Genetic and social structure of Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) in the Patos Lagoon estuary and adjacent coastal waters

By

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Thesis
Submitted to Flinders University
for the degree of

Doctor of Philosophy

College of Science and Engineering
30th July 2021

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Rodrigo Cezar Genoves

RODRIGO CEZAR GENOVES

29 March 2021

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"

Acknowledgements:

In general, I thank all partners and institutions that somehow helped the "Botos da Lagoa dos Patos" project throughout its history. Without this joint action, we would never reach the level of knowledge and influence in the management and conservation actions of this population.

To Lauro Barcellos, who welcomed me warmly at the Oceanographic Museum, providing me with an office and always helping me along this journey. It was in one of his lectures that the conservationism seed was planted in me.

To Eduardo Secchi, a friend and advisor, who opened the doors to the world of marine mammals, accompanying me throughout my academic life. Although often distant, because of his enormous dedication to the institution and to the environment, he was always present in the moments that I most needed.

To Pedro Fruet, a great friend, to whom I should write not just a paragraph but a book of acknowledgements, a person I admire immeasurably, the source of inspiration and example of character, professionalism and dedication.

To Juliana Di Tullio, for the friendship, companionship, for all help during those years and for having trusted me and consequently gave me the best field works of my life.

Luciana Möller, my advisor, and Luciano Beheregaray, for having received me very well in Australia and contributed a great deal both in structure and knowledge in the development of my thesis. I have no words to thank you for your help in the final stretch of this thesis.

To the friends of MELFU and CEBEL for having received me very well and helped me a lot during my stay in Australia, especially Yuma, Andrea Barceló, Chris and Cat, who helped me a lot in the data analysis.

To Altamir Bras Pinto (Miro), Eli Bernardino and Diego Fernandes for piloting the boat during field work and Vitor Hugo for driving the field cars.

To Fabio Daura-Jorge and Mauricio Cantor, who have always been available to talk about my research and to help in whatever was necessary.

To the laboratory friends, Projeto Botos, ECOMEGA and Molecular Ecology Laboratory, who contributed a lot over the years. In particular, I thank Silvina Botta, Elisa Seiboth and Camila

Carvalho for help me with stable isotope analyzes, as well as Luciana Medeiros, Lucas Rodrigues and Cintia Brito in genetic analyzes.

I thank Lorenzo von Fersen and Yaqu Pacha e.V., for their trust in our project and for being fundamental in the development of this thesis, allowing me to spend a year at Flinders University.

To my pillars in the form of mother, Regina Maria Seneda, and brother, Luis Carlos Genoves Junior, as well as all my family, who are always in my heart, giving me joy when we are together and support when we are apart.

To my friends, source of joy and love, which is fundamental in this chaotic world in which we are inserted. In particular, Christian Göbel and Maíra Proietti, great partners in this journey.

To all the institutions that have assisted the Botos Project over the years: Yaqu Pacha e.V., KAOSA, Rio Grande Port, Rio Grande Oil Refinery and Rio Grande City Foundation.

Finally, I thank the Brazilian Government and the Graduate Program in Biological Oceanography at FURG for granting my scholarship, through which it was possible to carry out this study, as well as the agreement with Flinders University, which exempted me from fees and allowed me to carry out this study in a shared way, which has greatly enriched its results.

CONTENTS

1 CHAPTER I

THESIS OVERVIEW

Abstract

The dolphin genus *Tursiops* has a cosmopolitan distribution, but the subspecies *Tursiops truncatus gephyreus*, which is restricted to the South Atlantic Ocean, between South Brazil and Argentina, has recently been revalidated. The Patos Lagoon estuary and its adjacent coastal waters are home to the largest known population of this subspecies, and better understanding this population can contribute to the evaluation of its conservation status. The main objectives of this thesis were to investigate the spatial use patterns of these individuals, how they are structured socially, if there is genetic structuring, as well as to understand the feeding ecology of these groups and which factors influence population structuring. In chapter I an overview of the thesis is presented, with a general introduction, hypotheses, objectives, and the main methods, results, conclusions and recommendations. In Appendix I, all the available individual data of photo-identified and cataloged dolphins of the population were gathered to, through social analyses, evaluate which factors influence social structure. Four social units were identified and these were strongly associated with the spatial and temporal use of each study area (estuary, southern adjacency and northern adjacency), and individual aggregation levels. Excluding these factors from the analysis, it was observed that the two units present in each adjacent area maintain their cohesion, while the large group that uses the estuary is subdivided into four social groups. In Appendix II, the genetic structure of the estuarine and coastal dolphins was investigated. It was found that there are two genetically distinct populations occupying different isotopic niches, one associated with the estuary and another with the adjacent coastal zone. Finally, this thesis shows that the two populations have distinct patterns of habitat use, and that this is a key factor in their structuring.

Keywords: Lahille's dolphin; social structure; population structure; genomics; habitat use; feeding ecology.

1.1 GENERAL INTRODUCTION

Understanding the genetic diversity of a species can provide important information for management and conservation strategies, such as the identification and evaluation of management units (Palsbøll et al. 2007). Due to this relevance, studies on conservation biology seek to understand the main factors responsible for population variation over time and space (Frankham et al. 2002, Toro & Caballero 2005, Paz-Vinas et al. 2018). Space-related variation in genetic diversity is strongly influenced by the presence of barriers to dispersion, which restrict or impede the genetic flow between groups. There are several factors that can act as barriers to dispersal, including features such as mountain ranges or rivers separating environments (leading to allopatric speciation); ecological factors, such as habitat differences in adjacent regions (parapatric speciation); or behavioral factors such as differences in reproductive behavior between groups living in the same environment (sympatric speciation) (reviewed by Coyne & Orr 2004). In addition to natural barriers, pressure and anthropogenic impacts, such as severe habitat reduction, may influence the genetic diversity of organisms (e.g. Guschanski et al. 2007). When these patterns of genetic variation arise from previously panmictic populations (random mating, without restrictions), it is said that the population is structured.

Cetaceans comprise organisms that, in general, present great capacity of movement, and their species are distributed over large areas and occupy a wide diversity of habitats (Hoelzel 1998). This high dispersion ability, coupled with the reduced amount of geographic barriers in the environment where they live, suggests that their populations have little or no population structure (Palumbi 1992, Bohonak 1999). However, genetically distinct populations are often found in cetacean species, even in the absence of obvious geographical barriers (Hoelzel 1998). For example, differentiated habitat use and social structure (synthesis of how individuals interact with one another) can act as nonphysical "barriers" to gene flow by reducing interactions with individuals who do not share the same area and/or behavior preferences, therefore leading to genetic differentiation (Hoelzel et al. 1998, Wiszniewski et al. 2009, Ansmann et al. 2012, Louis et al. 2014). Clusters may arise within populations, especially when animals benefit from the company of other individuals (Krause & Ruxton 2002). Social structure studies describe these patterns of interactions (or associations) between individuals (Hinde 1976), estimating the amount of time that pairs of individuals remain associated, as well as the duration and nature of these associations (Cairns & Schwager 1987, Whitehead 1995, 2008). In this context, the presence of socially stable groups in a population may cause individuals to stop reproducing at random, prioritizing socially close animals and, consequently, generating genetic differentiation (Riesch et al. 2012, Van Cise et al. 2017). Finally, social groups are often tied to individual preferences for distinct habitats, use of different resources or different feeding strategies, also resulting in ecological structuring (Marcoux et al. 2007, by Stephanis et al. 2008, Riesch et al. 2012).

Social, ecological and genetic structures are closely linked and often depend on each other to develop or perpetuate within a population. For this reason, research has generally involved multidisciplinary approaches, incorporating information on habitat, social structure and genetic composition (e.g. Wiszniewski et al. 2009, Louis et al. 2010). Concepts about the mechanisms involved in the structuring process, the target species, the background information that led to the formulation of the hypotheses, the methods used, the results obtained, and the implications of this study for future management and conservation actions will be discussed in the following sections.

1.1.1 Social structure

The most used concept to define the term social structure in marine mammals are based on interactions between individuals and suggests that the social structure of a population is a synthesis of the nature, quality and patterning of the relationships among its members (Hinde 1976). Therefore, to build a model of animal social structure, we must first investigate interactions and use them to describe relationships (Whitehead 2008). For cetaceans and many other animals these are difficult to access, and for this reason we use associations, which are observations of animals in circumstances where interactions are likely to occur (Whitehead 1997). Thus, the fundamental elements of this study of Lahille’s bottlenose dolphins social structure uses the photo-identification method to investigate their associations; visual estimation of body size to infer dolphins’ ages; genital photographs and genetic methods for sexing; geographic locations to infer spatiotemporal use; and several analytical techniques for modeling social structure.

Despite the logistical constraints imposed on the study of cetaceans, dolphins and whales are known to have a range of social systems due their high cognitive capability and diverse behavioral repertory (e.g. Connor et al. 2001, Rendell & Whitehead 2001, Lusseau et al. 2006, Gero et al. 2014, Beirão-Campos et al. 2016). In the last decades, there has been great advance in the study of their social structure from the use of analytically refined association indices (Cairns & Schwager 1987), to the development and compilation of various techniques for social analysis (Whitehead 1997) and the application of social network theories (Newman 2004, 2006). It has been observed that social groups arise through individuals who associate more by sharing some kind of similarity, a phenomenon known as homophily (McPherson et al. 2001), which could be to protect from predators, increase efficiency in capturing prey or even by empathy/aversion. More specifically, association patterns may be related to gender (reviewed by Ruckstuhl 2007), age class (Smith et al. 2002, Manno 2008), reproductive state (Sundaresan et al. 2007, Möller & Harcourt 2008), sociability (Lusseau et al. 2006, Manno 2008), feeding strategies (Chilvers and Corkeron 2002, Daura-Jorge et al. 2012), habitat use patterns (Möller et al. 2007, Ansmann et al. 2014), kinship relationships (Pinter-Wollman et al. 2009, Wiszniewski et al. 2010, Frère, Krützen et al. 2010, Van Cise et al. 2017), individual behavior (or personality; Highfill & Kuczaj II 2007, Krause et al. 2007, 2010) and social resistance (Armansin et al. 2019). Moreover, within a population, there may be a type of individual variation called gregariousness, which occurs when some individuals prefer to form smaller groups, while others prefer to form larger groups (Godde et al. 2013).

Many of these factors have a great influence on estimates of association indices, making it more difficult to distinguish which pairs associate for purely social reasons within a social network. This type of interaction, called affiliation, can be assessed by excluding the influence of these structural factors from analysis (Whitehead & James 2015). After the initial step of understanding the social organization of the target population (Genoves 2013), this is the next step to understand the social structure of this population and, in the future, be able to understand the causes for its existence and maintenance.

1.1.2 Ecological structure

One of the mechanisms that promote structuring in sympatry is habitat/resource specialization, which means that individuals in a population specialize in some type of habitat or resource (Futuyma & Moreno 1988, Dieckmann & Doebeli 1999). Specialization for a given resource may arise due to individual plasticity in either behavioral or morphological traits and temporal stability of feeding strategies (Knudsen et al. 2010). However, for this specialization event to result in genetic differentiation, it should be linked to selective breeding or reproductive isolation (Dieckmann & Doebeli 1999), which tends to occur since these specialized individuals are inclined to spend more time associating, and therefore results in social structuring. This type of structuring can be advantageous for the population if it reduces the competition for resources (Robinson et al. 1996). Resource specialization may also be a cultural behavior within the population, passed from generation to generation, often during parental care. This has been reported, for example, in dolphins from Shark Bay (Australia), which use sponges to supplement their diets (Smolker et al. 2010). Another example is the lobtail feeding in humpback whales from the Gulf of Maine, which has been passed among associating individuals for over three decades (Allen et al. 2013). Finally, variations in morphological traits may also be related to adaptation to different resources (Smith & Skúlason 1996, Foote et al. 2009).

A widely used technique to investigate the spatial and trophic ecology of top predators is the stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (see revisions of Hobson 1999, Kelly 2000, Newsome et al. 2010). $\delta^{13}C$ provides information on the base of the trophic chain and can be used to reveal patterns of spatial utilization, such as between coastal and oceanic environments (Hobson et al. 1994). On the other hand, $\delta^{15}N$ is widely used as an indicator of trophic position, as it enriches from one level to another within the trophic chain (DeNiro & Epstein 1981). In addition, it is possible to identify the proportion of contribution of the main prey to the isotopic signal of the consumers through Bayesian mixture models (Parnell et al. 2013) when prior knowledge of the main prey is available (Phillips et al. 2014). Therefore, several studies have used SIA to investigate the presence of niche partitioning within and among populations of, for example, seabirds (Hodum & Hobson 2000, Young et al. 2010) and elephant seals (*Mirounga leonina*; Lewis et al. 2006).

Within this study's concept, the analysis of stomach content is inefficient because it depends on newly stranded animals (which takes them out of the social analysis) that could be photo-identified (through their dorsal fins). SIA is the most robust method available and can be performed by collecting material from live animals, complementing the social structure model more effectively.

1.1.3 Genetic structure

Genetic methods have advanced rapidly and they allow extraordinary insights into marine mammal societies from very small samples that can be collected minimally invasively from live animals (Krutzen et al. 2001). Nowadays, genetics can be used to indicate sex (Gilson et al. 1998); maternal lineages (Dillon and Wright 1993); genetic relatedness (Valsecchi and Amos 1996); and parentage (Wiszniewski et al. 2012). The use of genetic methods is also very important in a variety of other context in cetacean studies, including population structure studies that evaluate the influence of environmental and social factors (e.g. Wiszniewski et al. 2010; Kopps et al. 2012; Louis et al. 2018).

The geographic variation between populations, both in morphological and genetic characteristics, is a result of the balance of forces that produce local genetic differentiation and homogeneity (Slatkin 1987). Barriers to gene flow may arise as a result of the environment in which animals are inserted, historical and behavioral processes, and generate genetic heterogeneity among populations. Cetaceans are faced with few geographical barriers given their great mobility and the characteristics of the environment they occupy. However, abrupt environmental and habitat variations, climate, or even particular oceanographic characteristics (e.g. currents, salinity and temperature) can reduce this dispersal capacity, generating reproductive isolation and genetic structuring (Fullard et al. 2000, Bilgmann et al. 2007, Fontaine et al. 2007, Möller et al. 2011, Louis et al. 2014). Recent studies have shown that there is genetic structuring at relatively small geographical scales in dolphin populations (Ansmann et al. 2012), including between an estuary and its adjacent coastal zone (Möller et al. 2007). Historical geographic barriers may also influence the current structure of populations. For example, after the last glaciation's ice melting, some previously allopatric populations became sympatric or parapatric, but still presented historic genetic differentiation (Hewitt 1996). Identifying the genetic structure of animal populations living in sympatry, as occurs in the present study, is often challenging; in this context, it is necessary to use a large number of molecular markers capable of detecting fine-scale genetic structure. Single-nucleotide polymorphisms (SNPs) have been shown to be very efficient for analyses of population structure, especially when used in large quantities (Liu et al. 2005, Gaughran et al. 2018).

1.1.4 Contextualizing the species and the target population

The common bottlenose dolphin, *Tursiops truncatus*, is cosmopolitan and inhabits coastal and oceanic regions in tropical and temperate regions (Wells & Scott 1999). Studies around the world have shown that their populations are mostly found in societies with fission-fusion dynamics (Connor et al. 2000), although some populations can present a more stable social structure (eg. Lusseau et al. 2003, Wells 2014). They are long-lived animals, with delayed maturation (between 5 and 14 years) and slow reproduction (one calf every 2 to 4 years, 12 months of gestation) through a polygamous mating system (Urian et al. 1996, Wells & Scott 1999, Connor et al. 2000, Fruet et al. 2015). Common bottlenose dolphins have a great plasticity in feeding strategies (Chilvers & Corkeron 2001, Smolker et al. 2010, Daura-Jorge et al. 2012), habitat use (Natoli et al. 2005, Wiszniewski et al. 2009, Ansmann et al. 2004) and resource utilization (Berens McCabe et al. 2009, Barros et al. 2010, Fernández et al. 2011, Rossman et al. 2015). Therefore, populations of common bottlenose dolphins around the world can have varying patterns of residency and home range (Welles et al. 1987, Simões-Lopes & Fabian 1999, Silva et al. 2009, Hwang et al. 2014, Laporta et al. 2016). Generally, populations with a higher degree of residency and smaller home ranges are associated with highly productive coastal environments such as river mouths, estuaries, bays and fjords (Wells et al. 1987, Simões-Lopes & Fabian 1999, Fruet et al. 2015). However, large-scale movements are common, including temporary emigration (Bearzi et al. 2008, Silva et al. 2009, Laporta et al. 2016). Individual identification through dorsal fin photographs of these animals (the basis for most of the studies cited), which often have long lasting marks, allows analytical evidence of this plasticity in different aspects of the animals' ecology (Würsig & Würsig 1977, Würsig & Jefferson 1990, Urian et al. 2015).

Until a few years ago, coastal populations found in southern Brazil, Uruguay and Argentina (Figure 1) were recognized as *T. truncatus*. However, recent studies have shown that these populations have unique morphological and genetic characteristics (Costa et al. 2016, Wickert et al. 2016, Fruet et al. 2017) and are now recognized as a subspecies, *Tursiops truncatus gephyreus* (Committee on Taxonomy 2019) (referenced only as Lahille's dolphin from here on). Several studies on the social and genetic structure of populations of this subspecies throughout its distribution had been carried out when it was not recognized. Daura-Jorge et al. (2012) and Zappes et al. (2011) described the cooperative interaction between dolphins and fishermen respectively in Laguna and Barra de Imbé/Tramandaí, south Brazil. In Laguna this feeding strategy is performed by only a portion of the population and is associated with social structuring. In Argentina, Vermeulen (2018) described a relatively homogeneous social structure in resident dolphins (Vermeulen & Cammareri 2009) of the Bay of Santo Antônio. Costa et al. (2015) found three genetic clusters in dolphins that are distributed between the north of Rio Grande do Sul and south of Santa Catarina, Brazil. These studies show that, in general, many characteristics of *Tursiops* are observed in Lahille's dolphin populations.

In this thesis I study the largest aggregation of resident Lahille's dolphins, which inhabit the Patos Lagoon estuary and its adjacent coastal waters (Figure 1). The area corresponds to the cities of Rio Grande and São José do Norte (Brazil) and the majority of the population uses this area throughout the year (Fruet et al. 2011, Fruet et al. 2015). There is evidence that females prefer the estuarine area (sex ratio ~2 females:1 male - Fruet et al. 2015), while males (mainly juveniles) apparently prefer the adjacent coast (Fruet et al. al. 2010). This population, which has been systematically monitored by photo-identification since 2005, preferably uses the mouth of the estuary and adjacent coastal waters, both subject to intense artisanal fishing especially in the spring and summer (Di Tullio et al. 2015). This period coincides with the population’s breeding period, which presents a birth pulse during warm months (November to April) (Fruet et al. 2015). Females reach sexual maturity between 7 and 8 years, generate only one calf after a gestational period of about 12 months, and present intense parental care during the first 2 to 3 years of the calf's life (Fruet et al. 2015). The diet of the population is based on teleost fish, mainly whitemouth croaker (*Micropogonias furnieri*), lebranche mullet (*Mugil lisa*), banded croaker (*Paralonchuros brasiliensis*), southern kingcroaker (*Menticirrhus sp*.) and cutlassfish (*Trichiurus lepturus*) (Secchi et al. 2016). Based on social analysis, it was recently shown that this population is structured into three distinct social units, one occupying the estuarine area, another occupying the coastal area to the south, and a third along the coast to the north of the estuary mouth (Genoves 2013).

These studies carried out over many years provide a level of knowledge about this population that is very difficult to reach about marine mammals. In this thesis, I propose to advance in essential points of social organization, spatiotemporal use, feeding ecology and genetics of this population. These main parameters to be analysed will allow elucidating whether there is competition for resources between these social groups, as well as understanding whether this social segregation and site fidelity has led to a reduction of gene flow among them (e.g. Möller et al. 2007, Rosel et al. 2009, Ansmann et al. 2012b). This multidisciplinary study is likely to reveal much more detail about the population organization than previously described, and will have direct implications for management and conservation measures, as postulated below.

Figure 1. Distribution area of the known populations of Lahille's dolphins, *Tursiops truncatus gephyreus*, which occupy estuaries and coastal zones of southern Brazil (BR), Uruguay (UR) and Argentina (AR).

1.1.5 Implications for conservation

The main issue in conservation biology is how to delineate suitable conservation units to maintain a species' adaptive potential and consequently its persistence in the environment (Moritz 1999, Fraser & Bernatchez 2001). The conservation of genetically differentiated populations of a species contributes to maintain its genetic diversity and maximize its evolutionary potential, minimizing its risks of extinction. However, recent studies have found relevant intra-population components and structures that can also have great importance in the conservation of the population as a whole. Therefore, it is necessary to discuss and define the types and levels of structure that is relevant to conservation efforts.

Genetic knowledge has been fundamental in the context of conservation plans, especially when integrated with information regarding population parameters, ecology and movements of individuals acquired by alternative methods (Lowe & Allendorf 2010). Among the mostly used designations for conservation units based on genetic characteristics are *Evolutionary Significant Units* (ESUs) and Management Units (MUs). An ESU (sensu Ryder 1986) is a population, or a group of populations, that have been historically isolated, requiring independent management actions aimed at maximizing the evolutionary potential in the face of climatic and environmental changes. ESUs are relevant to long-term approaches, which are the establishment of priority conservation strategies and measures (Moritz 1994). On the other hand, MUs (Moritz 1994) consider recent population structure, that is, factors such as allele frequency and adaptive variation are also addressed; MUs are suggested for short-term management actions. In some ways, MUs can be considered as subpopulations within a metapopulation, represented by an ESU. Therefore, MUs may represent populations that are important for the persistence of an ESU, or even a species, over time (Allendorf et al. 2007). Many dolphin populations have been shown to present relatively weak genetic structures that do not comprise ESUs, but are of great importance in maintaining the genetic diversity of populations (Möller et al. 2007, Ansmann et al. 2012), and should therefore be considered MUs.

Over the last decades, new concepts to improve conservation biology have been discussed, including the incorporation of information on animal behavior (Sutherland 1998, Caro 1999, 2007, Berger-Tal et al. 2011), such as social structure. In general, social units that use different areas (eg. Möller et al. 2007, Ansmann et al. 2014) may also differ in diet composition (Fernández et al. 2011, Monteiro et al. 2015) and, consequently, in their responses to environmental variations (Sutherland 1998). For example, sperm whales (*Physeter macrocephalus*) from the South Pacific Ocean show different levels of feeding efficacy according to environmental fluctuations (El Niño) and, therefore, present varied reproductive success (Whitehead & Rendell 2004) that likely affects the fitness of clans. Another relevant factor in these populations is the cultural component, which is usually restricted to groups within the population, and can lead to genetic structuring. This is the case of Shark Bay dolphins, which feed on sponges through a strategy passed from generation to generation by parental care and have genetically differentiated from dolphins that do not use the same technique (Kopps et al. 2014). Understanding mating systems can also help to identify if inbreeding depression could be a problem for a population (Sutherland, 1998). In addition, Whitehead and Rendell (2004) suggest that for some groups such as dolphins, whales and elephants, it is essential to include a cultural component in management and conservation actions of evolutionary units. Therefore, although many conservation-related actions can be effective without behavioral information, such information can contribute significantly when conservation strategies based on traditional methods are ineffective (Sutherland 1998, Caro 2007).

T. truncatus is listed as "least concern" in the IUCN Red List, but this is due to its cosmopolitan distribution and global estimate of about 600,000 individuals. However, there is a great concern for the conservation of smaller, local populations, mainly coastal that are exposed to various anthropogenic threats (see Reeves 2003). For this reason, there has been considerable concern for the conservation of the population that uses the Patos Lagoon estuary and adjacent coastal waters in southern Brazil. Although the abundance estimates of individuals using the estuary have remained constant over the last decades (Dalla Rosa 1999, Fruet et al. 2011, Fruet et al. 2015), mortality from bycatch in fishing nets has been concerning (Fruet et al. 2010), mainly for the dolphins that use the coastal zone. This bycatch is due to the overlap of fishing effort with the area preferentially used by the dolphins, especially during spring and summer (Di Tullio et al. 2015). This concern has increased with the recognition that these dolphins compose the subspecies *T. t. gephyreus* by the *Taxonomy Committee* of *The Society for Marine Mammalogy* in 2017. The dolphins that use the PLE and adjacent coasts therefore form a population of a coastal subspecies, subject to constant human impacts and with a distribution restricted to the western South Atlantic Ocean. If these individuals present population structuring, conservation strategies at the population level could be an important component for the long-term survival of this subspecies.

1.1.6 Hypothesis formulation

Given the introduction above, the hypotheses of this study are that: (1) the social units share at least parts of their home range, but differ temporally in their area use; (2) individual similarities (i.e. age class, sex and individual strategies) intensify social associations and spatial use patterns;

(3) dolphin’s genetic structure correlates with the social structure; and (4) differential habitat use (estuary vs. coastal) promote niche partitioning.

If my hypotheses are true, the Lahille’s bottlenose dolphins using this region will show reduced spatial overlap by not occupying the same area at the same time; they will associate more with individuals with whom they share some similarities; social segregation will lead to a level of genetic structuring due to reduced gene flow; and, finally, differential use of the habitat will lead to differences in isotope signals.

1.2 OBJECTIVES

The general objective of this thesis is to investigate impact of social relationships, spatiotemporal use patterns, genetic structure and/or feeding ecology on the fine-scale population structure of Lahille’s bottlenose dolphins inhabiting the Patos Lagoon estuary and adjacent coastal waters.

The specific objectives are to: (1) estimate spatiotemporal use of photo-identified individuals to enhance the social structure model; (2) identify factors that significantly influence social structure; (3); investigate whether genetic structuring exists; (4) investigate whether differential habitat use promotes isotopic niche partitioning; (5) identify the most relevant preys to the dolphins’ diet.

1.3 THESIS STRUCTURE

This thesis is structured in an introductory chapter (current Chapter I) and two appendices containing novel research results (Appendix I and II). The main methodologies used, the main results obtained, a general conclusion and recommendations for future studies are also presented in this chapter. Appendix I used 10 years of photo identification data and, apart from measuring the influence of spatiotemporal use pattern and gregariousness on the population’s association index, extracted the social network based exclusively on the "true social relationships". The results show that, in social terms, there are four groups within the unit that use the estuary, while there are two social units in the coastal zone. Appendix II arose from the need to understand if the dolphins that use the Patos Lagoon estuary and adjacent coastal waters in a differential pattern present genetic structuring, and occupy differentiated trophic niches. The results show that the individuals that use the Patos Lagoon estuary and adjacent coastal waters are structured in two populations that occupy different trophic niches. One of the populations uses the entire study area, but occurs mainly in the final portion of the estuary, and the other is restricted to the adjacent coastal zone.

1.4 MATERIAL AND METHODS

1.4.1 Study area and data collection

The study area comprises a total of 140km^2, divided into three subareas: the final portion of the Patos Lagoon estuary (PLE), which is sheltered and has 40 km^2; adjacent northern (NC) and southern (SC) coastal areas, both with 50 km^2 and more susceptible to variations in oceanographic conditions (Figure 2). The PLE is a subtropical system located in south Brazil, and acts as a drainage basin of approximately 200,000 km^2 (Möller et al. 2001), having a connection with the Atlantic Ocean via a canal limited by two jetties of approximately 4 km each. The estuary is one of the most productive coastal areas in Brazil, with large fish stocks occupying the area and adjacent coastal zone (Garcia et al. 2012). The coastal areas adjacent to the south and north of the jetties, in terms of abundance and richness of fish species, are very similar, both showing higher productivity in the warm months (Rodrigues & Vieira 2013). However, they differ slightly in terms of beach morphodynamics, with SC being characterized as a dissipative beach, dominated by muddy and sandy-mud sediments, originating from the lagoon plume (Marques et al. 2009). Meanwhile, NC is a more reflective beach, with a relatively larger grain size than SC (De Oliveira & Calliari 2006).

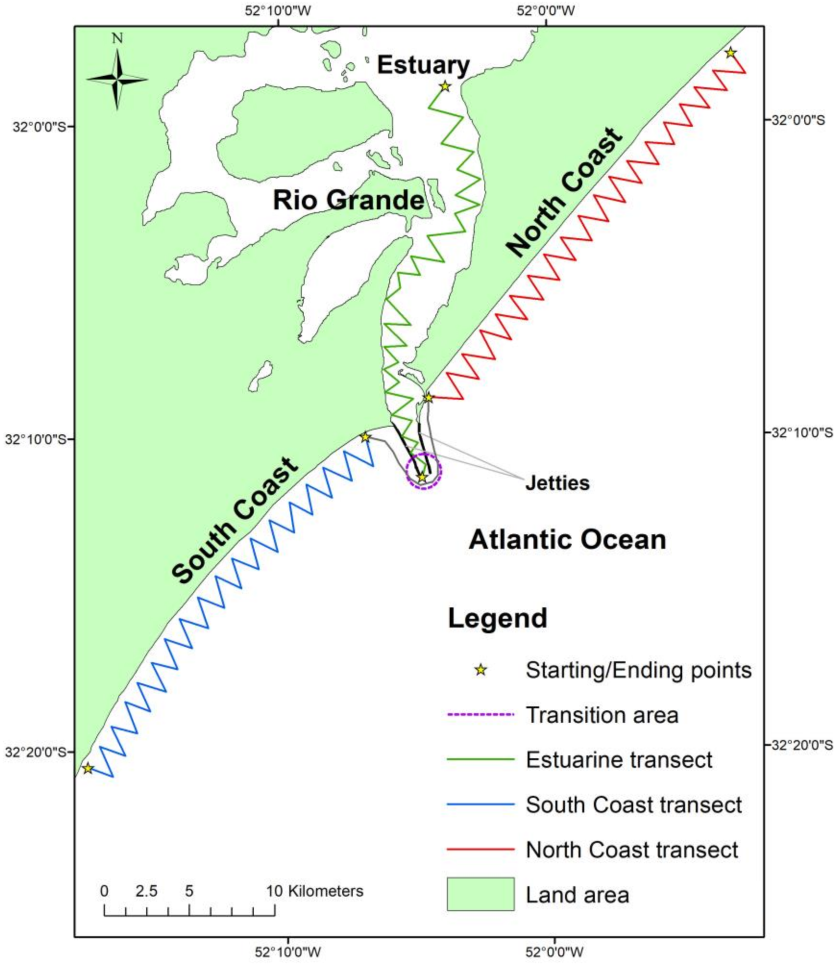

Figure 2. Study area and the zigzag transects performed during surveys for photo-identification and collection of biopsy samples from Lahille's dolphins, *Tursiops truncatus gephyreus*, in the Patos Lagoon estuary and adjacent coastal waters, south Brazil.

Field surveys were conducted between August 2005 and December 2015 under favorable weather conditions (e.g. good visibility, sea state < 3 on the Beaufort scale). Each survey was carried out to cover at least one of the sub-areas and, whenever possible, at least one survey was conducted per month, alternating the start of the course. Individuals with spatial cohesion (i.e. up to 100 m from each other) and involved in similar activities were defined as a group (Wells et al. 1987). Individuals were randomly photographed in order to identify them through the long-lasting marks present on their dorsal fins (Urian et al. 2015). Photographic analysis criteria to determine whether an animal had sufficient marks to be used in the analyzes, minimum number of sightings, as well as the spatial and temporal use characterization criteria are specified in Appendix I (Genoves et al. 2018).

1.4.2 Sample collection

Skin samples for isotopic and genetic analyses of Lahille's dolphins were collected during surveys from adult individuals, known for their long-lived marks on their dorsal fins. For such, a 120 lb crossbow was used with an arrow adapted specifically for the collection of small cetacean biopsies. This arrow contains a tip that penetrates the body of the animal and collects only a fragment of skin and fat (a circle of 6 mm radius), without reaching the muscles and, consequently, minimizing damages to the animals (Fruet et al. 2016).

For isotopic analyses, the main prey of the Lahille's dolphins were selected based on Secchi et al. (2016), who described the feeding ecology of these dolphins. The carbon and nitrogen isotopic signs of the main prey specimens are present in the database of the Marine Megafauna Ecology and Conservation Laboratory (ECOMEGA) and were analysed by Wiegand (2017).

1.4.3 Appendix I methods

Appendix I refers to the study of the social structure of the Lahille's dolphins that use the Patos Lagoon estuary and its adjacent coastal waters, aiming at identifying the presence of social units and describing how their individuals behave socially and use the area spatiotemporally. For social analyses, the half-weight index (HWI, Cairns & Schwager 1987) was corrected for influence of gregariousness (Godde et al. 2013), which was present in the population after the permutation tests (Bejder et al. 1998). Thus, a preliminary analysis was carried out, applying the Newman modularity (Newman 2004, 2006), without identifying the structural variables that could influence the association index. After this process, the HWI was used as a basis to investigate which of the predictive variables were significant through MRQAP (Multiple Regression Quadratic Assignment Procedure: Dekker et al. (2003, 2007)), with 20,000 permutations. The variables used were: gregariousness; spatial use pattern; home range overlap; temporal overlap; and classes such as sex, sub-area predominantly used, and preference periods. After removing the significant predictive variables, affiliation indices were calculated using generalized affiliation indices (GAIs: Whitehead & James 2015). To detect strong and avoidance relationships, the residues from this procedure were transformed into Anscombe residuals (Pierce & Schafer 1986). In addition, to aid in the interpretation of results, network metrics such as strength, cluster coefficient and affinity were calculated (Whitehead 2008). Finally, the temporal pattern of the associations was measured through the standardized lagged association rate (SLAR: Whitehead 1995). The software used were: SOCPROG, version 2.8 (Whitehead 2009), and UCINET (Borgatti et al. 2002), for social analyzes; R, version 3.4.3 (R Core Team 2017) and AdehabitatHR package (Calenge 2006), for the home range overlap estimation; and Arcview 9.3 (ESRI, Redlands, CA, U.S.A.) for map production.

1.4.4 Appendix II methods

Appendix II was designed to investigate possible genetic population structure in the Lahille's dolphins that use the Patos Lagoon estuary and adjacent coastal waters, given the different habitat use and social structure described previously. The study was restricted to those dolphins that were analysed socially in Appendix I and also had skin samples collected. We used 49 individuals in the genetic analyses and 40 in the carbon and nitrogen stable isotope analyses. These sample sizes are different because some individuals did not have skin samples for isotope analysis.

For genetic analyses, we analysed Single Nucleotide Polymorphisms (SNPs), with all laboratory and bioinformatics procedures based on Sandoval-Castillo et al. (2018), and described in detail in Appendix II. The genetic diversity of the social units was analysed through the means of nucleotide diversity (π), expected heterozygosity (H_E) and percentage of polymorphic loci, through the program ARLEQUIN 3.5 (Excoffier & Lischer 2010). The potential of the social units to reflect a possible genetic structure was examined through the Bayesian clustering algorithm implemented in the fastSTRUCTURE program (Raj et al. 2014). To determine the most probable number of clusters, the measure of complexity of the ideal model ($K^*\varepsilon$) and the number of relevant model components ($K^{*}{}_{\emptyset}{}^{C}$) were used (Raj et al. 2014). In addition, in case of discrepancy between these numbers, it was compared with the lowest cross-validation error (CV error) in the ADMIXTURE program (Alexander et al. 2009). In addition, a nonmetric multidimensional scaling (nMDS) was also performed in order to compare the topology of the genetic similarity of individuals with the social network topology. Finally, genetic differentiation among social units was investigated by computing pairwise F_{ST} values in ARLEQUIN 3.5, with its significance estimated through 10,000 permutations.

For the evaluation of the isotopic niche of the dolphins, stable isotopes of carbon and nitrogen were used. The laboratory procedure for this analysis is described in detail in Appendix II. In order to understand the contribution of the prey to the isotope signal of the dolphins, Bayesian mixture models (Layman et al. 2012) were used through the *simmr* package in R (Parnell 2016). In order to verify the significance of possible variables responsible for changes in the isotopic signal of the dolphins, generalized linear models (GLMs) were used. The variables tested were: social unit; preference for estuary or coastal zone; sex; and warm (November - April) or cold (May - October) seasons, assigning the samples to each season considering the turnover rates in the skin of common bottlenose dolphins reported by Giménez et al. (2016). The best model was selected through the lowest AIC value (Akaike's information criterion). The isotopic niches and their metrics were calculated through multivariate ellipses estimated by Bayesian inference corrected for small sample sizes. These analyses were done in the *SIBER* package (Jackson et al. 2011), in software R.

1.5 **RESULTS**

Between August 2005 and December 2015, 354 surveys were carried out, where 2,233 groups were found and 87,811 photos were analysed, resulting in the photo identification of 217 individuals. After data processing, 318 field trips and 51,920 good-quality photographs of 1,792 groups were used, resulting in the identification of 102 dolphins with evident long-lasting marks on their dorsal fins. A total of 154 skin samples were collected, of which 49 were used in the genetic analysis and 40 in the stable isotope analyses, and correspond to individuals analysed in terms of social structure and used in Appendix II.

1.5.1 Appendix I

Lahille's dolphins that use the Patos Lagoon estuary and adjacent coastal waters are socially well differentiated since the coefficient of variation of the true association index using the likelihood method was relatively high (S = 0.891 ± 0.015). The correlation between the true association index and its estimate (r = 0.642 ± 0.020) indicated that the database had a good power to represent the social system of these individuals. The social system has a gregarious influence, since the standard deviation of the 'typical group size' was higher than what would be expected at random (real = 0.89, random = 0.74, p = 0.001). Therefore, the representation relative to the index of association was corrected for gregariousness. The modularity was significant (Q_{max} = 0.36), indicating the presence of four social units, one strongly associated to the estuary (PLE), one to the southern area (SC), one to the northern area (NC), and a smaller social unit formed by individuals from different areas (GR4). For the affiliations analysis, the MRQAP indicated that the factors that most influence the association index of this population are the spatiotemporal use patterns and gregariousness of individuals. Therefore, GAIs were constructed excluding these variables. The modularity for this analysis was also significant (Q_{max} = 0.32), differing from the previous analysis because it indicated four subdivisions in the social unit that uses the estuary. This shows that the temporal component in the association index masked these stronger relations within the PLE unit, providing evidence for the importance of using this correction.

1.5.2 Appendix II

The bioinformatics filtering process resulted in 2,942 SNPs that were used in genetic analyses of the dolphin populations. Both Bayesian cluster analysis ($K^{*}\varepsilon$) and CV error (0.48) indicated the presence of two genetically distinct populations in the area. One population corresponds to the dolphins of the PLE social unit, which has been studied over the last several years. The other population consists of the coastal dolphins, represented by the social units SC and NC as indicated in Appendix I. The F_{ST} (0.054) value corroborates this division, indicating a moderate ($F_{ST} < 0.1$) but significant ($P < 0.0001$) genetic differentiation. In addition, F_{ST} values were also significant among social units, indicating that although there is a genetic differentiation between SC and NC units, their social and area use patterns reduces gene flow. These values were higher between the PLE and NC units, followed by PLE and SC, and finally SC and NC, corresponding to the degree of social proximity reported in Appendix I. Stable isotope values varied significantly by season (warm and cold) and between environments (estuary vs. coast). The preys used adequately described the isotopic composition of the dolphins of the PLE population, but not of the coastal population (SC and NC). The PLE dolphins presented a wider isotopic niche than the coastal dolphins. However, the latter seem to occupy a higher trophic position, with more enriched nitrogen isotopic values. Due to the low number of samples collected in the cold period for the coastal dolphins (only two for the SC unit), it was not possible to compare between units for this period. Finally, it is clear that habitat use greatly influences the structure of this population.

1.6 CONCLUSIONS

The major conclusion of this thesis is that there are two populations of Lahille's dolphins using the Patos Lagoon estuary (PLE) and its adjacent coastal waters (SC and NC), and these are structured in different social units with a strong spatiotemporal component and gregariousness. It is noteworthy that the presence of two genetic populations, revealed in Appendix II, introduces some bias to the social structure described in Appendix I. The social study showed that the social structure of Lahille's dolphins using this area is much more complex than previously described, which is a forewarning to those who perform this type of study. Although weak, there is a significant genetic difference between the dolphins of the social units (PLE, SC and NC). With this, it is evident that the dissolution of any of these groups, whether by anthropic (unnatural mortality and habitat alteration) or natural causes, could result in significant changes to the dynamics and viability of these units. In terms of feeding ecology, estuarine dolphins seem to prioritize prey from the inner estuary, while coastal dolphins feed exclusively on coastal preys, resulting in the occupation of distinct isotopic niches, which suggests that any fishing pressure above the capacity of the system in one of these habitats could generate greater competition between groups. Finally, these social units of Lahille's dolphins at the region have accumulated differences in genetic composition, feeding ecology and social associations, and should be considered in future studies and when deciding on management and conservation actions for the species in the region.

1.7 RECOMMENDATIONS

The most important recommendation for future studies arising from this thesis is that the presence of two populations occupying and sharing the same area must be considered. In social terms, it is still necessary to investigate whether the maintenance of these groups and differentiated spatiotemporal use is due to agonistic/aggressive behaviors. Furthermore, it may be that genetic relatedness, which was not measured in this thesis, promotes stronger associations and the formation of these social units. In relation to future studies on feeding ecology using stable isotopes, they should prioritize samples collected over a short period of time to minimize the potential effects of temporal variability of the isotopic landscape and the displacement of the organisms (prey and predator) between areas with different isotopic values. In addition, comparisons of the isotopic niche of coastal dolphins with dolphins from neighbouring populations could elucidate if they are feeding in the same fish stocks. Finally, we did not capture the relationship between individual behaviour and population-level processes, which must be further investigated.

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2 Appendix II

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

Short title: Spatiotemporal use predicts dolphin social units

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2.2 Abstract

Ranging behavior 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 modelling intra-population dynamics and viability, as well as for investigating patterns of gene flow among them.

Keywords: Social group; spatiotemporal dynamics; gregariousness; spatial distribution; affiliation; social division

2.3 **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 (e.g. Whitehead 2003), whereas in societies with fission-fusion dynamics a 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 et al. 2000), bats (Kerth et al. 2006), and elephants (Wittemyer et al. 2005).

Although there is much fluidity in the individual associations within populations with fission-fusion dynamics, on a fine-scale these populations can be structured into social units (Karczmarski et al. 2005; Urian et al. 2009; Best et al. 2013). 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 and Ruxton 2002). Therefore, social units are usually composed by individuals that are largely behaviorally self-contained, interacting more with each other than with others, sharing a similar living space, and that 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 (e.g. Cantor et al 2012).

Most studies about social networks of non-human 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, 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 et al. 1998; Croft et al. 2011; Godde et al. 2013; Whitehead and James 2015). These factors can be related, for example, to spatial overlap (e.g. Shizuka et al. 2014), temporal overlap (e.g. Cantor et al. 2012), gregariousness (Godde et al. 2013), and sex of individuals (Wiszniewski et al. 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 et al. 2008; Farine and Whitehead 2015).

Bottlenose dolphins, *Tursiops* spp., are cosmopolitan animals that inhabit coastal and oceanic waters of both tropical and temperate regions (Wells and 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 et al. 2009), ranging patterns (Rossbach and Herzing 1999; Urian et al. 2009), feeding strategies (Chilvers and Corkeron 2001; Mann et al. 2012; Daura-Jorge et al. 2012; Ansmann et al. 2012), habitat use (Laska et al. 2008; Baird et al. 2009), sex (Wiszniewski et al. 2012), and kinship relationships (Möller et al. 2001, 2006; 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 et al. 2016; Wickert et al. 2016; Fruet et al. 2017). 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 approximately 87 individuals inhabit the sheltered waters of the PLE in southern Brazil (Fruet et al. 2011; Fruet et al. 2015a). 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 overfishing and habitat degradation (Moraes et al. 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 ≤ 6m) coastal waters (Mattos et al. 2007; Di Tullio et al. 2015). 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* 250km 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 1) categorize and group individuals according to their patterns of spatial use and temporal fidelity to the area; 2) identify the most adequate analytical method to describe its social structure; and 3) verify the presence of social units and elucidate their role within the population’s social network.

2.4 **Methods**

2.4.1 Study area and data collection

The Patos Lagoon is a large coastal lagoon located between 30º 30'S and 32º 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 (Moller et al. 2001) and is connected to the Atlantic Ocean by two jetties of about 4km. Approximately 10% of the area is characterized as an estuary composed of shallow bays (80% of which are < 2m in depth), and a narrow navigation channel that can reach up to 20m 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 et al. 2012; Rodrigues and Vieira 2013). Our study area includes the lower part of the PLE and adjacent coastal waters (*ca* 140km^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 and Calliari 2006). For the purpose of survey design and due to some logistical limitations, the area was divided into three sub-areas: i) the estuary to the lagoon’s mouth (*ca* 40km^2); ii) the estuary’s adjacent northern coastal waters; and iii) the estuary’s adjacent southern coastal waters. The two coastal areas are approximately 50km^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 sub-areas, 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 circunference of 1000m 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 5m 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 < 2m). 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 1.5nm offshore for the remainder of the study (Figure 1a). Each survey covered at least one of the three sub-areas. At least one survey per month was conducted in each sub-area, 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 100m of each other) and that were engaged in similar activities were defined as a group (Wells et al. 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 300mm lens. In subsequent analysis, each photograph was graded for quality (Q1–Q3) (Wilson et al. 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 et al. 2000). Since this was a systematic study, we chose, besides the use of evident long-lasting marks, to use ancillary marks in 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 pre-defined route, and the photo-identification analysis was performed later by the two independent researchers.

2.4.2 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. less than two 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).

2.4.3 Data Classification

Sex Classification - The sex of individuals was obtained using (1) 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 et al. (1998); and (2) large dolphins (i.e. >3m) with a closely associated calf photographed on ≥3 independent sampling occasions were determined as females (Fruet et al. 2015b); and (3) 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 et al. 1985).

2.4.4 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 and 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 2008). The index is defined as: $HWI = x/(x + yab + 0.5(ya + yb))$, 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 et al.

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 and 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 greater than 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).

2.4.5 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 were 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 towards explaining the matrix of association indices. The MRQAP was performed with 20,000 permutations (using the ‘double-semi-partialing’ technique of Dekker et al. (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 and Schafer 1986). The calculated prediction measures were as follows:

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).

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 and Kochanny 2005), which is implemented in the package AdehabitatHR (Calenge 2006) for R v 3.4.3 (R Core Team 2017).

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.

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.

2.4.6 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 et al. 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 and 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.

2.4.7 Network metrics

Network metrics are statistical measures used to characterize properties of an individual or a network as a whole (Farine and Whitehead 2015). Three individual-based network statistics, calculated from the weighted network (association matrix), were averaged over and within the social units: 1) strength, which is a measure of gregariousness, and is the sum of the association indices for each individual (Barthélemy et al. 2005); 2) the clustering coefficient, which measures how well the partners of an individual are themselves associated (as calculated by Holme et al. 2007); and 3) 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 et al. 2008).

2.4.8 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, i.e. 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 quasi-Akaike information criterion (QAIC; Whitehead 2007), with additional support of QAIC weights and likelihood (Burnham and Anderson 2002).

2.5 **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 is presented in Table S1 (Appendix S1) and, for each area class, in Figures 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; Appendix S1: Table S1).

2.5.1 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 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 non-zero 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 non-preferred 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.

2.5.2 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).

2.5.3 Detecting social units

Based on the HWIG, the estimated modularity coefficient (*Qmax* = 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 (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 (*Qmax* = 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 sub-divided 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 area, but utilizing more the southern and northern coasts, respectively (Figure 4e and 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 individuals (Figure 2c). Avoidances occurred mostly between SUs 1-4 individuals (Figure 2d).

2.5.4 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 individuals 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, explain 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.

2.5.5 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 (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 and 3c, respectively), differing due the presence of preferred companions (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 (Figure 3b and c, respectively). The other social units (SUs, 2, 5 and 6) are composed of a smaller number of individuals, many of them with few sightings (compared with SUs-3-4) and, therefore, their results are not presented.

2.6 **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 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.

2.6.1 Ranging behavior

Spatial dynamics are important to consider when examining animal sociality, especially when studying animals which are capable of long-range movements (tens to thousands of kilometers) in short periods of time (days to months) such as dolphins (Irvine et al. 1981; Mate et al. 1995). In our study, we identified social units composed by individuals that: *i*) use the entire study area but mainly concentrate around the estuary mouth; *ii*) use mostly the inner estuary area but also use the coastal area; *iii*) use the entire coastal area; and *iv*) 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 (Urian et al. 2009; Wiszniewski et al. 2009; Louis et al. 2015; Titcomb et al. 2015). 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 (e.g. Castello and Pinedo 1977; Dalla Rosa 1999; Fruet et al. 2011; Fruet et al. 2015a). The PLE is a protected, highly productive environment (Seeliger and Odebrecht 2010), which provides favorable environmental conditions throughout the year for these dolphins, particularly for feeding and shelter (e.g. Mattos et al. 2007; Fruet et al. 2015a; 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 beaviour and is widely seen in other mammals (e.g. primates, Watts and Mitani 2001; Williams et al. 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.G. and P.F.F., personal observations).

2.6.2 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 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 (e.g. Shizuka et al. 2014) and individuals using the area at the same time are more likely to associate (e.g. 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 et al. 2011, respectively). However, as previously mentioned, this dolphin population appear 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 non-random 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 a 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 and Heithaus 1999; Lusseau et al. 2003; *Hyperoodon ampullatus*, Gowans et al. 2001; *Grampus griseus*, Hartman et al. 2008; *Globicephala macrorhynchus*, Mahaffy et al. 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 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 to 0.022) and temporal pattern (casual acquaintances and constant companions) to the *T. truncat*us population of Normano-Breton Gulf, France (Louis et al. 2015).

2.6.3 Social network

The connection between social units can occur through a few key individuals. These key individuals, known as brokers (*sensu* Lusseau and 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 and Whitehead 2001), assisting with gene flow within, but can also potentially lead to the spread of diseases (Newman 2002; Frère et al. 2010). 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 societies with fission-fusion dynamics such as that of Asian elephants (de Silva et al. 2011), spotted hyenas, *Crocuta crocuta* (Holekamp et al. 2012) and Galapagos sea lions, *Zalophus wollebaeki* (Wolf et al. 2007), where individuals tend to interact more 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 Lahille'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. In other delphinids, genetic relatedness between individuals can induce adult females striped dolphins (*Stenella coeruleoalba*) in small groups to associate preferentially with adult kin (Gaspari et al. 2007); it can influence female relationships of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Wiszniewski et al. 2010); and bottlenose dolphin (*Tursiops truncatus*) leaders can gain indirect benefits by leading relatives (Lewis et al. 2013). Therefore, genetic relatedness within the social units should be investigated. While we did not observe distinct feeding techniques in this population, the three sub-areas of the study show different ecological and physico-chemical characteristics making it possible that there are differences in their feeding ecology of the social units identified here, which means that they could be feeding on different preys or stocks, (as observed for bottlenose dolphins of Normano-Breton Gulf; Louis et al. 2018).

2.7 **Conclusion**

This Lahille's bottlenose dolphin population of the Patos Lagoon estuary and adjacent coast are structured into 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 towards 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.

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 24407-2, 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.

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.

Acknowledgments: We are very grateful to Cetacean Society International, Organization for the Conservation of South American Aquatic Mammals – YAQU PACHA e.V, Nuremberg Zoo, and Rancho Texas de Lancerote for funding support. We also thank all field assistants for helping data collection over the years and Lauro Barcellos, director of the Oceanographic Museum 'Prof Eliezer C. Rios' at FURG, and NGO KAOSA for providing logistical support to the fieldwork. Mauricio Cantor, Luciano Dalla Rosa, Silvina Botta and

Fabio Daura-Jorge provided useful advice in the early drafts of the manuscript. CNPq provided a research fellowship to E.R.S. (PQ 307846/2014-8). This article is part of RCG's Ph.D. thesis in Biological Oceanography and was supported by the National Council for Scientific and Technological Development (CNPq - Brazil) and Yaqu Pacha (German) scholarships, and is under a cotutelle agreement between Flinders University of South Australia and Universidade Federal do Rio Grande-FURG, under the supervision of E.R.S. and L.M.M. This study was part of the Brazilian Long Term Ecological Program (PELD – process 441492/2016-9), partially funded by CNPq (Ministry of Sciences, Technology, Innovation and Communication), and it is a contribution of the Research Group Ecologia e Conservação da Megafauna Marinha – EcoMega-FURG/CNPq.

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 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.

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2.9 Figures

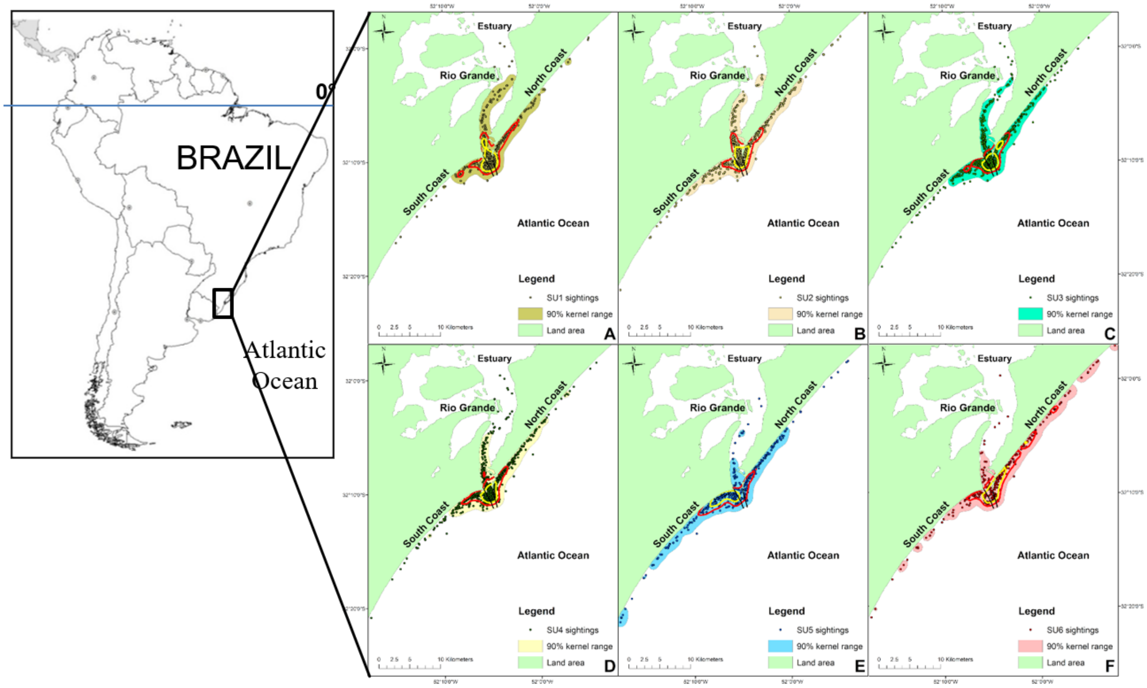

Figure 1. A: Area covered during boat surveys (sampling occasions) to search for Lahille’s bottlenose dolphins (*Tursiops truncatus gephyreus*) in the Patos Lagoon Estuary (green) and adjacent coastal waters (jetties transect = grey, south = blue and north = red) in southern Brazil. The dotted purple circle in the mouth of the estuary represents the transition area. B, C, D, E and 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).

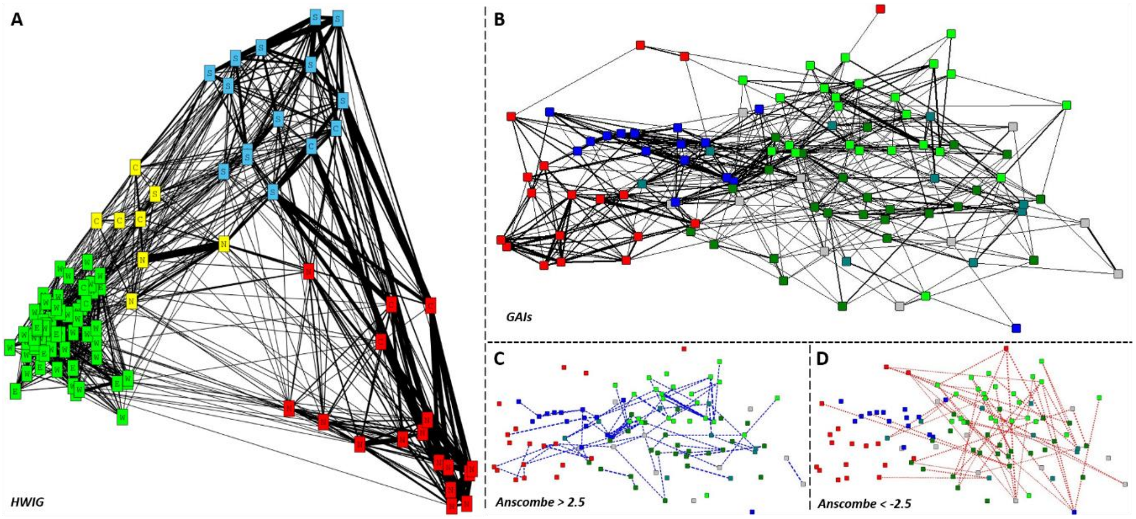

Figure 2. Network diagrams for 102 Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) that use the Patos Lagoon Estuary and adjacent coastal waters in southern Brazil, using the half-weight index corrected for gregariousness (A) and generalized affiliation indices (B). The thickness of the 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.

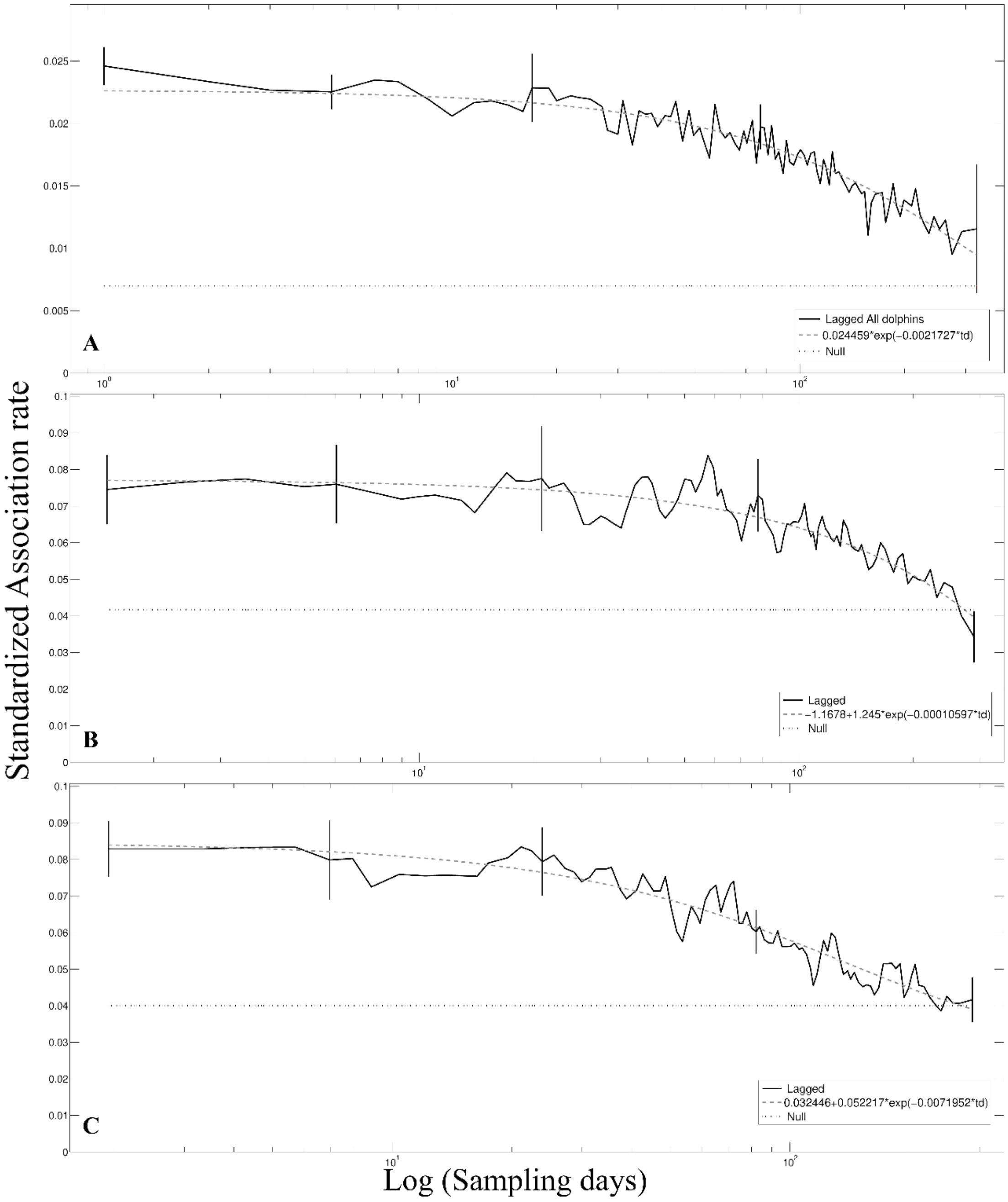

Figure 3. Standardized lagged association rate (solid line) compared to the best fitting model (dashed line) and standardized null association rate (dotted line) for all dolphins (A), within Social Unit 3 (B) and within Social Unit 4 (C) dolphins. Standard error bars (vertical lines) were computed by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

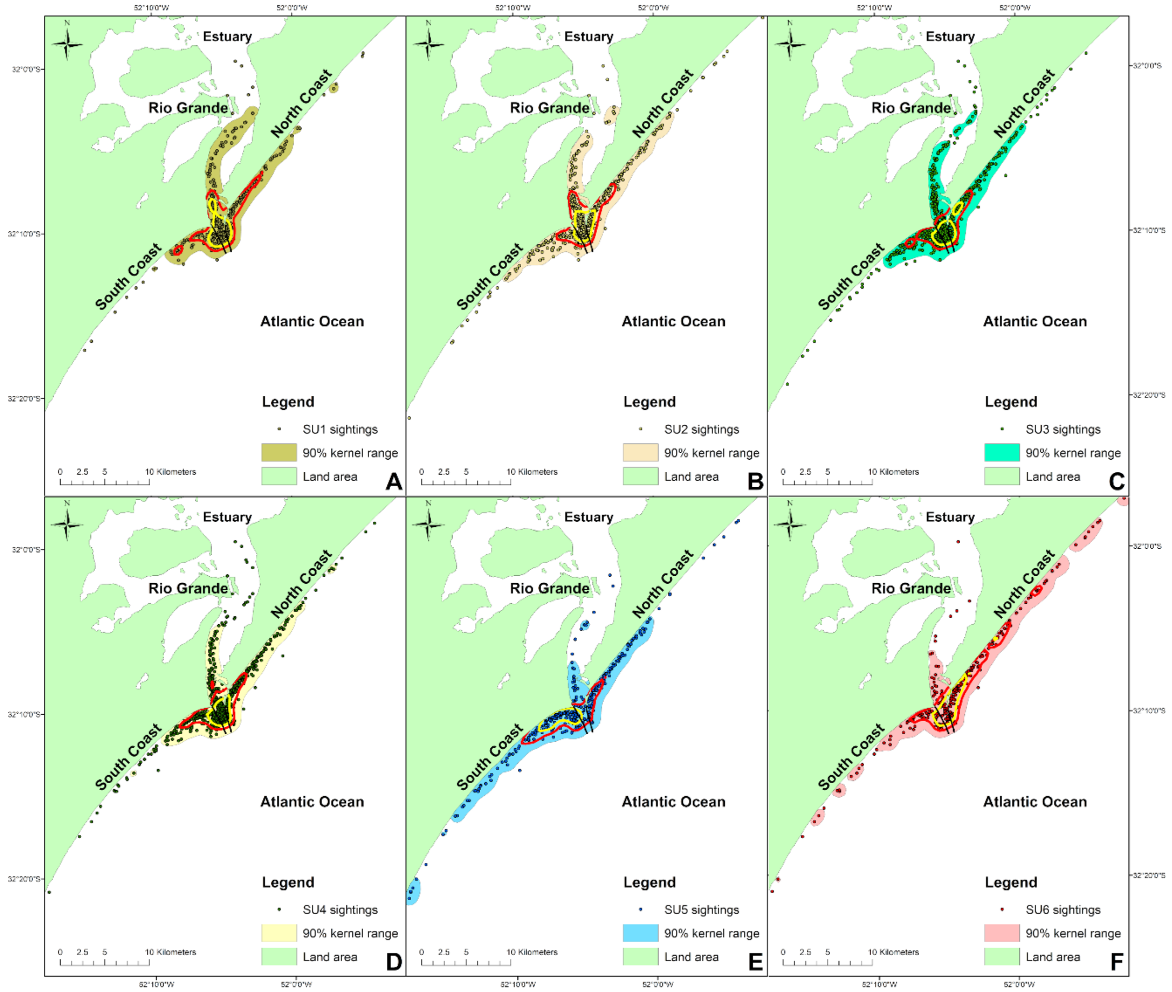

Figure 4. Locations of each social unit of Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*), proposed by community division and modularity based on generalized affiliation indices, with 90% (full color), 50% (red line) and 25% (yellow line) kernel isopleths. A = Social Unit 1, B = Social Unit 2, C = Social Unit 3, D = Social Unit 4, E = Social Unit 5 and F = Social Unit 6.

2.10 Tables

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 sub-areas (Estuary, South and North) and a transition area, in the Patos Lagoon estuary and adjacent coastal waters in southern Brazil.

Sub-Area	**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

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

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. The standard deviation, estimated by bootstrap, is in brackets.

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	0.02910

2.11 Supplementary material

Appendix S1: Table S 1. Characterization of the 102 Lahille’s bottlenose dolphins (Tursiops truncatus gephyreus), sorted and grouped based on the affiliation-based social units (CMD GAIs column), that fulfilled the prerequisites and were used in the social analyses. Abbreviation corresponds to: Females (F), Males (M) and Unknown (U) sex; DNA = Genetic method, PAC = Parental care method, DOF = dorsal fin method; Wanderer (WA), Estuary (ES), Southern (ES), Northern (NO) and Coastal (CO) dolphin; Cold = percentage of sightings in cold periods and Warm = percentage of sightings in warm periods; Resident (RES), Cold period (COL), Warm period (WAR), and cold period dolphin that was sighted in Uruguayan waters (COU); CMD-HWIG and GAIs = community division by modularity using the half-weight index corrected for gregariousness and generalized association indices (GAIs), respectively.

ID	Sex	Sexing Method	N. sampling periods	Estuary (%)	Southern Coast (%)	Northern Coast (%)	Area Class	N. Seasons	Cold (%)	Warm (%)	Period Class	CMD HWIG	CMD GAIs
ID004	F	DNA	39	56.4	25.6	17.9	ES	18	44	56	RES	GR1	SU1
ID013	F	DNA	63	34.9	30.2	34.9	WA	19	53	47	RES	GR1	SU1
ID029	M	DNA	100	37.0	31.0	32.0	WA	20	50	50	RES	GR1	SU1
ID033	F	DNA	80	38.8	21.3	40.0	WA	20	50	50	RES	GR1	SU1
ID043	F	PAC	18	5.6	22.2	72.2	NO	14	50	50	RES	GR4	SU1
ID078	F	DNA	13	0.0	27.3	72.7	NO	5	60	40	RES	GR4	SU1
ID125	U	---	31	51.6	25.8	22.6	ES	8	50	50	RES	GR1	SU1
ID300	F	PAC	62	53.2	21.0	25.8	ES	18	44	56	RES	GR1	SU1
ID301	F	PAC	73	60.3	13.7	26.0	ES	19	47	53	RES	GR1	SU1
ID001	F	DNA	121	62.8	11.6	25.6	ES	20	50	50	RES	GR1	SU2

ID002	F	PAC	88	65.9	10.2	23.9	ES	19	53	47	RES	GR1	SU2
ID008	M	DNA	78	34.6	35.9	29.5	WA	18	44	56	RES	GR1	SU2
ID025	F	DNA	90	50.0	23.3	26.7	ES	20	50	50	RES	GR1	SU2
ID056	F	PAC	59	37.3	18.6	44.1	WA	20	50	50	RES	GR1	SU2
ID063	M	DNA	48	0.0	0.0	100.0	NO	17	47	53	RES	GR3	SU2
ID090	M	DNA	47	0.0	0.0	100.0	NO	15	47	53	RES	GR3	SU2
ID092	U	---	62	1.6	83.9	14.5	SO	14	43	57	RES	GR2	SU2
ID120	M	DNA	94	46.8	13.8	39.4	WA	14	50	50	RES	GR1	SU2
ID196	M	DNA	33	15.2	36.4	48.5	WA	4	50	50	RES	GR1	SU2
ID006	F	DNA	69	75.4	1.4	23.2	ES	13	46	54	RES	GR1	SU3
ID007	F	PAC	62	27.4	43.5	29.0	WA	19	53	47	RES	GR1	SU3
ID012	F	DNA	75	24.0	33.3	42.7	WA	20	50	50	RES	GR1	SU3
ID014	F	PAC	65	56.9	14.1	29.0	ES	20	50	50	RES	GR1	SU3
ID016	M	DNA	48	22.9	39.6	37.5	WA	15	47	53	RES	GR1	SU3
ID019	F	DNA	66	25.8	25.8	48.5	WA	18	56	44	RES	GR1	SU3
ID023	M	DNA	64	15.6	50.0	34.4	WA	18	50	50	RES	GR1	SU3
ID024	F	DNA	41	34.1	31.7	34.1	WA	13	46	54	RES	GR1	SU3
ID037	F	DNA	47	0.0	40.4	59.6	CO	18	44	56	RES	GR4	SU3
ID038	M	DNA	58	5.2	39.7	55.2	WA	17	41	59	RES	GR1	SU3
ID039	F	DNA	76	43.4	19.7	36.8	WA	19	47	53	RES	GR1	SU3

ID047	F	DNA	78	25.6	38.5	35.9	WA	20	50	50	RES	GR1	SU3
ID059	F	DNA	89	42.7	14.6	42.7	WA	20	50	50	RES	GR1	SU3
ID066	M	DNA	72	0.0	44.4	55.6	CO	20	50	50	RES	GR1	SU3
ID079	U	---	64	28.1	26.6	45.3	WA	18	50	50	RES	GR1	SU3
ID105	M	DNA	74	24.3	31.1	44.6	WA	19	47	53	RES	GR1	SU3
ID107	F	DNA	97	47.4	20.6	32.0	WA	18	50	50	RES	GR1	SU3
ID118	U	---	82	57.3	14.6	28.0	ES	19	53	47	RES	GR1	SU3
ID127	F	DNA	68	0.0	39.7	60.3	CO	14	50	50	RES	GR4	SU3
ID131	U	---	40	20.0	42.5	37.5	WA	9	44	56	RES	GR1	SU3
ID142	F	DNA	63	15.9	42.9	41.3	WA	17	53	47	RES	GR1	SU3
ID172	M	DNA	47	8.5	40.4	51.1	WA	17	47	53	RES	GR1	SU3
ID177	M	DNA	46	0.0	54.3	45.7	CO	15	53	47	RES	GR1	SU3
ID198	U	---	55	30.9	29.1	40.0	WA	15	53	47	RES	GR1	SU3
ID005	M	DNA	72	40.3	27.8	31.9	WA	19	47	53	RES	GR1	SU4
ID010	F	DNA	54	46.3	16.7	37.0	WA	18	56	44	RES	GR1	SU4
ID011	F	PAC	69	23.2	21.7	55.1	WA	20	50	50	RES	GR1	SU4
ID015	F	DNA	64	65.6	14.1	20.3	ES	20	50	50	RES	GR1	SU4
ID017	M	DNA	51	19.6	31.4	49.0	WA	18	50	50	RES	GR1	SU4
ID020	M	DNA	71	29.6	42.3	28.2	WA	20	50	50	RES	GR1	SU4
ID022	F	DNA	65	18.5	40.0	41.5	WA	19	53	47	RES	GR1	SU4

ID027	M	DNA	58	34.5	29.3	36.2	WA	19	47	53	RES	GR1	SU4
ID031	F	DNA	46	17.4	45.7	37.0	WA	16	44	56	RES	GR1	SU4
ID034	M	DNA	58	31.0	34.5	34.5	WA	18	44	56	RES	GR1	SU4
ID041	F	PAC	101	36.6	31.7	31.7	WA	20	50	50	RES	GR1	SU4
ID045	F	DNA	66	28.8	28.8	42.4	WA	19	47	53	RES	GR1	SU4
ID048	F	DNA	76	32.9	39.5	27.6	WA	19	47	53	RES	GR1	SU4
ID055	F	DNA	65	35.4	24.6	40.0	WA	20	50	50	RES	GR1	SU4
ID058	F	PAC	38	5.3	28.9	65.8	WA	18	56	44	RES	GR1	SU4
ID071	F	PAC	46	0.0	69.6	30.4	CO	16	56	44	RES	GR4	SU4
ID091	F	PAC	118	63.6	19.5	16.9	ES	20	50	50	RES	GR1	SU4
ID104	F	PAC	63	9.5	49.2	41.3	WA	17	47	53	RES	GR1	SU4
ID115	U	---	41	0.0	61.0	39.0	CO	17	53	47	RES	GR4	SU4
ID116	F	DNA	46	39.1	15.2	45.7	WA	20	50	50	RES	GR1	SU4
ID176	M	DNA	58	29.3	32.8	37.9	WA	15	47	53	RES	GR1	SU4
ID179	M	DNA	45	62.2	20.0	17.8	ES	8	50	50	RES	GR1	SU4
ID197	F	DNA	30	23.3	36.7	40.0	WA	8	50	50	RES	GR1	SU4
ID214	F	DNA	74	21.6	35.1	43.2	WA	17	53	47	RES	GR1	SU4
ID302	F	PAC	31	3.2	9.7	87.1	NO	17	53	47	RES	GR4	SU4
ID030	M	DNA	95	38.9	27.4	33.7	WA	20	50	50	RES	GR1	SU5
ID054	M	DOF	47	19.1	61.7	19.1	SO	18	44	56	RES	GR4	SU5

ID062	U	---	19	0.0	47.4	52.6	CO	13	54	46	RES	GR2	SU5
ID067	F	DNA	19	0.0	84.2	15.8	SO	10	90	10	COU	GR2	SU5
ID070	M	DNA	41	0.0	70.7	29.3	SO	11	45	55	RES	GR2	SU5
ID072	U	---	28	0.0	100.0	0.0	SO	10	70	30	COU	GR2	SU5
ID074	U	---	29	0.0	100.0	0.0	SO	8	75	25	COL	GR2	SU5
ID084	U	---	20	0.0	100.0	0.0	SO	8	88	13	COL	GR2	SU5
ID085	U	---	30	0.0	70.0	30.0	SO	14	57	43	RES	GR2	SU5
ID089	M	DNA	29	0.0	100.0	0.0	SO	11	73	27	COU	GR2	SU5
ID093	M	DOF	34	0.0	61.8	38.2	CO	14	29	71	WAR	GR2	SU5
ID103	U	---	44	0.0	70.5	29.5	SO	12	50	50	RES	GR2	SU5
ID113	U	---	12	0.0	91.7	8.3	SO	7	71	29	COU	GR2	SU5
ID141	M	DNA	10	0.0	100.0	0.0	SO	7	71	29	COU	GR2	SU5
ID150	U	---	11	0.0	100.0	0.0	SO	4	75	25	COL	GR2	SU5
ID174	U	---	33	0.0	24.2	75.8	NO	9	44	56	RES	GR3	SU5
ID049	F	PAC	77	31.2	20.8	48.1	WA	20	50	50	RES	GR1	SU6
ID069	M	DNA	71	32.4	35.2	32.4	WA	19	47	53	RES	GR1	SU6
ID094	F	DNA	15	0.0	0.0	100.0	NO	12	42	58	RES	GR3	SU6
ID095	F	PAC	44	0.0	0.0	100.0	NO	16	44	56	RES	GR3	SU6
ID096	F	DNA	33	0.0	39.4	60.6	CO	15	47	53	RES	GR3	SU6
ID097	F	DNA	36	0.0	0.0	100.0	NO	14	29	71	WAR	GR3	SU6

ID123	M	DNA	58	25.9	24.1	50.0	WA	13	46	54	RES	GR1	SU6
ID143	M	DNA	11	0.0	36.4	63.6	CO	4	25	75	WAR	GR3	SU6
ID145	M	DNA	10	0.0	0.0	100.0	NO	5	20	80	WAR	GR3	SU6
ID147	U	---	10	0.0	0.0	100.0	NO	5	20	80	WAR	GR3	SU6
ID159	F	DNA	14	0.0	92.9	7.1	SO	4	50	50	RES	GR2	SU6
ID160	M	DNA	10	0.0	0.0	100.0	NO	4	25	75	WAR	GR3	SU6
ID166	U	---	13	0.0	53.8	46.2	CO	7	29	71	WAR	GR3	SU6
ID167	U	---	10	0.0	0.0	100.0	NO	4	25	75	WAR	GR3	SU6
ID169	M	DNA	10	0.0	0.0	100.0	NO	4	25	75	WAR	GR3	SU6
ID170	U	---	15	0.0	13.3	86.7	NO	7	29	71	WAR	GR3	SU6
ID171	U	---	13	0.0	0.0	100.0	NO	7	29	71	WAR	GR3	SU6
ID184	U	---	10	0.0	16.7	83.3	NO	4	25	75	WAR	GR3	SU6

Appendix S1: Table S 2. Models fit to Standardized Lagged Association Rates (SLAR) ranked by the lowest quasi-Akaike Information Criteria (QAIC) for all Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) and for each of the proposed social units (SUs) using generalized affiliation indices, of the Patos Lagoon Estuary and adjacent coastal waters in southern Brazil. Preferred companions (Pref. Comps), casual acquaintances (Casual acqs), both preferred companions and casual acquaintances present (Pref + casual) and two levels of casual acquaintances (Two levels) were the four models fitted. ΔQAIC, QAIC weights and Likelihood indicates the relative support for each model.

SLAR model	Model formula	QAIC	ΔQAIC	QAIC weight	Likelihood
All individuals					
Casual acqs	$0.024*e^{(-0.0022\cdot t)}$	405,410.13	0	0.88	1
Two levels	$-0.63*e^{(-0.0022\cdot t)}+0.66*e^{(-0.0022\cdot t)}$	405,414.13	4.0	0.12	0.13
Pref + casual	$0.019+0.029*e^{(-1.25\cdot t)}$	406,350.36	940.2	0	0
Pref. Comps	0.019974	406,403.52	993.4	0	0
SU1 x SU1					
Two levels	$0.343*e^{(-0.007*t)}+0.02*e^{(-0.007*t)}$	11,911.59	0	0.99	1
Pref + casual	$0.184+0.183*e^{(-0.017*t)}$	11,930.35	18.75	0	0
Casual acqs	$0.308*e^{(-0.002*t)}$	11,977.67	66.07	0	0
Pref. Comps	0.242	12,091.22	179	0	0
SU2 x SU2					
Two levels	$-1.142*e^{(-0.013*t)}+1.47*e^{(-0.009*t)}$	21,321.98	0	0.99	1

Pref + casual	$-6.79+7.18*e^{(-0.0001*t)}$	21,351.33	29.35	0	0
Casual acqs	$0.403*e^{(-0.004*t)}$	21,382.30	60.31	0	0
Pref. Comps	0.286	21,899.47	577	0	0
SU3 x SU3					
Pref + casual	$-1.167+1.24*e^{(-0.0001*t)}$	11,3542.20	0	0.93	1
Casual acqs	$0.078*e^{(-0.002*t)}$	11,3547.74	5.53	0.06	0.006
Two levels	$-0.0185*e^{(-1.9*t)}+0.078*e^{(-0.002*t)}$	11,3551.51	9.3	0.008	0.009
Pref. Comps	0.0651	11,3856.85	314	0	0
SU4 x SU4					
Pref + casual	$0.032+0.05*e^{(-0.007*t)}$	71,098.35	0	0.98	1
Casual acqs	$0.08*e^{(-0.003*t)}$	71,106.72	8.36	0.01	0.01
Two levels	$2.287*e^{(-6*t)}+0.08*e^{(-0.003*t)}$	71,110.16	11.8	0.002	0.002
Pref. Comps	0.0617	71,536.00	437	0	0
SU5 x SU5					
Casual acqs	$0.095*e^{(-0.005*t)}$	3,059.99	0	0.77	1
Two levels	$0.04*e^{(-0.99*t)}+0.091*e^{(-0.004*t)}$	3,063.27	3.3	0.15	0.19
Pref. Comps	0.079	3,065.97	6.0	0.038	0.05
Pref + casual	$0.077+0.058*e^{(-0.62*t)}$	3,066.14	6.2	0.035	0.04
SU6 x SU6					
Pref + casual	$0.13-0.206*e^{(-0.045*t)}$	2,756.23	0	0.91	1
Casual acqs	$0.29*e^{(-0.011*t)}$	2,761,51	5.3	0.064	0.07
Two levels	$0.241*e^{(-0.004*t)}+0.28*e^{(-0.011*t)}$	2,763.83	7.6	0.02	0.02

Pref. Comps	0.207		2,807.10	50.9	0	0

3 Appendix II

Manuscript submitted for publication to the Journal Marine Biology on 1st February 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, social resistance or even social preferences/avoidances 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 Lahille's 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, behavioral specializations and social barriers 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 personality or competition between individuals and groups, and favoring population structure (e.g. Wiszniewski et al. 2009; Möller et al. 2011; Daura-Jorge et al. 2012; Ansmann et al. 2014; Armansin et al. 2019). 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 due to habitat competition, which possibly produces resource partitioning 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 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), and make it possible to investigate the feeding ecology of free-living animals, that is, it does not depend on the collection of stranded animals. $\delta^{13}C$ 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 (DeNiro & 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 presents 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 these social units described by Genoves et al. (2018) are stable enough to generate or be the result of genetic structuring should 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 (π), 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 cluster value (K; tested from 1 to 10) were completed to ensure consistency. The most likely number of clusters 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, nonmetric multidimensional scaling (nMDS) analysis was performed with the 'metaMDS' function using the R packages 'ADEGENET' 2.1 (Jombart & Ahmed 2011) and 'vegan' (Oksanen et al. 2020). NMDS 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. Lipids are depleted in ^{13}C compared with other molecules and variability in lipid content of samples may result in undesirable variability in $\delta^{13}C$ values (DeNiro and Epstein 1978). However, Wilson et al. (2014) recommended that in the case of *Tursiops* skin, C/N ratios up to 4.5 do not require lipid extraction. The mean C/N mass ratio of all samples (3.6) thus indicated that no lipid extraction was required for the dolphin samples. Stable isotopes were analysed using an elemental analyzer coupled to a Thermo Scientific Delta V isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes (UNM–CSI). 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 ($\Delta^{13}C = 1.01 \pm 0.37$‰ and $\Delta^{15}N = 1.57 \pm 0.52$‰). 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 analysed using generalized linear models (GLMs). Separate GLMs were used to model the $\delta^{13}C$ and $\delta^{15}N$ values of the Lahille's bottlenose dolphins. The fit of the models to $\delta^{15}N$ values were performed using Gaussian distributions and identity link functions while for $\delta^{13}C$ values models were fit using Gamma distributions and log link functions. All models were optimized using a forward selection procedure and the model with the lowest AIC (Akaike Information Criterion) was chosen (Burnham and Anderson 2002).

GLMs 1 were run considering two explanatory variables: (1) main environment used: the estuary (PLE dolphins) and coastal zone (SC and NC combined into a single coastal unit) and (2) sex (males and females). Season (cold: May–October and warm: November–April) could not be considered as an explanatory variable in this sample set as most of the coastal dolphin samples were from the warm season. Therefore, only samples representing the warm season were used in these models. Considering the estimated skin half-life of bottlenose dolphins of 24 days (± 8) and 47 days (± 19) for carbon and nitrogen isotopes, respectively (Giménez et al. 2016), samples were attributed to seasons by subtracting 3 months from the day of the biopsy. This prevents, for example, that a dolphin sampled at the start of the season would be erroneously classified, since its tissue corresponds to the isotopic signal of the previous season.

GLMs 2 were run to model the isotopic values of PLE dolphins as a function of season, sex, and period of sampling (2009–2012 and 2013–2016). This last variable was added to investigate if any significant change of isotope niche occurred over 4-year periods. The restriction of this analysis to only PLE dolphins is due to their high residence to the area, which should be a good representation of a top predator isotopic niche of the study area.

3.4.9 Isotopic Niche of Social Units

Stable isotope niches of the dolphins were calculated for dolphins from the PLE and the coastal units (SC and NC) combined as “coastal” (see Fig. 1B). Only samples from the warm season were included due to low samples sizes in the cold season for the coastal group, as a minimum of five samples are needed to calculate the ellipse areas (Jackson et al. 2011). The isotopic niches of the dolphins from the PLE were generated by seasons, periods, and sexes. Isotopic niche ellipses 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) and Bayesian standard ellipse areas (SEAB) were calculated using individual δ^{13}C and δ^{15}N values from the dolphin groups. To test whether one group's SEAB is smaller (or larger) than another, the probability that its posterior distribution is smaller (or larger) was calculated between pairs. Furthermore, the magnitude of the isotopic overlap among the pairs was calculated as percentage of the SEAc that overlapped. 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 analysed 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 analysed 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 ($K^*\varepsilon$) 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 nMDS analysis, although at its threshold of acceptable representation (stress ≤ 0.2; Kruskal 1964), 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 nMDS 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 carried out for skin samples of 40 adult individuals used in the social structure study, including 17 females and 23 males. Number of samples collected in each period, for each unit, and their respective isotopic composition are summarized in the Supplemental Material Table S1. The $\delta^{13}C$ and $\delta^{15}N$ values ranged from − 15.7 to − 13.3‰, and from 15.6 to 18.4‰, respectively (Table 6). The mixing polygon approach showed that all the PLE dolphins analysed were within the 95% mixing region (formed by the TDF-corrected isotopic values of the prey) (Figure 4A). For dolphins of the SC and NC units, some individuals were in the limit of the 95% mixing region (the outermost contour), thus indicating that the model fitted was not as good as for the PLE dolphins (Figure 4B and C). The fact that no consumers occurred outside the 95% mixing polygon suggests that adjustments (e.g., consumer exclusion, parameter correction, and model rejection) are not necessary, and the model representation is relatively robust.

The GLM1 that best fitted the $\delta^{15}N$ data included only the environment preferentially used by the dolphins (PLE and coastal) as a significant explanatory variable, where coastal dolphins showed higher nitrogen isotope values (Table 7). In the case of the PLE dolphins, GLMs2 that included period and sex as explanatory variables were the best fitted in the case of $\delta^{13}C$ values. In the case of $\delta^{15}N$ data, season was the only explanatory variable that had a significant effect on these isotopes, where samples representing the warm season had more ^{15}N-enriched values (Table 7).

The relative contribution of prey sources to the diet of PLE dolphins for each season, 2009–2012/2013–2016 periods, and for the warm season for SC and NC dolphins is presented in Figure 5. The relative contribution of the analysed preys is very similar to the diet of the different social units. For PLE dolphins, the whitemouth croaker (MF), banded croaker (PB), and cutlassfish (TL) increased their importance in the second period (2013–2016).

The isotopic niche of the PLE dolphins in the warm months was larger than that of coastal dolphins with a probability of 0.96. The overlap between these two groups represents 14% and 28% of the SEAc of the PLE and coastal dolphins, respectively (Table 8, Fig. 6A). Among PLE dolphins, the isotopic niche area was slightly narrower during the warm than in the cold months, with a probability of 0.74. The overlap between these two ellipses encompassed 40% and 29% of the warm and cold SEAc areas, respectively (Table 8, Fig. 6B). Considering the two periods analysed for the PLE dolphins, the ellipse area of the first period (2009–2012) was larger than that of the second period (2013–2016), with a probability of 0.99, and niches were completely segregated in the δ-space (Table 8, Fig. 6C). Males and females from the PLE showed similar niche areas and a high overlap area, which represents 62% and 67% of the SEAc area of females and males, respectively (Table 8, Fig. 6D).

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 fastSTRUCTURE (Figure 2) and nMDS (Figure 3) graphs, 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 4), 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

Different from the genetic analyses, the feeding ecology analysis can be strongly influenced by seasonal and interannual variation, requiring a larger sample size for each period. In this context, our database was possibly insufficient to evaluate some aspects of dolphins' feeding ecology, especially for the cold period. However, despite sampling biases towards the PLE social unit and towards warm months, with little sampling of coastal dolphins in the cold season, the stable isotope analysis allowed us to identify resource partitioning in the social units that use the Patos Lagoon estuary and its adjacent coastal waters. It is noteworthy that this isotopic difference exists even with a large spatial overlap between PLE and coastal dolphins in the adjacent marine coast. The isotopic signs of the SC and NC social units are similar, possibly explained by the homogeneity in the adjacent southern and northern coasts regarding the richness and abundance of prey throughout the year (Rodrigues and Vieira 2013). Dolphins from the PLE social unit, on the other hand, showed wider variability in both $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, with significant lower values of $\delta^{15}N$ than dolphins from the coastal units (SC and NC). Probably, this greater isotopic range is due to the use of the entire study area by PLE dolphins, while coastal dolphins are restricted to the marine environment. Furthermore, it seems that some prey with higher $\delta^{15}N$ is likely missing from the model proposed (Figure 4B and C).

The Patos Lagoon estuary exhibits extreme temporal and spatial variability in physical and chemical processes, salinity in particular (Möller et al. 2001), which may influence the isotopic composition of dolphin prey. Among dolphins' preferred prey, there are estuarine dependent marine species, such as the whitemouth croaker and lebranche mullet, and opportunistic or facultative estuarine marine species, such as the southern kingcroaker, the banded croaker, and the cutlassfish (Vieira et al. 1998). Therefore, the isotopic niche differentiation detected may be related to the prey's life stage and habitat use, which would probably reflect on prey's food items and, consequently, on their isotopic composition. In addition, the study area includes only a fraction of the home range of the coastal dolphins, so they may be feeding in areas distant from the influence of the estuary. Therefore, the feeding ecology of the PLE population has been well explored, but the coastal population feeding ecology needs further investigation, increasing the number of samples and comparing the isotopic signatures of fish collected within with fish collected outside the study area.

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 differences possibly driven by habitat-use patterns. It is not possible to determine what factor(s) has initially shaped this structure, but the presence of two populations suggests that genetic differentiation among social units may influence the dolphin social structure analysis performed previously for this region. Despite the large spatial overlap between populations in the coastal area, variation in the isotopic composition related to this differential use of the habitat and prey preferences were observed. The genetic differentiation observed among social units is consistent with the social structure, emphasizing the importance of social relationships in the composition of the population. Despite the significant range overlap, the genetic differentiation among the dolphin social units has arisen over a very fine spatial scale, demonstrating that sociality is important in shaping the population structure and should be considered in conservation and management 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 (Southern 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 δ^{13}C and δ^{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**	**Δ^{13}C(‰)**	**Δ^{15}N(‰)**	**N**	**Δ^{15}N(‰)**	**Δ^{15}N(‰)**	**N**	**Δ^{15}N(‰)**	**Δ^{15}N(‰)**
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 Generalized Linear Models (GLMs) with environment (Patos Lagoon Estuary-PLE population vs. Coastal Zone-CZ population) and sex (females and males) as predictors of the stable isotopes of carbon and nitrogen in skin of Lahille’s bottlenose dolphins.

Model	**Intercept (*p* value)**	**Environment (PLE) (*p* value)**	**Season (warm) (*p* value)**	**Period (2013-2016) (*p* value)**	**Sex (male) (*p* value)**	***df***	**AIC**
δ^{15}N~Envitonment PLE dolphins	17.84 (0.00)	- 0.67(0.00)	-	-	-	28	46.033
δ^{13}C~Period+sex	2.66 (0.00)	-	-	0.06 (0.00)	- 0.03 (0.02)	26	38.051
δ^{15}N~Season	16.58 (0.00)	-	0.59 (0.13)	-	-	26	47.661

GLMs for δ^{13}C and δ^{15}N values of the PLE dolphins using season (Cold and Warm months), sex and periods (2009–2012 vs. 2013–2016) as explanatory variables are also presented. Degrees of freedom (*df*) and Akaike Information Criteria (AIC) are shown. Only the selected models (lowest AIC) results are presented

Table 8. Convex hulls (CH), standard ellipse areas for small sample sizes (SEAc) and Bayesian standard ellipse areas (SEAB) and their respective 95% credibility intervals (CI) of Lahille's bottlenose dolphins from Patos Lagoon estuary (PLE) and adjacent coastal waters (coastal: Southern coast—SC and Northern coast—NC) in southern Brazil.

Group	CH	SEAc	SEAb (95% CI)
PLE	2.59	1.03	0.91 (0.57-1.52)
Coastal	0.90	0.53	0.44 (0.24-0.87)
PLE dolphins			
Season			
Warm	2.59	1.03	0.92 (0.58-1.54)
Cold	2.41	1.40	1.14 (0.54-2.38)
Period			
2009-2012	2.32	1.28	1.10 (0.55-2.13)
2013-2016	0.97	0.45	0.41 (0.24-0.71)
Sex			
Female	2.80	1.27	1.10 (0.60-2.01)
Male	2.16	1.19	1.02 (0.61-1.87)

All metrics are in (‰^{2})

3.10 FIGURES

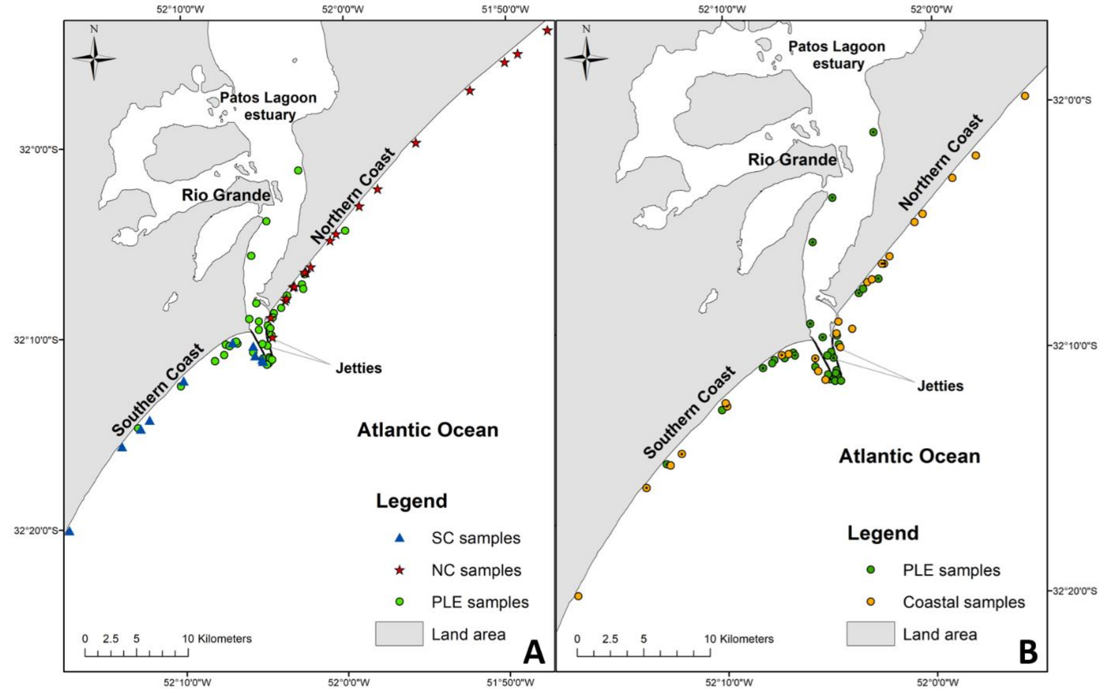

Figure 1. Biopsy sample locations of photo identified, adult, Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) in the Patos Lagoon estuary and adjacent coastal waters, southern Brazil, used for: A) genomic analysis (N= 49), specifying social unit memberships of sampled individuals, i.e., 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).

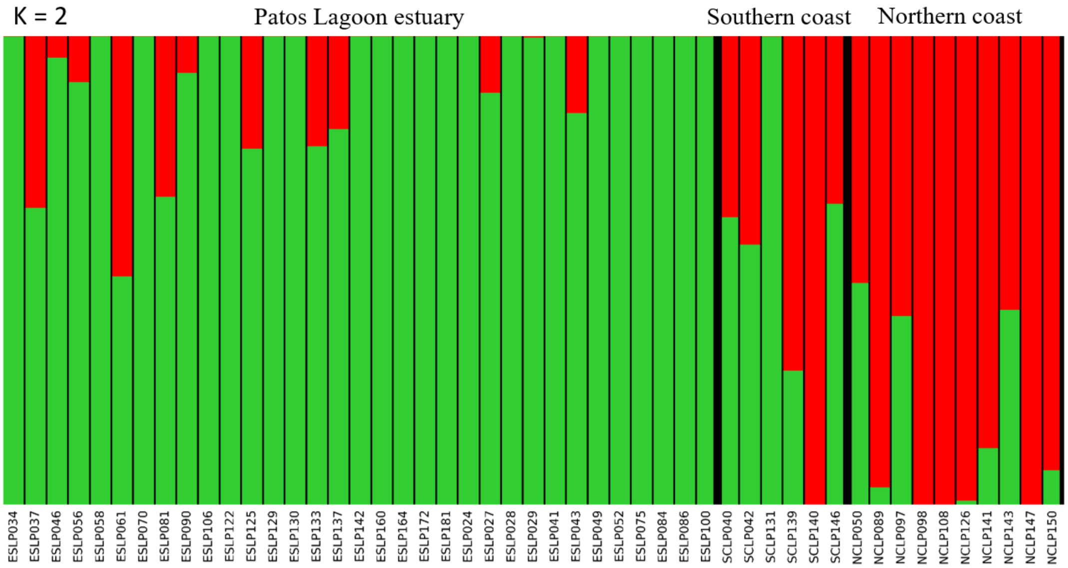

Figure 2. Bayesian clustering from fastSTRUCTURE for 49 Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) from three social units sampled in the Patos Lagoon estuary and its adjacent coastal waters, southern Brazil. The most likely number of genetic clusters in the data set was identified 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.

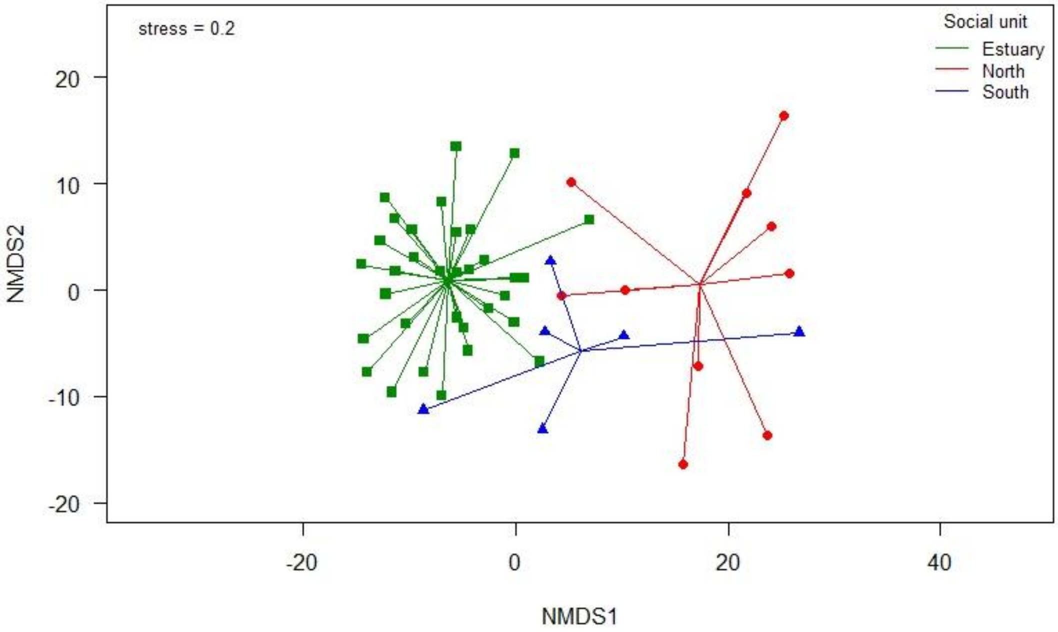

Figure 3. Nonmetric Multidimensional Scaling (nMDS) analysis based on 2,942 SNPs from 49 Lahille’s bottlenose dolphins (*Tursiops truncatus gephyreus*). Dots are colored according to the social unit which the individual belong to, i.e., Patos Lagoon estuary (green squares), Southern coast (blue triangles) and Northern coast (red circles).

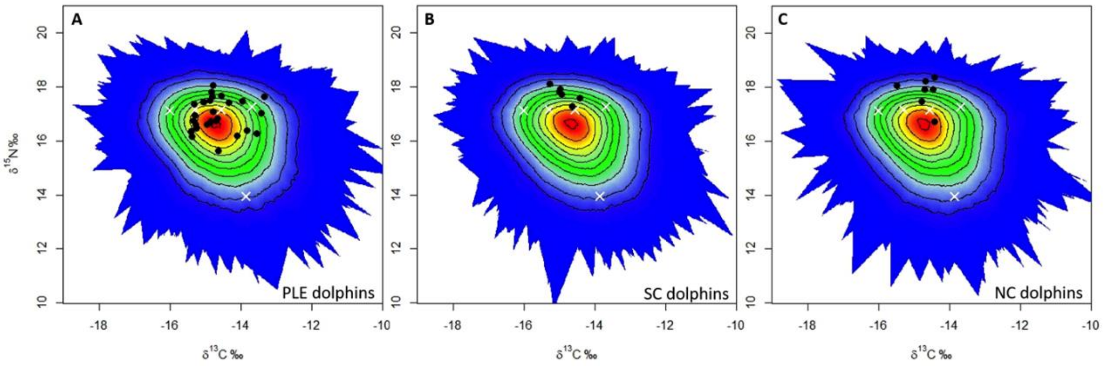

Figure 4. Simulated mixing polygons for Lahille's bottlenose dolphins sampled between 2009 and 2016 from A) the Patos Lagoon estuary social unit (PLE); B) the southern coast social unit (SC); and C) the northern coast social unit (NC). A set of trophic discrimination values for correcting prey isotopic 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.

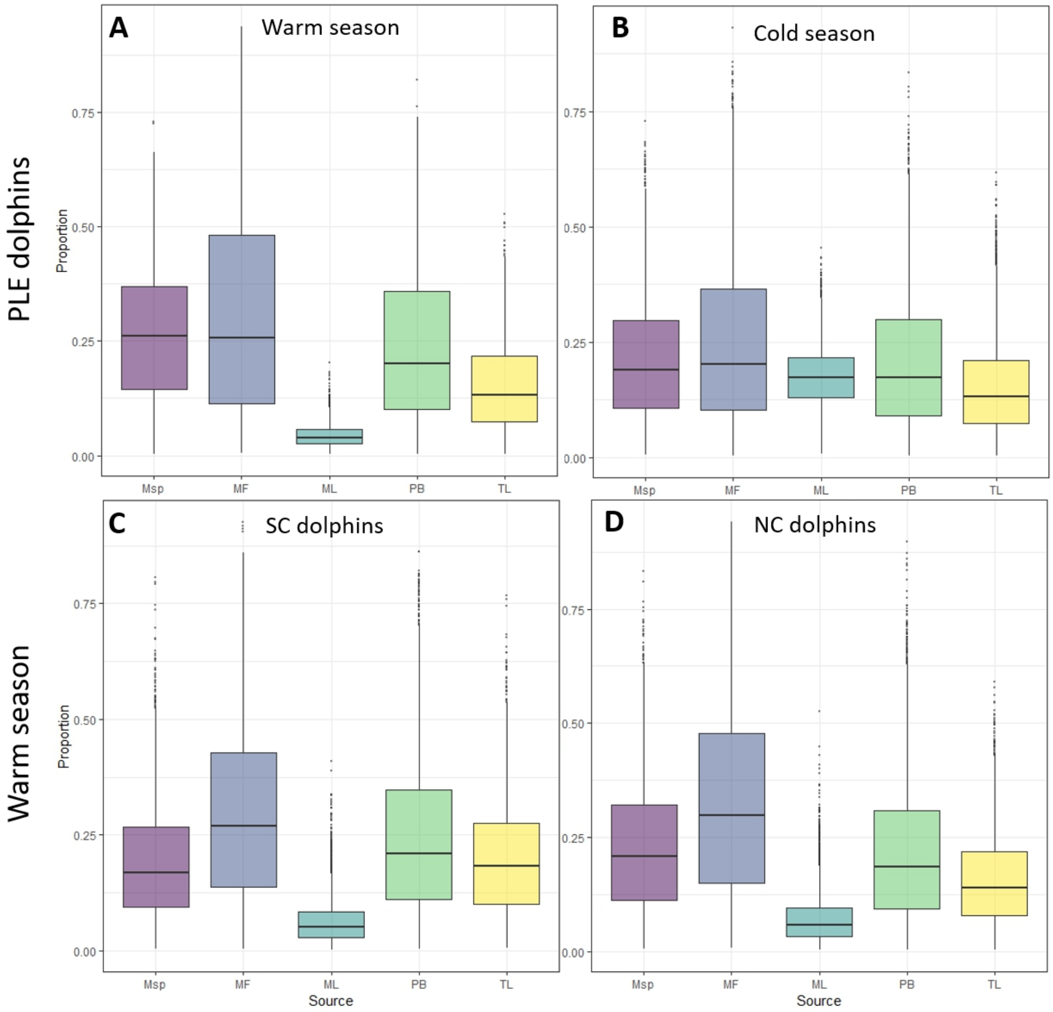

Figure 5. Relative contribution of prey sources to the diet of Lahille's bottlenose dolphin units that use the Patos Lagoon estuary (PLE social unit), adjacent southern (SC social unit) and northern coasts (NC social unit) for: Warm season (November – April) for PLE (A), SC (C), and NC (D) social units; 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*.

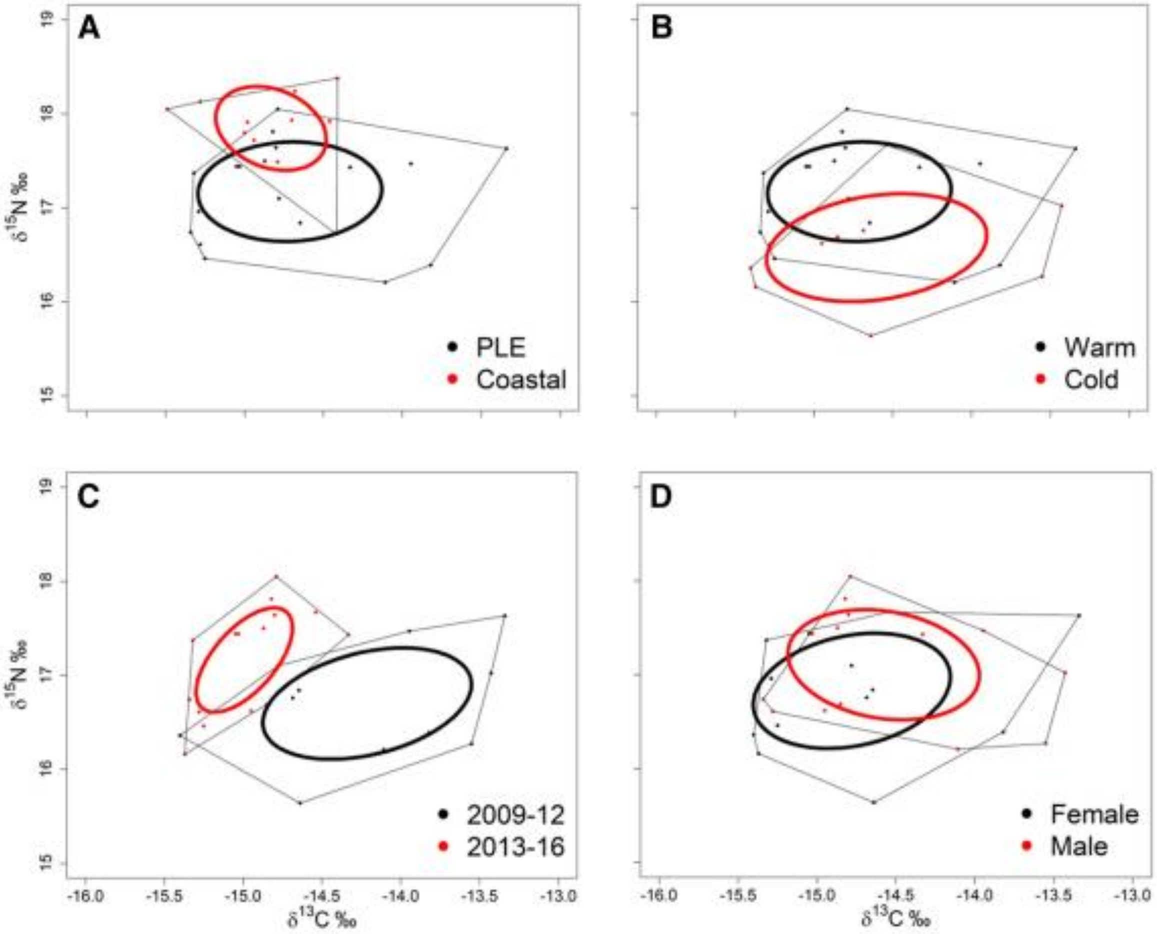

Figure 6. $\delta^{13}C$ and $\delta^{15}N$ isotopic niches of Lahille's bottlenose dolphins social units, *Tursiops truncatus gephyreus*, that use the Patos Lagoon estuary (PLE), southern (SC) and northern (NC) adjacent coastal waters, southern Brazil: A) for dolphins from social units that use the estuarine (PLE) and coastal adjacent areas (Coastal, including SC and NC) during the entire study (2009 – 2016) period and in the warm season (November – April); B) PLE dolphins in the cold (May – October) and warm seasons; C) PLE dolphins in the warm period of 2009-2012 and 2013-2016; and D) PLE females and males. 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).