.26
Characidae	x		50.58
Parapimelodus nigribarbis	x	x	1,071.01
Mugilidae	x	x	3,700.95
Atherinidae*	x	x	200,739.54
Odontesthes sp.	x	x	6,533.15
Hemiranphidae	x		1,567.86
Hyporhamphus kronei	x	x	1,237.06
Jenynsia sp.	x		2,013.15
Poecilia vivipara		x	21.48
Syngnatus folletti	x	x	1,165.98
Sciaenidae*	x	x	21,318.22
Cynoscion sp.	x	x	339.96
Macrodon atricauda	x	x	3,567.27
Menticirrhus sp.	x	x	564.24
Menticirrhus americanus	x	x	1,822.89
Micropogonias furnieri	x	x	4,280.77
Paralonchurus sp.		x	87.10
Paralonchurus brasiliensis	x	x	1,340.54
Blennidae	x	x	484.15
Blennius sp.		x	14.25
Gobiesox strumosus		x	270.01

Gobiidae	x	x	17,986.55
Gobionellus sp.	x		193.11
Trichiurus lepturus	x	x	1,031.49
Peprilus paru		x	61.09
Pleuronectiformes	x	x	494.85
Paralichthys sp.		x	5.01
Achirus garmani	x	x	258.52
Catathyridium jeninsy	x		9.92
NI	x	x	960.04

3.3.1 Abundance variation in relation to temperature and salinity

Considering the two most abundant taxa for eggs (*Brevoortia pectinata* and Sciaenidae) and larvae (Atherinidae and Sciaenidae), we analysed the variation in their abundance in relation of mean temperature and salinity within year (Figures 5 and 7) and month (Figures 6 and 8) at each sampling station. In general, *B. pectinata* and Sciaenidae eggs occurred throughout the study period, but showed high variability with years and months at different stations (Figures 5 and 6). Eggs of both taxa were more abundant at sampling station 1 and 5, and clearly associated with peaks of high salinity in the estuary. Years and months with low salinity were related to low abundance of the species or to no recruitment of eggs into sampling stations (Figures 5 and 6). Abundance of their eggs was also influenced by high temperature when considering the monthly variability (Figure 6), as high abundance was associated with warmer months.

In the case of larvae, Atherinidae occurred throughout the estuary and during the whole study period, meanwhile Sciaenidae larvae did not occur at sampling station 2 being mainly associated with station 1 (Figures 7 and 8). A clear pattern of variability for Atherinidae larvae abundance was not found, as high abundances were registered on different situations of high or low salinity and temperature (Figures 7 and 8). However, for Sciaenidae larvae, it was possible to establish a pattern associated with high salinity and warmer months (Figures 7 and 8).

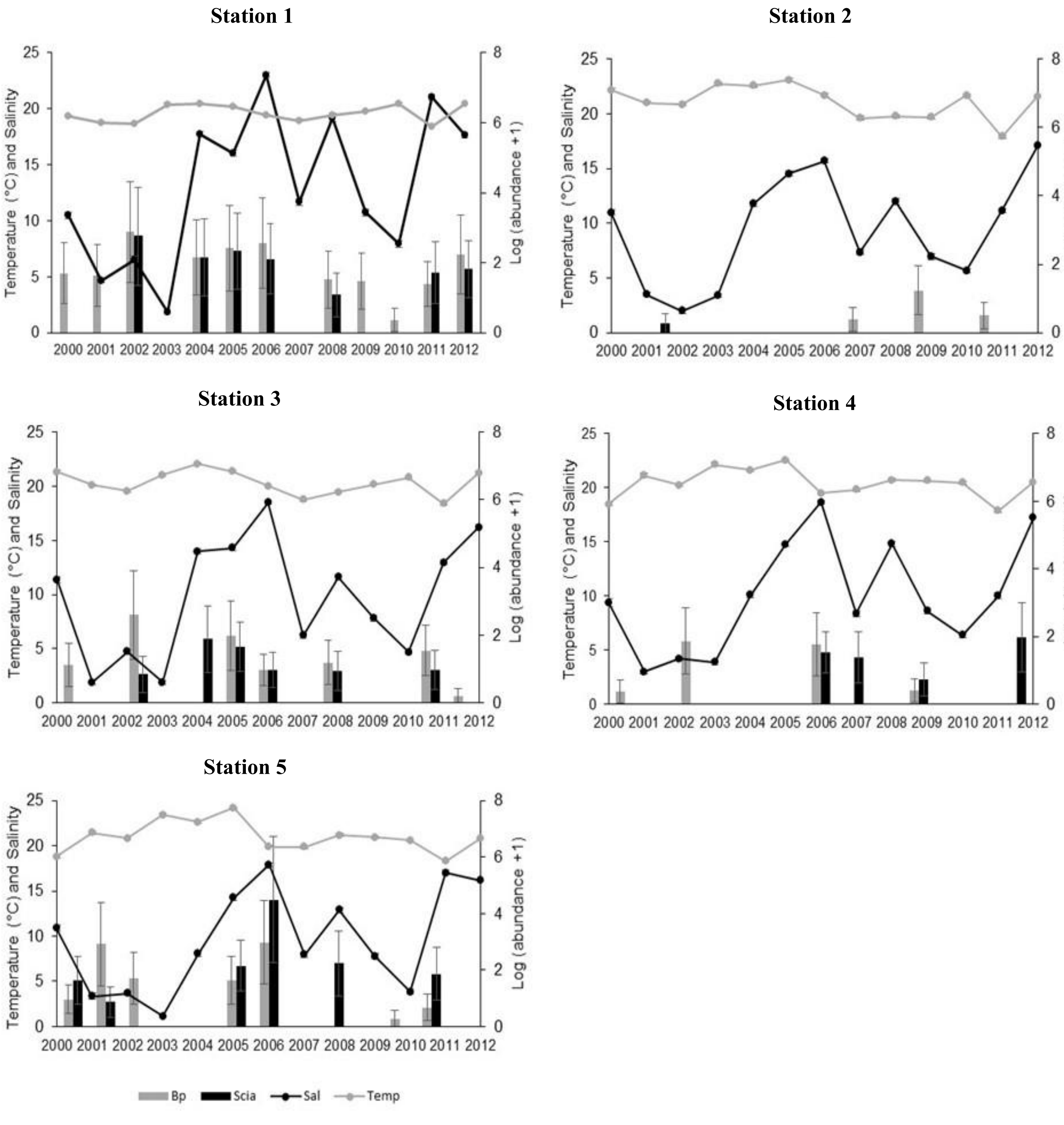
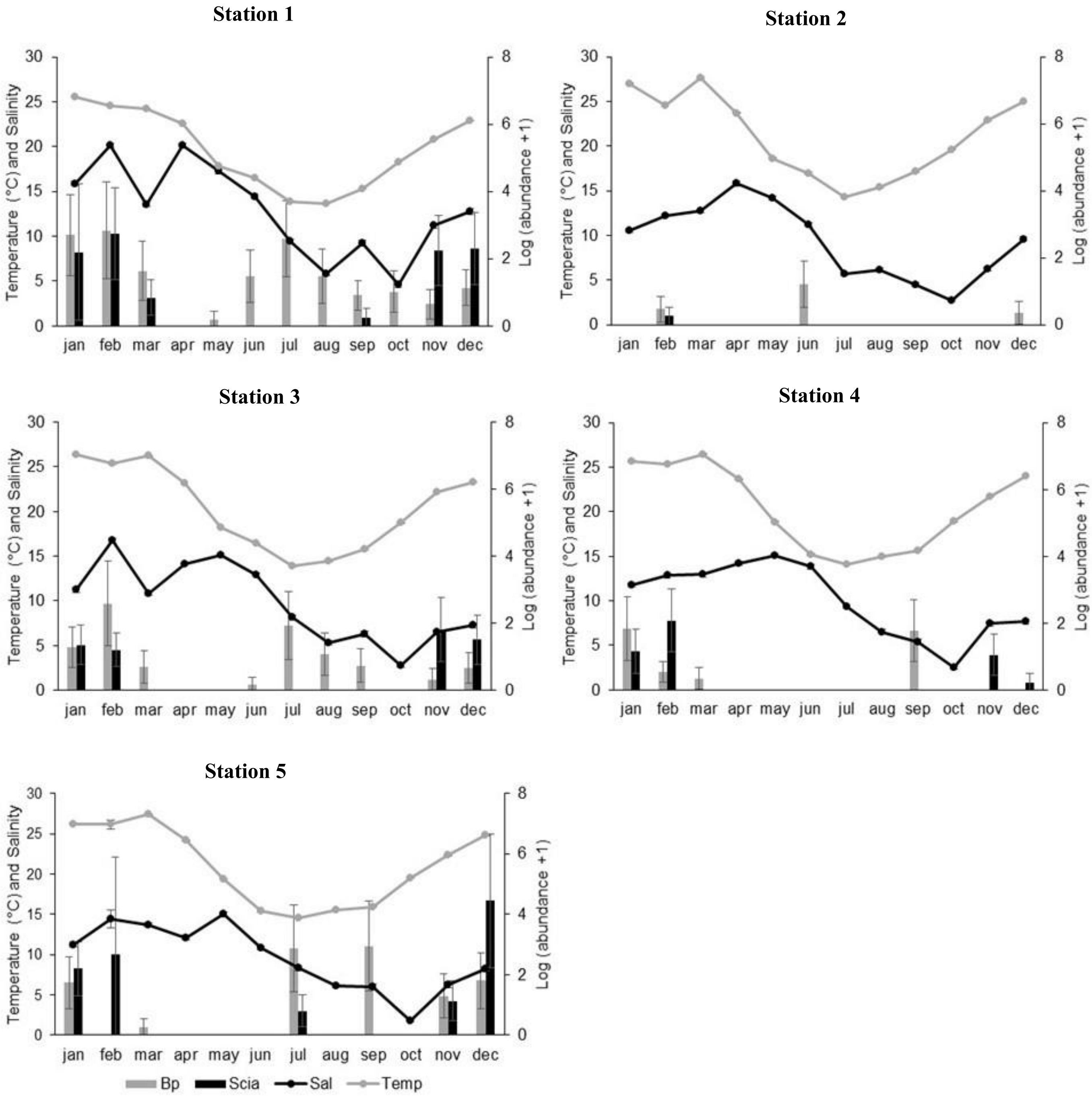


Figure 5: Inter-annual variability in the mean abundance of *Brevoortia pectinata* (Bp) and Sciaenidae (Scia) eggs from 2000 to 2012, for each sampling station, in relation to temperature and salinity at Patos Lagoon estuary (Brazil). Bars are ± standard error.

 Station 1

Station 2

Station 3

Station 4

Station 5

Figure 6: Monthly variability in the mean abundance of *Brevoortia pectinata* (Bp) and Sciaenidae (Scia) eggs from 2000 to 2012, at each sampling station in relation to temperature and salinity at Patos Lagoon estuary (Brazil). Bars are ± standard error.

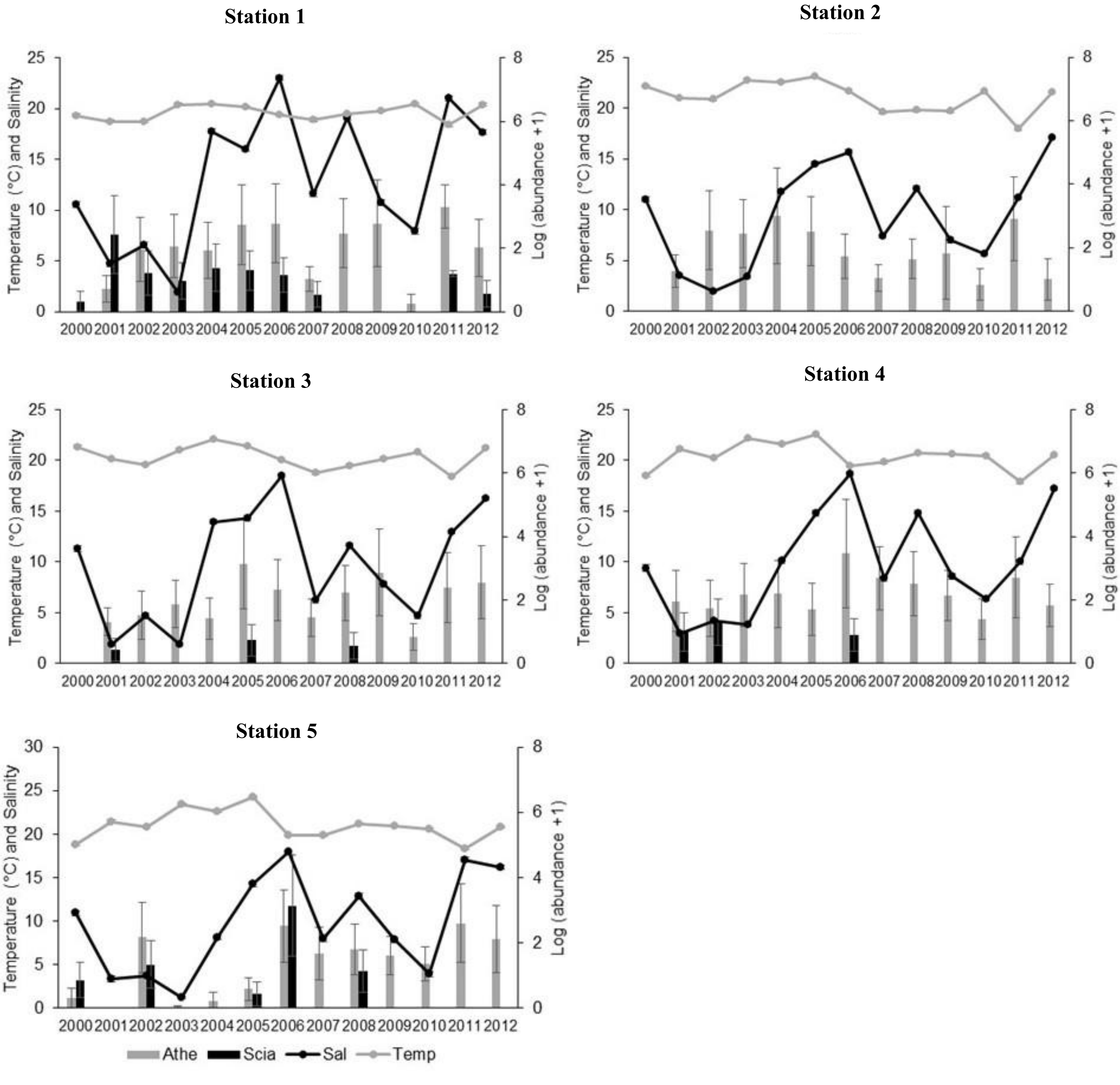


Figure 7: Inter-annual variability in the mean abundance of Atherinidae (Athe) and Sciaenidae (Scia) larvae from 2000 to 2012, at each sampling station in relation to temperature and salinity at Patos Lagoon estuary (Brazil). Bars are ± standard error.

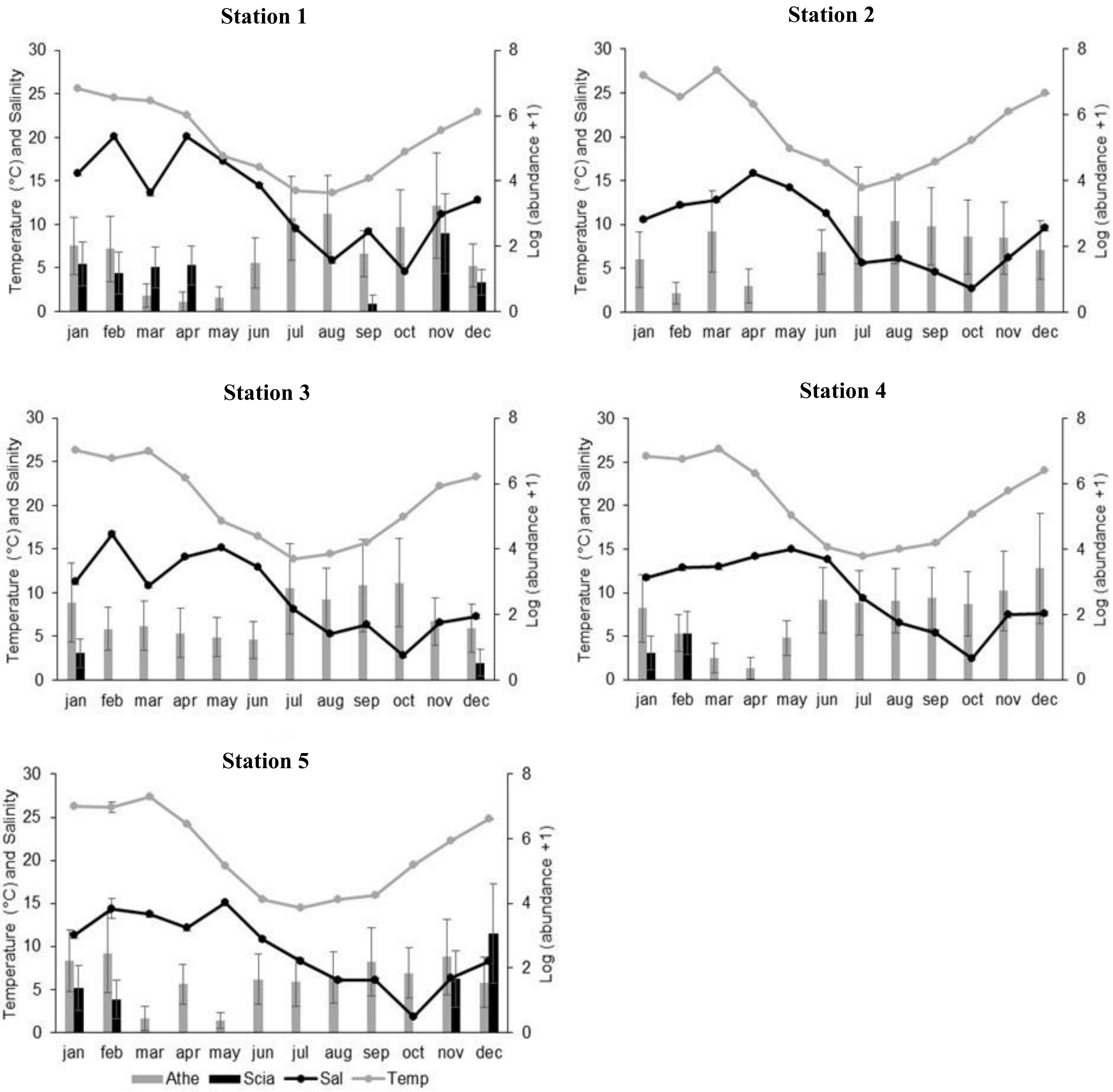


Figure 8: Monthly variability in the mean abundance of Atherinidae (Athe) and Sciaenidae (Scia) larvae from 2000 to 2012, at each sampling station in relation to temperature and salinity at Patos Lagoon estuary (Brazil). Bars are ± standard error.

3.4 Abundance variation of ichthyoplankton assemblage in relation to FWC

Cluster analysis considering fish eggs and larvae collected at shallow waters in PLE revealed a consistent spatial pattern when considering the whole study period (Figure 9). In this analysis, two groups were identified for both fish eggs (Figure 9A) and larvae (Figure 9B). Group I consisted of sampling station 2 in both cases, however, indicated by different taxa: *A. garmani* and *T. lepturus* in the case of fish eggs (Figure 9A) and by *L. grossidens*, *M. americanus* and *M. furnieri* in the case of fish larvae (Figure 9B). The second group was represented by other stations (1, 3, 4 and 5) with *B. pectinata* and Sciaenidae as the indicator taxa for fish eggs (Figure 9A) and Mugilidae, Gobiidae and *B. pectinata* for fish larvae (Figure 9B). Despite the high values found for each indicator taxa none of them were significant ($p < 0.05$).

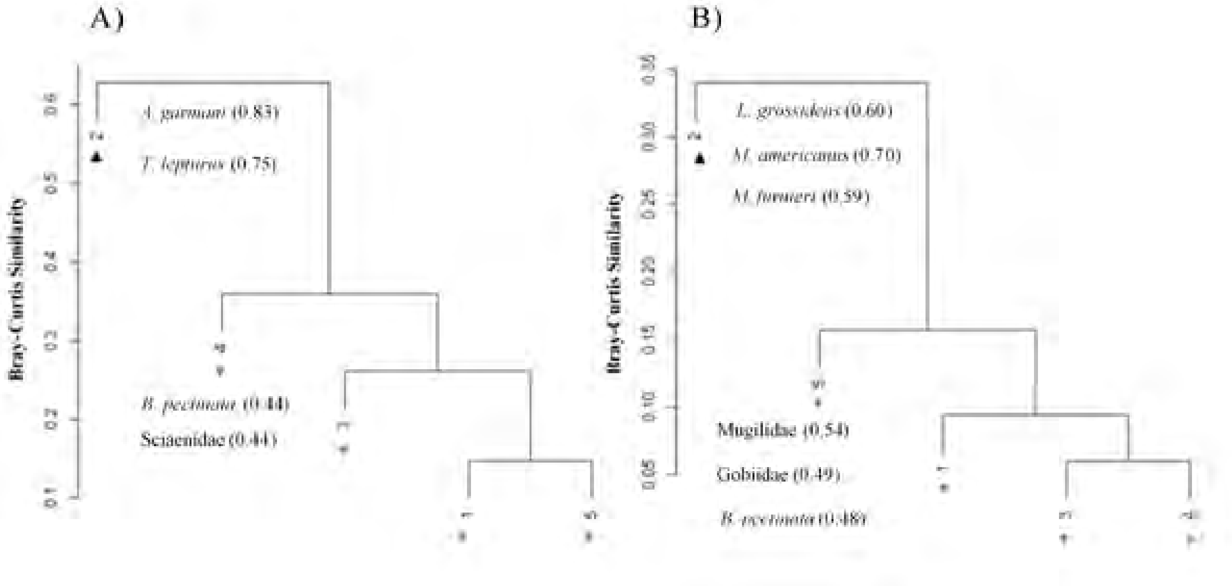


Figure 9: Spatial cluster analysis using Bray-Curtis similarity and the method of UPGMA over the transformed matrix of fish eggs (A) and larvae (B) total density with the respective indicator taxa (▲ : group, ▼ : group II).

However, when analysing the spatial pattern separately for each year, we observed that fish eggs assemblage shows a well-defined distribution pattern. In general, station 1 is separated from other stations and indicated mostly by Clupeidae and *B. pectinata* (Figures 10). Two exceptions of this pattern occurred on 2006 and 2011 (Figure 10). In 2006, station 2 was separated from other stations, whereas, in 2011, we observed one group composed by stations 1, 3 and 5, and the second group by stations 2 and 4 (Figure 10). Despite of the well-defined spatial pattern found for fish eggs, we observed that indicator species for each group had a high variability during years (Figures 10). In contrast, fish larvae assemblage did not exhibited a well-defined spatial pattern (Figures 11). In general, we observed two main groups composed by distinct stations and indicator species throughout time (Figures 11). One exception of this spatial pattern occurred on 2002 (Figure 11) when we found three groups: group I composed by station 2, group II by stations 4 and 5, and group III by stations 1 and 3.

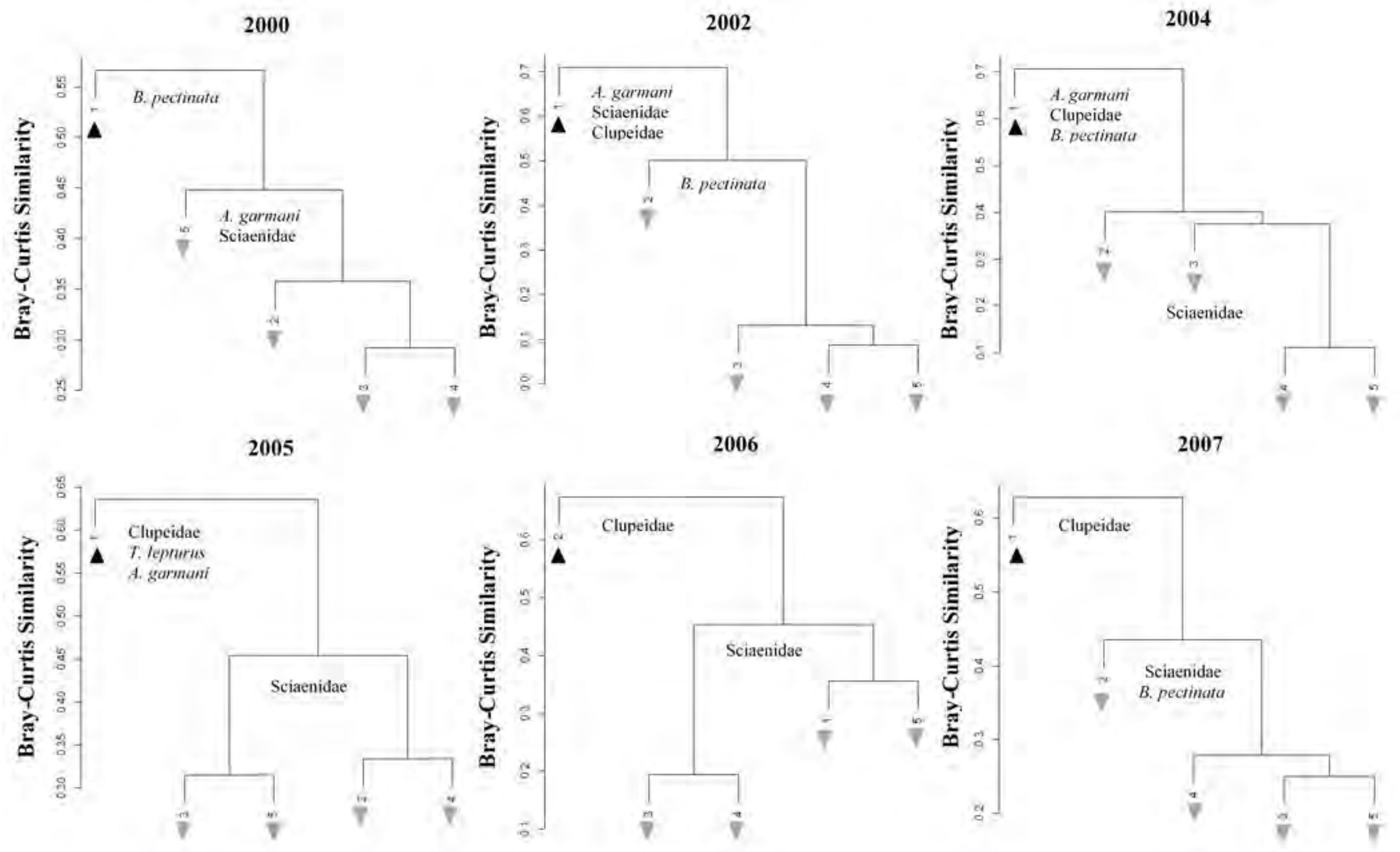
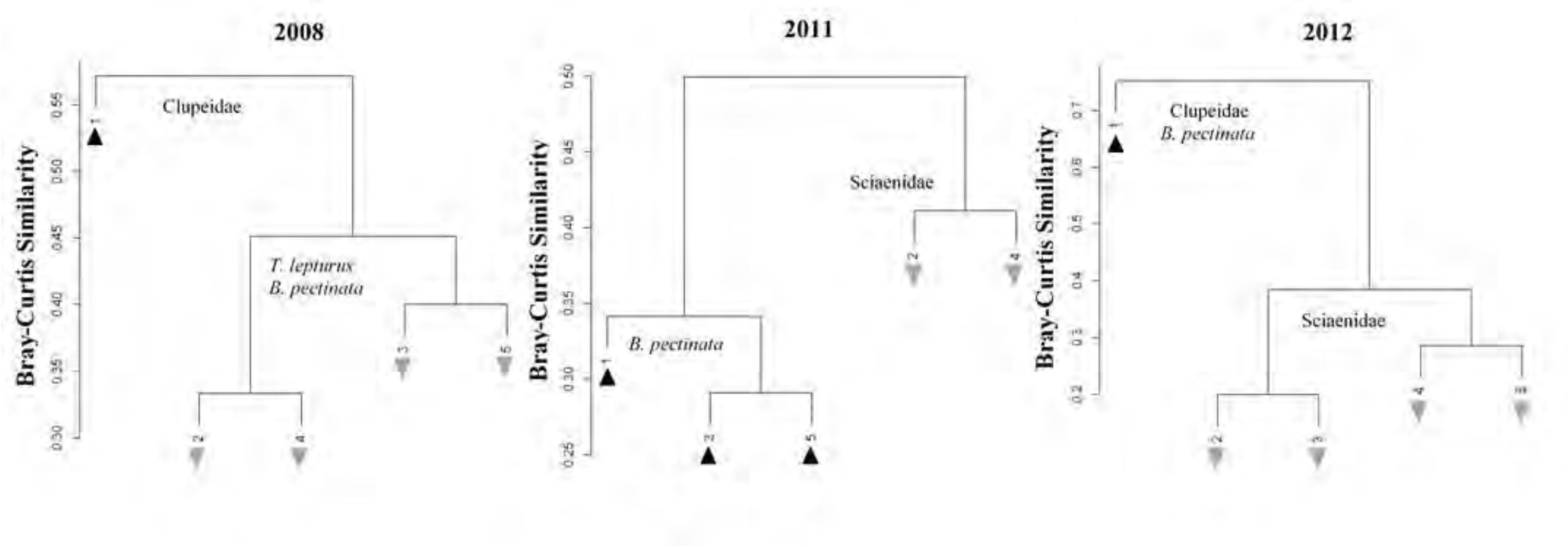


Figure 10: Spatial cluster analysis for each year during the study period, using Bray-Curtis similarity and the method of UPGMA over the transformed matrix of fish eggs density with the respective indicator taxa (▲ : group, ▼ : group II) (fish eggs were restricted to one or two

sampling stations on 2001, 2003, 2009, 2010. In this sense, these years were excluded from this spatial analysis). Taxa named in the figure represents indicator species with highest values.

Continuation of Figure 10



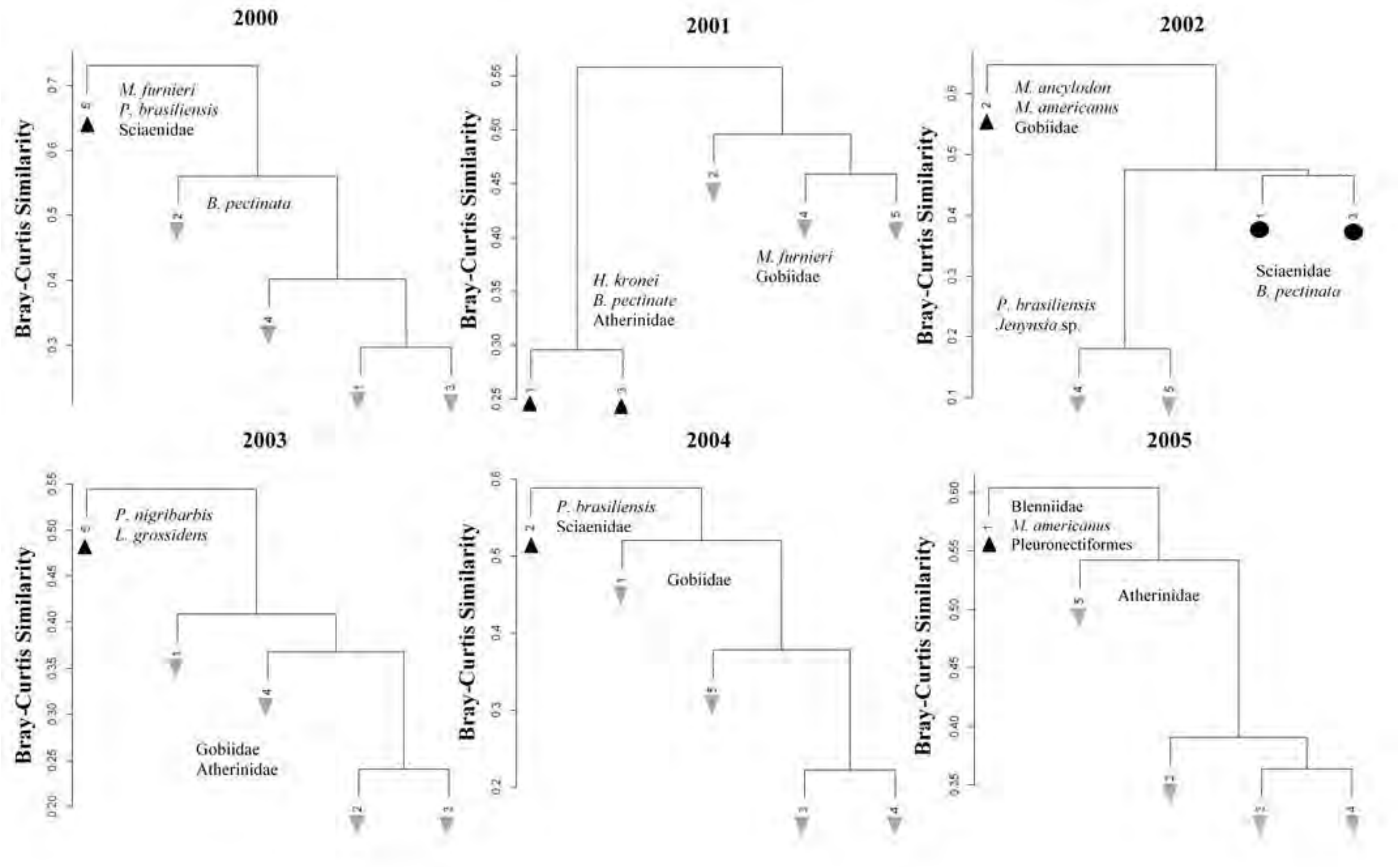
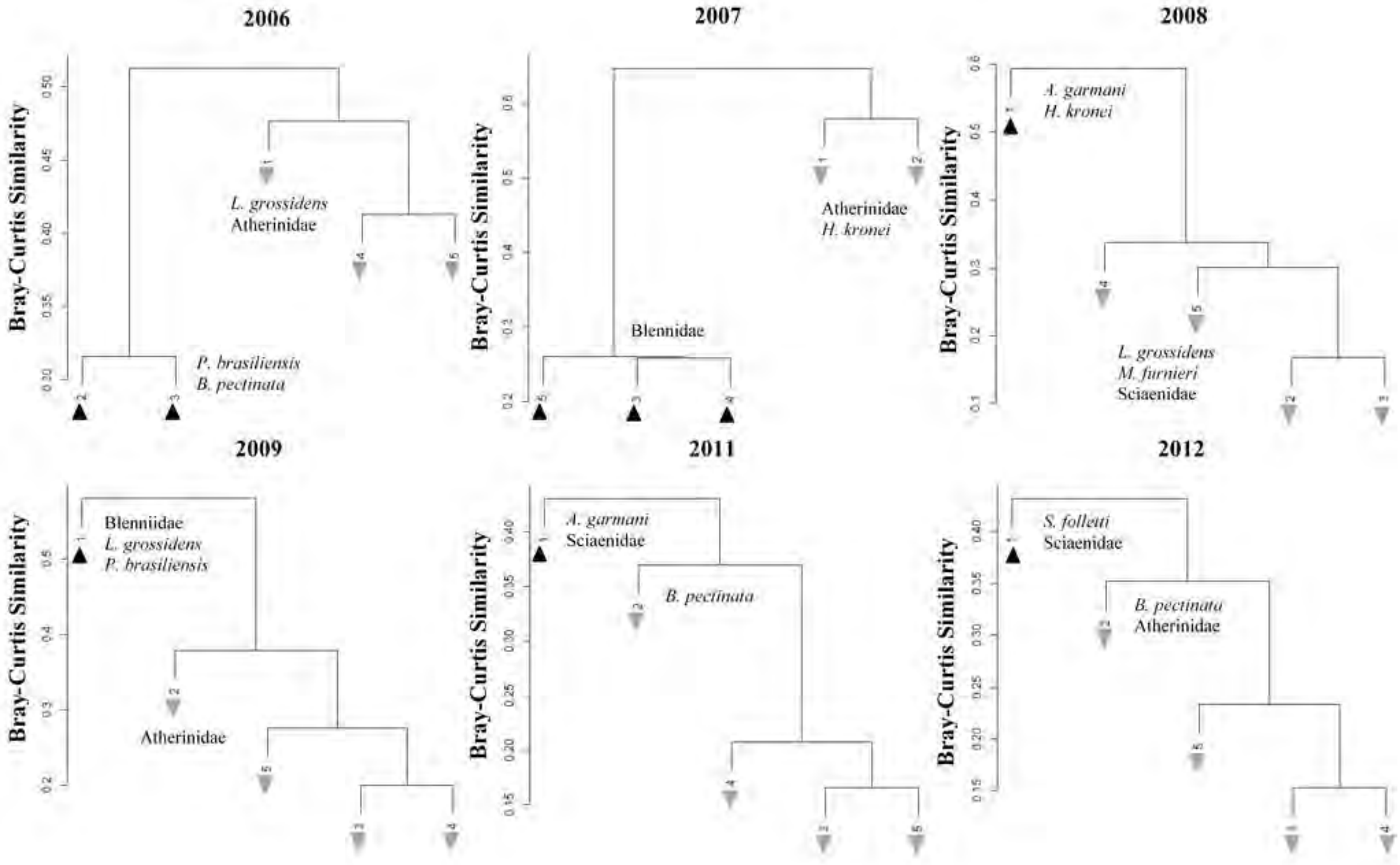


Figure 11: Spatial cluster analysis for each year during the study period, using Bray-Curtis similarity and the method of UPGMA over the transformed matrix of fish larvae density with the respective indicator taxa (▲ : group, ▼ : group II, ●: group III). 2010 was excluded from

this spatial analysis because we registered only 3 taxa during this year. Taxa named in the figure represents indicator species with highest values.

Continuation of Figure 11



Larvae assemblage of shallow waters in PLE was relatively diverse (Table III). In this sense, we also analysed the total abundance of different taxa of larvae in the different sampling stations in order to evaluate the FWC in relation to early life stages of fishes. Results showed a high variability of abundance and number of taxa among stations (Figure 12), reflecting their location in the estuary (Figure 1). In general, station 1, which is influenced by the adjacent ocean, exhibited higher abundance and number of taxa than other stations. In this case, marine species such as *A. marinii* and *T. lepturus* were present (Figure 12). Station 2, which is located in a degraded shallow embayment, showed less number of taxa and abundance than other stations (Figure 12). Stations 3, 4 and 5 showed distinct abundance and occurrence patterns, which can be related to the dynamic nature of the ecosystem (Figure 12). Comparing these results to the FWC applied to the PLE, it was possible to assume that FWC alone is not representative of the dynamic spatial pattern of estuarine early life stages of fishes at PLE, and might not be sufficient to conserve essential habitats for their development.

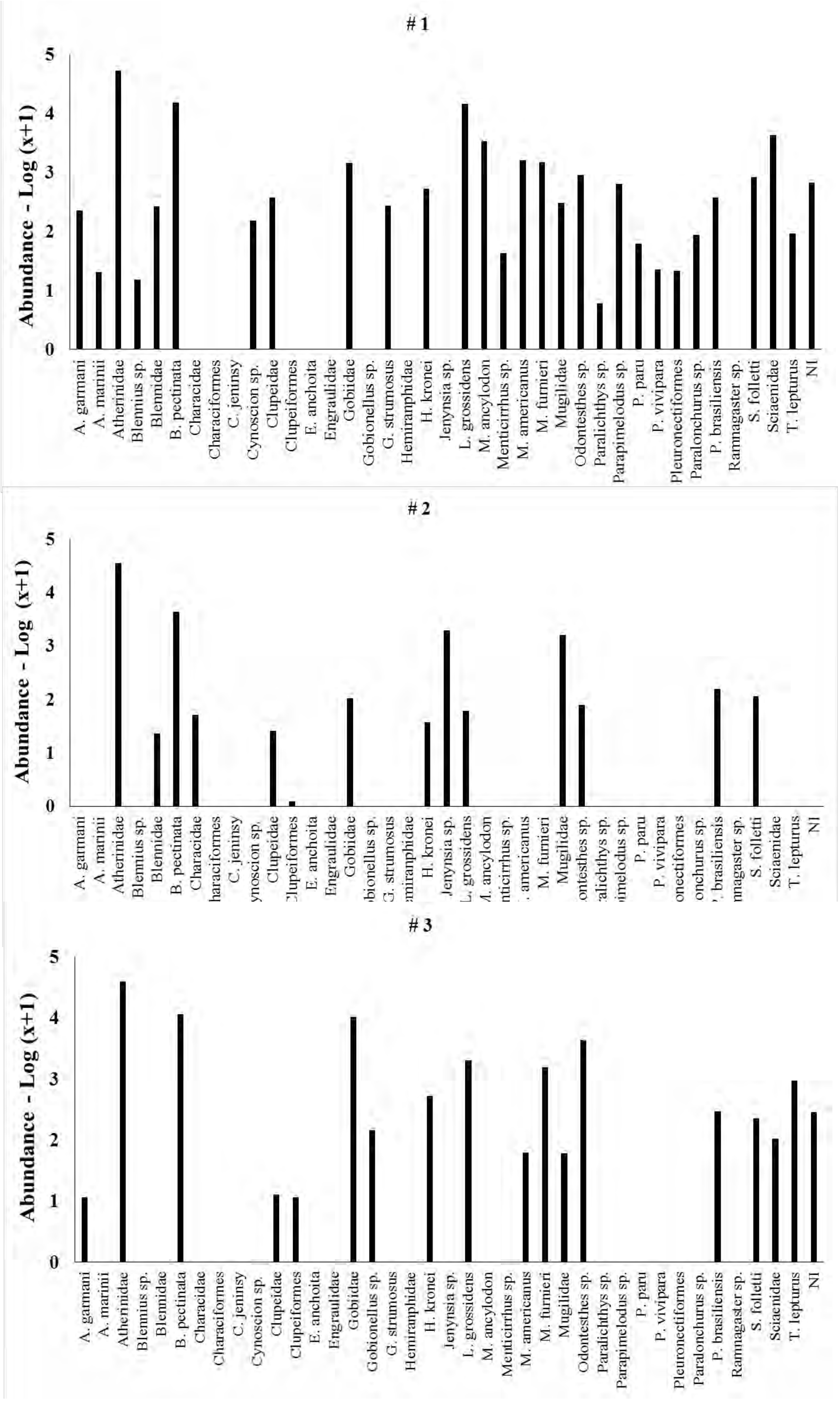
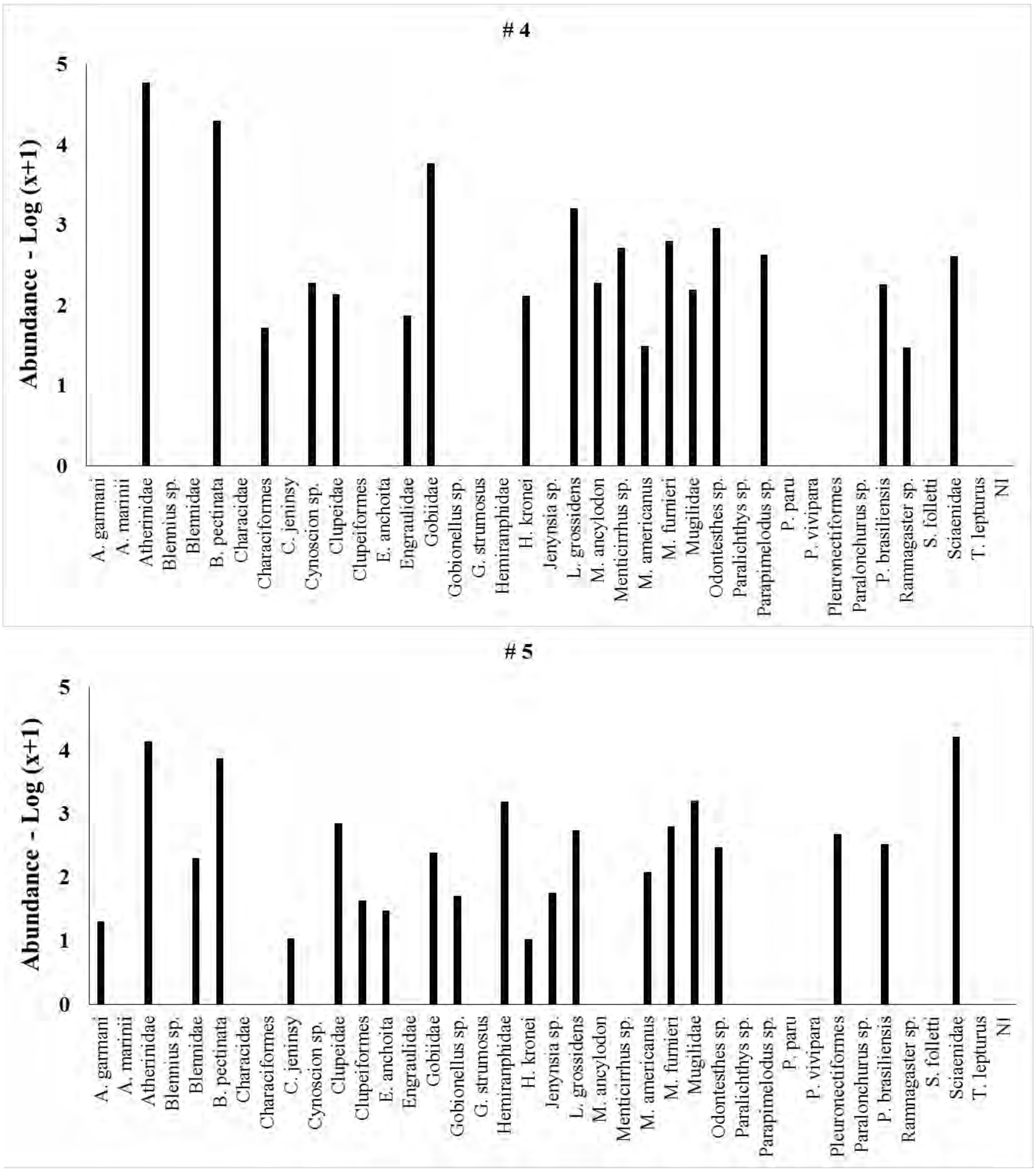


Figure 12: Total abundance variability in each sampling station at Patos Lagoon estuary.

Continuation of Figure 12



4. DISCUSSION

Long-term assessment of fish eggs and larvae abundance showed variability among sampling stations, month and year studied. Results showed that to effectively protect early life stage of fishes at PLE, temporal and spatial dynamics of fish eggs and larvae should be considered. Also, that it is essential to combine these information with other parameters (e.g.

water quality and human activities) in management plans. The main objective of the FWC is to guarantee the water quality based on its dominant use, without any consideration of the status of the biological community. In the city of Rio Grande, which is the main city near the PLE, the Framework is the only management action applied to PLE in the environmental plan of Rio Grande (Plano Ambiental de Rio Grande, 2007). However, aquatic resources management and biodiversity conservation have common objectives, as sustainable use of water and protection of the aquatic and coastal environments. Our results demonstrated that long-term studies can facilitate conservation actions, as it can help to identify temporal and spatial variation patterns that can be incorporate into planning. In this sense, especially when considering dynamic ecosystems such as estuaries, we should apply an integrate framework, also considering temporal and spatial scales, to achieve an efficient management plan.

Our results showed that abundance of fish eggs and larvae was influenced by the location of the sampling station. Besides that, we noticed variability within year and month. This spatial and temporal pattern is already well defined for PLE, where the occurrence and abundance of species are correlated with salinity and temperature distribution (Muelbert et al., 2010), and that highest abundance is related to areas close to the entrance of the estuary where spawning of many marine species occur (Muelbert and Weiss, 1991; Sinque and Muelbert, 1997). Despite the intra and inter annual variation, extreme climatic events as El Niño (related to high precipitation and low salinity) and La Niña (related to low precipitation and high salinity) can also influence the distribution and the recruitment of fish eggs and larvae into the PLE, and also influence fishing production (Möller et al., 2009; Muelbert et al., 2010; Odebrecht et al., 2010; Schroeder and Castello, 2010). Ichthyoplankton transport between adjacent Atlantic Ocean and PLE is also controlled by wind force and the morphology of the ecosystem (Martins et al., 2007).

Beyond influencing intra and inter annual abundance patterns, salinity and temperature variation can also influence which ichthyoplankton assemblage will be present at the estuary (Muelbert et al., 2010). Understand this variability and how it can contribute to conservation of estuarine habitats and biodiversity is extremely important. Our main results demonstrated that fish eggs and larvae from PLE have a high variability within year, month and sampling station. In this sense, the FWC is not sufficient to deal with the dynamic nature of PLE and to protect essential habitats for early life stages in PLE. Our study considered sampling stations only at zones 2 and 3 of the FWC, being both includes affected by human activities and associated with degraded or impacted waters. Fish eggs and larvae showed a high variation on spatial distribution patterns throughout study period (Figures 10 and 11), with distinct taxa inhabiting both zones (Table III and Figure 12).

Usually conservation planning is developed as a static problem, not accounting for feature dynamics in time and space (Moilanen et al., 2009). As estuaries are highly dynamic ecosystems, is necessary to incorporate temporal and spatial parameters that reflect their dynamic nature in order to achieve an efficient conservation plan. Our results showed that long-term ichthyoplankton studies can be very helpful in order to facilitate conservation actions, since it makes possible to understand the variability on abundance, composition and spatial distribution of species throughout time. In addition, the incorporation of information of early life stages of fishes into systematic conservation planning significantly alters spatial priorities for conservation in estuaries (Costa et al., unpublished results). In the case of PLE, we found that only the application of the FWC might not be sufficient to guarantee the protection of essential habitat for fish eggs and larvae development in the estuary. The maintenance of early life stages has a direct effect on the maintenance of adult populations, fishing production and consequently, economic and social benefits. In this sense, we strongly suggest that coastal management plans should combine habitat dynamics to economic and social data to achieve an efficient outcome. Adaptive management (Holling, 1978; Walters, 1986) can be an interesting strategy to achieve an efficient management plan in estuaries. This approach recognizes that conservation strategies needs to be flexible to changing social, environmental and economic perspectives, but also, susceptible to new information on biodiversity, costs, threats and how to incorporate them into conservation planning (Cinner et al., 2012; Weeks & Jupiter, 2013). The main advantage of this perspective is that adaptive management is a dynamic and iterative process, which recognizes that conservation objectives can change over time. In this process, new information can be incorporated in the management strategies as soon as they become available (Walters & Hilborn, 1978; Weeks & Jupiter, 2013). In the case of PLE, where there is a continuous long term study covering most of estuarine aspects and biota, implement an adaptive management plan can be a good strategy to combine previous information and the existing FWC with new knowledge. In conclusion, long-term studies can be a helpful strategy to assist conservation decision making. For dynamic estuaries, such as PLE, a management plan as the adaptive management plan that combines a fixed strategy, such as the Framework for Water Classification, with dynamics and long-term information obtained from the variability of the biological community is extremely useful to achieve the protection of the ecosystem and its biodiversity.

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