



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

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Abstract

Varying levels of population structure may arise from interactions between intrinsic behavioral and demographic factors and extrinsic environmental factors. Social organization, habitat use, resource partitioning, or even individual preferences are putative drivers of population genetic differentiation over fine spatial scales. 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 three social units of bottlenose dolphins with strong home range overlap in a relatively small geographic area in southern Brazil. Results from model-based and model-free analyses of population structure supported the delineation of two populations, one with preferences for estuarine waters and another strictly coastal, consistent with isotopic niche differentiation. These findings suggest that genetic and ecological structuring is mainly driven by habitat use. At finer scale, we also detected low but significant genetic differentiation among the three social units. The outcomes of this study provide new insights into population structure of Lahille's bottlenose dolphins in the Patos Lagoon estuary and its adjacent coastal waters, which are exposed to increasing levels of anthropogenic disturbances, such as intensive artisanal fisheries, pollution, and boat traffic. Although for demographic studies, the estuary and the coastal dolphins should be treated separately, for conservation purposes, we recommend that the three social units be regarded as different entities.

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Introduction

Knowledge of genetic structuring of populations is an important basis for ecological and behavioral studies, in addition to informing and facilitating conservation actions (Bossart and Prowell 1998; Palsbøll 1999). Isolation by distance, when genetic similarity decays with increasing geographic distance between individuals or populations (Wright 1943), is a commonly observed pattern of genetic structure

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in terrestrial and marine organisms (Teske et al. 2018). However, both demographic and environmental factors, such as life-history, dispersal, disturbance and vicariance events, population size, social organization, habitat connectivity, and resource distribution, are often drivers of fine-scale population structure (SurrIDGE et al. 1999; Beheregaray and Sunnucks 2001; Fraser et al. 2004; Archie et al. 2008; Worthington Wilmer et al. 2008; Grummer et al. 2019).

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

Estuaries, where fresh water meets seawater, are among the most productive and heterogenic ecosystems in the world and often host populations of bottlenose dolphins (e.g., *Tursiops truncatus gephyreus*: Simões-Lopes and Fabian 1999; *T. aduncus*: Fury and Harrison 2008; *T. truncatus*: Mazzoil et al. 2008). The unique environmental features of estuaries, by themselves, are known to drive adaptive divergence and genetic differentiation (e.g., Beheregaray and Sunnucks 2001; Watts and Johnson 2004). Furthermore, the estuarine environment differs significantly from its adjacent coastal environment, both in physicochemical properties and in abundance and diversity of prey, providing subsidies for genetic segregation (e.g., Möller et al. 2007). This habitat heterogeneity can also induce individuals that share the same preference for an area, resource, environment, feeding strategy, or even social preferences to segregate in social groups (e.g., Wiszniewski et al. 2009a; Möller et al. 2011; Daura-Jorge et al. 2012; Ansmann et al. 2014) and, therefore, reinforce the genetic differentiation. 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 powerful for this purpose (Liu et al. 2005; Gaughran et al. 2018).

Bottlenose dolphins, *Tursiops* spp., inhabit estuaries and their adjacencies worldwide, usually having small population

sizes and showing high degrees of site fidelity to these areas (Wells et al. 1987). These dolphins are top predators mainly classified as generalists, with individuals within populations showing plasticity in feeding habits according to spatial and temporal patterns of prey availability (e.g., Barros and Wells 1998). The Lahille's bottlenose dolphin (*T.t. gephyreus*) is a subspecies of bottlenose dolphins that inhabits estuaries and coastal waters of the southwestern Atlantic Ocean, from southern Brazil to Argentina. Lahille's bottlenose dolphins were delineated into several Management Units (MUs) throughout their range with different degrees of gene flow (Fruet et al. 2014), and negligible gene flow to common bottlenose dolphins (*T.t. truncatus*; Fruet et al. 2017).

Relatively large numbers of Lahille's bottlenose dolphins (203 individuals cataloged between 2005 and 2015) inhabit the Patos Lagoon estuary (PLE) and its adjacent coastal waters (CZ) in southern Brazil (Fruet et al. 2011, 2015a), where they present a complex pattern of social structure and habitat use. A recent study showed that in this region, the species is socially structured into different social units mainly driven by spatiotemporal use of the area and social gregariousness (Genoves et al. 2018). In an association-based perspective, there is a social unit of approximately 90 individuals (Fruet et al. 2015a) resident in the PLE and its adjacent marine coast, and two units strongly associated with the coastal zone, one in the southern coast (SC) and the other in the northern coast (NC). There are a 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, residents and temporary dolphins compose the coastal units. 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 period of mating activities and calving (Fruet et al. 2015b). 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. 2015b). Furthermore, it is likely that the differential habitat usage patterns reflect variation in resource access and utilization that reinforces within-population structuring, and possibly reduces competition among social units.

In recent years, the use of stable isotopes analysis (SIA) of carbon and nitrogen to investigate the trophic and spatial ecology of top predators has increased worldwide (see reviews by Hobson 1999; Kelly 2000; Newsome et al. 2010), including marine mammals in the Southwestern Atlantic (Seyboth et al. 2018). The $\delta^{13}\text{C}$ is informative of the base of the food chain, and since it does not change markedly between trophic levels (ca. 1‰), it 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, $\delta^{15}\text{N}$ vary approximately from 3 to 5‰ between trophic levels and, therefore, it is a useful indicator of trophic position (DeNiro and Epstein 1981) and feeding habitat (Chouvelon et al. 2012). 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 dolphins inhabiting the Patos Lagoon and adjacent marine coast. Based on stomach contents and stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) from tooth dentine of stranded individuals, but initially considering these individuals as a single social unit, clear seasonal and decadal variations (1977–1980 to 2002–2012) in their feeding ecology were demonstrated (Secchi et al. 2016). However, it is unknown whether differences in feeding ecology were due to individual variation or the inclusion of dolphins from different social units in the sample. In addition, the Patos Lagoon estuary presents a large interannual variation in productivity due to factors that affect its hydrological regime, which is mainly related to climatic phenomena (Garcia et al. 2003, 2007; Teixeira-Amaral et al. 2017). Resident dolphins using the estuary thus provide an excellent opportunity to investigate the isotopic niche of these near-top predators over the years.

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 test for fine-scale genetic structure and habitat segregation of known social units. Our assessment of population structure in this system provides an opportunity to understand links between habitat use, feeding preferences, social organization, and genetic differentiation in coastal dolphins. Clarifying these associations is particularly important for resident bottlenose dolphins inhabiting embayment and estuaries, since these environments are often under strong and localized anthropogenic pressures.

Materials and methods

Study area

The Patos Lagoon Estuary (PLE), located approximately between 31°58' S and 32°12' S, is characterized by shallow bays (< 2 m in depth), a narrow navigation channel that can reach up to 20 m deep, and it is connected to the Atlantic Ocean by two jetties of 4.6 and 3.8 km in length. The PLE and its adjacent marine coast is a very productive environment that hosts abundant assemblages of fish (Garcia et al. 2012; Rodrigues and Vieira 2013). The estuary is also an important nursery ground for several fish species that sustain

an extensive artisanal and commercial fishery (Haimovici and 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 and Calliari 2006).

Sample collection

Skin samples were collected from Lahille's bottlenose dolphins during photo-identification surveys carried out from January 2009 to September 2016 onboard a 5 m-long inflatable boat powered with a 90 hp outboard engine. Samples were taken in the estuary and the adjacent marine coast (Fig. 1). 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. Dolphins were sampled using modified darts specifically designed for small cetaceans (F. Larsen, Ceta-Dart) fired from a 120 lb draw weight crossbow. To minimize the wound, only individuals older than 3 years of age (i.e., independent individuals, see Fruet et al. 2015b) were biopsied as they hold large body mass and 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 (Amos and Hoelzel 1991) and stored at $-20\text{ }^{\circ}\text{C}$, and those for stable isotopes analysis (SIA) were frozen.

Assigning dolphins to units

Previous social and spatiotemporal analysis 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 analyzed 102-catalogued frequently sighted dolphins and identified three social divisions, based on associations with a strong spatial component, which clustered individuals that preferentially use the same subarea (PLE, SC, and NC). Thereby, approximately 65 of these individuals used the PLE and adjacent coastal waters and 37 of these 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 genomic and stable isotopes analyses were restricted to samples collected from these 102 dolphins and used their respective social characterizations.

Genomic methods and bioinformatics

Genomic DNA was extracted using a modified salting-out protocol (Sunnucks and Hales 1996). The DNA quality was

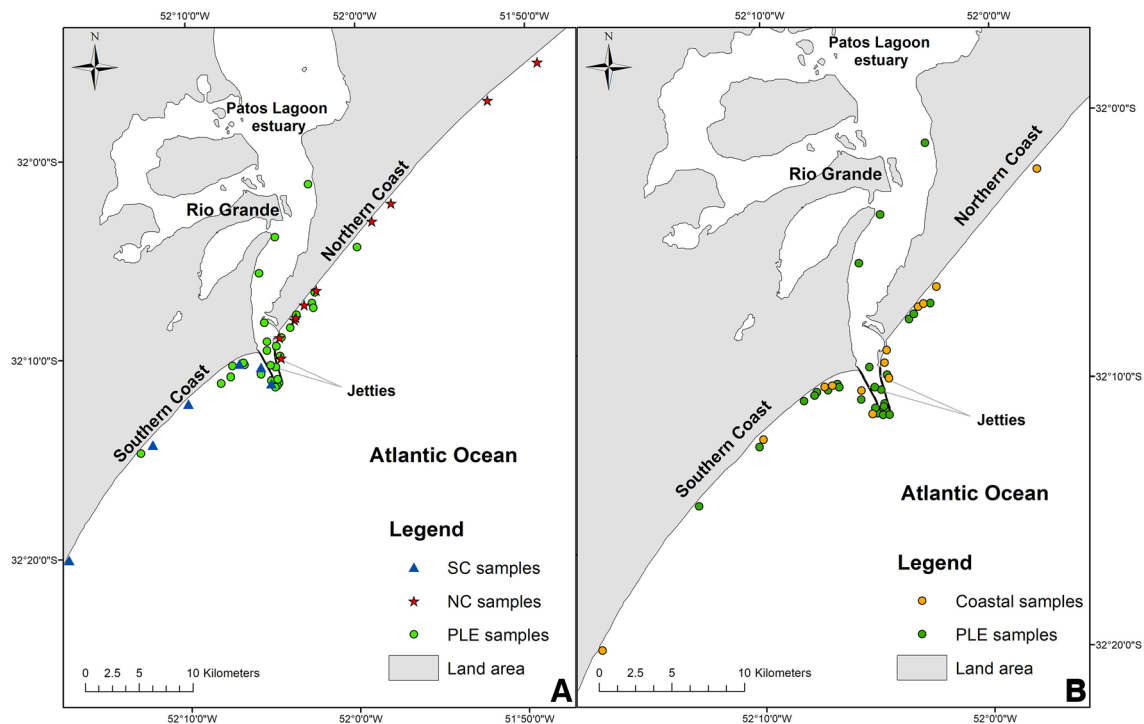


Fig. 1 Biopsy sample locations of photo-identified, adult, Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) in the Patos Lagoon estuary and adjacent coastal waters, southern Brazil, used for: **a** genomic analysis ($N=49$), specifying social unit memberships of

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)

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 ninety-six 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 between 250 and 800 bp were selected using a Pippin Prep (Sage Science) and amplified using PCR. Libraries were pooled in equimolar concentrations, and then, 96 samples per lane of Illumina HiSeq 2000 (100 bp, single end reads) were sequenced at the South Australian Health and Medical Research Institute (SAHMRI).

Raw sequences for all available samples were demultiplexed using the *process rad-tags* in STACKS 1.19 (Catchen et al. 2013), allowing a maximum of two mismatches in the RAD tags (restriction enzyme recognition sequence) and barcodes (barcodes are unique up to two mismatches). 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. This process used a minimum coverage of $15\times$ and a maximum of 12 mismatches were allowed to form reference contigs. A Bayesian-based variant detection approach, FREEBAYES (Garrison and 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 and coverage depth, and to control for Hardy–Weinberg (HW) equilibrium and Linkage disequilibrium (LD) of the SNPs in the data set (see Table 1), following the procedure detailed and justified in previous studies (Sandoval-Castillo et al. 2018; Batley et al. 2019). The final step retained only the samples from individuals of the 102 previously analyzed the social structure study.

Genomic diversity and population structure analysis

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

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	8067
2. Read depth (\leq mean depth + (2 * standard deviation))	6393
3. Read quality (ratio quality/coverage depth > 0.2)	5557
4. Allele balance ($> 20\%$ and $< 80\%$)	5370
5. Hardy–Weinberg equilibrium in at least 2 populations	4056
6. Present in 75% of individuals in 75% of populations	4052
7. Single SNP per locus	3047
8. Linkage disequilibrium ($r^2 < 0.8$)	2942

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 of their genotypes belonging to different populations. Using the simple prior model, ten independent runs for each K value (K tested from 1 to 10) were completed to ensure consistency. The most likely number of clusters (K) was chosen based on the optimal model complexity measure ($K^* \epsilon$) and the number of relevant model components ($K^* \phi^C$) (Raj et al. 2014). These values should match to the true K when population structure is strong (Raj et al. 2014). If a value greater than 1 was detected for K , it was verified if the genomic division corresponded to the social division of the population. In case of inconsistency between these K measures, the value of K based on the lowest cross-validation error (CV error), from ADMIXTURE (Alexander et al. 2009), was used. Additionally, principal component analysis (PCA) was performed using the R package ADEGENET 2.1 (Jombart and Ahmed 2011). PCA was used to visualize how much the genomic differentiation topology resembled 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.

C and N stable isotope analysis

For the stable isotope analysis (SIA), only 40 adults from the 102 dolphins used in the social structure study were included. Dolphin skin samples were rinsed with distilled water, dried at 60 °C for 48 h, 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 $\delta^{13}\text{C}$ 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 analyzed 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 ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) in each sample, as well as international standards, were calculated to obtain individual isotopic composition according to the formula:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1,$$

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 $< 0.2\text{‰}$ for both isotope values.

Prey contribution

Bayesian stable isotope mixing models are a tool used to identify proportional contributions of prey sources to the consumer diet 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 white-mouth croaker, *Micropogonias furnieri* (MF); the lebranche mullet, *Mugil liza* (ML); the banded croaker, *Paralichthys*

brasiliensis (PB); and the cutlassfish, *Trichiurus lepturus* (TL) (Secchi et al. 2016). The isotopic composition of these main preys was extracted from Secchi et al. (2016), supplemented with some samples collected between 2011 and 2015 and processed according to Secchi et al. (2016) (Table 2). There was no specific procedure for prey collection; however, samples were obtained from the local fishery that operates within the dolphins' core area of distribution. Estimates for trophic discrimination factors (TDFs) for skin samples of common bottlenose dolphins were described by Giménez et al. (2016) and were used in the models ($\Delta^{13}\text{C} = 1.01 \pm 0.37\text{‰}$ and $\Delta^{15}\text{N} = 1.57 \pm 0.52\text{‰}$). A total of 1500 iterations of a Monte Carlo simulation of mixing polygons (Smith et al. 2013), using the packages *sp* and *splanx* in R, were performed to verify the validity of the mixing model for describing the consumers' diet composition. Model validation is straightforward and accomplished when all consumers are within the 95% mixing region (Smith et al. 2013). Finally, mixing models were run to estimate the contribution of prey samples to the dolphins' diet using the *simmr* package (Parnell 2016).

Stable isotope data analysis

Variables affecting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the skin of adult Lahille's bottlenose dolphins were analyzed using generalized linear models (GLMs). Separate GLMs were used to model the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the Lahille's bottlenose dolphins. The fit of the models to $\delta^{15}\text{N}$ values were performed using Gaussian distributions and identity link functions while for $\delta^{13}\text{C}$ 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 use: 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 a 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.

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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 overlapped. All analyses were carried out in the R 3.4.3 statistical environment (R Core Team 2017).

Table 2 Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (\pm 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	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
		Mean \pm SD	Mean \pm SD
<i>Menticirrhus</i> sp.	26	-14.7 ± 0.9	15.7 ± 0.4
<i>Micropogonias furnieri</i>	17	-15.5 ± 0.5	15.6 ± 0.6
<i>Mugil liza</i>	18	-14.8 ± 1.1	12.4 ± 0.9
<i>Paralichthys brasiliensis</i>	26	-16.3 ± 0.8	15.6 ± 0.6
<i>Trichiurus lepturus</i>	15	-17.0 ± 0.7	15.5 ± 0.7

Results

A total of 148 biopsy samples were collected concomitantly with dorsal fin photographs. From the 102-catalogued dolphins, which were previously analyzed regarding the social structure, 62 were biopsied. Due to storage, extraction or sequencing issues, four of them were not included in the subsequent analyzes and it was not possible to use all the

remaining 58 samples for both procedures. Finally, 49 of these dolphins were used in the genomic analysis and 40 in the stable isotope analysis (Table 3; Fig. 1), with 34 dolphins subjected to both genetic and isotopic analyses.

From the Illumina sequencing of all samples with high-quality DNA extractions ($n = 108$), 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). The average coverage was 20.71, the final minimum read depth per locus was $8\times$ and the average error rate between replicates was 7.8%. Four samples were later removed from the data set, 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 HW and Linkage disequilibrium, 2942 SNPs were retained (Table 1). Finally, we excluded 55 individuals that could be mistakenly assigned to the proposed populations (PLE, SC, or NC) and retained 49 well-known photo-identified dolphins that were previously analyzed regarding the social structure and were subsequently used for population structure analysis.

Genomic diversity within social units

Estimates of genomic diversity based on the 2942 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. 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).

Population structure

The Bayesian clustering analysis inferred between two ($K^*\epsilon$) and three ($K^*\phi^C$) genetic clusters (Fig. 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 other coastal, represented by individuals from SC and NC social units, which exclusively inhabit the coastal zone (CZ population). Although the results suggest two populations, there is some level of admixture between PLE and CZ clusters (Fig. 2). The PCA analysis also corroborated this delineation (Fig. 3). Pairwise comparisons of genomic differentiation measured by F_{ST} showed a moderate (F_{ST} values < 0.1), significant genomic differentiation ($P < 0.0001$) between the two identified populations in the fastSTRUCTURE and PCA analysis (Table 5). F_{ST} values were also significant between social units, being higher between PLE and NC, followed by PLE and SC and, finally, SC and NC (see Table 5). The H_O was also higher than the H_E in the CZ population (SC and NC together) (Table 4).

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 analyzed were within the 95% mixing region (formed by the TDF-corrected isotopic values of the prey) (see Supplemental Material Fig. S1a). 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

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 2942 SNPs

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
SD	0.22/0.16	0.22/0.13	0.20/0.14	0.19/0.14

H_O observed heterozygosity, H_E expected heterozygosity, SD the standard deviation

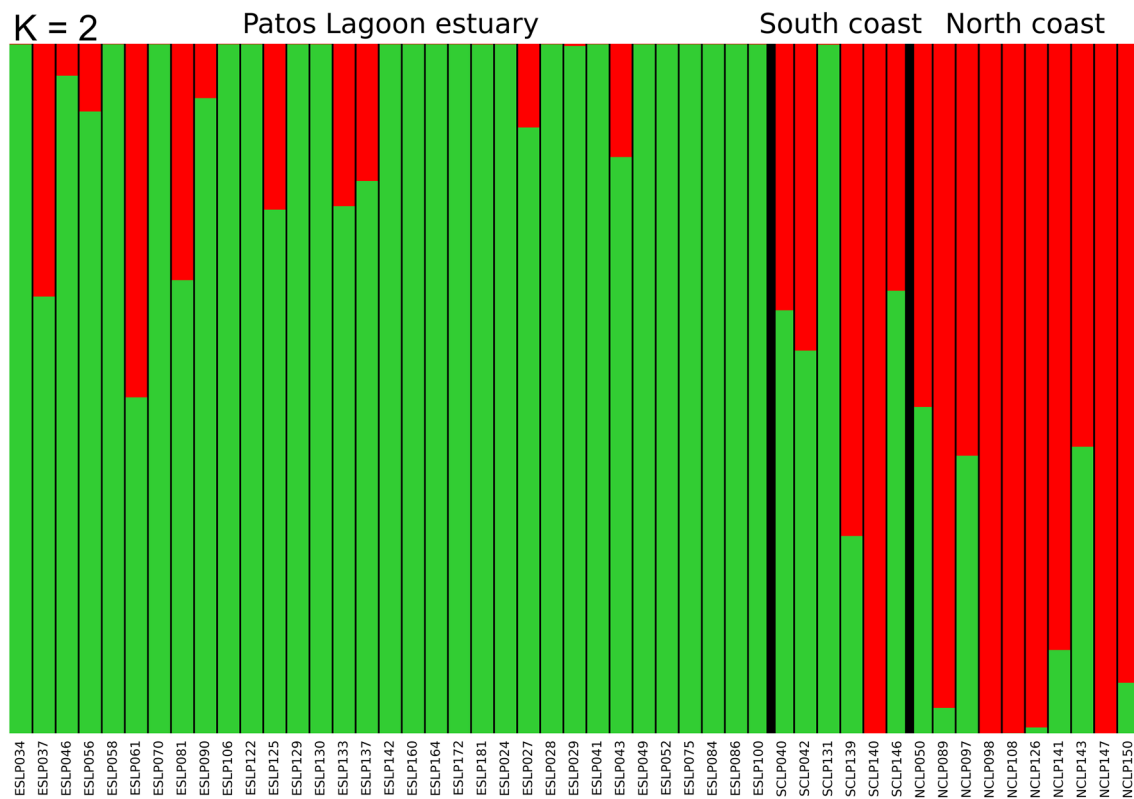


Fig. 2 Bayesian clustering from fastSTRUCTURE for 49 Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) from three social units sampled in the Patos Lagoon estuary and its adjacent coastal waters, southern Brazil. The most likely number of genetic clusters

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

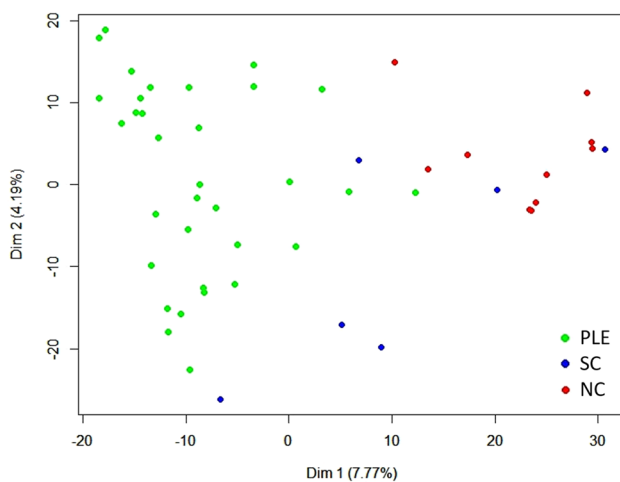


Fig. 3 Principal Component Analysis based on 2942 SNPs from 49 Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*). Dots are colored according to the social unit which the individual belongs to, i.e., Patos Lagoon estuary (PLE), Southern coast (SC), and Northern coast (NC)

not as good as for the PLE dolphins (Supplemental Material Fig. S1b 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 $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ values. In the case of $\delta^{15}\text{N}$ 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 Supplemental Material Fig. S2. The relative contribution of the analyzed preys is very similar to the diet of the different social units. For PLE dolphins, the whitemouth croaker (MF), banded croaker (PB), and

Table 5 Estimates of genomic differentiation (expressed as F_{ST}) of Lahille’s bottlenose dolphins (*Tursiops truncatus gephyreus*) based on 2942 SNPs between the population that use the estuary (PLE) and the population that use the coastal waters (CZ)

Populations comparison	PLE		CZ		Social units’ comparison		
	PLE	CZ	PLE	CZ	PLE	SC	NC
PLE	–	<0.00001	PLE	–	<0.00001	<0.00001	<0.00001
CZ	0.0538	–	SC	0.0368*	–	–	<0.00001
			NC	0.0628*	0.0184*	0.0184*	–

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

* $p < 0.1$

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

Unit	2009–2016			Cold season			Warm season		
	<i>N</i>	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	<i>N</i>	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	<i>N</i>	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
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 ± 0.3	0	–	–	7	– 14.7 ± 0.4	18.0 ± 0.3
Total	40	– 14.7 ± 0.5	17.2 ± 0.6	11	– 14.6 ± 0.6	17.3 ± 0.6	29	– 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 (<i>p</i> value)	Environment (PLE) (<i>p</i> value)	Season (warm) (<i>p</i> value)	Period (2013–2016) (<i>p</i> value)	Sex (male) (<i>p</i> value)	<i>df</i>	AIC
$\delta^{15}N \sim$ Environment	17.84 (0.00)	– 0.67(0.00)	–	–	–	28	46.033
PLE dolphins							
$\delta^{13}C \sim$ Period + sex	2.66 (0.00)	–	–	0.06 (0.00)	– 0.03 (0.02)	26	38.051
$\delta^{15}N \sim$ Season	16.58 (0.00)	–	0.59 (0.13)	–	–	26	47.661

GLMs for $\delta^{13}C$ and $\delta^{15}N$ 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

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. 4a). 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. 4b). Considering the two periods analyzed 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. 4c). 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. 4d).

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 Southwestern 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, particularly in coastal dolphins (Möller et al. 2007; Hollatz et al. 2011; Ansmann et al. 2014). We found evidence for two genetic populations of Lahille’s bottlenose

Table 8 Convex hulls (CH), standard ellipse areas for small sample sizes (SEAc) and Bayesian standard ellipse areas (SEA_B) 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	SEA _B (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 (%c²)

dolphins in southern Brazil primarily associated with 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 exchange of migrants and gene flow. The differential use of habitat by each population also resulted in distinct isotopic niches, with the coastal dolphins showing higher nitrogen isotopic values than those inhabiting the estuary.

Fine-scale population structure

About 60% of the individuals used in the social structure study were sampled: 33 out of 65 samples were collected from PLE, 6 out of 18 from SC, and 10 out of 19 from NC. This data set can be considered a good representation of the population structure analysis given the difficulty of collecting samples from specific animals (photo-identified), especially in an area of predominantly turbid waters, where dolphins can only be observed when out of the water. For the coastal dolphins, which inhabit an area of difficult effort sampling (wave incidence and more susceptible to high winds), are more boat-shy than the PLE dolphins and, most of them, are only sighted temporarily in the study area, it is even more difficult to collect samples. This population structure analysis was extremely conservative, using only adult individuals which underwent analysis from a robust social study, but it is noteworthy that we also run exploratory analyzes using all available samples and the results followed the same pattern. A potential influence could come from relatedness between some dolphins within populations.

However, our photo-identification control based on a data set that covers nearly uninterrupted 15 years of sampling did not detect any close kin relationship among the 49 dolphins analyzed and only four first-order relationships (3 PLE–PLE and 1 SC–SC) were found by exploratory relatedness analyzes.

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 adjacent 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 or frequent movements between units, 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 dolphins essential for maintaining a social link between units that could translate to gene flow and increase genetic diversity.

The home range of dolphins of the CZ population is 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 in Uruguay (URU) and another along to the coast to the north (NLP), which can 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 is almost the same as between the PLE and CZ populations ($F_{ST}=0.066$ versus $F_{ST}=0.054$). The PLE is a birth pulse population, with most births occurring during late spring and summer (Fruet et al. 2015b), the same period (warm period) in which temporary individuals appear in the northern coast. On the other hand, temporary individuals that appear in the southern coast, many of them belonging to the URU population (Laporta et al. 2016), do it during the cold period, which is when the PLE dolphins

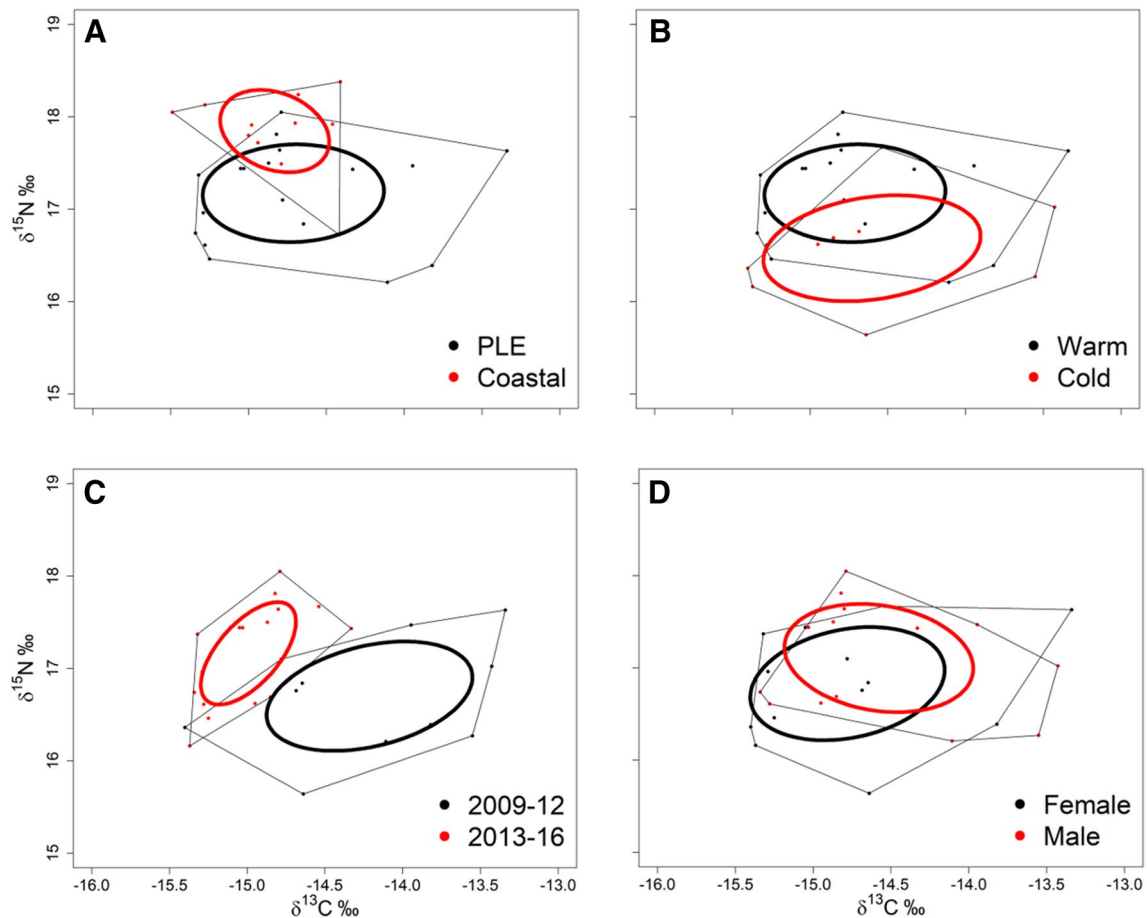


Fig. 4 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic niches of Lahille's bottlenose dolphins, *Tursiops truncatus gephyreus*, that use the Patos Lagoon estuary (PLE), southern (SC), and northern (NC) adjacent coastal waters, southern Brazil: **a** for dolphins from social units that use the 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 (Stable Isotope Bayesian Ellipses in R, Jackson et al. 2011)

are in apparent reproductive rest. This greater genetic differentiation between the PLE and URU populations may be due to the mismatch of reproductive periods. Nonetheless, it is important to highlight the role of the CZ population as potentially connecting the PLE population and these neighboring populations.

Genomic characterization of social units

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 km²) 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 are consistent with patterns observed

in the social structure analyses; that is, the NC unit is relatively more segregated from the other two populations, while the PLE unit has more associations with the SC unit. This sociogenetic pattern is highlighted in the PCA (Fig. 3) and fastSTRUCTURE graphs (Fig. 2), where SC dolphins present a closer genetic affinity to the PLE dolphins.

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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes, with significant lower values of $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ is likely missing from the model proposed (Supplemental Material Fig. S1b 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 comprehends 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 inside with fish collected outside the study area.

Ecology and population structure

Resource specialization may be one 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–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.

2012a; 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 and Rosenzweig 1981). More than that, niche partitioning is considered important for the maintenance of species diversity (Chesson 2000; Levine and HilleRisLambers 2009), and is an important driver of genetic differentiation (Möller et al. 2007; Wiszniewski et al. 2009b), including in populations from the Patos Lagoon and its adjacent coastal zone (Beheregaray and Sunnucks 2001). In a larger geographic scale, several studies have shown segregation in spatial and/or habitat-type use promoting significant genetic differentiation in dolphin populations (Natoli et al. 2005; Bilgmann et al. 2007; Wiszniewski et al. 2009b; Louis et al. 2018). However, in only a few populations (i.e., Möller et al. 2007; Ansmann et al. 2012b, 2014), this pattern was described for small spatial scales as observed in our study. The study area is characterized by high productivity and this niche partitioning between populations potentially contributes for the higher known concentrations of Lahille's bottlenose dolphins, including the capacity to receive temporary individuals into the region in both warm and cold periods.

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). Regarding sex, there is evidence that female dolphins prioritize the inner estuary use with approximately two PLE females to one PLE male (Fruet et al. 2015b), while the proportion of PLE males (mainly juveniles) is significantly higher among stranded dolphins along the adjacent marine coast (Fruet et al. 2010). Although non-significant, the small difference between PLE females and males may be related to females feeding more frequently in the inner estuary (increasing their carbon sign variance due to freshwater influence), while males might spend more time feeding in the open coast, increasing its $\delta^{15}\text{N}$ values. Furthermore, it was observed a difference in the isotopic niche of the PLE dolphins between 2009–2012 and 2013–2016 periods, which may be related to important changes in the composition of the producers due to the influence of meteorological events. 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. The first period of study was marked by a strong event of La Niña in 2010–2011, and the second by a very strong event of El Niño in 2015–2016. In addition, besides the warm and cold seasons driving the isotopic signal of dolphins, these meteorological events may also have a significant influence on the isotopic niche variation of the PLE dolphin social unit. Within the unit, it is expected that dolphins that are strongly associated will exhibit similar patterns of habitat use and feeding behavior, and hence show more ecological similarities (lower variance in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) if compared to dolphins with weak 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 the effects of confounding factors, such as variation in oceanographic conditions and prey dynamics (e.g., relative abundance and assemblage composition) that may influence stable isotopes values. As the present study found niche partitioning on a finer scale than the previous study (Secchi et al. 2016), it may be that these populations' feeding ecology is more complex than described to date.

Conclusion

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 in 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, probably, 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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Data accessibility Analysis reported in this article can be reproduced using the data provided in the Figshare repository: <https://figshare.com/s/2ddb4f0b338f5437e280>.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval 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.

References

- Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 19:1655–1664. <https://doi.org/10.1101/gr.094052.109>
- Amos B, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. *Rep Int Whal Comm (Spec Issue)* 13:99–103
- Ansmann I, Parra GJ, Chilvers B, Lanyon J (2012a) Dolphins restructure social system after reduction of commercial fisheries. *Anim Behav* 84:1–7. <https://doi.org/10.1016/j.anbehav.2012.06.009>
- Ansmann IC, Parra GJ, Lanyon JM, Seddon JM (2012b) Fine-scale genetic population structure in a mobile marine mammal: inshore bottlenose dolphins in Moreton Bay, Australia. *Mol Ecol* 21:1–14. <https://doi.org/10.1111/j.1365-294X.2012.05722.x>
- Ansmann IC, Lanyon JM, Seddon JM, Parra GJ (2014) Habitat and resource partitioning among Indo-Pacific bottlenose dolphins in Moreton Bay, Australia. *Mar Mamm Sci*. <https://doi.org/10.1111/mms.12153>

- Archie EA, Maldonado JE, Hollister-Smith J, Poole JH, Moss CJ, Fleischer RC, Alberts SC (2008) Fine-scale population genetic structure in a fission–fusion society. *Mol Ecol* 17:2666–2679. <https://doi.org/10.1111/j.1365-294X.2008.03797.x>
- Barros N, Wells R (1998) Prey and feeding patterns of resident bottlenose dolphins (*Tursiops truncatus*) in Sarasota bay, Florida. *J Mammal* 79:1045–1059. <https://doi.org/10.2307/1383114>
- Batley K, Sandoval-Castillo J, Kemper C, Attard C, Zanardo N, Tomo I, Beheregaray LB, Möller LM (2019) Genome-wide association study of an unusual dolphin mortality event reveals candidate genes for susceptibility and resistance to cetacean morbillivirus. *Evol Appl* 12:718–732. <https://doi.org/10.1111/eva.12747>
- Beheregaray LB, Sunnucks P (2001) Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odonesthes argentinensis*. *Mol Ecol* 10:2849–2866. <https://doi.org/10.1046/j.1365-294X.2001.t01-1-01406.x>
- Bilgmann K, Möller LM, Harcourt RG, Gibbs SE, Beheregaray LB (2007) Genetic differentiation in bottlenose dolphins from South Australia: association with local oceanography and coastal geography. *Mar Ecol Prog Ser* 341:265–276
- Bohonak AJ (1999) Dispersal, gene flow, and populations structure. *Q Rev Biol* 74:21–45
- Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Tree* 13:202–206
- Brauer CJ, Hammer MP, Beheregaray LB (2016) Riverscape genomics of a threatened fish across a hydroclimatically heterogeneous river basin. *Mol Ecol* 25:5093–5113. <https://doi.org/10.1111/mec.13830>
- Burnham KP, Anderson DR (2002) model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York
- Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. *Mol Ecol* 22:3124–3140. <https://doi.org/10.1111/mec.12354>
- Chesson P (2000) Mechanisms of maintenance of species diversity. *Annu Rev Ecol Syst* 31:343–366. <https://doi.org/10.1146/annurev.ecolsys.31.1.343>
- Chilvers B, Corkeron P (2001) Trawling and bottlenose dolphins' social structure. *Proc R Soc Lond B Biol Sci* 268:1901–1905. <https://doi.org/10.1098/rspb.2001.1732>
- Chouvelon T, Spitz J, Caurant F, Mèndez-fernandez P, Chappuis A, Laugier F, Le Goff E, Bustamante P (2012) Revisiting the use of $\delta^{15}N$ in meso-scale studies of marine food webs by considering spatio-temporal variations in stable isotopic signatures—the case of an open ecosystem: the Bay of Biscay (North-East Atlantic). *Prog Oceanogr* 101:92–105. <https://doi.org/10.1016/j.pocena.2012.01.004>
- Connor R, Wells RS, Mann J, Read AJ (2000) The bottlenose dolphin: social relationships in a fission–fusion society. In: Mann J, Conner RC, Tyack PL, Whitehead H (eds) Cetacean societies, field studies of dolphins and whales. University of Chicago Press, Chicago, pp 91–126
- Daura-Jorge F, Cantor M, Ingram SN, Lusseau D, Simões-Lopes P (2012) The structure of a bottlenose dolphin society is coupled to a unique foraging cooperation with artisanal fishermen. *Biol Lett*. <https://doi.org/10.1098/rsbl.2012.0174>
- Day JW, Crump BC, Kemp WM, Yanez-Arancibia A (2012) Estuarine ecology, 2nd edn. Wiley, New York
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 45:341–351. [https://doi.org/10.1016/0016-7037\(78\)90199-0](https://doi.org/10.1016/0016-7037(78)90199-0)
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10:564–567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Figueiredo SA, Calliari LJ (2006) Sedimentologia e suas Implicações na Morfodinâmica das Praias Adjacentes às Desembocaduras da Linha de Costa do Rio Grande do Sul pp 73–87
- Fraser DJ, Lippé C, Bernatchez L (2004) Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). *Mol Ecol* 13:67–80. <https://doi.org/10.1046/j.1365-294X.2003.02038.x>
- Fruet PF, Kinas PG, Da Silva KG, Di Tullio JC, Monteiro DS, Dalla Rosa L, Estima SC, Secchi ER (2010) Temporal trends in mortality and effects of by-catch on common bottlenose dolphins, *Tursiops truncatus*, in southern Brazil. *J Mar Biol Assoc UK* 98:1–12. <https://doi.org/10.1017/S0025315410001888>
- Fruet PF, Secchi ER, Di Tullio JC, Kinas PG (2011) Abundance of bottlenose dolphins, *Tursiops truncatus* (Cetacea: Delphinidae), inhabiting the Patos Lagoon estuary, southern Brazil: implications for conservation. *Zoologia (Curitiba, Impresso)* 28:23–30. <https://doi.org/10.1590/S1984-46702011000100004>
- Fruet PF, Secchi ER, Daura-Jorge F, Vermeulen E, Flores PA, Simões-Lopes PC, Genoves RC, Laporta P, Di Tullio JC, Freitas TRO, Dalla Rosa L, Valiati VH, Beheregaray LB, Möller LM (2014) Remarkably low genetic diversity and strong population structure in common bottlenose dolphins (*Tursiops truncatus*) from coastal waters of the Southwestern Atlantic Ocean. *Conserv Genet* 15:879–895. <https://doi.org/10.1007/s10592-014-0586-z>
- Fruet PF, Daura-Jorge FG, Möller LM, Genoves RC, Secchi ER (2015a) Abundance and demography of bottlenose dolphins inhabiting a subtropical estuary in the Southwestern Atlantic Ocean. *J Mammal* 96:332–343. <https://doi.org/10.1093/jmammal/gyv035>
- Fruet PF, Genoves RC, Möller LM, Botta S, Secchi ER (2015b) Using mark-recapture and stranding data to estimate reproductive traits in female bottlenose dolphins (*Tursiops truncatus*) of the Southwestern Atlantic Ocean. *Mar Biol* 162:661–673. <https://doi.org/10.1007/s00227-015-2613-0>
- Fruet PF, Dalla Rosa L, Genoves RC, Valiati VH, De Freitas TRO, Möller LM (2016) Biopsy darting of common bottlenose dolphins (*Tursiops truncatus*) in southern Brazil: evaluating effectiveness, short-term responses and wound healing. *Lat Am J Aquat Mamm* 11:121–132. <https://doi.org/10.5597/lajam00221>
- Fruet PF, Secchi ER, Di Tullio JC, Simões-Lopes PC, Daura-Jorge F, Costa APB, Vermeulen E, Flores PAC, Genoves RC, Laporta P, Beheregaray LB, Möller LM (2017) Genetic divergence between two phenotypically distinct bottlenose dolphin ecotypes suggests separate evolutionary trajectories. *Ecol Evol*. <https://doi.org/10.1002/ece3.3335>
- Fury CA, Harrison PL (2008) Abundance, site fidelity and range patterns of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in two Australian subtropical estuaries. *Mar Freshw Res* 59:1015–1027. <https://doi.org/10.1071/MF08109>
- Garcia A, Raseira M, Vieira J, Winemiller KO, Grimm A (2003) Spatiotemporal variation in shallow-water freshwater fish distribution and abundance in a large subtropical coastal lagoon. *Biol Fishes* 68:215–228
- Garcia AM, Hoehinghaus DJ, Vieira JP, Winemiller KO (2007) Isotopic variation of fishes in freshwater and estuarine zones of a large subtropical coastal lagoon. *Estuar Coast Shelf Sci* 73:399–408. <https://doi.org/10.1016/j.ecss.2007.02.003>
- Garcia AM, Vieira JP, Winemiller KO, Moraes LE, Paes ET (2012) Factoring scales of spatial and temporal variation in fish abundance in a subtropical estuary. *Mar Ecol Prog Ser* 461:121–135. <https://doi.org/10.3354/meps09798>

- Garrison E, Marth G (2012) Haplotype-based variant detection from short-read sequencing. arXiv preprint arXiv:12073907. <https://doi.org/10.1093/bioinformatics/btw709>
- Gaughran SJ, Quinzin MC, Miller JM, Garrick RC, Edwards DL, Russello MA, Poulakakis N, Ciofi C, Beheregaray LB, Caccione A (2018) Theory, practice, and conservation in the age of genomics: the Galápagos giant tortoise as a case study. *Evol Appl* 11:1084–1093. <https://doi.org/10.1111/eva.12551>
- Genoves RC, Fruet PF, Di Tullio JC, Möller LM, Secchi ER (2018) Spatiotemporal use predicts social partitioning of bottlenose dolphins with strong home range overlap. *Ecol Evol* 8:12597. <https://doi.org/10.1002/ece3.4681>
- Giménez J, Ramírez F, Almunia J, Forero MG, de Stephanis R (2016) From the pool to the sea: applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*). *J Exp Mar Biol Ecol* 475:54–61. <https://doi.org/10.1016/j.jembe.2015.11.001>
- Grummer JA, Beheregaray LB, Bernatchez L, Hand BK, Luikart G, Narum SR, Taylor EB (2019) Aquatic landscape genomics and environmental effects on genetic variation. *Trends Ecol Evol* 34:641–654. <https://doi.org/10.1016/j.tree.2019.02.013>
- Haimovici M, Cardoso LG (2017) Long-term changes in the fisheries in the Patos Lagoon estuary and adjacent coastal waters in Southern Brazil. *Mar Biol Res* 13:135–150. <https://doi.org/10.1080/17451000.2016.1228978>
- Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314–326
- Hobson KA, Piatt JF, Pitocchelli J (1994) Using stable isotopes to determine seabird trophic relationships. *J Anim Ecol* 63:786. <https://doi.org/10.2307/5256>
- Hoelzel AR (1998) Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. *J Hered* 89:451–458. <https://doi.org/10.1093/jhered/89.5.451>
- Hoelzel AR, Dahlheim M, Stern SJ (1998) Low genetic variation among killer whales (*Orcinus orca*) in the eastern north Pacific and genetic differentiation between foraging specialists. *J Hered* 89:121–128
- Hollatz C, Flach L, Baker CS, Santos R (2011) Microsatellite data reveal fine genetic structure in male Guiana dolphins (*Sotalia guianensis*) in two geographically close embayments at southeastern coast of Brazil. *Mar Biol* 158:927–933. <https://doi.org/10.1007/s00227-010-1619-x>
- Irvine AB, Scott MD, Wells RS, Kaufmann JH (1981) Movements and activities of the atlantic bottlenose dolphin, *Tursiops truncatus*, near Sarasota, Florida. *Fish Bull* 79:671–688
- Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SiBER—stable isotope Bayesian Ellipses in R. *J Anim Ecol* 80:595–602. <https://doi.org/10.1111/j.1365-2656.2011.01806.x>
- Jombart T, Ahmed I (2011) adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics* 27:3070–3071. <https://doi.org/10.1093/bioinformatics/btr521>
- Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can J Zool* 78:1–27
- Krützen M, Mann J, Heithaus M, Connor R, Bejder L, Sherwin W (2005) Cultural transmission of tool use in bottlenose dolphins. *Proc Natl Acad Sci* 102:8939–8943. <https://doi.org/10.1073/pnas.0500232102>
- Laporta P, Martins CCA, Lodi L, Domit C, Vermeulen E, Di Tullio JC (2016) Report of the working group on habitat use of *Tursiops truncatus* in the Southwest Atlantic Ocean. *Lat Am J Aquat Mamm* 11:47–61. <https://doi.org/10.5597/lajam00215>
- Levine JM, HilleRisLambers J (2009) The importance of niches for the maintenance of species diversity. *Nature* 461:254–257. <https://doi.org/10.1038/nature08251>
- Liu N, Chen L, Wang S, Oh C, Zhao H (2005) Comparison of single-nucleotide polymorphisms and microsatellites in inference of population structure. *BMC Genet* 6:1–5. <https://doi.org/10.1186/1471-2156-6-S1-S26>
- Louis M, Viricel A, Lucas T, Peltier H, Alfonsi E, Berrow S, Brownlow A, Covelo P, Dabin W, Deaville R, de Stephanis R, Gally F, Gauffier P, Penrose R, Silva MA, Guinet C, Simon-Bouhet B (2014) Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic. *Mol Ecol* 23:857–874. <https://doi.org/10.1111/mec.12653>
- Louis M, Simon-Bouhet B, Viricel A, Lucas T, Gally F, Chérel Y, Guinet C (2018) Evaluating the influence of ecology, sex and kinship on the social structure of resident coastal bottlenose dolphins. *Mar Biol*. <https://doi.org/10.1007/s00227-018-3341-z>
- Lusseau D, Newman MEJ (2004) Identifying the role that animals play in their social networks. *Proc R Soc Lond Ser B Biol Sci* 271:477–481. <https://doi.org/10.1098/rsbl.2004.0225>
- Mate BR, Rossbach KA, Nieuirkirk SL, Wells RS, Irvine AB, Scott MD, Read AJ (1995) Satellite-monitored movements and dive behavior of a bottlenose dolphin (*Tursiops truncatus*) in Tampa Bay, Florida. *Mar Mamm Sci* 11:452–463. <https://doi.org/10.1111/j.1748-7692.1995.tb00669.x>
- Mazzoil M, Reif JS, Youngbluth M, Murdoch ME, Bechdel SE, Howells E, McCulloch SD, Hansen LJ, Bossart GD (2008) Home ranges of bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida: environmental correlates and implications for management strategies. *EcoHealth* 5:278–288. <https://doi.org/10.1007/s10393-008-0194-9>
- Möller OO, Castaing P, Salomon J-C, Lazure P (2001) The influence of local and non-local forcing effects on the subtidal circulation of Patos Lagoon. *Estuaries* 24:297. <https://doi.org/10.2307/1352953>
- Möller LM, Wiszniewski J, Allen S, Beheregaray L (2007) Habitat type promotes rapid and extremely localised genetic differentiation in dolphins. *Mar Freshw Res* 58:640–648
- Möller LM, Valdez FP, Allen S, Bilgmann K, Corrigan S, Beheregaray LB (2011) Fine-scale genetic structure in short-beaked common dolphins (*Delphinus delphis*) along the East Australian Current. *Mar Biol* 158:113–126. <https://doi.org/10.1007/s00227-010-1546-x>
- Natoli A, Birkun A, Aguilar A, Lopez A, Hoelzel AR (2005) Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). *Proc R Soc Lond Ser B Biol Sci* 272:1217–1226. <https://doi.org/10.1098/rspb.2005.3076>
- Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. *Mar Mamm Sci* 26:509–572. <https://doi.org/10.1111/j.1748-7692.2009.00354.x>
- Palsbøll PJ (1999) Genetic tagging: contemporary molecular ecology. *Biol J Linn Soc* 68:3–22. <https://doi.org/10.1006/bjil.1999.0327>
- Palumbi SR (1992) Marine speciation on a small planet. *Trends Ecol Evol* 7:114–118
- Parnell A (2016) simmr: a stable isotope mixing model. R package. Version 0.3. <https://CRAN.R-project.org/package=simmr>. Accessed 15 July 2017
- Parnell AC, Phillips DL, Bearhop S, Semmens BX, Ward EJ, Moore JW, Jackson AL, Grey J, Kelly DJ, Inger R (2013) Bayesian stable isotope mixing models. *Environmetrics* 24:387–399. <https://doi.org/10.1002/env.2221>
- Pérez-Alvarez MJ, Olavarría C, Moraga R, Baker CS, Hamner RM, Poulin E (2015) Microsatellite markers reveal strong genetic structure in the endemic Chilean dolphin. *PLoS ONE* 10:1–15. <https://doi.org/10.1371/journal.pone.0123956>
- Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0037135>

- Phillips DL, Newsome SD, Gregg JW (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia* 144:520–527. <https://doi.org/10.1007/s00442-004-1816-8>
- Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. *Can J Zool* 92:823–835. <https://doi.org/10.1139/cjz-2014-0127>
- Pimm SL, Rosenzweig ML (1981) Competitors and habitat use. *Oikos* 37:1. <https://doi.org/10.2307/3544067>
- Puritz JB, Hollenbeck CM, Gold JR (2014) dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ* 2:e431. <https://doi.org/10.7717/peerj.431>
- R Core Team (2017) R: a language and environment for statistical computing. R Core Team, Geneva
- Raj A, Stephens M, Pritchard JK (2014) fastSTRUCTURE: variational inference of population structure in large SNP data sets. *Genetics* 197:573–589. <https://doi.org/10.1534/genetics.114.164350>
- Rodrigues FL, Vieira JP (2013) Surf zone fish abundance and diversity at two sandy beaches separated by long rocky jetties. *J Mar Biol Assoc UK* 93:867–875. <https://doi.org/10.1017/s0025315412001531>
- Sandoval-Castillo J, Robinson NA, Hart AM, Strain LWS, Beheregaray LB (2018) Seascape genomics reveals adaptive divergence in a connected and commercially important mollusc, the greenlip abalone (*Haliotis laevis*), along a longitudinal environmental gradient. *Mol Ecol* 27:1603–1620. <https://doi.org/10.1111/mec.14526>
- Secchi ER, Botta S, Wiegand MM, Lopez LA, Fruet PF, Genoves RC, Di Tullio JC (2016) Long-term and gender-related variation in the feeding ecology of common bottlenose dolphins inhabiting a subtropical estuary and the adjacent marine coast in the western South Atlantic. *Mar Biol Res* 13:121–134. <https://doi.org/10.1080/17451000.2016.1213398>
- Seyboth E, Botta S, Secchi ER (2018) Using chemical elements to the study of trophic and spatial ecology in marine mammals of the Southwestern Atlantic Ocean. In: Rossi-Santos MR, Finkl CW (eds) *Advances in marine vertebrate research in Latin America*, vol 22. Coastal Research Library, New York, pp 221–248
- Simões-Lopes PC, Fabian ME (1999) Residence patterns and site fidelity in bottlenose dolphins, *Tursiops truncatus* (Montagu) (Cetacea, Delphinidae) off Southern Brazil. *Rev Bras Zool* 16:1017–1024. <https://doi.org/10.1590/S0101-81751999000400012>
- Smith JA, Mazumder D, Suthers IM, Taylor MD (2013) To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. *Methods Ecol Evol* 4:612–618. <https://doi.org/10.1111/2041-210X.12048>
- Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Mol Biol Evol* 13:510–524. <https://doi.org/10.1093/oxfordjournals.molbev.a025612>
- Surridge A, Ibrahim K, Bell D, Webb N, Rico C, Hewitt G (1999) Fine-scale genetic structuring in a natural population of European wild rabbits (*Oryctolagus cuniculus*). *Mol Ecol* 8:299–307
- Teixeira-Amaral P, Amaral WJA, de Ortiz DO, Agostini VO, Muxagata E (2017) The mesozooplankton of the Patos Lagoon Estuary, Brazil: trends in community structure and secondary production. *Mar Biol Res* 13:48–61. <https://doi.org/10.1080/17451000.2016.1248850>
- Teske PR, Golla TR, Sandoval-Castillo J, Emami-Khoyi A, van der Lingen C, von der Heyden S, Chiazzari B, van Vuuren BJ, Beheregaray LB (2018) Mitochondrial DNA is unsuitable to test for isolation by distance. *Sci Rep* 8:8448
- Vachon F, Whitehead H, Frasier TR (2017) What factors shape genetic diversity in cetaceans? *Ecol Evol*. <https://doi.org/10.1002/ece3.3727>
- Van Cise AM, Martien KK, Mahaffy SD, Baird RW, Webster DL, Fowler JH, Oleson EM, Morin PA (2017) Familial social structure and socially driven genetic differentiation in Hawaiian short-finned pilot whales. *Mol Ecol* 26:6730–6741. <https://doi.org/10.1111/mec.14397>
- Vieira JP, Garcia AM, Moraes LE (1998) Ictiofauna. In: Seeliger U, Odebrecht C (eds) *O Estuário da Lagoa dos Patos: um século de transformações*. Editora da FURG, Rio Grande, pp 56–59
- Watts RJ, Johnson MS (2004) Estuaries, lagoons and enclosed embayments: habitats that enhance population subdivision of inshore fishes. *Mar Freshw Res* 55:641–651. <https://doi.org/10.1071/MF04051>
- Wells R, Scott M, Irvine A (1987) The social structure of freeranging bottlenose dolphins. In: Genoways H (ed) *Current mammalogy*. Plenum Press, New York, pp 247–305
- Wilson RM, Chanton JP, Balmer BC, Nowacek DP (2014) An evaluation of lipid extraction techniques for interpretation of carbon and nitrogen isotope values in bottlenose dolphin (*Tursiops truncatus*) skin tissue. *Mar Mamm Sci* 30:85–103. <https://doi.org/10.1111/mms.12018>
- Wiszniewski J, Allen SSJ, Möller LM (2009a) Social cohesion in a hierarchically structured embayment population of Indo-Pacific bottlenose dolphins. *Anim Behav* 77:1449–1457. <https://doi.org/10.1016/j.anbehav.2009.02.025>
- Wiszniewski J, Beheregaray LB, Allen SJ, Möller LM (2009b) Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia. *Conserv Genet* 11:1405–1419. <https://doi.org/10.1007/s10592-009-9968-z>
- Worthington Wilmer J, Elkin C, Wilcox C, Murray C, Niejalkes D, Possingham H (2008) The influence of multiple dispersal mechanisms and landscape structure on population clustering and connectivity in fragmented artesian spring snail populations. *Mol Ecol* 17:3733–3751. <https://doi.org/10.1111/j.1365-294X.2008.03861.x>
- Wright S (1943) Isolation by distance. *Genetics* 28:114–138

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