

**UNIVERSIDADE FEDERAL DO RIO GRANDE
PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA**

**ESTRUTURA GENÉTICA, DINÂMICA E
VIABILIDADE POPULACIONAL DO BOTO,
Tursiops truncatus, DO ESTUÁRIO DA
LAGOA DOS PATOS, SUL DO BRASIL**

PEDRO FRIEDRICH FRUET

Tese apresentada ao Programa de Pós-graduação em Oceanografia Biológica da Universidade Federal do Rio Grande e Flinders University of South Australia sob acordo de “co-tutela” entre as duas universidades como requisito parcial à obtenção do título de DOUTOR

Orientador: Dr. Eduardo R. Secchi (FURG)
Co-Orientador: Dra. Luciana Möller (Flinders University of South Australia)

**RIO GRANDE
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*Eu dedico esta tese à minha família e a todas as
pessoas do bem que propagam o amor e doam-se na busca
de um mundo melhor*

“De que adianta se comover e não mover um músculo?”

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RESUMO

Apesar de bem estudado em todo o mundo, o conhecimento sobre a ecologia do boto (*Tursiops truncatus*) ainda é escasso no Oceano Atlântico Sul Ocidental (ASO). O aumento das taxas de capturas acidentais em algumas áreas costeiras do sul do Brasil tem levantado suspeitas sobre um potencial declínio de algumas comunidades locais de botos, mas a falta de dados relevantes dificultam uma avaliação adequada sobre o seu status de conservação. Os principais objetivos desta tese foram investigar a estrutura genética do boto no ASO, assim como compreender a dinâmica e avaliar a viabilidade da pequena comunidade de botos que habita o estuário da Lagoa dos Patos (ELP) no sul do Brasil. No capítulo II (ANEXO I) combinou-se uma análise de 16 loci de microsatélites e sequências de região controle do DNA mitocondrial para investigar a diversidade genética, estrutura e conectividade de seis comunidades de botos amostradas ao longo da costa do ASO. Foram encontrados níveis extremamente baixos de diversidade genética e forte estruturação populacional entre algumas comunidades, o que sugere que os botos da Baía San Antonio (BSA), Argentina, e do sul do Brasil e Uruguai (SB-U) representam duas Unidades Evolutivamente Significativas (*ESUs*), e que as comunidades de botos do SB-U compõe cinco Unidades de Manejo (*MUs*) distintas para fins de manejo e conservação. Nos capítulos III (ANEXO II) e IV (ANEXO III) combinou-se uma série de informações coletadas sistematicamente através de foto-identificação e monitoramento da mortalidade dos botos para estimar parâmetros demográficos e de história de vida dos botos do ELP e águas adjacentes. Aplicando-se os dados de foto-identificação a modelos de marcação-recaptura do Desenho Robusto de Pollock e Commack-Jolly-Seber, foram encontradas taxas de sobrevivência mais altas para fêmeas adultas (0,97, 95% CI: 0,91-0,99) do que para os machos adultos (0,88, 95% CI: 0,75-0,94), juvenis (0,83, 95% CI: 0,64-0,93) e filhotes de 0,84 (IC 95% = 0,72-0,90). Estimativas anuais de abundância foram altamente precisas (maior CV = 0,053) e não ultrapassaram 88 indivíduos, sem detectar claras tendências temporais na abundância. Os resultados sustentam uma comunidade de botos com reprodução em pulso, com a maioria dos nascimentos ocorrendo durante o início do verão austral. As fêmeas começam a reproduzir com idade mínima de 8 anos. A média de intervalo entre nascimentos foi de 3 anos (moda = 2), e a fecundidade foi estimada em 0,11. A partir da análise de dentes de animais encalhados observou-se clara mudança nos perfis de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ próximos a idade de 2 anos, indicando a idade mais provável de desmame. Fêmeas mais velhas reproduziram-se a taxas mais baixas, sugerindo uma diminuição na aptidão reprodutiva relacionada a idade. Este padrão pode explicar a marcada variação individual no sucesso reprodutivo observada. No capítulo V (ANEXO IV), utilizando-se das informações demográficas obtidas nos capítulos anteriores, contrui-se um modelo estocástico estruturado por estágios para investigar a dinâmica e a viabilidade da comunidade de botos do ELP sob diferentes cenários de impactos de pesca e incertezas nos parâmetros do modelo. Na ausência de capturas acidentais, estimou-se uma taxa anual de crescimento próxima a 3% (IC 95%: 1,2%-5,8%) e baixas probabilidades de declínio nos próximos 60 anos sob os atuais impactos da pesca. No entanto, as simulações de viabilidade mostraram que um pequeno aumento nas taxas de captura, especialmente de fêmeas maduras, pode levar a comunidade do ELP a declinar rapidamente. A viabilidade, contudo, seria substancialmente beneficiada com um aumento na sobrevivência de juvenis/sub-adultos, o que pode ser alcançado através da área de proteção para o boto recentemente implementada no sul do Brasil, a qual proíbe a pesca de emalhe por embarcações na área preferencial dos botos do ELP.

1. INTRODUÇÃO GERAL

1.1 *Variação interespecífica e intraespecífica em cetáceos*

Cetáceos são mamíferos totalmente adaptados à vida aquática. Distribuídos de polo a polo e ocupando os habitats fluviais e marinhos (incluindo águas costeiras e oceânicas) (Bowen & Sniff 1999), este grupo foi exposto a diversas pressões ecológicas durante sua evolução, ocupação e expansão para o ambiente aquático (Berta & Sumich 1999). Atualmente, a diversidade dos cetáceos consiste em 90 espécies reconhecidas (e uma funcionalmente extinta - ver Turvey *et al.* 2007), das quais 14 são baleias-de-barbatana (Misticetos) e 76 são baleias-dentadas (Odontocetos) (Comitee on Taxonomy 2014). O crescente número de amostras biológicas disponíveis para análises moleculares e morfológicas, em conjunto com o surgimento de novas tecnologias de sequenciamento de DNA e potentes programas computacionais de análise de dados, estão levando a rápidas mudanças na classificação taxonômica atual dos cetáceos. Na última década, por exemplo, foi reivindicado o reconhecimento de nove novas espécies de Odontocetos (*e.g.* Beasley *et al.* 2005; Cunha *et al.* 2005; Caballero *et al.* 2007; Charlton-Robb *et al.* 2011; Mendez *et al.* 2013; Hrbek *et al.* 2014). Abaixo do nível de espécie, variações intraespecíficas que surgem a partir de adaptações a condições ecológicas específicas (p.ex. especialização comportamental na captura de presas) estão ganhando crescente reconhecimento como constituintes de grande relevância para a conservação da biodiversidade deste grupo (Morin & Dizon 2009).

Durante sua história evolutiva, os cetáceos têm demonstrado uma grande capacidade de adaptarem-se rapidamente a diferentes condições ambientais (Berta 2012). Por exemplo, eles invadiram águas fluviais, estuarinas e oceânicas, explorando todos os oceanos e uma variedade de habitats, como a zona de arrebentação (*e.g.* botos,

Tursiops spp.; boto-cinza, *Sotalia guianensis*), plataforma continental (*e.g.* franciscana, *Pontoporia blainvillei*) e águas oceânicas profundas (*e.g.* baleias-bicudas) (Bowen & Sniff 1999). Como resultado, os cetáceos representam um grupo heterogêneo no que diz respeito a sua história de vida (Chivers 2009). Em geral, as espécies que investem muita energia em reprodução (*i.e.* maturação sexual precoce e curtos intervalos de nascimento) têm crescimento físico e sobrevivência limitados (*e.g.* franciscana e golfinho-do-porto, *Phocoena phocoena*), enquanto espécies com menor capacidade reprodutiva (ou seja, maturação sexual mais tardia e intervalos de nascimento mais prolongados) alcançam maiores tamanhos e longevidade (*e.g.* orcas, *Orcinus orca*; baleia-da-Groenlândia, *Balaena mysticetus*) (revisado em Chivers 2009). Apesar de heterogêneos, todos os cetáceos têm um baixo potencial de crescimento populacional, o qual é maior em espécies com maior biomassa. Muitos mysticetos possuem uma potencial taxa de crescimento, próxima ou ligeiramente superior a 10% ao ano, enquanto pequenos odontocetos exibem taxas intrínsecas de crescimento próximas a 4%, caracterizando os cetáceos como um grupo de baixa resiliência (Reilly & Barlow 1996; Wade 1998).

1.2 Ameaças passadas e atuais para as populações de cetáceos

Historicamente, atividades humanas tais como a degradação e destruição do habitat, poluição do ar e da água, caça comercial e as capturas acidentais têm ameaçado os cetáceos em escala local, regional e global (Reeves *et al.* 2003). Várias unidades populacionais de grandes baleias foram dizimadas pela indústria baleeira, mas após décadas de proteção legal e cumprimento das leis algumas começaram a mostrar sinais de recuperação (*e.g.* baleia-franca-austral, *Eubalaena australis*, Groch *et al.* 2005). Por outro lado, outras permaneceram em baixa densidade e ainda encontram-se em perigo

após décadas de proteção (*e.g.* baleia-franca-do-Atlântico-Norte, *Eubalaena glacialis*, e baleia-franca-do-Pacífico-Norte, *Eubalaena japonica*, Clapham *et al.* 2008).

Atualmente, as populações de cetáceos que habitam áreas costeiras estão enfrentando ameaças frequentes pois estas são geralmente pequenas e habitam áreas geográficas restritas, onde concentram-se uma série de atividades humanas que sabidamente impactam sua sobrevivência (Reeves *et al.* 2003). A captura acidental é reconhecida como uma das principais causas da mortalidade de cetáceos, embora capturas intencionais também ocorram em algumas localidades (Reeves *et al.* 2003). Estimativas globais sugerem que as capturas acidentais matam aproximadamente 308 mil cetáceos no mundo a cada ano, sendo a maioria das mortes decorrentes de interações com a pesca de emalhe costeira (Read *et al.* 2006). Juntamente com a modificação do habitat (p.ex. utilização das águas para fins comerciais, práticas de uso da terra, poluição), as capturas acidentais são responsáveis pelo declínio de várias populações de cetáceos no mundo inteiro (*e.g.* golfinho-sem-dorsal-do-Rio-Amarelo, *Neophocaena asiaeorientalis asiaeorientalis*, Mei *et al.* 2012; população de botos de Fiordland, Nova Zelândia, Currey *et al.* 2009), e a viabilidade de algumas espécies encontra-se em perigo (*e.g.* vaquita, *Phocoena sinus*, Rojas-Bracho *et al.* 2006).

1.3 Análise da viabilidade populacional para a conservação dos cetáceos

Declínios populacionais subsequentes e extinções locais são aspectos típicos que precedem o início de uma extinção regional ou mesmo de uma espécie (*e.g.* Ceballos & Ehrlich 2002). Identificar populações ameaçadas e quantificar a sua vulnerabilidade é, portanto, crucial para auxiliar a estabelecer prioridades de conservação e também para subsidiar processos de tomadas de decisão (*e.g.* Morris & Doak 2003). À medida em que o tamanho da população diminui, aumenta o risco de extinção devido à perda da

diversidade genética, maior suscetibilidade à endogamia e aos efeitos de estocasticidade ambiental e demográfica (*e.g.* Fowler & Baker 1991). Análise de viabilidade de populações de pequenos cetáceos costeiros (*e.g.* boto e golfinhos-de-Hector, *Cephalorhynchus hectori*) sugerem que populações compostas por menos de 100 indivíduos têm elevadas chances de extinção mesmo quando as taxas de mortalidade não-natural são relativamente baixas (Thompson *et al.* 2000; Slooten 2007). Assim, fornecer sinais de alerta antes que uma população sofra um declínio severo na abundância pode ter importantes consequências para que as medidas de conservação sejam eficazes.

Contudo, classificar precisamente as populações de acordo com seus níveis de vulnerabilidade é um desafio. A União Internacional para a Conservação da Natureza e Recursos Naturais (IUCN) desenvolveu critérios e categorias para classificar os níveis de ameaças de populações em nível global, regional e local (IUCN 2012). De acordo com os métodos adotados pela IUCN, a vulnerabilidade é medida em termos de risco de extinção, o qual baseia-se em diversos critérios tais como o tamanho e tendências populacionais, número de indivíduos maduros na população, ocupação e necessidades espaciais de habitat, ou análise de viabilidade populacional.

A análise de viabilidade populacional (AVP) engloba uma série de métodos analíticos e de modelagem numérica utilizados para projetar as populações no futuro com base em modelos de dinâmica populacional (Beissinger 2002). Este método permite incluir inúmeros fatores que afetam o crescimento e persistência das populações, incluindo estocasticidade ambiental e demográfica, catástrofes, e fatores determinísticos (*e.g.* cotas de caça), assim como incertezas nas estimativas dos parâmetros de entrada dos modelos (*e.g.* Gilpin & Soulé 1986; Possingham *et al.* 1993;

Morris & Doak 2003). Não existe um processo único que constitui uma AVP, mas um de seus maiores elementos é estimar a probabilidade de extinção (ou *quasi*-extinção) de uma população ao longo de um período pré-determinado e sob circunstâncias particulares (Possingham *et al.* 1993). Além disso, a contribuição relativa dos parâmetros vitais (*e.g.* sobrevivência, crescimento e fecundidade) para o crescimento populacional pode ser quantificado (Morris & Doak 2003). Esta informação pode ser utilizada para identificar prioridades de pesquisa e guiar ações de conservação e manejo para proteger populações ameaçadas (Possingham *et al.* 1993).

A AVP surgiu no início dos anos 80, mas sofreu profundas transformações ao longo do tempo (Beissinger 2002). Novos rumos para utilização de modelos matemáticos na biologia da conservação surgiram com a abordagem apresentada por Shaffer (1981), que desenvolveu o primeiro modelo de AVP incorporando eventos ao acaso (estocasticidade) nas probabilidades de persistência das populações. Após isso, a complexidade dos modelos cresceu rapidamente, em conjunto com o surgimento de ferramentas computacionais poderosas, e também com a necessidade de considerar uma variedade de processos e escalas que afetam tanto os organismos quanto as decisões de manejo (Beissinger 2002).

Desde sua criação, modelos de AVP têm sido empregados em diversos táxons, e somente no final da década de 1990 a sua aplicação foi estendida aos cetáceos (*e.g.* Caswell *et al.* 1998; Slooten *et al.* 2000; Secchi 2006). Atualmente, a falta de dados básicos sobre demografia e estrutura populacional ainda limita sua aplicação a algumas poucas espécies de cetáceos (*e.g.* golfinho-de-Hector, Slooten *et al.* 2000; boto, Currey *et al.* 2009; golfinho-corcunda-do-Indo-Pacífico, *Sousa chinensis*, Huang *et al.* 2012; golfinho-sem-dorsal, *Neophocoena asiaeorientalis*, Hashimoto *et al.* 2013).

A acurácia da AVP é muito sensível à qualidade dos dados. Se a população é bem estudada, a AVP pode fornecer previsões válidas e precisas (Brook *et al.* 2000); caso contrário, os resultados devem ser interpretados com muito cuidado ou até mesmo ignorados (Coulson *et al.* 2001). Assim, traduzir os resultados da AVP em recomendações úteis de manejo requer uma investigação detalhada sobre os fatores impactantes e estimativas robustas dos seus parâmetros demográficos.

1.4 Taxonomia dos botos

O boto (*Tursiops* spp.) é encontrado em todos os oceanos tropicais e temperados, e é capaz de ocupar, explorar e adaptar-se localmente a diferentes ecossistemas (Wells & Scott 1999). Tal plasticidade resultou em uma clara variação geográfica nos seus traços morfológicos, o que fez da taxonomia do gênero *Tursiops* uma das mais controversas dentre os cetáceos (Wang *et al.* 1999). Inicialmente, baseados em dados limitados, mais de 20 espécies nominais foram descritas (Hershkovitz 1966). Contudo, posteriormente, uma visão mais conservadora foi adotada e por décadas considerou-se apenas uma espécie globalmente distribuída (Rice 1998). Atualmente, duas espécies são reconhecidas: o boto-do-Indo-Pacífico (*T. aduncus*), distribuído descontinuamente ao longo das águas costeiras da região Indo-Pacífica; e o boto-comum (*T. truncatus*) (daqui para diante mencionado apenas como boto), ocorrendo em todos os oceanos exceto em regiões polares (Fig. 1). Recentemente, uma terceira espécie (boto-de-Burrunan, *T. australis*) foi descrita para as águas costeiras do sul da Austrália (Charlton-Robb *et al.* 2011). Contudo, sua validade como nova espécie é ainda incerta pois, de acordo com o Comitê de Taxonomia (Committee on Taxonomy 2014), uma "reavaliação rigorosa dos dados e argumentos relevantes é necessária".

Abaixo do nível de espécie, diferenças significativas na morfologia, coloração,

ecologia e genética encontradas entre os botos do Mar Negro e *T. truncatus* de outras bacias oceânicas (por exemplo, Pacífico e Atlântico) e de dentro da mesma bacia oceânica (costa leste e oeste do Mediterrâneo) suportam o reconhecimento de pelo menos uma subespécie, *T. t. ponticus* (Birkin 2006). Adicionalmente, a existência de dois ecótipos, um costeiro e outro oceânico, é reconhecida para algumas regiões do mundo. Estudos realizados no Oceano Atlântico Norte encontraram diferenças marcantes entre os ecótipos no que tange a várias características ecológicas e biológicas, incluindo diferenças genéticas fixadas (*e.g.* Hersh & Duffield 1990; Hoelzel *et al.* 1998; Natoli *et al.* 2004). No entanto, se estas diferenças traduzem-se em distintas unidades taxonômicas ainda permanece sob debate (Hoelzel 1998; Hoelzel *et al.* 1998; Reeves *et al.* 2003). Portanto, é possível que outras espécies/subespécies sejam reconhecidas no futuro, o que pode trazer grandes implicações para a conservação dos botos em um nível mundial.

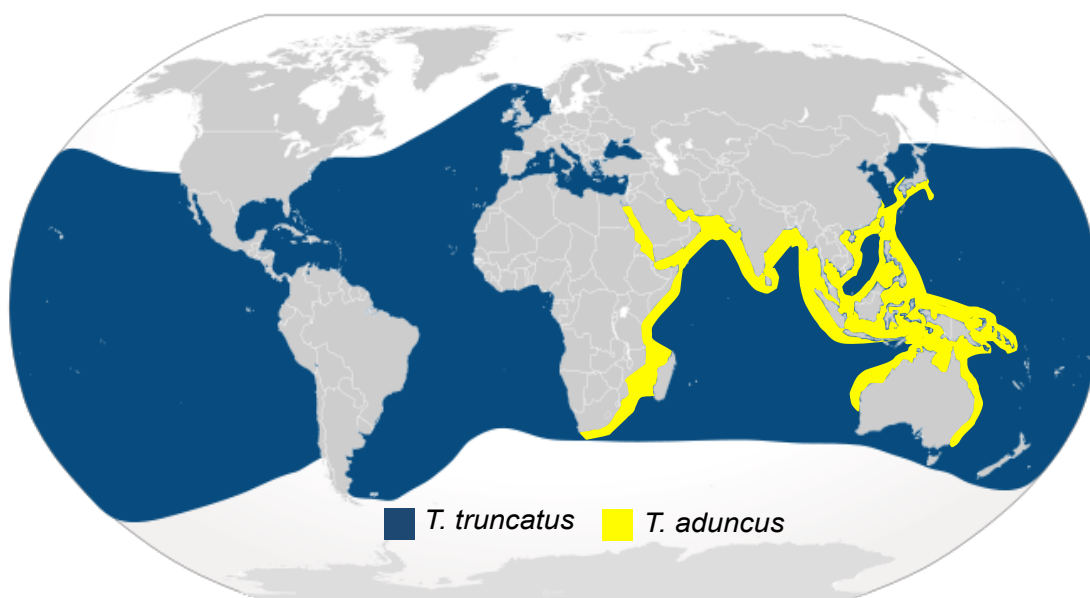


Figura 1. Mapa mostrando a distribuição global do boto (*Tursiops* spp.). Esta figura foi adaptada a partir de um mapa produzido por The Emirrr/MapLab/Cypron ®

1.5 Status e ameaças aos botos em nível global

Tursiops truncatus é considerado o cetáceo mais bem estudado (Wells & Scott 2009). A espécie está listada como “menor preocupação” na Lista Vermelha da UICN, com uma estimativa global de cerca de 600.000 indivíduos, mas sem informações a respeito de tendências na abundância (Hammond *et al.* 2012). Contudo, populações costeiras estão expostas a uma série de impactos causados pelo homem, os quais incluem captura direta para exposição pública, morte intencional, capturas acidentais, poluição, modificação do habitat e redução da disponibilidade de espécies de presas importantes, como consequência da sobrepesca e degradação do meio ambiente (Reeves *et al.* 2003). Catástrofes, como por exemplo um aumento abrupto na densidade de algas nocivas e surtos de morbilivírus também ameaçam os botos. Uma epizootia no final de 1980 levou a um declínio da população migratória de botos no oeste do Atlântico Norte estimado em até 50% do seu tamanho original (*e.g.* Eguchi 2002). Da mesma forma, há evidências de que algumas populações regionais e locais estão declinando devido a taxas elevadas de impactos antrópicos (*e.g.* população residente do Banco da Bahamas, Fearnbach *et al.* 2012; população de Bay of Islands, Nova Zelândia, Tezanos-Pinto *et al.* 2013). Em alguns casos, reporta-se elevados riscos de extinção, como para a população de botos dos Fiordland, Nova Zelândia, a qual foi recentemente listada como criticamente ameaçada na última avaliação da Lista Vermelha da UICN (Currey *et al.* 2013). Outras, como a comunidade* de botos de Sarasota Bay, EUA, têm se mantido relativamente estável ao longo de décadas, embora também sujeita a impactos humanos (*e.g.* Wells & Scott 1990; Powell & Wells 2011).

* daqui por diante adoto o termo “comunidade” no *strictu sensu* de sua definição publicada por Wells *et al.* (1987) para fazer referência a unidades de botos locais, uma vez que a definição enfatiza as relações geográficas e sociais dos indivíduos. Uma comunidade é então composta por aqueles indivíduos que compartilham grande parte de sua área de vida e interagem uns com os outros em uma intensidade muito maior do que com membros de unidades similares em águas adjacentes. O termo população é utilizado para fazer referência a unidades maiores, as quais podem ser compostas por várias comunidades ou encontram-se isoladas.

1.6 Estudos de marcação-recaptura de longo prazo com os botos

Até o início da década de 1970 a maioria das informações disponíveis sobre a biologia dos botos era obtida a partir de pesquisa com indivíduos em cativeiro (*e.g.* McBride & Kritzler 1951; Tavalga & Essapian 1957; Essapian 1963). Reavistagens e observações de alguns botos marcados artificialmente na costa da Flórida forneceram algumas ideias iniciais sobre o comportamento, distribuição e movimentos dos botos (Irvine & Wells 1972). Mais tarde, a descoberta de que os botos poderiam ser individualmente identificados através de fotografias de marcas naturais presentes nas suas nadadeiras dorsais (Würsig & Würsig 1977), combinados com tecnologias aprimoradas como a telemetria, análises genéticas e bioquímicas (*e.g.* Duffield & Wells 1991; Wells *et al.* 1999; Wells *et al.* 2005) revolucionaram o nosso conhecimento sobre a biologia e ecologia desta espécie no ambiente natural. Isto porque os dados de foto-identificação fornecem um modo eficiente, a custos relativamente baixos, que permite seguir indivíduos durante longos períodos de tempo sem a necessidade de sujeitá-los a procedimentos de captura e manipulação para marcá-los. Deformações, cortes e cicatrizes na nadadeira dorsal, na sua grande maioria adquiridas através de interações sociais ao longo da vida dos animais, permitem identificar os indivíduos a longo prazo (*i.e.* anos a décadas) (Würsig & Würsig 1977), enquanto que arranhões e outras marcas superficiais no corpo podem ser utilizados para rastreá-los e diferenciá-los dentro de um curto espaço de tempo (*i.e.* alguns meses) (*e.g.* Wilson *et al.* 1999) (Fig. 2).

Além de ser possível derivar importantes informações sobre os padrões de residência, fidelidade local e movimento dos indivíduos (*e.g.* Wells & Scott 1999), o uso de dados de foto-identificação em modelos de marcação-recaptura (MR) permite obter estimativas confiáveis de uma série de parâmetros populacionais (Hammond *et al.*

1990). Se a natureza dos dados é de curto prazo (*i.e.* semanas, meses), apenas alguns parâmetros podem ser estimados, tal como o tamanho da população. Se recapturas estão disponíveis ao longo de períodos de tempo prolongados (*i.e.* meses ou estações ao longo de múltiplos anos) outros parâmetros relevantes tais como a sobrevivência e taxas reprodutivas podem ser estimados, e as tendências na abundância analisadas (Hammond *et al.* 1990).

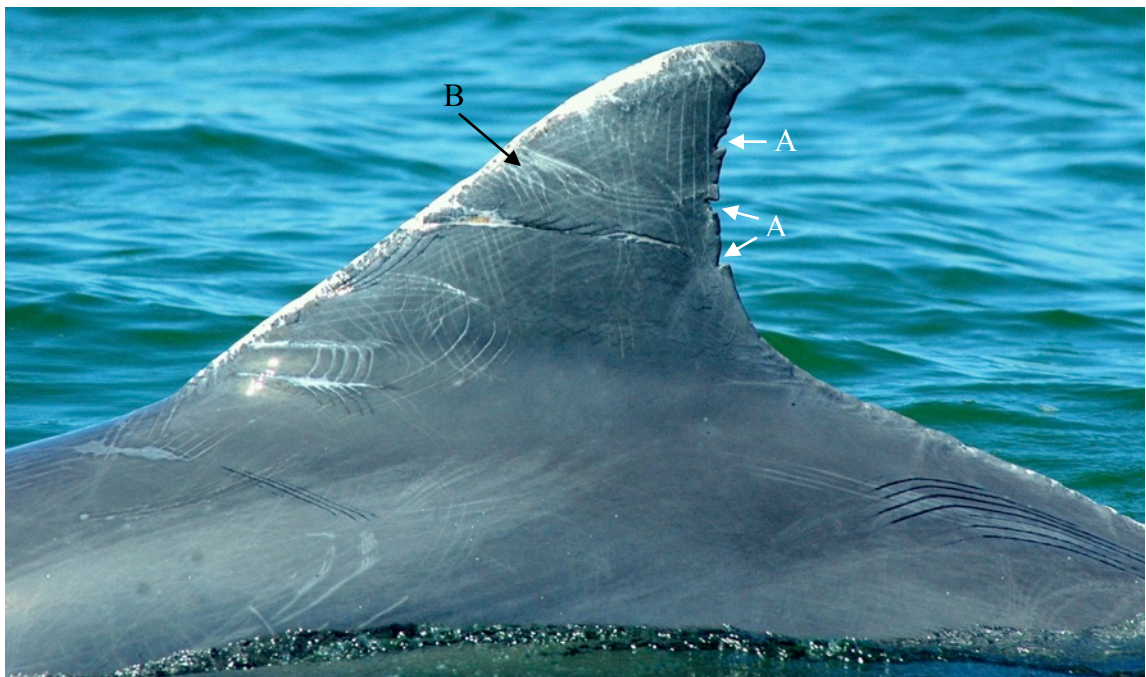


Figura 2. Típicas marcas naturais utilizadas na identificação individual dos botos (*Tursiops truncatus*), a partir de fotografias das nadadeiras dorsais dos indivíduos. (A) Marcas de longa-duração (cortes); (B) Marcas temporárias (arranhões). Foto: Pedro Fruet / Arquivo Projeto Botos da Lagoa dos Patos.

Em Sarasota Bay, onde há mais de quatro décadas pesquisas sistemáticas estão sendo conduzidas com uma comunidade residente de botos, a história de vida da espécie foi estudada em detalhe (*e.g.* Wells & Scott 1990; Wells 2000). A partir da fotointificação a longo prazo, muitos indivíduos são acompanhados desde o nascimento até a morte, fornecendo informações consistentes sobre idade dos indivíduos, sazonalidade de nascimentos, idade de primeira reprodução das fêmeas, taxas reprodutivas, fecundidade, intervalos de nascimento e sucesso reprodutivo (Wells &

Scott 1990; Wells 2000). Além disso, tais dados fornecem o único meio para compreender os padrões de relações sociais de um animal de vida longa como os botos. Apenas alguns estudos sistemáticos de longo prazo têm sido realizados para essa espécie em outros lugares do mundo (*e.g.* Morey Firth, Escócia, Wilson *et al.* 1999; Doubtful Sound, Nova Zelândia, Currey *et al.* 2009).

1.7 História de vida e ecologia dos botos

Os botos são animais de vida longa que se reproduzem lentamente através de um sistema de acasalamento poligâmico (Wells & Scott 1990; Duffield & Wells 2002). As fêmeas atingem a maturação sexual entre 5 e 12 anos, geram apenas um filhote após um período de gestação de 12 meses, e investem pesadamente no cuidado parental durante os primeiros anos de vida do filhote (Wells & Scott 1999; Wells 2000). Intervalos de nascimentos prolongados (entre 3 e 4 anos) são comuns, e a separação entre o par mãe-filhote ocorre geralmente antes do nascimento do próximo filhote (Wells & Scott 1999; Wells 2000; Henderson *et al.* 2014). Os machos tendem a maturar sexualmente mais tarde do que as fêmeas, normalmente entre 9 e 14 anos de idade (Wells *et al.* 1987). Dados de longo prazo obtidos da comunidade de botos de Sarasota Bay sugerem uma prolongada vida reprodutiva para as fêmeas, uma vez que indivíduos com até 48 anos de idade já foram registrados com neonatos (Wells & Scott 1999). Os botos podem viver mais de 50 anos, com as fêmeas atingindo normalmente idades mais avançadas do que os machos (Wells & Scott 1999). As taxas de sobrevivência variam entre regiões e populações, dependendo das características ambientais e de diferenças populacionais específicas no fitness dos indivíduos, mas em geral é menor em classes etárias mais jovens (Wells & Scott 1990; Stolen & Barlow 2003; Fruet *et al.* 2012).

Estudos de marcação-recaptura (MR) têm demonstrado que as comunidades de

botos incluem indivíduos com padrões variáveis de residência e área de vida (*e.g.* Wells *et al.* 1987; Simões-Lopes & Fábian 1999; Silva *et al.* 2009; Hwang *et al.* 2014). Botos associados a ambientes costeiros produtivos (p.ex. desembocaduras de rios e estuários, baías e fiordes) tendem a ser residentes a áreas relativamente pequenas, onde procuram abrigo para evitar predadores e beneficiarem-se de recursos alimentares mais previsíveis no espaço e tempo (*e.g.* Wells *et al.* 1987; Simões-Lopes & Fábian 1999). Estas comunidades são frequentemente pequenas, possivelmente como resultado de limitado espaço e disponibilidade de alimento (Gowans *et al.* 2007). Em Sarasota Bay, taxas de imigração e emigração foram estimadas em menos de 3% ao ano (Wells & Scott 1990), e dados de MR de outras comunidades de botos associados a estuários suportam baixas taxas de emigração (*e.g.* Daura-Jorge *et al.* 2013). Embora eventos de emigração permanente sejam improváveis para estas comunidades estuarinas, alguns indivíduos podem abandonar temporariamente suas áreas preferenciais e movimentarem-se para área distantes (*i.e.* eventos de emigração temporária) (*e.g.* Silva *et al.* 2009; Bearzi *et al.* 2011). A passagem ocasional de alguns botos de comunidades adjacentes nas proximidades destas áreas mais produtivas pode ocorrer (*i.e.* eventos de transiência), especialmente se os indivíduos fazem parte de comunidades costeiras (*e.g.* Wood 1998; Hwang *et al.* 2012). Botos de comunidades costeiras também podem realizar movimentos em maiores escalas (*e.g.* movimento de centenas de indivíduos ao longo de centenas/milhares de km), os quais são muitas vezes desencadeados como respostas às flutuações significativas nas condições oceanográficas (*e.g.* Wells *et al.* 1990). Esta complexidade de movimentos e sobreposição espaço-temporal de áreas de vida estabelece dificuldades para estudar os botos. Em primeiro lugar, efeitos de transiência/emigração temporária devem ser incorporados nos modelos estatísticos para fornecer estimativas robustas dos parâmetros populacionais de interesse. Em segundo

lugar, o contato entre indivíduos de diferentes comunidades fora das suas áreas preferenciais pode traduzir-se em troca genética, podendo resultar em diferentes conceitos de população (*e.g.* população discreta, meta-população, população panmítica). Este dinamismo têm implicações importantes para a compreensão da dinâmica populacional, avaliação de risco e para a tomada de decisões de conservação, especialmente quando meta-populações são formadas por múltiplas pequenas unidades de botos que estão sujeitas a impactos humanos localizados (*e.g.* Rosel *et al.* 2009).

1.8 Diversidade genética e estrutura populacional dos botos

Marcadores moleculares têm sido utilizados com êxito para estimar taxas de dispersão sob diferentes escalas espaciais, assim como para estimar a diversidade genética das populações de botos (*e.g.* Hoelzel *et al.* 1998; Natoli *et al.* 2004; Sellas *et al.* 2005; Tezanos-Pinto *et al.* 2009). Estudos genéticos direcionados para fins de conservação e manejo têm utilizado principalmente a genotipagem de regiões do DNA nuclear (DNA_n) como os microssatélites, ou dados de sequenciamento de segmentos do genoma do DNA mitocondrial (DNA_{mt}) (ver revisão realizada por Morin & Dizon 2009), embora isso possa modificar-se no futuro próximo com o advento dos estudos genômicos (Allendorf *et al.* 2010).

Através destes estudos, diferenciações genéticas significativas vêm sendo detectadas entre botos de bacias oceânicas distintas (Natoli *et al.* 2004; Tezanos-Pinto *et al.* 2009) e entre os ecótipos costeiro e oceânico amostrados no Atlântico Norte (Hoelzel *et al.* 1998) e no Caribe (Caballero *et al.* 2012). Botos do ecótipo oceânico parecem fazer parte de grandes populações distribuídas ao largo de milhares de quilômetros, as quais apresentam intenso fluxo gênico e fraca estruturação populacional geográfica em larga escala (Quérrouil *et al.* 2007; Tezanos-Pinto *et al.* 2009). Em ambos

os casos, descontinuidades genéticas parecem coincidir com fatores de quebras ecológicas, como distintas massas de água, correntes e contornos de profundidade (Hoelzel *et al.* 1998; Natoli *et al.* 2004).

Em escalas geográficas menores (*i.e.* poucas dezenas de km), estudos genéticos têm revelado dispersão restrita e diferenciação genética significativa entre várias comunidades de botos do ecótipo costeiro, suportando as hipóteses de estruturação sugerida por estudos de residência e estrutura social baseados em dados de foto-identificação (*e.g.* Golfo do México, Sellas *et al.* 2005; Bahamas, Parsons *et al.* 2006; Atlântico Norte ocidental, Rosel *et al.* 2009; Atlântico Norte oriental, Mirimin *et al.* 2011; Pacífico Sul ocidental, Tezanos-Pinto *et al.* 2009). Além disso, estes estudos revelaram que comunidades costeiras geralmente possuem diversidade genética bem inferior (tanto no DNA_{mt} quanto DNA_n) quando comparado ao ecótipo oceânico (Natoli *et al.* 2004; Quéroutil *et al.* 2007; Tezanos-Pinto *et al.* 2009). Especula-se que muitas populações costeiras teriam se originado através de sucessivos eventos fundadores resultantes da invasão de indivíduos de populações pelágicas a ambientes costeiros e posteriormente tornando-se filopátricos a áreas específicas. Isso teria resultado em baixos níveis de diversidade e alta diferenciação genética em microescalas geográficas (Hoelzel 1998; Natoli *et al.* 2004).

Neste contexto, estudos genéticos também podem fornecer informações a serem usadas na identificação de unidades biologicamente relevantes para a conservação, especialmente se integrados com informações sobre a estrutura das populações e movimento de indivíduos adquiridas por métodos alternativos (p.ex. marcação-recaptura, rastreamento satelital) (*e.g.* Lowe & Allendorf 2010). O termo "unidades de conservação" refere-se a unidades intraespecíficas identificadas ao longo da distribuição

das espécies as quais são utilizadas para orientar pesquisa, programas de monitoramento e esforços de conservação e manejo (ver revisão feita por Morin & Dizon 2009). Dentre outras, unidades evolutivamente significativas (*ESUs*) e unidades de manejo (*MUs*) são designações comuns de unidades de conservação. Uma *ESU* (*sensu* Ryder 1986) é uma população ou um grupo composto de várias populações que historicamente foram isoladas, necessitando de ações de manejo independentes, visando maximizar o potencial evolutivo da espécie em decorrência das mudanças ambientais, e que abordam questões de conservação de longo-prazo e tendências populacionais históricas (*e.g.* Moritz 1994). Por outro lado, *MUs* (*sensu* Moritz 1994) são consideradas unidades demograficamente independentes, nas quais a dinâmica populacional é afetada sobretudo por nascimentos e mortes, ao invés de eventos de migração (ver também Funk *et al.* 2012 para uma perspectiva recente sobre *ESUs* e *MUs*). Estas *MUs* são geralmente adequadas para o manejo com metas de curto-prazo e, tipicamente, são utilizadas para orientar planos de monitoramento e regular os impactos atuais de atividades humanas sobre populações (Schwartz *et al.* 2007).

1.9 Botos no Sul do Brasil

O status taxonômico dos botos do Atlântico Sul Ocidental (ASO), incluindo indivíduos do sul do Brasil, ainda é motivo de debate. Barreto (2000) analisou as métricas do crânio e sequências do DNA_{mt} de botos encalhados coletados nas costas do Brasil, Uruguai e Argentina. O autor encontrou um gradiente latitudinal na variação morfológica dos crânios e, juntamente com os resultados preliminares das sequências de DNA_{mt}, propôs duas formas geográficas para a região, uma ocorrendo ao sul e outra ao norte de 27°30'S, recomendando a adoção da subespécie *T. truncatus geophyreus* para a forma sul. Mais recentemente, Wickert (2013) realizou uma análise morfométrica

similar, porém um pouco mais abrangente, e propôs que a subespécie *T. truncatus gephyreus* deve de fato ser elevada a nível de espécie. No entanto, a falta de dados genéticos robustos e de amostras de maior abrangência geográfica ainda dificulta uma avaliação adequada do seu status taxonômico.

Na costa sul do Brasil, o ecótipo costeiro está amplamente distribuído entre 27°21'S e 33°70'S, com registros de ecótipos oceânicos ocorrendo também ao longo desta região (Zerbini *et al.* 2004). No entanto, parece haver uma separação espacial e ecológica entre os ecótipos (*e.g.* Botta *et al.* 2012). A ampla extensão da plataforma continental do sul do Brasil potencialmente reduz a possibilidade de contato entre os ecótipos, já que não existem registros do ecótipo oceânico em águas com profundidades inferiores a 20 m. Há pouca informação sobre a ecologia dos botos que habitam águas oceânicas, mas sugere-se que eles tenham uma morfologia externa e craniana distinta do ecótipo costeiro (Simões-Lopes 1996; Barreto 2000). Registros ocasionais indicam que botos oceânicos ocorrem em grandes grupos e em profundidades entre 50 e 250 m no sul do Brasil (Zerbini *et al.* 2004; Wedekin *et al.* 2008). Botta *et al.* (2012) identificou diferenças expressivas nos sinais de isótopos estáveis (em ambos $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$) de um indivíduo encalhado no sul do Brasil (agora confirmado como ecótipo oceânico através de análise genética – Fruet *et al.* dados não publicados) quando comparado a botos costeiros. Além disso, os resultados preliminares de um estudo filogeográfico comparativo sustentam uma alta diferenciação genética entre os ecótipos para o ASO (Fruet *et al.* dados não publicados).

Nas águas costeiras do sul do Brasil, a espécie distribui-se amplamente, porém concentra-se em pequenas comunidades associadas a lagunas, desembocaduras de rios e estuários, como Laguna de Santo Antônio (estado de Santa Catarina), desembocaduras

dos rios Mampituba e Tramandaí, e estuário da Lagoa dos Patos (ELP) (estado do Rio Grande do Sul) (Castello & Pinedo 1977; Simões-Lopes & Fábian 1999) (Fig. 3). Dados de indivíduos encalhados e avistagens confirmam a existência de comunidades costeiras ao longo da costa, margeando as comunidades residentes (Laporta 2009; Fruet *et al.* 2011; Genoves 2013). Estudos sistemáticos de MR realizados nestas áreas revelaram que cada comunidade é muito pequena (<100 indivíduos – ver revisão feita por Fruet *et al. no prelo*), mas com graus variáveis de residência e fidelidade local entre indivíduos (Simões-Lopes & Fabian 1999; Laporta 2009; Fruet *et al.* 2011).

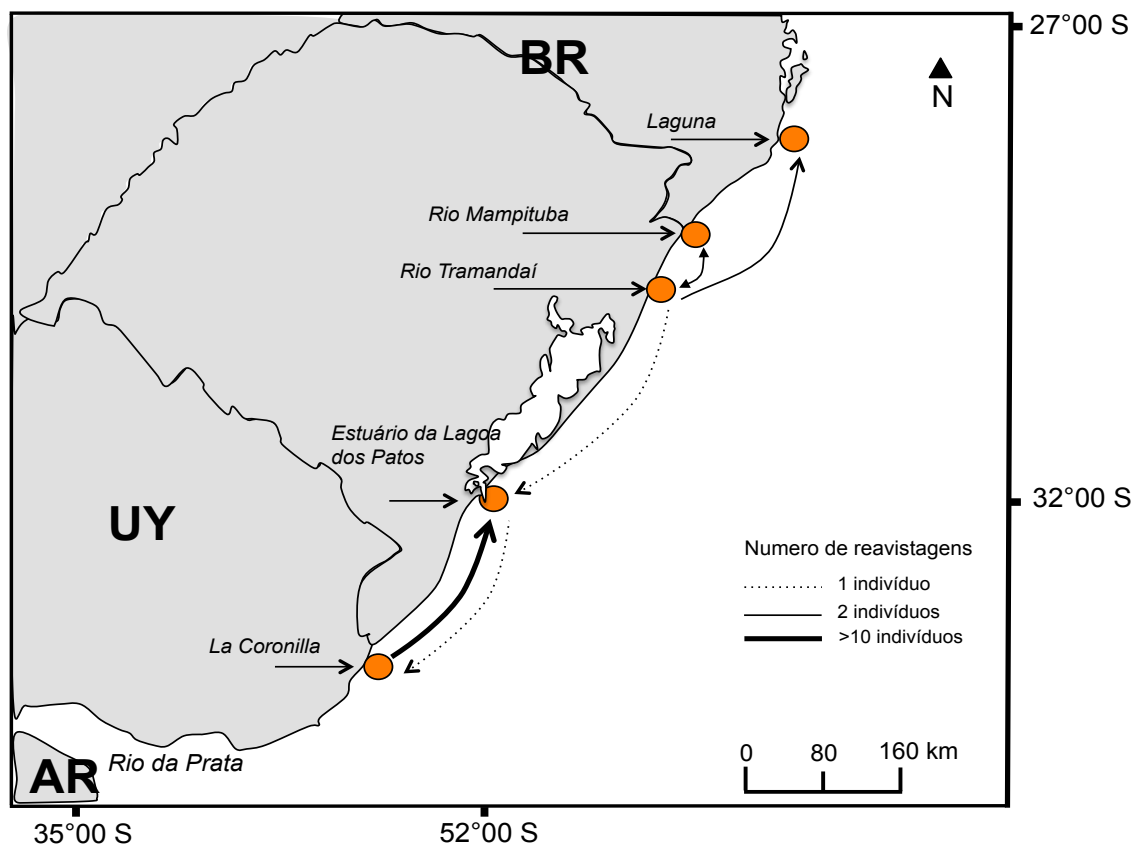


Figura 3. Comunidades de botos (*Tursiops truncatus*) associadas a estuários e rios no sul do Brasil (BR) e a área preferencial da comunidade costeira de botos na costa uruguaia (UY) (círculos laranja). Setas indicam a direção dos movimentos e o número de indivíduos reavistados entre as áreas durante estudos anteriores de marcação-recaptura (Möller *et al.* 1994; Simões-Lopes & Fábian 1999; Hoffmann 2004; Laporta 2009).

Têm sido registradas altas taxas de reavistagens para quase todos os indivíduos da comunidade de botos de Laguna, com baixas probabilidades de emigração temporária (Daura-Jorge *et al.* 2013). Em contraste, embora os botos de Tramandaí e Torres tenham um certo grau de fidelidade local, eles parecem abandonar as áreas em determinadas épocas do ano por períodos mais longos do que registrado em outras localidades, provavelmente devido à realização de movimentos sazonais entre estas áreas ou para áreas mais distantes (*e.g.* Möller *et al.* 1994; Simões-Lopes & Fábian 1999; Hoffmann 2004). Além disso, alguns indivíduos avistados regularmente na costa oceânica uruguaia parecem mover-se sazonalmente para as águas costeiras próximas ao ELP durante os meses mais frios, enquanto que movimentos de indivíduos na direção oposta são raros (Laporta 2009) (Fig. 3).

1.10 Ecologia de botos do Estuário da Lagoa dos Patos

Possivelmente a maior comunidade de botos no sul do Brasil habita o Estuário da Lagoa dos Patos (ELP) e sistema marinho adjacente (Fruet *et al. no prelo*). Esta comunidade tem sido estudada desde meados de 1970 (Castello & Pinedo 1977) e alguns indivíduos foto-identificados na época ainda são regularmente registrados na mesma área (Fruet *et al.* 2011). Informações sobre a abundância, adquiridas em 1998 e 2005 por meio de estudos de MR, sugerem que esta é uma comunidade pequena (< 90 indivíduos) e residente durante todo o ano (Dalla Rosa 1999; Fruet *et al.* 2011). Os botos do ELP têm preferências claras por águas estuarinas (Di Tullio *et al. no prelo*). O tamanho médio dos grupos é de 4 botos ($SD = 2$), e indivíduos solitários representaram <10% das avistagens (Mattos *et al.* 2007). Eles usam as águas estuarinas para suas atividades diárias, mas concentram-se na desembocadura do estuário durante todo o ano para a alimentação (Mattos *et al.* 2007). Quando na região costeira, os botos

concentram-se entre a zona de arrebentação e em distâncias inferiores a 2 km da costa, com registros ocasionais entre 2 - 4 km, diminuindo a probabilidade de encontro à medida que a distância do estuário aumenta (Di Tullio *et al. no prelo*). Associações entre os indivíduos são predominantemente de curta duração (dinâmica de fissão-fusão) (Dalla Rosa 1999), apesar do registro de algumas associações de longa duração (Genoves 2013). A predação por tubarões ou orcas parece não ser uma ameaça para os botos nesta área (Fruet *et al.* 2012).

Os botos predam uma variedade de peixes, com preferência por scianídeos e outras espécies estuarinas-dependentes, as quais incluem a corvina (*Micropogonias furnieri*), peixe-espada (*Trichiurus lepturus*), maria-luísia (*Paralonchurus brasiliensis*), papa-terra (*Menticirrhus* spp.) e a tainha (*Mugil liza*) (Lopez 2014). Análises de isótopos estáveis a partir dos dentes de indivíduos encalhados no sul do Brasil, incluindo na amostra alguns botos residentes do ELP, identificaram dois grupos de botos que diferem na sua assinatura isotópica de $\delta^{15}\text{N}$, sugerindo haver uma partição na exploração dos recursos pelos botos costeiros no sul do Brasil (Botta *et al.* 2012).

1.11 O Estuário da Lagoa dos Patos

O estuário da Lagoa dos Patos é o maior estuário do tipo “estrangulado” do mundo, com uma superfície de aproximadamente 10.360 km² (Kjerfve 1986). Ela está conectada ao Oceano Atlântico através de um estreito canal com largura de 0,5 a 3 km. As variações de temperatura da água são típicas dos sistemas temperados, com valores mais baixos (10° C a 15° C) durante o inverno austral e valores mais elevados durante os meses de verão (22° C a 30° C) (Pesquisa Ecológica de Longa Duração – PELD - FURG). Sua hidrodinâmica é impulsionada principalmente pela força e direção dos ventos e entrada de água proveniente de grandes bacias de drenagem (Möller *et al.*

2009). Este processo dinâmico de circulação de água desempenha um papel fundamental no controle da salinidade e variabilidade da produtividade no estuário da Lagoa dos Patos em várias escalas de tempo e espaço (Möller *et al.* 2009; Fujita & Odebrecht 2007; Abreu *et al.* 2010). Como tal, a composição e abundância de espécies de peixes estão sujeitas a grandes oscilações sazonais, anuais e interanuais (Garcia *et al.* 2012).

Embora o ecossistema estuarino compreenda apenas 5% da área total da Lagoa dos Patos, este ambiente possui diversos habitats, incluindo marismas, pradarias submersas e áreas rasas que proporcionam habitats críticos para o estabelecimento e desenvolvimento de várias espécies de peixes e crustáceos (Costa *et al.* 1997), os quais têm sido explorados na região pela pesca artesanal e industrial desde o final do século XIX (von Ihering 1885). A produção da pesca artesanal dentro da Lagoa dos Patos sofreu um colapso devido ao esforço pesqueiro excessivo e ao uso de equipamentos de pesca não seletivos (Reis 1992). Isto resultou na perda de biodiversidade, pobreza e perda da identidade cultural das comunidades pesqueiras locais (Kalikoski *et al.* 2002). A crescente escassez de recursos na Lagoa dos Patos e de aperfeiçoamento das tecnologias de pesca propiciou a intensificação do uso das águas costeiras rasas pelos pescadores artesanais (Kalikoski 2002).

Além da influência da pressão da pesca, o ELP sofreu mudanças significativas em sua morfologia e hidrodinâmica devido à expansão urbana, industrial e portuária no último século (Tagliani *et al.* 2003; Odebrecht *et al.* 2010). Este estuário abriga um porto de grande capacidade operacional em uma região estratégica do Brasil, e uma série de grandes obras de infraestrutura portuária (por exemplo, a expansão dos molhes e dragagem do canal do estuário) estão agora em andamento. Recentemente, ampliou-se

a extensão dos molhes e intensificaram-se as atividades de dragagem. Como consequência, novas mudanças no regime hidrológico do estuário são esperadas (*e.g.* intensificação dos fluxos de saída de água - Fernandes *et al.* 2005).

1.12 Ameaças enfrentadas pelos botos no ELP e a necessidade de obter informações prioritárias para sua conservação

Apesar de sua potencial vulnerabilidade aos impactos humanos, nenhuma análise robusta para classificar o status de conservação da comunidade dos botos do ELP foi realizada até hoje. As capturas incidentais em redes de pesca foram reportadas como esporádicas nas décadas passadas (Pinedo 1986). Recentemente, Fruet *et al.* (2012) avaliou a mortalidade dos botos baseado em dados de encalhes de 914 saídas de monitoramento de praia conduzidas entre 1969 e 2006 (incluindo os dados registrados por Pinedo 1986). Trinta e seis de 188 carcaças apresentaram evidências claras de emalhamento em redes de pesca (Fig. 4). A taxa de captura acidental permaneceu baixa durante 30 anos (1969-1999), mas tornou-se frequente na década passada nas áreas costeiras próximas à desembocadura do ELP.

Do número total de botos capturados pelas redes de pesca nas áreas próximas ao estuário ($n = 32$) durante os 40 anos de amostragem, 64% foram registrados entre os anos de 2002 e 2006. Durante o período supracitado o número mínimo de botos encontrados mortos anualmente variou de dois a nove, sendo a captura acidental responsável por, pelo menos, 43% dos registros nas áreas próximas ao estuário. A maioria das capturas acidentais ocorreu durante os meses de verão, quando o esforço pesqueiro com redes de emalhe é intenso no estuário e águas costeiras adjacentes (Klippel *et al.* 2005). Capturas acidentais foram maiores para os machos (3,5M:1F), sendo a maioria (57,1%) indivíduos imaturos (Fruet *et al.* 2012). Uma análise

preliminar do Potencial Biológico de Remoção (PBR) (Wade 1998) sugeriu que tais níveis de captura accidental para os botos da comunidade do ELP poderiam ser insustentáveis (Fruet *et al.* 2012). Entretanto, a análise foi realizada pressupondo-se que todos os botos encalhados próximo ao estuário pertenciam à comunidade do ELP (Fruet *et al.* 2012).



Figura 4. Carcaça de um boto (*Tursiops truncatus*) encontrada na costa sul do Brasil com evidências de ter sido capturado por rede de pesca. Esquerda: nadadeira caudal amputada por corte de faca. Direita: marcas de rede na nadadeira peitoral.

Estudos de MR baseados em saídas sistemáticas e intensas na área costeira desde 2007, revelaram a presença de indivíduos que nunca tinham sido registrados nas águas estuarinas (Fruet *et al.* 2011). Em uma análise de redes sociais baseada em dados de

foto-identificação, determinou-se que esses botos costeiros formam duas unidades sociais distintas e que ocupam as áreas costeiras ao sul e ao norte do ELP (Genoves 2013). Estas comunidades, em conjunto com a que habita o ELP, formam um mosaico de três unidades sociais que envolvem um complexo padrão de movimentos e sobreposição das áreas de vida em diferentes escalas temporais e geográficas (ver Fig. 3). Esta configuração implica em uma potencial conectividade reprodutiva, o que pode ter implicações para conservação. Além disso, caso carcaças de botos das comunidades costeiras estejam misturadas nos registros de monitoramento de praia, os efeitos da mortalidade não-natural sob a comunidade do ELP (Fruet *et al.* 2012) podem estar superestimados.

2. OBJETIVOS E ESTRUTURA DA TESE

A partir das informações apresentadas acima, fica claro que vários aspectos da biologia e ecologia dos botos do ELP permanecem desconhecidos, sendo necessário investigar os impactos das capturas acidentais na sua viabilidade populacional a longo prazo. Por exemplo, a comunidade do ELP deve ser tratada como uma unidade discreta para fins de manejo? Qual o grau de conectividade entre os botos do ELP e as comunidades de botos adjacentes? A captura acidental impacta a sobrevivência de todos os estágios de vida homogeneamente? As taxas de reprodução dos botos do ELP são suficientemente altas para permitir a recuperação das pressões determinísticas como a captura acidental por redes de pesca? Qual o efeito dos níveis atuais de mortalidade não-natural na sua persistência a longo prazo?

O objetivo geral desta tese é avaliar quantitativamente o status de conservação da comunidade de botos residente no estuário da Lagoa dos Patos, no sul do Brasil. Os objetivos específicos estão detalhados em cada capítulo da tese.

Esta tese está estruturada em um capítulo introdutório (este capítulo) e quatro capítulos contendo os resultados de pesquisas inéditas (ANEXOS I - IV). Apesar de estarem sendo apresentados como itens independentes, todos estão inter-relacionados e apresentados de acordo com a ordem cronológica de suas realizações. Esses capítulos foram escritos em forma de manuscritos que foram, ou serão, submetidos para posterior publicação em periódicos científicos internacionais. Os dados utilizados foram adquiridos a partir de estudos de longo-prazo que envolveram muitos pesquisadores e instituições de pesquisas. Portanto, eu compartilho co-autorias com pessoas que contribuíram substancialmente com a coleta e análise de dados, e que também tenham prestado assistência durante o planejamento e escrita dos artigos. Abaixo deixo explícito o racional para a estruturação da tese.

No capítulo 2 (ANEXO I), combinando a análise molecular de 16 loci de microssatélites e sequências da região controle do DNA_{mt}, foi investigada a diversidade genética e estrutura populacional do boto no Oceano Atlântico Sul Ocidental, com o objetivo de determinar o grau de conectividade entre a comunidade do ELP e outras comunidade de botos em áreas adjacentes. Nos capítulos 3 e 4 (ANEXOS II e III) estimaram-se parâmetros demográficos específicos (*e.g.* abundância, taxas de sobrevivência, razão sexual de adultos, fecundidade, sazonalidade reprodutiva) necessários para subsequente modelagem demográfica. Estes estudos basearam-se em análises de dados de marcação-recaptura e mortalidade, os quais foram obtidos a partir de estudos sistemáticos de longo prazo (2005-2012) conduzidos no estuário da Lagoa dos Patos e áreas costeiras adjacentes. No capítulo 5 (ANEXO IV) construiu-se uma matriz de modelo populacional estruturado por estágios de vida para analisar a demografia da comunidade dos botos do ELP, utilizando-se como parâmetros de entrada do modelo os dados de história de vida estimadas nos capítulos anteriores.

Finalmente, baseado no modelo demográfico, uma análise de viabilidade populacional foi realizada para simular os riscos da comunidade de botos do ELP sob diferentes cenários de capturas acidentais, considerando-se efeitos de incertezas nas estimativas dos parâmetros e de estocasticidade nas projeções. Abaixo, apresento os principais resultados para cada capítulo subsequente, os quais serão incluídos nesta tese como anexos, assim como o status das publicações.

3. SÍNTESE DOS CAPÍTULOS

3.1 CAPÍTULO 2 (ANEXO 1) - Baixa diversidade genética e forte estruturação populacional nos botos (*Tursiops truncatus*) de águas costeiras do Oceano Atlântico Sul Ocidental (Fruet *et al.* 2014, *Conservation Genetics* 15:879-895). Análises de 16 loci de microssatélites e sequências de um segmento de aproximadamente 550pb da região controle do DNA mitocondrial (DNA_{mt}) foram combinados para investigar a diversidade genética, estrutura populacional e conectividade dos botos costeiros do Oceano Atlântico Sul Ocidental (ASO). Foram coletadas um total de 124 amostras de pele de botos fotograficamente identificados ao longo de seis comunidades costeiras do sul do Brasil, Uruguai e Argentina central. Níveis de diversidade genética nuclear foram notavelmente baixos (valores médios de diversidade alélica e heterozigosidade em todos os loci foram de 3,6 e 0,21, respectivamente), um resultado que possivelmente reflete o pequeno tamanho das comunidades locais de botos costeiros ao longo do ASO. Em uma larga escala geográfica, foi encontrada forte e significativa estruturação genética entre os botos amostrados no sul do Brasil e Uruguai (SB-U) e aqueles na Bahía San Antonio (BSA), Argentina (AMOVA DNA_{mt} Φ_{ST} = 0,43; F_{ST} microssatélites = 0,46), detectando-se insignificante fluxo gênico contemporâneo, baseado em estimativas Bayesianas. Em

uma menor escala geográfica, diferenciação moderada, mas significativa (AMOVA DNA_{mt} $\Phi_{ST} = 0,29$; F_{ST} microssatélites = 0,13) e fluxo gênico assimétrico foi detectada entre as cinco comunidades de botos vizinhas no SB-U. O isolamento por distância, ao invés do tipo de habitat (costeiro versus estuarino), explicou melhor os padrões de estruturação encontrados. Com base nestes resultados, sugere-se que BSA e SB-U representam distintas unidades evolutivamente significantes (*ESUs*), e que as cinco comunidades de botos amostradas ao longo da costa do SB-U representam independentes Unidades de Manejo (*MUs*), as quais configuram-se como uma metapopulação. Sob este cenário, recomenda-se que os esforços de conservação devam priorizar as áreas do sul do Brasil, onde botos de no mínimo três *MUs* sobrepõem suas áreas de vida e onde as taxas de capturas acidentais são mais elevadas.

3.2. CAPÍTULO 3 (ANEXO II) - Abundância e demografia de uma comunidade de botos residente em um estuário subtropical do Oceano Atlântico Sul Ocidental (manuscrito aceito para publicação no periódico Journal of Mammalogy). Neste trabalho estimaram-se diversos parâmetros demográficos de uma comunidade de boto (*Tursiops truncatus*) que habita o estuário da Lagoa dos Patos e costa marítima adjacente, no sul do Brasil. Para tanto, realizou-se uma análise estatística de dados de marcação-recaptura obtidos ao longo de 8 anos (2005-2012) que foram coletados sistematicamente por meio da aplicação da técnica de foto-identificação (reconhecimento individual dos botos por meio de fotografias das nadadeiras dorsais). Sob o modelo mais parcimonioso de Desenho Robusto de Pollock, o qual desconsiderou os efeitos da emigração temporária, a estimativa de sobrevivência anual aparente foi maior para fêmeas adultas (0,97; 95% CI: 0,91-0,99) do que para machos adultos (0,88; 95% CI: 0,75-0,94) e juvenis (0,83; 95% CI: 0,64-0,93), o que pode explicar a razão sexual observada (1M:2F) para a parcela de indivíduos adultos desta comunidade. Uma

tendência de aumento da abundância de indivíduos marcados foi observada durante os primeiros seis anos de amostragem, quando o número de novos recrutas superou a mortalidade, seguido por uma diminuição notável nos últimos dois anos, quando uma relação inversa de recrutas/mortes ocorreu. Alterações anuais na abundância (λ_t) variaram de -0,1 a 0,07. Estimativas de abundância de toda a comunidade (incluindo indivíduos marcados e não marcados) tiveram alta precisão (o maior CV foi 0,053) e não excederam 88 indivíduos (95% IC = 70 - 94), confirmando o pequeno tamanho da comunidade de botos do estuário da Lagoa dos Patos. As estimativas de abundância obtidas neste estudo foram semelhantes àquela obtida em um estudo anterior realizado na mesma área quase uma década antes, o que sugere uma relativa estabilidade ao longo dos últimos 14 anos. A aparente estabilidade na abundância, no entanto, deve ser vista com cautela, pois esta comunidade precisaria sofrer uma mortalidade significativa de 10% antes de que um declínio no seu tamanho pudesse ser detectado com o poder estatístico desejável de 90%.

3.3 CAPÍTULO 4 (ANEXO III) – Integrando dados de marcação-recaptura e encalhes para estimar as características reprodutivas das fêmeas de botos (*Tursiops truncatus*) no Oceano Atlântico Sul Ocidental (Manuscrito em revisão no periódico “*Marine Biology*”; submetido em 5 de junho de 2014). Apesar de bem estudado em várias regiões, não há informação sobre a reprodução dos botos (*Tursiops truncatus*) do Oceano Atlântico Sul Ocidental (ASO). Dados de marcação-recaptura e de indivíduos encalhados obtidos através de programas de monitoramento sistemáticos foram utilizados para estimar alguns parâmetros da história de vida das fêmeas de botos que habitam o estuário da Lagoa dos Patos (ELP). A partir da análise de 32.296 fotografias de alta qualidade das nadadeiras dorsais dos animais foi possível identificar e monitorar 37 fêmeas adultas e um total de 66 filhotes. Os resultados apontam

nascimentos ocorrendo em pulso, concentrados durante a primavera e o final do verão, associados com o aumento da temperatura da água e maior oferta de alimento. As fêmeas desta comunidade reproduziram com idade mínima de 8 anos. A média de intervalo de tempo entre sucessivos nascimentos para uma mesma fêmea foi estimada em 3 anos (moda = 2) e a fecundidade estimada em 0,11. Ao analisar os isótopos estáveis nos dentes de carcaças de botos encalhados, observou-se uma clara mudança nos perfis de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ próximos à idade de 2 anos, indicando a idade mais provável de desmame. Aplicando-se os dados de foto-identificação ao modelo de marcação-recaptura de Cormack-Jolly-Seber, as taxas de sobrevivência de filhotes entre 0-1 ano e 1-2 anos foram estimadas em 0,84 (IC 95% = 0,72-0,90) e 0,86 (IC 95% = 0,74-0,94), respectivamente. Fêmeas mais velhas parecem reproduzir em menores frequências do que fêmeas mais jovens, o que sugere uma diminuição na aptidão reprodutiva relacionada à idade dos indivíduos. Em nível individual, foi observada uma marcada variação no sucesso reprodutivo (*i.e.* a geração de um filhote e a garantia de sua sobrevivência até que ele atinja 2 anos de idade – a idade mínima estimada para o desmame). Os resultados sugerem que, logo após a maturidade sexual, as fêmeas de botos da comunidade do ELP tendem a alocar mais energia para a reprodução do que para o cuidado parental. Isto é seguido por uma diminuição nas taxas reprodutivas e um potencial aumento no investimento de cuidado parental durante o último terço de sua vida reprodutiva.

CAPÍTULO 5 (ANEXO IV) – Dinâmica e viabilidade de uma comunidade residente de botos (*Tursiops truncatus*) no sul do Brasil (Manuscrito não submetido para publicação). Identificar populações ameaçadas e quantificar a sua vulnerabilidade é crucial para o estabelecimento de prioridades de conservação e para fornecer informações confiáveis durante os processos de tomada de decisão. Os botos (*Tursiops*

truncatus) têm sido vítimas de capturas acidentais na pesca com redes de emalhe ao longo das águas costeiras do sul do Brasil, principalmente no estuário da Lagoa dos Patos (ELP) e águas costeiras adjacentes. Um estudo anterior sugeriu que estes níveis de mortalidade não-natural relacionadas diretamente com atividades pesqueiras poderia ser insustentável para a pequena comunidade residente de botos do ELP. No entanto, uma avaliação contundente do impacto das capturas acidentais sobre a viabilidade desta comunidade ainda não foi realizada. Neste estudo foi utilizada uma matriz de modelo populacional estruturada por estágios de vida para realizar uma análise demográfica da comunidade de botos do ELP, utilizando-se dados de história de vida estimados especificamente durante um estudo de longo prazo de marcação-recaptura com esses animais. Uma análise de viabilidade populacional foi utilizada para executar uma série de simulações, onde o risco foi avaliado sob diferentes cenários de capturas acidentais, considerando-se os efeitos das incertezas dos parâmetros e de estocasticidade ambiental e demográfica nas projeções. Na ausência de capturas acidentais, foi estimado que a comunidade de botos do ELP teria um crescimento de cerca de 3% ao ano (IC 95%: 1,2% - 5,8%). Sob os efeitos atuais das taxas de capturas acidentais e incertezas nas estimativas dos parâmetros, prognósticos indicaram altas probabilidades de viabilidade desta comunidade ao longo dos próximos 60 anos. Estes prognósticos otimistas parecem estar associados com as elevadas taxas de sobrevivência de fêmeas adultas e um ambiente relativamente estável fornecido pelo ELP. No entanto, a potencial remoção de algumas fêmeas adultas (uma por ano ou uma a cada dois anos) resultaria em uma eminente probabilidade de declínio em todos os níveis pré-especificados (redução de 30%, 50%, 80% do seu tamanho inicial). A viabilidade da comunidade de botos do ELP seria substancialmente beneficiada caso ocorra um aumento na sobrevivência de juvenis/sub-adultos. Isto pode ser atingido através da recente implementação da área de

proteção para o boto no sul do Brasil, a qual proíbe a pesca de emalhe por embarcações na área preferencial dos botos do ELP. Caso a área seja efetiva (*i.e.* reduza a mortalidade dos botos juvenis, os mais suscetíveis) existe uma considerável chance de aumentar a abundância da comunidade em 20% de seu tamanho atual em menos de 60 anos.

4. CONCLUSÕES

- Os botos costeiros do Atlântico Sul Ocidental possuem variabilidade genética extremamente reduzida;
- Os botos do sul do Brasil e Uruguai estão reprodutivamente isolados dos botos da Baía de San Antonio, Argentina central;
- No sul do Brasil, as comunidades de botos formam, no mínimo, cinco Unidades de Manejo distintas;
- A comunidade de botos do estuário da Lagos dos Patos pode ser considerada como uma unidade demograficamente discreta, composta por indivíduos com alto grau de residência onde sua dinâmica é substancialmente afetada por nascimentos e mortes, e não por eventos de imigração/emigração;
- A sobrevivência das fêmeas adultas é superior à dos machos adultos e de botos juvenis, como resultado de vieses nas capturas acidentais para estas últimas classes;
- A comunidade de botos tem uma abundância relativamente estável, mas com desequilíbrio na razão sexual de adultos (2F:1M);
- A reprodução dos indivíduos é lenta, e os nascimentos ocorrem em pulso, entre o final da primavera e o final do verão;
- As fêmeas começam a reproduzir com idade mínima de 7 anos e reproduzem-se

em taxas mais elevadas durante seus primeiros anos de vida reprodutiva;

- A comunidade do ELP suporta os níveis atuais de capturas acidentais, porém um pequeno aumento das capturas, especialmente de fêmeas adultas, pode levar esta comunidade a um rápido declínio;
- A manutenção da área de proteção ao boto projeta cenários otimistas para o futuro da comunidade de botos do ELP, caso seja efetiva na diminuição da mortalidade de botos juvenis nas redes de pesca de emalhe.

5. CONSIDERAÇÕES FINAIS

5.1 Conservação dos botos costeiros no Oceano Atlântico Sul Ocidental

Combinando-se os resultados obtidos nesta tese com as informações atualmente disponíveis na bibliografia, espera-se proporcionar novos rumos para a conservação dos botos costeiros do ASO. A ampla distribuição geográfica da espécie não deve ser utilizada como motivo para subestimar as suas necessidades de conservação. Comunidades de botos locais em todo o mundo estão em perigo (*e.g.* Currey *et al.* 2009; Tezanos-Pinto *et al.* 2013), incluindo os botos costeiros no ASO. Nesta região, as comunidades ocorrem em baixas densidades (revisada por Fruet *et al.* no prelo), possuem níveis extremamente reduzidos de diversidade genética (Fruet *et al.* 2014), e restringem-se ao uso de águas rasas costeiras (revisado por Laporta *et al.* no prelo), tornando-os particularmente vulneráveis a impactos humanos. As áreas centrais de distribuição do boto (por exemplo, foz dos rios e águas costeiras rasas) são sujeitas a diferentes níveis de atividades de pesca, tráfego de embarcações, poluição sonora e química, e vários estoques populacionais de peixes que fazem parte de sua dieta estão considerados exauridos (*e.g.* Reis & D'Incao 2000; Haimovici *et al.* 2006). É uma fonte adicional de preocupação o aumento na incidência de botos afetados por graves doenças

de pele no sul do Brasil (*e.g.* Moreno *et al.* 2008, Daura-Jorge & Simões-Lopes 2011). Todas estas ameaças tornam-se ainda mais críticas com a recente descoberta de que a pequena comunidade de botos da Argentina central está isolada dos coespecíficos da costa sul do Brasil-Uruguai (SB-U), onde os botos subdividem-se em pelo menos cinco unidades de manejo independentes (Fruet *et al.* 2014). Apesar dos avanços notáveis no conhecimento sobre vários aspectos ecológicos e ameaças enfrentadas pelos botos no ASO, o nível de informação atual não permite avaliar o status de conservação de todas as unidade de manejo ao longo da distribuição da espécie nesta região. Os objetivos e metas de conservação devem ser urgentemente traçados para garantir a viabilidade destas unidades discretas a longo-prazo, impedindo uma maior redução na abundância e permitindo com que elas cresçam e expandam suas áreas de vida e, se possível, aumentem o fluxo gênico e variabilidade genética. O manejo adaptativo (*e.g.* Holling 1978; Hilborn 1992; Walters 1997) deve ser adotado para evitar que a demora da implementação de um plano “ideal” de manejo (*i.e.* baseado no “conhecimento científico necessário”) acarrete efeitos ecológicos difíceis de serem revertidos. Além disso, adotando-se o manejo adaptativo, as estratégias de conservação para os botos no sul do Brasil-Uruguai podem ser reavaliadas e ajustadas à medida que novas informações sobre o status dessas unidades sejam obtidas.

O governo brasileiro recentemente estabeleceu uma área de proteção ao boto, onde a pesca de emalhe a partir de embarcações fica proibida ao longo de todo ano no estuário da Lagoa dos Patos e áreas costeiras adjacentes (Brasil 2012). Esta área de proteção, se respeitada, deverá reduzir as taxas de captura acidentais no sul do Brasil, uma vez que abrange a área central de distribuição da comunidade de botos do ELP e parte da distribuição de duas comunidades costeiras (Genoves 2013). No entanto, a área de proteção não é suficiente para evitar a captura acidental em outras áreas utilizadas

pelos botos ou até mesmo dentro da área de proteção, uma vez que outros tipos de atividades de pesca que sabidamente matam os botos (*p.ex.* redes de emalhe de calão fixados na praia) ainda são permitidos dentro da área de proteção (Brasil 2012).

5.2 Recomendações para futuras pesquisas e monitoramento

Com base no conhecimento atual e necessidades de conservação, recomenda-se:

- Realizar um estudo ecológico abrangente, a fim de investigar a conectividade genética e ecológica entre ecótipos costeiros e oceânicos dos botos ao longo do ASO;
- Reavaliar a estrutura genética dos botos costeiros, incluindo nas análises biópsias de pele de botos de regiões que não foram incluídas neste estudo, com atenção especial para a costa norte da Argentina e águas costeiras do sul do Brasil e Uruguai;
- Identificar o limite norte da Unidade Evolutivamente Significante de botos do Sul do Brasil-Uruguai (*ESU SB-U*) com base em dados genéticos adicionais e de distribuição.

Para cada unidade de manejo identificada dentro da ESU SB-U, recomenda-se:

- Utilizar uma abordagem de marcação-recaptura para estimar a abundância e parâmetros da história de vida estratificada por sexo e estágio de vida dos indivíduos;
- Determinar as suas áreas de vida;
- Realizar uma avaliação de risco utilizando uma abordagem semelhante à utilizada neste estudo.

Especificamente para a comunidade de botos do ELP, recomenda-se:

- Monitorar a eficácia da área de proteção recentemente implementada através de um processo contínuo de acompanhamento de tendências na abundância e outros parâmetros de história de vida;
- Utilizar dados de marcação-recaptura para estimar a área de vida dos indivíduos por sexo e estágio de vida;
- Determinar a sobreposição espacial e temporal do uso de habitat com as comunidades costeiras adjacentes.

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Remarkably low genetic diversity and strong population structure in common bottlenose dolphins (*Tursiops truncatus*) from coastal waters of the Southwestern Atlantic Ocean

Pedro F. Fruet · Eduardo R. Secchi · Fábio Daura-Jorge · Els Vermeulen · Paulo A. C. Flores · Paulo César Simões-Lopes · Rodrigo César Genoves · Paula Laporta · Juliana C. Di Tullio · Thales Renato O. Freitas · Luciano Dalla Rosa · Victor Hugo Valiati · Luciano B. Beheregaray · Luciana M. Möller

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Abstract Knowledge about the ecology of bottlenose dolphins in the Southwestern Atlantic Ocean is scarce. Increased by-catch rates over the last decade in coastal waters of southern Brazil have raised concerns about the decline in abundance of local dolphin communities. Lack of relevant data, including information on population structure and connectivity, have hampered an assessment of the conservation status of bottlenose dolphin communities in this region. Here we combined analyses of 16 microsatellite loci and mitochondrial DNA (mtDNA) control region sequences to investigate genetic diversity, structure and

connectivity in 124 biopsy samples collected over six communities of photographically identified coastal bottlenose dolphins in southern Brazil, Uruguay and central Argentina. Levels of nuclear genetic diversity were remarkably low (mean values of allelic diversity and heterozygosity across all loci were 3.6 and 0.21, respectively), a result that possibly reflects the small size of local dolphin communities. On a broad geographical scale, strong and significant genetic differentiation was found between bottlenose dolphins from southern Brazil–Uruguay (SB–U) and Bahía San Antonio (BSA), Argentina (AMOVA mtDNA $\Phi_{ST} = 0.43$; nuclear $F_{ST} = 0.46$), with negligible contemporary gene flow detected based on Bayesian estimates. On a finer scale, moderate but significant differentiation (AMOVA mtDNA $\Phi_{ST} = 0.29$; nuclear $F_{ST} = 0.13$) and

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P. F. Fruet (✉)
 Programa de Pós-Graduação em Oceanografia Biológica, FURG,
 Rio Grande, Brazil
 e-mail: pfruet@gmail.com

P. F. Fruet · L. B. Beheregaray · L. M. Möller
 Molecular Ecology Laboratory, School of Biological Sciences,
 Flinders University, Bedford Park, SA, Australia

P. F. Fruet · E. R. Secchi · R. C. Genoves ·
 J. C. Di Tullio · L. D. Rosa
 Museu Oceanográfico “Prof. Eliézer C. Rios”, FURG,
 Rio Grande, Brazil

P. F. Fruet · E. R. Secchi · R. C. Genoves ·
 J. C. Di Tullio · L. D. Rosa
 Laboratório de Ecologia e Conservação da Megafauna Marinha,
 Instituto de Oceanografia, FURG, Rio Grande, Brazil

P. F. Fruet · L. M. Möller
 Cetacean Ecology, Behaviour and Evolution Lab, School of
 Biological Sciences, Flinders University, Bedford Park, SA,
 Australia

F. Daura-Jorge · P. C. Simões-Lopes
 Laboratório de Mamíferos Aquáticos (LAMAQ), UFSC,
 Florianópolis, Brazil

E. Vermeulen
 Laboratory of Oceanology - MARE Research Centre, University
 of Liege, Liège, Belgium

P. A. C. Flores
 Centro Nacional de Pesquisa e Conservação de Mamíferos
 Aquáticos - CMA, ICMBio, MMA, Florianópolis, Brazil

P. Laporta
 Yaqu-pacha Uruguay, Punta del Diablo, Rocha, Uruguay

T. R. O. Freitas
 Departamento de Genética, Universidade Federal do Rio Grande
 do Sul, Porto Alegre, Brazil

V. H. Valiati
 Laboratório de Biologia Molecular, Unisinos, São Leopoldo,
 Brazil

asymmetric gene flow was detected between five neighbouring communities in SB–U. Based on the results we propose that BSA and SB–U represent two distinct evolutionarily significant units, and that communities from SB–U comprise five distinct Management Units (MUs). Under this scenario, conservation efforts should prioritize the areas in southern Brazil where dolphins from three MUs overlap in their home ranges and where by-catch rates are reportedly higher.

Keywords Cetacean · Conservation · Connectivity · Population genetics · Microsatellite · Mitochondrial DNA

Introduction

Bottlenose dolphins (*Tursiops* spp.) are cetaceans able to explore, occupy and adapt to different marine environments, with the exception of polar regions. Many genetic studies of bottlenose dolphins around the globe have reported moderate genetic differentiation among regional populations, despite some reproductive exchange (Sellas et al. 2005; Rosel et al. 2009; Tezanos-Pinto et al. 2009; Urian et al. 2009; Mirimin et al. 2011). Over large spatial scales, genetic discontinuities appear to coincide with ecological and topographic breaks, such as distinct water masses, currents and depth contours (Hoelzel et al. 1998a; Natoli et al. 2004; Bilgmann et al. 2007). On the other hand, habitat selection (e.g. open coast vs. estuarine ecosystems) and local adaptation to prey resources are believed to shape population structure over small spatial scales (Möller et al. 2007; Wyszniowski et al. 2010). Therefore, a combination of environmental, geomorphological and evolutionary factors appears to influence the genetic structure of bottlenose dolphin populations, although some may represent cryptic species-level differences (e.g. Natoli et al. 2004; Rosel et al. 2009).

Despite being extensively studied in many regions of the world, limited information is available for bottlenose dolphins of the Southwestern Atlantic Ocean (SWA); particularly scarce are details of their genetic diversity and population structure. Understanding population sub-divisions and connectivity provides information critical to the identification of relevant biological units to be conserved. These include evolutionary significant units (ESUs)—a group of historically isolated populations with unique genealogical and adaptive legacy—and Management Units (MUs)—demographically distinct populations that should be managed separately to ensure the viability of the larger metapopulation (see Funk et al. 2012 for definitions and a recent perspective on ESUs and MUs). This is especially important in cases where populations are restricted in distribution, have small population sizes and are subject to

human induced mortality, which is the case for bottlenose dolphins of the SWA. It has been reported that in the SWA coastal bottlenose dolphins are mainly found between Santa Catarina State, in southern Brazil, and Central Argentina—and particularly along a narrow coastal corridor between southern Brazil and Uruguay (SB–U) (Laporta et al. in press). In this region, bottlenose dolphins occur in bays and estuaries, and between the surf zone and 2 km from the coastline when in the open-coast, with occasional records between 2 and 4 km (Laporta 2009; Di Tullio 2009). The distribution of coastal and offshore bottlenose dolphins apparently does not overlap and their feeding ecology is distinct, at least in part of the SWA (e.g. Botta et al. 2012). Concerns about the conservation of coastal bottlenose dolphins in SWA has recently emerged due to their relatively small population sizes (Laporta 2009; Fruet et al. 2011; Daura-Jorge et al. 2013), vulnerability to by-catch (Fruet et al. 2012) and substantial coastal development, particularly in southern Brazil (Tagliani et al. 2007). A long-term study of dolphin strandings has revealed high levels of mortality along Brazil's southernmost coastline, mainly in areas adjacent to the Patos Lagoon estuary where by-catch seems to be the main cause of death (Fruet et al. 2012).

Systematic photo-identification studies have shown that coastal bottlenose dolphins of the SWA consist of small communities with high site fidelity to estuaries and river mouths (and each community not exceeding 90 individuals, Fruet et al. in press a). These are often bordered by other small bottlenose dolphin communities that show more extensive movements along the coast, in contrast to estuarine communities (Laporta et al. in press). Photo-identification efforts in the two main estuaries of southern Brazil suggest that bottlenose dolphins exhibit long-term residency in these areas (Fruet et al. 2011; Daura-Jorge et al. 2013). Although there is distribution overlap of dolphins from these estuarine-associated and the adjacent coastal communities, no information is available on the levels of genetic connectivity among them. For example, social network analyses has revealed the existence of at least three distinct communities, which partially overlap in range near the Patos Lagoon estuary, in southern Brazil (Genoves 2013). This includes the year-round resident community of the Patos Lagoon estuary and two coastal communities: one that regularly moves from Uruguay to southern Brazil during winter and spring (Laporta 2009) and another which appears to inhabit the adjacent coastal waters of the Patos Lagoon estuary year-round. Such range overlap suggests potential for interbreeding among individuals of these communities, which would have implications for MUs classification and conservation management efforts. Given the assumption of demographic independence between different MUs, their delineation requires a

direct or indirect estimate of current dispersal rates (Palsbøll et al. 2007). However, dispersal rates can be difficult to estimate, particularly in the marine environment, which lacks marked physical barriers and where many organisms are not easily accessible for long-term field studies of identifiable or tagged individuals. In these cases, genetic methods generally offer a suitable alternative to assess dispersal rates and other indicators of demographic independence, as well as for estimating genetic diversity.

In this study we investigate the genetic diversity and population structure of bottlenose dolphins along the SWA coast using data from nuclear microsatellite markers and mtDNA control region sequences. We use this information to assess the strength and directionality of genetic connectivity over a range of spatial scales. Our sampling design allows comparisons among neighbouring coastal communities in southern Brazil-Uruguay (SB-U), and between these and a community inhabiting Bahía San Antonio (BSA) in the Patagonian coast—the most southern resident bottlenose dolphin community known for the SWA and located in a different marine biogeographical region to southern Brazil-Uruguay. We hypothesize that specialization for, or association with particular habitat types such as estuaries and open coasts may promote genetic differentiation on small spatial scales, while the biogeographical disjunction may influence differentiation at broad scale. The adjacent dolphin communities sampled in SB-U include two estuarine and three open coast communities. If habitat type specialization or, association with, drives genetic structure, we might expect to find lower genetic differentiation between communities inhabiting the contiguous open coast habitat than those living in sheltered estuarine environments, irrespective of geographical distances. We also expect that greater differentiation would characterize communities from different biogeographical regions. By delineating conservation units for coastal bottlenose dolphins in the SWA we expect to provide scientific support to guide strategies for population monitoring efforts, conservation status assessment and short-term management goals.

Methods

Sampling scheme

The study area covers approximately 2,112 km of linear distance along the coast. It extends from Florianópolis, in southern Brazil, to Bahía San Antonio, in the Patagonian Argentina. Along this region we surveyed six locations between 2004 and 2012 and collected 135 samples (Fig. 1). Samples consisted primarily of skin tissue obtained from free-ranging coastal bottlenose dolphins (common bottlenose

dolphins, *Tursiops truncatus*—see Wang et al. (1999) for southern Brazil bottlenose dolphins molecular taxonomic identification) belonging to communities inhabiting a variety of habitat types: Florianópolis (FLN, coastal, $n = 9$), Laguna (LGN, estuarine, $n = 11$), north of Patos Lagoon (NPL, coastal, $n = 21$), Patos Lagoon estuary (PLE, estuarine, $n = 71$), south of Patos Lagoon/Uruguay (SPL/URU, coastal, $n = 14$) and Bahía San Antonio, Argentina (BSA, coastal bay, $n = 12$) (Table 1). Samples were collected using a crossbow with 150 lb (68 kg) draw weight and darts and tips especially designed for sampling small cetaceans (Ceta-Dart, Copenhagen, Denmark). We attempted to individually identify sampled dolphins through simultaneous photo-identification (see Fruet et al. in press b for details). Samples were grouped according to the sampled location. For those collected in the adjacent coastal areas of Patos Lagoon estuary, where three distinct communities live in close proximity and overlap in their range, identified individuals were grouped according to the social unit to which they were previously assigned based on social network analysis (Genoves 2013). Our dataset also included four samples from freshly stranded carcasses, two collected in La Coronilla, Uruguay, and two in southern Brazil from animals known to belong to the NPL community as photo-identified based on their natural marks prior to their death. Samples were preserved in 20 % dimethyl sulphoxide (DMSO) saturated with sodium chloride (Amos and Hoelzel 1991) or 98 % ethanol.

Genetic methods

Genomic DNA was extracted from all samples following a salting-out protocol (Sunnucks and Hales 1996). Sex of each biopsy sample was determined by the amplification of fragments of the *SRY* and *ZFX* genes through the polymerase chain reaction (PCR) (Gilson et al. 1998), with PCR conditions described in Möller et al. (2001). Samples were genotyped at 16 microsatellite loci (Online Resource 1) and a fragment of approximately 550 bp of the control region was sequenced using primers Dlp-1.5 and Dlp-5 (Baker et al. 1993) on an ABI 3730 (Applied Biosystems) with GenScan 500 LIZ 3130 internal size standard. Procedures for microsatellite PCR and genotyping are found in Möller and Beheregaray (2004), and for mtDNA PCR and sequencing in Möller and Beheregaray (2001). For microsatellites, bins for each locus were determined and genotypes scored in GENE-MAPPER 4.0 (Applied Biosystems). Rare alleles (i.e. frequency < 0.05) or alleles that fell in between two bins were re-genotyped. Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) was used to check for potential scoring errors, the presence of null alleles, stuttering and large allelic drop out. Genotyping error rates were estimated by re-genotyping 30 randomly selected samples, representing 22 % of the total sample size used in this study. We used GENALEX 6.5

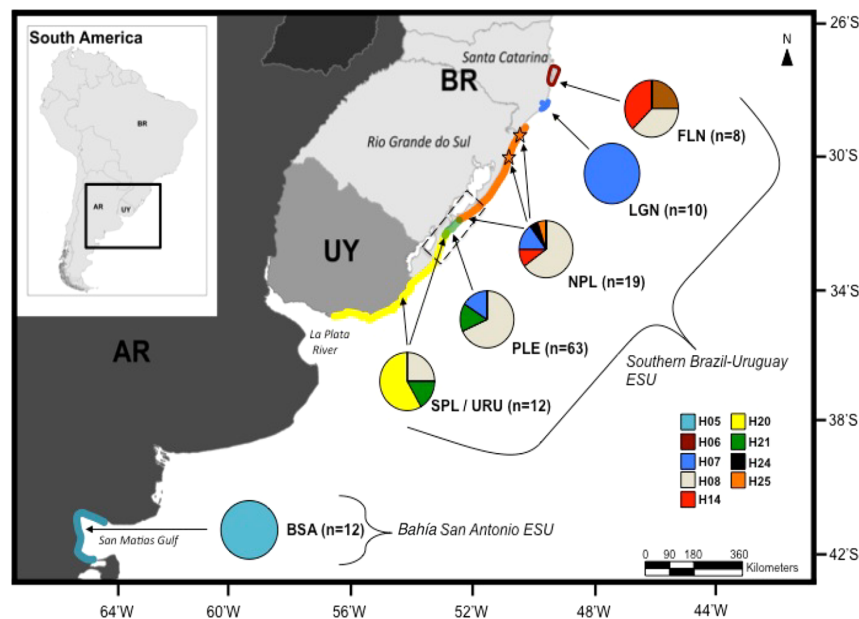


Fig. 1 Study area in the Southwestern Atlantic Ocean showing the proposed evolutionary significant units (ESUs) and management units (MUs) (color counter lines) for coastal common bottlenose dolphins (*Tursiops truncatus*), and the respective frequencies of mitochondrial control region haplotypes (pie charts). Arrows indicate the main sampling locations for each dolphin community. Approximate geographic boundaries of management units were built combining the results of this study with current knowledge on residency, social structure and movement patterns of bottlenose dolphins along this

region. Specifically for NPL, the genetic assignment of some individuals regularly sighted approximately 400 km north of Patos Lagoon estuary (represented by stars) to NPL community were used as a proxy to define the northern limit of the community range. The dashed rectangle highlights the area of heightened conservation concern proposed by this study (see “[Conservation implications](#)” section for details). FLN Florianópolis, LGN Laguna, NPL north of Patos Lagoon, PLE Patos Lagoon estuary, SPL/URU south of Patos Lagoon/Uruguay, BSA Bahía San Antonio. (Color figure online)

(Peakall and Smouse 2012) to find potential matches between genotypes and to estimate the probability of identity as an indicator of the power of the 16 markers to distinguish between two sampled individuals. Samples matching at all genotypes or those mismatching at only a few alleles (1–2) were double-checked for potential scoring errors. Sequences of the mtDNA were edited using SEQUENCHER 3.0 (Gene Codes Corporation, Ann Arbor, MI) and aligned using the ClustalW algorithm in MEGA 5.05 (Tamura et al. 2011). Haplotypes were defined using DNASP 5.0 (Librado and Rozas 2009). After careful examination, samples sharing identical genotypes at all loci, same mtDNA haplotype and sex were considered as re-sampled individuals and one of each pair was removed. Re-sampled individuals identified by photo-identification ($n = 7$) were also confirmed through genetic methods.

Data analysis

Population structure

We used 10,000 permutations in SPAGEDI to test for the relative importance of a stepwise mutation model as a

contributor to genetic diversity and structure (Hardy and Vekemans 2002). This provides a way to assess whether F_{ST} or R_{ST} potentially provides a more appropriate statistic to estimate genetic structure since R_{ST} accounts for divergence times between microsatellite alleles and is thus expected to better reflect older divergences (Hardy et al. 2003). Allele size permutation test in SPAGEDI were non significant for all loci. This suggests that F_{ST} is likely the most appropriate estimator, and only F_{ST} values are therefore reported hereafter. ARLEQUIN 3.5.1.2 was used for an analysis of molecular variance (AMOVA) to evaluate differentiation between SB–U and BSA dolphins, and among SB–U communities, for both nuclear and mtDNA datasets. Degree of genetic differentiation among locations was also assessed using ARLEQUIN to calculate F_{ST} (Weir and Cockerham 1984) for microsatellites, and both F_{ST} and Φ_{ST} measures for mtDNA. For each of these measures we used the Tamura and Nei (1993) model with a gamma correction of 0.5. Significance was tested based on 10,000 permutations. We also estimated the statistical power to detect nuclear differentiation using POWSIM (Ryman and Palm 2006) by simulating six populations with samples sizes of each sampled community (8, 10, 19, 63, 12, 12) with F_{ST} of 0.05 (combining generation, time

Table 1 Ecological information and summary of genetic diversity for the six communities and the two proposed evolutionary significant units (ESUs) of coastal common bottlenose dolphins (*Tursiops truncatus*) based on mtDNA control region sequences and 15 microsatellite loci

	<i>N</i> (f:m)	Pop. size (95 % CI)	Habitat type	mtDNA		Microsatellites						<i>PI_U</i>	<i>PI_{SBS}</i>
				<i>h</i>	π	PA	NA	AR	<i>H_E</i>	<i>H_O</i>	<i>F_{IS}</i>		
Southern Brazil– Uruguay ESU	FLN	8 (6:2)	Unknown	Coastal	0.7500 (0.0965)	0.0045 (0.0032)	0	1.6	1.6	0.19	0.23	1.5×10^{-3}	4.3×10^{-2}
	LGN	10 (2:8)	59 (49–72) ^a	Estuarine	0.0000 (0.0000)	0.0000 (0.0000)	0	1.6	1.5	0.21	0.15	1.3×10^{-3}	3.6×10^{-2}
	NPL	19 (8:11)	Unknown	Coastal	0.5425 (0.1231)	0.0067 (0.0041)	2	2.3	1.9	0.20	0.19	7.5×10^{-4}	3.5×10^{-2}
	PLE	63 (38:25)	86 (78–95) ^b	Estuarine	0.4808 (0.0621)	0.0072 (0.0042)	9	3.0	2.0	0.26	0.26	4.6×10^{-5}	9.7×10^{-3}
	SPL/ URU	12 (5:7)	Unknown	Coastal	0.6484 (0.1163)	0.0067 (0.0041)	5	2.1	1.9	0.20	0.23	3.5×10^{-4}	2.4×10^{-2}
Bahía San Antonio ESU	Total	112 (59:53)	–	–	0.6457 (0.0404)	0.0096 (0.0053)	16	3.7	2.2	0.22	0.22	–	–
	BSA	12 (2:10)	76 (70–97) ^c	Coastal Bays	0.0000 (0.0000)	0.0000 (0.0000)	1	1.76	1.76	0.19	0.18	2.6×10^{-3}	5.4×10^{-2}
	Total	124 (61:63)	–	–	0.7022 (0.0352)	0.0195 (0.0100)	–	3.6	–	0.28	0.23	0.194 [*]	–

N total number of individuals (separated by sex); *PA* number of private alleles; *NA* mean number of alleles per locus; *AR* mean allelic richness; *H_E* mean expected heterozygosity; *H_O* mean observed heterozygosity; *F_{IS}* inbreeding coefficient; *PI_U*, *PI_{SBS}* probabilities of identity for unbiased samples and samples of full-sibs, respectively

^{*} Significant multi-locus *P* value ($P < 0.001$)

^a Daura-Jorge et al. (2013), ^b Fruet et al. (2011), ^c Vermeulen and Cammareri (2009)

$t = 25$ with effective population size, $N_e = 500$), which approximates the lowest empirical fixation index found based on 15 loci (see “Results” section). The α (Type I) error was assessed running the same simulated scenario, but sampling directly from the base population (i.e. setting drift time $t = 0$). A thousand replicates were run and the significance of the tests was assessed with Fisher’s exact tests and Chi square tests.

The Bayesian clustering method implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000) was also used for inferring population structure based on the microsatellite data. We assumed correlated allele frequencies and an admixture model using sampling location as prior information (LOCPRIOR function) (Hubisz et al. 2009). Simulations were performed using a 200,000 step burn-in period and 10^6 repetitions of the Markov Chain Monte Carlo (MCMC) search, assuming number of clusters (*K*) varying between 1 and 6. We performed 20 independent runs to limit the influence of stochasticity, to increase the precision of the parameter estimates, and to provide an estimate of experimental reproducibility (Gilbert et al. 2012). The most likely *K* was explicitly determined by examining ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER (Earl and vonHoldt 2012). Following the recommendations of Evanno et al. (2005), we ran an iterative process where, for each most likely *K* detected by STRUCTURE, we independently re-analyzed the data to test for further sub-division. This process was repeated until the most likely *K* was 1.

Isolation by distance (IBD) was assessed by conducting Mantel tests (Mantel 1967) between matrices of *F_{ST}* genetic distances and geographical distances measured as the shortest marine coastal distance between two locations. Given the large geographical distance between the southernmost sampling site (BSA) and others, we excluded BSA from the IBD analysis. We also used partial Mantel tests to test for an association between habitat type (estuarine versus coastal) and genetic distance, while controlling for the effect of geographical distance. Both tests were run with 1,000 random permutations in GENODIVE 2.0.

Gene flow

Magnitude and direction of contemporary gene flow among the six sampled communities was estimated using BAYESASS 3.0 (Wilson and Rannala 2003). The software uses a MCMC algorithm to estimate the posterior probability distribution of the proportion of migrants from one population to another. This was conducted with ten independent MCMC runs of 10^7 steps, with the first 10^6 repetitions discarded as burn-in. To reach the recommended acceptance rates of total iterations between 20 and 40 % we adjusted the values of continuous parameters such as

migration rates (Δ_M), allele frequencies (Δ_A) and inbreeding coefficient (Δ_F) to 0.9, 0.6 and 0.8, respectively. Samples were collected every 200 iterations to infer the posterior probability distributions of parameters. Trace files were monitored for convergence and runs with potential problems were discarded. Additionally, convergence was checked by comparing the migration rate profile between the runs according to their average total likelihood and associated credible confidence interval (CI).

Genetic diversity

For microsatellites, genetic diversity, expressed as number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosity, as well as the inbreeding coefficient (F_{IS}) were estimated for each community in GENODIVE 2.0 (Meirmans and Van Tienderen 2004). Departures from Hardy–Weinberg equilibrium and linkage disequilibrium were tested using the Fisher's exact test and a Markov chain method with 1,000 iterations in GENEPOP 4.2 (Rousset 2008). Allelic richness (AR) was estimated in FSTAT 2.9.3.2 (Goudet 1995). All statistical tests followed sequential Bonferroni correction to address type I errors associated with multiple comparisons (Rice 1989). For the mtDNA sequences, we used ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) to estimate haplotypic and nucleotide diversities. A median-joining network from the mtDNA haplotypes was constructed using NETWORK 4.6.1.1 (Bandelt et al. 1999).

Results

Summary statistics

A total of 134 biopsy samples and four samples from stranded carcasses were used. All samples were successfully amplified at 16 microsatellite loci and sequenced for approximately 550 bp of the mtDNA control region. Only eight out of 450 repeated genotypes (1.7 %) did not match but were resolved by re-genotyping. The probability of two unrelated individuals or siblings sharing the same genotypes was very low for all communities (Table 1). Multiple lines of evidence (identical genotype, same mtDNA sequence and sex) suggested that 14 biopsied individuals were sampled twice, including seven individuals that were suspected re-samples based on photo-identification. All re-sampled animals were biopsied in the same location: eight in PLE, two in SPL/URU, two in NPL, one in LGN, and one in FLN. After removal of duplicates, 124 samples were included in the final dataset analyzed. From these, 61 samples were males and 63 were females (Table 1).

The microsatellite locus Tur91 was monomorphic and therefore excluded from further analysis. We found no

evidence for effects of large allelic dropout in any locus. Null alleles were detected for two loci but these were not consistent among sampled locations (locus TUR80 in PLE and Ttr04 in BSA), and therefore the loci were kept for all analyses. One locus pair (TUR105 and EV37) showed evidence of linkage disequilibrium. However, because similar results were obtained when analyses were run both with and without TUR105 this locus was kept in the dataset. Laguna was the only sample location that showed significant deviation from Hardy–Weinberg equilibrium when averaged across all loci, likely due to inbreeding ($F_{IS} = 0.28$) in this small community. Inbreeding coefficient was low and non-significant for all other communities (Table 1).

Genetic structure

The AMOVA results showed strong differentiation between SB–U and BSA for both microsatellites ($F_{ST} = 0.46$, $P < 0.001$) and mtDNA ($\Phi_{ST} = 0.43$, $P < 0.0001$). On a smaller spatial scale, the AMOVA indicated moderate differentiation among SB–U communities, for both microsatellites ($F_{ST} = 0.13$, $P < 0.0001$) and mtDNA ($\Phi_{ST} = 0.29$, $P < 0.0001$). Accordingly, significant differentiation was observed for all pairwise comparisons using microsatellites (Table 2), but over a wide range of F_{ST} values (0.066–0.617). Excluding BSA, which was by far the most differentiated (average F_{ST} of 0.51 for all comparisons with other communities), moderate but significant differentiation was found between all other pairwise comparisons, with the two geographically closest communities (PLE and NPL) having the lowest value of F_{ST} ($F_{ST} = 0.06$; $P < 0.001$). POWSIM simulations for 15 microsatellite loci and the sample sizes used in this study suggested a 100 % probability of detecting differentiation above the lowest empirical F_{ST} level of differentiation, indicating satisfactory statistical power for our analyses. The estimated type I error varied from 0.041 with Fisher's exact tests to 0.083 with χ^2 tests, which approximates the conventional 5 % limit for significance testing.

Results of pairwise comparisons using mtDNA were generally congruent with results from the microsatellite analyses, albeit with higher levels of differentiation between communities. The exceptions were NPL and PLE (for both F_{ST} and Φ_{ST}), and NPL and FLN (for Φ_{ST} only), which showed no significant differentiation (Table 3). All three of these communities are dominated by the most common mtDNA haplotype (H08). Pairwise significant F_{ST} values ranged between 0.097 (NPL–FLN) to 1 (LGN–BSA), with BSA the most differentiated community across all comparisons.

Mantel tests revealed a positive and significant correlation between microsatellites and mtDNA fixation indices

Table 2 Estimates of microsatellite differentiation among six coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along the Southwestern Atlantic Ocean

	FLN	LGN	NPL	PLE	SPL/ URU	BSA
FLN	–					
LGN	0.131**	–				
NPL	0.147**	0.169**	–			
PLE	0.144**	0.101**	0.066**	–		
SPL/ URU	0.289**	0.250**	0.156**	0.101**	–	
BSA	0.617**	0.502**	0.538**	0.423**	0.477**	–

Differentiation is expressed as F_{ST} based on 15 microsatellites loci
FLN Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPL/URU* south of Patos Lagoon/Uruguay, *BSA* Bahía San Antonio

* $P < 0.05$; ** $P < 0.01$

and geographical distances, suggesting a pattern of IBD (Fig. 2). For the mtDNA data, the correlation was not as strong ($r^2 = 0.428$) as for the microsatellites ($r^2 = 0.934$), but still significant. Results of partial Mantel tests (details not shown) suggested that differentiation was more likely influenced by distance than by habitat type (estuarine versus coastal). When controlling for geographical distances, non-significant relationships between locations and clusters (cluster 1 and 2: estuarine and coastal communities, respectively) were found for both microsatellites ($r^2 = -0.437$; $P = 0.51$) and mtDNA ($r^2 = -0.525$; $P = 0.52$).

Bayesian posterior probabilities indicated that the dataset is best explained by the clustering of samples into two genetic populations ($K = 2$), with all individuals from BSA placed in one cluster and remaining individuals sampled in SB–U placed in a second cluster (Fig. 3a). Negligible admixture appears to exist between these two clusters, with assignment estimates of all individuals to

their respective clusters above 0.99 and 0.98, respectively. Testing for further sub-division by running STRUCTURE for the set of northern communities led to the identification of additional partitioning within SB–U most consistent with five populations (Fig. 3b–d). No sub-division was detected within BSA (data not shown).

Gene flow

Estimates of contemporary gene flow inferred in BAYESASS suggested very low gene flow from BSA to SB–U communities (2.2 %) and negligible gene flow in the opposite direction (0.3 %). Within the SB–U region, BAYESASS revealed moderate and complex asymmetrical migration rates (Table 4; Fig. 4) consistent with the inferred pattern of IBD. Generally, higher migration occurred between neighbouring communities than between those separated by greater geographic distances, with the exception of LGN, which seems to exchange more migrants with more distant communities than with its closest neighbouring community (FLN). Migration estimates between sampling locations at the extremities of the sampling distribution was low. Estimated migration rates from FLN to NPL and from SPL/URU to PLE were at least twice the rates between all other community pairs (Fig. 4). For the estuarine communities, PLE seems to act as a sink with a considerable rate of migrants coming from LGN, NPL and SPL/URU, and negligible migration in the opposite direction. In contrast, LGN seems to be more closed to immigration while contributing genetic migrants to PLE and NPL.

Genetic diversity

Levels of genetic variation were remarkably low for all samples as measured by both allelic richness (AR) and expected heterozygosity (H_E) (Table 1; Appendix). Observed heterozygosity (H_O) ranged from 0.15 to 0.26, with a mean across all loci of 0.21. AR ranged from 1.5 to

Table 3 Estimates of mitochondrial differentiation among six coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along the Southwestern Atlantic Ocean

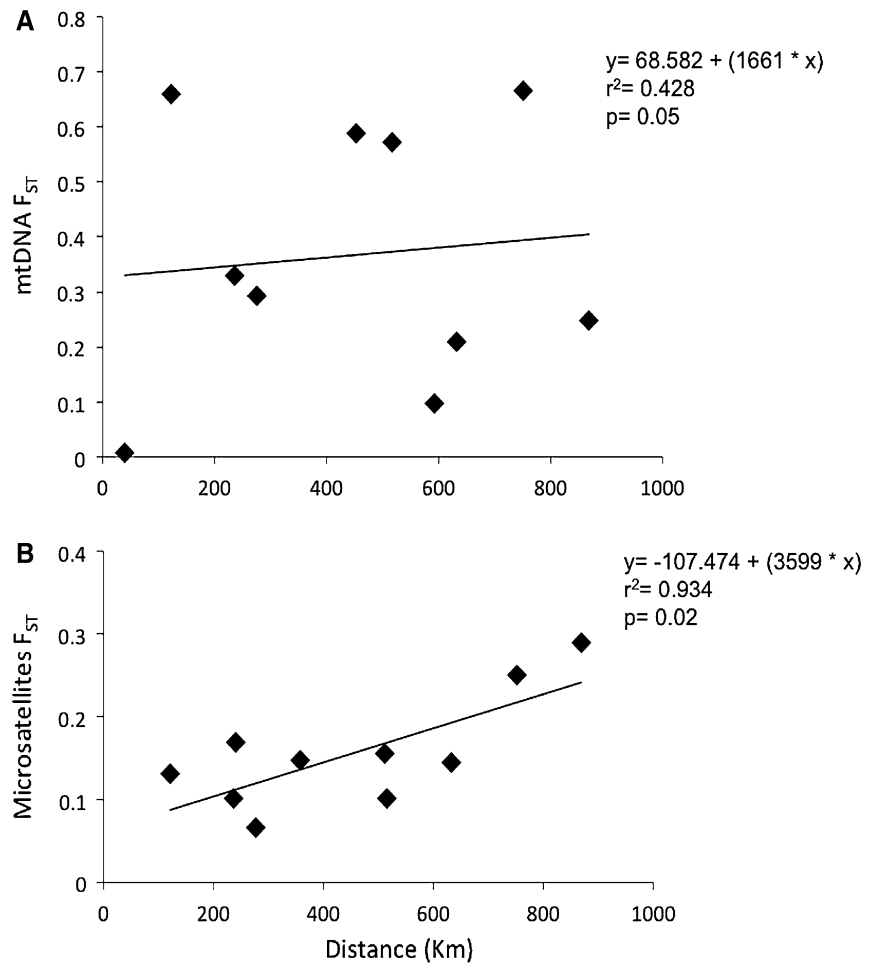
	FLN	LGN	NPL	PLE	SPL/URU	BSA
FLN	–	0.659**	0.100*	0.209**	0.249**	0.687**
LGN	0.893**	–	0.622**	0.572**	0.666**	1.000**
NPL	0.040	0.744**	–	0.009	0.297**	0.679**
PLE	0.198*	0.489**	0.06	–	0.329**	0.638**
SPL/URU	0.531**	0.466**	0.392**	0.230**	–	0.689**
BSA	0.639**	1.000**	0.399**	0.340**	0.609**	–

Differentiation is expressed as Φ_{ST} (above diagonal) and F_{ST} (below diagonal) based on 457-bp of the mtDNA control region

FLN Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPL/URU* south of Patos Lagoon/Uruguay, *BSA* Bahía San Antonio

* $P < 0.05$; ** $P < 0.01$

Fig. 2 Isolation by distance plots using Euclidean distance (km) and genetic distance (F_{ST}) among five coastal communities of common bottlenose dolphins (*Tursiops truncatus*) inhabiting southern Brazil–Uruguay based on **a** mtDNA control region and **b** 15 microsatellite loci (lower box)



2.0, being higher in PLE, NPL and SPL/URU, and lower in LGN and BSA. Number of alleles per locus ranged from two to seven (Appendix) with a mean across all loci of 3.6, while the mean number of alleles per community was two. Out of 17 “private” (unique) alleles identified, nine were found in PLE, five in SPL/URU, two in NPL and one in BSA (Table 1). The only private allele in BSA was found in high frequency in that community, while in all other communities unique alleles had low frequencies.

After sequence alignment and editing, 457 bp of the mtDNA control region could be analyzed for the same 124 individuals used for the microsatellite analysis. Thirteen polymorphic sites (all transitional mutations) revealed nine distinct haplotypes. The number of haplotypes detected in each sampled location varied from one to five, and haplotype diversity ranged from 0 to 0.75. Overall, nucleotide diversity among all individuals was low ($\pi = 0.009$), and haplotype diversity moderate ($h = 0.712$), although values varied among communities. FLN community displayed the highest level of haplotype diversity, while PLE had the highest nucleotide diversity (Table 1). The most common

and widely dispersed haplotype (H8) was found in 49.6 % of the individuals and across all locations, except in LGN and BSA where all dolphins shared the same haplotypes (H7 for LGN and H4 for BSA). Private haplotypes were found in four of the six communities (FLN, $n = 1$; NPL, $n = 1$; SPL/URU, $n = 2$; BSA, $n = 1$) (Fig. 1).

The median-joining network showed two main groups of haplotypes separated by a minimum of five mutational steps (Fig. 5). Individuals from PLE, NPL and SPL/URU communities were present in both groups while individuals from LGN, BSA and FLN were represented in only one of the groups. Bahía San Antonio retains a unique haplotype (H05), which is fixed for this location and differs from the most common haplotype (H08) by one mutational step.

Discussion

This study comprises the first comprehensive assessment of population structure and genetic diversity of coastal

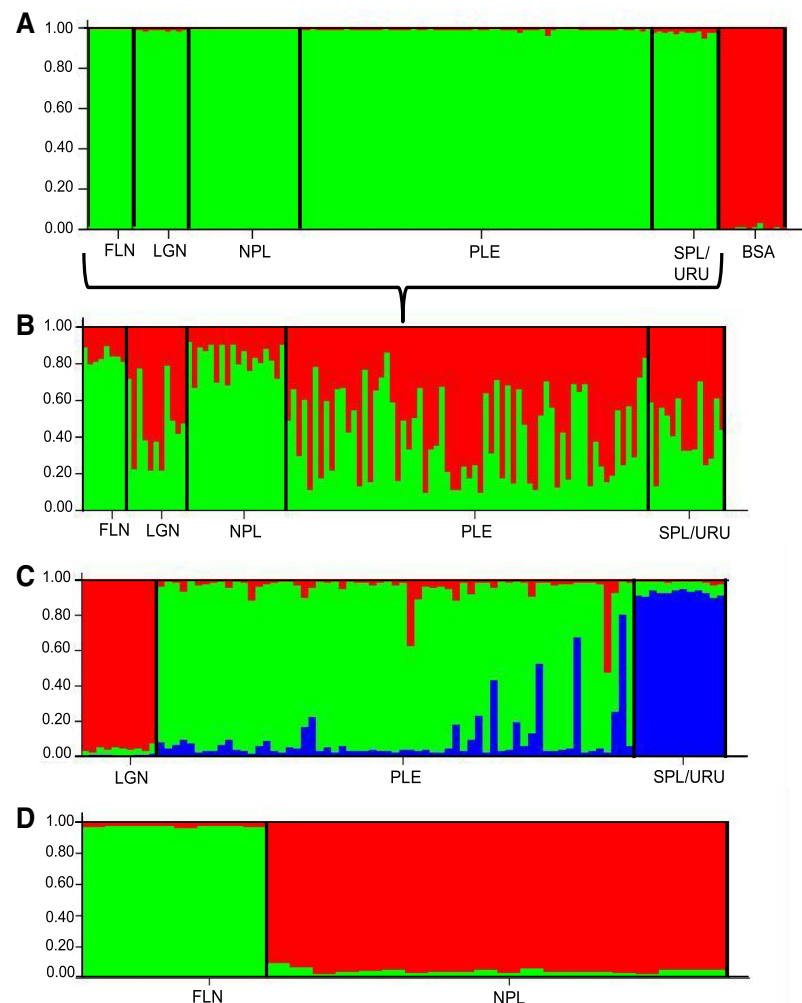


Fig. 3 STRUCTURE Bayesian assignment probabilities for common bottlenose dolphins (*Tursiops truncatus*) based on 15 microsatellite loci. Each vertical line represents one individual dolphin and vertical black lines separate the sampled communities. We run an iterative process where for each most likely K detected by STRUCTURE we independently re-analyzed the data to test for further sub-division (Evanno et al. 2005; Pritchard et al. 2007). This process was repeated iteratively until the highest likelihood values resulted in K = 1. When all samples were analyzed together, STRUCTURE clearly separated

individuals sampled in BSA from all those sampled in southern Brazil/Uruguay, resulting in K = 2 (a). The highest ΔK for the next run within southern Brazil/Uruguay communities was for K = 2, clustering LGN, PLE and SPL/URU, and FLN and NPL (b). When we run STRUCTURE independently for the above-mentioned clusters, the highest ΔK resulted for K = 3 (c) and K = 2 (d), respectively. FLN Florianópolis, LGN Laguna, NPL north of Patos Lagoon, PLE Patos Lagoon estuary, SPL/URU south of Patos Lagoon/Uruguay, BSA Bahía San Antonio. (Color figure online)

bottlenose dolphins (*Tursiops truncatus*) along the SWA. On a large spatial scale, we report on two genetic populations (SB–U and BSA) that are highly differentiated and show very low level of gene flow. On a smaller spatial scale, we detected low to moderate levels of asymmetric gene flow between communities within the SB–U population and an influence of geographic distance in shaping patterns of connectivity, perhaps with the exception of Laguna. Here we also show that coastal bottlenose dolphins in the SWA have very low levels of genetic diversity. This reduced gene flow and genetic diversity, combined with the

small size and probable demographic independence of communities, limit the likelihood of replenishment if they undergo a genetic or demographic decline, highlighting the need to implement local-based monitoring and conservation plans.

Large-scale population structure in SWA bottlenose dolphins

On a broad geographical scale, our results indicate that bottlenose dolphins in coastal Argentinean Patagonia (BSA

Table 4 Estimates of recent migration rates among six coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along the Southwestern Atlantic Ocean

From	To	FLN	LGN	NPL	PLE	SPL/URU	BSA
FLN		0.6915 (0.646–0.736)	0.0232 (0.019–0.066)	0.2152 (0.133–0.296)	0.0237 (0.019–0.067)	0.0232 (0.019–0.065)	0.0232 (0.019–0.063)
LGN		0.0209 (0.017–0.058)	0.6887 (0.648–0.728)	0.1289 (0.016–0.241)	0.1197 (0.007–0.232)	0.0209 (0.017–0.058)	0.0210 (0.017–0.059)
NPL		0.0126 (0.011–0.036)	0.0127 (0.011–0.036)	0.8454 (0.738–0.952)	0.1036 (0.001–0.208)	0.0127 (0.012–0.037)	0.0129 (0.010–0.036)
PLE		0.0050 (0.004–0.015)	0.0054 (0.004–0.015)	0.0455 (0.003–0.094)	0.9343 (0.883–0.985)	0.0049 (0.010–0.019)	0.0049 (0.004–0.014)
SPL/URU		0.0181 (0.015–0.051)	0.0179 (0.016–0.052)	0.0237 (0.029–0.076)	0.2367 (0.141–0.331)	0.6855 (0.621–0.749)	0.0180 (0.015–0.051)
BSA		0.0182 (0.015–0.051)	0.0183 (0.015–0.051)	0.0182 (0.015–0.052)	0.0185 (0.015–0.052)	0.0183 (0.015–0.052)	0.9084 (0.841–0.975)

Bold denotes the proportion of non-migrants in each dolphin community. 95 % CI values are given in brackets

FLN Florianópolis, LGN Laguna, NPL Patos Lagoon estuary, SPL/URU south of Patos Lagoon/Uruguay; BSA Bahía San Antonio

community) are highly differentiated from those sampled along the southern Brazil–Uruguay (SB–U) coast, likely reflecting a combination of IBD and environmental differentiation. Several studies have argued that bottlenose dolphins are capable of specialization for a variety of habitats and prey types, and that such specialization could promote genetic divergence (Hoelzel et al. 1998a; Natoli et al. 2004; Möller et al. 2007; Tezanos-Pinto et al. 2009; Wiszniewski et al. 2010; Möller 2012). Bahía San Antonio is located in the San Matías Gulf (Fig. 1), which is part of the Northern Patagonian gulfs of Argentina. Geomorphological characteristics (bathymetry and coastal complexity), oceanographic processes (upwelling, nutrient input, sea surface temperature regimes and currents), and biological community structure biogeographically distinguishes the Patagonian region from the rest of the Atlantic coast (Balech and Ehrlich 2008; Tonini 2010). For example, archaeozoological evidence suggests that one of the main prey species of bottlenose dolphins in SB–U, the white croaker (*Micropogonias furnieri*) (Pinedo, 1982; Mehnen et al. 2005), is currently absent from BSA (Scartascini and Volpedo 2013), which is the northernmost limit for many prey species confirmed to be part of the diet of bottlenose dolphins in Patagonia (e.g. pouched lamprey (*Geotria australis*), Patagonian octopus (*Octopus tehuelchus*), Argentine Hake (*Merluccius hubbsi*) (Crespo et al. 2008), as it is located at the boundary between two biogeographic regions (Galván et al. 2009). Regional differences in prey distribution and abundance are thought to play a role on the genetic structuring of bottlenose dolphins elsewhere (e.g. Bilgmann et al. 2007). Therefore, BSA bottlenose dolphins may have different foraging adaptations compared to SB–U bottlenose dolphins. The high degree of differentiation at neutral markers and the results from the Bayesian analysis of migration rates imply negligible gene flow between bottlenose dolphin communities of these two regions. Future studies combining morphological, genetic, environmental, and ecological data are needed to better clarify the taxonomic status between BSA and SB–U coastal bottlenose dolphins.

Fine-scale population structure in SWA bottlenose dolphins

In spite of their high dispersal potential, several empirical studies have shown that coastal bottlenose dolphins often form discrete population units, even at very small geographical scales (e.g. Sellas et al. 2005; Möller et al. 2007; Rosel et al. 2009; Ansmann et al. 2012). Our results from both fixation indices and the Bayesian clustering analysis confirmed that the five studied communities within the SB–U population are genetically distinct, indicating higher genetic differentiation than expected over small

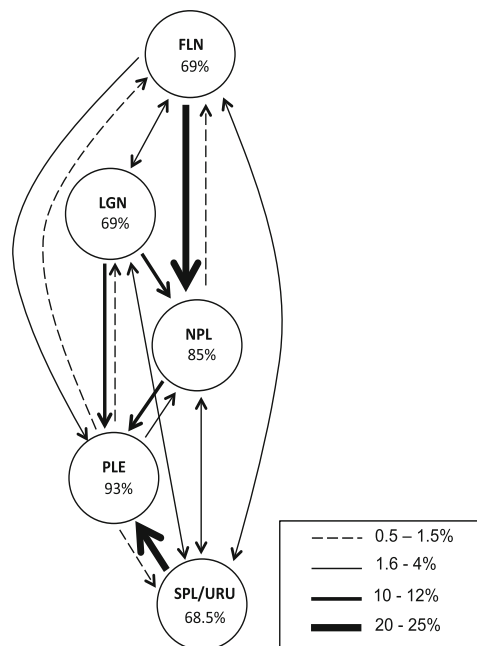


Fig. 4 Schematic diagram showing the recent asymmetric migration rates estimated between five coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along southern Brazil and Uruguay. The width of the arrows corresponds to the rates of gene flow between putative populations

geographical scales. Relatively lower degrees of nuclear genetic differentiation are commonly reported for bottlenose dolphins over comparable spatial scales with the exception of the high differentiation found among the neighbouring communities of *T. truncatus* in Irish coastal waters (Shannon estuary and Connemara–Mayo communities $F_{ST} = 0.179$; Mirimin et al. 2011). For instance, lower differentiation was found between neighbouring communities of *T. truncatus* along the coast of the western North Atlantic (minimum and maximum reported F_{ST} values of 0.002 and 0.015, respectively; Rosel et al. 2009) and Bahamas ($F_{ST} = 0.048$; total distance between two sampling sites was 116 km; Parsons et al. 2006).

For highly mobile, long-lived animals with low reproductive rates such as cetaceans, it is well accepted that a combination of mechanisms including habitat selection, specialized foraging behaviours, social structure and natal philopatry can drive population differentiation across small spatial scales (Hoelzel 2009; Möller 2012). For a closely related species, the Indo-Pacific bottlenose dolphins, restricted gene flow between some coastal and estuarine communities appears to have occurred after coastal dolphins colonized the embayment, as a consequence of high site fidelity and resource and behavioural specializations

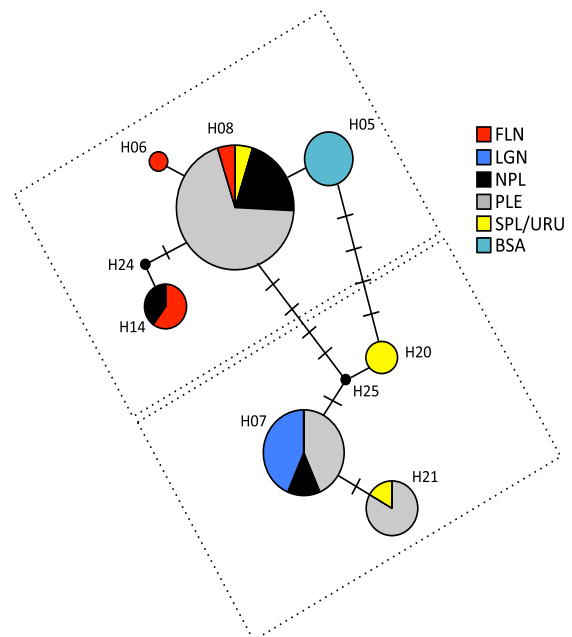


Fig. 5 Median-joining network of mtDNA control region haplotypes in coastal common bottlenose dolphins (*Tursiops truncatus*). The size of the circles is proportional to the total number of individuals bearing that haplotype. Dashed lines separate the two main groups of haplotypes. Different colors denote the different sampled communities: FLN Florianópolis, LGN Laguna, NPL north of Patos Lagoon, PLE Patos Lagoon estuary, SPE/URU south of Patos Lagoon/Uruguay, BSA Bahía San Antonio. Dashed lines represent extinct or unsampled haplotypes. (Color figure online)

(Möller et al. 2007). In our study, however, we actually found similar levels of genetic differentiation when comparing coastal and estuarine communities or among coastal communities of the common bottlenose dolphin in SWA. This pattern is contrary to what would be expected if habitat type was a main driver of bottlenose dolphin population structure in the region. Instead, for most communities, structure appeared to follow an isolation-by-distance model, where exchange of individuals seems to more likely occur between adjacent communities, irrespective of habitat type. The only exception was Laguna, which appeared as an outlier to the IBD model. In Laguna, a unique foraging tactic involving cooperative interactions between dolphins and beach-casting fishermen has evolved. It has been suggested that the propagation of such behaviour through social learning has a matrilineal origin, where the mother–calf relationship might create conditions suitable for behavioural information exchange (Daura-Jorge et al. 2012). In such special conditions, the costs to individuals of leaving a suitable habitat is likely greater than the risk of searching for more profitable locations. In contrast, some

PLE dolphins frequently interact with animals from other communities in the coastal zone, and there is no evidence of particular feeding specializations compared to LGN. Thus, it appears that feeding specializations (LGN) and sociality (PLE), instead of habitat type *per se*, may play a role in shaping genetic structure of bottlenose dolphins in these regions.

The contemporary asymmetric gene flow found in our study system suggests moderate levels of connectivity among communities in SB–U ESU, which are consistent with a metapopulation. Gene flow is particularly mediated by coastal communities, especially FLN and SPL/URU, although estuarine communities exchange genes as well. It seems that PLE potentially acts as a sink, receiving low to moderate number of migrants while not contributing substantially to other communities. In contrast, LGN showed much lower gene flow with adjacent communities, apparently constituting a more closed genetic unit. This pattern is also supported by mitochondrial data, which suggested high connectivity between PLE and the adjacent coastal community (NPL), but high maternal philopatry and restricted dispersal of LGN dolphins.

Remarkably low levels of genetic diversity in SWA bottlenose dolphins

Low genetic variation was detected with both mitochondrial and nuclear DNA markers across all communities. Levels of variation at the mtDNA control region were similar to those reported for *T. truncatus* in other parts of the world. In contrast, nuclear DNA variation for all communities was much lower than that reported for other local coastal communities elsewhere (see Online Resource 2 for comparisons with studies of Parsons et al. 2006; Rosel et al. 2009; Tezanos-Pinto et al. 2009; Mirimin et al. 2011; Caballero et al. 2012). This is supported by the low numbers of alleles, reduced allelic richness and reduced heterozygosity. For LGN and BSA communities in particular, the remarkably low variation at both marker types fall within the range observed for cetaceans with extremely small populations sizes (i.e. <100 individuals), such as the subspecies of Hector's dolphins, *Cephalorhynchus hectori mauii* (Hamner et al. 2012), and the Black Sea subspecies of the harbour porpoise, *Phocoena phocoena relicta* (Rosel et al. 1995). These findings are consistent with the current abundance estimates of less than 90 individuals for the BSA, PLE, and LGN communities (Vermeulen and Cammareri 2009; Fruet et al. 2011; Daura-Jorge et al. 2013) and may also reflect the potential small size of the other

communities (such as FLN, NPL and SPL/URU) for which estimates of abundance are not currently available. Several authors have suggested that coastal populations of bottlenose dolphin elsewhere might have originated via independent founder events from offshore populations, followed by local adaptation and natal philopatry (Hoelzel et al. 1998a; Natoli et al. 2004; Sellas et al. 2005; Möller et al. 2007; Tezanos-Pinto et al. 2009), leading to a reduction in genetic diversity.

Conservation implications

On a large geographical scale our results strongly support that SB–U and BSA dolphins constitute at least two distinct ESUs, and these warrant separate conservation and management strategies. The SB–U ESU comprises a set of communities (or sub-populations) distributed along a narrow strip of the coast between Florianópolis (27°21'S) in southern Brazil, and the southern limit of the Uruguayan coast (34°55'S). The BSA ESU geographical range goes possibly from the northern border of Rio Negro Province, at the Rio Negro estuary (41°01'S), to southern Golfo Nuevo (43°05'S), as suggested by sightings of bottlenose dolphins in northern Patagonia (Vermeulen and Cammareri 2009; Coscarella et al. 2012). Our results indicate that these two ESUs are genetically isolated which has important implications for future conservation plans. It is fundamental that managers design appropriate conservation strategies for each ESU, taking into account their respective threats, genetic and ecological processes shaping structure, and geographical distribution in space and time, as their responses to future environmental changes may possibly differ. This is of particular relevance for BSA dolphins since they apparently constitute the only population within that ESU with reduced abundance and signs of historical decline (Bastida and Rodríguez 2003; Coscarella et al. 2012).

The most serious and continuous threats for bottlenose dolphins along the SWA coast are found within the SB–U ESU, where they have experienced increased rates of human-related mortalities during the past decade (Fruet et al. 2012). These animals also face considerable coastal habitat degradation as a consequence of ongoing industrial and port development activities (Tagliani et al. 2007). Based on this study we suggest that these dolphin communities within SB–U are functionally independent, and therefore should be treated as separate MUs for conservation purposes. We advocate for managers to adopt the proposed MUs reported here (see Fig. 1), while

recognizing that their boundaries may change as more information on dolphin home ranges and population genetic structure becomes available. Under this proposed management scenario, conservation programs should be directed towards the Patos Lagoon estuary and adjacent coastal waters where dolphins from distinct communities (PLE, NPL and SPL/URU) show overlapping home ranges, and where by-catch rates are reportedly higher (Fig. 1). Protecting dolphins in this region would reduce the risk of disrupting connectivity between MUs and increase the chances of long-term viability. Strategies should reduce the impact of by-catch and maximize the protection of “corridors” in coastal areas for maintaining connectivity between adjacent dolphin communities.

The very low levels of genetic diversity in coastal bottlenose dolphins from SWA could be a source for concern. The importance of genetic variation relates to multiple aspects of population resilience and persistence, and is usually assumed to be critical for long-term fitness and adaptation (Franklin 1980; Charlesworth and Willis 2009), although some studies have shown that minimal genetic variation is not always a reliable predictor of extinction risk in wild populations (e.g. Schultz et al. 2009). We propose, however, the adoption of a precautionary approach for coastal bottlenose dolphins in SWA. Although there is no evidence of inbreeding depression for bottlenose dolphins in this region, the possibility of inbreeding in the small LGN community (Table 1) may, in the long-term, be detrimental to its viability since inbreeding can increase vulnerability to environmental stressors (O’Brien et al. 1985; Frankham 1995; Spielman et al. 2004; Hale and Briskie 2007). Bottlenose dolphins from Laguna and their neighbouring community (FLN) are being affected by a chronic dermal infection, the fungal Lobomycosis, and Lobomycosis-like disease (LLD) (Van Bressen et al. 2007, Daura-Jorge and Simões-Lopes 2011), with evidence of an increase in the number of affected animals in recent years (Daura-Jorge and Simões-Lopes 2011). While our results suggest restricted dispersal of LGN dolphins, which may limit the spread of the disease, the isolated nature of this community can potentially accelerate fungal transmission among resident dolphins.

Conclusions

Common bottlenose dolphins from coastal waters of the SWA are characterized by unprecedentedly low mitochondrial and nuclear DNA diversity. Moderate to strong

levels of population differentiation at both marker types were also disclosed and are likely associated with a combination of geographical, environmental and social factors. The pattern of genetic differentiation and the negligible migration rates detected suggest two distinct lineages, or evolutionarily significant units, one in Argentina and the other in southern Brazil-Uruguay. In addition, five distinct communities, or Management Units, characterized by low to moderate asymmetrical gene flow were identified in southern Brazil-Uruguay—a region where human activities negatively impact upon common bottlenose dolphins. We propose that policies and practices relevant to conservation management of common bottlenose dolphins in coastal waters of the SWA should recognize the existence of two lineages, as well as promote connectivity between the estuarine and open-coast populations in southern Brazil and Uruguay to ensure their long-term persistence.

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Appendix

See Table 5.

Table 5 Genetic diversity screened at 16 microsatellite loci in six coastal communities of common bottlenose dolphin sampled along the Southwestern Atlantic

	FLN (<i>n</i> = 8)				LGN (<i>n</i> = 10)				NPL (<i>n</i> = 19)				PLE (<i>n</i> = 63)				SPL/URU (<i>n</i> = 12)				BSA (<i>n</i> = 12)			
	NA	H _O	H _E	P	NA	H _O	H _E	P	NA	H _O	H _E	P	NA	H _O	H _E	P	NA	H _O	H _E	P	NA	H _O	H _E	P
Tur4_142 ^a	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	2	0.01	0.01	1.00	1	0.00	0.00	NA	1	0.00	0.00	NA
Tur4_91 ^a	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA
Tur4_141 ^a	2	0.25	0.23	1.00	1	0.00	0.00	NA	1	0.00	0.00	NA	2	0.06	0.06	1.00	2	0.08	0.08	1.00	2	0.08	0.08	1.00
Tur4_F10 ^a	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	3	0.06	0.09	0.05	2	0.08	0.08	1.00	2	0.25	0.23	1.00
Tur4_E12 ^a	3	0.75	0.66	0.77	3	0.30	0.59	0.02*	3	0.45	0.53	0.15	4	0.68	0.65	0.85	3	0.67	0.68	0.21	2	0.33	0.39	1.00
Tur4_105 ^a	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	4	0.04	0.04	1.00	1	0.00	0.00	NA	2	0.25	0.23	1.00
Tur4_80 ^a	1	0.00	0.00	NA	2	0.10	0.10	1.00	2	0.05	0.05	1.00	5	0.03	0.08	0*	2	0.08	0.23	0.13	1	0.00	0.00	NA
Tur4_87 ^a	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	3	0.03	0.03	1.00	1	0.00	0.00	NA	1	0.00	0.00	NA
Mk6 ^b	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	2	0.58	0.52	1.00
Mk8 ^b	3	0.62	0.62	0.73	2	0.60	0.53	1.00	5	0.50	0.45	0.13	4	0.43	0.46	0.03*	4	0.75	0.69	0.45	2	0.42	0.43	1.00
Kw2 ^c	2	0.75	0.50	0.43	2	0.20	0.50	0.08	5	0.60	0.62	0.92	5	0.55	0.67	0.15	3	0.08	0.70	0.55	1	0.00	0.00	NA
Kw12a ^c	1	0.00	0.00	NA	2	0.30	0.39	0.48	2	0.15	0.14	1.00	2	0.46	0.39	0.20	1	0.00	0.00	NA	2	0.08	0.08	1.00
Ev37mn ^d	2	0.62	0.46	0.48	2	0.20	0.50	0.08	3	0.25	0.23	1.00	3	0.44	0.43	1.00	4	0.17	0.30	0.09	1	0.00	0.00	NA
TexVet5 ^e	2	0.12	0.12	1.00	1	0.00	0.00	NA	2	0.05	0.05	1.00	1	0.00	0.00	NA	2	0.08	0.08	1.00	2	0.25	0.23	1.00
Tr63 ^f	2	0.12	0.12	1.00	1	0.00	0.00	NA	3	0.35	0.50	0.23	3	0.63	0.51	0.06	2	0.33	0.29	1.00	1	0.00	0.00	NA
Tr04 ^f	2	0.50	0.40	1.00	3	0.70	0.65	0.37	4	0.65	0.66	0.37	5	0.78	0.75	0.69	4	0.58	0.47	1.00	3	0.42	0.68	0.28

NA number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, P P-value of exact test using Markov chain, NA not available

* Significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$)

^a Nater et al. (2009), ^b Krützen et al. (2001), ^c Hoelzel et al. (1998b), ^d Valsecchi and Amos (1996), ^e Rooney et al. (1999), ^f Rosel et al. (2005)

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Online Resource 1 Details of the 16 microsatellites screened in this study and their polymorphism in 124 common bottlenose dolphin samples. Colors depict co-loaded loci

Locus	Isolated from	Repeat motif	Primer sequence 5' - 3'	PCR Condition	Multiplex	Label	ASR (bp)	NA	H _O	H _E
1 <i>Tur4_142</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₉	F: GGCCCCCTTTCCATCCTCA R: CCAGCCCCAAAATCACGAGT	Wiszniewski et al. 2012	1	FAM	350-354	2	0.003	0.003
2 <i>Tur4_91</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₁₄	F: GTTGGCTCTCCAGCTCTCAGGT R: CAGTGGCTCCCATCTGTATTAGTCA	Wiszniewski et al. 2012	1	FAM	235	1	NA	NA
3 <i>Tur4_141</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₉	F: CACAAGCCTCAACCTGGTGT R: CTAGTCTGCCAATCTGCCCTACAG	Wiszniewski et al. 2012	2	PET	234-258	3	0.080	0.078
4 <i>Tur4_F10</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₉	F: TCTTGATGGCTCAGAGGATGATTTTAC R: AGCCAACTGAAGATGCAACTGACTAC	Wiszniewski et al. 2012	2	PET	406-414	3	0.066	0.068
5 <i>Tur4_E12</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₉	F: CTGGGCACTGTCTCTGAACATC R: AGGAACGGCACATAAAGCACTGA	Wiszniewski et al. 2012	3	NED	284-292	4	0.530	0.587
6 <i>Tur4_105</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₁₁	F: CCCCAGCCTGCTTACCTCTG R: CCGCCCCCTCCCCAAGTC	Wiszniewski et al. 2012	3	NED	410-418	4	0.050	0.046
7 <i>Tur4_80</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₁₀	F: AGCCAATGTCAGGGTCTGGAT R: GGGGCTTCTTGGCCTCTGTAA	Wiszniewski et al. 2012	4	VIC	326-342	5	0.044	0.078
8 <i>Tur4_87</i> ¹	<i>Tursiops aduncus</i>	(GATA) ₈	F: CCCCATATGATGCCTTTGTAAGTCC R: AATTCCTTGTAACAAACCTCTTATCT	Wiszniewski et al. 2012	4	VIC	192-220	3	0.005	0.005
9 <i>Mk6</i> ²	<i>Tursiops aduncus</i>	(GT) ₁₇	F: GTCCTCTTTCCAGGTGTAGCC R: GCCACTAAGTATGTTGCAGC	Möller and Beheregaray (2004)	single	VIC	188-190	2	0.097	0.086
10 <i>Mk8</i> ²	<i>Tursiops aduncus</i>	(CA) ₂₃	F: TCCTGGAGCATCTTATAGTGGC R: CTCTTTGACATGCCCTCACC	Möller and Beheregaray (2004)	single	NED	113-123	7	0.553	0.528
11 <i>Kw2</i> ³	<i>Orcinus orca</i>	-	F: GCTGTGAAAATTAATGT R: CACTGTGGACAAATGTAA	Möller and Beheregaray (2004)	single	FAM	164-176	6	0.490	0.500
12 <i>Kw12a</i> ³	<i>Orcinus orca</i>	-	F: CCATACAATCCAGCAGTC R: CACTGCAGAATGATGACC	Möller and Beheregaray (2004)	single	PET	192-194	2	0.166	0.170
13 <i>Ev37mn</i> ⁴	<i>Megaptera novaeangliae</i>	(AC) ₂₇	F: AGCTTGATTTGGAAGTCATGA R: TAGTAGAGCCGTGATAAAGTGC	Same conditions as KW2 and KW12	single	FAM	219-231	5	0.281	0.324
14 <i>TexVet</i> ⁵	<i>Tursiops truncatus</i>	(CA) ₂₄	F: GATTGTGCAATGGAGACA R: TTGAGATGACTCCTGTGGG	Same conditions as KW2 and KW12	single	VIC	219-223	3	0.085	0.080
15 <i>Ttr63</i> ⁶	<i>Tursiops truncatus</i>	(CA) ₃₄	F: CAGCTTACAGCCAAATGAGAG R: GTTCTCATGGCTGAGTCATCA	Wiszniewski et al. 2012	single	FAM	130-134	3	0.241	0.240
16 <i>Ttr04</i> ⁶	<i>Tursiops truncatus</i>	(CA) ₂₅	F: CTGACCAGGCACCTTTCCAC R: GTTTGTTTCCAGGATTTAGTGC	Same conditions as KW2 and KW12	single	NED	132-140	5	0.605	0.604

¹Nater et al. (2009); ²Krützen et al. (2001); ³Hoelzel et al. (1998a); ⁴Valsecchi and Amos (1996); ⁵Rooney et al. (1999); ⁶Rosel et al. (2005)

Online Resource 2 Summary of genetic variation for mtDNA control region sequences and nuclear microsatellite for coastal common bottlenose dolphins (*Tursiops truncatus*) worldwide, including findings from this study. N= total number of individuals; h=haplotypic diversity; π = nucleotide diversity; NA= mean number of alleles per locus; AR= mean allelic richness; H_O= mean observed heterozygosity; H_E= mean expected heterozygosity. Note: values can slightly differ from original source because they were adjusted to standardize decimal places

			mtDNA					Microsatellite									
Region	Source	Sampling site	N	h		π		N	Loci	NA		AR		H _O		H _E	
USA	Rosel et al. 2009	Virginia and North	100	0.761 (0.022)		0.013 (0.007)		87	18	8.3		7.4		0.66		0.68	
	Rosel et al. 2009	Southern North Carolina	51	0.756 (0.029)		0.003 (0.002)		50	18	7.3		7.0		0.62		0.64	
	Rosel et al. 2009	Charleston, SC and surrounding area	110	0.498 (0.053)		0.002 (0.001)		100	18	7.6		6.8		0.63		0.65	
	Rosel et al. 2009	Georgia	40	0.573 (0.067)		0.002 (0.002)		40	18	7.1		7.1		0.67		0.68	
	Rosel et al. 2009	Jacksonville	78	0.558 (0.039)		0.002 (0.002)		77	18	7.8		7		0.67		0.69	
	Rosel et al. 2009	Florida panhandle, Gulf of Mexico	72	0.754 (0.027)		0.009 (0.005)		77	18	7		6.4		0.62		0.65	
Ireland	Mirimin et al. 2011	Shannon Estuary	44	0.274 (0.076)		0.005 (0.003)		46	15	1.6		1.2		0.20		0.18	
	Mirimin et al. 2011	Connemara–Mayo	12	0.530 (0.136)		0.008 (0.005)		12	15	3.3		1		0.46		0.48	
New Zealand	Tezanos-Pinto et al. 2011	Northland	127	0.880 (0.01)		0.019 (0.010)		-	-	-		-		-		-	
	Tezanos-Pinto et al. 2011	Fiordland	24	0.760 (0.07)		0.015 (0.008)		-	-	-		-		-		-	
Caribbean	Caballero et al. 2013	Bahamas, Cuba, Mexico, Puerto	112	0.578 (0.049)		0.009 (0.005)		-	-	-		-		-		-	
Bahamas	Parsons et al. 2006	East and South Abbaco, White Sand Ridge	56	0.763 (0.046)		0.007 (0.004)		56	16	5.1		-		0.60		0.65	
Southern Brazil - Uruguay	This study	Florianópolis	8	0.750 (0.096)	0.646 (0.040)	0.004 (0.003)	0.019 (0.010)	8	15	1.6	3.7	1.6	2.2	0.23	0.22	0.19	0.22
	This study	Laguna	10	0.000 (0.000)		0.000 (0.000)		10	15	1.6		1.5		0.15		0.21	
	This study	North Patos Lagoon	19	0.542 (0.123)		0.007 (0.004)		19	15	2.3		1.9		0.19		0.20	
	This study	Patos Lagoon estuary	63	0.481 (0.062)		0.007 (0.004)		63	15	3.0		2.0		0.26		0.26	
	This study	South Patos Lagoon/ Uruguay	12	0.648 (0.116)		0.007 (0.004)		12	15	2.1		1.9		0.23		0.20	
Argentina	This study	Baia San Antonio	12	0.000 (0.000)		0.000 (0.000)		12	15	3.6		1.8		0.19		0.20	

ANEXO II

(Em revisão no periódico “*Journal of Mammalogy*”; submetido em 24 Abril de 2014)

Pedro Fruet (pfruet@gmail.com)

Laboratório de Ecologia e Conservação da Megafauna Marinha (EcoMega)

Instituto de Oceanografia, Universidade Federal do Rio Grande/FURG, Cx.P. 474 Rio Grande - RS,
Brazil. CEP: 96203-900

Mobile: ++ 55 53 99285204

Running Heading: Demography of bottlenose dolphins

**Abundance and demography of a resident dolphin community inhabiting a sub-tropical estuary in
Southwestern Atlantic**

Pedro F. Fruet*, Fábio Daura-Jorge, Luciana M. Möller, Rodrigo César Genoves, and
Eduardo R. Secchi

Programa de Pós-Graduação em Oceanografia Biológica, FURG, Brazil (PFF)

Museu Oceanográfico “Prof. Eliézer C. Rios”, FURG, Brazil (PFF, RCG, ERS)

*Laboratório de Ecologia e Conservação da Megafauna Marinha (EcoMega), Instituto
de Oceanografia, Universidade Federal do Rio Grande - FURG, Brazil (PFF, RCG,
ERS)*

*Cetacean Ecology, Behaviour and Evolution Lab, School of Biological Sciences,
Flinders University, Australia (PFF, LMM)*

*Molecular Ecology Laboratory, School of Biological Sciences, Flinders University,
Australia (PFF, LMM)*

*Laboratório de Ecologia e Conservação, Universidade Estadual de Santa Catarina -
UDESC, Brazil (FDJ)*

We conducted a mark-recapture analysis from 8 yrs (2005-2012) of photo-identification data collected systematically to investigate demographic parameters of a community of common bottlenose dolphins (*Tursiops truncatus*) inhabiting the Patos Lagoon estuary

and adjacent marine coast, southern Brazil. Under the most parsimonious model of Pollock's Robust Design, which disregarded the effects of temporary emigration, the estimate of annual apparent survival was higher for adult females (0.97, 95% CI: 0.91-0.99) than for adult males (0.88, 95% CI: 0.75-0.94) and juveniles (0.83, 95% CI: 0.64-0.93), which may explain an observed bias in sex ratio (1M: 2F) of adult dolphins in this community. An increasing trend in abundance of the marked individuals was observed during the first six years of sampling when the number of new recruits surpassed mortality, followed by a remarkable decrease in the last two years when an inverse ratio of recruits/deaths occurred. Yearly changes in abundance ($\hat{\lambda}_t$) varied from -0.1 to 0.07. Total abundance estimates were highly precise (the highest CV was 0.053) and did not exceed 88 individuals, confirming the small size of the Patos Lagoon estuary community of dolphins. Abundance estimates were similar to a previous mark-recapture study conducted in the same area almost a decade earlier, suggesting a relative stability over the last 14 yrs. The apparent stability in abundance, however, should be viewed with caution since this community would need to experience a substantial mortality of 10% before a declining trend in population size would be detected with a desirable power of 90%.

Key words: cetaceans, mark-recapture, population trends, survival, sex-ratio

*Correspondent: pfruet@gmail.com

Reliable estimates of demographic parameters of wild populations are the baseline for understanding the population dynamics and ecology of any species, allowing for a proper assessment of the impacts of non-natural mortality and trends (e.g. Thompson et al. 1998). Besides the intrinsic biological differences, there are many extrinsic natural factors that may cause inter and even intra-specific variability in vital parameters. For species exposed to long-lasting and complex life-history processes, survival is expected

to vary according to age, sex and possibly the individual genetic makeup (Ralls et al. 1980; Promislow 1992). Extrinsically, natural oscillations in ecosystem productivity at various temporal and spatial scales, generally impact demographic parameters such as survival and fecundity rates. This is also true for large marine vertebrates (e.g. Trillmich and Ono 1991; Soto et al. 2004; McMahon et al. 2005; Forcada et al. 2005; Leaper et al. 2006; Ward et al. 2009).

Relatively accurate and unbiased temporal estimates of cetacean population parameters can be obtained from longitudinal studies using data from ‘mark’ and ‘recapture’ (e.g. photographs of natural marks and photographic re-sightings) of individuals and analyzing them under the mark-recapture (MR) statistical framework (Seber 1982; Pollock et al. 1990; Kendall et al. 1995). Advances in MR analysis have enhanced our knowledge about population parameters of wild animals (see Sandercock 2006 for a review) and today, we are able to model multiple life history stages and individuals with states of uncertainty, greatly increasing the range of plausible biological hypotheses to be tested.

Despite the notoriously difficulty of fulfilling all the assumptions of classical open or closed MR population models, especially when dealing with highly mobile animals such as cetaceans, analytical methods offer the opportunity to relax some assumptions and explicitly model biological conditions that were previously ignored (Hammond 2009). A common problem of mark-recapture studies applied to cetaceans is our inability to survey the entire distributional range of the studied population. Movements of individuals in and out of the sampling area are expected to occur, potentially confounding mortality with temporary or permanent emigration, which may result in imprecise and, in some circumstances, biased estimates (e.g. Kendall et al. 1997).

Pollock’s Robust Design (Pollock 1982; Kendall et al. 1997), however, which combines

open and closed population models in an integrated framework, explicitly deals with issues of temporary emigration and offers a more biologically sounding scenario (e.g. Smith et al. 2013).

The common bottlenose dolphin *Tursiops truncatus* (hereafter simply termed as bottlenose dolphin) is one of the most studied cetacean species and is listed as “least concern” in the International Union for Conservation of Nature Red List (Hammond et al. 2012). However, only a few studies have comprehensively quantified its demographic parameters in the wild (e.g. Wells and Scott 1999; Currey et al. 2007). Despite many estimates of local abundance, population trends are often lacking (e.g. Currey et al. 2007; Fernbach et al. 2012; Tezanos-Pinto et al. 2013). Survival rate, the parameter for which estimates of intrinsic population growth rate of large vertebrates is most sensitive (Crouse et al. 1987; Caswell 2001), is rarely reported, especially when stratified by age or sex. Exceptions come from a few mark-recapture (Wells and Scott 1990; Currey et al. 2008) and other cross-sectioned studies that derived survivorship from the age-at-death distribution of stranded animals (for *T. truncatus* life table see Stolen and Barlow 2003). However, no variability of individual demographics can be estimated from cross-sectioned studies, and they have proven to be problematic because they rely on assumptions that in most circumstances are unrealistic (e.g. constant vital rates over time or accurate aging of recovered carcasses – Caughley 1977; Messier 1990).

The bottlenose dolphins inhabiting the Patos Lagoon estuary and adjacent marine coast are part of a genetically depauperated metapopulation that is comprised by a few small resident dolphin communities[†] of southern Brazil in the southwestern Atlantic Ocean (Fruet et al. 2014). The Patos Lagoon estuary and adjacent coastal waters were recently

[†] The term “community” was adapted from Wells et al. (1987) to make reference to resident dolphins that share large portions of their ranges, socially interact with each other to a much greater extent than with dolphins in adjacent zones and exhibit similar genetic profiles.

proposed as the most important areas for the conservation of bottlenose dolphins in the region (Fruet et al. 2014), where three communities share their ranges in coastal waters (Genoves 2013), and where bycatch mortality is known to be high (Fruet et al. 2012). The Patos Lagoon estuary (PLE) dolphin community is ideal for mark-recapture studies due to its presence in inshore, protected habitat, elevated rates of naturally marked individuals and high site fidelity (Castello and Pinedo 1977; Dalla Rosa 1999; Fruet et al. 2011). This is one of the few small, coastal and resident dolphin communities inhabiting lagoons and estuaries of southern Brazil (Fruet et al. in press). There are numerous evidences suggesting that these dolphins have been impacted by fisheries since the late 1970s, with a sudden increase in bycatch mortality reported for some years of the last decade (e.g. 2002-2004) (Fruet et al. 2012), primarily as a consequence of an extensive overlap with artisanal gillnet fishing (Di Tullio et al. in press). Since then, the possibility of a population decline has emerged as a central debate over future management actions for the conservation of the species in this area (Fruet et al. 2012; Di Tullio et al. in press). Nevertheless, reliable estimates of demographic parameters are needed for a proper assessment of the conservation status of these dolphin communities. A systematic monitoring program was established in 2005 in order to collect data to assess the conservation status of PLE dolphins. The intensive long-term monitoring between 2005 and 2012, coupled with previous non-systematic photo-identification data collected since 1974 (Pinedo and Castello 1977), allowed to track a number of individuals for many years, offering an excellent opportunity to quantify the relevant vital parameters of this community. Abundance estimates from 1998 and 2005 reported a small (numbering less than 90 individuals) community (Dalla Rosa 1999; Fruet et al. 2011). A comprehensive analysis of continued long-term mark-recapture history of the PLE bottlenose dolphin community can provide baseline parameter estimates for

describing its dynamics and conservation status. Therefore, the aims of this 8-year study were to use a sampling design that accounted for the complexity of this biological system to (1) estimate annual abundance and life-history parameters such as age and sex-specific survival rates, and (2) examine whether the data are sufficient to detect significant trends in abundance.

Material and Methods

Survey design and data collection.—Between August 2005 and December 2012, year-round boat-based surveys were conducted on the main core area of the studied dolphin community (Di Tullio et al. in press). The sampled area is 85 km² and encompasses the lower portion of the Patos Lagoon estuary (40 km²) and adjacent marine coast (45 km²). Resident well-marked dolphins can be reliably found in this area and are easily distinguishable from dolphins from other communities because they have been observed in the studied area for decades (Castello and Pinedo 1977; Fruet et al. 2011). Until December 2006, surveys were concentrated primarily in estuarine waters and followed predefined zig-zag transects. Since then, the surveys were extended to the adjacent marine coast and followed either zig-zag or parallel-to-shore transects up to 20 km north and south of the estuary mouth (Fig. 1). After transects were surveyed, dolphins were randomly searched in the estuary mouth (2005-2006) or along the coastal zone (2007-2012), depending on sea conditions. Surveys were curtailed when Beaufort Sea states reached 3. Transects were run at speeds between 18 and 22 km/h onboard a 5.3 m aluminum boat equipped with a 60 hp outboard engine until 2008, when it was replaced by a 5.5 m semi-rigid inflatable boat with 90 hp outboard motor. Two observers were positioned in the bow to perform 180° wide scans with naked eyes. Whenever a dolphin group was sighted, the survey route was abandoned for approaching the animals for photo-identification (see Fruet et al. 2011 for details). After ensuring that a sufficient

number of good quality digital photographs from the dorsal fins of all animals were taken, the survey was resumed.

Scoring dorsal fin photographs and individual identification.—Each photograph was scored with a quality grade between 1 and 3 (Q_1 - Q_3) (Hammond et al. 1990). Lower quality photographs (Q_2 and Q_3) were not considered for further mark-recapture analysis. “Marks” such as nicks, cuts and deformities are unique patterns in the dorsal fin which persist for a long time (years) (Würsig and Würsig 1977). Other mark types (e.g. tooth rakes, epidermal disease) were only used to assist in differentiating individuals without long-lasting marks within a short time frame (months). Two trained and experienced researchers made positive re-sightings of catalogued individuals by comparing all the digital images. The sighting history of each marked individual was translated into a numerical binary matrix of presence (1) and absence (0).

Determining the sex and life stage of individuals.—The sex of individuals was determined through genetic methods using biopsy samples (see Fruet et al. 2014), by simultaneous photographs from dorsal fin marks and the genital slit, or from records of adult individuals repeatedly found in close association with calves. The longitudinal photo-id data and biological characteristics of individuals were used for determining dolphin life stages. For both sexes we considered those >7 yrs old as adult individuals, as this is the minimum age at which some individuals from this community reach sexual maturity based on photo-identification data. Large dolphins sighted in close association with a calf in more than two independent sampling occasions (days), or based on molecular sexing, were considered as adult females. Adult males in this dolphin community are distinguished from others by its larger body size, notably higher dorsal fin, robustness, heavy tooth rakes on the body and no close association with a calf. Individuals with body size of approximately 2/3 of an adult, noticeably less robust than

an adult, light-gray and often (but not always) associated with an adult were considered juveniles. All juveniles (between 3 and 7 yrs of age) reported here acquired long-term marks in the dorsal fin during their first two years of life, allowing for a precise ageing and tracking since their separation from their mothers.

Mark-recapture analysis for abundance, survival and temporary emigration.—Mark-recapture analysis was conducted using the Pollock's Robust Design (RD) (Pollock 1982; Kendall et al. 1997), implemented in the MARK program 6.2 (White and Burnham 1999). The robust design combines both open and closed population models, and explicitly allows for temporary emigration (Williams et al. 2002). Longer intervals among marking sessions are defined as “primary periods”, when demographic changes can occur. In contrast, the shorter sampling intervals are referred to as “secondary periods”, and assume an effectively closed unit (i.e. events of births and deaths, immigration and emigration do not occur during this period) (Kendal et al. 1995, 1997). To obtain life-stage and sex-specific apparent survival probabilities we used individual data sets for parameter estimation of each of these components (hereafter termed “groups”) of the PLE dolphin community. Following notation of Kendall et al. (1995, 1997) we organized our sampling into two hierarchical periods: the primary periods were composed of 8 yrs with 3-4.5 mo of effort each year; and the secondary periods were represented by several sampling occasions within each primary period (between 10 and 13 survey trips conducted between late March and early November). This approach minimizes violation of closure assumption since bycatch mortality in this area occurs primarily between December and March (Fruet et al. 2012). For obtaining annual parameter estimates, time intervals between primary periods were quantified as decimal years between their last and first days of sampling (Δt). Primary periods were separated by a minimum of 4.9 mo (0.41 decimal yrs) and maximum of 12 mo (Table 1). In order

to decrease the effects of pseudoreplication (Wilson et al. 1999), data from consecutive surveys were excluded.

Model assumptions.—The following assumptions were made under the RD (following Williams et al. 2002): (1) marks are not lost during the study period; (2) marked individuals are correctly recognized when recaptured; (3) individuals are instantly released after being marked; (4) intervals between sampling occasions are longer than the duration of the sampling; (5) all individuals observed during a given sampling occasion have the same probability of surviving to the next occasion; (6) study area does not vary; (7) marked and unmarked individuals have the same probability of being captured; (8) the population remains closed (i.e., events of births and deaths, immigration and emigration do not occur) within primary periods; and (9) the capture of an individual does not affect its subsequent recapture probability during the secondary period. While our sampling design accounted for all these assumptions any potential violation may cause an extra binomial variation (Williams et al. 2002), which is a common characteristic of cetacean data (discussed later).

Building and selecting models.—Our RD models contain the following parameters: ϕ = apparent survival probability between primary periods; p = the probability of first capture; c = the probability of re-capture; γ' and γ'' = the probability that an individual would be unavailable for capture during primary period t , given that it was available or unavailable, respectively, for capture in period $t - 1$ (the probability of temporary emigration), and abundance (N) which is estimated as a derived parameter by Huggins's parameterization method (Huggins 1991). We considered several statistical models to evaluate different movement patterns, considering completely random ($\gamma'' = \gamma'$) or Markovian ($\gamma' \neq \gamma''$) emigration models, or no emigration models ($\gamma'' = \gamma' = 0$) (Kendall et al. 1997). Candidate models were also constructed assuming, or not, the

time-dependence (t) and/or groups (g) (sex and life-stage) effects in the parameters of interest (p' , p , ϕ , γ). The most parsimonious model was selected by the AICc (Akaike's Information Criterion adjusted for small sample size; Burnham and Anderson 2002). In our first round of modeling we ignored temporary emigration ($\gamma' = \gamma'' = 0$) and considered time (t) and group effect (g) in survival to explore both time ('t' for between primary periods; 's' for within primary periods) and group (g) effects in capture probabilities. For the second round we adjusted the apparent survival parameter evaluating time and group effects, considering the most parsimonious model for capture probabilities. We then explored the effects of temporary emigration fitting random ($\gamma' = \gamma''$) and Markovian ($\gamma' \neq \gamma''$) models with or without time effect on these parameters. To account for model selection uncertainty, abundance estimates and confidence intervals were obtained by averaging the results from models that supported the data (Burnham and Anderson 2002). Variation in survey effort may have affected capture probabilities in two ways: 1) changes in surveyed area (in 2005/2006 surveys were carried out primarily in estuarine waters, while in subsequent years they were extended to the adjacent coastal zone); 2) a decrease in survey effort in 2012 due to logistical issues. To account for potential effects due to these two changes in sampling effort we also built models where capture probabilities were constrained to vary between 2005-2006 and 2007-2012 seasons (p - season) and to vary between 2005-2011 and 2012 (p - 2012).

Because there are no appropriate methods available in MARK for testing the goodness-of-fit in RD models (White and Burnham 1999), the overall model fit could not be completely evaluated. Alternatively, we collapsed our data from the secondary period of the RD and fitted CJS models to evaluate if the open part of the RD model fitted our data. Goodness-of-fit tests were run using RELEASE in MARK. Extra binomial

variation (\hat{c}) was estimated by dividing the chi-square statistics of goodness of fit tests by the number of degrees of freedom, and by a parametric bootstrapping approach with 1,000 iterations. To be conservative, the higher \hat{c} values were used to adjust lack of fit of the models for both approaches (cf. Silva et al. 2009). Closure assumption underlying secondary periods were verified by visual inspection of discovery curves. The plateau of the discovery curve was defined as the survey where 95% of all observed dolphins could be photo-identified.

The rate of change in population size, sex ratio of marked individuals, yearly number of recruits to the marked population and total population size (which included the non-marked dolphins in their calculation) were computed from the same binomial dataset and/or parameters estimated by the RD modeling. Annual abundance of marked dolphins in the population was estimated separately in RD analysis for adult females (N_1), adult males (N_2) and juveniles (N_3) as derived parameters. The sum of abundance estimates per group represented the total abundance (\hat{N}_T) of all marked individuals ($\hat{N}_{(1+2+3)}$). The rate of change in abundance ($\hat{\lambda}_t$) between sampling periods was calculated as:

$$\hat{\lambda}_t = \frac{N_{T(t+1)}}{N_{T(t)}}$$

An index of recruitment was obtained by determining the number of new ‘captured’ individuals between sampling sessions. Adult sex ratio was determined for each year.

Estimating total population size — Total abundance (\hat{N}_T) of bottlenose dolphins was calculated using the ratio $\hat{N}/\hat{\theta}$, where theta ($\hat{\theta}$) is the proportion of marked individuals

in the population. For each primary period $\hat{\theta}$ was estimated as: $\hat{\theta} = \frac{\sum_{i=1}^k \frac{I_i}{T_i}}{k}$ and its

variance expressed as:

$$\text{var}(\hat{\theta}) = \left(\sum_{i=1}^k \frac{\theta_i(1 - \theta_i)}{T_i} \right) / k^2,$$

where I_i is the total number of dolphins with long-lasting marks photographed in the group i ; T_i is the total number of dolphins photographed in group i ; k is the total number of groups sampled (cf. Fruet et al. 2011).

This approach was used because marked dolphins compose a large proportion of the population, mean group size is small (= 4) (Mattos et al. 2007) and a variety of skin markings made it possible to distinguish unmarked dolphins in most of sampled groups. Large groups (>8 individuals) were excluded from this analysis because the number of unmarked individuals could not be determined with certainty.

The coefficient of variation for the total population size was expressed as a combination of CVs of \hat{N} and $\hat{\theta}$ ($CV(N_T) = \sqrt{(CV(\hat{N}))^2 + (CV(\hat{\theta}))^2}$), while the 95% confidence interval was constructed assuming a lognormal approximation as recommended by Burnham et al. (1987).

Power Analysis.—The program TRENDS (Gerrodette 1993) was used to run a power analysis in order to investigate the probability of our eight years of monitoring being able to detect a linear significant trend in the marked population, and also to assess the required time for detecting population changes with high statistical power (i.e. $\geq 90\%$). Three scenarios were run in order to simulate the detection of population decline of -5%, -10% and -15%. We assumed a linear model (as there is no evidence that this population is in recovering process), a one-tailed test (the focus here is on a decreasing

trend), a CV constant with abundance (since no clear relationship was detected plotting annual CVs against $\sqrt{\hat{N}}$ and $(\sqrt{\hat{N}})^{-1}$ and a t -student distribution (Gerrodette 1987). We fixed the probability of Type I and II errors as 0.05 and used the averaged CV of 3% obtained from our estimates. We also evaluated the effect of increasing estimation uncertainty by increasing CV to 5% (the highest CV obtained in this study for the total abundance estimation).

Results

Photo-identification.—After constraining the data to fit Pollock’s Robust Design models, we analyzed information from 103 boat-based photo-identification surveys (totaling 752.5 h of sampling) of bottlenose dolphins carried out in the Patos Lagoon estuary and adjacent marine coast. The dolphins were seen in all surveys and a total of 701 groups were sampled (Table 1). Sightings were made throughout the study area but most (about 71.4%) took place in the Patos Lagoon estuary or close to its entrance. On average, group size was small (mean = 5 dolphins; SD = 4), but groups larger than 8 individuals represented 14.9% of the encounters.

A total of 21,639 photographs were analyzed, from which 13,726 fitted in the higher quality (Q_1) category. After constraining for individual distinctiveness, 76 dolphins were considered for the mark-recapture analysis and a maximum of 62 marked individuals were sighted in a single year. This number fluctuated only slightly across seasons, suggesting an effective intra-annual sampling (Table 1). The cumulative number of identified dolphins experienced a pronounced increase during the first four surveys and then gradually decreased as survey effort progressed, suggesting a closed population within sampling seasons and that nearly all marked individuals in the PLE community were captured during the experiment (Fig. 2; Supplement 1). New marked dolphins have continually appeared at low numbers each year, resulting in an average

recruitment of three individuals per year ($SD = 2$) (Fig. 2). Many (95%) of these recruits have subsequently returned to the study area. Re-sighting rates were high, with an average of 84% ($SD = 5\%$) of marked dolphins subsequently sighted within, and 93% ($SD = 4\%$) between sampling seasons, demonstrating remarkable site fidelity. The adult sex ratio was biased towards females in all sampling seasons (Table 2).

Goodness-of-fit test and c-hat adjustment.—The goodness-of-fit test showed that the assumption of equal capture (TEST 2) and survival (TEST 3) probabilities were not violated and that the global CJS model fitted to the data (TEST 2 + TEST 3; $\chi^2 = 11.318$, $p = 0.254$, $df = 9$). Indeed, when estimating c-hat with RELEASE (= 1.25) and the Bootstrapping approach (= 1.42), no significant overdispersion was detected. Adjusting the models by the higher estimated c-hat did not change AIC ranking, but redefined models weight, which slightly modified the parameters estimated by the model averaging procedure.

Model selection.—QAIC_c (quasi-Akaike's Information Criterion with a second-order correction for small sample sizes) values indicated that during our first round of modeling the most parsimonious model (model 9), which disregarded temporary emigration and considered time variation between primary periods on the capture probability, fitted the data better than models allowing for a series of variation in capture probabilities, including group effect (models 11, 13 and 14). Models incorporating potential effects of effort (models 10 and 12) also had a poor fit (Table 3). Our second round of modelling favoured models with constant survival varying among groups. The model selection procedures indicated that the best-fitting model considered non time-dependence and group effect in survival probabilities, with no temporary emigration and with time-dependent capture probabilities among primary periods. A model considering Markovian constant temporary emigration (model 2) also adequately

fitted the data ($\Delta\text{QAICc} < 2$) (Table 3). Models considering temporary emigration, however, did not provide adequate parameter estimation (unrealistic confidence intervals and upward biased survival) and therefore results from these models were not considered further.

Apparent survival and capture probabilities.—The final data set included sighting histories of 58 adults (21 males and 37 females) and 18 juveniles. Overall apparent survival (considering model 5 that disregarded the group effect on survival) was 0.93 (95% CI: 0.89-0.95). Nevertheless, when considering the best fitted model, which takes into account group effect, the averaged annual apparent survival estimate was higher for adult females (0.97, 95% CI: 0.91-0.99) than for adult males (0.88, 95% CI: 0.75-0.94) and juveniles (0.83, 95% CI: 0.64-0.93). Mean annual capture probabilities among primary periods were moderate, with the highest obtained during 2005 season (0.33) and the lowest in 2007 (0.22) (Table 2).

Trends in abundance and power analysis.—Within each primary period, the plateau of the discovery curve was reached before the final survey (data not shown), suggesting that the population remained closed for immigration during the abundance estimates of marked individuals. Marked population size estimations were highly precise (CVs from 0.8% to 3.2%) and fluctuated around 50-60 individuals along the study period (Figs. 3 and 4). An increasing trend in abundance of marked individuals was observed during the first six years of sampling when the number of recruits surpassed mortality, followed by a remarkable decrease in the last two years when an inverse ratio of recruits/deaths was observed. Yearly changes in abundance ($\hat{\lambda}_t$) varied from -10% to 7% and were most evident from a high number of 63 dolphins in 2010 to a low of 54 at the end of the sampling period (Table 3; Fig. 3).

Despite the high precision obtained to estimate the number of marked individuals in the

community ($CV = 0.03$), power analysis showed that subtle changes in abundance (-5%) cannot be statistically detected over short periods of time. Nevertheless, our eight years of monitoring provided a satisfactory power of 0.89 and 1.00 to detect an overall population decline of 10% and 15%, respectively (Fig. 5). Additional three years of sampling effort would provide a desirable 0.95 likelihood of detecting a 10% decline in population size. However, a slightly increase in abundance uncertainty ($CV = 0.05$) greatly inflated the minimum number of years required to detect a negative trend under the target power of 90% (Fig. 5).

Mark rate and total abundance.—The estimated proportion of marked dolphins in the PLE community remained relatively constant over the years, ranging between 0.68 and 0.72 (Table 2), exerting a small influence on total abundance variation (Fig. 3). The highest theta values were estimated for years with the highest recruitment of juveniles into the marked population (2007, 2008 and 2009). Total abundance ranged from 75 (2012) to 88 (2011), and even considering the highest of all upper confidence limits, maximum abundance did not exceed 94 dolphins.

Discussion

This study represents one of the very few longitudinal studies designed for estimating dolphin demographic parameters along the Southwestern Atlantic Ocean. It differs from others by its remarkably consistent survey effort throughout an 8 yr period and by deriving life-stage and sex-specific survival rates. Our results indicate that this female sex-biased dolphin community is relatively stable despite the incidental mortality in fisheries reported for the last decade in adjacent areas (Fruet et al. 2012). The high adult female survival contrasts with the poor survival of adult males and juveniles, possibly reflecting a distinct vulnerability of these population components to local human-induced impacts.

Robust Design model assumptions.—Assumption of demographic closure was supposedly met by using data collected on the main distribution area of the PLE community over a relatively short time (considering that bottlenose dolphins can live for more than four decades – Wells and Scott (1999) - 4 mo. would represent only 1% of their life time) and during a period when the probability of human-induced mortality and births is low (Fruet et al. 2012). To ensure mark recognition we constrained our analysis to well-marked dolphins and used only high quality photographs. Geographical closure was probably not fully satisfied since individuals may move in and out of the study area, although the high residence pattern of the dolphins suggests a negligible influence of such movements on the results. Closure assumption is supported by the plateau of the discovery curve of marked dolphins within each primary period (Fig. 2), the high re-sighting rates of individuals (see Supplement 1), and the negligible number of carcasses found on coastal beaches close to the estuary mouth during the sampling periods of our MR experiment (2010, $n = 1$; 2011, $n = 1$). Equal capture probabilities among individuals, a critical assumption for most mark-recapture procedures, can be violated by a number of ways, especially when dealing with photo-identification (e.g. geographic sampling bias, differences in marks' distinctiveness). We explored Pledger's mixture model for individual heterogeneity (Pledger 2000), but this model suffered from over parameterization. Systematically covering the entire sampling area, stratifying our data by sex and age and using only well-marked animals potentially minimize violation of this assumption. The non-random associations between individuals can also increase the magnitude of bias caused by heterogeneity of capture probabilities. GOF test, however, did not suggest heterogeneity and the overdispersion estimates were not outstanding.

Survival.—The overall adult survival rate estimated here (0.93; 95% CI: 0.89-0.95) is

similar to those reported for other wild coastal communities of common bottlenose dolphins (e.g. Sarasota Bay, United States of America (0.96 ± 0.01 SD; Wells and Scott 1990), Morey Firth, Scotland (0.93 ± 0.29 ; Corkrey et al. 2008), Doubtful Sound (0.94, 95% CI: 0.92-0.95; Currey et al. 2008) and Bay of Islands, New Zealand (0.93, 95% CI: 0.91-0.94; Tezanos-Pinto et al. 2013), Little Bahama Banks, Bahamas (0.94, 95% HPDI: 0.82-0.99; Fearnbach et al. 2012) and the nearest community from our study area, in Laguna, southern Brazil (0.92, 95% CI: 0.88 – 0.96; Daura-Jorge et al. 2013)). Early studies have proposed that differences in survival estimates likely reflect differences in ecological characteristics of the study sites (Currey et al. 2008). Variations could also be due to uneven sampled ages to derive adult survival and different levels of human-induced impacts experienced by local dolphin communities (Silva et al. 2009).

One of the important features of our study was the ability to estimate sex (for adults only) and stage-specific (adults and juveniles) survival rates. These stratified estimates represent an advance for understanding processes shaping the dynamics of this bottlenose dolphin community. Models with group effect were strongly supported (cumulative QAIC_c weight 99.9%), from which constant survival rates for juveniles (0.83, 95% CI: 0.64-0.93), adult males (0.88, 95% CI: 0.75-0.94) and females (0.97, 95% CI: 0.91-0.99) were estimated. These noteworthy disparities in survival rates imply that selective forces are significantly constraining the survival of juveniles and adult males in this dolphin community. Species life history characteristics should be considered as potential sources driving the disparate survival rates. Bottlenose dolphins from the PLE are known to be sexually dimorphic, with males reaching larger sizes than females (Fruet et al. 2012). In sexually dimorphic species with a polygyny mating system, survival is expected to vary by sex due to differential energy investment in

growth and reproduction (i.e. male aggressive behaviour towards other conspecifics for mating access might constrain survival – e.g. Ralls et al. 1980; Promislow 1992).

Sex and life-stage biased bycatch mortality affecting the PLE community is certainly an important factor influencing survival estimates. Analysis of a dataset consisting of carcasses recovered during systematic beach surveys carried out between 1974 and 2006 in this region has shown that mortality of bottlenose dolphins near PLE was relatively high for immature and adult males and low for adult females (Fruet et al. 2012), corroborating with our survival estimates and the skewed adult sex ratio of live dolphins (*ca* 1M:2F). The potentially larger home-range of adult male bottlenose dolphins (e.g. Wells et al. 1987; Scott et al. 1990, Quintana-Rizzo and Wells 2001), may increase energetic requirements and susceptibility to predation and fatal interactions with human activities, such as fisheries. The inexperience and prolonged period that juveniles are exposed to natural (e.g. lower foraging skills for energy intake and protection against conspecifics; predation risk) and anthropogenic (e.g. boat strike; bycatch) threats could also lead to lower survival rates. Several studies have reported high juvenile bottlenose dolphin mortality associated with such threats (Hersh et al. 1990; Wells and Scott 1997; Stolen and Barlow 2003).

The lower vulnerability of females has yet to be determined. Fishing net densities were not evenly distributed throughout the studied area (Di Tullio et al. in press) and the sex ratio of biopsied dolphins (marked and unmarked individuals) inside the estuary was skewed towards females (Fruet et al. 2014). One possibility could be that fishing net densities are lower in areas preferred by females. Disentangling non-natural (bycatch) from natural mortality would allow for explicitly testing a wide range of biological hypotheses for these differences in survival rates. Individual genetic tagging offers a promising alternative to address this question, since the advanced decomposition state

of many carcasses of dolphins killed in fisheries makes it difficult to verify whether or not they belonged to the marked population.

Abundance estimates and trends.—The abundance estimates were highly precise (highest CV for total population size was 5.3% in 2011) and resulted in low numbers of dolphins using PLE in a yearly basis, corroborating previous estimates conducted using similar protocols (Dalla-Rosa 1999 – estimates for 1998; Fruet et al. 2011 – estimate for 2005). It has been proposed that communities of bottlenose dolphins associated with coastal, protected habitats such as estuaries and river mouths are generally small and resident (Wells and Scott 1990; Berrow et al. 1996; Wilson et al. 1999; Currey et al. 2007), but they tend to increase in numbers and range when inhabiting primarily open coasts (e.g. Defran and Weller 1999; Gubbins et al. 2003). In southern Brazil and Uruguay small communities numbering less than 90 individuals seem to be common in both relatively protected habitat (Daura-Jorge et al. 2013) and open coast (for a review, see Fruet et al. in press). Although the PLE community is one of the largest known for this region, there are reasons for conservation concern. Viability of small demographically independent units is naturally constrained due to environmental and demographic stochastic factors (e.g. Caswell 2001) and can be seriously jeopardized if survival is reduced by non-natural deterministic factors, such as bycatch.

The relatively stable abundance and high re-sighting rates of individuals within and between primary periods demonstrate marked fidelity to the study area, revealing the importance of the PLE for this bottlenose dolphin community. In many mammal species, including some communities of a closely related species, the Indo-Pacific bottlenose dolphin (*T. aduncus*), females tend to remain with their natal groups, whereas males often disperse (Möller and Beheregaray 2004). Our mark-recapture modeling, however, revealed that group had a non-significant effect on both temporary emigration

and capture probabilities, which would be expected in case of a substantial variation in habitat preferences and dispersal between sexes or life-stages.

As in a large number of populations of long-lived vertebrates, the annual rate of increase fluctuates only slightly, with no obvious changes in population size (Caughley and Sinclair 1994). A similar pattern was observed for the recruitment of individual dolphins to the marked population, as evidenced by a slightly variation in mark rate ($\hat{\theta}$) (Table 3). Observed shifts in population size were closely associated with number of recruits to and disappearances from the marked population between primary periods (see Figs. 3 and 4). Records from stranding carcasses systematically collected along the surveyed area during 2005-2012 corroborate the observed inverse relationship between mortality and population size estimation. These findings suggest that fluctuations were not only due to sampling variation (or movement in and out of the study area) but also to non-natural mortality that is possibly affecting the dynamics of this dolphin community. In addition, when mortality rates were low, abundance estimates increased, suggesting that PLE community may have a solid resilience under its current age and sex structure.

The abundance estimate and its precision were similar to a previous mark-recapture study carried out with the Patos Lagoon dolphin community ($N_T = 83$ (79-88); M_{th} model - Dalla Rosa 1999), suggesting a stable population over the last 15 yrs. Given the importance of mature females for reproduction and population viability, the high estimated survival rates of this component is probably the main factor buffering non-natural mortality and providing stability to the PLE. Alternatively, it could be going through a very slow process of decline, which may require a longer data set to detect significant changes in abundance (e.g. 20 yrs of data would not be sufficient to detect a 5% population decline with high statistical power - see Fig. 5).

As variation in abundance and other demographic parameters in large marine predators are potentially driven by both large and small-scale ecosystem changes, our results of relatively even inter-annual abundance estimates and high survival rates of PLE bottlenose dolphins may suggest a stable and healthy ecosystem. Although a recent analysis using a 30-year long database has revealed substantial changes in fish assemblage in PLE as a result of human pressure such as fishing and habitat loss (Moraes et al. 2012), which has apparently affected important prey items of bottlenose dolphins (Lopes 2014), such changes do not seem to have affected the dolphin's community demography. The generalist habits of bottlenose dolphins and their ability to adapt to variation in prey availability (Lopes 2014) could play an important role for their persistence in face of changing environments, such as depletion of fish stocks.

Future research.—The long-term monitoring of this bottlenose dolphin community is particularly important because the Brazilian government has recently approved a fishing closure area in the southern portion of the PLE and adjacent marine coast. This closure, which includes the core area used by this dolphin community, was specifically designed for banning gillnets in places of high overlap between dolphins and fisheries (Di Tullio et al. in press). Thus, the continued monitoring of PLE dolphins will provide data for quantifying and assessing the effectiveness of this conservation measure. Extending the monitoring period will also increase the likelihood of detecting potential trends in abundance and thus allow understanding of how this dolphin community may respond to environmental stress. Other demographic parameters, such as age at first reproduction, fecundity, calf survival and longevity should be prioritized in future research. These parameters are relevant for building stage- or age-structured population models to describe the dynamics of this community and its viability in face of current and future human impacts and environmental change.

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Table 1.—Sampling scheme and survey effort after constrained the dataset to fit Pollock’s Robust Design (see methods). Δ_t = Time between sampling seasons (duration of primary periods); Q_1 = number of excellent graded quality photographs; M_{t+1} = number of marked dolphins sighted.

Year	Sampling season (secondary period)	Sampling Duration (Mo.)	Surveys (N)	Δ_t (Mo.)	Sampled Area	Effort (h:mm)	Number of encounters	Q_1	M_{t+1}
2005	04 Aug. – 13 Nov.	3.4	13	-	40 km ²	98:21	122	1473	56
2006	06 June – 08 Oct.	4.1	12	6.8	40 km ²	96:35	118	1469	56
2007	10 Apr. – 20 July	3.4	15	6.1	85 km ²	106:48	83	1514	58
2008	26 Mar. – 14 July	3.7	11	8.3	85 km ²	93:20	92	1204	58
2009	11 May – 11 Aug.	3.0	13	10.0	85 km ²	111:23	89	1479	62
2010	06 Aug. – 05 Nov.	3.0	11	12.0	85 km ²	98:01	57	1388	60
2011	01 Apr. – 06 Aug.	4.2	11	4.9	85 km ²	81:59	73	2322	59
2012	03 Mar. – 17 July	4.5	10	7.0	85 km ²	66:02	67	1677	50

Table 2.—Mark-recapture parameters estimated for the Patos Lagoon dolphin community in 2005-2012: annual survival, capture probabilities ($\hat{\rho}$), abundance of marked dolphins (\hat{N}) and their proportion in the population ($\hat{\theta}$), rate of change in abundance (λ), and annual adult sex ratio. Values between brackets are the associated measurements of parameter estimate uncertainty.

Year	Annual survival (95% CI)			$\hat{\rho}$ (95%CI)	$\hat{N}_{g1+g2+g3}$ (CV)	$\hat{\theta}$	λ	Adult sex ratio (M:F)
	Juveniles (n = 18)	Adult Males (n = 21)	Adult Females (n = 37)					
2005				0.33 (0.29-0.37)	56 (0.01)	0.69	1	17:33
2006				0.31 (0.27-0.35)	56 (0.01)	0.69	1.05	18:35
2007				0.22 (0.19-0.25)	59 (0.02)	0.72	1	20:34
2008	0.83 (0.64-0.93)	0.88 (0.75-0.94)	0.97 (0.91-0.99)	0.27 (0.24-0.32)	59 (0.02)	0.70	1.07	20:35
2009				0.25 (0.22-0.29)	63 (0.01)	0.72	1	18:37
2010				0.24 (0.20-0.28)	63 (0.02)	0.73	0.95	16:37
2011				0.28 (0.24-0.32)	60 (0.02)	0.68	0.90	16:35
2012				0.22 (0.18-0.27)	54 (0.03)	0.69	-	13:31

Table 3.— Details of mark-recapture fitted models and model selection statistics obtained in MARK's analysis of the 2005-2012 sighting history data from the Patos Lagoon bottlenose dolphin community using the Pollock's Robust Design. For each model, number of parameters (No. Par), a relative measure of fit (QDEV- the relative deviance) and the quasi-Akaike's Information Criterion with a second-order correction for small sample sizes (QAIC_c) are shown. Notation: (ϕ) apparent survival, (ρ) capture probability, (.) constant, (t) time-dependence, (g) group effect, (γ'') probability of temporary emigration, (γ') probability of remaining outside the study area, ($\gamma'' = \gamma' = 0$) no emigration, ($\gamma'' = \gamma'$) random emigration, ($\gamma''\gamma'$) Markovian emigration. Recapture probability (c) is not shown in the model description because it was set equal to (ρ) for all candidate models.

	Model	QAIC _c	Δ QAIC _c	QAIC _c Weight	Model Likelihood	No. Par.	QDev
1.	$\{\phi_{(g)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(t)}\}$	4703.6451	0.0000	0.44124	1.0000	11	5773.9503
2.	$\{\phi_{(g)} \gamma'_{(.)} \gamma''_{(.)} \rho_{(t)}\}$	4703.9417	0.2966	0.38042	0.8622	13	5770.1795
3.	$\{\phi_{(g)} \gamma'_{(.)} = \gamma''_{(.)} \rho_{(t)}\}$	4705.6774	2.0323	0.15972	0.3620	12	5773.9503
4.	$\{\phi_{(g)} \gamma'_{(g)} \gamma''_{(g)} \rho_{(t)}\}$	4710.1242	6.4791	0.01729	0.0392	17	5768.1946
5.	$\{\phi_{(.)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(t)}\}$	4713.3807	8.0478	0.00970	0.0179	9	5775.1666
6.	$\{\phi_{(g)} \gamma'_{(t)} = \gamma''_{(t)} \rho_{(t)}\}$	4716.6136	12.9685	0.00067	0.0015	18	5772.6354
7.	$\{\phi_{(t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(t)}\}$	4718.1081	14.4630	0.00032	0.0007	15	5780.2677
8.	$\{\phi_{(g)} \gamma'_{(t)} \gamma''_{(t)} \rho_{(t)}\}$	4718.1781	14.5330	0.00031	0.0007	24	5761.8493
9.	$\{\phi_{(g*t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(t)}\}$	4723.1938	19.5487	0.00003	0.0001	29	5756.4962
10.	$\{\phi_{(g*t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(season)}\}$	4734.1080	30.4629	0.00000	0.0000	23	5779.8445
11.	$\{\phi_{(g*t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(.)}\}$	4734.9985	31.3534	0.00000	0.0000	22	5782.7977
12.	$\{\phi_{(g*t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(2012)}\}$	4735.3378	31.6927	0.00000	0.0000	23	5781.0744
13.	$\{\phi_{(g*t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(g)}\}$	4738.3102	34.6651	0.00000	0.0000	24	5781.9814
14.	$\{\phi_{(g*t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(g*t)}\}$	4747.1994	43.5543	0.00000	0.0000	45	5746.8439
15.	$\{\phi_{(g*t)} \gamma'_{(.)} = \gamma''_{(.)} = 0 \rho_{(g*t*s)}\}$	4990.5170	286.8719	0.00000	0.0000	309	5304.6811

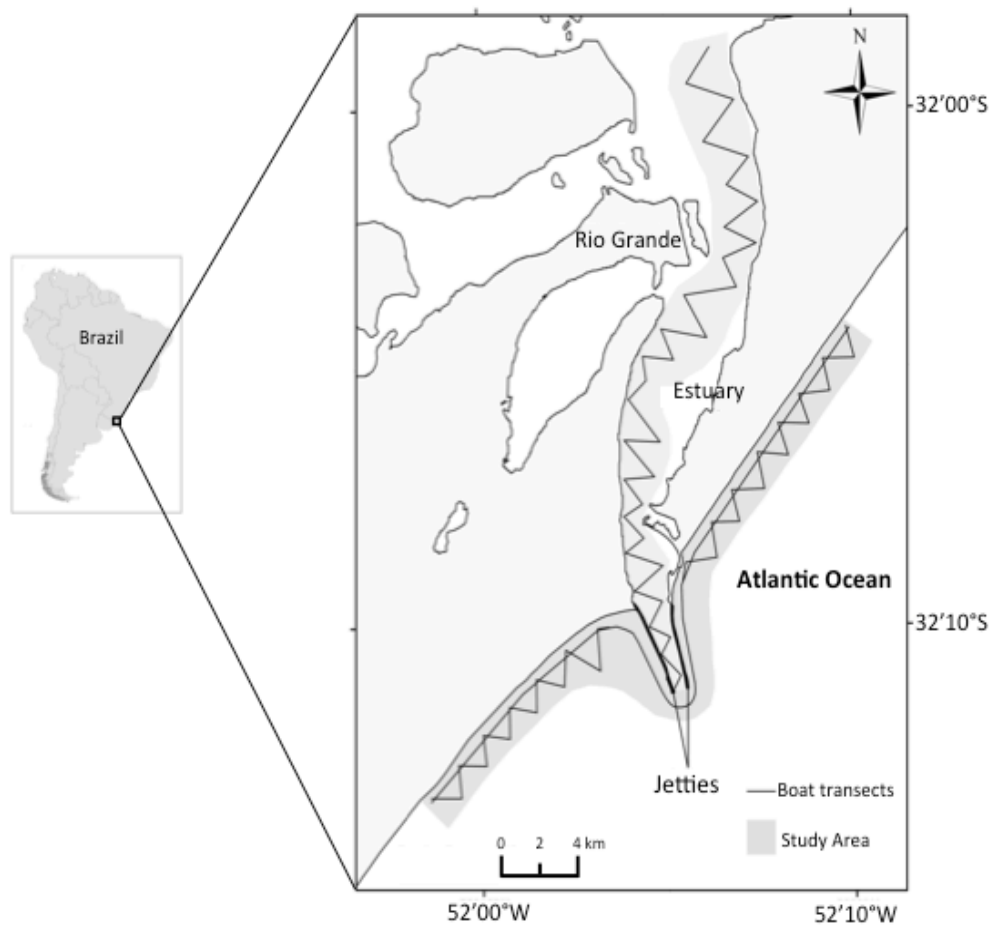


Fig. 1.—Map of the study site showing transects (solid lines) designed for surveying common bottlenose dolphins (*Tursiops truncatus*) in the Patos Lagoon estuary and surrounding coastal areas, southern Brazil. Dark-gray shaded area delimits the surveyed area.

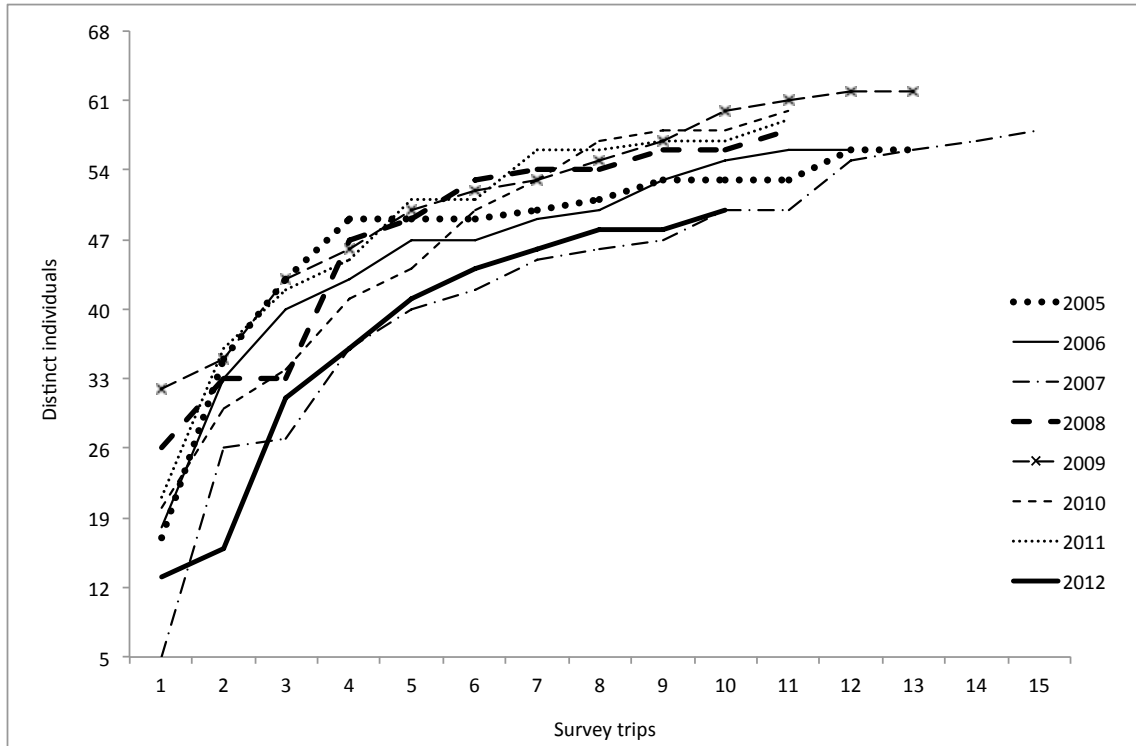


Fig. 2.—Discovery curve plot showing the yearly cumulative number of photo-identified bottlenose dolphins (*Tursiops truncatus*) in the Patos Lagoon Estuary, southern Brazil, against the sequential surveys for each sampling period (2005-2012).

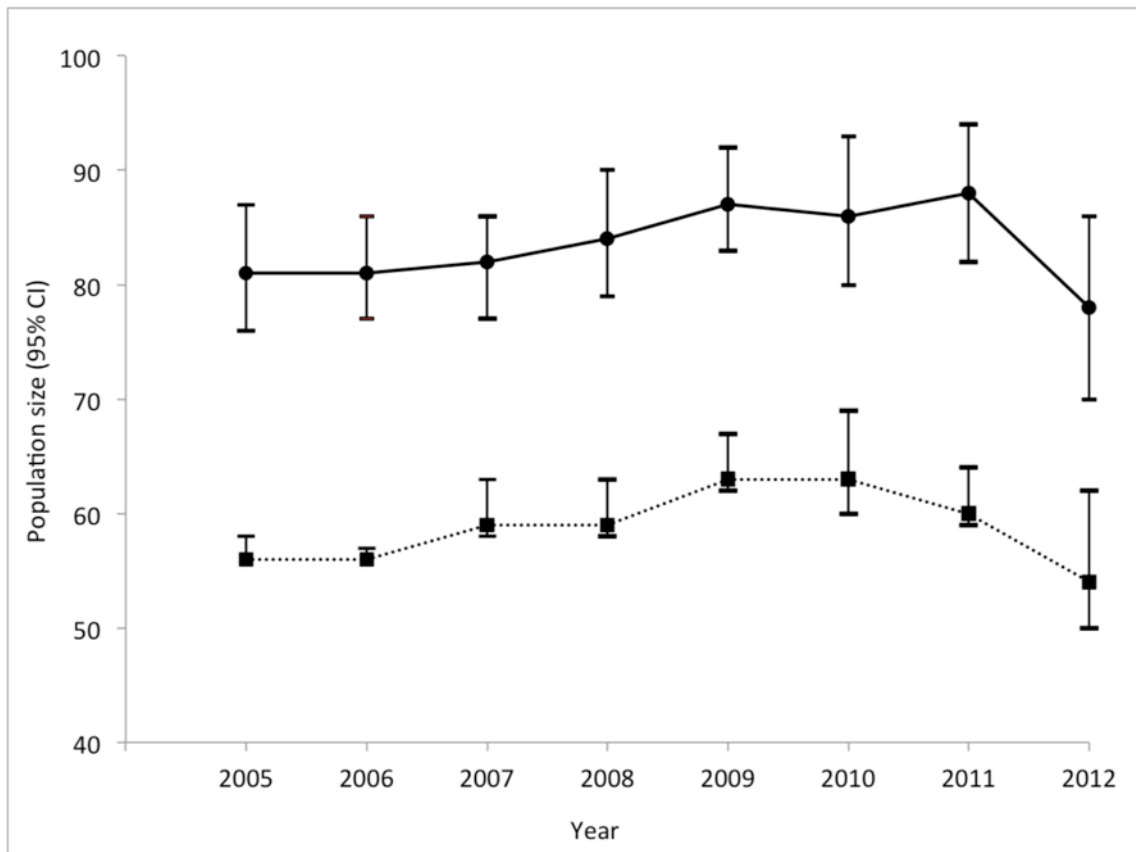


Fig. 3.—Annual abundance estimates for the Patos Lagoon bottlenose dolphin (*Tursiops truncatus*) community in 2005-2012 and their associated levels of uncertainty. Dashed and continuous lines are estimates for marked and total population size (corrected by the proportion of marked individuals in the population), respectively. Vertical lines are the 95% confidence intervals.

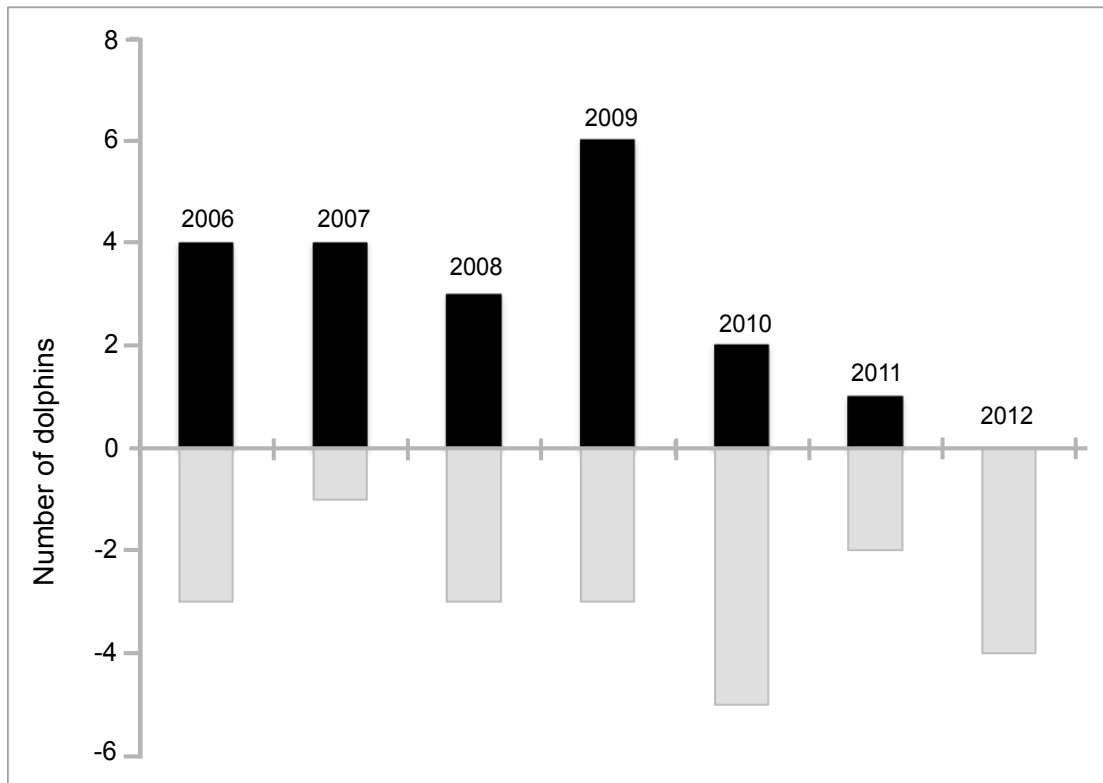


Fig. 4.—Gross annual recruitment (black bars above the x-axis) and apparent mortality (grey bars, below the x-axis) extracted from the 2005-2012 sighting history data of naturally marked resident bottlenose dolphins (*Tursiops truncatus*) from the Patos Lagoon estuary, southern Brazil.

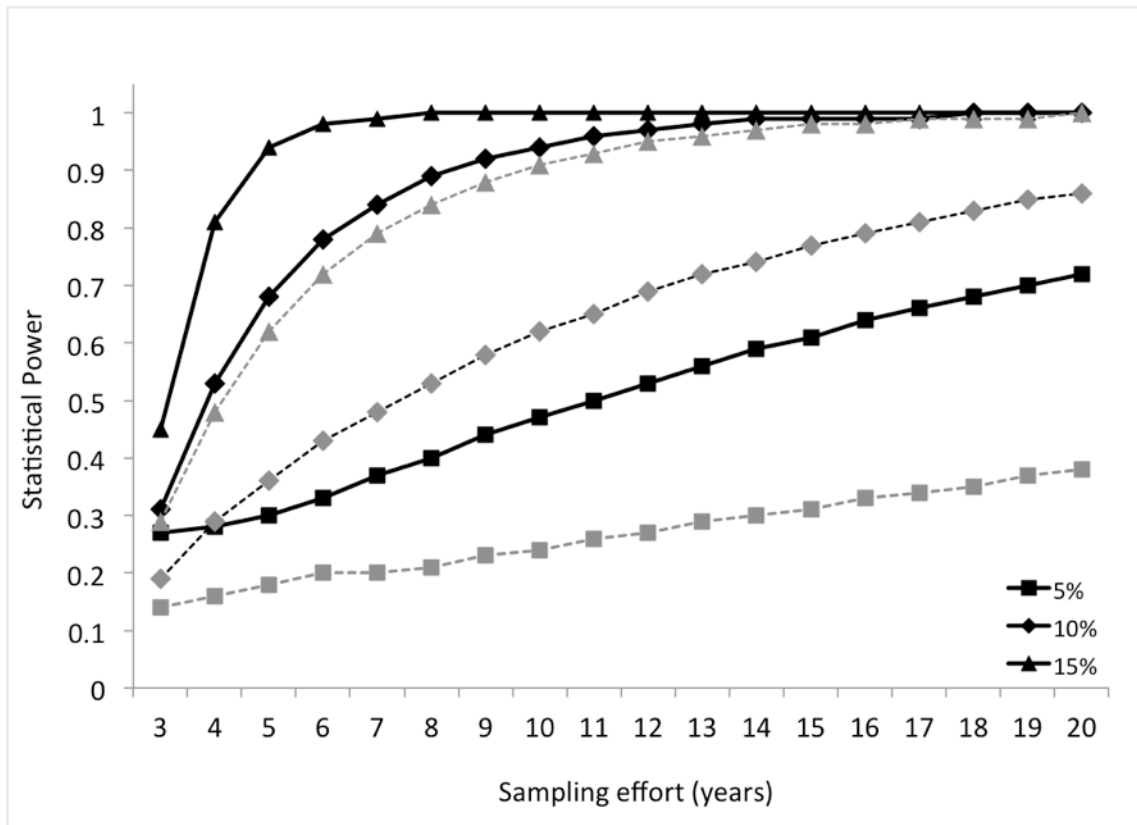
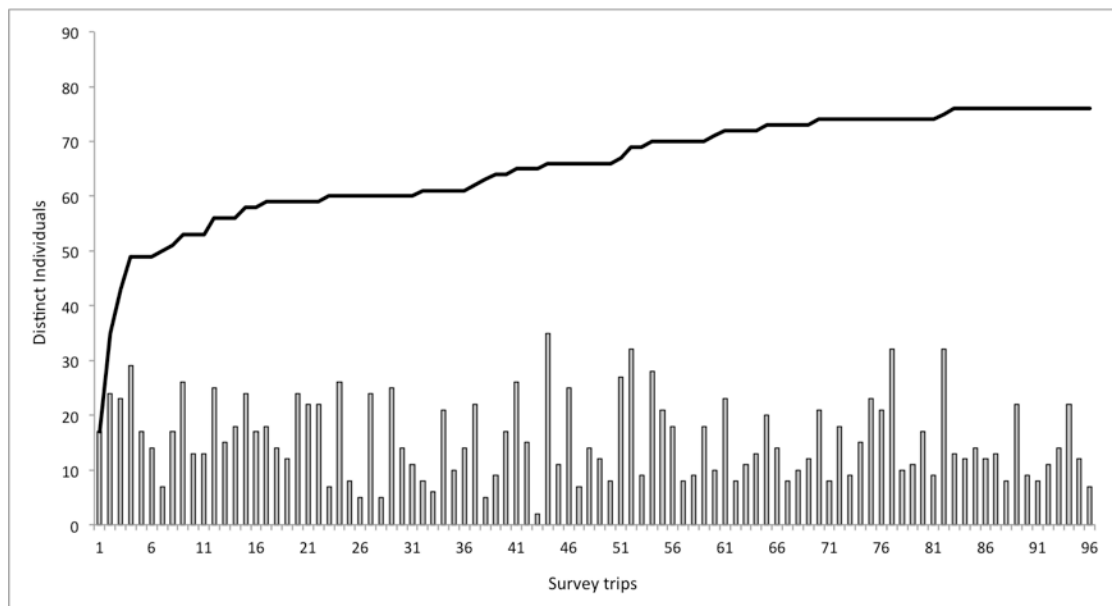


Fig. 5.—Power analysis plot showing the relationship between statistical power and time (represented as sampling effort) to detect a significant overall population decline of 5%, 10% and 15% in the face of two levels of precision found during our eight years of monitoring: the average (CV= 0.03 - continuous line) and highest (CV of 0.05 - dashed lines). For this analysis, we assumed a linear model, a one-tailed test, a CV constant with abundance, and a *t*-student distribution, fixing the probability of Type I and II errors as 0.05.



Supplement 1. Discovery curve plot showing the cumulative number of photo-identified bottlenose dolphins (*Tursiops truncatus*) in the Patos Lagoon estuary against the sequential survey effort (black line) and the respective number of individuals sighted during each survey (columns).

ANEXO III

(Em revisão no periódico “*Marine Biology*”; submetido em 05 Junho de 2014).

Integrating mark-recapture and stranding data to estimate reproductive traits in female bottlenose dolphins (*Tursiops truncatus*) of the Southwestern Atlantic Ocean

Pedro F. Fruet^{1,2,3,4,5*}, Rodrigo César Genoves^{2,3}, Luciana M. Möller^{4,5}, Silvina Botta³
and Eduardo R. Secchi^{2,3}

¹ Programa de Pós-Graduação em Oceanografia Biológica, Universidade Federal do Rio Grande - FURG, Brazil

² Museu Oceanográfico “Prof. Eliézer C. Rios”, Universidade Federal do Rio Grande - FURG, Brazil

³ Laboratório de Ecologia e Conservação da Megafauna Marinha (EcoMega), Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Brazil

⁴ Cetacean Ecology, Behaviour and Evolution Lab, School of Biological Sciences, Flinders University, Australia

⁵ Molecular Ecology Laboratory, School of Biological Sciences, Flinders University, Australia

Corresponding Author: Pedro F. Fruet (pfruet@gmail.com)

Phone: +55 (53) 32329107

Abstract

Despite well-studied in several regions, no information is available about the reproduction of bottlenose dolphins (*Tursiops truncatus*) of the Southwestern Atlantic Ocean (SWA). Data from a long-term mark-recapture and stranding monitoring program were used to estimate life history traits of female bottlenose dolphins inhabiting the Patos Lagoon estuary (PLE), a sub-tropical coastal region in the SWA. From the analysis of 32,296 high qualities dorsal fin photographs, the fate of 37 individual females and 66 of their calves were tracked. Results supported a birth pulse dolphin community, with most births occurring during late spring and summer, in association with increased water temperature and food supply. Female bottlenose dolphins first reproduced at a minimum age of 8 years. Mean inter-birth interval was 3 years (mode = 2) and fecundity was 0.11. A clear change in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiles in teeth from stranded carcasses near age 2 indicated the most probable weaning age. First and second-year annual calf survival estimates were 0.84 (95% CI = 0.72–0.90) and 0.86 (95% CI = 0.74–0.94), respectively. Older females reproduced at lower rates, suggesting an age-related decrease in reproductive output. At an individual level, marked variation in reproductive success was observed. Our findings suggest that soon after sexual maturity PLE female bottlenose dolphins tend to allocate more energy into offspring production than in parental care. This is followed by a decrease in reproductive rates and potential increase in parental investment during the last quarter of their reproductive life.

Introduction

The rate at which population abundance changes largely depends on individuals' ability to reproduce. In slow-reproductive species this is primarily governed by females' survival and reproductive potential, as most population dynamic models assume that some variation in male availability does not necessarily limit female reproduction in polygamous or promiscuous systems (Caswell 2001). Age at first reproduction, survival, fecundity, inter-birth interval, longevity and senescence are all important parameters that define a female's reproductive potential. For mammals, all these components are influenced by individual fitness, which in turn underlies complex interactions between biological, ecological and social factors (e.g., Lindström 1999; Stanton and Mann 2012). Therefore, fitness (or its associated constituents, survival and reproduction) is expected to vary among closely related species, and between and within populations of the same species, as local populations are exposed to specific environmental conditions, social systems, and individual variation in coping with these forces.

The common bottlenose dolphin (*Tursiops truncatus*) (hereafter "bottlenose dolphin") is widely distributed and inhabits several kinds of environments such as open oceans, river mouths, estuaries and tidal creeks (e.g., Wells and Scott 1999). As a result of such plasticity, their life history parameters and reproduction onset can vary at different scales along its distribution. For example, female bottlenose dolphins apparently start to reproduce early in life in the Sarasota Bay community, Northwest Atlantic (minimum age at first reproduction is 6 years; mean = 9; Wells and Scott 1990; Wells 2000), in contrast to individuals studied in Doubtful Sound, South Pacific (mean = 11.6 years; Henderson 2012). Generally, bottlenose dolphins inhabiting high latitudes have a well-

defined birth season coincident with periods of high levels of prey availability (e.g., Henderson et al. 2014) in contrast to a diffuse birth period reported for those in lower latitudes (e.g., Urian et al. 1996), where resources are less predictable in space and time. Average inter-birth interval also varies among regions and female reproductive success can vary greatly between individuals within populations (Wells 2000; Henderson et al. 2014). Thus, understanding the parameters and factors affecting them at regional, populational and individual levels is important for a better understanding of the basic biology of the species, how it interacts with the environment, and how variations in the environment impact on population dynamics.

A common approach for estimating life history parameters in cetaceans is based on age estimation and histological or gross observation of gonadal tissue from stranded or bycaught individuals (e.g., Perrin and Donovan 1984). This method has been widely used to estimate reproductive parameters of bottlenose dolphins in several regions of the world (e.g., Cockcroft and Ross 1990; Stolen and Barlow 2003). However, such cross-sectional studies often provide only a “snapshot” of some parameters at a specific point in time with no individual and temporal variability.

Alternatively, information on life history traits can be obtained through the use of mark-recapture (MR) data to track naturally marked dolphins over time (i.e., longitudinal studies). When repeated sightings of marked females over multiple years are available, it is possible to build individual reproductive histories from which fertility, birth season, birth intervals and age at first reproduction can be assessed (Hammond et al. 1990). This type of data has the advantage over cross-sectioned studies by allowing for the description of reproduction patterns over time and also providing the opportunity to investigate individual variation in reproductive rates, which is often lacking in cetacean

studies.

Bottlenose dolphins are common in coastal waters of southern Brazil. In this area they are typically found forming small and year-round resident communities (*sensu* Wells et al. 1987) associated to estuaries and river mouths (Simões-Lopes and Fabian 1999; Fruet et al. 2011; Daura-Jorge et al. 2013), with restricted dispersal (Fruet et al. 2014).

The small and resident community of bottlenose dolphins inhabiting the Patos Lagoon estuary (PLE) and adjacent coast has been studied since the mid-1970s (Castello and Pinedo 1977). Mark-recapture data collected non-systematically before 2005 and systematically since then made possible to track several individuals for many years, allowing determination of their sex, age and some key life history parameters. Presently, approximately 70% of the individuals, including all adult females, are recognized by natural marks in their dorsal fins (Fruet et al. 2011). In addition, the regular beach surveys conducted along the core area of the community (Fruet et al. 2012) make possible to collect stranding carcasses of some marked individuals with known reproductive histories from which relevant demographic parameters can be estimated.

While substantial information about reproductive parameters of female bottlenose dolphin has emerged in the last decades, no information is available for bottlenose dolphins from the SWA. In this study we combined 8 years of data collected from a systematic MR study and stranding monitoring program to estimate life history traits of female bottlenose dolphins of the PLE community, including calving seasonality, inter-birth intervals, weaning onset and age at first reproduction. We also investigate temporal and individual variation in female reproductive success and estimated calf survival rates. As life history theory predicts that births should pulse during better environmental conditions in areas subjected to substantial seasonal changes in

biological conditions, such as the PLE, we hypothesized that calves born during the pulse should have greater chances of survival. The results of this study will provide crucial information for future comparative analysis aiming at understanding how this top-predator interacts with distinct habitat types subjected to divergent environmental conditions along its distribution.

Methods

Study area and surveys

Boat-based surveys for monitoring bottlenose dolphins were conducted regularly between December 2004 and March 2013 in the PLE and adjacent coast, encompassing an area of approximately 85 km² (Fig. 1). This is the core area of occurrence of this dolphin community (Mattos et al. 2007; Di Tullio 2009). The area is characterized by high anthropogenic influence (such as boat traffic and industrial activities) and turbid, though highly productive waters, being considered one of the most productive fishing grounds in Brazil (e.g., Reis and D'Incao 2000; Odebrecht et al. 2010). As typical of sub-tropical regions, this area is subject to strong seasonal variation in temporal and spatial patterns of resources, including the occurrence and abundance of fish assemblages (Garcia et al. 2012; Rodrigues and Vieira 2013).

Surveys were run throughout the year using *ca.* 5 m-long boats powered by 60 or 90 hp outboard engines. These were photo-identification surveys that generally followed pre-determined routes consisting of zigzag or linear transect inside the estuary and/or perpendicular transects to the adjacent coast. As a standard protocol for this boat-based monitoring program (see Fruet et al. 2011 for more details) photo-identification took place for every dolphin or group of dolphins encountered using digital SLR cameras

equipped with 300 mm (f 2.1) or 70–300 mm (f 4.5–5.6) lens. After selecting only good/excellent quality photographs (i.e. dorsal fin occupying a large proportion of the frame, in sharp focus, without water drops, orientated perpendicular to photographer) (Hammond et al. 1990), individuals were identified by the presence of evident long-lasting marks in their dorsal fins, such as cuts, nicks and/or deformities.

Identification of mothers and calves

Adult dolphins regularly sighted in close association with calves (small size, light grey in coloration) were assigned as "mothers". As calves generally do not acquire long-lasting marks in the dorsal fin in their first years of life, they were tracked by following their well-marked (supposedly) mothers until weaning. Temporary marks in the dorsal fin (e.g., scratches) were also used to assist in identifying calves without long-lasting marks.

Age at first reproduction

Information regarding age at first reproduction was documented from females born during the study period (2005–2013), and two others that had the year of their birth back calculated from their first sighting in 2005 when they were between 1 and 2 years of age.

Annual crude birth rate and fecundity

Number of births was obtained by counting individual newborns on an annual basis. The annual crude birth rate was calculated for each year as $\frac{N_b}{\hat{N}_T}$, where N_b is the number of births and \hat{N}_T is the abundance estimate (Fruet et al. in press).

We estimated fecundity (the number of offspring produced by mature females in a given period, assuming the expected calf sex ratio of 1:1 for mammals; Caughley 1977) as a measure of the potential reproductive capacity of the PLE dolphin community. We used longitudinal photo-identification data to estimate the minimum number of mature females in the community in each given year. We considered all individuals that have been frequently recorded with a closely associated calf along the study period as mature females. Non-systematic photo-identification data collected in the PLE between 1976 and 2004 were also used to assist our analysis (Castello and Pinedo 1977; Dalla Rosa 1999). Using these data, individuals that have never been seen with a calf during the 2005–2012 period, but that were classified as "mothers" in previous years, were also included as mature females. Thus, mean fecundity was calculated as

$$\hat{F}_i = \frac{1}{2n} * \sum_{i=1}^n Nci / Nmi$$

where \hat{F}_i is the estimated fecundity in year i ; Nci is the number of calves born in year i ; Nmi is the number of mature females alive in year i .

Seasonality in reproduction

Seasonal patterns in reproduction were investigated based on the estimated month of births. The month of a calf's birth was estimated as the midpoint between the day of the last sighting of its mother without the newborn and the date of her first sighting with the calf (adapted from Wells et al. 1987). If this time exceeded 45 days, the estimated date of birth was not included in the analyses, except when calves presented clear characteristics of newborns (dark-grey coloration, prominent foetal folds, floppy dorsal fin; Fig. 2), with birth assigned to the month of their first sighting. Additionally, calving

seasonality was investigated by analysing stranding data collected from the beach surveys carried out monthly by experienced personnel along the coastal beaches adjacent to the Patos Lagoon estuary between 2005 and 2012. Fresh to moderately decomposed carcasses (conditions code ≤ 3 of Geraci and Lounsbury 2005) with total length ≤ 140 cm and having any characteristic of a newborn mentioned above were assigned as neonates (Fig. 2). From these combined dataset we constructed a frequency distribution of birth dates and defined the birth pulse (based on MR data) as the shortest period where $\geq 70\%$ of births had occurred. Seasons were defined as follow: spring (October–December), summer (January–March), autumn (April–June) and winter (July–September). We used monthly surface water temperature data in the estuary collected between December 2004 and March 2013 to investigate for a potential correlation of this variable with calving. These data have been systematically collected since 1998 by *Universidade Federal do Rio Grande* (FURG) at a portion of the Patos Lagoon estuary frequently used by dolphins, as part of the Brazilian Long Term Ecological Research (BR_LTER) (Seeliger and Odebrecht 2010).

Calf survival

We used the Cormack-Jolly-Seber (CJS) open population models (Cormack 1964; Jolly 1965; Seber 1965; Lebreton et al. 1992) to estimate annual calf survival rates from calves born to mothers with conspicuous dorsal fin marks that gave birth between 2005 and 2011 ($n = 30$). As the minimum weaning age was estimated to be around 2 years for this dolphin community (see results) we assumed that a calf had died if it was no longer seen in association with its regularly sighted mother until the end of the calf's second year of life. Analyses were performed in program MARK 7.1 (White and Burnham 1999). We estimated age-specific survival by fitting age models to calf sighting history

data, letting survival (Φ) be constant (.) or to vary with time (t), with constant recapture probabilities for all candidate models (justified by intense field effort and high rates of encounter of adult females in this community; Fruet et al. 2011). Our models included the following age classes: 0–1, 1–2 and >2 years. The potential effect of timing of birth on calf survival until weaning was investigated by fitting models where the calf's sighting history data were split into two groups: dolphins born during the pulse birth season (December–February, $n = 34$) and born out-of-pulse birth season (March–November, $n = 10$). Dolphins born after 2011 were excluded from this analysis because not enough time has elapsed to estimate survival until weaning. The LRT (Likelihood Ratio Test) was used to test this biological hypothesis between nested models. We used the Akaike Information Criterion (AIC) to select the most parsimonious model (Burnham and Anderson 1992). As the CJS goodness-of-fit (GOF) test neglects age effect, model fit was assessed by a parametric bootstrapping approach with 1 000 iterations (White et al. 2001).

Inter-birth interval

The inter-birth interval (IBI) was estimated using two approaches (adapted from Mann et al. 2000). Firstly, we used the entire reproductive history dataset available that included 37 well-marked mature females in the PLE community. This approach included IBI of females with large gaps between weaning of one calf and the birth of the next, therefore potentially biasing IBI upwards by failing to detect births that might have occurred in between. In the second approach, females with larger IBI (>2 years) were considered only if they remained in close association with the surviving calf until the known subsequent birth ($n = 30$). In this case, downward bias in IBI estimates may occur.

Age at weaning

The weaning age was estimated by stable isotope analysis (e.g., Newsome et al. 2006; 2009). Carbon and nitrogen isotopes in teeth from stranded carcasses were used to investigate the age at weaning. The rationale for this approach is based on the fact that offspring still nursing on their mother's milk, which is produced by catabolism of her own tissues, will present higher $\delta^{15}\text{N}$ relative to the mother's signal. Likewise, they should also present lower $\delta^{13}\text{C}$ values due to the influence of high lipid content, and thus ^{13}C depleted milk (Hobson and Sease 1998; Newsome et al. 2009). In this context, time of weaning was defined as the age when a change in trend of $\delta^{13}\text{C}$ (i.e., a marked increase followed by a stabilizing or reduction phase) and $\delta^{15}\text{N}$ (i.e., a marked decrease followed by a stabilizing or increasing phase) was detected (Newsome et al. 2009).

Reported weaning ages for bottlenose dolphins vary between 1.5 to 2 years of age (Wells and Scott 2009). In our study, we selected only individuals from 0 to 5 years ($n = 49$) to include the most probable weaning age classes. Dolphins aging <1 year found stranded during the birth pulse period that presented a considerable amount of post-natal dentine deposition (dentine deposited after birth) were considered as 0.5 years as they were probably almost 1 year-old (Hohn et al. 1989). Teeth of each age group were processed for stable isotopes analysis following Botta et al. (2012). Isotopic results are expressed in delta notation ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) in parts per thousand (‰). Repeated analysis of an internal standard yielded a within-run standard deviation of 0.2 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Differences in dentine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among ages were assessed using a one-way analysis of variance (ANOVA). When significant differences were detected this was followed by a *post hoc* Tukey's Honest Significant Difference pairwise comparison (Zar

2010). Generalized additive models (GAMs) were used to describe the age-related changes in isotopic composition. Models were performed using R (R Development Core Team 2006), and GAMs were fitted with the mgcv package in R (Wood 2006).

Reproductive success (RS)

We assumed that a female reproduced successfully if her calf survived from birth to the minimum age at weaning estimated for PLE bottlenose dolphins (i.e., 2 years – see Results). Otherwise, we considered she has failed that reproductive attempt. We estimated average female RS of PLE bottlenose dolphin community and also explore the potential effects of time (cohort) and individual differences in RS frequencies. Individual analysis was restricted to females with ≥ 3 documented births to reduce the influence of small sample sizes. Females that gave birth during the end of the sampling period (2011–2012) were not included, as their offspring would not have completed the minimum estimated age of weaning.

Results

Between December 2004 and March 2013 we spent 284 days (1 890h) collecting data from bottlenose dolphins in the PLE and surrounding areas. Boat-based survey effort varied slightly along the year, peaking in fall (28.5% of sampling days; $n = 81$) and being lowest during the winter months (21.5% of sampling days; $n = 61$). Bottlenose dolphins were found in all surveys. From the analysis of 32 296 high quality dorsal fin photographs we could recognize and track the reproductive history of 37 well-marked females. Maximum number of documented births per female was four (median = 2). Nine (25%) reproduced only once, from which four were primiparous and five multiparous mothers based on long-term observations. Three females (8%) were not

documented with calves between 2005 and 2013, two of them (LP#028 and LP#021) died in 2010 and had their ages estimated at 40 and 44 years by counting growth layer groups in the teeth dentine and cement (EcoMega Research Group, unpubl data). The third one (LP#050), frequently sighted in the studied area since 1977, suddenly disappeared and supposedly died in 2012 with a minimum age of 40 years, as it was considered adult when first sighted.

Age at first reproduction (AFR)

We had the opportunity to track four females since birth. Two of them (LP#091 and LP#107) gave birth for the first time at age 8. The calves of both females survived their first year of life. A 9 years-old female (LP#116) did not give birth until the end of the study. Another female (YO#021) that gave birth in the summer of 2013 AFR was estimated at 10 years, as it was aged 2 years when first sighted in 2005. Despite the limited sample size and the possibility of undetected births, it seems that PLE females first reproduce at about 8 years of age.

Annual crude birth rate and fecundity

Fifty-nine births were documented along the study period. Seven 1 year-old calves first sighted in 2005 were also documented. The annual number of documented births varied from five in 2006 up to nine in 2008 and 2011 ($\text{mean} \pm \text{SD} = 7.4 \pm 1.6$), with slight variations in the number of mature females among years. These correspond to an average crude birth rate of 0.09 ($\text{SD} = \pm 0.02$) and an estimated fecundity of 0.11 ($\text{SD} = \pm 0.02$) (Table 1).

Calving seasonality

Through MR analysis we assigned the month of birth for 57 neonates of 32 females. Calving was clearly seasonal, with births occurring mainly in late spring and summer months (Fig. 3). Most births ($n = 45$, 78.9%, December–February) occurred in a period of high surface water temperatures in the estuary (range 21.7–25.3°C), with a sudden drop in births recorded when the water temperature started to decrease. Stranding records ($n = 16$) mirrored this pattern, with higher number of dead neonates occurring between December–January ($n = 10$; 62.5%).

Calf survival

Mark-recapture parameter saturated-model, which incorporates time-variation in survival and recapture probabilities for all age classes (model 5), indicated a good model fit, as it showed no deviance. Therefore, no adjustment in AICc scores for overdispersion was needed. The most parsimonious model (model 1; Table 2) had constant survival and recapture probabilities and disregarded age effect. This model estimated an apparent overall survival of 0.85 (95% CI = 0.77–0.91) from birth to weaning. The stratified age-class model also had a good fit (model 3, $\Delta\text{AICc} < 2$) and gave a first and second-year calf survival estimates of 0.84 (95% CI = 0.72–0.90) and 0.86 (95% CI = 0.74–0.94), respectively. Overlapping confidence intervals suggest that this difference is not statistically different. Models accounting for timing of birth were also parsimonious (Table 2) and estimated survival probabilities of 0.86 (95% CI = 0.78–0.92) and 0.78 (95% CI = 0.55–0.92) from birth to weaning for dolphins born within and outside the birth pulse season, respectively. Overlapping confidence intervals and the LRT test, however, suggest that time of birth were not significantly affecting calf survival probabilities (LRT for model $\{\Phi(c_{0-2\text{yr}(\cdot)} * g/c_{>2\text{yr}(\cdot)}) p_{(\cdot)}\}$ versus $\{\Phi(c_{0-2\text{yr}(\cdot)}/c_{>2\text{yr}(\cdot)}) p_{(\cdot)}\}$: Chi-square test, $\chi^2 = 0.66$, $P = 0.42$). For all candidate models

capture probability was estimated to be 1.

Inter-birth interval (IBI)

Thirty-seven inter-birth intervals (IBI) were documented for 24 individual females. Two females had a 1-year calving interval, possibly induced by conception after losing their calves within the first weeks of life. IBI for females with surviving calves ranged from two to six years (mean \pm SD = 3.3 ± 1.2 ; mode = 2) (Fig. 4). Similar results were obtained by excluding possible gaps between successive births of females with prolonged inter-birth intervals (>3 years) (see methods for details) (mean \pm SD = 3 ± 1.2 ; mode = 2).

Age at weaning

Carbon isotope values in 0-year old dolphins were significantly lower than all other age classes (ANOVA, $F_{(5)} = 38$, $P < 0.0001$). In the case of $\delta^{15}\text{N}$, significant differences were found between the 0-year old individuals and the remaining age classes (ANOVA, $F_{(5)} = 38$, $P < 0.0001$), except between 0 and 0.5 year-old dolphins (Tukey's HSD, $P = 0.06$) (Table 3). GAM analysis (54.6% $\delta^{13}\text{C}$ and 47.7% $\delta^{15}\text{N}$ explained deviance and $P < 0.00001$ for the smoothed functions) showed a sharp increase/decrease in teeth $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values during the first year, followed by a slightly increase/decrease in year 2 and remained relatively constant with increasing ages, respectively (Fig. 5).

Female reproductive success

Thirty-one (76%) of 41 calves born to 27 females between 2005 and 2010 were observed to have survived until weaning. Yearly observed RS varied from 100% for neonates born in the 2007 cohort ($n = 5$), when the number of surveys during birth

season was highest, to a low of 63% from those born in the 2010 cohort ($n = 8$), with a decline in observed RS during the last three years of available sample (Fig. 6). This temporal analysis resulted in a mean observed RS rate of 0.78 ($SD = \pm 1.5$). Marked individual variation in observed RS was found. Some females had 100% of observed RS while others never succeed (Fig. 7).

Discussion

We described for the first time multiple reproductive parameters for female bottlenose dolphins from the southwestern Atlantic Ocean. The dolphin community of the PLE presents a birth-pulse strategy, with calving concentrated in the warmer months. Females presented a relatively early sexual maturation and shorter inter-birth intervals when compared to other regions (Table 4), with evidence of reproductive senescence and long post-reproductive life. At an individual level, highly variable reproductive success was observed. These findings highlight the importance of long-term studies to yield individual and population level parameters for demographic analyses, especially for a widely distributed species that are subjected to distinct environmental and ecological conditions throughout its distribution range.

Relative early reproduction and evidence for senescence in bottlenose dolphins of the southwestern Atlantic Ocean

Although based on a reduced sample size our results showed that PLE bottlenose dolphins attain sexual maturation early in life. Two primiparous females were 8 and 10 years, suggesting that sexual maturity was attained, respectively, at ages of 7 and 9, or earlier. Eight years is the most common age observed for first reproduction of female bottlenose dolphins in Sarasota Bay, Northwest Atlantic, although females as young as

6 or 7 years old have given birth (Wells et al. 1987; Wells and Scott 1999; Wells 2000). Later sexual maturation was observed for bottlenose dolphins elsewhere (9–14 years: Perrin and Reilly (1984) in the Northeastern Pacific; Mead and Potter (1990) in the Northwest Atlantic; Henderson (2012) in the South Pacific).

Bottlenose dolphins have not yet been demonstrated to exhibit menopause (i.e., termination of reproductive function before death), which is reported to occur in some cetacean species with matrilineal based social systems such as short-finned pilot (*Globicephala macrorhynchus*) and killer whales (*Orcinus orca*) (e.g., Marsh and Kasuya 1984; Foot 2008; Foster et al. 2012). However, bottlenose dolphins show clear evidence of reproductive senescence in the Sarasota community, as older females appear to invest less in reproduction to improve reproductive success (Wells 2000).

In our study three old, frequently sighted females were never seen with a calf during the intensive survey effort (2005–2012: average number of surveys during birth season was 15; SD = ± 4). The minimum age of one of these females (LP#050) was estimated at around 40 years when she died in 2012, suggesting that at least during her last 8 years of life no successful reproduction was observed. The other two females (LP#028 and LP#021) that died in 2010 at estimated ages of 40 and 44 years (EcoMega Research Group, unpubl data) gave birth for the last time in 2000 and 2003, respectively. Although it is possible that undetected abortions or newborn deaths may have occurred, our intensive survey effort during the breeding season minimizes this possibility. Therefore, it is likely that these three females reproduced successfully for the last time in their life when they were >32 and approximately 30 and 36 years, respectively. Whether these females terminated their reproductive function or died before the chance of giving birth after a long resting period (i.e., >8 years) is still uncertain, but opens to

the possibility that females of PLE community could have a long post-reproductive life span (about 19–25% of their lives). In addition, five multiparous females (>20 years) that had produced just one calf early in the study period (2005/2006 season) and were still alive in 2013 (data not shown) indicate an age-related decrease in reproductive fitness. This suggests that aging PLE bottlenose females may change their role from ‘breeding’ to ‘nursing’ females, compensating their negative effect on average fecundity by increasing the reproductive success of the community, as predicted by classical life-history theories for species with low adult mortality (Clutton-Brock 1984). The fact that two old living females that reproduced for the last time just prior to the implementation of our systematic monitoring have carried out parental care duties for 8 years is a supporting evidence of this change in female’s ecological role within the community (EcoMega Research Group, unpubl data).

Several adaptive functional theories (e.g., grand-mother theory) have been proposed to explain the role of old individuals in species living in stable social groups with overlapping generations (e.g., Norris and Pryor 1991; Marsh and Kasuya 1991; Whitehead and Rendell 2004), but all are still inconclusive. However, there is increasing evidence to suggest that non-reproductive females possibly play an important role for increasing survival probabilities of several dependent young and perhaps other close relatives (Marsh and Kasuya 1991; Foster et al. 2012).

Inter-birth interval, crude birth rate and fecundity

With the exception of fecundity rate (0.11), which fall within the limits reported for bottlenose dolphins elsewhere, we found higher annual crude birth rates (0.09) and shorter (mode = 2 years) inter-birth intervals (IBI) for PLE bottlenose dolphins. The mean IBI of 3 years found in this study is similar, but at the lower range to what is

reported for several other regions (see Table 4 for comparisons). Wherever the reproduction of bottlenose dolphins has been studied in the wild, shorter IBI (1–2 years) were likely induced by calf loss during the first year of life. In our study, however, two years between successive births was common regardless of calf's fate, an unusual pattern for bottlenose dolphins. The lack of (or minimal) predatory pressure (Fruet et al. 2012) and the abundant and predictable distribution of feeding resources in our study system may provide an optimal condition for females to reduce maternal investment without adding substantial costs for young survival after weaning.

Best et al. (1984) found a shorter IBI for sperm whales (*Physeter macrocephalus*) in the high productive waters off the Humboldt Current than in other, less productive study areas. Several other studies with aquatic mammals have found relationship between top-down and bottom-up effects in demographic parameters after sudden changes in food availability and/or predator abundance (southern right whales, *Eubalaena australis*, Leaper et al. 2006; killer whales, Ward et al. 2009; Antarctic fur seals, *Arctocephalus gazella*, Schwarz et al. 2013). Thus, the minor year-to-year variation in fecundity and crude birth rates (and other parameters such as abundance and adult survival from this dolphin community; Fruet et al. in press) potentially suggest a relatively stable environment over the course of this study period, or the ability of PLE bottlenose dolphins to respond to fluctuations in species-specific abundance of prey through feeding plasticity (Lopes 2014).

Birth seasonality

Our analysis of 8 years of MR and stranding data revealed that calving of PLE bottlenose dolphins is highly seasonal and is associated with warmer surface waters in the estuary. The Patos Lagoon estuary and adjacent coast are subjected to substantial

seasonal oscillations in physical and biological processes (Möller et al. 2001; Seeliger and Odebrecht 2010; Garcia et al. 2012; Moraes et al. 2012). Abundance of adults of several estuarine-dependent fishes that are important prey species for PLE bottlenose dolphins (e.g., *Micropogonias furnieri*; *Paralichthys brasiliensis*; Lopes 2014) pulse during spring and summer months with a drastic reduction during winter (Garcia et al. 2012; Rodrigues and Vieira 2013). As prey abundance decrease, time and, therefore, energy spent to search for sparse food likely increases. Hence, synchronizing time of birth with high food abundance and warmer temperatures is probably favouring infant survival by minimizing thermoregulation-related stress and maximizing the opportunity of mothers to explore the optimal levels of resources during early-mid lactation, the most energetically expensive time of reproduction (e.g., Rutberg 1987; Gaillard et al. 1993; Rechsteiner et al. 2013). In addition, as lower energetic demand of thermoregulation is need for the mother in warmer waters (Williams et al. 1992) calving during this period may improve quality of maternal milk and promote a rapid calf growth, thus potentially enhancing calf survival (see Whitehead and Mann 2000 for review). Several other studies on bottlenose dolphins and other small dolphin species have reported birth pulse in areas of similar or higher latitudes (e.g., *T. truncatus*, Urian et al. 1996; Thayer et al. 2003; Henderson et al. 2014; *Pontoporia blainvillei*, Danilewicz 2003; *Delphinus delphis*, Westgate and Read 2007), where they are also subjected to intra-annual changes in prey availability and water temperature.

Calf Survival

The first-year survival rate estimated in this study (mean = 0.84, 95% CI = 0.72–0.90) falls within the range of values obtained from others long-term studies on bottlenose dolphins that derived estimates from MR studies (Sarasota Bay, mean \pm SD = 0.81 \pm

0.06, Wells and Scott 1990; Doubtful Sound, mean = 0.86, 95 %CI = 0.69–0.95, Currey et al. 2008). It was also similar to the first-year survival rate estimates for Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) at Mikura Islands (mean = 0.86; Kogi et al. 2004) (Table 4). However, as in other MR studies, first-year survival is possibly overestimated. As most deaths probably occur during the first month of life (e.g., Henderson et al. 2014), this narrow time interval coupled with sampling limitations increase the chances of missing an early death and thus overestimating first-year survival rate. This could be the reason why we did not find significant differences in apparent survival rates between age-classes of 0-1 and 1-2.

As we found that PLE bottlenose dolphins have a narrow birth pulse coinciding with optimal environmental conditions (warmer water temperature and potentially high food supply), we predicted that the time of birth should play a critical role in calf survival, with infants born out-of-pulse birth season having lower survival rates. Nevertheless, we did not found a significant difference between these two groups to support this hypothesis. This indicates that the short-term variability in water temperature and food resources within calving season is not so strong to the point of affect survival of calves born at or out-of-pulse birth season.

Weaning

Findings from field studies suggest that lactation can last between 1.5 and 2 years in bottlenose dolphins (see review in Wells and Scott 2009). As separation between mother and calf tends to occur before the birth of the next calf (Wells 2000; Mann et al. 2000; this study), we deduced that the most typical inter-birth interval in this study (mode = 2) should at least roughly coincide with the time of weaning in PLE bottlenose dolphins. C and N stable isotopes analysis provided congruent results with estimated

IBIs. The clear change in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiles near age 2 indicated the most probable weaning age in this bottlenose dolphin community. Low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ isotopic values were found in <1 year-old individuals, when gradually started to increase and decrease up to age 1.5-2 years. These results suggest higher nutritional investment by mothers (through lactation) during the calf's first year of life, and a gradual incorporation of solids concomitant with a decreasing milk intake during the next year as part of the weaning process. This may be necessary to both promote a fast development so the calf became independent and to allow a recovery period for the female by the time her next calf is born.

Reproductive success

The observed reproductive success (RS) of the PLE female bottlenose dolphins revealed a high individual and community level variation, with some multiparous females failing at all observed reproductive attempts while others successfully weaning all their observed calves. Only few cetacean studies have assessed female reproductive success at an individual level based on a longitudinal observational sampling, but it seems that such a high individual variability may be common in wild bottlenose dolphins (Wells 2000; Mann et al. 2000; Frère et al. 2010; Henderson et al. 2014; this study). Henderson et al. (2014) found that mother identity is crucial to calf survival and when these “good mothers” have calves influence the temporal variation in abundance of the Doubtful Sound bottlenose dolphin community. Whereas the detrimental effects that demographic stochasticity plays in the dynamics of small populations is well known (Lande et al. 2003), the mechanisms influencing variations at individual level are still not well understood.

Age-dependent effects possibly affect female RS, as experienced mothers should invest

more in parental care than in reproduction (Wells 2000; this study). However, social factors should also impact female RS in highly social species with prolonged period of nursing such as bottlenose dolphins. It has been proposed choice of female alliances and stability of associates may improve foraging access and protection against conspecifics (Wells et al. 1987; Mann et al. 2000). In our study system there is increasing evidence that some nursing females have preferences for the inner estuarine waters and form larger groups during the critical period of calving in contrast to other females that tend to form smaller groups and use larger areas. If habitat and choice of group size and composition improve foraging access and protection, it is therefore possible that differences in female grouping strategies are affecting RS in this dolphin community.

Protection against harassment of males, especially close-relatives, could play an important role in RS as there is strong evidence suggesting that inbreeding brings detrimental costs for female reproduction in several wild mammalian species (e.g., Mainguy et al. 2009; Cohas et al. 2009; Frère et al. 2010). Frère et al. (2010) reported that inbreeding is more common than previously thought in Shark Bay, one of the largest known inshore populations of Indo-Pacific bottlenose dolphins (>2000 individuals, Preen et al. 1997). There, inbred females have reduced reproductive fitness (lower fecundity) as females with inbred calves had lower reproductive success and prolonged weaning age than non-inbred females (Frère et al. 2010). As PLE bottlenose dolphins constitute a very small and resident social unit with remarkably low-levels of genetic variation (Fruet et al. 2011, 2014; Genoves 2013) and evidence of philopatry by both sexes (EcoMega Research Group, unpubl data), females are expected to be vulnerable to inbreeding, especially if non-natural mortality come to cause further reduction in population size.

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Table 1 Summary of reproductive rates from common bottlenose dolphins (*Tursiops truncatus*) of the Patos Lagoon estuary community estimated from the long-term mark-recapture program; Fecundity = ratio between the number of females calves (assuming a calf sex ratio of 1:1) and the number of mature females in the PLE community

	2004	2005	2006	2007	2008	2009	2010	2011	2012	Average (SD)
¹ Abundance	-	81	81	82	84	87	86	88	78	83 (3)
Photo-id surveys	23	30	45	38	49	39	34	19	7	31 (13)
Adult females	-	33	35	34	35	37	37	35	31	35 (2)
Births	7 (7)	7 (7)	5 (5)	6 (5)	9 (9)	8 (7)	8 (8)	8 (7)	8 (8)	7.3 (1.2)
Survivors to age 1	7	6	5	5	7	7	6	5	-	6 (1)
Crude birth rate	-	0.09	0.06	0.07	0.11	0.09	0.09	0.09	0.10	0.09 (0.01)
Fecundity	-	0.11	0.07	0.09	0.13	0.11	0.11	0.11	0.13	0.11 (0.02)

¹Extracted from Fruet et al. (in press).

Table 2 Summary of age-structured modification of CJS models for survival (Φ) and recapture (p) probabilities of calves (c) from common bottlenose dolphins (*Tursiops truncatus*) of the Patos Lagoon estuary community based on a long-term mark–recapture study (2005–2013). Models are ranked according to the lowest AIC. Notation: (.) constant, (t) time-dependence, (g) group effect (dolphins born during the pulse of calving season and dolphins born out-of-pulse birth season. Slash distinguishes age-classes

	Model	AIC	Δ AIC	AIC weight	Model Likelihood	N parameters
1.	$\{\Phi(c_{0na2yr(.)}/c_{>2yr(.)}) p_{(.)}\}$	208.615	0.00	0.54	1	3
2.	$\{\Phi(c_{0na2yr(.)} * g/c_{>2yr(.)}) p_{(.)}\}$	210.037	1.42	0.27	0.49	4
3.	$\{\Phi(c_{0na1yr(.)}/c_{1na2yr(.)}/c_{>2yr(.)}) p_{(.)}\}$	210.694	2.07	0.19	0.35	4
4.	$\{\Phi(c_{0na1yr(t)}/c_{1na2yr(t)}/c_{>2yr(.)}) p_{(.)}\}$	232.558	23.94	0.00	0.00	17

Table 3 Mean (\pm SD) values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in teeth of common bottlenose dolphins (*Tursiops truncatus*) of the Patos Lagoon estuary community

Age class	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	N
0	-13.45 (\pm 0.98)	21.50 (\pm 1.91)	4
0.5	-11.45 (\pm 0.87)	19.14 (\pm 1.49)	3
1	-11.40 (\pm 0.58)	18.48 (\pm 1.20)	8
2	-11.22 (\pm 0.70)	18.30 (\pm 0.65)	14
3	-10.99 (\pm 0.45)	17.96 (\pm 0.87)	6
4	-11.13 (\pm 0.37)	18.51 (\pm 0.57)	7
5	-11.32 (\pm 0.33)	18.57 (\pm 1.08)	5

Table 4 Reproductive parameters of bottlenose dolphins (*Tursiops* spp.) estimated from long-term mark-recapture studies around the world. Fecundity = ratio between the number of female calves (assuming a calf sex ratio of 1:1) and the number of mature females in the PLE dolphin community. Original values of fecundity extracted from the literature were divided by 2 for adjusting the fecundity definition used in this study where only female calves were considered

Species	Site	Survival to 1yr	Fecundity (SD)	Crude birth rate (SD)	IBI (yrs)	
					Mean	Mode
<i>T. aduncus</i>	Mikura island, Japan	¹ 0.86	¹ 0.136 (0.04)	¹ 0.071 (0.024)	¹ 3.4	-
<i>T. aduncus</i>	Shark Bay, Australia	² 0.71	-	-	² 4.1	4
<i>T. aduncus</i>	Port River, Australia	³ 0.70	-	³ 0.064	³ 3.8	-
<i>T. truncatus</i>	Sarasota Bay, USA	⁴ 0.81	⁴ 0.09 (0.04)	⁴ 0.055 (0.009)	⁵ 4	⁵ 4-5
<i>T. truncatus</i>	Doubtful Sound, NZ	⁶ 0.86	-	⁷ 0.040 (0.04)	⁸ 5.3	⁸ 4-6
<i>T. truncatus</i>	Scotland	-	⁹ 0.11 (0.2)	¹⁰ 0.046	⁹ 3.8	⁹ 3-6
<i>T. truncatus</i>	Patos Lagoon estuary, Brazil	¹¹ 0.84	¹¹ 0.11 (0.2)	¹¹ 0.090 (0.01)	¹¹ 3	¹¹ 2

¹ Kogi et al. (2004); ² Mann et al. (2000); ³ Steiner and Bossley (2008); ⁴ Wells and Scott (1990);
⁵ Wells (2000); ⁶ Currey et al. (2008); ⁷ Haase and Schneider (2001); ⁸ Henderson et al. (2014); ⁹
Mitcheson (2008); ¹⁰ Wilson et al. (1999); ¹¹ This study

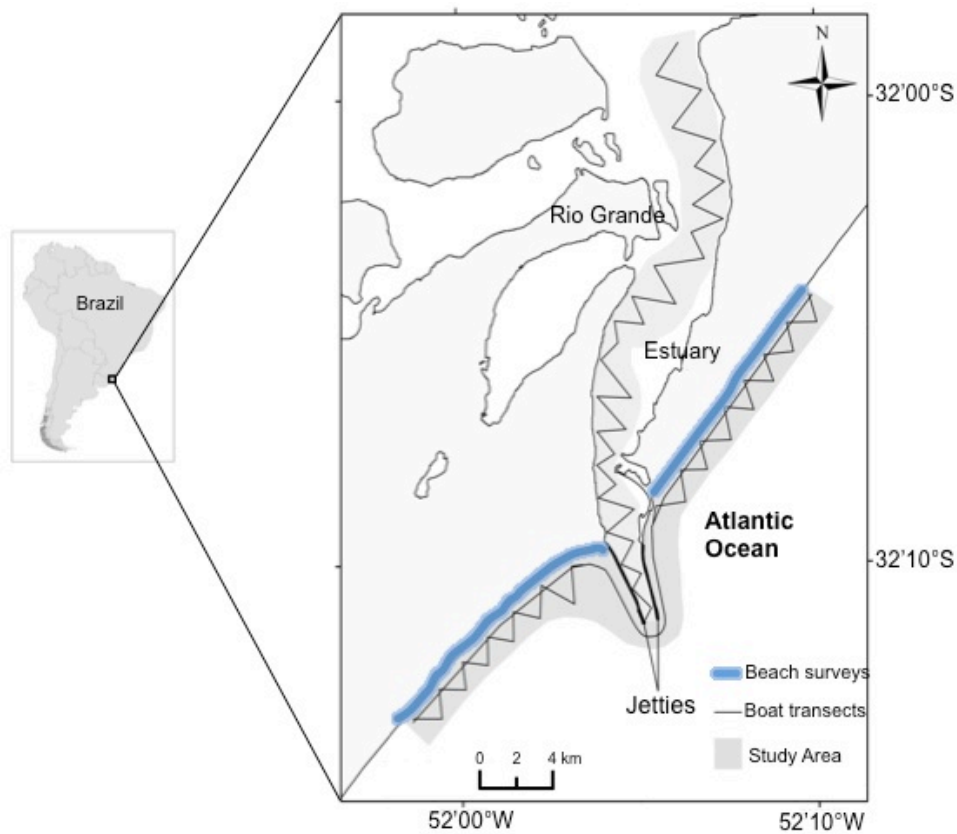


Fig. 1 Study area in the southwestern Atlantic Ocean (SWA) showing transects (solid lines) designed for mark-recapture (black lines) and beach surveys (blue lines) for common bottlenose dolphins (*Tursiops truncatus*) in the Patos Lagoon estuary and surrounding coastal areas in southern Brazil.



Fig. 2 Adult bottlenose dolphin (*Tursiops truncatus*) accompanied by a small dark-gray calf was one of the typical criteria to identify mothers during mark-recapture surveys (left). Freshly stranded carcass (right) found during beach surveys presenting clear characteristics of newborn such as prominent fetal folds (red arrows) and small size.

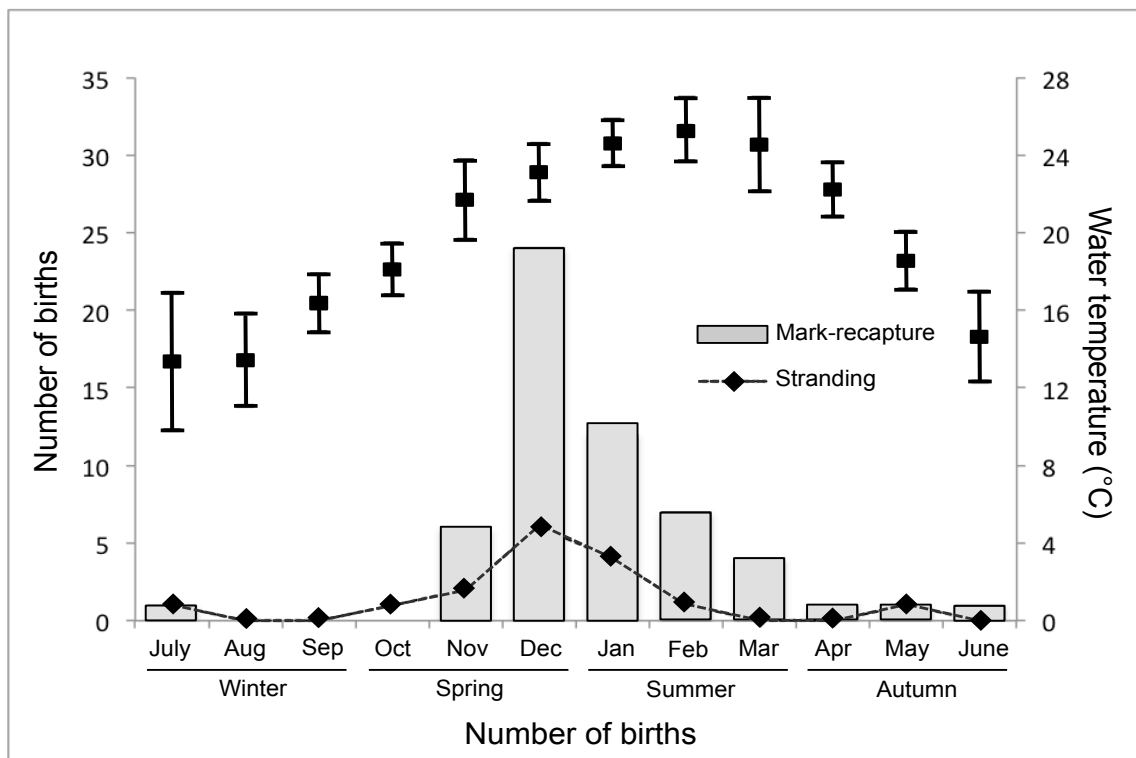


Fig. 3 Birth seasonality for common bottlenose dolphins (*Tursiops truncatus*) of the Patos Lagoon estuary community based on a long-term monitoring study (2005–2013). Gray bars express the number of births estimated from mark-recapture surveys conducted in the estuary and adjacent coastal waters, while dashed line shows the number of neonates found stranded during beach surveys. Black squares and error bars are the averaged monthly water temperature and its associated standard error.

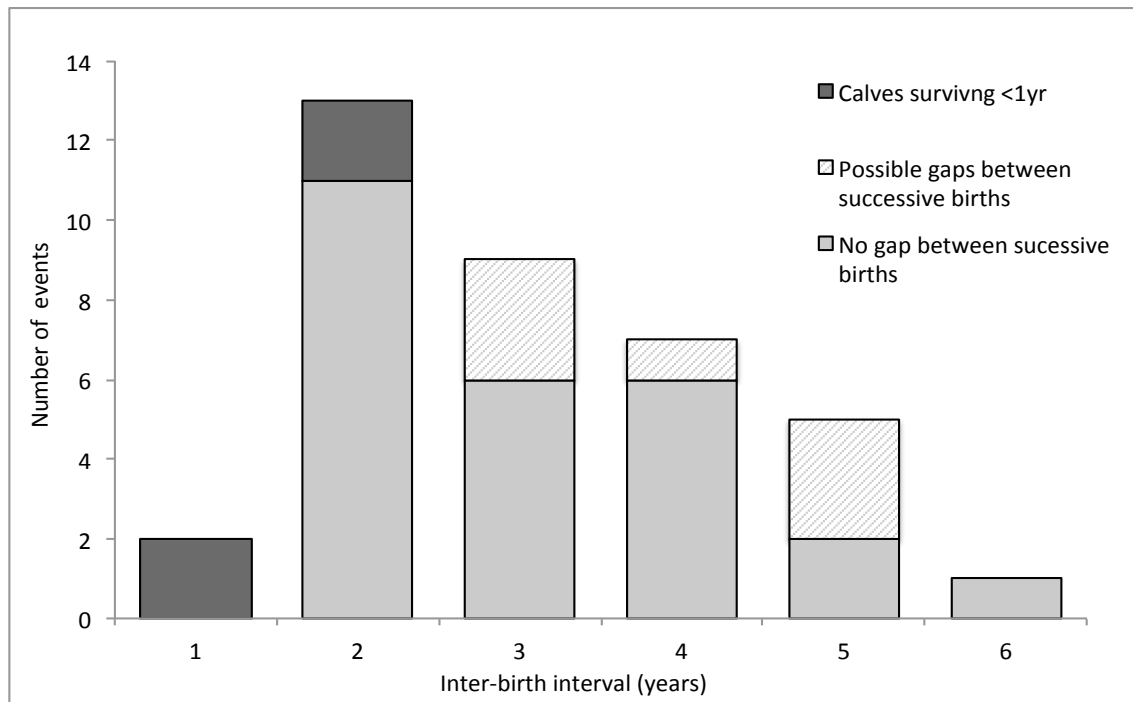


Fig. 4 Estimated inter-birth intervals for common bottlenose dolphins (*Tursiops truncatus*) of the Patos Lagoon estuary community (PLE). “Possible gaps” include inter-birth intervals with large gaps between weaning of the first calf and the birth of the second calf, with chances of undetecting a birth in between ($n = 37$). “No gaps” included larger inter-birth intervals (>2 years) only for females that remained in close association with a surviving calf until a known subsequent birth ($n = 30$). Dark-gray boxes show the inter-birth interval for females that have lost their calf during the calf’s first year of life.

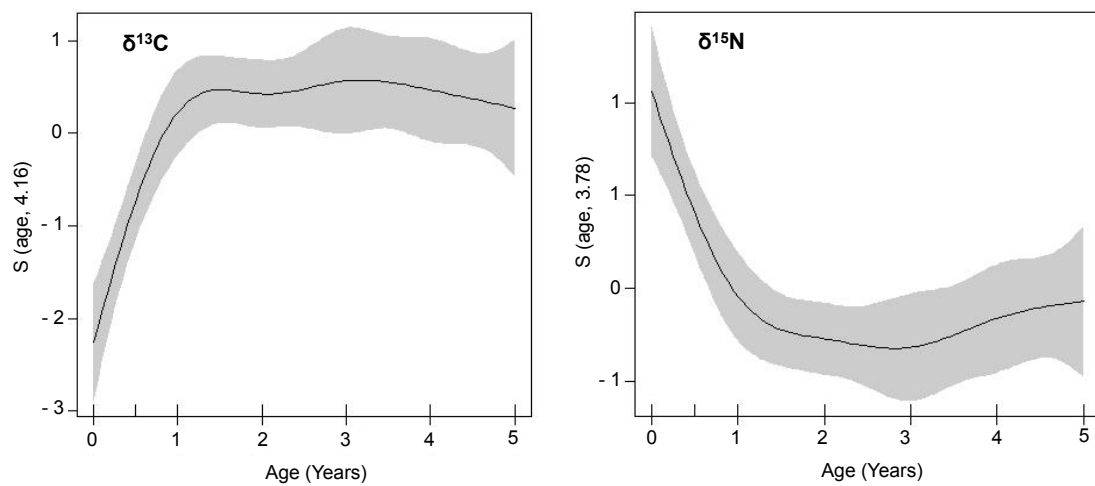


Fig. 5 Plots of Generalized Additive Models (GAMs) of $\delta^{13}\text{C}$ (left) and $\delta^{15}\text{N}$ (right) by age in teeth from common bottlenose dolphins (*Tursiops truncatus*) of the Patos Lagoon estuary community (PLE). X-axis denotes age (in years) and Y-axis denotes relative distance from the mean. Gray envelopes correspond to the 95% confidence interval.

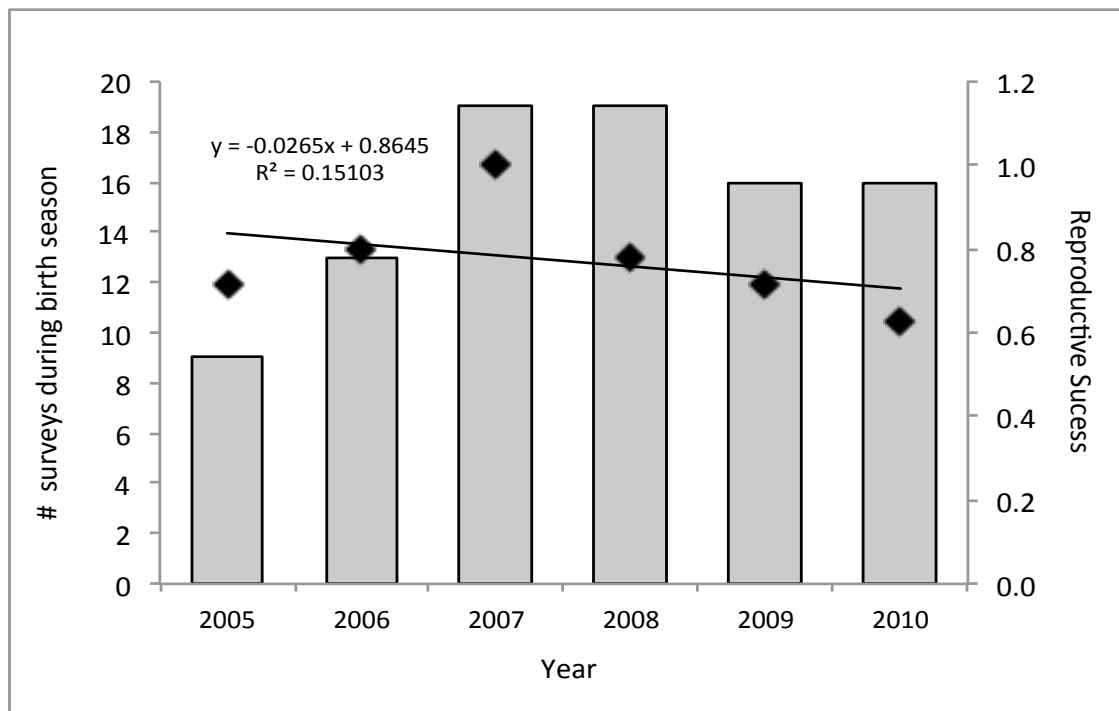


Fig. 6. Yearly changes on reproductive success (black diamonds) of female common bottlenose dolphins (*Tursiops truncatus*) of the Patos Lagoon estuary community (PLE) over six years (2005–2010). Gray bars are the number of summer surveys per year. Data from calves born in the end of sampling period (2011–2012) were not included in this analysis as they did not have the minimum estimated age to be weaned (≥ 2 years).

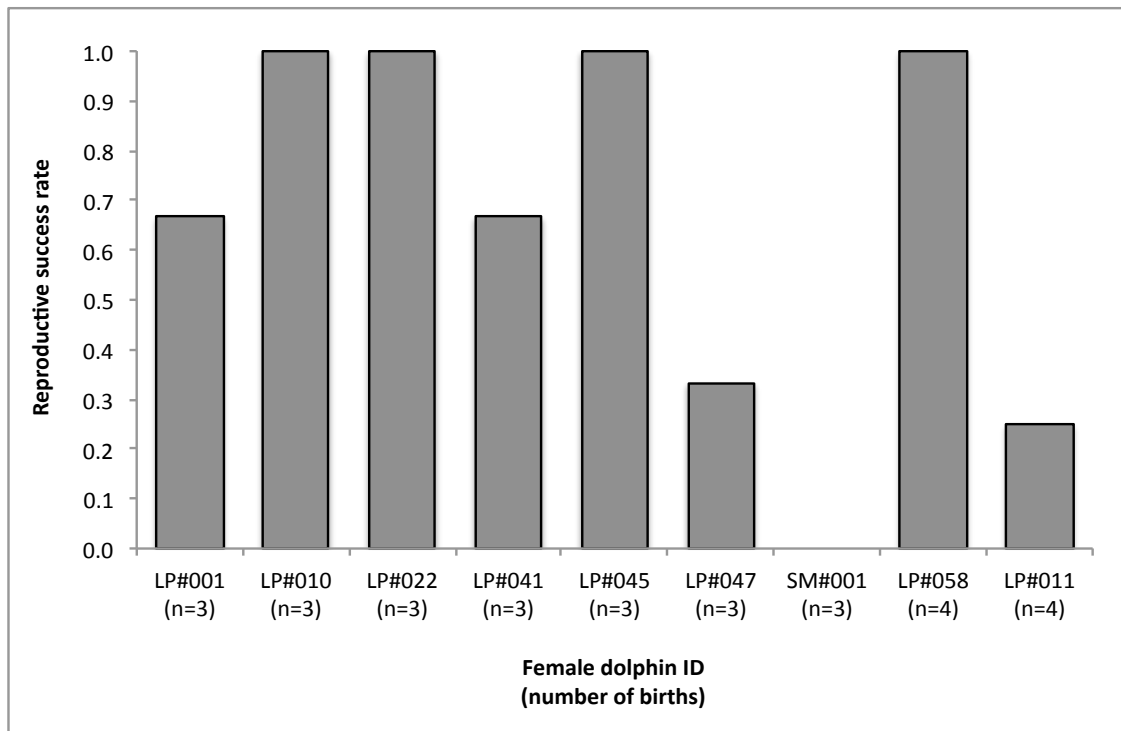


Fig. 7. Individual variation in reproductive success for female common bottlenose dolphins (*Tursiops truncatus*) with ≥ 3 documented births in the Patos Lagoon estuary community (PLE). Data from calves born at the end of the sampling period (2011–2012) were not included in this analysis as they did not have the minimum estimated age to be weaned (≥ 2 years).

ANEXO IV

(Não submetido para publicação em periódico).

Modelling the dynamics and viability of a small, estuarine-resident community of common bottlenose dolphins (*Tursiops truncatus*) from southern Brazil

Pedro F. Fruet ^{1,2,3,4,5*}, Luciana M. Möller ^{4,5} and Eduardo R. Secchi ^{2,3}

¹ Programa de Pós-Graduação em Oceanografia Biológica, Universidade Federal do Rio Grande - FURG, Brazil

² Museu Oceanográfico “Prof. Eliézer C. Rios”, Universidade Federal do Rio Grande - FURG, Brazil

³ Laboratório de Ecologia e Conservação da Megafauna Marinha (EcoMega), Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Brazil

⁴ Cetacean Ecology, Behaviour and Evolution Lab, School of Biological Sciences, Flinders University, Australia

⁵ Molecular Ecology Laboratory, School of Biological Sciences, Flinders University, Australia

*Corresponding Author: Pedro F. Fruet (pfruet@gmail.com)

Phone: +55 (53) 32329107

Abstract

Identifying threatened populations and quantifying their vulnerability is crucial for establishing priorities for conservation and providing reliable information for decision-making. Common bottlenose dolphins (*Tursiops truncatus*) have been subjected to by-catch mortality in gillnet fisheries along the coastal waters of southern Brazil, particularly in the Patos Lagoon estuary (PLE) and adjacent coastal waters. A previous study suggested that this fishing-related mortality could be unsustainable to the small, resident PLE dolphin community. However, a comprehensive assessment of how this non-natural mortality impacts upon population viability is still lacking. In this study we used a stage-classified matrix population model to conduct a demographic analysis of the PLE dolphin community with life-history data estimated during a long-term mark-recapture study of these animals. A population viability analysis was used to run a series of simulations where the risk of this dolphin community was assessed under different by-catch scenarios, taking into account the effects of parameter uncertainty and stochasticity in the projections. In the absence of by-catch, we estimated that the PLE dolphin community would grow about 3% annually (95% CI: 1.2% - 5.8%). Under current by-catch rates and uncertainties in parameter estimates, prognoses indicated high probabilities of viability of this community over the next 60 years. These optimistic prognoses appear to be associated with the high survival of adult females and a relatively stable environment in the PLE. However, the potential removal of a few mature females (one every 1 or 2 years) would result in an eminent likelihood of decline from its current abundance at all pre-specified levels. The viability of the PLE dolphin community would be substantially improved if the survival of juveniles/sub-adults could be increased. This may be achieved through the recently implemented dolphin protected area in southern Brazil, which prohibits gillnetting fisheries in the core area of the PLE dolphin community. If the protection area works (i.e. reduce the entanglement rates of juvenile dolphins/sub-adults) there will be a substantial chance of increase the PLE dolphin community above 20% of its current size.

Introduction

The central aim of conservation biology is to establish principles and tools for preserving biological diversity from excessive rates of extinction and the processes that sustain it (Soulé 1985). Identifying threatened populations and quantifying their vulnerability is therefore crucial for establishing priorities for conservation and providing reliable information for decision-making (i.e. to decide if intervention is needed with minimal delay). Finding early warning signs before severe decline of a given population should have important implications for successful conservation attempts. However, objectively classifying populations according to their precise levels of vulnerability is challenging (Ralls and Taylor 1997).

Population viability analysis (PVA) is a process widely used in conservation biology and management decision-making (e.g. Morris and Doak 2003). PVA is a procedure that projects the population into the future based on models of population dynamics, allowing the incorporation of elements that can affect their likelihood of persistence, including environmental and demographic stochasticity, catastrophes, deterministic pressures (e.g. annual hunting quotas), uncertainties in parameters estimates, and control variables representing conservation strategies (e.g. Gilpin and Soulé 1986; Possingham et al. 1993; Morris and Doak 2003). Probably the most important use of these models are for investigating the extinction probabilities of populations within pre-specified periods of time and under particular circumstances (e.g. Possingham et al. 1993), as well as for identifying the most important factors affecting the likelihood of extinction (Reed et al. 2002). This information can then be used to identify research priorities and guide conservation and management actions for protecting threatened populations (e.g. Possingham et al. 1993; Caswell 2001).

The common bottlenose dolphin (*Tursiops truncatus*) is a small delphinid widely distributed, and probably the best known of all cetaceans (Wells and Scott 2009). Bottlenose dolphins in Brazil are found in both coastal and offshore waters, but coastal bottlenose dolphins are regularly sighted along a narrow stretch in southern Brazil. In this area resident bottlenose dolphin communities are typically found associated with estuaries and river mouths (Castello and Pinedo 1977; Simões-Lopes and Fabian 1999).

A recent study suggested that bottlenose dolphins in southern Brazil and Uruguay (SB-U) are part of an Evolutionary Significant Unit (ESU), genetically isolated from bottlenose dolphins found in central Argentina (Fruet et al. 2014). This ESU is comprised by at least five communities of dolphins with very low levels of genetic diversity and restricted dispersal between them (Fruet et al. 2014) (Fig. 1). There are no abundance estimates available for the entire ESU, but mark-recapture studies conducted at several sites indicate that each of these communities is very small, and some exhibit strong site fidelity and year round residency (e.g. Simões-Lopes and Fabian 1999; Fruet et al. 2011; Daura-Jorge et al. 2013). By-catch in the gillnet fishery is recognized as the main threat for these local communities, and it is known to occur throughout their range (Fruet et al. 2012; submitted a), but other agents such as skin-diseases, boat strikes, chemical pollution and underwater noise also impact upon dolphins in southern Brazil (e.g. Lago 2006; Daura-Jorge and Simões-Lopes 2011). The bottlenose dolphin community of the Patos Lagoon estuary (PLE) is possibly the largest of all five within the SB-U ESU, numbering approximately 90 individuals (Fruet et al. 2011; in press). By-catch of animals from this community used to occur sporadically, and was previously not considered a reason for concern (e.g. Pinedo 1986). In the last decade, however, the PLE dolphin community suffered an increase in gillnet entanglement rates, with young males particularly vulnerable (Fruet et al. 2012). The

number of resident dolphins with evidence of interaction with fisheries (piece of nets attached to the body and rostrum, mutilated appendices), and the significant increase in mortality in adjacent coastal beaches, including some known resident PLE dolphins, raised concern about their conservation (Fruet et al. 2012; Fruet et al. 2014). Lower survival rates of adult males and immatures compared to adult females from the PLE community (Fruet et al. submitted b) suggest that the two former age-sex classes may be more vulnerable to non-natural sources of mortality, particularly by-catch (Fruet et al. 2012).

A preliminary analysis of the sustainability of the PLE bottlenose dolphin community was carried out (Fruet et al. 2012) using the Potential Biological Removal (PBR) approach of Wade (1998). Results suggested that the recent by-catch levels would be unsustainable in the most optimistic scenario if by-catch was exclusively affecting individuals from the PLE dolphin community (Fruet et al. 2012). In August 2012, in response to this vulnerability and the large overlap in dolphin distribution and the gillnet fishery, the Brazilian Ministry of Fisheries and Aquaculture and the Ministry of Environment published a joint norm to regulate the gillnet fishery. This norm included a dolphin protection area that prohibits gillnetting in the core area of the PLE dolphin community (Brasil 2012). If effective, this fishery ban is expected to increase dolphin survival and the growth rate of this bottlenose dolphin community.

The adoption of the PBR-based framework represented an important step for measuring the sustainability of the PLE bottlenose dolphins in the absence of suitable biological data. However, the level of understanding about the population dynamics and impacts of fishery by-catch to its viability was trivial. A comprehensive demographic modeling analysis using current life-history data estimated from the PLE dolphins

would certainly provide a better baseline for understanding their dynamics and conservation needs. In this study we used a stage-classified matrix population model for analysing the demography of the PLE dolphin community, estimating population growth, projecting it into the future, and assessing the risk of decline based on current species-specific life-history data. Effects of parameter uncertainty, demographic and environmental stochasticity, and by-catch mortality were also included. Finally, we assessed the potential effectiveness of the new dolphin protection area for the PLE dolphin community by modelling the effects of increasing survival rates as a result of an expected reduction in by-catch rates.

Methods

The Model

A stochastic stage-structured matrix population model developed to model the viability of the franciscana dolphin (*Pontoporia blainvillei*) and the hector's dolphin (*Cephalorhynchus hectori*) (Secchi 2006) was adapted to depict the dynamics and estimate the viability of the PLE bottlenose dolphins. Because population growth rates in large mammals are mainly driven by female vital rates (e.g. Brault and Caswell 1993; Morris and Doak 2003), only females were included in the model. The model assumes that the population is closed to immigration and emigration processes. We used a stage-structured model because estimation of survival and fertility parameters are essentially stage-specific for the PLE bottlenose dolphins. We grouped individuals in three life-stages following the classification used by Fruet et al. (submitted b and c) to estimate the life history data for the PLE dolphin community: Stage 1 is represented by calves (0 – 2 yr), stage 2 by juveniles/sub-adults (>2 yr up to 8 yr; all the immature non-calves),

and stage 3 by adults (> 8 yr; all the mature individuals) (Eq. 1). For details on the model structure and definitions refer to Caswell (2001).

$$\begin{bmatrix} n_1(t+1) \\ n_2(t+1) \\ n_3(t+1) \end{bmatrix} = \begin{bmatrix} 0 & F_2 & F_3 \\ G_1 & P_2 & 0 \\ 0 & G_2 & P_3 \end{bmatrix} \begin{bmatrix} n_1(t) \\ n_2(t) \\ n_3(t) \end{bmatrix} \quad (\text{eq. 1})$$

where:

n_i is the number of individuals in state class i ;

P_i is the probability of surviving and staying in stage i ($P_1=0$; $i=2,3$);

G_i is the probability of surviving from t to $t+1$ and moving to the stage $i+1$ ($i=1, 2$); and

F_i is the number of female offspring at time $t+1$ per adult female in stage i at time t ($F_1=0$; $i=2,3$).

Because the PLE dolphin community has a well-defined breeding season with most births occurring during late spring and summer (Fruet et al. submitted c), we used a birth-pulse model. In this case the model will represent the post-birth census (see definitions in Caswell 2001).

For a birth-pulse population,

$$P_i = \sigma_i (1-\gamma_i)$$

$$G_i = \sigma_i \gamma_i$$

$$F_i = P_i m_i + G_i m_{i+1}$$

where, for stage i :

σ_i is the stage-specific survival probability;

γ_i is the transition probability or growth probability; and

m_i is the mean reproductive output of females (see Brault and Caswell 1993; Caswell 2001, p.171-173).

Hence, for a three-stage birth-pulse population:

$$F_2 = (1-\gamma_2) \sigma_2 m_2 + \gamma_2 \sigma_2 m_3 = F_2 ((1-\gamma_2) m_2 + \gamma_2 m_3)$$

$$F_3 = \sigma_3 m_3$$

$$G_1 = \sigma_1$$

$$G_2 = \gamma_2 \sigma_2$$

$$P_1 = 0$$

$$P_2 = (1-\gamma_2) \sigma_2$$

$$P_3 = \sigma_3$$

Finally, equation 1 can be rewritten as

$$\begin{bmatrix} n_1(t+1) \\ n_2(t+1) \\ n_3(t+1) \end{bmatrix} = \begin{bmatrix} 0 & (1-\gamma_2)\sigma_2 m_2 + \gamma_2 \sigma_2 m_3 & \sigma_3 m_3 \\ \sigma_1 & \sigma_2(1-\gamma_2) & 0 \\ 0 & \sigma_2 \gamma_2 & \sigma_3 \end{bmatrix} \begin{bmatrix} n_1(t) \\ n_2(t) \\ n_3(t) \end{bmatrix} \quad (\text{eq. 2})$$

to represent the dynamic of a birth-pulse population.

To estimate the transition probability we used a “variable stage duration” (T_i) (Caswell 2001, p. 164).

$$\gamma_i \approx \left(\frac{1}{\bar{T}_i} \right) \exp \left(-a \left(\frac{\bar{T}_i}{2} - \frac{V(T_i)}{2\bar{T}_i} \right) \right) \quad (\text{eq. 3})$$

where,

$$a = \ln \left(\frac{\lambda}{\sigma_i} \right) \quad (\text{eq. 4})$$

\bar{T}_i is the mean stage duration and $V(T_i)$ is its variance.

Equation 3 depends on λ , the largest eigenvalue of the matrix, whose entries are being estimated and it cannot be calculated before estimation is completed. However, it can be done through an iterative approach that requires an initial value of λ (for details see Caswell 2001, p.164).

Initial models - input parameters and uncertainty

Model input parameters were all obtained from the PLE dolphin community throughout our long-term photo-identification and monitoring program (Fruet et al. submitted b, c). Uncertainty in parameter estimates was allowed using Monte Carlo methods (Manly 1997) by running the model 1000 times and randomly selecting

parameter values from probability distributions representing parameter uncertainties. Whenever appropriate data were available, they were used to estimate statistical distributions for the model parameters. When poor data were available, a uniform distribution was chosen to represent parameter uncertainty. With the exception of age at first reproduction (AFR) and abundance, for which values were fixed (abundance estimates are precise and model output is not overly sensitive to variations in AFR and initial abundance), uncertainties were incorporated into all remaining model parameters.

Initial abundance was set as 44 females based on the last and more precise mark-recapture abundance estimation available ($N_{T\ 2011} = 88$ individuals, 95% CI = 82-94; Fruet et al. submitted b). Stage-specific survival rates were obtained from recent studies that have applied mark-recapture models to 8 yr of photo-identification data (2005-2012) to estimate demographic parameters of the PLE dolphin community (Fruet et al. submitted b, c). For adults, female survival rates were used, but for juveniles and calves the available estimation was not sex-specific. It was assumed that survival rates of all immature dolphins were sex invariant. For survival rates, we chose a beta distribution for incorporating uncertainty into the estimates (White 2000).

Female AFR varied from 8 to 10 yr in the PLE dolphin community (Fruet et al. submitted c). Thus, AFR was assumed to be 9 yr. Such estimation is similar to the available estimate of AFR for Sarasota bottlenose dolphins (Wells 2000), the best-studied bottlenose dolphin community in the world. Fruet et al. (submitted c) estimated fecundity as the number of female calves born per known mature female in the PLE dolphin community. However, the nature of the data analysed is expected to produce a downward bias in the estimation due to the potential of non-detected births before calves die. In addition, we derived fecundity as the reciprocal of the average calving

interval estimated by Fruet et al. (submitted c), assuming an even sex ratio at birth. This estimate is likely to bias fecundity upwards as longer calving intervals may not be detected due to the relative short-term nature of the data relative to the life expectancy of this long-lived species. These estimates were used to set the lower and upper bound values of fecundity to represent parameter uncertainty, which was expressed through a uniform distribution. The parameter estimates and the distributions that represented our level of uncertainty for different modeling scenarios are summarized in Table 1.

Incorporating effects of stochasticity

Year-to-year variation in survival rates, expressed as the standard deviation for each life-stage estimate, was obtained using sampling variance component analysis in Program Mark (White and Burnham 1999). For fecundity, the standard deviation value was obtained from the year-to-year variation of the estimate for this parameter (Fruet et al. submitted c). Demographic stochasticity is important only for small populations (e.g. Shaffer 1981; Goodman 1987). It is the variation in the average chance of population being made up of a finite, integer number of individuals. Demographic stochasticity was modelled using a binomial distribution from which we drew a random number for each individual. If the random number exceeded the stage-specific survival rate for that year, the individual died; otherwise the individual remained alive. Likewise, the number of newborns in a given year was a random variable from a binomial distribution including the reproductive rate and the number of breeding females.

All projections were run for 60 yrs, corresponding to about three generation-periods

(one generation¹ is estimated to be 21.1 years for common bottlenose dolphins in a stable population – see Taylor et al. 2007). This is probably a reasonable length of time for management purposes. The parameters were selected according to the following steps to represent parameter uncertainty and/or stochasticity (adapted from Secchi 2006):

1. For one 3-generation run, select a mean survival rate for each stage class and a mean reproductive rate from distributions that represent parameter uncertainty.
2. Select the value of each parameter in each year by selecting at random from a distribution representing temporal environmental stochasticity for that parameter. The mean of the distribution is that chosen in step 1. The standard deviation is fixed at a pre-specified estimated value. For one scenario (see below), the model was set to be deterministic, in which case the standard deviations were set to zero.
3. Incorporate demographic stochasticity using a binomial distribution, involving i) a survival rate and the relevant number of individuals from the previous year, or ii) the reproductive rate and the number of breeding females.
4. Repeat steps 1 to 3 many times (*e.g.* 1000 times in this study).

The model and simulations were written in Matlab following Secchi (2006).

Simulation scenarios for demography

The model-scenarios we considered are specified below:

- *Scenario 1*: Deterministic under current levels of by-catch;
- *Scenario 2*: Stochastic under current levels of by-catch;

¹ Generation length is the average age of parents of the current cohort (i.e. newborn individuals in the population), reflecting the turnover rate of breeding individuals in a population (Taylor et al. 2007).

- *Scenario 3*: Stochastic under current levels of by-catch and unbalanced adult sex ratio;
- *Scenario 4*: Decreasing survival; removal of one mature female per year from the PLE dolphin community;
- *Scenario 5*: Decreasing survival; removing one mature female every two years from the PLE dolphin community;
- *Scenario 6*: Increasing survival; adding one juvenile every two years;
- *Scenario 7*: Increasing survival; adding two juveniles every three years;

In scenarios 1 and 2 we used current stage-specific estimates of survival rates for estimating the population growth rate and risk of extinction and for exploring the effects of stochasticity in projections under the current fishery impact. This is because the mark-recapture survival rates contain both natural and non-natural dolphin mortality (Fruet et al. submitted b) with by-catch as the main source of human-related mortality for this dolphin community (Fruet et al. 2012). In scenario 3 we modeled the effects of the unbalanced adult sex ratio reported for the PLE dolphin community (2F:1M) (Fruet et al. submitted b). We fixed the number of calves and juveniles estimated in scenario 2 (calculated assuming a stable age-structure distribution and initial abundance of 44 females) and used the number of mature females known to be alive in the PLE dolphin community ($N = 37$) as the adult female abundance. This resulted in an initial population size of 53 as opposed to 44 females used in scenarios 1 and 2, which assumed a 1:1 sex ratio. The effects of potential changes in by-catch rates were simulated by artificially increasing or decreasing entanglement, which would affect survival rates (scenarios 4-7). We simulated the increase in survival of immature animals because it is possible that the by-catch mortality of this most affected life-stage will be reduced if the dolphin protection area turns out to be effective. However, as

some types of artisanal and recreational gillnetting are still allowed within the protection area and are known to catch and kill bottlenose dolphins, simulating a slightly increase in by-catch of adult females is also justifiable.

The effects of changes in survival rates was simulated by manipulating the fate of known individuals directly in the mark-recapture matrix used by Fruet et al. (submitted b) for estimating the current survival rates of the PLE bottlenose dolphins. For example, the effects of additional by-catch was assessed by simulating a decrease in the survival rate of adult females (scenarios 4 and 5), by manually setting the last year of the survival history at zero for some individuals that had survived until the end of the study period (2005-2012). On the other hand, for scenarios simulating the effectiveness of the dolphin protection area (scenarios 6 and 7), some immature dolphins were resurrected by setting, for some individuals that were already dead, the capture history at one until the end of the period. In scenario 6, three individuals were added back to the community. This represents the minimum number of juvenile resident dolphins know to have been caught during the period of the mark-recapture study. In scenario 7, four dolphins were resurrected, as it is likely that some incidentally killed individuals do not show clear signs of fishing-related mortality. Thus, the survival rates estimated from these simulations potentially provide a reasonable approximation of the expected juvenile survival in the absence of by-catch (i.e. after the implementation of the dolphin protection area). All mark-recapture simulations were run in Program MARK (White and Burnham 1999) using the most parsimonious model of the Robust Design approach reported by Fruet et al. (submitted b).

Risk assessment and management goals

Following the IUCN Red List categories and criteria (criterion A3) (IUCN 2012) the risk was measured as the probability of the PLE dolphin community declining below 80% (Critically Endangered - CR), 50% (Endangered - EN) and 30% (Vulnerable - VU) of the current abundance. If PLE community declines below the CR, EN or VU thresholds in 5%, 10% and 20% of the runs, respectively, it will be recommended to be classified in the corresponding IUCN Red List category. Conservation goal should ensure the long-term viability and ecological function of bottlenose dolphins in their environments. Thus, the management goal of the protected area assumed here is to promote a 20% increase in abundance of the PLE bottlenose dolphins over 60 yrs. This seems to be a satisfactory assumption from both biological and management perspectives. First, because it symbolizes a reasonable increase over such long-term period and because this community potentially is operating near or slightly below to its carrying capacity. And second because, if achieved, it expected to allow to increase the chances of the community to expand its range and intermixing at higher rates with the adjacent communities, thus enhancing genetic variability. The goal of the dolphin protection area will be considered achieved only if at least 90% of the runs predicted the population to be at a level 20% higher than its current size.

Results

Under current by-catch rates and uncertainties in parameter estimates, the effects of stochasticity and skewed adult female sex ratio in the PLE community had low impact in estimates of r , probably as a consequence of high adult female survival and environmental stability (Table 2, Fig. 2). Current by-catch appears to have a modest effect on the dynamics of the PLE dolphin community. All simulations that took into account current by-catch rates indicated this community is growing at a rate of

approximately 1.2 - 1.3% per year, and projected low probabilities of decline and high likelihood of an increase of 20% above their current size over three generations (Table 2). According to this scenario, the PLE dolphin community would be classified as Least Concern.

The removal of a few adult females from the PLE dolphin community would have severe consequences for its dynamics and likelihood of future persistence. Model runs indicated that if one adult female was captured every year or every two years, the PLE dolphin community would experience very high probabilities of decline at all pre-specified levels (Table 2; Fig. 3). According to these scenarios, the PLE community would decline at a rate of 2.3 and 0.5% per year, respectively, suggesting that such by-catch rates would be unsustainable.

Reducing by-catch of juvenile/sub-adult dolphins (scenarios 6 and 7) would result in enhanced chances of an increase of 20% above the current size in 60 yrs, with the conservation goals achieved. Since scenario 6 used survival rates obtained from the simulation of the survival of the three by-caught resident juvenile dolphins, this scenario is probably the most realistic for representing the expected “natural” juvenile survival. Under this scenario, the PLE bottlenose dolphin community would have a mean annual potential intrinsic growth rate of 2.9%, with its maximum estimated at 5.1%.

Discussion

Several other studies and approaches have been carried out to estimate the potential intrinsic growth rate of cetacean species and model population viability, although many of these demographic analyses were conducted with limited data or overly simplistic model structure. For example, survival rates (the parameter to which

population growth rate is most sensitive in long-lived and slow-reproducing species) (e.g. Caswell 2001) is lacking for most cetacean species and for several threatened species this information is obtained by constructing life-table models using survival curves from other species with similar life histories (e.g. Caswell et al. 1998; Secchi 2006; Hashimoto et al. 2013). Although valid, the accuracy of these estimations is constructed under a series of assumptions and also depends on appropriate selection of the model species and the time scaling procedures (Hashimoto et al. 2013). This is particularly relevant when using structured models because λ is influenced disproportionately by variations in demographic traits of each life-stage (Morris and Doak 2003). Despite uncertainties, our structured model was tailored and fed according to the best and up to date stage-specific parameters estimated through a systematic mark-recapture study of the PLE bottlenose dolphin community.

Potential intrinsic growth rates of the PLE bottlenose dolphin community

Our results suggested that the PLE dolphin community would growth to about 3% annually if by-catch of juveniles could be reduced to levels we predicted to approximate their natural survival (scenarios 6 and 7). The maximum estimated potential annual growth rate was 5.1%. Despite parameter uncertainties, this estimate is consistent with other cetacean studies. Reilly and Barlow (1986) have suggested that the intrinsic growth rate of small cetaceans is likely to be approximately 4%. Secchi (2006) estimated an annual growth rate ranging between 0.8% and 3.8% for multiple populations of the franciscana dolphin. Hashimoto et al. (2013) estimated the potential intrinsic growth rate of finless porpoises (*Neophocoena asiaorientalis*) between 4.1% and 5.6%. For bottlenose dolphins, there is only one available potential intrinsic growth rate estimation. A demographic analysis based on age-at-death life-table data suggested

that the population inhabiting the Indian River Lagoon system in Florida have the potential to grow up to 4.7% per year (Stolen and Barlow 2003).

Effects of stochasticity

Stochastic effects and initial number of adult females appear to have little or no influence on the population growth rate and viability of the PLE bottlenose dolphin community. Stochasticity arises from intrinsic or extrinsic forces that change individual fitness and lead to variations in population size, which is independent of the average growth rate of the population (Burgman et al. 1993). In our study, stochasticity had minimal effects in the projections of long-term viability. Projections were similar for both stochastic and deterministic models. These results suggest that year-to-year fluctuations in the environmental conditions of the Patos Lagoon estuary during the study period did not significantly affected reproduction and survival rates of individual females. Our data from the PLE dolphin community showed that the number of births, fecundity and survival rates were relatively consistent over the last 8 yr (Fruet et al. submitted b, c). This does not mean that the PLE dolphin community is invulnerable to stochastic effects. The time-scale used to obtain year-to-year variation in reproduction and vital rates probably was not enough to allow for the detection of extreme events (e.g. strong La Niña, El Niño) that could have severely impacted upon these parameters (e.g. Trillmich and Ono 1991; Leaper et al. 2006). The time scale of this study is probably insufficient to detect important environment-related variation in life-history traits. Given the low abundance of the PLE bottlenose dolphin community, vulnerability to environmental and demographic stochasticity in the long-term should not be overlooked (e.g. Lande 1988).

Viability of the PLE bottlenose dolphin community

An early study used a simple logistic model to determine the Potential Biological Removal (*sensu* Wade 1998) for the PLE dolphin community (Fruet et al. 2012). Simulations indicated this community would decline if by-catch levels reported after 2002 remained at those levels (Fruet et al. 2012). However, the results of the present study suggested that entanglements have a lower effect on the demographic processes of this community than previously thought (scenarios 1 – 3). The mean annual growth rate of the PLE bottlenose dolphins under current levels of by-catch was low but positive (1.2% per year), with a probability of decline <10%.

The discrepancies between these two studies possibly arose because the PBR approach tends to be conservative (Hall and Donovan 2001). The PBR model did not take the sex and life-stage of by-caught individuals into account, and the number of dolphins killed in the fishery used in the simulations was overestimated (Fruet et al. 2012). Any dolphin found dead along the coast near the PLE was assumed to belong to the PLE dolphin community (Fruet et al. 2012). Although reasonable at that time, we now know that there are two coastal bottlenose dolphin communities transiting nearby this area (Genoves 2013). Hence animals stranded in the area may belong to any of the three communities. In addition, PBR considers minimum abundance estimates (i.e. the 20th percentile of a log-normal distribution based on an absolute estimate of the number of animals in the population - see Wade 1998), and that the population cannot grow more than half of the default maximum population growth of 4% suggested for impacted small cetacean species (Reilly and Barlow 1986). Also this approach uses a recovery factor for populations lacking good demographic data (see Wade 1998). In contrast, our stage-structured model used specific life history parameters estimated from mark-recapture data of photo-identified individuals from the dolphin community of interest. Hence, effects of fishing related mortality was implicitly considered as a

component of survival rates, which were estimated specifically for the PLE resident dolphins. In fact, the results found in the present study are similar to the mark-recapture estimates of yearly changes in abundance ($\hat{\lambda}$) (Fruet et al. submitted b). Between 2005 and 2012 the abundance of dolphin community remained relatively stable, with an average $\hat{\lambda}$ estimated at 0.00 (Fruet et al. submitted b).

Differential stage-specific vulnerability to entanglements is likely to maintain the PLE dolphin community relatively stable and with a high likelihood of growing if current levels of by-catch is maintained or reduced. While immature dolphins are particularly susceptible to entanglements, fishing-related mortality of adult females appears to be a rare event (Fruet et al. 2012; Fruet et al. submitted b). Previous studies using sensitivity and elasticity analyses to compare the life histories across several taxa have suggested that adult survival have greater influence on fitness of long-lived and slow-reproducing species than other parameters (e.g. Caswell 2001; Morris and Doak 2003). Therefore, the high adult female survival rate of the PLE dolphin community appears to buffer the current low survival of juvenile dolphins.

The dolphin protection area and the future of the PLE bottlenose dolphin community

Although the abundance of the PLE bottlenose dolphin community is likely to increase under current by-catch rates, this does not mean that the community can persist over the long-term. Predictions based on PVAs are generally optimistic unless all potential threats are included in the model (e.g. Lacy 1993; Young 1994; Ralls and Taylor 1997). Thus, the PLE dolphin community would persist only if its habitat and other potential sources that could affect their vital rates remains the same during the projected time (i.e. no substantial changes in habitat quality or impacts that would cause additional deaths).

Our simulations indicated that a minimal reduction in current by-catch would maximize the chances (>99%) of the PLE bottlenose dolphin community increasing 20% above its current size in three generations. Increasing abundance could potentially increase gene flow among the three adjacent communities, which are genetically differentiated and depauperated (Fruet et al. 2014). In turn this could lead to an increase of their genetic diversity, and potential resilience to local environmental changes (e.g. Frankham 1995; Reed and Frankham 2003). In addition, excluding gillnet fisheries from the dolphin protection area may also promote habitat quality by increasing prey densities, as estuarine waters provide critical habitat for various fish and crustacean species (e.g. Costa et al. 1997; Haimovici et al. 2006; Vieira et al. 2010). Nevertheless, if enforcement fails, fishing effort may increase, or could shift to other areas or fishing gears, such as beach fixed trammel gillnetting, which is still allowed within the protection area (Brasil 2012). The consequences of such changes cannot be foreseen, but could lead to high risk of entanglements to both coastal and estuarine dolphins. These potential changes are a source of concern because these communities are small and its viability highly susceptible to non-natural mortality or catastrophic events. For example, if only a few mature females were removed from the PLE dolphin community, the effect on its viability would be severe (Table 2; Figs. 2 and 3). Therefore, continued systematic monitoring of these dolphin communities is highly recommended in order to evaluate the effectiveness of the dolphin protection area for their long-term viability. Monitoring schemes should be designed according to the ecological characteristics of each community and consider home-ranges, movements, habitat use and residency patterns.

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Table 1. Input parameters for all scenarios used to model the dynamics and viability of the PLE bottlenose dolphin (*Tursiops truncatus*) community. The table includes parameter estimates, uncertainty and the distribution used to represent the uncertainty. AFR is the age at first reproduction (in years). The table also includes year-to-year variation and the distribution used to model the environmental stochasticity.

Parameter	Scenario	Estimate	Variation	Distribution	Year-to-year variation	Distribution
N_T	1,2,4-7	44	Fixed	-	-	-
N_T	3	53	Fixed	-	-	-
AFR	All	9	Fixed	-	-	-
Fecundity (m_3)	All	0.106 ¹ 0.156 ²	0.106 – 0.156	Uniform	0.020	Beta
σ_3	1,2,3,6,7	0.973	0.014	Beta	0.030	Beta
σ_2	1,2,3,4,5	0.832	0.061	Beta	0.099	Beta
σ_1	All	0.851	0.034	Beta	0.115	Beta
σ_3	4	0.926	0.022	Beta	0.030	Beta
σ_3	5	0.949	0.018	Beta	0.030	Beta
σ_2	6	0.928	0.039	Beta	0.099	Beta
σ_2	7	0.952	0.032	Beta	0.099	Beta

¹ Mean fecundity of the PLE bottlenose dolphin community estimated as the total number of females calves born each year by the minimum number of mature females;

² Fecundity estimated as the reciprocal of the average inter-birth interval of the PLE bottlenose dolphin community.

Table 2. Results of simulations used to estimate the growth rates and viability of the PLE bottlenose dolphin community under several scenarios of by-catch. Mean (r), lower (Low) and upper (Up) confidence interval for probabilities (as a proportion of number of model runs) of declining below its initial size (P decline), below 80% (P-80%) (Critically Endangered), 50% (P-50%) (Endangered) and 30% (P-30%) (Vulnerable) of and increasing 20% (P+20%) above its initial size ($N_T = 44$).

Scenario	r	Low	Up	P decline (%)	P - 80%	P - 50%	P - 30%	P +20%
1) Det. Curr. By-catch	0.0136	-0.019	0.040	17.3	1.0	5.3	9.6	77.8
2) Stoch. Curr. by-catch	0.0121	-0.022	0.038	8.8	0	1.0	2.7	83.0
3) 2:1 adult sex ratio	0.0129	-0.017	0.037	6.7	0	0.2	1.1	86.2
4) σ_3 : 1 adult/yr	-0.0234	-0.066	0.012	99.8	66.3	94.9	98.9	0.1
5) σ_3 : 1 adult/2yr	-0.0051	-0.040	0.025	86.8	7.5	44.3	65.1	5.5
6) σ_{2j} : 1 sub-ad/2yr	0.0292	-0.002	0.051	0	0	0	0	99.7
7) σ_{32} : 1 sub-ad/1.5yr	0.0305	0.052	0.052	0	0	0	0	99.9

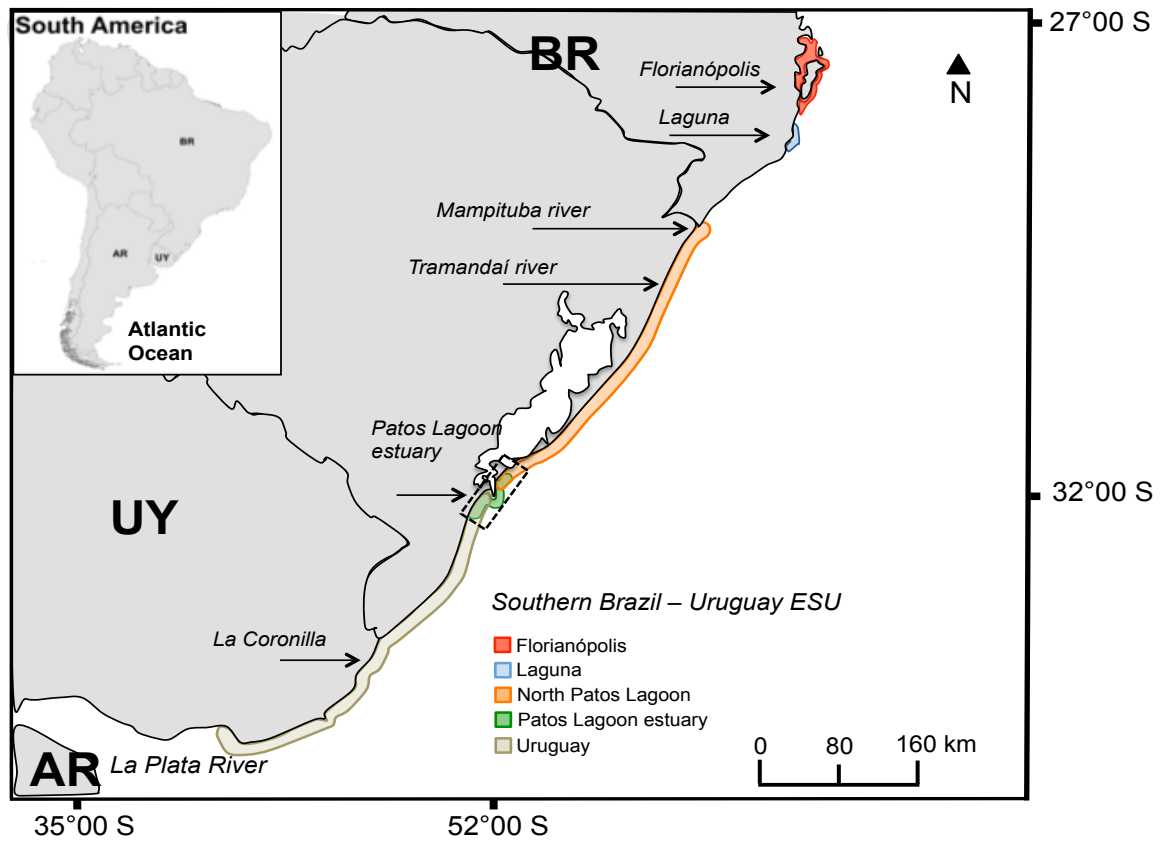


Figure 1. Map showing the approximate geographic boundaries of the five bottlenose dolphin communities (colour contoured lines) identified along the Southern-Brazil/Uruguay Evolutionary Significant Unit (SB-U ESU) (see Fruet et al. 2014 for details), and the recently implemented dolphin protection area in southern Brazil (denoted by dashed lines). Boat-based gillnet fishery is banned within the protection area (Brasil 2012).

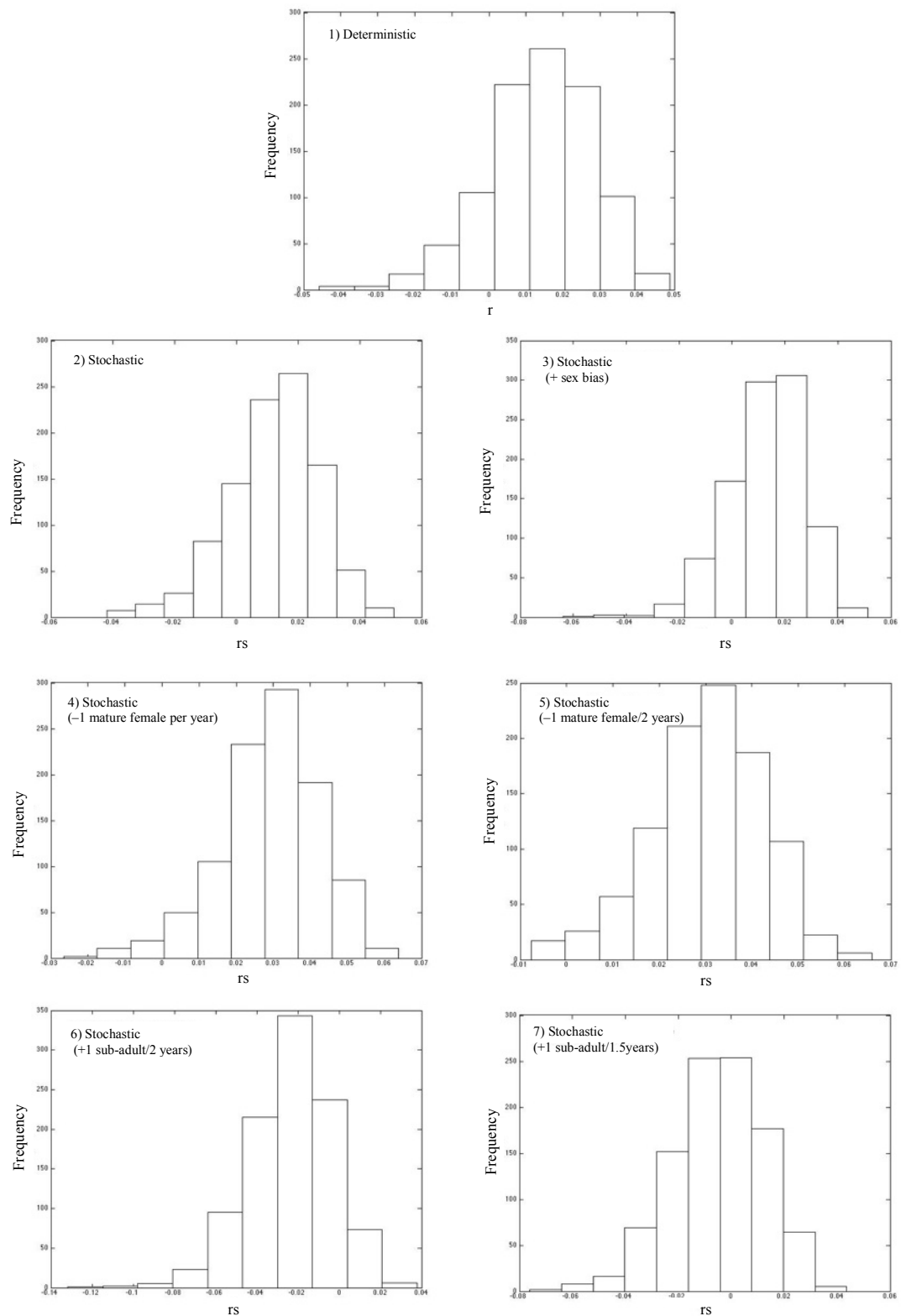


Figure 2. Uncertainties in population growth rates (r: deterministic; rs: stochastic) estimations for the bottlenose dolphin (*Tursiops truncatus*) community of the Patos Lagoon estuary, southern Brazil, under different levels of by-catch and initial number of females (scenario 3).

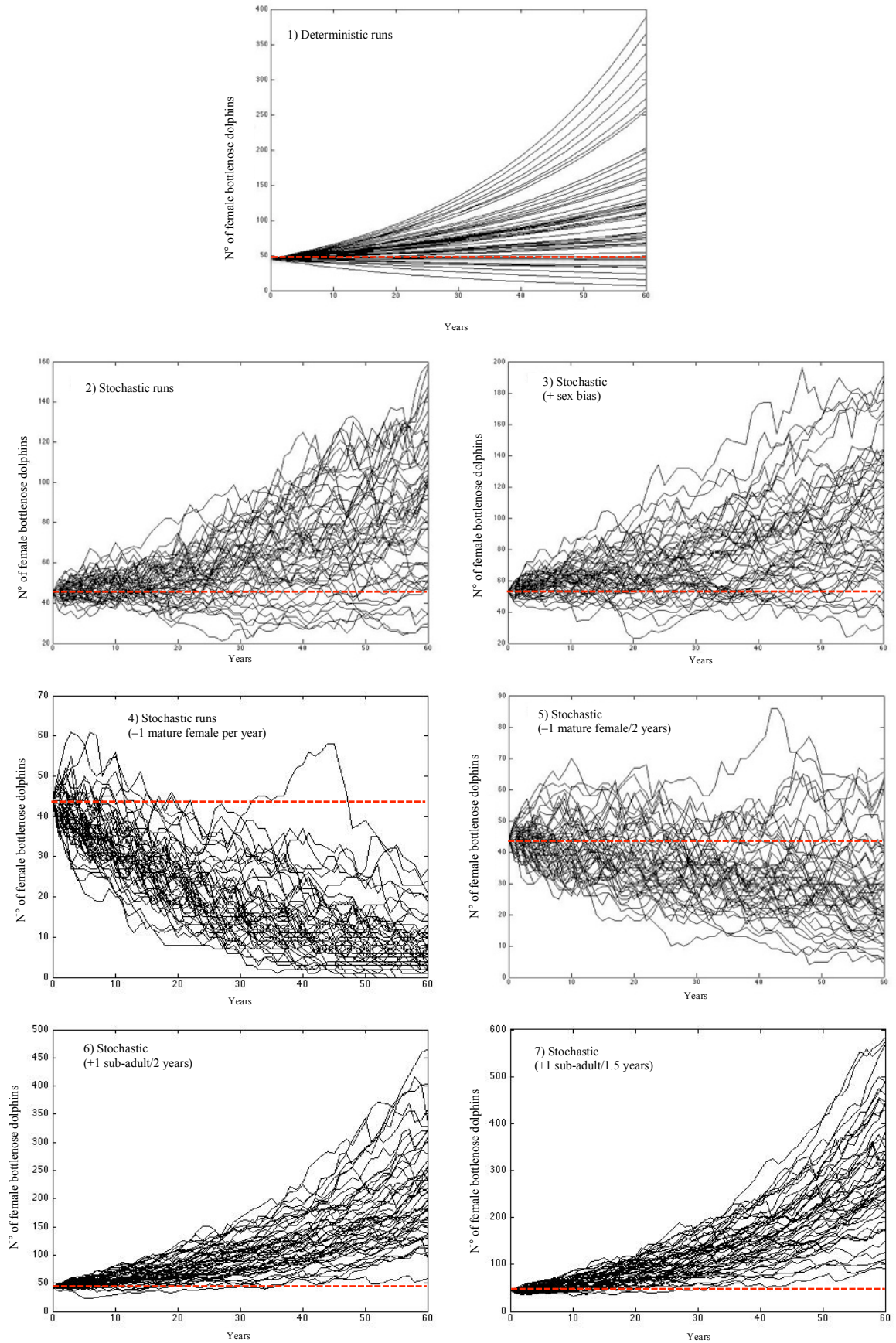


Figure 3. Samples ($N = 50$) from 1000 projections of the size of the bottlenose dolphin (*Tursiops truncatus*) community size of the Patos Lagoon estuary, southern Brazil, for a period of three generations under different scenarios of by-catch and initial community size considering parameter uncertainty in the estimates of stochastic population growth rate. Red dotted line highlights the initial female population size.