**UNIVERSIDADE FEDERAL DO RIO GRANDE-FURG**
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# Estrutura Genética e Social do Boto (*Tursiops truncatus gephyreus*) no Estuário da Lagoa dos Patos e Águas Costeiras Adjacentes

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*Eu dedico esta tese para minha mãe, peça fundamental na engrenagem que me move e me motiva*

*“Quando você atinge seu limite, deve buscar forças para superá-lo”*

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# SUMÁRIO

# 1 CAPÍTULO I

## PANORAMA GERAL DA TESE

## RESUMO

Apesar do gênero *Tursiops* spp. ter distribuição cosmopolita, recentemente revalidou-se a subespécie, *Tursiops truncatus gephyreus*, que é restrita ao Oceano Atlântico Sul Ocidental, entre Sul do Brasil a Argentina. O estuário da Lagoa dos Patos e suas águas costeiras adjacentes abrigam a maior população conhecida desta subespécie e o conhecimento sobre esta população pode contribuir para avaliação de seu status de conservação. Os principais objetivos desta tese foram investigar o padrão de uso espacial destes indivíduos, a forma como estão estruturados socialmente, se existe estruturação genética, assim como entender a ecologia alimentar destes grupos e entender qual fator é preponderante para a estruturação populacional. No capítulo I é apresentado um panorama geral da tese, com uma introdução geral, as hipóteses, objetivos, e os principais métodos, resultados, conclusões e recomendações. No anexo I, levantaram-se todos os dados individuais disponíveis dos golfinhos foto-identificados e catalogados da população para, através de análises sociais, desvendar quais desses fatores influenciam na estrutura social da população. Foram encontrados quatro agrupamentos sociais fortemente relacionados ao uso espacial e temporal de cada área de estudo (estuário, adjacência sul e adjacência norte) e nível de agregação individual. Excluindo estes fatores da análise, para observar apenas as associações puramente sociais, observou-se que as duas unidades presentes em cada área adjacente mantêm sua coesão, enquanto que o grande grupo que utiliza o estuário se subdivide em quatro grupos sociais. No anexo II, investigou-se a estrutura genética dos botos do estuário e costa marinha adjacente. Evidenciou-se que existem duas populações geneticamente distintas que ocupam diferentes nichos isotópicos, uma associada ao estuário e outra à zona costeira. Por fim, esta tese mostra que as duas populações têm um padrão de uso de habitat distintos e que isto é um fator chave de sua estruturação.

Palavras-chave: Boto de Lahille; estrutura social; estrutura populacional; genômica; uso de habitat; ecologia alimentar.

## 1.1 *INTRODUÇÃO GERAL*

Entender a diversidade genética das espécies pode fornecer importantes informações para as estratégias de manejo e conservação como, por exemplo, para identificação e avaliação de unidades de manejo (Palsbøll et al. 2007). Por esta relevância, estudos voltados para biologia da conservação têm demonstrado interesse em entender os fatores responsáveis por esta variação ao longo do tempo e espaço (Frankham et al. 2002, Toro & Caballero 2005, Paz-Vinas et al. 2018). A variação na diversidade genética em relação ao espaço sofre uma forte influência da presença de barreiras para dispersão, que restringem ou inviabilizam o fluxo genético entre grupos. Existem diversos fatores que podem atuar como barreiras para dispersão, incluindo fatores geográficos, como cadeias de montanhas ou rios separando ambientes (exemplo de especiação alopátrica); fatores ecológicos, como diferenças de habitat em regiões adjacentes (exemplo de especiação parapátrica); ou mesmo fatores comportamentais, como diferenças no comportamento reprodutivo entre grupos convivendo no mesmo ambiente (exemplo de especiação simpátrica) (revisado por Coyne & Orr 2004). Além das barreiras naturais, a pressão e impactos antrópicos, como a redução severa de habitat, podem influenciar na diversidade genética dos organismos (e.g., Guschanski et al. 2007). Quando estes padrões de variação genética surgem de populações anteriormente panmíticas (cruzamentos ao acaso, sem restrições), diz-se que a população está estruturada.

A ordem dos cetáceos compreende organismos que, de forma geral, apresentam grande capacidade de movimentação, e suas espécies estão distribuídas por grandes áreas e ocupam uma ampla diversidade de habitats (Hoelzel 1998). Esta alta capacidade de dispersão, aliada à reduzida quantidade de barreiras para tal no ambiente em que estão inseridos, sugere que suas populações apresentem pouca ou nenhuma estruturação populacional (Palumbi 1992, Bohonak 1999). Porém, populações geneticamente distintas são frequentemente encontradas em espécies de cetáceos, mesmo na ausência de barreiras geográficas evidentes (Hoelzel 1998). Por exemplo, o uso diferenciado de habitat e a estrutura social (síntese de como os indivíduos interagem uns com os outros) podem atuar como “barreiras” não-físicas ao fluxo genético ao reduzir as interações com indivíduos que não partilham das mesmas preferencias de área e de comportamento e, portanto, gerando diferenciação genética (Hoelzel et al. 1998, Wiszniewski et al. 2009, Ansmann et al. 2012, Louis et al. 2014). Agrupamentos podem surgir dentro de populações, principalmente quando os animais se beneficiem da companhia de outros indivíduos (Krause & Ruxton 2002). A estrutura social descreve esses padrões de interações (ou associações) entre indivíduos (Hinde 1976), sendo possível estimar a proporção de tempo que os pares de indivíduos permanecem associados, assim como a duração e natureza destas associações (Cairns & Schwager 1987, Whitehead 1995, 2008). Neste contexto, a presença de grupos de grande estabilidade social numa população pode fazer com que os indivíduos parem de se reproduzir aleatoriamente entre si, priorizando animais socialmente próximos e, consequentemente, podendo gerar diferenciação genética (Riesch et al. 2012, Van Cise et al. 2017). Por fim, muitas vezes os agrupamentos sociais estão atrelados à preferências dos indivíduos por habitats distintos, uso de diferentes recursos ou estratégias distintas de alimentação, resultando, além da estruturação social, numa estruturação ecológica (Marcoux et al. 2007, de Stephanis et al. 2008, Riesch et al. 2012).

Estruturas sociais, ecológicas e genéticas são intimamente conectadas e, muitas vezes, dependem da existência umas das outras para se desenvolverem ou se perpetuarem dentro de uma população. Por este motivo, pesquisas têm deixado de investigar estas estruturas separadamente, partindo para abordagens multidisciplinares, incorporando informações sobre o habitat, estrutura social e composição genética (e.g., Wiszniewski et al. 2009, Frère et al. 2010, Louis et al. 2018). A seguir serão abordados os conceitos sobre os mecanismos evolvidos no processo de estruturação, a espécie alvo e os antecedentes que levaram à formulação da hipótese, os métodos utilizados, resultados obtidos e as implicações deste estudo em futuras ações de manejo e conservação.

### *1.1.1 Estrutura social*

Nas últimas décadas houve um grande avanço no estudo da estrutura social das populações, desde a formulação do seu conceito por Hinde (1976), à escolha de um índice de associação adequado (Cairns & Schwager 1987), até o desenvolvimento e compilação de diversas técnicas de análise social (Whitehead 1997) e de redes sociais (Newman 2004, 2006). Com isso, percebeu-se que grupos sociais surgem através de indivíduos que se associam mais por compartilharem algum tipo de similaridade, um fenômeno conhecido como homofilia (McPherson et al. 2001). Estas similaridades podem estar relacionadas ao sexo (revisado por Ruckstuhl 2007), classe etária (Smith et al. 2002, Manno 2008), estado reprodutivo (Sundaresan et al. 2007, Möller & Harcourt 2008), sociabilidade (Lusseau et al. 2006, Manno 2008), estratégias de forrageio (Chilvers & Corkeron 2002, Daura-Jorge et al. 2012, Mann et al. 2012), uso differenciado do habitat (Möller et al. 2007, Ansmann et al. 2014), relações de parentesco dos indivíduos (Pinter-Wollman et al. 2009, Wiszniewski et al. 2010, Frère, Krützen et al. 2010, Van Cise et al. 2017) e também o comportamento individual (ou personalidade; Highfill & Kuczaj II 2007, Krause et al. 2007, 2010). Além disso, dentro de uma população pode haver um tipo de variação individual denominada gregarismo, que é quando alguns indivíduos preferem formar grupos menores, ou possuir uma menor quantidade de associados, enquanto outros preferem formar grupos grandes, ou terem mais associados (Godde et al. 2013). Muitos destes fatores têm uma influência muito grande nas estimativas dos índices de associação e, portanto, dificultam distinguir dentro de uma rede social quais pares se associam por motivos puramente sociais. Este tipo de interação, denominado afiliação, pode ser acessado excluindo-se a influência destes fatores estruturais (Whitehead & James 2015).

### *1.1.2 Estrutura ecológica*

Um dos mecanismos que promovem estruturação em simpatria é a especialização por habitat/recurso, que significa que os indivíduos de uma população se especializaram em algum tipo de habitat ou recurso (Futuyma & Moreno 1988, Dieckmann & Doebeli 1999). A especialização por um determinado recurso pode surgir devido a plasticidade dos indivíduos e da estabilidade temporal de suas estratégias de alimentação (Knudsen et al. 2010). Entretanto, para que este evento de especialização resulte em diferenciação genética, deve estar atrelado à reprodução seletiva ou isolamento reprodutivo (Dieckmann & Doebeli 1999), o que tende a acontecer uma vez que estes indivíduos especializados tendem a passar mais tempo associados, resultando em estruturação social. Este tipo de estruturação é vantajosa para a população, pois reduz a competição por recursos (Robinson et al. 1996). A especialização por recurso também pode ser um comportamento cultural dentro da população, passado de geração para geração, muitas vezes durante o cuidado parental. Isto já foi reportado, por exemplo, nos golfinhos de Shark Bay, na Austrália, que utilizam esponjas para se alimentarem (Smolker et al. 2010). Outro caso semelhante é a interação entre os golfinhos e pescadores de tarrafa em Laguna, Santa Catarina, que realizam uma pesca cooperativa (Daura-Jorge et al. 2012). Por fim, variações nos traços morfológicos também podem estar relacionados à adaptação por recursos distintos (Smith & Skúlason 1996, Foote et al. 2009).

Atualmente, uma técnica amplamente utilizada para investigar a ecologia espacial e trófica dos predadores de topo tem sido a análise de isótopos estáveis (*stable isotope analysis* – SIA) de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) (ver revisões de Hobson 1999, Kelly 2000, Newsome et al. 2010). O $\delta^{13}C$ é informativo quanto à base da cadeia trófica, podendo ser utilizado para revelar padrões de utilização espacial, como entre um ambiente costeiro e outro oceânico (Hobson et al. 1994). Por outro lado, o $\delta^{15}N$ é muito utilizado como indicador da posição trófica, visto que enriquece de um nível para outro, dentro da cadeia trófica (de Niro & Epstein 1981). Além disso, é possível identificar a proporção de contribuição das principais presas para o sinal isotópico dos consumidores através dos modelos de mistura Bayesianos (Parnell et al. 2013), embora estes modelos exijam o conhecimento pretérito destas presas principais (Phillips et al. 2005, 2014). Por isso, diversos estudos têm utilizado a SAI para investigar a presença de partição de nicho dentro e entre populações de, por exemplo, aves marinhas (Hodum & Hobson 2000, Young et al. 2010), elefantes-marinhos (*Mirounga leonina*; Lewis et al. 2006) e golfinhos (Barros et al. 2010, Fernández et al. 2011, Wilson et al. 2017).

### *1.1.3 Estrutura genética*

A variação geográfica entre populações, tanto em características morfológicas quanto genéticas, é resultado do balanço das forças que produzem diferenciação e homogeneidade genética local (Slatkin 1987). Barreiras para o fluxo genético podem surgir como resultado do ambiente no qual os animais estão inseridos, processos históricos e comportamentais, e gerar heterogeneidade genética entre populações. Existem poucas barreiras físicas para os cetáceos, dada sua grande mobilidade e as características do ambiente que ocupam. Entretanto, variações ambientais e de habitat abruptas, clima, ou mesmo características oceanográficas particulares (e.g. correntes, salinidade e temperatura) podem reduzir esta capacidade de dispersão, gerando isolamento reprodutivo e estruturação genética (Fullard et al. 2000, Bilgmann et al. 2007, Fontaine et al. 2007, Möller et al. 2011, Louis et al. 2014). Estudos recentes têm mostrado que também existe estruturação genética em escalas geográficas relativamente pequenas em populações de golfinhos (Ansmann et al. 2012), inclusive entre um estuário e sua zona costeira adjacente (Möller et al. 2007). Barreiras geográficas históricas também podem ter influência na estrutura atual das populações. Por exemplo, após a última glaciação, com o derretimento do gelo, algumas populações anteriormente alopátricas passaram a ser simpátricas ou parapátricas, trazendo uma diferenciação genética anterior para os dias atuais (Hewitt 1996). Por fim, identificar uma estruturação genética em animais que vivem em simpatria é, muitas vezes, desafiador. Para tanto, faz-se necessário a utilização de um grande número de marcadores moleculares capazes de detectar uma estruturação em uma fina escala. Os polimorfismos de nucleotídeo único (*single-nucleotide polymorphisms* - SNPs) têm se mostrado muito eficientes para análises de estrutura populacional quando utilizados em grande quantidade (Liu et al. 2005, Gaughran et al. 2018).

### *1.1.4 Contextualizando a espécie e a população alvo*

O golfinho nariz-de-garrafa, *Tursiops* spp., é cosmopolita, habitando regiões costeiras e oceânicas, tanto em clima tropical quanto temperado (Wells & Scott 1999). Estudos ao redor do mundo têm mostrado que suas populações são sociedades majoritariamente do tipo fissão-fusão (Connor et al. 2000), embora algumas populações apresentem uma estrutura social com relações mais estáveis (e.g., Lusseau et al. 2003, Wells 2014). São animais de vida longa, maturação tardia (entre 5 e 14 anos) que se reproduzem lentamente (um filhote a cada 2 a 4 anos, 12 meses de gestação) através de um sistema de acasalamento poligâmico (Urian et al. 1996, Wells & Scott 1999, Connor et al. 2000, Fruet et al. 2015). Os golfinhos nariz-de-garrafa possuem uma grande plasticidade em estratégias de alimentação (Chilvers & Corkeron 2001, Smolker et al. 2010, Daura-Jorge et al. 2012), uso de habitat (Natoli et al. 2005, Wiszniewski et al. 2009, Ansmann et al. 2014) e utilização de recursos (Berens McCabe et al. 2009, Barros et al. 2010, Fernández et al. 2011, Rossman et al. 2015). Por isso, estudos tem mostrado que as populações de golfinhos nariz-de-garrafa ao redor do mundo possuem padrões variáveis de residência e área de vida (Wells et al. 1987, Simões-Lopes & Fabian 1999, Silva et al. 2009, Hwang et al. 2014, Laporta et al. 2016). Geralmente, as populações que apresentam maior grau de residência e menor área de vida estão associadas a ambientes costeiros de alta produtividade como desembocaduras de rios e estuários, baías e fiordes (Wells et al. 1987, Simões-Lopes & Fabian 1999, Fruet et al. 2015). Entretanto, movimentos de larga escala também são frequentemente observados, inclusive de emigração temporária (Bearzi et al. 2008, Silva et al. 2009, Laporta et al. 2016). No que tange a ecologia destes animais, eles possuem uma grande plasticidade em estratégias de alimentação (Chilvers & Corkeron 2001, Smolker et al. 2010, Daura-Jorge et al. 2012), uso de habitat (Natoli et al. 2005, Wiszniewski et al. 2009, Ansmann et al. 2014) e utilização de recursos (Berens McCabe et al. 2009, Barros et al. 2010, Fernández et al. 2011, Rossman et al. 2015). A identificação individual através de fotografias das nadadeiras dorsais destes animais (base para maioria dos estudos citados), que muitas vezes apresentam marcas de longa duração evidentes, permite que esta plasticidade em diferentes aspectos da ecologia desse animais seja analiticamente comprovada (Würsig & Würsig 1977, Würsig & Jefferson 1990, Urian et al. 2015).

Até poucos anos atrás, as populações costeiras encontradas no sul do Brasil, Uruguai e Argentina (Figura 1) eram reconhecidas como da espécie *Tursiops truncatus*. Porém, estudos recentes mostraram que estas populações possuem características morfológicas e genéticas únicas (Costa et al. 2016, Wickert et al. 2016, Fruet et al. 2017), sendo agora reconhecidas como populações da subespécie *Tursiops truncatus gephyreus* (Committee on Taxonomy 2019) (referenciado apenas como boto daqui em diante). Diversos estudos haviam sido realizados com populações desta subespécie, quando ainda era reconhecida como *Tursiops truncatus*, no que tange a estrutura social e genética ao longo de sua distribuição. Daura-Jorge et al. (2012) e Zappes et al. (2011) descreveram a interação cooperativa entre botos e pescadores de tarrafa em Laguna e Barra de Imbé/Tramandaí, respectivamente. O estudo de Laguna também mostrou que esta estratégia de alimentação é desempenhada por uma parcela da população, estando associada a uma estruturação social. Na Argentina, Vermeulen (2018) descreveu uma estrutura social relativamente homogênea nos botos residentes (Vermeulen & Cammareri 2009) da Baía de Santo Antônio. Costa et al. (2015) encontraram três agrupamentos genéticos nos botos que estão distribuídos entre o norte do Rio Grande do Sul e Laguna, em Santa Catarina. Estes estudos mostram que, em linhas gerais, muitos aspectos do gênero *Tursiops* são observados nas populações de botos.

O presente estudo se refere à maior população residente de botos (*Tursiops truncatus gephyreus*), a qual habita o estuário da Lagoa dos Patos e águas costeiras adjacentes (Figura 1). A área corresponde aos municípios de Rio Grande e São José do Norte (RS) e a grande maioria da população utiliza esta área durante todo o ano (Fruet et al. 2011, Fruet et al. 2015). Há evidências de que a área estuarina é priorizada pelas fêmeas (razão sexual ~2fêmea: 1macho – Fruet et al. 2015), enquanto a proporção de machos (principalmente juvenis) é significativamente maior entre os animais encalhados ao longo da costa adjacente (Fruet et al. 2010). Esta população, que vem sendo monitorada sistematicamente por foto-identificação desde 2005, utiliza preferencialmente a desembocadura do estuário e águas costeiras adjacentes, ambas sujeitas a uma intensa pesca artesanal, especialmente na primavera e verão (Di Tullio et al. 2015). Esta época que coincide com o período de reprodução da população, que apresenta nascimentos em pulso, os quais ocorrem durante os meses quentes (Fruet et al. 2015). As fêmeas da população atingem maturação sexual entre 7 e 8 anos, geram apenas um filhote após um período de gestação de 12 meses, e investem intensamente no cuidado parental durante os primeiros 2 a 3 anos de vida do filhote (Fruet et al. 2015). A dieta da população é baseada em peixes teleósteos, principalmente a corvina (*Micropogonias furnieri*), a tainha (*Mugil lisa*), a maria-luíza (*Paralonchuros brasiliensis*), o papa-terra (*Menticirrhus* sp.) e o peixe-espada (*Trichiurus lepturus*) (Secchi et al. 2016).

Recentemente, baseado em um estudo de análise social, demonstrou-se que esta população está estruturada em três unidades sociais distintas, uma ocupando a área estuarina, outra ocupando a área ao sul e a terceira ao norte da desembocadura do estuário (Genoves 2013). Este uso diferenciado da área, com animais utilizando preferencialmente um estuário e outros exclusivamente um ambiente marinho, pode resultar em uma partição dos recursos da área, diminuindo, indiretamente, a competição intraespecífica. Além disso, populações costeiras associadas a ambientes altamente produtivos e com alto grau de fidelidade local, como no caso do presente estudo, podem apresentar unidades fortemente estruturadas e com reduzido fluxo gênico (e.g., Möller et al. 2007, Rosel et al. 2009, Ansmann et al. 2012b). Finalmente, alguns indivíduos podem vir a exercer funções chave dentro do sistema social da população, como servir de ligação entre unidades sociais distintas (Lusseau & Newman 2004) e gerar um certo grau de “diluição” em possíveis estruturações. Portanto, essa segregação social pode estar atrelada ao uso de habitat, composição genética, ou mesmo diferenças de ecologia alimentar entre seus indivíduos.

**Figura 1. Área de distribuição das populações conhecidas do boto, *Tursiops truncatus gephyreus*, que compreende zonas costeiras do sul do Brasil (BR), Uruguai (UR) e Argentina (AR).**

### *1.1.5 Implicações para a conservação*

Uma questão fundamental da biologia da conservação é como delinear unidades de conservação adequadas para manter o potencial adaptativo das espécies e, consequentemente, sua persistência (Moritz 1999, Fraser & Bernatchez 2001). Por muito tempo convencionou-se que a conservação de populações de uma espécie contribuiria para manter sua diversidade genética e maximizar o seu potencial evolutivo, minimizando seus riscos de extinção. Porém, estudos recentes têm encontrado componentes e estruturas intra-populacionais relevantes que, muitas vezes, têm grande importância na conservação da população como um todo. Por isso, tem se discutido qual tipo de estrutura e em que nível é relevante se conservar.

O uso da genética tem sido fundamental no âmbito dos planos da conservação, especialmente se integrados com informações sobre parâmetros populacionais, ecologia e movimentos de indivíduos adquiridas por métodos alternativos (Lowe & Allendorf 2010). Dentre as designações mais utilizadas para unidades de conservação com base genética estão as unidades evolutivamente significativas (*Evolutionary Significant Unit* – ESUs) e as unidades de manejo (*Management Units* – MUs). A ESU (sensu Ryder 1986) é uma população, ou um conjunto de populações, que historicamente foram isoladas, necessitando de ações de manejo independentes, visando maximizar o potencial evolutivo frente a mudanças climáticas e ambientais. As ESUs são relevantes para abordagens de longo prazo, que consiste no estabelecimento de estratégias e medidas prioritárias de conservação (Moritz 1994). Por outro lado, as MUs (Moritz 1994) consideram a estrutura populacional atual, ou seja, fatores como frequência alélica e variação adaptativa também são abordados, sendo indicadas para ações de manejo de curto prazo. De certa forma, as MUs podem ser consideradas como subpopulações dentro de uma metapopulação, representada por uma ESU. Por isso, as MUs podem representar populações que são importantes para persistência de uma ESU, ou mesmo uma espécie, ao longo do tempo (Allendorf et al. 2007). Estudos recentes têm encontrado estruturações genéticas relativamente mais fracas em populações de golfinhos, que não são englobadas pela caracterização de ESU, mas têm grande importância na manutenção da diversidade genética das populações (Möller et al. 2007, Ansmann et al. 2012), podendo ser consideradas MUs.

Nas últimas décadas tem-se discutido a necessidade e benefícios da inserção de um novo conceito visando melhorar as ações voltadas à biologia da conservação, que é a conservação baseada no estudo do comportamento dos animais (Sutherland 1998, Caro 1999, 2007, Berger-Tal et al. 2011). O estudo da estrutura social está inserido nesse contexto. Geralmente as unidades sociais que utilizam áreas diferentes (e.g. Möller et al. 2007, Ansmann et al. 2014) também podem contrastar na forma e tipo de alimentação (Fernández et al. 2011, Monteiro et al. 2015) e, consequentemente, podem diferir em respostas às variações ambientais (Sutherland 1998). Por exemplo, os clãs de cachalote (*Physeter macrocephalus*) do oceano Pacífico Sul, diferem na efetividade de alimentação de acordo com as oscilações ambientais (El Niño) e, consequentemente, diferem no sucesso reprodutivo (Whitehead & Rendell 2004), provavelmente afetando o sucesso reprodutivo e aptidão dos clãs menos aptos. Outro fator relevante nestas populações é o componente cultural, que geralmente se restringe a grupos dentro da população e pode levar à estruturação genética. É o caso supracitado dos golfinhos de Shark Bay, que utilizam esponjas para alimentação, estratégia que passa entre gerações por cuidado parental, e acabaram por se diferenciar geneticamente dos golfinhos que não utilizam da mesma técnica (Kopps et al. 2014). Outro fator elencado por Sutherland (1998) é que entender o sistema de acasalamento ajuda a compreender quando e se a depressão por endogamia pode vir a ser um problema para uma população. Além disso, Whitehead e Rendell (2004) sugerem que para algumas espécies como golfinhos, baleias e elefantes, seria fundamental incluir esse componente cultural em ações voltadas para a conservação de unidades evolutivas. Portanto, embora muitos ações relacionadas à conservação não necessitem do componente comportamental para serem efetivos, estudos comportamentais podem contribuir significativamente quando as estratégias de conservação através dos métodos tradicionais não são efetivas (Sutherland 1998, Caro 2007).

*Tursiops truncatus*, a espécie previamente descrita para a população do estuário da Lagoa dos Patos e águas costeiras adjacentes, está listada como “menor preocupação” na Lista Vermelha da UICN), mas isso se deve a sua distribuição cosmopolita e estimativa global de cerca de 600.000 indivíduos. Entretanto, existe uma grande preocupação na conservação de diversas populações, principalmente as costeiras, que estão expostas a uma série de impactos causados pelo ser humano (ver Reeves 2003). Por este motivo, sempre houve uma grande preocupação com o estado de conservação da população que utiliza o estuário da Lagoa dos Patos e águas costeiras adjacentes. Embora as estimativas de abundância dos indivíduos que utilizam o estuário tenham se mantido constantes nas últimas décadas (Dalla Rosa 1999, Fruet et al. 2011, Fruet et al. 2015), a mortalidade oriunda de capturas acidentais em redes de pesca tem sido motivo de muita preocupação (Fruet et al. 2010), principalmente quanto aos botos que utilizam a zona costeira. Esta captura se deve à sobreposição do esforço de pesca com a área preferencialmente utilizadas pelos botos, principalmente durante a primavera e verão (Di Tullio et al. 2015). Esta preocupação aumentou com o reconhecimento dessa população como pertencente à subespécie *Tursiops truncatus gephyreus* pelo Comitê de Taxonomia da "*The Society for Marine Mammalogy*" em 2017. Este reconhecimento confere aos botos que utilizam o PLE e as costas adjacentes uma grande relevância por se tratarem de uma população de uma subespécie costeira, portanto sujeita constantemente a impactos antrópicos, e de distribuição restrita ao oceano Atlântico Sul ocidental. Caso exista estruturação genética e/ou social nestes indivíduos, sua conservação poderá se tornar um importante componente para a sobrevivência da subespécie em longo prazo.

### *1.1.6 Formulação das hipóteses*

Dado o exposto acima, as hipóteses deste estudo são: (1) as unidades sociais identificadas possuem grande sobreposição espacial, mas não costumam frequentar a mesma área no mesmo espaço de tempo; (2) algumas das similaridades (e.g., classe etária, sexo e estratégias individuais) intensificam as relações sociais e a distribuição espacial dos indivíduos; (3) associado à estrutura social, existe uma estrutura genética; e (4) o uso diferenciado de habitat (estuário vs. zona costeira) promove partição de nicho.

## 1.2 ***OBJETIVOS***

O objetivo geral desta tese é analisar numa fina escala a estrutura social dos botos que utilizam o estuário da Lagoa dos Patos e águas costeiras adjacentes e investigar se ela está associada a uma estruturação populacional genética e/ou ecológica.

Os objetivos específicos são: (1) verificar a necessidade de se corrigir o índice de associação quanto à presença de gregarismo; (2) avaliar o uso temporal dos indivíduos em cada uma das áreas de estudo; (3) identificar e excluir os possíveis fatores que influenciam significativamente a análise social antes da estimativa dos índices de afiliação generalizados; (4); investigar se existe estruturação genética; (5) investigar se o uso diferenciado de habitat promove partição de nicho isotópico; (6) determinar contribuição das principais presas do boto; (7) identificar o fator determinante dessa estrutura genética.

## 1.3 *ESTRUTURA DA TESE*

Esta tese está estruturada em um capítulo introdutório (este Capítulo I) e dois anexos contendo os resultados inéditos de pesquisa (ANEXOS I e II). A seguir, neste capítulo, serão apresentadas as principais metodologias utilizadas, os principais resultados obtidos, uma conclusão geral e recomendações para futuros estudos. O anexo I utilizou-se de 10 anos de foto-identificação e, além de mensurar a influência que o uso espaço-temporal e o gregarismo têm sobre o índice de associação na população, extraiu a rede social baseada exclusivamente em “relações verdadeiras”. Os resultados mostram que, em termos sociais, existem quatro grupos dentro da unidade que utiliza o estuário, enquanto que nas áreas costeiras segue-se o mesmo padrão encontrado com a influência do uso da área, com a presença de duas unidades sociais. O anexo II surgiu da necessidade de se entender se estes botos que utilizam o estuário da Lagoa dos Patos e águas costeiras adjacentes de forma diferenciada apresentam estruturação genética, assim como uma ocupação de nicho trófico diferenciada. Os resultados mostram que os indivíduos que utilizam o estuário da Lagoa dos Patos e águas costeiras adjacentes estão estruturados em duas populações, que ocupam nichos tróficos distintos. Uma das populações utiliza toda a área de estudo, mas ocorre principalmente na porção final do estuário, e outra está restrita à zona costeira adjacente.

## 1.4 *MATERIAL E MÉTODOS*

### *1.4.1 Área de estudo e coleta dos dados:*

A área de estudo compreende um total de 140km$^2$, divididos em três subáreas: a porção final do estuário da Lagoa dos Patos (PLE), mais abrigada, com 40km$^2$; área costeira adjacente norte (NC) e área costeira adjacente sul (SC), ambas com 50km$^2$ de área e mais suscetíveis às variações nas condições oceanográficas (Figura 2). O PLE é um sistema subtropical, situado no sul do Brasil, que atua como bacia de drenagem de aproximadamente 200.000 km$^2$ (Möller et al. 2001) e possui conexão com o oceano Atlântico por dois molhes de aproximadamente 4km cada. O estuário é uma das principais áreas em termos de produtividade no Brasil, com abundante presença de peixes dentro do estuário e em suas praias adjacentes (Garcia et al. 2012). As praias adjacentes ao sul e ao norte dos molhes da barra, em termos de abundância e riqueza de espécies de peixes, são muito similares, ambas apresentando maior produtividade nos meses quentes (Rodrigues & Vieira 2013). Entretanto, diferem um pouco em relação à morfodinâmica praial, com a SC sendo caracterizada como uma praia dissipativa, dominada por sedimentos lamosos e areno-lamosos, oriundos da pluma da lagoa (Marques et al. 2009). Já a NC é uma praia mais refletiva, com tamanho de grão relativamente maior do que a SC (De Oliveira & Calliari 2006).

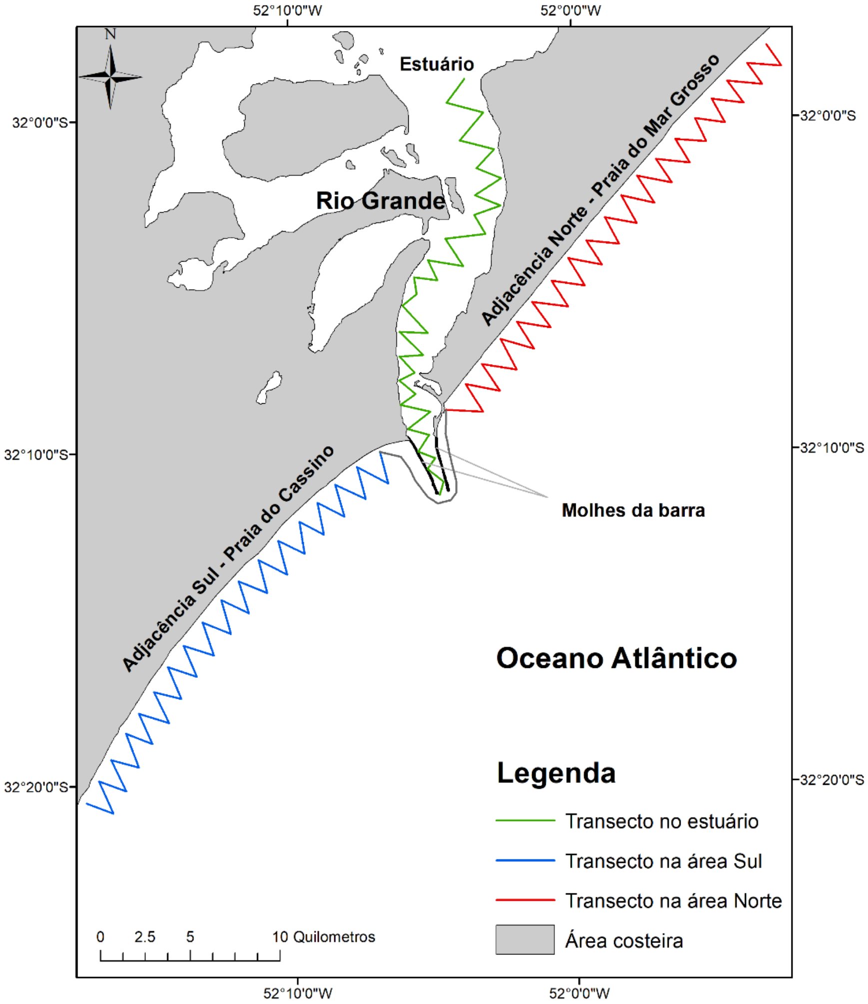

**Figura 2. Área de estudo e transecções em zigue-zague realizadas nas saídas para foto-identificação e coleta de biópsias dos botos, *Tursiops truncatus gephyreus*, no estuário da Lagoa dos Patos e águas costeiras adjacentes, sul do Brasil.**

As saídas de campo foram realizadas entre agosto de 2005 e dezembro de 2015, somente sob condições climáticas favoráveis (e.g. boa visibilidade, estado do mar <3 na escala Beaufort). Cada saída foi destinada a cobrir ao menos uma das subáreas e, na medida do possível, realizou-se ao menos uma saída por mês, alternando-se o início do percurso. Indivíduos com coesão espacial (i.e. até 100m um do outro) e que estavam envolvidos em atividades similares foram definidos como um grupo (Wells et al. 1987). Os indivíduos foram fotografados aleatoriamente, a fim de identificá-los através das marcas de longa duração presentes em suas nadadeiras dorsais (Urian et al. 2015). Os critérios de análise fotográfica, para determinar se um animal possuía marcas suficientes para ser utilizado nas análises, número mínimo de avistagens, assim como os critérios de caracterização de uso espacial e temporal estão especificados no anexo I (Genoves et al. 2018).

### *1.4.2 Coleta de amostras*

As amostras de pele para análises isotópicas e genéticas dos botos foram coletadas de indivíduos adultos e juvenis, conhecidos por suas marcas de longa duração presentes na nadadeira dorsal, durante as saídas de foto-identificação. Para tanto, utilizou-se uma balestra de 120lb com uma flecha adaptada especificamente para coleta de biópsias de pequenos cetáceos. Esta flecha contém uma ponteira que penetra no corpo do animal e coleta apenas um fragmento de pele (um círculo de 6mm de raio) e gordura, sem atingir os músculos e, consequentemente, causar maiores danos aos animais (Fruet et al. 2016).

Para análises isotópicas, as principais presas do boto foram selecionadas com base em Secchi et al. (2016), que descreveram a ecologia alimentar da população, e os respectivos sinais isotópicos de carbono e nitrogênio de amostras de músculo dos exemplares presentes no banco de dados do Laboratório de Ecologia e Conservação da Megafauna Marinha (ECOMEGA) foram analisados por Wiegand (2017).

### *1.4.3 Métodos do Anexo I*

O anexo I se refere ao estudo da estrutura social da população de botos, visando identificar a presença de unidades sociais e descrever como seus indivíduos se comportam socialmente e como utilizam a área no tempo e espaço. Para as análises sociais, utilizou-se o índice de pesomédio (HWI, Cairns & Schwager 1987) corrigido para influência de gregarismo (Godde et al. 2013), que se mostrou presente na população após os teste de permutação (Bejder et al. 1998). Com isso, realizou-se uma análise preliminar, aplicando a modularidade de Newman (2004, 2006), sem identificar as variáveis estruturais que poderiam influenciar no índice de associação. Após este processo, utilizou-se o HWI como base e investigou-se quais das variáveis preditivas são significativas através do MRQAP (*Multiple Regression Quadratic Assignment Procedure*: Dekker et al. (2003, 2007)), com 20.000 permutações. As variáveis utilizadas foram: gregarismo; padrão de uso espacial; sobreposição de área de vida; sobreposição temporal; e classes como sexo, sub-área e períodos preferencialmente utilizados. Após remover as variáveis preditivas significativas, as relações de afiliação foram calculadas através dos Índices de afiliação generalizados (GAIs: Whitehead & James 2015). Para detectar as relações muito fortes e muito fracas, transformou-se os resíduos deste procedimento em resíduos de Anscombe (Pierce & Schafer 1986). Além disso, para auxiliar na interpretação dos resultados, calcularam-se métricas de rede como força, coeficiente de agrupamento e afinidade (Whitehead 2008). Por fim, o padrão temporal das associações foram mensurados através da taxa de decaimento do índice de associação padronizada (SLAR: Whitehead 1995). As plataformas e softwares utilizados foram: SOCPROG, versão 2.8 (Whitehead 2009), e UCINET (Borgatti et al. 2002), para as análises sociais; R, versão 3.4.3 (R Core Team 2017) e pacote AdehabitatHR (Calenge 2006), para a estimativa de área de vida; e Arcview 9.3 (ESRI, Redlands, CA, U.S.A.), para produção dos mapas.

### *1.4.4 Métodos do Anexo II*

O anexo II foi destinado a investigar uma possível estruturação populacional genética nos botos que utilizam o estuário da Lagoa dos Patos e águas costeiras adjacentes, dado o diferente uso de habitat e estrutura social descritos anteriormente. O estudo se restringiu aos botos que foram analisados socialmente no anexo I e que tiveram amostras de pele coletadas através de biópsias. Foram utilizados 49 indivíduos nas análise genéticas e 40 nas análises de isótopos estáveis de carbono e nitrogênio. Os números não são iguais pois alguns indivíduos não tiveram amostras de pele destinadas à análise de isótopos.

Para as análises genéticas da população de botos, foram utilizados os marcadores genéticos Polimorfismos de Nucleotídeo Único (*Single Nucleotide Polymorphisms* – SNPs), com todos os procedimentos laboratoriais e de bioinformática baseados em Sandoval-Castillo et al. (2018) e descritos com detalhes no anexo II. A diversidade genética das unidades sociais foram analisadas através das médias de diversidade dos nucleotídeos ($\pi$), médias de heterozigosidade esperada ($H_E$) e a porcentagem de loci polimórficos, através do programa ARLEQUIN 3.5 (Excoffier & Lischer 2010). O potencial das unidades sociais encontradas refletirem uma possível estrutura genética foi examinado através do algoritmo de agrupamento Bayesiano implementado no programa fastSTRUCTURE (Raj et al. 2014). Para determinar o número mais provável de agrupamentos, utilizou-se a medida de complexidade do modelo ideal ($K^*\varepsilon$) e o número de componentes de modelos relevante ($K^*{}_{\emptyset}{}^{C}$) (Raj et al. 2014). Além disso, em caso de discrepância entre esses números, comparou-se com o menor erro de validação cruzada (erro CV) no programa ADMIXTURE (Alexander et al. 2009). Adicionalmente, uma análise de componentes principais (ACP) também foi realizada de forma a comparar a topologia da similaridade genética dos indivíduos com a topologia da rede social. Por fim, a diferenciação genética entre as unidades sociais foi investigada computando valores emparelhados de $F_{ST}$ no ARLEQUIN 3.5, com sua significância estimada através de 10.000 permutações.

Para avaliação do nicho isotópico dos botos, isótopos de carbono e nitrogênio foram utilizados. O procedimento laboratorial para isto encontra-se descrito em detalhes no anexo II. Para entender a contribuição das presas para o sinal isotópico dos botos, foram utilizados modelos de mistura Bayesianos (Layman et al. 2012) através do pacote *simmr* no programa R (Parnell 2016). Para verificar a significância das possíveis variáveis responsáveis por alterações no sinal isotópico dos golfinhos, foram usados modelos lineares generalizados (GLMs). As variáveis testadas foram: unidade social; preferência por estuário ou zona costeira; sexo; e estação quente (novembro – abril) ou fria (maio – outubro), atribuindo as amostras às estações considerando a taxa de recâmbio da pele reportado em Giménez et al. (2016). Além disso, fizemos um GLM separado, incluindo dois períodos (2009 – 2012 e 2013 – 2016) e restrito para os botos da PLE (que são residentes), para investigar se houve alteração significativa de nicho isotópico na área durante o período de estudo. Em ambos os casos, o melhor modelo foi selecionado através do menor valor de AIC (critério de informação de Akaike). Os nichos isotópicos e suas métricas foram calculados através de elipses multivariadas estimadas por inferência Bayesiana corrigidas para pequenos tamanhos amostrais. Estas análises foram feitas no pacote *SIBER* (Jackson et al. 2011), no software R.

## 1.5 ***RESULTADOS***

Entre agosto de 2005 e dezembro de 2015 foram realizadas 354 saídas de campo, onde 2.233 grupos foram encontrados e 87.811 fotos foram analisadas, resultando na foto-identificação de 217 indivíduos. Após o tratamento dos dados, foram utilizadas 318 saídas de campo e 51.920 fotografias de boa qualidade de 1.792 grupos, resultando na identificação de 102 botos com marcas de longa duração evidentes em suas nadadeiras dorsais. Também foram coletadas 154 amostras de pele, das quais 49 foram utilizadas nas análises de genética e 40 nas análises de isótopos estáveis e correspondem a indivíduos analisados quanto a estrutura social e utilizadas no anexo II.

### *1.5.1 Anexo I*

Os botos que utilizam o estuário da Lagoa dos Patos e águas costeiras adjacentes são socialmente bem diferenciados, visto que o coeficiente de variação do índice de associação verdadeiro, utilizando-se do método de verossimilhança, foi relativamente alto ($S = 0.891 \pm 0.015$). A correlação entre o índice de associação verdadeiro e sua estimativa ($r = 0.642 \pm 0.020$) indicou que o banco de dados utilizado teve um bom poder para representar o sistema social destes indivíduos. O sistema social tem influência de gregarismo, visto que o desvio padrão do ‘tamanho típico de grupo’ foi maior do que o esperado ao acaso (real = 0,89, aleatório = 0,74, p = 0,001). Por isso, a representação relativa ao índice de associação foi corrigida para gregarismo. A modularidade foi significativa ($Q_{max} = 0,36$), indicando a presença de quatro unidades sociais, uma fortemente associada ao estuário (PLE), uma na área sul (SC), outra na área norte (NC), e por fim uma unidade social menor e transicional, formada por indivíduos de diferentes áreas (GR4). Já para a análise de afiliações, o MRQAP indicou que os fatores que mais influenciam o índice de associação desta população são: o padrão de uso espaço temporal dos indivíduos e o gregarismo. Com isso, os GAIs foram construídos excluindo estas variáveis. A modularidade para esta análise também foi significativa ($Q_{max} = 0,32$), diferindo da análise anterior por indicar quatro subdivisões na unidade social que utiliza o estuário. Isto indica que o componente espaço temporal no índice de associação mascarava estas relações mais fortes dentro da unidade PLE, evidenciando a importância do uso dessa correção.

### *1.5.2 Anexo II*

O processo de filtragem por bioinformática resultou em 2.942 SNPs que foram utilizados nas análises genéticas da população de botos. Tanto a análise de agrupamento Bayesiano (K*ε) quanto o erro de CV (0.48) indicaram a presença de duas populações geneticamente distintas utilizando a área. Uma população corresponde aos botos da unidade social PLE, que vem sendo estudada ao longo dos últimos anos. A outra população é composta pelos golfinhos costeiros, representados pelas unidades sociais SC e NC, indicados pelo anexo I. O valor de $F_{ST}$ (0.54) corrobora com esta divisão, indicando uma diferenciação genética moderada (valores < 0.1), mas significativa (P < 0.0001). Além disso, os valores de $F_{ST}$ também foram significativos entre as unidades sociais, indicando que, embora exista uma diferenciação genérica entre as unidades SC e NC, seu comportamento social e de uso de área reduz o fluxo gênico. Esses valores foram maiores entre as unidades PLE e NC, seguido da PLE e SC e, finalmente, SC e NC, o que corresponde ao grau de proximidade social relatada no anexo I. Os valores de isótopos estáveis variaram significativamente de acordo com a estação (quente e fria) e entre ambientes (botos de estuário vs. costa). Além disso, o teste entre períodos (2009 – 2012 e 2013 – 2016) mostrou que houve mudança de nicho dos botos da PLE entre um período e outro. As presas utilizadas descreveram bem a composição isotópica dos golfinhos da população PLE, mas ficou evidente a ausência de presas para explicar melhor o sinal isotópico dos botos da população costeira (SC e NC). Os botos da PLE apresentaram um nicho isotópico mais amplo do que os botos costeiros. Porém, estes últimos parecem ocupar uma posição trófica superior, com valores isotópicos de nitrogênio mais enriquecidos em $\delta^{15}N$. Devido ao baixo número de amostras coletadas no período frio para os botos costeiros (apenas 2 para unidade SC) não foi possível fazer uma comparação entre unidades neste período, mas os resultados com base apenas no período quente são evidentes. Por fim, ficou clara a forte influência de uso de habitat na estrutura desta população.

## 1.6 *CONCLUSÕES*

- As populações de botos que utilizam o estuário da Lagoa dos Patos (PLE) e águas costeiras adjacentes (SC e NC) estão estruturados em diferentes unidades sociais com um forte componente espaço-temporal e de gregarismo;

- A presença de duas populações é um viés importante na estrutura social descrita no anexo I;

- As análises de estrutura genética indicam a presença de duas populações na área de estudo: uma associada ao estuário e outra à zona costeira adjacente;

- Em termos puramente sociais, a população do PLE possuí quatro unidades sociais e a população da zona costeira, duas;

- Existe diferenciação genética fraca mas significativa entre os botos das unidades sociais PLE, SC e NC;

- Os botos da população PLE ocupam um nicho isotópico diferente dos botos costeiros;

- Os botos da população PLE se alimentam preferencialmente no estuário;

- As unidades sociais encontradas acumulam características diferenciadas quanto à composição genética, ecologia alimentar e interações sociais que são governadas por diferenciação no uso de habitat por parte de seus indivíduos;

## 1.7 *RECOMENDAÇÕES*

- Investigar a estrutura social da população do estuário, sem a influência dos indivíduos da população costeira;

- Analisar se o estado reprodutivo das fêmeas da população PLE influencia em suas relações sociais;

- Verificar se há variação temporal em curto e longo prazo no padrão de uso do habitat pela população estuarina e se o padrão é similar entre unidades sociais;

- Ampliar o conhecimento a respeito do padrão de movimentação dos botos da população costeira, com o uso de novas tecnologias (e.g. transmissores satelitais);

- Determinar se os indivíduos da unidade social SC representam uma conexão genética entre a população PLE e a população que vive no Uruguai;

- Investigar se as relações de parentesco promovem associações mais fortes e a formação das unidades sociais da população PLE;

- Agregar informações oriundas de outros marcadores moleculares no estudo da estrutura populacional;

- Futuros estudos de ecologia alimentar utilizando análises de isótopos estáveis devem priorizar amostras coletadas num curto período para minimizar os efeitos da variabilidade temporal da paisagem isotópica e do deslocamento dos organismos (presas e predador) entre áreas com valores isotópicos distintos;

- Comparar o nicho isotópico dos botos que vivem no Uruguai com os botos que vivem na zona costeira para ambos os períodos: quando estão separados, de novembro a abril, e quando estão no mesmo local, maio a outubro;

- Futuros estudos e ações de conservação destes botos que utilizam o estuário da Lagoa dos Patos e águas costeiras adjacentes devem considerar a existência de duas populações.

## 1.8 ***REFERÊNCIAS***

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# 2 ANEXO II

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ORIGINAL RESEARCH

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## Spatiotemporal use predicts social partitioning of bottlenose dolphins with strong home range overlap

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### Abstract

Ranging behaviour and temporal patterns of individuals are known to be fundamental sources of variation in social networks. Spatiotemporal dynamics can both provide and inhibit opportunities for individuals to associate, and should therefore be considered in social analysis. This study investigated the social structure of a Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*) population, which shows different spatiotemporal patterns of use and gregariousness between individuals. For this, we constructed an initial social network using association indices corrected for gregariousness and then uncovered affiliations from this social network using generalized affiliation indices. The association-based social network strongly supported that this dolphin population consists of four social units highly correlated to spatiotemporal use patterns. Excluding the effects of gregariousness and spatiotemporal patterns, the affiliation-based social network suggested an additional two social units. Although the affiliation-based social units shared a large part of their core areas, space and/or time use by individuals of the different units were generally distinct. Four of the units were strongly associated with both estuarine and shallow coastal areas, while the other two units were restricted to shallow coastal waters to the south (SC) and north of the estuary (NC), respectively. Interactions between individuals of different social units also occurred, but dolphins from the NC were relatively more isolated and mainly connected to SC dolphins. From a conservation management perspective, it is recommended that information about the dolphin social units should be incorporated in modeling intrapopulation dynamics and viability, as well as for investigating patterns of gene flow among them.

KEYWORDS
affiliation, gregariousness, social division, social group, spatial distribution, spatiotemporal dynamics

## 1 | INTRODUCTION

Social structure is a synthesis of the nature, quality, and patterning of the relationships among members of a population (Hinde, 1976). Therefore, the way that a population is structured is a key component of its biology, genetics and spatiotemporal dynamics, representing an important factor in management and conservation of wildlife (Whitehead, 2008a). Regarding social organization, individuals can associate with either the same or with several different individuals over time. In mammals, stable groups are usually observed in matrilineal societies (Whitehead, 2003), whereas in fission–fusion societies wide variation in group size and/or composition is usually observed, along with temporal variation in spatial cohesion (Aureli et al., 2008). Fission–fusion social dynamics are commonly found in some societies of primates (van Schaik, 1999), dolphins (Connor, Wells, Mann, & Read, 2000), bats (Kerth, Ebert, & Schmidtke, 2006), and elephants (Wittemyer, Douglas-Hamilton, & Getz, 2005).

Although there is much fluidity in the individual associations within populations governed by fission–fusion dynamics, on a fine-scale these populations can be structured into social units (Best, Seddon, Dwyer, & Goldizen, 2013; Karczmarski, Würsig, Gailey, Larson, & Vanderlip, 2005; Urian, Hofmann, Wells, & Read, 2009). Social segregation of individuals may be related to common biological and behavioral factors such as sex, age, feeding strategy, behavior, habitat use, or preferential/avoided companions (Krause & Ruxton, 2002). Therefore, social units usually arise when some individuals of a population are largely behaviorally self-contained, interacting more with each other than with others, sharing a similar living space, and generally use this space at the same time (Whitehead, 2008a). These imply that in a population with social units, individuals can present different spatiotemporal use patterns. The challenge when describing this kind of social system is thus to define an appropriate spatiotemporal scale within which the social patterns can be adequately described (Cantor et al, 2012).

Most studies about social networks of nonhuman populations have been based on matrices of association indices, which estimates the proportion of time pairs of individuals stay associated, and these are used to define social units (Whitehead, 2008a). However, to access preferred and avoided dyadic relationships from association data (also called true affiliations), and the structural factors that may affect associations, have been a major challenge for behavioral ecologists (Bejder, Fletcher, & Bräger, 1998; Croft, Madden, Franks, & James, 2011; Godde, Humbert, Côté, Réale, & Whitehead, 2013; Whitehead & James, 2015). These factors can be related, for example, to spatial overlap (Shizuka et al., 2014), temporal overlap (Cantor et al., 2012), gregariousness (Godde et al., 2013), and sex of individuals (Wiszniewski, Lusseau, & Möller, 2010). To deal with multiple structural factors affecting association indices, Whitehead and James (2015) proposed the use of residuals following a multiple regression on the association indices and on structural variables using generalized linear models, which they called generalized affiliation indices (GAIs). Both GAIs and association indices can be used for network analysis to understand the social structure of animals, either at an individual or population level (Croft, James, & Krause, 2008; Farine & Whitehead, 2015).

Bottlenose dolphins, *Tursiops* spp., are cosmopolitan animals that inhabit coastal and oceanic waters of both tropical and temperate regions (Wells & Scott, 1999). Studies around the world, mainly on coastal animals, have demonstrated that fission–fusion social dynamics appear to be the rule for bottlenose dolphins (Connor et al., 2000), although some populations contain stable components (Lusseau et al., 2003; Wells, 2014). Factors that can be associated to the structuring of social units within bottlenose dolphin populations include the association patterns of individuals (Lusseau et al., 2006; Wiszniewski, Allen, & Möller, 2009), ranging patterns (Rossbach & Herzing, 1999; Urian et al., 2009), feeding strategies (Ansmann, Parra, Chilvers, & Lanyon, 2012; Chilvers & Corkeron, 2001; Daura-Jorge, Cantor, Ingram, Lusseau, & Simões-Lopes, 2012; Mann, Stanton, Patterson, Bienenstock, & Singh, 2012), habitat use (Baird et al., 2009; Laska, Speakman, & Fair, 2008), sex (Wiszniewski, Brown, & Möller, 2012), and kinship relationships (Möller, Beheregaray, Allen, & Harcourt, 2006; Möller, Castaing, Salomon, & Lazure, 2001; Parsons et al., 2003).

Bottlenose dolphins from subtropical coastal waters of the western South Atlantic hold unique morphological and genetic characteristics compared to their offshore counterparts (Costa, Rosel, Daura-Jorge, & Simões-Lopes, 2016; Fruet et al., 2017; Wickert, Eye, Oliveira, & Moreno, 2016). These dolphins were recently recognized as a new dolphin subspecies, the Lahille's bottlenose dolphin, *Tursiops truncatus gephyreus* (Committee on Taxonomy, 2017) (although these characteristics have been argued to be indicative of species-level differences by some authors; Wickert et al., 2016). Some populations of the Lahille's bottlenose dolphins have also been proposed as discrete management units, such as in the Patos Lagoon Estuary (PLE) and adjacent coastal waters (Fruet et al., 2014, 2017 ). Recent mark-recapture studies using photo-identification (photo-ID) to individually recognize dolphins through natural marks on their dorsal fins have demonstrated that a small, relatively stable, resident population of ~87 individuals inhabit the sheltered waters of the PLE in southern Brazil (Fruet, Daura-Jorge, Möller, Genoves, & Secchi, 2015a; Fruet, Secchi, Tullio, & Kinas, 2011). It is noteworthy that these studies were restricted to resident individuals using PLE and did not include individuals sighted using adjacent coastal waters. Although this portion of the population has remained stable, the population as a whole has over the years suffered unnatural mortality associated with fishing activities (Fruet et al., 2012), and changed its feeding ecology (Secchi et al., 2016) due to overfishing and habitat degradation (Moraes, Paes, Garcia, Möller, & Vieira, 2012). Studies on spatial use patterns of this population, considering both the PLE and adjacent coastal waters, showed a preference of individuals for waters around the estuary mouth and its vicinities, as well as adjacent shallow (depth ≤ 6 m) coastal waters (Di Tullio, Fruet, & Secchi, 2015; Mattos, Dalla Rosa, & Fruet, 2007). Di Tullio et al. (2015) also found a decrease in dolphin densities in the southern coastal area during warmer months, possibly associated with increased anthropogenic disturbance during this period. However, these studies show spatiotemporal use patterns at the population level, which is unlikely to be enough for effective conservation management of socially structured populations. On an individual scale, preliminary analyses revealed that some individuals appear to not enter estuarine waters. Among dolphins that were never observed inside the estuary, some appear to travel during the colder months from Uruguay to PLE's adjacent southern coast (*ca* 250 km southward; Laporta et al., 2016), while others, tend to use the area immediately to the north of the PLE during warmer months (R.C.G., personal observation).

The objectives of this long-term study on this Lahille's bottlenose dolphin population were to (a) categorize and group individuals according to their patterns of spatial use and temporal fidelity to the area; (b) identify the most adequate analytical method to describe its social structure; and (c) verify the presence of social units and elucidate their role within the population's social network.

## 2 | METHODS

### 2.1 | Study area and data collection

The Patos Lagoon is a large coastal lagoon located between 30°30′S and 32°12′S (*ca* 10,000 km$^2$). It is a subtropical system that receives freshwater input from a drainage basin of about 200,000 km$^2$ in southern Brazil (Möller et al., 2001), and is connected to the Atlantic Ocean by two jetties of about 4 km. Approximately 10% of the area is characterized as an estuary composed of shallow bays (80% of which are <2 m in depth), and a narrow navigation channel that can reach up to 20 m deep. The Patos Lagoon Estuary (PLE) is one of the most productive fishing grounds in Brazil, with abundant assemblages of fish in the estuary and adjacent coastal waters (Garcia, Vieira, Winemiller, Moraes, & Paes, 2012; Rodrigues & Vieira, 2013). Our study area includes the lower part of the PLE and adjacent coastal waters (*ca* 140 km$^2$) (Figure 1a). The area immediately south of the estuary mouth consists of a dissipative beach, with mainly mud and sandy mud originated from the estuarine plume. The beach to the north is characterized as more reflective and with larger particle sizes compared to the south (Figueiredo & Calliari, 2006). For the purpose of survey design and due to some logistical limitations, the area was divided into three subareas: (a) the estuary to the lagoon's mouth (*ca* 40 km$^2$); (b) the estuary's adjacent northern coastal waters; and iii) the estuary's adjacent southern coastal waters. The two coastal areas are ~50 km$^2$ each and are strongly influenced by the surf zone (Figure 1a). Furthermore, due to the characteristics of the area, with a triple intersection of subareas, a transition area was created, mainly to prevent individuals transiting between the coastal areas in front of the estuary mouth to be designated as "sighted in the Estuary". This transition area was defined as a circumference of 1,000 m radius, centered on the median of an imaginary line between the end of the two jetties of the PLE (Figure 1a).

The surveys were conducted between January 2006 and December 2015 onboard a 5 m boat powered with a 90 hp outboard engine, with at least three people on board: a skipper, a photographer, and a note taker. All three were responsible for estimating the minimum (the lower value among them), maximum (highest value among them), and best group size (through a consensus decision). Surveys were restricted to favorable weather conditions (i.e., Beaufort ≤3, good visibility, and swell <2 m). Zig-zag transects were run through the estuary in all sampling occasions (Figure 1a). The coastal areas were initially surveyed through transects perpendicular to the coastline, in order to investigate the width of the population's spatial use patterns on the coast. During these surveys, it was observed that bottlenose dolphins were only rarely found beyond two nautical miles from the shore (Di Tullio et al., 2015). Therefore, after the identification of this core coastal area in February 2012, the southern and northern coastal areas were surveyed with zig-zag transects from the coastline to 1.5 nm offshore for the remainder of the study (Figure 1a). Each survey covered at least one of the three subareas. At least one survey per month was conducted in each subarea, and each of them had two different starting points, closest or farthest from the estuary's mouth (see Figure 1a). These were alternated to diversify the route and reduce possible bias in the data collection due to sampling design.

Dolphins exhibiting spatial cohesion (i.e., within 100 m of each other) and that were engaged in similar activities were defined as a group (Wells, Scott, & Irvine, 1987). Time of sighting, group size, and geographic position (through a GPS) were recorded for each group sighted. In addition, individuals in a group were identified through evident long-lasting marks (cuts and mutilations) and ancillary long-lasting marks (nicks and deformities) in their dorsal fins using standard photo-identification protocols (Urian et al., 2015). Other types of marks (e.g., tooth rakes, skin alterations) which are not long-lasting were only used to assist in estimating the number of individuals in a group. Photographs were taken using a Nikon D300 digital camera equipped with a 300 mm lens. In subsequent analysis, each photograph was graded for quality (Q1–Q3; Wilson, Hammond, & Thompson, 1999). In excellent (Q1) photos, the dorsal fin was clearly visible (completely exposed), on sharp focus, oriented perpendicularly to the photographer and large enough to allow the detection of minor identifiable details. The use of lower quality photos (Q2 and Q3), where the fin is not fully visible, focus is somewhat blurry, and the angle not perpendicular, reduces the efficacy of the use of ancillary marks (e.g., minor cuts and deformities) and increases the probability of misidentification (false positive/negative; Friday, Smith, Stevick, & Allen, 2000). Since this was a systematic study, we chose, besides the use of evident long-lasting marks, to use ancillary marks in

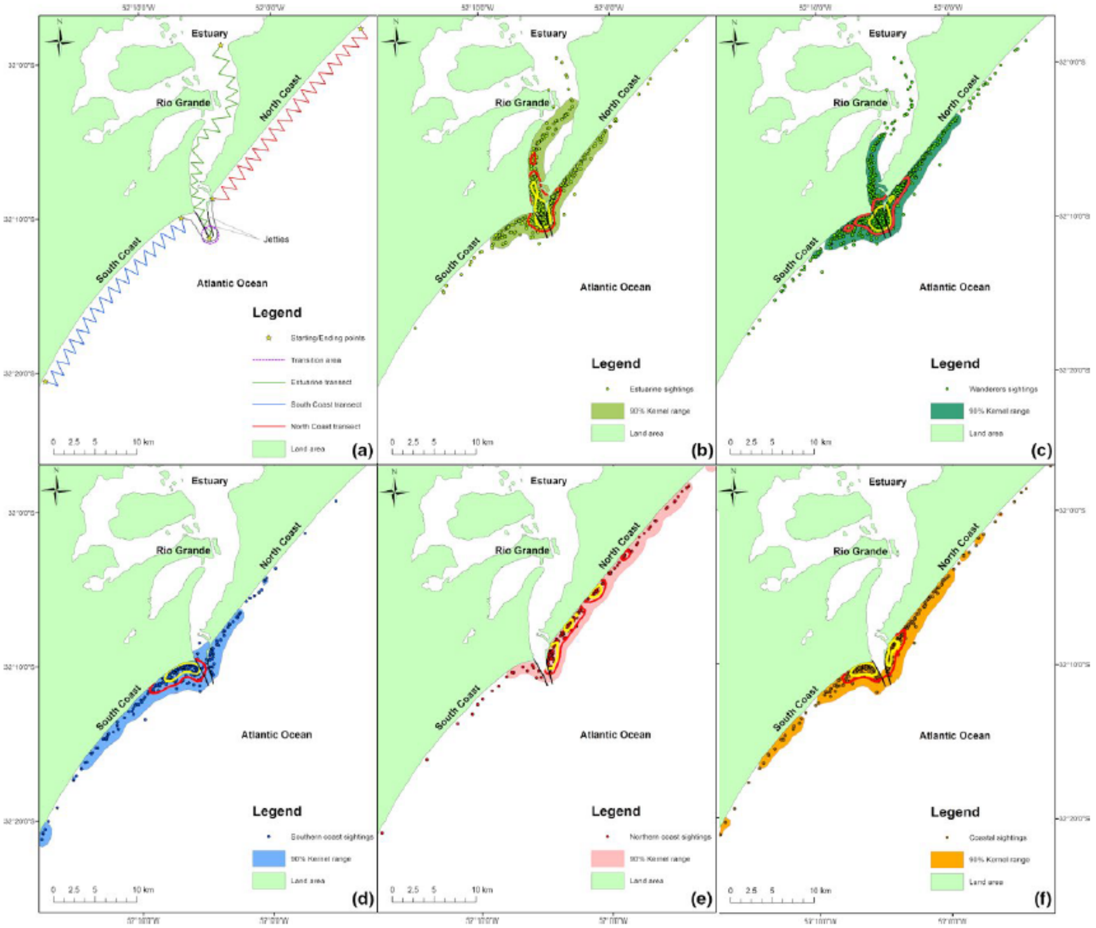

**FIGURE 1** (a) Area covered during boat surveys (sampling occasions) to search for Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) in the Patos Lagoon Estuary (green) and adjacent coastal waters (jetties transect = gray, south = blue and north = red) in southern Brazil. The dotted purple circle in the mouth of the estuary represents the transition area. (b–f) Locations where dolphins (grouped by their spatial preferences) were photographed within the study area are plotted separately, with the 90% (full color), 50% (red line) and 25% (yellow line) kernel isopleths for each group (estuary (b), wanderers (c), south coast (d), north coast (e), and coastal (f) dolphins)

the identification, increasing its reliability and allowing the use of individuals with only one evident long-lasting mark (detailed further). For this reason, only Q1 photographs were considered in further analyses. Finally, two trained and experienced researchers independently identified all individuals "captured" (and "recaptured") in these Q1 photographs, and then compared their results. In divergent events (two different IDs for one individual), both researchers repeated the process, comparing the photograph under analysis with the capture history (whole study period) of the two suggested individuals, until they reached a consensus. These primary data were recorded blindly because groups were photographed randomly, found within a predefined route, and the photo-identification analysis was performed later by the two independent researchers.

## 2.2 | Data treatment

The following analyses were restricted to dolphins with significant long-lasting marks (i.e., at least two evident long-lasting marks [cuts and/or mutilations], or one evident long-lasting mark with at least two ancillary marks [nicks and/or deformities]) (allowing consistent matching between sampling periods), and that were photographed in at least ten sampling occasions, with at least five in the first half of the study (2006–2010) and five in the second half (2011–2015). Dolphins known to have died over the course of the study (i.e., found stranded on the beach) were excluded from analyses. These restrictions were adopted to ensure accurate identification, minimize the effects of sample size, to control for demographic effects and/or to control for the presence of rarely encountered individuals.

Each survey which covered at least all transects of one of the areas (Figure 1a) was defined as a sampling occasion. Calves (e.g., <2 years old) were excluded from analyses as their association patterns cannot be considered independent from that of their mother. Groups where the number of individuals estimated in the laboratory, by photo-id using only Q1 photographs, exceeded the maximum number of individuals estimated in the field (proving control of group size in the field), and groups in which the number of individuals estimated in the laboratory was less than half of the best-estimated group size in the field (consensus decision among observers) were excluded from analysis (Lusseau et al., 2006).

### 2.2.1 | Data classification

**Sex classification**

The sex of individuals was obtained using (a) genetic sex determination from biopsy samples (only adult animals were sampled using modified darts specifically designed for small cetaceans (F. Larsen, Ceta-Dart) fired from a 120-lb draw weight crossbow, which has caused minor physical and behavioral disturbance in this population (see Fruet et al., 2016), following the protocol developed by Gilson, Syvanen, Levine, and Banks (1998); and (b) large dolphins (i.e., >3 m) with a closely associated calf photographed on ≥3 independent sampling occasions were determined as females (Fruet, Genoves, Möller, Botta, & Secchi, 2015b); and (c) large dolphins with several long-lasting marks and scars in the dorsal fin which were first identified as adults in the first year of the study (2006) and never seen in close association with calves were determined as males.

**Area classification**

Each individually identified dolphin was classified as preferring a particular area (estuary—E, southern coast—S, or northern coast—N) based on where it was predominantly found (i.e.,>50% of all sightings in an area and <30% in the other two), excluding the transition area. This restriction on the frequency of sightings in other areas is to prevent an individual from being classified as, for example, an individual who predominantly uses the estuary, when in fact it also uses the southern area at similar frequency (e.g., 51% and 49%, respectively). In the case of coastal dolphins that do not enter the estuary and use only two areas, it was necessary for them to have more than 70% of sightings in one area to be classified as S or N dolphin. If an individual did not match any of these criteria, it was classified as a wanderer dolphin (W) (i.e., use all areas but has no area preference), or a coastal dolphin (C) if the individual did not use the estuary and showed no particular preference to one of the two coastal areas.

**Period classification**

In order to identify transient individuals, the study period was divided into Cold period (May to October) and Warm period (November to April). Dolphins sighted more than 70% of sampling periods (same criterion of two times adopted in the spatial class) in one of these periods were classified as transients (cold or warm) and those dolphins without a period preference as residents.

In order to verify the relevance of these classes as candidates for predictive variables of the GAIs, a Mantel test was conducted using SOCPROG 2.8 (Whitehead, 2009) to test if association indices were significantly higher between dolphins of the same class than between dolphins of other classes (Schnell, Watt, & Douglas, 1985).

## 2.3 | Social analysis

The associations between individuals were based on group membership, such that dolphins present in the same group were assumed to be associated. The half-weight index (HWI; Cairns & Schwager, 1987) was used to measure the intensity of the relationship between pairs of individuals. This index estimates the proportion of time that a given pair remains associated, is symmetric and varies between zero and one. It also enables comparisons between populations, and minimizes possible bias in the sample (e.g., misidentifications); therefore, it has been largely used in cetacean research (e.g., Whitehead, 2008b). The index is defined as: $\mathrm{HWI} = x/(x + yab + 0.5(ya + yb))$, where, $x$ is the number of sampling occasions in which the individuals $a$ and $b$ were observed in the same group; $yab$ is the number of sampling occasions that $a$ and $b$ were identified in different groups; $ya$ and $yb$, respectively, are the number of sampling occasions in which only the individuals $a$ and $b$ were identified. Unfortunately, the HWI does not account for differences in sociality or gregariousness among individuals in the population. Gregariousness exists when some individuals are found in consistently larger, or smaller, groups than others (Whitehead, Bejder, & Ottensmeyer, 2005), and this should be corrected because it can strongly affect the HWI (Godde et al., 2013). Typically, the presence of gregariousness can be tested by the Bejder et al. (1998) modification of the Manly (1995) procedure, which takes into account the standard deviation of the typical group size, which is the group size experienced by individuals (Jarman, 1974). High and significant values of this statistic, compared with those from random data sets, suggest the presence of individuals that are found in consistently larger or smaller groups than that of other individuals. Here, the HWI corrected by gregariousness, referred to as HWIG (Godde et al., 2013) was used. In the HWIG, the HWI between individuals $a$ and $b$ is divided by the sum of the HWIs involving $a$ and the sum of those involving $b$, and multiplied by the sum of all association indices. This correction also changes the index interpretation because it is no longer restricted to between zero and one. A HWIG equals one means that a pair of individuals associate at random; a HWIG lower than one indicates that a pair associate less often than expected, and a HWIG higher than one indicates that a pair associate more often than expected, given their gregariousness (Godde et al., 2013).

Monte Carlo simulations were performed following the methodology proposed by Bejder et al. (1998) and modified by Whitehead et al. (2005), to verify if the associations between individuals of this population occur more frequently than expected by chance, and to find potential significant levels of association (preferred/avoided) between pairs of individuals. The sampling periods were defined as sampling occasions, which corresponded to one day, to avoid the influence of demographic effects during the study period (i.e., births, deaths, immigration, and emigration) (Whitehead & Dufault, 1999). The original matrix of association was randomized until the *p* value stabilized (in our case at 40,000 iterations), with 1,000 flips per permutation. This test suggests long-term preferred companionships when the standard deviation (*SD*) of the real association indices are significantly higher than those expected by chance, whereas if mean of the real association indices is significantly lower than the random mean, this indicates short-term preferred companionships (Whitehead, 2009). To verify if the collected data were sufficient for a good description of the social structure of this population, the social differentiation (*S*) and the correlation coefficient between the true association indices and their estimated values (*r*) were calculated using the methods described by Whitehead (2008b). The social differentiation indicates the variability of the association index within the population: if *S* is near 0, the relationships within the population are homogeneous; if *S* is close to or >1, the associations are highly variable and fewer associations are needed for detecting the preferred companionships (Whitehead, 2008b). The correlation coefficient between the true association indices and the calculated association indices (*r*) is a measure of precision of the representation to describe the social structure (the matrix of the association index) of a population, indicating how close it is to reality. Values of *r* near 1 indicate an excellent representation, whereas values close to 0 indicate a poor representation (Whitehead, 2008b). The standard errors were calculated through 10,000 bootstrap replications. All social and network structure analyses were run in SOCPROG, version 2.8 (Whitehead, 2009).

## 2.4 | Constructing generalized affiliation indices (GAIS)

The GAIs were constructed using the half-weight index (with gregariousness entered as one of the predictor measures) with a binomial model. The significance of the predictor variables was examined using the multiple regression quadratic assignment procedure (MRQAP). This test considers whether each of the predictor matrices, controlling for the presence of the other predictors, makes a significant contribution toward explaining the matrix of association indices. The MRQAP was performed with 20,000 permutations (using the "double-semi-partialing" technique of Dekker, Krackhardt, and Snijders 2007), and the effective contribution of each predictor was measured by the partial correlation coefficients. To identify particularly large positive or negative affiliations (greater/smaller than ±2.5; Whitehead and James 2015), the residuals of this procedure were transformed into Anscombe residuals (Pierce & Schafer, 1986). The calculated prediction measures were as follows:

### 2.4.1 | Gregariousness

Differently of the correction made in the HWI, gregariousness as a predictor variable was calculated following Whitehead and James's (2015) correction, where the gregariousness predictor between two individuals (*a* and *b*) is the log of the sum of the association indices involving *a* (except the *ab* index) multiplied by the sum of those involving *b* (except the *ba* index).

### 2.4.2 | Spatial and home range overlap

Individuals using the same area tend to associate more often with each other. To investigate spatial overlap, we calculated the proportion of those months in which both individuals in a pair were identified in the same area (estuary, northern coast, southern coast). Month was chosen as a period because of the survey procedure, which was intended to monitor all areas at least once every month. The home range overlap between pairs of individuals were estimated following the kernel-based utilization distribution overlap index method (Fieberg & Kochanny, 2005), which is implemented in the package AdehabitatHR (Calenge, 2006) for R v 3.4.3 (R Core Team, 2013).

### 2.4.3 | Temporal overlap

Individuals using an area at the same time are more likely to be associated with each other. The study period corresponds to a total of ten years, which equates to 120 months. The temporal overlap was calculated as the sum of months that at least one individual of a pair was identified, divided by the sum of months that both were identified.

### 2.4.4 | Sex, area and period classes

Predictors were calculated for each class that was used in the Mantel tests with the HWIG. For that, it was constructed a $x(\text{attribute class})_{ij}$ matrix for each class, where 1 is given if *i* and *j* have the same attribute and zero if they have a different attribute.

## 2.5 | Detecting social units

The detection of social units was performed through modularity, which is the difference between the proportion of the total associations within clusters and the expected proportion, given the summed associations of the different individuals (Newman, 2004). In order to find the best delineation, Newman (2006) suggests an eigenvector-based method as being generally efficient and this was implemented by SOCPROG and UCINET (Borgatti, Everett, & Freeman, 2002). This method is based on defining a parsimonious division of the individuals, which maximizes the weight and the number of associations within the units and consequently minimizes the associations between them. The modularity coefficient (*Q*) measures the quality of the division, observing if individuals are designated to clusters with many internal connections and few connections with other clusters, indicating a good division when *Q* is greater or equal to 0.3 (Newman & Girvan, 2004). The coefficient *Q* is the sum of all pairs of associations belonging to the same cluster, minus the expected value if the pairs were randomly associated, given the strength of the connection between the individuals. The spring embedding layout was used in NetDraw (Borgatti, 2002) to draw the social network diagram, showing only associations with HWIG > 1.

### 2.6 | Network metrics

Network metrics are statistical measures used to characterize properties of an individual or a network as a whole (Farine & Whitehead, 2015). Three individual-based network statistics, calculated from the weighted network (association matrix), were averaged over and within the social units: (a) strength, which is a measure of gregariousness, and is the sum of the association indices for each individual (Barthélemy, Barrat, Pastor-Satorras, & Vespignani, 2005); (b) the clustering coefficient, which measures how well the partners of an individual are themselves associated (as calculated by Holme, Park, Kim, & Edling, 2007); and (c) affinity, which is higher when individuals are connected to other individuals with high strength (Whitehead, 2009). To verify whether the network structure was influenced by individual association preferences and/or whether association patterns differed significantly between social units, the calculated network metrics for each unit were compared to those of an expected network based on 10,000 permutations (Lusseau, Whitehead, & Gero, 2008).

### 2.7 | Temporal patterns of association

Association indices represent the proportion of time that pairs of individuals were associated, but it does not distinguish whether and when associations were interrupted over a certain period of time. Thus, to assess temporal stability of associations, we calculated the standardized lagged association rate (SLAR) within the disclosed social units using the HWIG. SLAR is the estimated probability that a previously associated pair will be found in association after a given time lag, accounting for the fact that not all individuals within the groups were identified (Whitehead, 1995). We estimated the standard error of SLAR using a Jackknife procedure with 1,000 replications omitting 10 sampling periods each time (Whitehead, 2008b). As a theoretical benchmark, we compared the empirical SLAR with the null expectation, that is, when individuals associate at random (called standardized null association rate: SNAR). Results were plotted in a log-scale of the sampling periods to better visualize decays.

In addition, we fitted four exponential decay models to the observed SLAR to possibly identify patterns in the association decay over time. These models contain parameters that can be interpreted as follows: preferred companions, where pairs of individuals have a preference for associating, which is constant over time; casual acquaintances, where pairs associate for some time, disassociate, and may reassociate; both preferred companions and casual acquaintances present; and two levels of casual acquaintances, where, for example, a stability of a pair changes from a short time scale to a longer one (Whitehead, 2008a). The most parsimonious model was selected based on the lowest value of the quasiAkaike information criterion (QAIC; Whitehead, 2007), with additional support of QAIC weights and likelihood (Burnham & Anderson, 2002).

## 3 | RESULTS

During the study period, a total of 2,014 dolphin groups were encountered across 339 sampling occasions. During these encounters, 85,254 dorsal fin photographs were obtained, of which 51,920 were of Q1 quality, resulting in the identification of 217 individual dolphins. The mean observed group size was similar between the two coastal areas and the transition area, but slightly smaller in the estuary (Table 1). After data treatment for social analysis, 318 sampling occasions were considered; 1,792 groups fulfilled our requirements for inclusion (control of group size and minimum percentage of dolphins photographed in each group), with 102 dolphins used for further analysis based on established criteria. Data on the area classification, period classification and sex of the individuals used for analyses are presented in Supporting information Appendix S1: Table S1 and, for each area class, in Figure 1b–f. The classification of area created was suitable, since there were no cases of individuals who preferred two of the areas other than the coastal areas. In relation to the sexing of individuals, it was possible to determine the sex of 80 individuals (48 females and 32 males; Supporting information Appendix S1: Table S1).

### 3.1 | Social analysis

The coefficient of variation of the true association index using the likelihood method was relatively high ($S = 0.891 \pm 0.015$), indicating a socially well-differentiated population in which the relationships among individuals of the population are not necessarily homogeneous. The correlation between the true association index and the estimated association index ($r = 0.642 \pm 0.020$) indicated that the analysis using association data among individuals had relatively good power to represent

**TABLE 1** Group characteristics of Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) sighted in 339 boat surveys realized between January 2006 and December 2015 in three subareas (Estuary, South, and North) and a transition area, in the Patos Lagoon estuary and adjacent coastal waters in southern Brazil

| Subarea | No. of groups | Mean group size (*SD*) | Minimum and maximum number of individuals | Group size mode |
|---|---|---|---|---|
| Estuary | 515 | 4.63 ± 4.13 | 1–27 | 2 |
| South | 393 | 7.27 ± 5.92 | 1–44 | 4 |
| North | 487 | 6.79 ± 5.08 | 1–29 | 3 |
| Transition area | 619 | 5.79 ± 4.92 | 1–35 | 3 |
| Total | 2014 | 6.02 ± 5.09 | 1–44 | 3 |

the true social system of this dolphin population. The "*SD* of the typical group size" was higher than expected by chance (real = 0.89, random = 0.74, *p*-value = 0.0018). Therefore, the initial network was constructed using the HWIG, to avoid bias from the gregariousness of individuals. The association index among all pairs of individuals had a mean of 1.08 (*SD* = 0.27), with a maximum value of 39.98 (mean = 9.97, *SD* = 9.94). The permutation tests using the HWIG indicated that there is no long-term (between sampling period) preferred companionships ($SD_{real} = 2.01 < SD_{random} = 2.34$ and $CV_{real} = 1.92 < CV_{random} = 2.17$, $p = 0.999$), but the lower proportion of nonzero association indices (real = 0.644, random = 0.705, $p < 0.0001$), which was significant, suggested that some individuals avoid others. Regarding the spatial (estuary, southern coast, northern coast, and nonpreferred area), period (cold, warm, and residents) and sex classification, which were used as covariates, the Mantel tests of these classes indicated that individuals with similar patterns of area use, period, and sex tended to associate more often with each other than with individuals with different patterns ($t > 0$ and $p < 0.0001$ for all three tests). This justifies the use of these classifications as predictors variables in the MRQAP.

## 3.2 | Affiliation indices and predictors of social structure

Multiple regression quadratic assignment tests indicated that gregariousness, spatial overlap, and temporal overlap were useful predictors for explaining patterns of associations in this dolphin population (Table 2), but area class (significant *p*-value ($p = 0.0016$), but with a low partial correlation), home range overlap, sex, and period were removed by the stepwise procedure. Therefore, GAIs were calculated using gregariousness, spatial overlap, and temporal overlap as predictor variables. The GAIs among all pairs of individuals had a mean 0.00 (*SD* = 0.01), with a maximum value of 0.55 (mean = 0.18, *SD* = 0.11). The permutation tests indicated that the mean association rate among all pairs of individuals (real = 0.00251, random = 0.00099, $p < 0.0001$) and the standard deviation (real = 0.038, random = 0.028, $p < 0.0001$) were significantly higher than expected, indicating the presence of long-term preferred associations in the population. Large deviance residuals indicated 88 strongly affiliated associations, and low deviance residuals indicated 48 pairs with strong avoidance. Regarding the use of area classification, there were strong affiliations mostly within individuals of the same area class, and between southern and northern individuals (Figure 2c). Avoidances occurred mostly within wanderers, and between estuary and wanderer individuals (Figure 2d).

**TABLE 2** Efficiency of predictor variables in explaining association indices between Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*), indicated by partial correlation coefficients and results of multiple regression quadratic assignment procedures (MQRAP) tests (10,000 replications)

| Predictor | Partial correlation | MRQAP *p-value* |
|---|---|---|
| Gregariousness | −0.1722 | 0.0000 |
| Temporal overlap | 0.3383 | 0.0000 |
| Spatial overlap | 0.3457 | 0.0000 |
| Home range overlap | 0.0098 | 0.7322 |
| Area class | −0.0788 | 0.0016 |
| Sex class | 0.0255 | 0.1746 |
| Period class | 0.0089 | 0.7712 |

The used predictors are highlighted in bold.

## 3.3 | Detecting social units

Based on the HWIG, the estimated modularity coefficient ($Q_{max} = 0.364$) suggests a reasonable division of the population into social units. The application of Newman's modularity (Newman, 2006) indicated four divisions in the population (Figure 2a), here called GRs units, and these were consistent with our area classification (Supporting information Appendix S1: Table S1). One unit was composed by at least 62 individuals that used the entire study area, though predominantly in the vicinities of the transition area (GR1). Two units were strongly associated with the coastal area; one in the southern coast (GR2) and one in the northern coast (GR3), with at least 15 and 17 dolphins, respectively. The uniqueness of these units is that most of the individuals do not use the inner estuary. The last unit is composed by at least 8 individuals that have preferences for the entire coastal area, but occasionally use the mouth of the estuary (GR4).

Removing spatiotemporal dynamics and gregariousness of the association index using GAIs, the estimated modularity coefficient was similar ($Q_{max} = 0.32$), but instead of four, indicated six divisions (Figure 2b), here called social units (SUs). Although this index suggested a larger number of divisions in the population, the division mainly subdivided and reorganized individuals of the GR1 and GR4 units into four social units (SU1, SU2, SU3, and SU4). This implies that, in a scenario where spatiotemporal influence is excluded, individuals which composes the GR4 unit are no longer considered as important "connectors" between estuarine/wanderers and coastal individuals. The two social units associated with the coastal areas, SU5 and SU6, remained almost unchanged as the GR2 and GR3, respectively, with only three individuals designated to another social unit, and other three from other social units now designated as belonging to the coastal units. The SU6 maintained a clear separation from the other units and strong relationships among its individuals. On the other hand, the SU5, in the affiliation-based diagram, seems to act as "connectors" between coastal and estuarine/wanderer dolphins. In terms of spatial and temporal patterns, the SUs 1, 2, 3, and 4 have almost the same home range and core areas, which correspond to the estuary mouth and coastal waters adjacent to the jetties (Figure 4a, b, c, and d, respectively), and are composed only by resident individuals. The SU5 and SU6 have distinct home ranges, with core areas adjacent to the transition

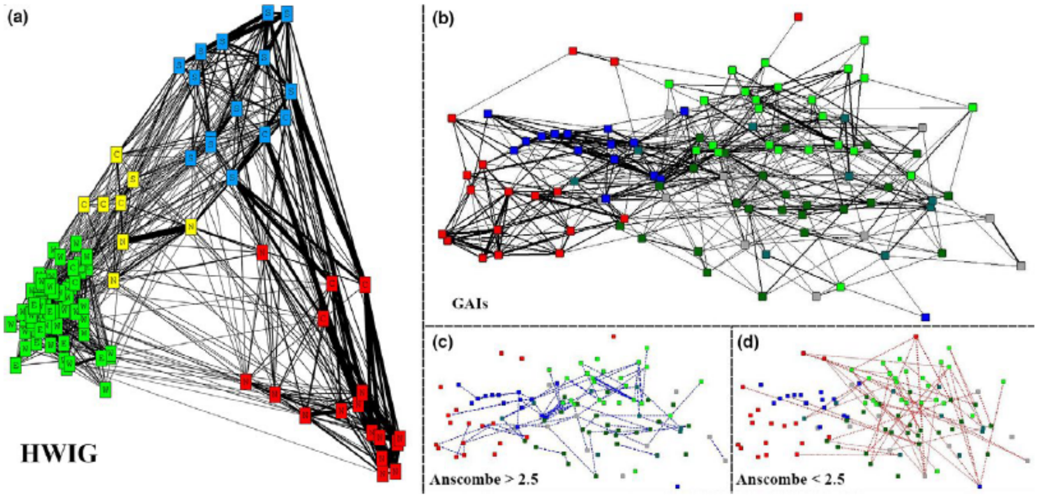

**FIGURE 2** Network diagrams of 102 Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) that use the Patos Lagoon Estuary and adjacent coastal waters in southern Brazil, using the half-weight index corrected for gregariousness (a) and generalized affiliation indices (b). The thickness of the lines connecting each pair of individuals indicates the strength of their associations, and each node corresponds to an individual and their social unit (GR = social units proposed using HWIG; SU = social units proposed using GAIs; green variations = GR1/SUs1–4 individuals, yellow = GR4 individuals, blue = GR2/SU5 individuals, and red = GR3/SU6 individuals). Node labels correspond to the first letter of each spatial class: Wanderers, Estuary, South coast, North coast, and Coastal dolphins. High affiliations (Anscombe residuals > 2.5) and strong avoidance (Anscombe residuals < −2.5) were highlighted in (c) and (d), respectively.

area, but utilizing more the southern and northern coasts, respectively (Figure 4e, f). These units are composed by resident individuals that prefer the coastal areas and those transient individuals mostly found in the Cold or Warm periods. Regarding preferred affiliations in the social units, there were strong affiliations mostly within SU5 and SU6 (Figure 2c). Avoidances occurred mostly between SUs 1–4 individuals (Figure 2d).

### 3.4 | Network metrics between social units

Using the HWIG and its putative units, both social units associated with the coastal area (GR2 and GR3) had similar and higher mean measures of strength, eigenvector centrality, clustering coefficient and affinity, than the overall means (Table 3). On the other hand, the GR1 and GR4, in general, presented lower mean measures than the overall means. Strength and eigenvector centrality measures using GAIs and their proposed units presented very similar results (Table 3). Unfortunately, the clustering coefficient and affinity measures using GAIs presented unreasonable standard errors, diminishing their interpretation. The lower mean strength and high eigenvector centrality in SU6 individuals, compared with the association-based unit (GR3), reflect what is shown in the network diagrams (Figure 2). The strength within the SU6 is strong (mean = 0.94 ± 0.26), but its weaker relationships with the SUs1–4 individuals reduced its overall mean. This higher internal strength, in addition to the relationships with individuals of the SU5, which also have high strength values, explains the higher value of eigenvector centrality in the SU6. Differently to the SU6, the SU5 has more of a connector role inside the network and some individuals also associate with many individuals of the SUs1–4, which in turn have more fluid relationships. This likely explains the lower eigenvector centrality in the SU5.

### 3.5 | Temporal patterns of association

The SLAR for all dolphins combined showed that the probability of recapture of individuals associated over time was low, decayed over time, but was still higher than expected by chance throughout the entire study period (Figure 3a). The error bars were relatively small, indicating the considerable precision of the estimates. The best fitting model consisted of casual acquaintances (Supporting information Appendix S1: Table S2). Despite the low probability of association between pairs, they still associated more often than expected by chance over more than 200 sampling periods (days) later. Considering the units suggested based on the GAIs separately, the SU3 and SU4 presented a similar pattern observed for the population (Figure 3b, c, respectively), differing due to the presence of preferred companions (Supporting information Appendix S1: Table S2). The probability of association between pairs is slightly higher (0.078), compared to the entire population (0.026), and the tendency of the pairs to dissociate is observed after 150 days (Figures 3b, c, respectively). The other social units (SUs, 2, 5, and 6) are composed of

**TABLE 3** Mean strength, eigenvector centrality, clustering coefficient and affinity of individuals of each social unit, proposed using half-weight index correct for gregariousness (HWIG; four GRs units) and generalized affiliation indices (GAIs; six SUs units), of the Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*) population that uses the Patos Lagoon Estuary and adjacent coastal waters in southern Brazil

| Social Unit | Index | No. of ind. | Strength | Eigenvector centrality | Clustering coefficient | Affinity |
|---|---|---|---|---|---|---|
| GR1 | HWIG | 62 | 92.93 (3.39) | 0.03 (0.01) | 0.04 (0.001) | 96.83 (1.92) |
| GR2 | HWIG | 15 | 127.26 (13.27) | 0.11 (0.03) | 0.10 (0.04) | 121.68 (6.59) |
| GR3 | HWIG | 17 | 133.68 (12.11) | 0.20 (0.06) | 0.17 (0.09) | 129.96 (8.93) |
| GR4 | HWIG | 8 | 106.26(9.75) | 0.05 (0.01) | 0.05 (0.01) | 105.88 (3.76) |
| Overall means | HWIG | 102 | **105.82 (19.03)** | **0.07 (0.06)** | **0.07 (0.06)** | **106.72(14.35)** |
| SU1 | GAIs | 9 | 0.12 (0.10) | 0.03 (0.01) | −0.60 (8.38) | −0.90 (5.23) |
| SU2 | GAIs | 10 | −0.14 (0.06) | 0.01 (0.04) | 0.01 (1.26) | 0.17 (3.64) |
| SU3 | GAIs | 24 | 0.18 (0.06) | 0.05 (0.02) | −0.08 (7.50) | −1.33 (6.23) |
| SU4 | GAIs | 25 | 0.20 (0.08) | 0.04 (0.02) | −0.29 (3.16) | −0.64 (7.39) |
| SU5 | GAIs | 16 | 0.81(0.42) | 0.01 (0.03) | −0.12(1.84) | 0.38(4.18) |
| SU6 | GAIs | 18 | 0.23(0.18) | 0.17 (0.07) | −0.22(4.44) | 0.96(2.62) |
| Overall means | GAIs | 102 | **0.25 (0.04)** | **0.06 (0.01)** | **−0.20 (2.12)** | **−0.30 (4.21)** |

| Correlation coefficients | HWIG 4 divisions | GAIs 6 divisions |
|---|---|---|
| Strength by clustering coefficient: | 0.8268 | 0.0613 |
| Strength by affinity: | 0.9743 | 0.0291 |

*Note*. The standard deviation, estimated by bootstrap, is in brackets.
Overall means were highlighted in bold.

a smaller number of individuals, many of them with few sightings (compared with SUs3–4) and, therefore, their results are not presented.

## 4 | DISCUSSION

Using ten years of photo-ID data and social network analyses, this study showed that the Lahille's bottlenose dolphins inhabiting the Patos Lagoon estuary and adjacent coastal waters in southern Brazil show preferred and/or avoided associations and form social units likely driven by their gregariousness, spatiotemporal use patterns and social preferences. This pattern of social relationships and space/time use led to the identification of three major dolphin units or subpopulations based on spatial use patterns: a large unit composed by four affiliation-based social units (SUs1–4) composed by resident individuals which use the entire study area but are mostly found in the estuary mouth and its adjacencies; and two coastal affiliation-based social units (SU5 and SU6) composed by some residents, but with seasonal inputs from transient individuals, which, in general, do not use the inner estuary; one preferentially using the southern area, and the other the northern area. The detection of transient individuals, as well as the differentiated spatiotemporal use of individuals in this population made affiliations (GAIs) the most appropriate method to describe the social network of this population. Overall, this population presented a typical fission–fusion social dynamics, which was predominantly composed of pairs of casual acquaintances that maintained associations over a few days, as well as some long-lasting associations and preferred companionships.

### 4.1 | Ranging behavior

Spatial dynamics are important to consider when examining animal sociality, especially when studying animals which are capable of long-range movements (10s–1,000s of km) in short periods of time (days–months) such as dolphins (Irvine, Scott, Wells, & Kaufmann, 1981; Mate et al., 1995). In our study, we identified social units composed by individuals that: (a) use the entire study area but mainly concentrate around the estuary mouth; (b) use mostly the inner estuary area but also use the coastal area; (c) use the entire coastal area; and (d) use mostly the coastal area north or south to the estuary mouth. This differentiated use of areas was reflected in the structure revealed by the association-based (HWIG) network (Figure 2), which does not control for the effect of spatial overlap. This bias, by itself, justifies the use of GAIs to understand the true affiliations of this population. However, even with distinct spatial use, the core areas of the coastal units are very close to the estuary mouth, resulting in high spatial overlap between all units (Figure 4). Because of this high spatial overlap, we tested the frequency of occurrence of pairs of individuals in the same area as a predictor measure of "spatial overlap", which proved to explain better the social network of this population than the home range overlap itself. The presence of social units that share large parts of their core areas reinforces the importance of the temporal overlap as a predictor variable.

There are some examples of bottlenose dolphin populations where, differently from this study, present social structuring with little or even no core area overlap between units (Louis et al., 2015; Titcomb, O'Corry-Crowe, Hartel, & Mazzoil, 2015; Urian et al., 2009; Wiszniewski et al., 2009). However, a similar pattern of social units with high spatial overlap emerging due to social preferences in other dolphin populations can be seen, for example, in bottlenose dolphins in the east coast of Scotland (Lusseau et al., 2006), and Guiana dolphins in the eastern coast of Brazil (Cantor et al., 2012). The large part of the population which frequently uses the PLE, the SUs1–4, is very well studied in terms of their population parameters and has remained stable over the last decades (Castello & Pinedo, 1977; Dalla Rosa, 1999; Fruet et al., 2011; Fruet, Daura-Jorge, et al., 2015a). The PLE is a protected, highly productive environment (Seeliger & Odebrecht, 2010), which provides favorable environmental conditions throughout the year for these dolphins, particularly for feeding and shelter (Fruet, Daura-Jorge, et al., 2015a; Mattos et al., 2007; Secchi et al., 2016). The fact that the coastal dolphins were not observed to enter this area, with such favorable characteristics, is noteworthy. Intraspecific territoriality, which could explain this kind of behavior and is widely seen in other mammals (e.g., primates, Watts & Mitani, 2001; Williams, Pusey, Carlis, Farms, & Goddall, 2002; carnivores, Heinsohn, 1997; rodents, Gurnell, 1984), is absent in most marine mammal species and has been poorly reported in resident *Tursiops* populations (Pearson, 2011). For some unknown reason, it seems that most of the SUs1–4 and SU6 dolphins avoid using the same area (in the northern coast) at the same time. This became evident on two occasions where we observed that the approach of SU6 dolphins to areas nearby the estuary triggered porpoising of dolphins from SUs1–4 to the estuary area (R. C. Genoves and P. F. Fruet, personal observations).

## 4.2 | Space and time matters

Combining the spatial behavior with the temporal measure, we revealed that spatiotemporal dynamics is a key structural variable in this social network. This is the major difference between

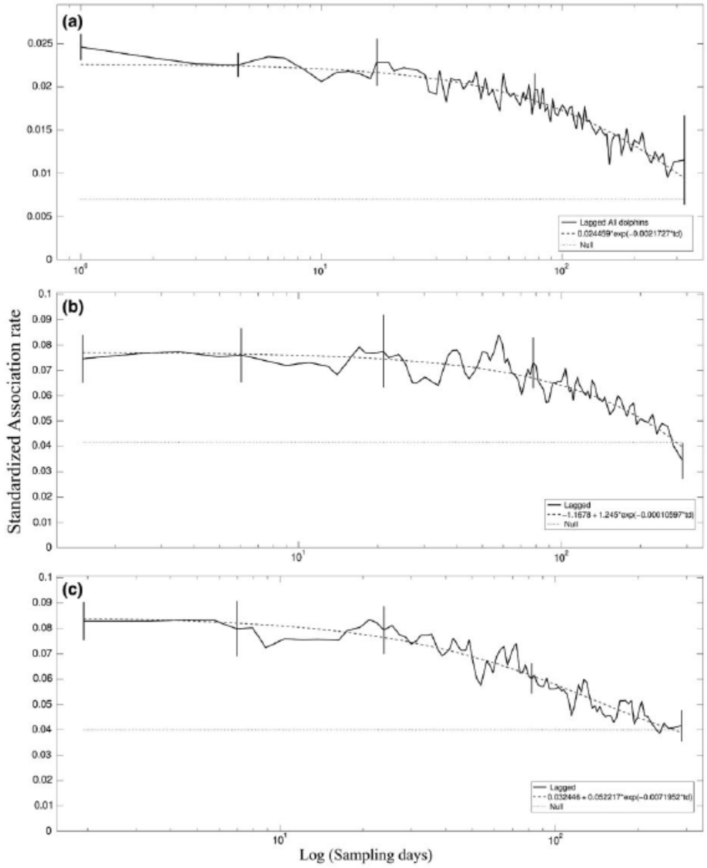

**FIGURE 3** Standardized lagged association rate (solid line) compared to the best fitting model (dashed line) and standardized null association rate (dotted line) for all dolphins (a), within Social Unit 3 (b) and within Social Unit 4 (c) dolphins. Standard error bars (vertical lines) were computed by jackknifing and SLAR curves were smoothed with moving averages of 8,000 (a) and 5,000 (b, c) associations

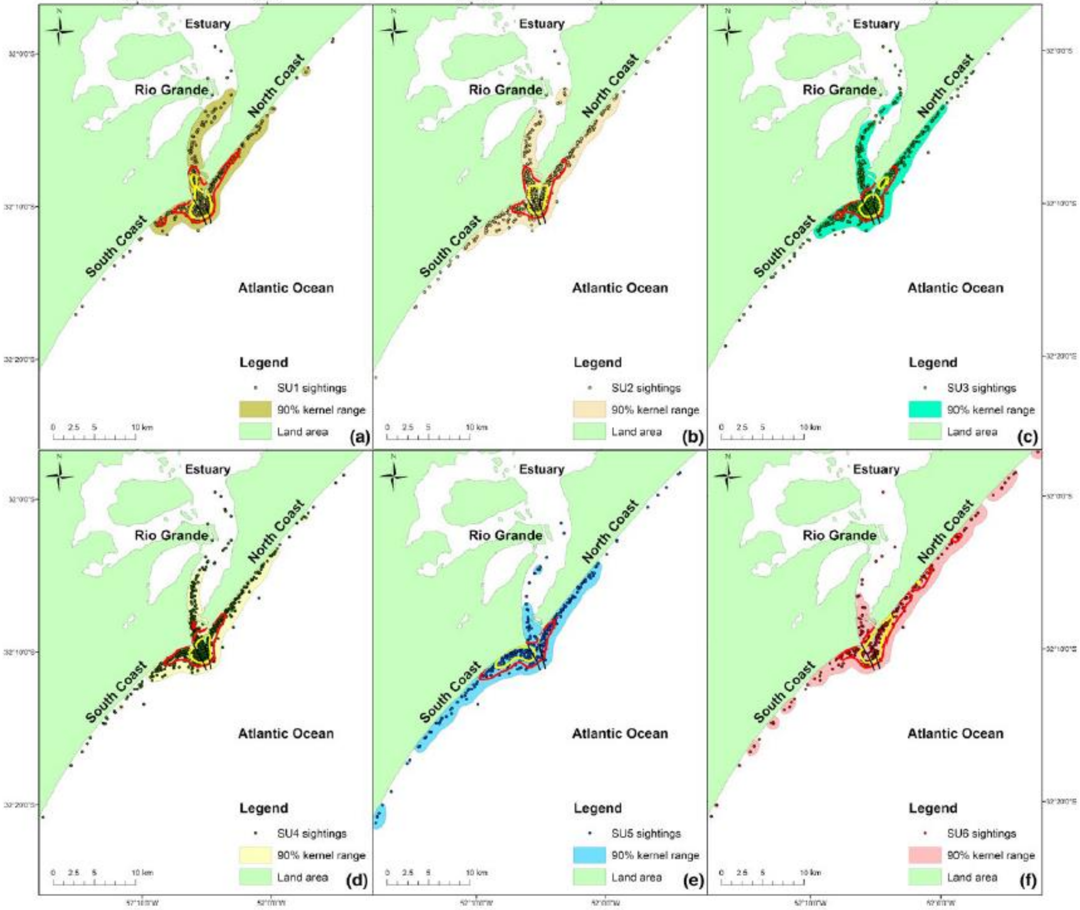

**FIGURE 4** Locations of each social unit of Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*), proposed by community division and modularity based on generalized affiliation indices, with 90% (full color), 50% (red line), and 25% (yellow line) kernel isopleths. (a) Social Unit 1, (b) Social Unit 2, (c) Social Unit 3, (d) Social Unit 4, (e) Social Unit 5, and (f) Social Unit 6

the association-based network, which is biased by spatiotemporal dynamics, and the affiliation-based network structure observed, which exclude this source of bias. It is known that individuals using the same area associate more often (Shizuka et al., 2014) and individuals using the area at the same time are more likely to associate (Cantor et al., 2012). Therefore, the HWIG probably overestimated associations between pairs of individuals of the same GR unit, resulting in a clearer division in the association-based compared to affiliation-based network. In other words, if it were not for the use of GAIs, the social divisions present in dolphins that use the estuary (estuarine and wanderers) would not be detected. Regarding some factors that can potentially affect the temporal patterns, population growth and seasonal variability were identified as the major factors affecting the temporal variability in African and Asian elephant societies (Wittemyer et al., 2005; de Silva, Ranjeewa, & Kryazhimskiy, 2011, respectively). However, as previously mentioned, this dolphin population appears to have remained stable during the study period. Data treatment was controlled for death and the presence of newly marked individuals, and there were no observations of migration or emigration into the area. Furthermore, the number of transient individuals at each period was very similar, with 8 individuals in the "Cold period" and 11 in the "Warm period," confirming that there was no evidence of demographic effect over the years or between periods.

The temporal analysis considering all individuals showed that associations were nonrandom and characterized by short-term relationships (casual acquaintances), consistent with the presence of social units, which are segregated from each other to a certain degree. Furthermore, permutation and SLAR tests indicated the presence of some long-term associations within the social units of the study population. In cetacean populations governed by fission–fusion dynamics, associations between individuals could range from short-term associations with little or no structure (e.g., *Cephalorhynchus hectori*, Bräger, 1999; *Tursiops* spp., Vermeulen, 2018) to strong long-term sex and/or age-related alliances (e.g., *Tursiops* spp., Wells, 1991; Connor & Heithaus, 1999; Lusseau et al., 2003; *Hyperoodon ampullatus*, Gowans, Whitehead, & Hooker, 2001; *Grampus griseus*, Hartman, Visser, & Hendriks, 2008; *Globicephala macrorhynchus*, Mahaffy, Baird, Mcsweeney, Webster, & Schorr, 2015). This Lahille's bottlenose dolphin population appears to be between these two extremes, exhibiting a complex mix of social stability and change in both space and time. This dynamic is not exclusive to this population and is similar to its "neighbor" Lahille's bottlenose dolphin population, which also presents social units with high spatial overlap but, differently from this population, has a strong influence of social preferences due to feeding specialization (Daura-Jorge et al., 2012). Furthermore, disregarding the comparatively lower spatial overlap between units, it is very similar in terms of habitat specialization, probability of association (0.026–0.022) and temporal pattern (casual acquaintances and constant companions) to the *T. truncatus* population of Normano-Breton Gulf, France (Louis et al., 2015).

### 4.3 | Social network

The connection between social units can occur through a few key individuals. These key individuals, known as brokers (sensu Lusseau & Newman, 2004), form relationships with individuals of different social units and thus can play a crucial role in maintaining the cohesion of the population's social network as a whole. They are important for transferring information at different levels of the population (Rendell & Whitehead, 2001), assisting with gene flow within, but can also potentially lead to the spread of diseases (Frère et al., 2010; Newman, 2002). Considering only the association-based social network (Figure 2a), the GR4 individuals appeared to act as brokers in this population. However, the affiliation-based social network suggests that the SU5 individuals are more important for connecting SU6 dolphins to the SUs1–4 dolphins (Figure 2b). SU5 presented several moderate affiliative relationships with individuals from the other units and showed stable and long-lasting associations with some SU6 dolphins. The reason for this greater social proximity with the SU6 may be due to their greater use of the northern area during the warm period. This behavior increases the opportunities for these individuals to associate and may explain the decrease in the density of individuals that use the southern area during the warm period, as detected by Di Tullio et al (2015). The northern coastal unit showed stable and long-lasting associations mostly between individuals of their own unit, demonstrating that this unit is more socially segregated than the others are to each other in the population.

The modular network configuration of this Lahille's bottlenose dolphin population, structured by social units, is comparable to other fission–fusion societies such as that of Asian elephants, *Elephas maximus* (de Silva et al., 2011), spotted hyenas, *Crocuta crocuta* (Holekamp, Smith, Strelioff, Horn, & Watts, 2012) and Galapagos sea lions, *Zalophus wollebaeki* (Wolf, Mawdsley, Trillmich, & James, 2007), where individuals tend to interact more with each other to cope with environment changes and social pressures. However, the presence of transient individuals in this population resembles the pattern observed in a population of Guiana dolphins from Brazil (Cantor et al., 2012), where social units were composed by long-term resident individuals and others by transient individuals. Although the structure between this Guiana dolphin population and ours is generally similar, an important difference is that the transient Guiana dolphins occupied a peripheral position in their network and were more closely and strongly connected among themselves. In our population, the cold period individuals were strongly associated to the southern coast residents, composing the SU5, and the warm period individuals were strongly associated to the northern coast residents, composing the SU6. In addition, dolphins that use the entire area (SUs 1, 2, 3, and 4) are more closely associated to the southern dolphins (SU5) than to the northern coast dolphins (SU6). This scenario suggests that transient cold period dolphins (that include some individuals sighted in Uruguayan waters by Laporta et al. 2016), which associated with SU5 individuals, are more socially connected to SUs1–4 than warm period transient individuals, who are more socially connected to SU6 dolphins. While this pattern can be mainly driven by social preferences, this hypothesis needs to be further explored by longer-term studies including additional sightings of transient individuals. This could be achieved over the next few years but may be enhanced by increasing the survey effort and size of the area sampled in the coastal zone. The lower deviance residuals identified several avoidance relationships, mostly between individuals that use the estuary waters (estuarine and wanderer dolphins). This helps to explain why, even using almost the same area, these individuals compose four social units (SUs1–4). On the other hand, preferred relationships seem to be particularly important for the maintenance of the SU5 and SU6. Network metrics corroborated this, since dolphins that preferentially use the coastal area tend to have stronger relationships among themselves compared to dolphins that use the estuary or the entire area. Dolphins that were observed to use the inner estuary, but also use the coastal area, and those which use the entire study area (without particular area preference) have a greater chance of meeting and associating with other dolphins compared to those that show space use preferences over a smaller area (in relation to the study area; e.g., SU5 and SU6); this could explain the lower values of strength estimated for the SUs1–4. Another important characteristic was the low clustering coefficient (<0.2) for the population as a whole, which was particularly low for the SUs1–4 (Table 3), but similar to the Lahille's neighbor bottlenose dolphin population of Laguna (Daura-Jorge et al., 2012) and an Indo-Pacific botlenose dolphin population of Port Stephens, eastern Australia (Wiszniewski et al., 2009). Clustering coefficients are lower in territorial societies where individuals only associate with their neighbors, who, in turn, may not associate with each other (Whitehead, 2008a), which relates to the segregation by area observed in our study.

Our study on this Lahile's bottlenose dolphin population provides a better understanding of the impact of spatiotemporal dynamics and gregariousness on the patterns of social connections, but there are other structural variables that can also affect the social network. Genetic relatedness between individuals, for example, is a factor that is known to affect associations between individuals in many mammalian societies (e.g., spotted hyaenas, Wahaj et al., 2004; African elephants, *Loxodonta africana*, Archie, Moss, & Alberts, 2006; and Indo-Pacific bottlenose dolphins, Wiszniewski et al., 2010), and should therefore be investigated. While we did not observe distinct feeding techniques in this population, the three subareas of the study show different ecological and physicochemical characteristics so it is possible that there are differences in the feeding ecology of the social units identified here (as observed for bottlenose dolphins of Normano-Breton Gulf; Louis et al., 2018).

## 5 | CONCLUSION

The Lahille's bottlenose dolphin population of the Patos Lagoon estuary and adjacent coast in Southern Brazil has a society which combines the fluid associations of a fission–fusion system with the affiliative structure of six social units and these appear to be mainly driven by social and spatiotemporal patterns. Our results demonstrate that even with high home range overlap, including core areas, individuals can use the same area at different times. This, added to the presence of transient individuals in different seasons (cold and warm), led the generalized affiliations indices to be the best choice to describe this complex social network. Preferred relationships between individuals had an important impact on the social network, increasing the cohesion of individuals in each social unit, particularly in the coastal units. Avoided relationships occurred mostly between resident dolphins, impacting on their subdivision. Transient individuals mostly associated with coastal residents when they were using the same area. Until other structural variables are not tested, the compilation of these results suggests that the social network of this population is mainly governed by social relationships impacted by spatiotemporal use patterns. Future studies including structural variables such as genetic relatedness and "feeding ecology" will contribute toward a better understanding of the drivers of this social structure. We recommend that the social units identified here should be used as a framework for modeling the dynamics and viability of this population, as well as for investigating patterns of gene flow within and between social units.

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## ETHICAL STATEMENT

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. We only sampled adult animals and biopsy sampling procedures followed international guidelines, in accordance with ethical standards and under regional permits (Brazil's SISBIO 24,407-2, issued to P.F.F.). This article does not contain any studies with human participants performed by any of the authors.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Rodrigo C. Genoves contributed substantially to the conception, data collection, analysis, interpretation, and drafting the work; Pedro F. Fruet and Juliana C. Di Tullio contributed substantially to the conception, data collection, drafting, and revising it critically for important intellectual content; Eduardo R. Secchi and Luciana Möller contributed substantially to the conception, drafting, and revising it critically for important intellectual content.

## DATA ACCESSIBILITY

Analysis reported in this article can be reproduced using the data provided in the Dryad Digital Repository: https://doi.org/10.5061/dryad.r8f277f.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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# 3 ANEXO II

*Artigo submetido para o periódico Marine Biology dia 1 de fevereiro de 2019.*

## 3.1 *TITLE - Fine-scale genetic structure in Lahille's bottlenose dolphins (Tursiops truncatus gephyreus) from Southern Brazil is associated with social structure and feeding ecology*

## 3.2 *ABSTRACT*

Social organization, habitat use, resource partitioning, or even individual preferences are important drivers of population genetic differentiation at small geographic scales. A recent study showed that Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) that inhabit the Patos Lagoon estuary and its adjacent southern and northern coastal waters are socially structured in three social units strongly associated to each of these areas, named as PLE, SC and NC, respectively. Here genome-wide data from single-nucleotide polymorphisms (SNPs) and carbon and nitrogen stable isotope data were used to examine population structure and niche partitioning among the three social units. Results from model-based and model-free analyses of population structure supported the delineation of two populations, a result consistent with isotopic niche differentiation that appears strongly driven by habitat use preferences. The populations are represented by dolphins that use the estuary (PLE social unit) and dolphins that inhabit coastal waters (SC and NC social units). We also detected low but significant genetic differentiation among the three social units following a similar pattern as the social structure. The resilience of a population to anthropogenic or ecological disturbances is thought to be positively correlated with genetic diversity and population size. Current conservation actions of bottlenose dolphins at the region are based on investigations of the small and impacted Patos Lagoon population. This study highlights the importance of managing it in association with the coastal population for effective conservation action.

**Keywords:** population genomics; stable isotopes; ecological niche; common bottlenose dolphin

## 3.3 *INTRODUCTION*

Cetaceans are highly mobile and generally live in environments with few or no geographic barriers to dispersal. These characteristics are known to reduce intra-specific genetic differentiation in several populations due high levels of gene flow (Palumbi 1992, Bohonak 1999). However, population genetic studies have shown strong genetic subdivision among populations of several continuously distributed cetacean species, even over small geographic scales where physical barriers to gene flow are absent (Hoelzel 1998, Vachon et al. 2017). These population genetic studies have helped identify mechanisms leading to fine-scale genetic structuring. Social organization, habitat preferences, resource partitioning and behavioral specializations are some of the key drivers shaping patterns of gene flow and population structure in cetaceans (Hoelzel 1998; Möller et al. 2007; Ansmann et al. 2012; Van Cise et al. 2017).

Estuaries and their adjacent regions, where freshwater meets seawater, are among the most productive ecosystems in the world and often host populations of small cetacean species (e.g. *Sotalia guianensis*: Rossi-Santos et al. 2007; *Tursiops truncatus gephyreus*: Simões-Lopes and Fabian 1999; *Tursiops aduncus*: Fury and Harrison 2008; *Tursiops truncatus*: Mazzoil et al. 2008). The unique environmental features of estuaries are known to drive adaptive divergence and genetic differentiation in a range of coastal marine organisms (e.g. Beheregaray and Sunnucks 2001; Watts and Johnson 2004). The estuarine environment differs significantly from its adjacent coastal environment, both in physicochemical properties and in abundance and diversity of prey, providing subsidies for resident dolphin populations and influencing genetic segregation (e.g., Möller et al. 2007). This habitat heterogeneity can be reflected in the behavior of the population, grouping individuals that share the same preference for area, resource, environment and feeding strategy, leading to social preferences (social units) that can also be influenced by individual personality, and favoring population structure (e.g. Wiszniewski et al. 2009; Möller et al. 2011; Daura-Jorge et al. 2012; Ansmann et al. 2014). However, identifying whether social groups are sufficiently segregated to generate genetic differentiation is challenging. This requires the use of a large number of genetic markers capable of detecting fine-scale population structure, such as genome-wide single-nucleotide polymorphisms (SNPs) that have been proven to be powerful for this purpose (Liu et al. 2005, Gaughran et al. 2018)

Bottlenose dolphins, *Tursiops* spp., inhabit estuaries and adjacencies worldwide, usually having small population sizes and showing high degrees of site fidelity to these areas (Wells et al. 1987). This is the case for Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) that inhabit the Patos Lagoon estuary (PLE) and its adjacent coastal waters, in southern Brazil (Fruet et al. 2011; Fruet et al. 2015). A recent study showed that at this region the species is socially structured into different units mainly driven by spatiotemporal use of the area and social gregariousness (Genoves et al. 2018). In an association-based perspective, there is a large social unit of approximately 90 individuals (Fruet et al. 2015) resident in the PLE, and two relatively small units strongly associated with the coastal zone, one in the southern coast (SC) and the other in the northern coast (NC). There are few movements recorded between coasts, with some SC dolphins eventually been sighted in the northern coast and a few NC dolphins been sighted in the southern coast, but both have never been seen in the inner estuary during 15 years of systematic dolphin monitoring. Furthermore, temporary dolphins also compose the coastal units along with residents. The SC unit receives visitors mainly in the cold period (May-October) and the NC in the warm period (November-April), the last coinciding with the period of mating activities and offspring birth (Fruet et al. 2015). This differential use of habitats associated with preferred companions suggests the potential for some degree of genetic structuring within the population, especially because mating activity and offspring births are seasonally well defined in late spring and summer in the area (Fruet et al. 2015). Furthermore, it is likely that the differential habitat usage patterns reflect variation in resource access and utilization that reinforces within-population structuring, and possibly reduces competition among social units.

The genus *Tursiops* is classified as generalist, with individuals within populations showing plasticity in feeding habits according to spatial and temporal patterns of prey availability (e.g. Barros and Wells 1998). This plasticity was also observed in Lahille's bottlenose dolphins from the Patos Lagoon estuary and adjacent marine coast, based on stomach contents and stable isotopes from tooth dentine of stranded individuals ($\delta^{13}C$ and $\delta^{15}N$), with clear seasonal and temporal variations (1977-1980 to 2002-2012) (Secchi et al. 2016). However, this study did not take into account the complex social system of the population and regarded it as a single unit. Therefore, it could not be determined whether differences in feeding ecology were due to individual variation or the sampling of dolphins from different social units.

In recent years, the use of stable isotopes analysis (SIA) of $\delta^{13}C$ and $\delta^{15}N$ to investigate the trophic and spatial ecology of top predators has increased worldwide (see reviews by Hobson 1999; Kelly 2000; Newsome et al. 2010), including for marine mammals in the western South Atlantic (Seyboth et al. 2018). $\delta^{13}C$ is informative of the base of the food chain, and since it does not change markedly between trophic levels (*ca.* 1‰), can reveal spatial patterns of resource utilization, such as inshore versus offshore, or high versus low latitude feeding sites (Hobson et al. 1994). On the other hand, $\delta^{15}N$ varies approximately from 3 to 5‰ between trophic levels and, therefore, it is a useful indicator of trophic position (de Niro & Epstein 1981). For this reason, SIA of both carbon and nitrogen can be excellent tools to investigate preferential area for feeding (estuary or coastal waters), as well as trophic position of each social unit of the Lahille’s bottlenose dolphin population inhabiting the Patos Lagoon and adjacent marine coast. In addition, this estuary present a large interannual variation in productivity due to factors that affect its hydrological regime, mainly related to climatic phenomena (Garcia et al. 2003, 2007, Teixeira-Amaral et al.

2017). Resident individuals using the estuary provide an excellent opportunity to investigate the isotopic niche of these top predators over the years.

Lahille’s bottlenose dolphin populations inhabiting coastal waters of the southwestern Atlantic Ocean are structured into several Management Units (MUs) throughout their range with different degrees of gene flow among them (Fruet et al. 2014). These MUs, on the other hand, show negligible gene flow with the offshore populations of common bottlenose dolphins (*Tursiops trucatus*; Fruet et al. 2017). At a finer spatial scale, whether the social units described by Genoves et al. (2018) are related to genetic structuring has yet to be investigated. Here, we used genome-wide SNPs and SIA of carbon and nitrogen from skin samples of photo-identified, free-ranging, adult Lahille’s bottlenose dolphins to investigate the degree of genetic differentiation and habitat segregation of social units (*sensu* Genoves et al. 2018). Our assessment of population structuring in this system provides an opportunity to understand links between habitat use, feeding preferences, social organization and genetic differentiation in coastal cetaceans. Clarifying these associations is particularly important for resident bottlenose dolphins inhabiting embayments and estuaries since these environments are often under strong and localized anthropogenic pressure.

## 3.4 *MATERIALS AND METHODS*

### *3.4.1 Study area*

The Patos Lagoon Estuary (PLE), located approximately between 31º58’S and 32º12’S, is characterized by shallow bays (< 2 m in depth), a narrow navigation channel that can reach up to 20 m deep, and is connected to the Atlantic Ocean by two jetties of 4.6 and 3.8 km of length. The PLE and its adjacent marine coast is a very productive environment that hosts abundant assemblages of fish (Garcia et al. 2012, Rodrigues & Vieira 2013). The estuary is also an important nursery ground for several fish species that sustain an extensive artisanal and commercial fishery (Haimovici & Cardoso 2017). The area immediately south of the estuary mouth (South Coast -

SC) consists of a dissipative beach composed mostly of sand and mud transported by the estuarine plume. To the north (North Coast - NC) the beach is more reflective, composed of larger sand grains when compared to the south (Figueiredo & Calliari 2006).

### *3.4.2 Assigning dolphins to social units*

Previous social and spatiotemporal analyses highlighted that Lahille's bottlenose dolphins from southern Brazil have different preferences for the three subareas of this study (Genoves et al. 2018). These authors identified three social divisions, based on associations with strong spatial and temporal components, which clustered individuals that preferentially use the same subarea (PLE, SC and NC). Thereby, approximately 65 individuals used the PLE and adjacent coastal waters and 37 individuals regularly used the southern (SC, n=18) and northern coast (NC, n= 19) between 2006 and 2015 (see Genoves et al. 2018). The following analyzes were restricted to these dolphins.

### *3.4.3 Sample collection*

Skin samples were collected from Lahille's bottlenose dolphins via biopsy dart during photo-identification surveys carried out from January 2009 to September 2016 onboard a 5m long inflatable boat powered with a 90 hp outboard engine. Samples were taken in the estuary and the adjacent marine coast (Figure 1). In order to minimize risk of double sampling, biopsies were taken from recognizable individuals (i.e. with evident natural marks on their dorsal fin) that were photo-identified at the time of sampling. Modified darts specifically designed for small cetaceans (F. Larsen, Ceta-Dart) were fired using a 120 lb draw weight crossbow. In order to minimize the wound, only individuals older than three years of age (i.e. independent individuals, see Fruet et al. 2015) were biopsied as they have large body mass and a thick blubber layer (see Fruet et al. 2016). Darts never reached the muscle and collected only skin and fat tissues. Sub-samples for genetic analyses were preserved in 20% dimethylsulfoxide saturated with NaCl and stored at -20°C, and those for stable isotopes analysis (SIA) were frozen.

### *3.4.4 Genomic methods and bioinformatics*

Genomic DNA was extracted from dolphin tissue using a modified salting-out protocol (Sunnucks & Hales 1996). DNA quality was checked using three parameters: 1) purity, using a spectrophotometer (NanoDrop, Thermo Scientific); 2) integrity, using 2% agarose gels; and 3) quantity, using a fluorometer (Qubit, Life Technologies). Double-digest Restriction-site Associated DNA (ddRAD) sequencing libraries were constructed following the protocol of Peterson et al. (2012), with modifications as described in Brauer et al. (2016) and Sandoval-Castillo et al. (2018). Briefly, 300 ng of genomic DNA was digested per sample using the restriction enzymes *SbfI-HF* and *MseI* (New England Biolabs), and one of 96 unique six base pair barcodes was ligated to each individual library. Replicates of five samples were included to estimate sequencing and genotyping errors. Libraries were pooled into groups of 12 samples, and then fragments of between 250-800 bp were selected using a Pippin Prep (Sage Science). Libraries of 96 samples were pooled in equimolar concentrations, and then sequenced on a lane of Illumina HiSeq 2000 (100bp, single-end reads) at the South Australian Health & Medical Research Institute (SAHMRI).

Raw sequences were demultiplexed using the *process_rad-tags* in STACKS 1.19 (Catchen et al. 2013). Then, the dDocent 2.2.19 pipeline (Puritz et al. 2014) was used to remove low quality bases and to construct a *de novo* assembly of putative RAD reads. A Bayesian-based variant detection approach, FREEBAYES (Garrison & Marth 2012) was used to detect putative single nucleotide polymorphisms (SNPs) from the aligned reads of all individuals. After that, a series of data filtering steps were performed to ensure quality, coverage depth and to control for Hardy-Weinberg (HW) disequilibrium and Linkage disequilibrium (LD) of the SNPs in the dataset. Detailed filtering procedures and number of SNPs retained after each step is presented in Table 1.

### 3.4.5 *Genomic diversity and population structure analysis*

Genomic diversity within each unit sample was assessed as mean nucleotide diversity ($\pi$), mean expected heterozygosity (He), and percentage of polymorphic loci using ARLEQUIN 3.5 (Excoffier & Lischer 2010).

The potential for fine-scale population genomic structure based on social division (Genoves et al. 2018) was examined throughout both model-based and model-free approaches. First, we used the Bayesian clustering algorithm implemented in fastSTRUCTURE (Raj et al. 2014). This model-based method assumes that allelic frequencies are in HW equilibrium and assign individuals to one or more groups based on the probability that their genotypes belonging to different populations. Using the simple prior model, ten independent runs for each K value (K tested from 1 to 10) were completed to ensure consistency. The most likely number of clusters (K) was chosen based on the optimal model complexity measure ($K^*\varepsilon$) and the number of relevant model components ($K^*_{\emptyset}{}^{C}$) (Raj et al. 2014). These values should match to the true K when population structure is strong (Raj et al. 2014). If a value greater than 1 was detected for K, it was verified if the genomic division corresponded to the social division of the population. In case of inconsistency between these K measures, the value of K based on the lowest cross-validation error (CV error), from ADMIXTURE (Alexander et al. 2009), was used. Additionally, principal component analysis (PCA) was performed using the R package ADEGENET 2.1 (Jombart & Ahmed 2011). PCA was used to visualize how much the genomic differentiation topology resembles the social network. Genomic differentiation between social units was also investigated by computing pairwise $F_{ST}$ values in ARLEQUIN 3.5, with their significance assessed with 10,000 permutations.

### 3.4.6 *C and N Stable Isotope Analysis*

For the stable isotope analysis (SIA), dolphin skin samples were rinsed with distilled water, dried at 60°C for 48 hours, grounded with a mortar and pestle to obtain a fine powder, and then stored in tin capsules for analyzing the isotopic ratios of C and N. The isotopic ratio (R) of each element ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in each sample, as well as international standards, were calculated in order to obtain individual isotopic composition according to the formula: $\delta^{13}C$ or $\delta^{15}N$ (‰) = ($R_{sample}/R_{standard}$) - 1, where the natural isotope ratios of C and N found in the tissues are related to those found in the standard (VPDB - Vienna Peedee Belemnite limestone - for carbon, and atmospheric air for nitrogen). Analytical precision (SD) was assessed by an analysis of internal reference standards, and was measured to be <0.2‰ for both isotope values.

### *3.4.7 Prey contribution*

Bayesian stable isotope mixing models is a tool used to identify proportional contributions of prey sources to consumer diets using stable isotopic compositions (Parnell et al. 2013). Mixing models require a background knowledge of consumer diet to choose appropriate food sources to fit into the model (Phillips et al. 2005, 2014). The main consumed prey species by dolphins of this population are: the southern kingcroaker, *Menticirrhus* sp. (Msp); the whitemouth croaker, *Micropogonias furnieri* (MF); the lebranche mullet, *Mugil liza* (ML); the banded croaker, *Paralonchurus brasiliensis* (PB); and the cutlassfish, *Trichiurus lepturus* (TL) (Secchi et al. 2016). The isotopic composition of these main preys were extracted from Secchi et al. (2016), supplemented with some samples collected between 2011 and 2015, and processed according to these authors (Table 2). Estimates for trophic discrimination factors for skin samples of common bottlenose dolphins were described by Giménez et al. (2016) and used in the models ($\Delta^{13}C$ = 1.01 ± 0.37‰ and $\Delta^{15}N$ = 1.57 ± 0.52‰). Simulations of mixing polygons (Smith et al. 2013), using packages *sp* and *splancs* in R, were performed to assess the accuracy of the prey dataset to explain the isotopic signal of the dolphins sampled (consumers). Finally, mixing models were run to estimate the contribution of prey samples to the dolphins' diet using the simmr package (Parnell 2016).

### *3.4.8 Stable isotope data analysis*

Variables affecting $\delta^{13}C$ and $\delta^{15}N$ values in the skin of adult Lahille's bottlenose dolphins were analyzed using generalized linear models (GLMs). The explanatory variables considered were based on parameters or behaviors of this population that are able to generate isotopic niche differentiation between groups. They were: i) the three dolphin social units (PLE, SC and NC); ii) dolphins that use the two environment types, estuary (PLE dolphins) and coastal zone (SC and NC combined into a single Coastal unit); iii) sex (males and females); iv) seasons, Cold (May – October) and Warm (November – April). In the latter, samples were attributed to seasons considering the turnover time reported for skin of bottlenose dolphins (i.e., $t_{50\%}$ = 24 and 47 days for carbon and nitrogen, respectively; Gimenez et al. 2016). Considering these turnover rates, the date of each sample was recalculated to three months earlier. This prevents, for example, that a dolphin sampled at the start of a season would be erroneously classified, since its tissue corresponds to the isotopic signal of the previous season. All models were optimized using a forward selection procedure and the model with the lowest AIC (Akaike Information Criterion) was chosen. Furthermore, in order to exclude the influence of sampling size, if the number of samples differed among explanatory variable classes, a GLM with the same number of samples randomly selected was also run.

### *3.4.9 Isotopic Niche of Social Units*

Stable isotope niches of the social groups were estimated using multivariate, ellipse-based metrics through the SIBER package (Stable Isotope Bayesian Ellipses in R, Jackson et al. 2011). The standard ellipse areas corrected for small sample sizes (SEAc) were calculated using individual $\delta^{13}C$ and $\delta^{15}N$ values from the social groups. All analyses were carried out in the R 3.4.3 statistical environment (R Core Team 2017).

## 3.5 *RESULTS*

A total of 148 biopsy samples were collected concomitantly with dorsal fin photographs, corresponding to 99 catalogued and 32 unmarked dolphins. Among the catalogued dolphins, 58 were previously analyzed regarding the social structure (Table 3; Fig. 1). Seventeen dolphins were sampled more than once. Due to storage, extraction or sequencing issues, not all samples were subjected to both genetic and isotopic analyses.

From the Illumina sequencing of 108 individuals, a total of 231,104,429 forward reads and 88,995 raw SNPs were generated, from which 34,495 SNPs were obtained with the dDocent pipeline (Table 1). Four samples were later removed from the dataset because they had more than 15% missing data. The remaining 104 individuals had an average of 6.5% missing data. After filtering with stringent criteria, including for HWE and Linkage disequilibrium, 2,942 SNPs were retained (Table 1). Among the 104 samples, 49 corresponded to photo-identified dolphins that were analyzed regarding the social structure and were subsequently used for population structure analysis.

### *3.5.1 Genomic diversity within social units*

Estimates of genomic diversity based on the 2,942 SNPs in the Patos Lagoon estuary unit (PLE) differed slightly from the Southern (SC) and Northern coast (NC) units, which had similar levels of genomic variation (Table 4). There were more than 80% of polymorphic loci and the mean observed heterozygosity ($H_O$) was higher than the mean expected heterozygosity ($H_E$) in the three social units (Table 4).

### *3.5.2 Population structure*

The Bayesian clustering analysis inferred between two (K*ε) and three (K*$\emptyset^{C}$) genetic clusters (Figure 2) as the most likely number of populations. Cross-validation error from

Admixture indicated two populations (CV error = 0.480): one estuarine, consisting of dolphins from the PLE social unit (PLE population) and another coastal, represented by individuals from SC and NC social units, which exclusively inhabit the coastal zone (CZ population). The PCA analysis also corroborated with this delineation, with only a few individuals showing signs of admixture (Figure 3). Pairwise comparisons of genomic differentiation measured by $F_{ST}$ showed a moderate ($F_{ST}$ values $< 0.1$), significant genomic differentiation ($P < 0.0001$) between the two identified populations in the fastSTRUCTURE and PCA analysis (Table 5). $F_{ST}$ values were also significant between social units, being higher between PLE and NC, followed by PLE and SC and, finally, SC and NC (See Table 5). The $H_O$ was also higher than the $H_E$ in the CZ population (SC and NC together) (Table 4).

### 3.5.3 Isotopic Composition

SIA was applied to skin samples of 40 adult individuals used in the social structure study, represented by 17 females and 23 males. $\delta^{13}C$ and $\delta^{15}N$ values ranged from -15.7‰ to -13.3‰, and from 15.6‰ to 18.4‰, respectively. The mixing polygon approach shows that all the PLE dolphins are within more than 90% of possible mixing polygons, and most of them are surrounded by the main prey (see Figure 4A). For dolphins of the SC and NC units, some individuals are within the limit of the 95% mixing region (the outermost contour), but the model fit was inferior to the PLE dolphins' model (Figure 4B and C).

GLMs showed a significant and important relationship between $\delta^{15}N$ and the environment that is preferentially used by the dolphins (Table 7). Season was also a significant variable for PLE dolphins and nitrogen isotope values, with dolphins presenting higher values of $\delta^{15}N$ in Warm seasons (Table 7). Seasonal influence was not evaluated in coastal dolphins because only two samples of the SC dolphins and none for the NC unit were collected during the Cold season. Finally, similar results were obtained when using the same number of samples for each explanatory variable class, suggesting that there was no influence of sample size on the results.

The relative contribution of prey sources to the diet of PLE dolphins for both seasons and to SC and NC dolphins for the Warm season, are presented in Figure 5. Whitemouth croaker (MF) appears as an important prey for all units, mainly during the Warm season for PLE dolphins. Southern kingcroaker (Msp), banded croaker (PB) and cutlassfish (TL) are also important prey. Lebranche mullet's (ML) contribution to the diet of the coastal dolphins appears negligible, but it seems to be an important complement to the diet of PLE dolphins in the cold months.

The stable isotope Bayesian ellipses (Figure 6) show that the PLE social unit has a wider isotopic niche, and occupies a lower trophic level than the SC and NC units (Figure 6A). Between coastal units, the NC dolphins have the wider isotopic niche. The isotopic niche of the PLE dolphins was more $^{15}$N-enriched in the Warm season (Figure 6B), but not as much as the niche of the coastal dolphins in the same period..

## 3.6 *DISCUSSION*

Different habitat types and niche specializations have been suggested as important drivers of population structure in various cetaceans (Hoelzel et al. 1998, Natoli et al. 2005, Bilgmann et al. 2007, Louis et al. 2014, Pérez-Alvarez et al. 2015), including bottlenose dolphins from the western South Atlantic (Fruet et al. 2017). Despite the capacity for long-distance movements and range overlap of cetaceans, small-scale habitat variation (i.e., an enclosed embayment and its adjacent coast) can also promote extremely localized genetic differentiation (Möller et al. 2007, Hollatz et al. 2011, Ansmann et al. 2014). We found evidence for two genetic populations of Lahille's bottlenose dolphins in southern Brazil primarily associated to differences in habitat use and social structure. This genetic structure occurs over a relatively small geographic area without geographical barriers to dispersal and includes strong spatial overlap among populations, allowing migrants exchange. The differential use of habitat by each population also resulted in distinct isotopic niches, with the coastal dolphins occupying a higher trophic level than those inhabiting the estuary.

### *3.6.1 Fine-scale population structure*

The genomic structure analysis indicated two populations (estuary vs. coastal zone) that show home range overlap in the Patos Lagoon Estuary and adjacent coastal waters, and genetic admixture between them. Regarding the social organization, dolphins that preferentially use the Patos Lagoon estuary (PLE) and those that are restricted to the coastal zone (CZ: Southern coast and Northern coast social units – *sensu* Genoves et al. 2018) composed these two different clusters. Möller et al. (2007) found similar fine-scale structuring among an inshore and two adjacent populations ($F_{ST}$ = 0.066 and 0.073) of Indo-Pacific bottlenose dolphins (*T. aduncus*) inhabiting Port Stephens and its proximal coastal waters, in eastern Australia. In a slightly different environment but over similar spatial scale, Ansmann et al. (2012b) identified two genetic clusters with significant genetic differentiation ($F_{ST}$ = 0.05) in *T. aduncus* inhabiting Moreton Bay, also in eastern Australia. Our study also found evidence for exchange of migrants probably related to individuals that have affinity for more than one social unit. These dolphins, known as brokers (Lusseau and Newman 2004), are individuals that belong to a given unit and are often sighted with some individuals from other units and/or in their areas (see Genoves et al. 2018). The relationships between dolphins of different units tend to be generally weak, which makes these brokers essential for maintaining a social link between units that could translate to gene flow and increased genetic diversity.

The home range of dolphins of the CZ population is still unknown and, given the high mobility of these dolphins, they can overlap with neighboring populations. There are two known neighboring populations (Fruet et al. 2014), one to the south in Uruguay (URU) and another to the north of Patos lagoon (NLP), which have potential to overlap spatially with the CZ population. Fruet et al. (2014), using microsatellite markers, compared the PLE population with the URU and

NLP populations. They found that genetic differentiation between the PLE and the URU population is greater than between the PLE and CZ populations ($F_{ST}$ = 0.101 *versus* $F_{ST}$ = 0.054). On the other hand, the genetic differentiation between PLE and the NLP populations are almost the same as between the PLE and CZ populations ($F_{ST}$ = 0.066 *versus* $F_{ST}$ = 0.054). Apparently, the CZ population is genetically similar to the NLP population and could be a transitional component between the PLE and the population using Uruguayan waters. However, SC temporary dolphins visit the southern coast in the cold season, out of the breeding season, while NC temporary dolphins visit the northern coast in the warm season, the peak of mating activities. The genetic results do not match this description, probably because there are movements not yet described that allow the encounter between SC and PLE dolphins during the warm season.

Two aspects addressed in this study have been reported in the literature as potential factors influencing the genetic diversity of cetacean populations: habitat type and social structure (reviewed by Vachon et al. 2017). Despite the relatively small geographic area (*ca* 140 $km^2$) and large range overlap, the three previously described association-based social units (Genoves et al. 2018) also presented low but significant genetic differentiation. Moreover, the levels of $F_{ST}$ values among units is consistent with patterns observed in the social analyses described by these authors; that is, the NC unit is relatively more segregated, while the PLE unit has more associations with the SC unit. The PCA (Figure 2) and fastSTRUCTURE graphs (Figure 3B), evidence this socio-genetic pattern of higher association between the PLE and SC units, reinforcing the suggestion that SC dolphins could be functioning as mixing agents. The average observed ($H_O$) was higher than expected ($H_E$) heterozygosity and $F_{IS}$ values were negative (data not shown) in both populations, or even between the three social units (**Table**), suggesting that there are excess of heterozygotes and no inbreeding in these populations. Thus, this structuration in two populations with relative weak genetic differentiation and high spatial overlap decreases the chances of inbreeding depression.

### *3.6.2 Resource partitioning*

Stable isotope analysis allowed us to identify resource partitioning in the social units that use the Patos Lagoon estuary and its adjacent coastal waters. Dolphins from the PLE social unit showed more variability in both stable isotopes, and higher values of $\delta^{13}C$ and lower values of $\delta^{15}N$ than dolphins from the coastal units (SC and NC). This differentiation may be related to the prey's life stage and habitat use, as well as the relative contribution of the different preys that were consumed. For example, the whitemouth croaker, the most important prey in terms of frequency of occurrence in stomach contents of bottlenose dolphins in this area (Secchi et al. 2016), remains in sheltered waters of the estuary as juveniles, and then migrate to reproduce in the marine environment as adults (Haimovici & Cardoso 2017). The Patos Lagoon estuary exhibits extreme temporal and spatial variability in physical and chemical processes, mainly salinity (Möller et al. 2001). For instance, Mont'Alverne et al. (2016) provided lab-based evidence of salinity effects on diet-tissue discrimination of the whitemouth croaker, with a higher trophic discrimination factor for carbon isotopes in the marine environment. In addition, Garcia et al. (2007) showed that the isotopic composition of fish found in the estuarine and freshwater regions of the Patos Lagoon differ from each other due to differences in the average isotope values of primary producers of each location ($^{13}C$-depleted carbon signatures in freshwater and $^{13}C$-enriched signatures in the estuary zone). Although the adjacent coastal waters are influenced by the estuary plume (Marques et al. 2009), only main prey of the PLE dolphins are constantly exposed to this oscillation because those from the SC and NC units are restricted to the coastal waters. These relatively broader isotopic values in the PLE dolphins, the stomach content analysis of the populations (Secchi et al. 2016), and the mixing models from this study, seem to be related to their more diversified use of habitat (estuary and coastal zone) and isotopic variation of prey, rather than to differences in prey composition. Although not significant, the small difference between PLE females and males may be related to females feeding more frequently in the inner estuary, since their larger variation in $\delta^{13}C$ could be due to freshwater influence, while males might spend more time feeding in the open coast, or feeding upon higher trophic level prey, increasing its $\delta^{15}N$ values.

The three social units appear to feed on the same prey species, with slight differences in their contribution to the diet of the dolphins in each unit. Whitemouth croaker, southern kingcroaker and banded croaker occupy a similar trophic level, with little variation in $\delta^{13}$C values. Cutlassfish and lebranche mullet, on the other hand, presented, respectively, the lowest mean $\delta^{13}$C and $\delta^{15}$N values, hence differences in the relative consumption of these preys by dolphins from the three social units is probably the reason for their isotopic niche differences. PLE dolphins also probably feed more on lebranche mullet than coastal dolphins. In contrast, coastal dolphins may be feeding more on cutlassfish, a voracious predator that may have more $^{15}$N-enriched stocks in the region, which were not present in the prey dataset used in the mixing models. Given the adjustments of the mixing models, it is likely that the prey dataset comes from the feeding area of the PLE dolphins (estuary), while coastal dolphins, mainly NC dolphins, are feeding upon other fish that are more widespread along the coast, thus reducing the accuracy of the models. The adjacent beaches are very similar in relation to total fish abundance and diversity between seasons, with lower abundance in colder months and higher abundance in warmer months in both beaches (Rodrigues & Vieira 2013). Therefore, PLE dolphins may feed more frequently in the coastal zone in this period of higher productivity. For this reason, in order to increase the accuracy of the models, future studies on the contribution of different prey species to the diet of dolphins from the estuary must collect prey not only inside the estuary, but also in nearby coastal waters.

### *3.6.3 Ecology and Population Structure*

Resource specialization may be an important mechanism whereby cetacean populations differentiate in sympatry and parapatry (Hoelzel 1998). Delphinids (family Delphinidae) are capable of long-range movements (tens to thousands of kilometers) in short periods of time (days to months) (i.e. Irvine et al. 1981; Mate et al. 1995). Bottlenose dolphins are widespread across the globe and occupy a wide variety of environments, showing a high degree of behavioral and ecological plasticity (Connor et al. 2000). There are several reported studies revealing highly specialized foraging techniques, both for capturing specific prey or in cooperation with human activities, resulting in social structure (Chilvers and Corkeron 2001; Krützen et al. 2005; Ansmann et al. 2012; Daura-Jorge et al. 2012). Despite the absence of visually distinct feeding techniques and strong spatial overlap, the stable isotope analysis allowed to identify fine-scale resource partitioning for the social units that use the Patos Lagoon estuary and its adjacent coastal waters. Ansmann et al. (2014) also detected habitat and resource partitioning without apparent feeding specialization among the *T. aduncus* population units of Moreton Bay.

Niche partitioning allows species, or even groups of individuals within a population, to reduce competition and promote co-existence (Pimm & Rosenzweig 1981). Moreover, niche partitioning is considered important for the maintenance of species diversity (Chesson 2000, Levine & HilleRisLambers 2009), as the formation of different social units can be an important driver of genetic differentiation due to reproductive isolation (Möller et al. 2007; Wiszniewski et al. 2009a), including for populations from the Patos Lagoon and its adjacent coastal zone (Beheregaray and Sunnucks 2001). In a larger geographic scale, there are several studies showing segregated spatial and/or habitat type use promoting significant genetic differentiation in dolphin populations (Natoli et al. 2005, Bilgmann et al. 2007, Wiszniewski, Beheregaray, et al. 2009, Louis et al. 2018). However, few populations (i.e., Möller et al. 2007; Ansmann et al. 2012b; Ansmann et al. 2014) display this pattern in such small spatial scales as the one observed in this study. At present, it is impossible to discern how and when this niche partitioning has emerged. Fruet et al. (2014) suggested that the PLE unit potentially acts as a sink, receiving low to moderate number of migrants, while not contributing substantially to other populations. Additionally, our findings highlight the importance of the SC unit both for the social structure of these populations and for gene flow among the social units.

### *3.6.4 Ecology of the PLE during the study period*

Estuaries are very dynamic environments, with large fluctuations in their primary production and trophic chains in each season and over the years (Day et al. 2012). . Teixeira-

Amaral et al. (2017) observed that the mean secondary production at the mouth of the Patos Lagoon estuary was drastically reduced (from 700 mg to 284 C $m^{-3}$ $day^{-1}$) in La Niña years compared to neutral and El Niño years between 2009 and 2013. This natural oscillation can generate interannual variations in the isotopic composition of the organisms inserted in this environment, including dolphins. Throughout the study period, the isotopic signal of the PLE dolphins should have been influenced by this variation, but given the proximity of the adjacent area and the prey dynamics, the coastal dolphins signal can also be influenced. Therefore, besides the warm and cold seasons driving the isotopic signal of dolphins, these events may also have a significant influence on the isotopic niche variation of the PLE dolphin social unit and must be considered in the future. Additionally, within the unit, it is expected that dolphins that are strongly associated exhibit similar patterns of habitat use and feeding behavior, and hence show higher ecological similarities (lower variance in $\delta^{13}C$ and $\delta^{15}N$ values) when compared to dolphins with weaker associations. Testing this hypothesis may help explain factors determining patterns of social sub-structure within the units. Therefore, for such study it is recommended that dolphins be sampled in a short time frame to minimize effects of confounding factors, such as variation in oceanographic and climatic conditions and prey dynamics (e.g. relative abundance, assemblage composition) that may influence stable isotopes values. As the present study found niche partitioning at a finer scale than the previous study conducted by Secchi et al. (2016), it may be that the feeding ecology of these populations is more complex than described to date.

## 3.7 *CONCLUSIONS*

The Lahille’s bottlenose dolphins inhabiting the Patos Lagoon estuary and adjacent coastal waters exhibit genetic structure and isotopic niche partitioning associated with habitat use, foraging patterns and social structure. Genetic and isotopic results appear coupled, in a second order, with social structure between dolphins that preferentially use the estuary and dolphins that only use the coastal zone. The genetic differentiation among social units is consistent with social structure, emphasizing the importance of social relationships in the composition of the population and in the delimitation of management units. It is worth noting that despite the significant range overlap, this genetic differentiation has arisen over a very fine spatial scale. This study shows that the presence of two populations is relevant for future studies and must be preserved through unit-oriented conservation strategies.

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## 3.9 *TABLES*

**Table 1**. Number of SNPs retained after each filtering step for the Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) that use de Patos Lagoon estuary and adjacent coastal waters, southern Brazil.

| **Step** | **SNP count** |
|---|---|
| **Raw SNP catalogue** | 88,995 |

| | |
|---|---|
| **Genotyped in** | |
| 80% of individuals, base quality ≥30, minor allele frequency >0.03 | 34,495 |
| **Sequencing errors, paralogs, multicopy loci and artefacts of library preparation** | |
| (1) Remove indels SNPs | 8,067 |
| (2) Read depth (≤ mean depth + (2 * standard deviation)) | 6,393 |
| (3) Read quality (ratio quality/coverage depth > 0.2) | 5,557 |
| (4) Allele balance (> 20% and < 80%) | 5,370 |
| (5) Hardy–Weinberg equilibrium in at least 2 populations | 4,056 |
| (6) Present in 75% of individuals in 75% of populations | 4,052 |
| (7) Single SNP per locus | 3,047 |
| (8) Linkage disequilibrium ($r^2 < 0.8$) | 2,942 |

**Table 2**. Mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD) from main preys collected between 2011 and 2015 of Lahille’s bottlenose dolphins that use the Patos Lagoon estuary and adjacent coastal waters, southern Brazil. Source: Secchi et al. 2016.

| Prey species | n | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) |
|---|---|---|---|
| | | mean ± SD | mean ± SD |
| *Menticirrhus* sp | 26 | -14.7 ± 0.9 | 15.7 ± 0.4 |
| *Micropogonias furnieri* | 17 | -15.5 ± 0.5 | 15.6 ± 0.6 |
| *Mugil liza* | 18 | -14.8 ± 1.1 | 12.4 ± 0.9 |
| *Paralonchurus brasiliensis* | 26 | -16.3 ± 0.8 | 15.6 ± 0.6 |
| *Trichiurus lepturus* | 15 | -17.0 ± 0.7 | 15.5 ± 0.7 |

**Table 3.** Number of biopsy samples of photo identified Lahille's bottlenose dolphins collected from each social unit (Patos Lagoon estuary – PLE, Southern coast – SC and Northern coast – NC), including sex class proportion (females – F and males – M), used for each analysis.

| SOCIAL UNIT | N GENOMIC | F : M GENOMIC | N ISOTOPES | F : M ISOTOPES |
|---|---|---|---|---|
| **PLE** | 33 | 21 : 12 | 27 | 13 : 14 |
| **SC** | 6 | 1 : 5 | 6 | 1 : 5 |
| **NC** | 10 | 4 : 6 | 7 | 3 : 4 |

**Table 4**. Basic property statistics and estimates of genomic diversity for Lahille's bottlenose dolphins from three social units (Patos Lagoon estuary - PLE, Southern coast – SC and Northern coast - NC) based on 2,942 SNPs. $H_O$ is the observed heterozygosity, $H_E$ is the expected heterozygosity and S.D. the standard deviation.

| STATISTICS | PLE | SC | NC | CZ (SC+NC) |
|---|---|---|---|---|
| **Num. of individuals** | 33 | 6 | 10 | 16 |
| **Num. of usable loci** | 2747 | 2541 | 2700 | 2736 |
| **Num. of polymorphic loci** | 2642 | 2100 | 2400 | 2611 |
| **% of polymorphic loci** | 98.2% | 82.6% | 88.8% | 95.4% |
| **Results for polymorphic loci** | | | | |
| **$H_O$** | 0.3461 | 0.4269 | 0.3651 | 0.3530 |
| **$H_E$** | 0.3044 | 0.3661 | 0.3337 | 0.3243 |
| **S.D.** | 0.22 / 0.16 | 0.22 / 0.13 | 0.20 / 0.14 | 0.19/0.14 |

**Table 5**. Estimates of genomic differentiation (expressed as $F_{ST}$) of Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) based on 2,942 SNPs among the population that use the estuary (PLE) and the population that use the coastal waters (CZ). $F_{ST}$ values between each social

unity (Southen coast –SC and Northern coast –NC) are also presented. $F_{ST}$ values are at the lower matrix and P values are at the upper matrix.

**Populations comparison**

| | PLE | CZ |
|---|---|---|
| **PLE** | **-** | < 0.00001 |
| **CZ** | **0.0538** | **-** |

**Social units comparison**

| | PLE | SC | NC |
|---|---|---|---|
| **PLE** | - | < 0.00001 | < 0.00001 |
| **SC** | 0.0368* | - | < 0.00001 |
| **NC** | 0.0628* | 0.0184* | - |

**Table 6**. Skin $\delta^{13}C$ and $\delta^{15}N$ values (‰) of Lahille's bottlenose dolphins from the three social units that use the Patos Lagoon estuary (PLE) and adjacent coastal waters (Southern coast – SC and Northern coast –NC) in southern Brazil.

| | **2009 - 2016** | | | **COLD SEASON** | | | **WARM SEASON** | | |
|---|---|---|---|---|---|---|---|---|---|
| **UNIT** | **N** | **Δ13C(‰)** | **Δ15N(‰)** | **N** | **Δ15N(‰)** | **Δ15N(‰)** | **N** | **Δ15N(‰)** | **Δ15N(‰)** |
| PLE | 27 | -14.6 ± 0.6 | 16.9 ± 0.6 | 9 | -14.6 ± 0.7 | 16.6 ± 0.6 | 18 | -14.7 ± 0.5 | 17.1 ± 0.5 |
| SC | 6 | -14.8 ± 0.3 | 17.7 ± 0.3 | 2 | -14.5 ± 0.1 | 17.4 ± 0.2 | 4 | -15 ± 0.1 | 17.9 ± 0.2 |
| NC | 7 | -14.7 ± 0.4 | 18 ± 0.3 | 0 | | | 7 | -14.7 ± 0.4 | 18 ± 0.3 |
| Total | 40 | -14.7 ± 0.5 | 17.2 ± 0.6 | 40 | -14.6 ± 0.6 | 17.3 ± 0.6 | 40 | -14.7 ± 0.5 | 17.4 ± 0.6 |

**Table 7**. Results from Generalised Linear Models (GLMs) with environment (PLE population vs. Coastal zone population) and Season (Cold and Warm months) as predictors of the stable isotopes of carbon and nitrogen in skin of Lahille's bottlenose dolphins. Degrees of freedom (df), Akaike Information Criteria (AIC) and the delta AIC (Δi). Best fitting models given in bold and only the significant models were presented ($p < 0.001$).

| | Model | df | AIC | $\Delta_i$ |
|---|---|---|---|---|
| $\delta^{15}N$ | **Environment (PLE vs. CZ) and Season** | **39** | **63.92** | **0.00** |

| | | | |
|---|---|---|---|
| Environment (PLE vs. CZ) | 39 | 70.83 | 6.91 |

## 3.10 *FIGURES*

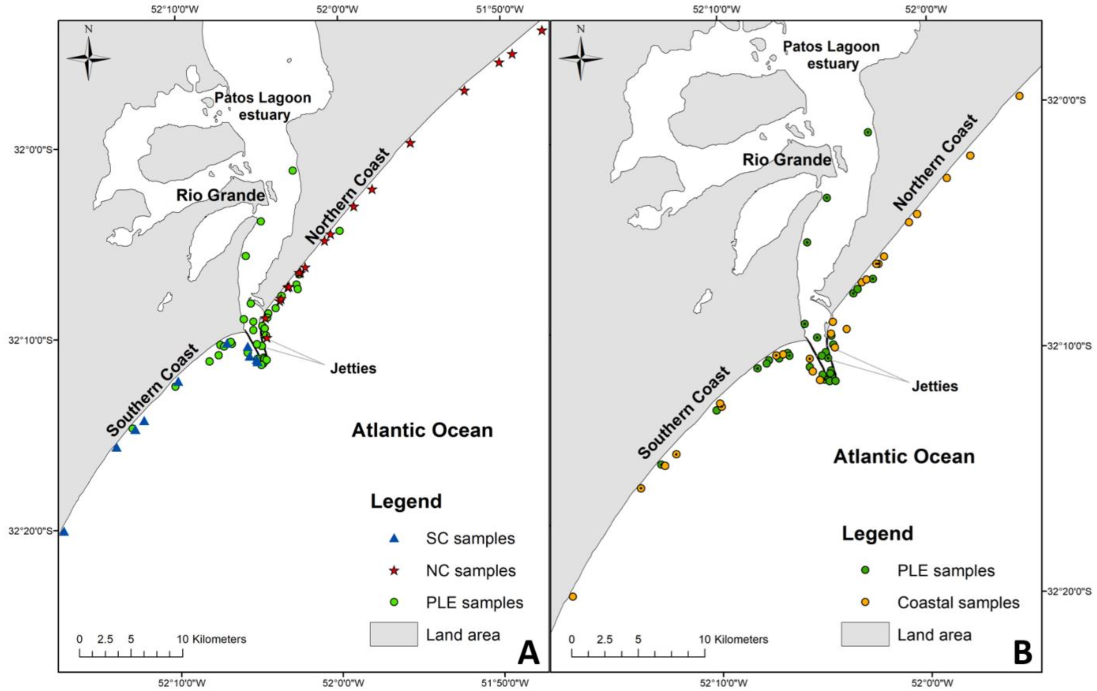

**Figure 1**. Biopsy sample locations of photo identified, adult, Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) in the Patos Lagoon estuary and adjacent coastal waters, southern Brazil, used for: A) genomic analysis (N= 49), specifying social unit memberships of sampled individuals, i.e., Patos Lagoon estuary (PLE – green circles), Southern coast (SC – blue triangles) and Northern coast (NC – red stars); and B) stable isotopes analysis (N= 40), where SC and NC individuals were grouped as Coastal dolphins (orange circles).

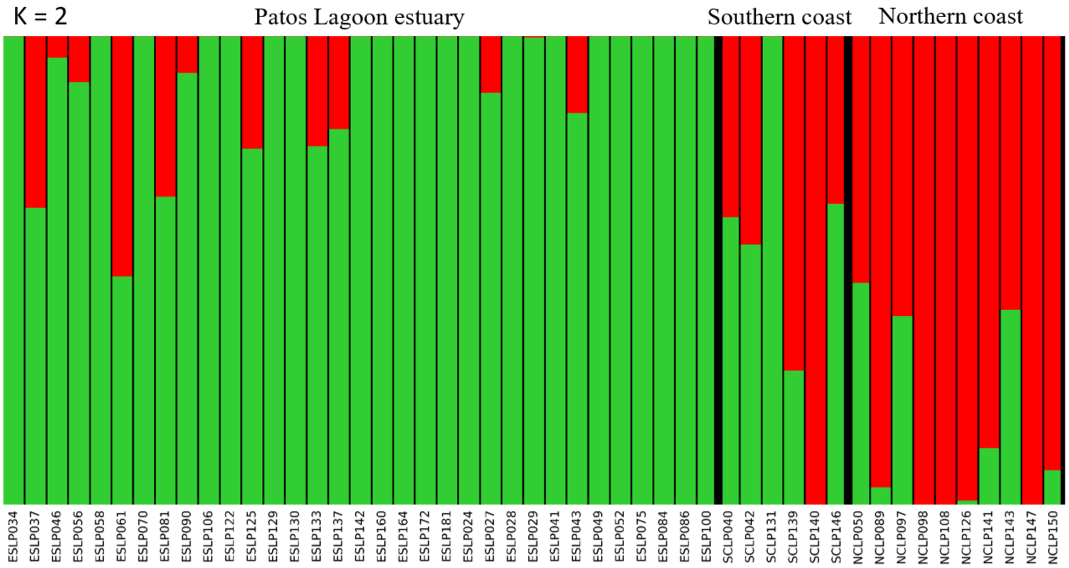

**Figure 2**. Bayesian clustering from fastSTRUCTURE for 49 Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) from three social units sampled in the Patos Lagoon estuary and its adjacent coastal waters, southern Brazil. The most likely number of genetic clusters in the data set was identified as two. Each individual is represented by a vertical column partitioned into two colored segments, with the length proportional to the individual’s estimated membership coefficient.

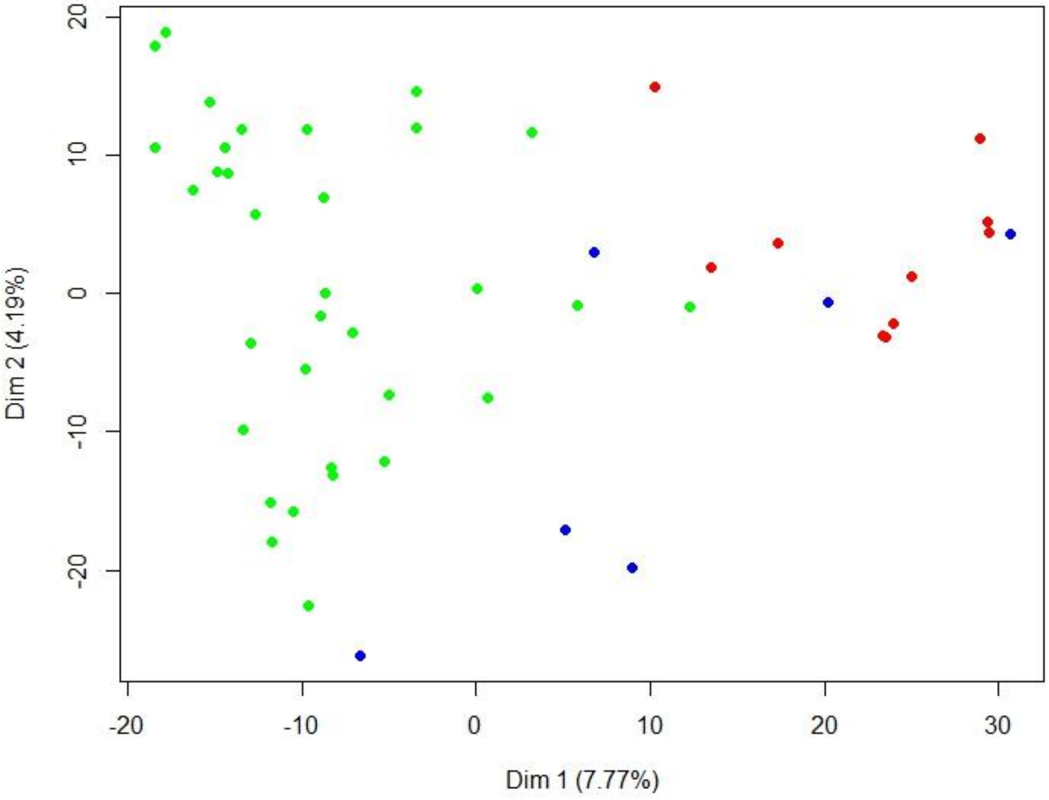

**Figure 3**. Principal Component Analysis based on 2,942 SNPs from 49 Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*). Dots are colored according to the social unit which the individual belong to, i.e., Patos Lagoon estuary (green), Southern coast (blue) and Northern coast (red).

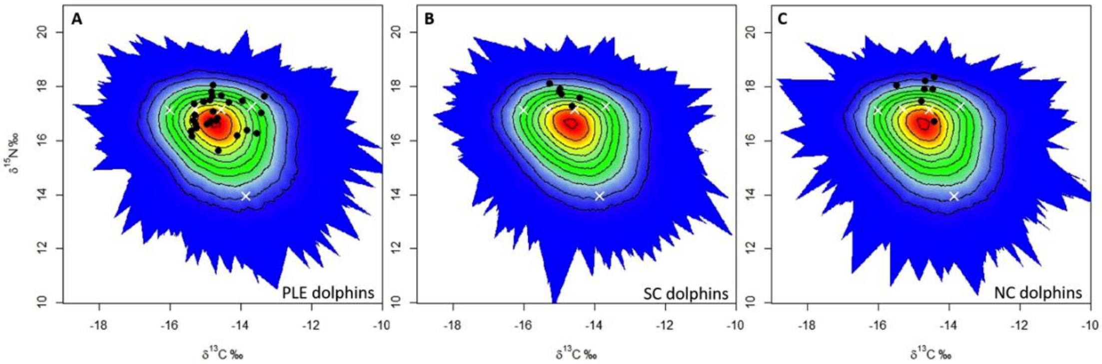

**Figure 4**. Simulated mixing polygons for Lahille's bottlenose dolphins sampled between 2009 and 2016 from A) the Patos Lagoon estuary social unit (PLE); B) the southern coast social unit (SC); and C) the northern coast social unit (NC). A set of trophic discrimination values for correcting prey isotopic values reported in Gimenez et al. (2016) was applied. The position of the consumers (black dots) and the average source signatures (white crosses) are shown. Probability contours (black lines) are at the 5% level (outermost line) and at every 10% level.

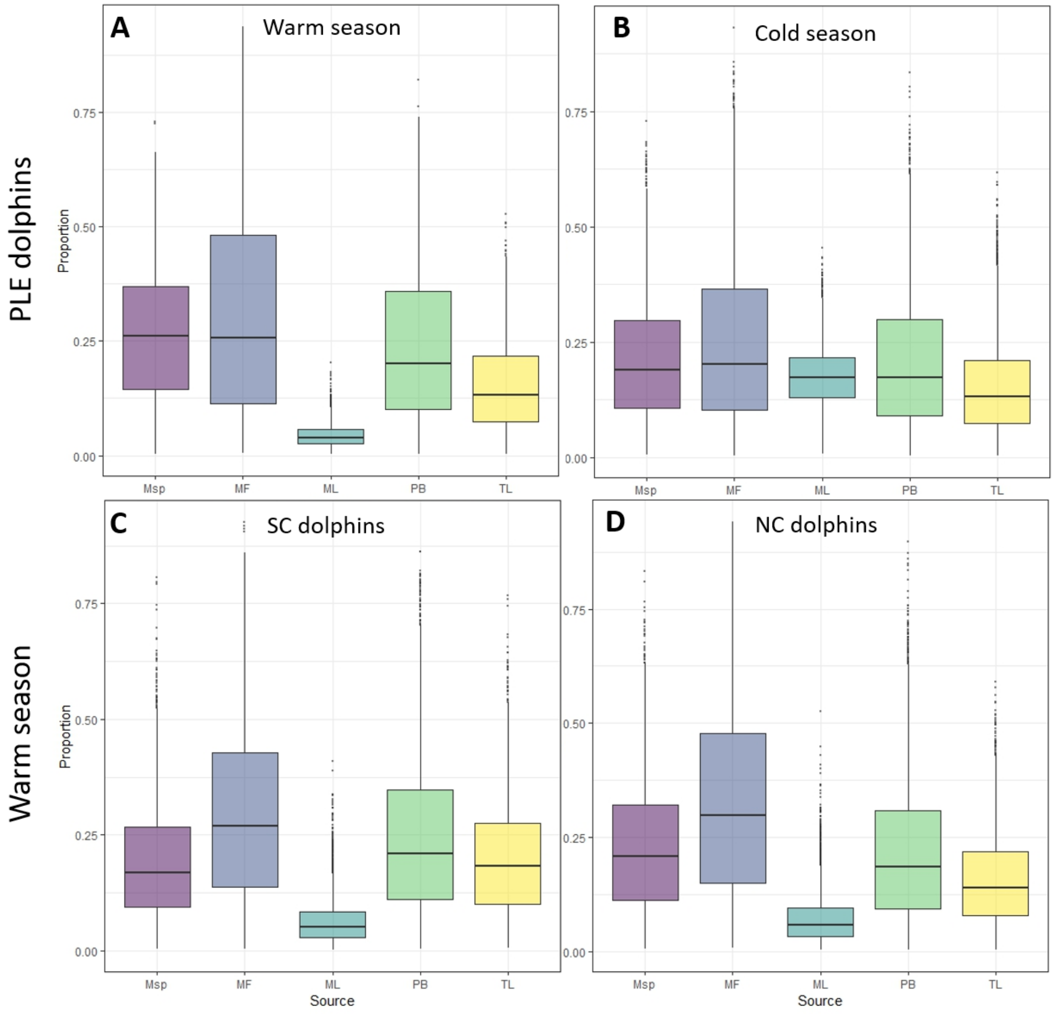

**Figure 5**. Relative contribution of prey sources to the diet of Lahille's bottlenose dolphin units that use the Patos Lagoon estuary (PLE social unit), adjacent southern (SC social unit) and northern coasts (NC social unit) for: Warm season (November – April) for PLE (A), SC (C), and NC (D) social units; and Cold season (May – October) for PLE dolphins (B). Proportions of each fish species are shown as box plots showing 50%, 75% and 90% credibility intervals. Msp: *Menticirrhus* sp.; MF: *Micropogonias furnieri;* ML: *Mugil liza*; PB: *Paralonchurus brasiliensis*; TL: *Trichiurus lepturus*.

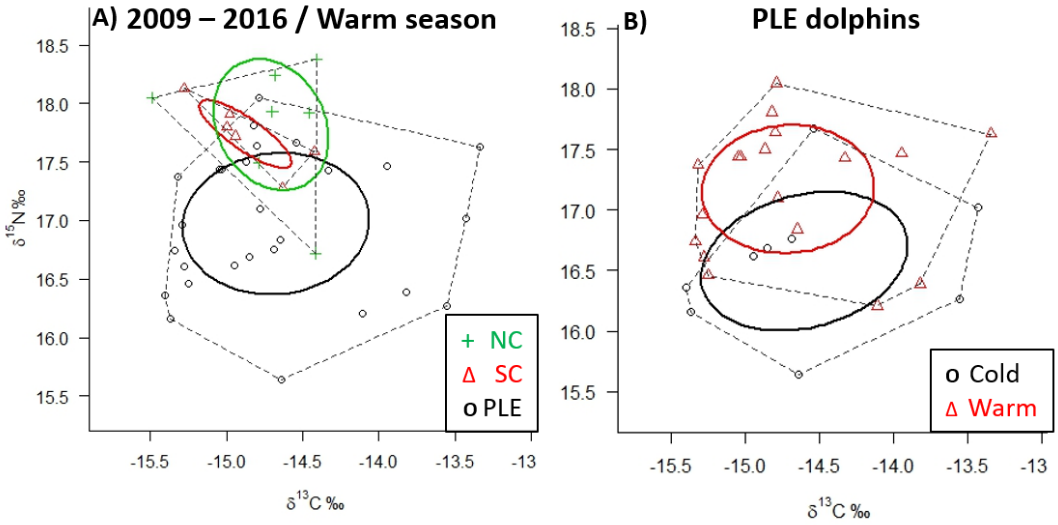

**Figure 6**. $\delta^{13}C$ and $\delta^{15}N$ isotopic niches of Lahille's bottlenose dolphins social units, *Tursiops truncatus gephyreus*, that use the Patos Lagoon estuary (PLE), southern (SC) and northern (NC) adjacent coastal waters, southern Brazil: A) for dolphins from each social unit (PLE, SC and NC) during the entire study (2009 – 2016) period and in the Warm season (November – April) and B) PLE dolphin in the Cold (May – October) and Warm seasons. The colored lines enclose the standard ellipse area (SEAc) for each group estimated by SIBER analysis (Stable Isotope Bayesian Ellipses in R, Jackson et al., 2011).