



UNIVERSIDADE ESTADUAL DE SANTA CRUZ - UESC
PROOGRAMA DE POS-GRADUAÇÃO EM ZOOLOGIA



PAMELA SOLEDAD ACTIS

**SINCRONIZAÇÃO DA RESPIRAÇÃO DA TONINHA (*Pontoporia blainvillii*) E DO
BOTO-CINZA (*Sotalia guianensis*) (MAMMALIA: CETARTIODACTYLA) NO SUL
DO BRASIL**

**ILHÉUS-BAHIA
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Dissertação apresentada ao Programa de Pós-Graduação em Zoologia Aplicada da Universidade Estadual de Santa Cruz como parte dos requisitos para obtenção do grau de Mestre em Zoologia Aplicada.

Área de concentração: Zoologia Aplicada

Orientador: Dr. Daniel Danilewicz Schiavon

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SUL DO BRASIL**

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não mediu esforços para que eu cumprisse mais esta etapa
de minha vida.*

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Resumo

Quando estudamos o comportamento de cetáceos, a respiração sincronizada é observada em conjunto com atividades coordenadas, como alimentação cooperativa, descanso, encontros inter e intra-específicas, acasalamento e namoro. Este estudo foi realizado na Baía da Babitonga, sul do Brasil, a fim de estudar a sincronia da respiração em duas pequenas populações de toninhas (*Pontoporia blainvilliei*) e boto-cinza (*Sotalia guianensis*). A área de estudo está altamente impactada por diferentes fontes de perturbação antrópica. Aqui nós analisamos quantitativamente a respiração síncrona de toninhas e do boto-cinza. Assumimos que a sincronia é representada pelo inverso do tempo entre a respiração de dois golfinhos num par. A relação entre sincronia e variáveis ambientais e/ou variáveis relacionadas com os animais foi avaliada com modelos lineares generalizados e modelos aditivos generalizados. A sincronia respiração no boto-cinza foi correlacionada positivamente com a proximidade entre animais, variou significativamente dependendo do barco de pesquisa utilizado, e mostrou uma tendência positiva com o tamanho dos grupos grandes. Para toninhas, a sincronia da respiração apenas foi positivamente correlacionada com a distância entre os animais. características sociais de cada espécie parece ser mais importante na determinação da sincronia de respiração do que os fatores externos.

Abstract

When studying cetacean behavior, synchronous breathing is observed together with coordinated activities, such as cooperative foraging, resting, inter and intraspecific encounters, mating and courtship. This study was conducted in Babitonga Bay, Southern Brazil, in order to study the synchrony of breathing in two small populations of franciscanas (*Pontoporia blainvillii*) and Guiana dolphins (*Sotalia guianensis*). The study area is highly impacted by different sources of anthropogenic disturbance. Here we quantitatively analysed synchronous breathing of franciscanas and Guiana dolphins. Synchrony was assumed to be represented by the inverse of the time between breathing surfacing displays of dolphins in a pair. The relationship between synchrony and environmental and/or animal-related variables was evaluated with Generalized Linear models and Generalized Additive Models. The breathing synchrony in Guiana dolphins was positively correlated to the proximity between animals, varied significantly depending on the research boat used, and showed a positive trend with higher group sizes. For franciscanas, breathing synchrony was only positively correlated to the distance between animals. Social characteristics of each species seems to be more important in determining breathing synchrony than external factors.

Sumario

Resumo	6
Abstract	7
Sumario	8
1 Introdução	9
1.1 Estudo do comportamento animal.....	9
1.2 Sincronia animal.....	9
1.3 Sincronia em cetáceos.....	10
1.4 Sincronia da respiração em cetáceos	11
1.5 A toninha	13
1.6 O boto-cinza.....	15
2 Justificativa.....	18
3 Bibliografia	19
4 Breathing synchrony in franciscana (<i>Pontoporia blainvilliei</i>) and Guiana dolphin (<i>Sotalia guianensis</i>) (MAMMALIA: CETARTIODACTYLA) in southern Brazil	26
4.1 Abstract.....	26
4.2 Introduction	26
4.3 Materials and methods	29
4.3.1 Study area	29
4.3.2 Data collection	30
4.3.3 Hypothesis and variables.....	32
4.3.4 Statistical analysis	32
4.3.5 <i>Guiana dolphins</i>	34
4.3.6 <i>Franciscana dolphins</i>	35
4.4 Discussion.....	36
4.5 Conclusion	39
4.6 Acknowledgements	39
4.7 Bibliography	40
5 Atigo Aceito	46

1 Introdução

1.1 Estudo do comportamento animal

Compreender o comportamento dos animais é essencial para o conhecimento de sua ecologia e as questões relacionadas com sua conservação (SUTHERLAND, 1996; CARO, 1998). Variações dos fatores ambientais podem influenciar padrões de comportamento de indivíduos, grupos sociais ou uma população que habita uma determinada área (KREBS; DAVIES, 1996). A identificação destes padrões ainda é um desafio em cetáceos em seu ambiente natural, uma vez que são animais altamente móveis e que passam a maior parte de sua vida submersos, dificultando a observação a partir da superfície (PERRIN; WÜRSIG; THEWISSEN, 2008).

1.2 Sincronia animal

Comportamento sincrônico ocorre quando dois ou mais animais exibem o mesmo comportamento simultaneamente (CONNOR; SMOLKER; BEJDER, 2006). Esta classe de comportamento tem sido estudada em um amplo espectro de grupos animais e relacionada a diversos outros comportamentos (ENGEL; LAMPRECHT, 1997), como, por exemplo, no mergulho de pinguins (TAKAHASHI; SATO; NISHIKSWA, 2004), nas vocalizações dos sapos (GRAFE, 2003), nos complexos padrões de movimentos nos cardumes de peixes e de bandos de aves (PARRISH; VISCIDO; GRUNBAUM, 2002; BALLERINI et al., 2008).

A sincronia comportamental pode ser causada por processos sociais, como pela comunicação, facilitação e inibição. Entretanto, a sincronia pode ser causada por fatores não sociais, categorizados como "coincidência temporal" (o comportamento de vários indivíduos que se ativa para o mesmo evento externo), ou "coincidência espacial" (a proximidade entre indivíduos conduz estes a permanecerem expostos a similares condições ambientais) (ENGEL; LAMPRECHT, 1997).

Para os animais que sincronizam, podem existir tanto benefícios quanto prejuízos. Estes podem contribuir, em parte, na escolha do indivíduo em permanecer ou não no grupo (PITCHER; PARRISH, 1993; AIVAZ; RUCKSTUHL, 2011), afetando sua estabilidade e composição, e comprometendo por fim sua organização social (CONRADT; ROPER, 2000). Ainda, a sincronização de atividades pode ser custosa para os indivíduos se esta requiser postergar uma atividade que seria mais vantajosa

individualmente, a fim de acompanhar o comportamento que o resto do grupo está realizando. Tais custos podem ser particularmente elevados em grupos cujos membros pertencem a diferentes classes de idade, tamanho ou sexo (CONRADT; ROPER, 2000).

1.3 Sincronia em cetáceos

Dentre diversos padrões comportamentais que os cetáceos apresentam, o sincronismo dentro dos grupos possui diversas particularidades. Em cetáceos o termo “sincrônico” tipicamente refere-se a animais em proximidade uns de outros, realizando o mesmo comportamento ao mesmo tempo, usualmente em orientação paralela (FELLNER; BAUER; HARLEY, 2006).

Em estudos de cetáceos o termo sincrônico tem sido definido de duas maneiras principais: distribuição temporal não aleatória dos indivíduos, onde o grau de sincronia temporal é medido e analisado (e.g. WURSIG, 1978; WHITEHEAD, 1996; HASTIE et al., 2003); e comportamento simultâneo realizado por membros de um grupo, onde a correspondência temporal é assumida e a coleta de dados aborda questões como identidade dos indivíduos e circunstâncias sociais e ambientais (MANN; SMUTS, 1999; CONNOR; SMOLKER; BEJDER, 2006). O uso de cada abordagem reflete a ênfase da pesquisa em questão. Dessa forma ambas as classes de estudos denotam a função do comportamento sincrônico.

A sincronia foi estudada em cetáceos em diferentes situações tais como no deslocamento e em situações antipredatórias (e.g. HEIMLICH-BORAN, 1988; HASTIE et al., 2003; SENIGAGLIA et al., 2012); em interações sociais afiliativas e agonísticas (e.g. JESSICA, 2012); no cuidado dos filhotes (e.g. WHITEHEAD, 1996; FELLNER et al., 2013); na aprendizagem social (e.g. FELLNER; BAUER; HARLEY, 2006); e na respiração (e.g. SAKAI et al., 2010; SENIGAGLIA; WHITEHEAD, 2012); entre outras. Ainda, existem evidências de que sincronia de dois ou mais animais aquáticos traz benefícios hidrodinâmicos, reduzindo o arrasto durante o nado (e.g. WEIHS, 2004; NOREN, 2008; NOREN; BIEDENBACH; REDFERN, 2008). Deste modo, através da proximidade e do nado sincrônico, golfinhos adultos podem ajudar filhotes a reduzir as forças necessárias para o deslocamento, minimizando seu gasto energético.

A sincronia também está relacionada com o "sistema de integração sensorial" (SIS), que permite ao grupo de animais se comportar como um organismo hipersensível (NORRIS; DOHL, 1980; NORRIS; SCHILT, 1988). Este sistema consiste na transmissão

rápida de informações, incluindo informações sonoras, a partir de cada indivíduo no grupo para todos os outros. Enquanto os membros do grupo estão em contacto visual ou táctil uns com os outros, os desvios sutis nos movimentos sincrônicos de um ou dois membros podem transmitir informação útil para o resto do grupo a uma velocidade mais rápida, o que é conhecido como “efeito Trafalgar” (TREHERNE; FOSTER, 1981).

Além disso, a sincronia pode ser um meio de transmissão de informação cultural (BAUER; HARLEY, 2001; WHITEN, 2001; FELLNER; BAUER; HARLEY, 2006). Como subproduto do contínuo comportamento sincrônico, é esperado que os filhotes fiquem expostos a todas as experiências de sua mãe, tendo oportunidade de aprender via suas interações com o meio ambiente e por meio da imitação do comportamento materno (FELLNER *et al.*, 2012).

1.4 Sincronia da respiração em cetáceos

A sincronia da respiração não representa uma categoria especial ou distintiva de comportamento, mas é observada quando os animais executam uma outra atividade de maneira coordenada, como a alimentação cooperativa, descanso, encontros inter e intra específicos, encontros de acasalamento e brincadeiras (PERELBERG; SCHUSTER, 2008). Estudos de sincronização da respiração têm sido realizados com o objetivo de avaliar as possíveis causas e funções próximas deste comportamento. Hastie *et al.* (2003) quantificaram os padrões de sincronização na respiração em golfinhos-nariz-de-garrafa (*Tursiops truncatus*) no norte da Escócia, e constataram que a sincronização na respiração possui uma correlação positiva com o tamanho do grupo e com a presença de embarcações, porém negativa com a presença de filhotes. Os autores consideram algumas possíveis hipóteses para explicar o aumento da sincronia na respiração na presença de embarcações. A sincronia poderia desempenhar um papel fundamental na coesão social durante períodos de comunicação pouco eficientes decorrentes do ruído dos motores. As hipóteses alternativas são que os golfinhos poderiam identificar as embarcações como possíveis predadores, ou que as mudanças no comportamento dos animais poderiam ser um reflexo do movimentos dos peixes ao redor das embarcações.

Por outro lado, Connor; Smolker; Bejder (2006) notaram que respirações sincrônicas em membros de alianças de machos de *Tursiops aduncus* são comuns especialmente durante encontros sociais. No mesmo trabalho é proposta uma hipótese sugerindo que os mamíferos marinhos precisam sincronizar fora da água para

consequentemente sincronizar também nas atividades dentro da água. Assim os indivíduos mantêm proximidade e, portanto, a sincronização da respiração em benefício do grupo.

Perelberg e Schuster (2008), ao estudar a respiração coordenada de 13 indivíduos de golfinhos-nariz-de-garrafa (*T. truncatus*) num semi-cativeiro marinho, sugeriram que a respiração coordenada de golfinhos representa um ato de cooperação que é influenciada não só por resultados materiais imediatos para os indivíduos, mas também pelas dimensões sociais de cooperação. Por outro lado, Sakai et al. (2010) estudaram a sincronia da respiração de *Tursiops aduncus* na ilha Mikura, Japão. Seus resultados revelaram que esta sincronia é um componente do comportamento social associada a dupla de nado e influenciado pela relação afiliativa, pela idade e pelo gênero/sexo, onde os golfinhos tendem a formar duplas com indivíduos do mesmo sexo e mesma idade.

Senigaglia e Whitehead (2012) estudaram a sincronia da respiração em baleia-piloto-de-peitorais-longas (*Globicephala melas*) na Nova Escócia, Canadá. Os autores concluíram que a sincronia era inferior em grupos socializando que em grupos em deslocamento, descanso ou alimentação. Foi verificado também que há uma tendência geral de maior sincronia em situações complexas e potencialmente estressantes como: tamanhos maiores do grupo, presença de filhote que acompanha dois adultos e mais de uma embarcação presente. Os autores concluíram que seus dados apoiam a ideia de que a função mais geral da sincronia é de reforçar laços sociais. Em um estudo similar, Senigaglia et al. (2012) compararam duas populações geneticamente diferentes de baleias-piloto expostas a diferentes condições sócio-ecológicas. O estudo forneceu evidências de que a respiração sincronizada é um importante componente da dupla de natação e tem um papel funcional no comportamento afiliativo. Também foi detectada a influência do estado comportamental e do tamanho do grupo sobre a sincronização, porém seu efeito diferiu entre as duas populações. Por isso, enquanto a sincronização da respiração pode ser uma resposta anti-predatória geral a um risco percebido, os dados suportam a hipótese de que sua importância na manutenção de agregações difere de acordo com o cenário social da população (ambiente geográfico particular, com suas influências antropicas, climatológicas e históricas próprias do lugar). Desta forma, a sincronização da respiração poderia funcionar como resposta anti-predatória nas duplas de animais nas quais a relação de afiliação é bem estabelecida (e.g. duplas mãe-filhote ou alianças de longo prazo).

1.5 A toninha

A toninha, *Pontoporia blainvilliei* (GERVAIS; D'ORBIGNY, 1844) (Figura 1), família Pontoporiidae, também chamada de franciscana, é um pequeno cetáceo com coloração dorsal cinza amarronzado (CREMER; SIMÕES-LOPES, 2005).



Figura 1. Toninha, *Pontoporia blainvilliei*.

A espécie é endêmica do Oceano Atlântico Sul Ocidental, ocorrendo desde Itaúnas ($18^{\circ}25'$ S), Estado do Espírito Santo, Brasil (SICILIANO; SANTOS, 1994), até Golfo San Matias ($42^{\circ}35'$ S), Província de Chubut, Argentina (SICILIANO; SANTOS, 1994; CRESPO; HARRIS; GONZÁLEZ, 1998). Habita áreas desde a linha da costa até 30 metros de profundidade ou 24 milhas náuticas mar adentro (PRADERI; PINEDO; CRESPO, 1989; DANILEWICZ et al., 2009).

A toninha tem o ciclo de vida mais curto conhecido dentre os cetáceos, com pouca diferença na idade de maturação entre os sexos, e dimorfismo sexual reverso (i.e. fêmeas maiores que machos). As toninhas tornam-se sexualmente maduras aos dois anos de idade com aproximadamente 115 cm de comprimento, para machos, e três anos e 130 cm para fêmeas (RAMOS; DI BENEDITTO; LIMA, 2000). Possuem relação sexual 1:1 ao nascimento (BROWNELL, 1984; DANILEWICZ, 2003).

A alimentação se compõe principalmente de peixes das famílias *Scianidae*, *Batrachoididae*, *Trichiuridae*, *Engraulidae*, *Stromateidae*, *Ariidae* e *Clupeidae* (RIVERO; BASTIDA, 2002; BASSOI et al., 2000). Estudos da ecologia trófica em diferentes áreas geográficas indicam que não há diferenças significativas na dieta entre sexos e classes de idade, a exceção dos filhotes (PINEDO; PRADERI; BROWNELL, 1989; BASSOI, 1997; RODRIGUEZ; RIVERO; BASTIDA, 2002). Os filhotes são desmamados gradualmente, atingindo a independência alimentar aproximadamente aos nove meses de idade. Durante o período de transição para a dieta sólida, os camarões são itens especialmente importantes na alimentação das toninhas (RIVERO; BASTIDA, 2002).

A espécie não exibe comportamentos aéreos (BORDINO; THOMPSON; IÑIGUEZ, 1999). Tipicamente, quando um animal emerge, expõe seu rostro e depois curva seu corpo, expondo a nadadeira dorsal. O rosto é exposto em 84% das saídas para a superfície. Padrões de respiração sincrônica foram registrados em 67% dos indivíduos adultos do grupo, e em 100% das duplas mãe-filhote (BORDINO; THOMPSON; IÑIGUEZ, 1999). Brownell (1989) e Pinedo; Praderi; Brownell (1989) sugerem que a espécie não é gregária, porém a espécie não é solitária e pode formar pequenos grupos. Por exemplo, enquanto no litoral sul de Rio de Janeiro o tamanho do grupo encontrado normalmente é de 1 a 10 indivíduos (DI BENEDITTO; RAMOS; LIMA, 2001), na Baía Anegada, Argentina (BORDINO; THOMPSON; IÑIGUEZ, 1999) foram registrados de 2 a 6 animais por grupo. Na Baía Babitonga, litoral Norte do estado de Santa Catarina, Sul do Brasil, observou-se que indivíduos solitários não são frequentes, encontrando-se 7 indivíduos por grupo em media (CREMER; SIMÕES-LOPEZ, 2005).

Apesar das variações regionais nos parâmetros vitais (taxa de sobrevivência, fecundidade, estimativa de abundância) e as incertezas associadas as estimativas, a toninha, em geral, apresenta um baixo potencial para crescimento populacional anual (SECCHI, 2006; DANILEWICZ et al., 2010; SECCHI, 2006). Toninhas são naturalmente predadas tanto por tubarões quanto por orcas (PRADERI, 1985; OTT; DANILEWICZ, 1998; SANTOS; NETTO, 2005).

Em 2008 a toninha foi classificada como Vulnerável (VU) na lista vermelha de animais ameaçados de extinção da “*International Union for Conservation of Nature*” (IUCN, 2012). Esta categorização justifica-se sob o critério A3d, devido a um declínio projetado de mais de 30% em três gerações (36 anos, TAYLOR et al. 2007), tendo como base os resultados de uma análise de simulação populacional (SECCHI, 2006), utilizando os níveis atuais e potenciais de mortalidade pela pesca. A espécie também

está incluída na Lista Nacional das Espécies da Fauna Brasileira Ameaçada de Extinção. Além disso, está atualmente classificada como criticamente em perigo no Plano de Ação dos Mamíferos Aquáticos do Brasil e listada no Apêndice II da Convenção sobre o Comércio Internacional de Espécies da Fauna e Flora Silvestres Ameaçadas de Extinção (CITES)(ICMBIO, 2010).

A toninha é considerada uma das espécies com o maior número de capturas acidentais em sua área de ocorrência (PRADERI; PINEDO; CRESPO, 1989; PINEDO, 1991). A distribuição da espécie tem uma grande sobreposição com a atividade pesqueira e a captura em redes de pesca tem comprometido sua conservação (DANILEWICZ et al., 2010; PRADO; SECCHI; KINAS, 2013). A análise do conteúdo estomacal da toninha tem mostrado que a espécie é também vulnerável à ingestão de vários tipos de dejetos humanos, incluindo pedaços de redes e linhas de pesca (BASSOI, 1997; BASTIDA; RIVERO; RODRÍGUEZ, 2000).

A presença de toninhas ao longo dos anos na Baía da Babitonga sugere que a espécie faz uso contínuo da área. No entanto, esta é usada seletivamente, com uma preferência marcada por áreas particulares (CREMER; SIMÕES-LOPES, 2005; CREMER; SIMÕES-LOPES, 2008). Esta distribuição de toninhas na Baía provavelmente esteja influenciada pela disponibilidade de peixes na área (SCHOENER, 1971; CREMER; SIMÕES-LOPES, 2005). A Baía da Babitonga é uma área de grande importância para esta população como sítio de alimentação, descanso e reprodução, pois trata-se de um estuário protegido com águas calmas e pouco profundas, ausência de grandes predadores, e alta produtividade (CREMER; SIMÕES-LOPES, 2005). Contudo, a Baía da Babitonga também tem sido impactada pela contaminação de poluentes químicos. Isto, somado a possibilidade desta população ser considerada residente no local (CREMER; SIMÕES-LOPES, 2008) e à proximidade da Baía com o maior polo industrial do estado de Santa Catarina, coloca esta população numa evidente situação de risco (CREMER; SIMÕES-LOPES, 2008).

1.6 O boto-cinza

Sotalia guianensis (VAN BÉNÉDEN, 1864) (Figura 2), também conhecido como boto-cinza, pertence à família Delphinidae. O gênero *Sotalia* foi dividido recentemente em *S. fluviatilis* e *S. guianensis*, com base em análises morfológicas e genéticas (MONTEIRO-FILHO; MONTEIRO; REIS, 2002; CUNHA et al., 2005; CABALLERO et al.,

2007; CUNHA; SILVA; SOLÉ-CAVA, 2010). *S. guianensis* ocorre na costa atlântica das Américas Central e do Sul, desde Honduras (EDWARDS; SCHNELL, 2001) até Baia Norte em Santa Catarina, no sul do Brasil (SIMÕES-LOPES, 1988; WEDEKIN et al., 2005).



Figura 2. *Sotalia guianensis*.

O boto-cinza tem coloração cinza-escuro no dorso e cinza-clara a branca na região ventral, com linha lateral pouco demarcada e estendendo-se desde o olho até a nadadeira peitoral. O bico é moderadamente longo e fino, e o melão é pequeno e arredondado. A nadadeira dorsal é triangular e as nadadeiras peitorais são longas (PERRIN; WÜRSIG; THEWISSEN, 2008). Trata se de uma das menores espécies de delfinídeos existentes, tendo em média 1,7 metros e atingindo no máximo 2,2 metros de comprimento (HETZEL; LODI, 1993; SILVA; BEST, 1996).

Apesar da ampla distribuição ao longo da costa da América do Sul, a espécie é considerada como “dados insuficientes” pela IUCN (*International Union for Conservation of Nature and Natural Resources*, 2010) para fins de conservação. E como espécie vulnerável pela ICMBio (ICMBIO, 2014)

No sul do Brasil, os machos atingem a maturidade sexual em uma idade estimada de 7 anos e as fêmeas entre 5 e 8 anos. Tem ciclo reprodutivo de dois anos, sendo que o período de gestação estimado é de 11,6 meses. Os filhotes nascem com comprimento total de 89,1–95,0 centímetros e tem período de lactação/amamentação de 8,7 meses aproximadamente. A promiscuidade com a “competição do esperma” como sistema de acasalamento é o mais provável para os golfinhos do gênero *Sotalia*, no qual ambos os sexos copulam com mais de um indivíduo (ROSAS; MONTEIRO-FILHO, 2002).

Na maioria das áreas onde esta espécie foi estudada, os sítios de uso são águas rasas, perto da costa e frequentemente em profundidades menores de 15 metros (BAZZALO; FLORES; PEREIRA, 2008; CREMER, 2000; DI BENEDITTO; RAMOS; LIMA, 2001).

O boto-cinza alimentam-se de diversas espécies de peixes, assim como de lulas e camarões (BOROBIA; BARROS, 1989; SANTOS et al., 2002; GURJÃO et al., 2003). A alimentação é o comportamento mais frequentemente observado em *S. guianensis* (GEISE, 1991; ARAÚJO; PASSAVANTE; SOUTO, 2001; BAZZALO; FLORES; PEREIRA, 2008), seguido pelo deslocamento (BAZZALO; FLORES; PEREIRA, 2008). Sua atividade alimentar contém um variado repertório de comportamentos, incluindo alimentação individual e estratégias cooperativas (ROSSI-SANTOS; FLORES, 1998).

S. guianensis é considerada uma espécie que evita embarcações (SILVA; BEST, 1994; SANTOS; ACUÑA; ROSSO, 2001). Porém, a ocorrência da espécie em ambientes que sofreram impacto significativo de trânsito náutico, como a Baía de Guanabara, estado do Rio de Janeiro (GEISE, 1991), a Baía da Babitonga (CREMER, 2000; CREMER et al., 2009) e a Baía Norte, em Santa Catarina, (WEDEKIN et al., 2005; FLORES, 1999) e o porto de Ilhéus, estado da Bahia (IZIDORO; LE PENDU, 2012), por exemplo, indicaram que a espécie apresenta uma considerável tolerância a perturbações antrópicas (CREMER; SIMÕES-LOPES; PIRES, 2009).

Na Baía Babitonga habita uma população de *S. guianensis* com elevados níveis de residência (CREMER, 2000; HARDT, 2005; SCHULZE, 2012). A utilização da Baía é de 87,01 km², correspondentes a 53,24% da área (CREMER, 2000). O tamanho dos grupos na região é de 1 a 32 indivíduos, com média de 6,5 animais por grupo (CREMER, 2000).

2 Justificativa

Animais são importantes indicadores ecológicos e o acompanhamento de seu comportamento permite avaliar os riscos ao ambiente. Do mesmo modo, as medidas conservacionistas precisam além do conhecimento sobre a ecologia e a genética das espécies ameaçadas, levar em conta como o animal se comporta (YAMAMOTO, 2007). Assim estudos do comportamento animal podem fornecer uma contribuição importante para a resolução de problemas de conservação (SUTHERLAND, 1998).

Por ser a toninha uma espécie *vulnerável* e devido à escassez de informações sobre seu comportamento e ecologia, o presente estudo pretende constituir uma fonte de incremento ao conhecimento da espécie em um aspecto de seu comportamento até agora desconhecido. A toninha é uma espécie de difícil observação, portanto, para área de estudo optamos pela Baía da Babitonga, uma das únicas regiões com uma população residente em águas estuarinas, facilitando a realização de estudos com esta espécie animal em seu meio natural. Da mesma maneira, o presente trabalho pretende ampliar o conhecimento sobre um aspecto não estudado do comportamento *S. guianensis* na Baía de Babitonga. Assim, almeja-se contribuir para o entendimento geral da sincronia da respiração em cetáceos, adicionando conhecimentos em populações singulares.

3 Bibliografia

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4 Breathing synchrony in franciscana (*Pontoporia blainvilliei*) and Guiana dolphin (*Sotalia guianensis*) (MAMMALIA: CETARTIODACTYLA) in southern Brazil

Pamela Soledad Actis et al.

4.1 Abstract

When studying cetacean behavior, synchronous breathing is observed together with coordinated activities, such as cooperative foraging, resting, inter and intraspecific encounters, mating and courtship. This study was conducted in Babitonga Bay, Southern Brazil, in order to study the synchrony of breathing in two small populations of franciscanas (*Pontoporia blainvilliei*) and Guiana dolphins (*Sotalia guianensis*). The study area is highly impacted by different sources of anthropogenic disturbance. Here we quantitatively analysed synchronous breathing of franciscanas and Guiana dolphins. Synchrony was assumed to be represented by the inverse of the time between breathing surfacing displays of dolphins in a pair. The relationship between synchrony and environmental and/or animal-related variables was evaluated with Generalized Linear models and Generalized Additive Models. The breathing synchrony in Guiana dolphins was positively correlated to the proximity between animals, varied significantly depending on the research boat used, and showed a positive trend with higher group sizes. For franciscanas, breathing synchrony was only positively correlated to the distance between animals. Social characteristics of each species seems to be more important in determining breathing synchrony than external factors.

Keywords: Babitonga Bay; Breathing synchrony; Generalized Linear models; *Pontoporia blainvilliei*; *Sotalia guianensis*.

4.2 Introduction

Synchronous behavior is when two or more animals perform the same activity at the same time (Connor et al., 2006) and it has been described for a wide spectrum of animal groups, e.g. for penguins diving (Takahashi et al., 2004), for frogs calling in chorus (Grafe, 2003), for fish and birds during complex movement (Ballerini et al., 2008; Parrish et al., 2002), among many others.

For cetacean species, synchrony is a term typically referred to when animals are in close proximity to each other and in parallel swimming direction (Fellener et al., 2006). Because cetaceans spend most of the time underwater, behavioral observations are restricted to what can be seen from the surface (e.g. Connor et al., 2006; Hastie et al., 2003; Senigaglia and Whitehead, 2012) or to what is registered with underwater devices (e.g. submersible cameras, acoustic hydrophones) (Aoki et al., 2013). For this reason, research on synchronous breathing is a relatively more accessible option when studying cetacean behavior, since it is discrete, unambiguous and easily observed from above water (Hastie et al., 2003). Synchronous breathing itself is not a special or distinctive behavior category, but it is closely linked to other coordinated activities, such as cooperative foraging and feeding, resting, inter and intraspecific encounters, mating and courtship (Perelberg and Schuster, 2008).

Breathing synchrony has been described for several cetacean species and many studies evaluated the potential proximate factors that may influence this behavior (Connor et al., 2006; Hastie et al., 2003; Senigaglia and Whitehead, 2012). In general, studies showed that group size, population stability, social behavior type and presence of vessels in the surroundings, are positively correlated to breathing synchrony. On the other hand, the presence of calves was negatively correlated with synchronized breathing within a group (Hastie et al., 2003). Nevertheless, when only mother-calf pair is considered, substantial higher synchronies on breathing were observed (Fellener et al., 2006).

As a more general function, the synchronization of breathing may also take part on strengthening social ties, especially in stressful situations such as in large group, for calves accompanying more than one adult and in the presence of boats (Senigaglia and Whitehead, 2012). It has a functional role in the maintenance of the affiliative relationship within swimming pairs and can be influenced by the social structure of the population (Senigaglia et al., 2012), level of kinship, age and gender (Sakai et al., 2010).

In habitats where human disturbance is present, breathing synchrony may have a key role in social cohesion during periods of inefficient communication caused by boat engine noise, and dolphins may possibly identify boats as potential predators (Hastie et al., 2003). The synchronization of breathing for cetaceans can help maintaining close proximity in favor of alliances. It follows that animals need to synchronize their surfacing in order to avoid separating from its pair, since one animal could be breathing at the surface while the other could be submerged (Connor et al., 2006). It was suggested that the

coordinated breathing in dolphins is a cooperation effort, influenced not only by immediate individual outcomes (e.g. food, protection, etc.), but also by social factors (e.g. strengthen social bonds) (Perelberg and Schuster, 2008).

The Babitonga Bay, state of Santa Catarina, southern Brazil, is an important area for resident populations of two small cetacean species: the franciscana dolphin (*Pontoporia blainvillii*) and the Guiana dolphin (*Sotalia guianensis*) (Cremer, 2000; Cremer and Simões-Lopes, 2008; Hardt, 2005). The abundance estimates for franciscanas inhabiting the bay were computed at 50 individuals (95% CI: 28–89) in 2003 (Cremer and Simões-Lopes, 2008), and varying from 52 to 72 between 2011 and 2013 (Sartori, 2014). For Guiana dolphins, existing estimates were computed at 245 (95% CI = 142–422, CV% = 27.9) animals in 2000–2001, 186 (95% CI = 36.2, CV% = 93–374) in 2002–2003 and 179 (95% CI = 93–344, CV% = 33.9) animals in 2003 (Cremer et al., 2011), and 209 (95% CI = 174–252) in 2011 (Schulze, 2012).

The franciscana dolphin is endemic of coastal waters of the Western South Atlantic Ocean (Crespo, 2009) and is currently classified as “vulnerable” in the red list of the International Union for Conservation of Nature (IUCN) (Reeves et al., 2012). The distribution of the Guiana dolphin spans from the Atlantic coast of Central America (Edwards and Schnell, 2001) to Florianópolis, Southern Brazil (Simões-Lopes, 1988), 150 km further south from Babitonga Bay. Despite its wide distribution along the Eastern coasts of South and Central Americas, information regarding density/abundance and vital parameters of Guiana dolphin populations are still scarce and the species is considered as "data deficient" by the IUCN (Secchi, 2012). Both dolphin populations in Babitonga Bay show a high degree of residency (Cremer and Simões-Lopes, 2005; Cremer and Simões-Lopes, 2008; Hardt et al., 2010). Because the area is a protected estuary with calm and shallow waters, absence of large predators, and high productivity, it presents a high quality habitat where dolphins feed, rest and mate (Cremer and Simões-Lopes, 2005).

About 500 thousand people inhabit the surrounding municipalities of Babitonga Bay, which results in serious water contamination, constant deforestation, construction of harbors, overfishing and illegal occupation of banks of water bodies (Cremer, 2006; IBAMA, 1998). The ineffective implementation of management strategies in the area enhances the environmental impacts from human disturbance. The Babitonga Bay is in the list of “Priority Areas for the Conservation of Marine Mammals Biodiversity” and is

considered as of "extremely high" biological importance by the Brazilian Institute of Environment and Renewable Natural Resources (MMA-IBAMA, 2007).

In order to better understand how cetaceans respond to habitat heterogeneity and external factors, such as human disturbance, it is essential to develop feasible ways to measure such adaptations. In this study, we evaluate the time between breathing surfacing displays of dolphin pairs in relation to environmental and animal-related variables, for franciscana and Guiana dolphins inhabiting Babitonga Bay.

4.3 Materials and methods

4.3.1 Study area

The present study was conducted in Babitonga bay ($48^{\circ}38'W$; $26^{\circ}14'S$), Southern Brazil (Figure 1). The bay covers an area of 160 km^2 and is characterized by the fluvial influence of Cubatão, Cachoeira, Palmital and Parati rivers. Sources of direct anthropogenic pressure comes from six cities in its surroundings: Joinville, Araquari, São Francisco do Sul, Garuva, Itapoá and Barra do Sul. In its eastern portion, the bay is connected to the Atlantic Ocean by a 1.7 km wide channel, with mean depth of 6 m (max = 28 m). This bay is under micro tidal regime domain (maximum amplitude variation of less than 2 m), with an average height of 0.84 m (max = 1.9 m) (DHN, 2007). Babitonga Bay borders consist of mangroves, rocky shores and muddy sand beaches, and there are 24 small islands in the inner waters of the bay. Two big harbors are present in the area (Figure 1) and therefore contribute with considerable maritime traffic of commercial boats, recreational fishing, touristic and ferry boats.

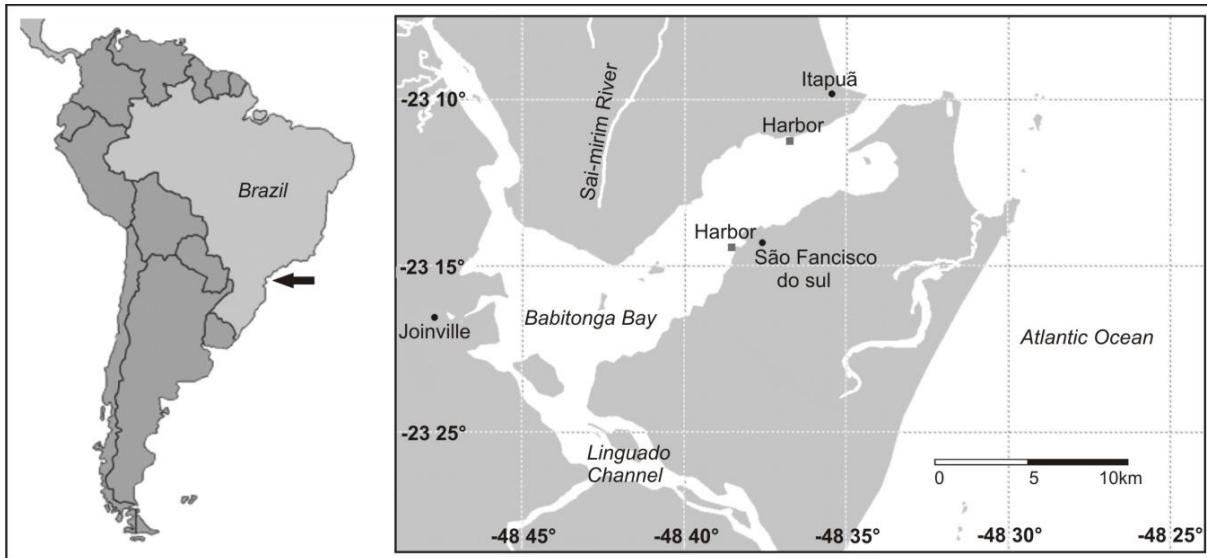


Figure 1. Babitonga Bay and its location in the Southern coast of Brazil, as indicated by a black arrow.

4.3.2 Data collection

Data on breathing synchrony of franciscana and Guiana dolphins were collected between October 2013 and September 2014. Surveys were conducted only in good weather conditions (i.e. Beaufort sea state > 3) and no rain. For this reason, the majority of the surveys (92.6 %) took place in the morning period. Dolphins were searched from small boats (up to 6 m long) at constant speed (20 km/h). Research teams included a pilot, a video recorder and a data recorder. When a dolphin group was detected, boat speed was reduced and animals were followed at a distance of not less than 15 meters. Three different boats were used, due to boat availability: (1) a 6-meter long rigid inflatable boat with a four-stroke engine and outboard motor of 200 HP; (2) a 4-meter long aluminum boat with two-stroke engine and outboard motor of 60 HP; and (3) a 6-meter long wooden boat with two-stroke engine of 60 HP. The boats were always equipped with GPS in order to collect position and a Secchi disk to measure the water visibility.

Data were organized in “encounters”. An encounter was defined as the period from when a group was first sighted to 30 min after the beginning of the encounter, or 10 min after animals remained permanently out of sight. Surveys frequently included more than one encounter. Each encounter was recorded with a digital Panasonic HC-V100 video recorder (full HD 1920×1080, 42x optical zoom). Video recording aimed mainly to focus on dolphin pairs or small groups, and preferentially on those closer to the sighting platform. However, due to wide dispersion of animals in the area or geographical obstacles that

prevented approach, such as islands, video recordings occasionally included more than two animals at the same time. Individual identification was not possible due to the high turbidity of Babitonga Bay (visibility mean = 1.2 m during this study) and due to video resolution limitations.

Each video file was analyzed frame by frame on Windows Live Movie Maker software (version 2.6), measuring the time between the start of the surfacing/breathing movement of any two individuals in an encounter. This interval was measured in seconds and labeled “lag”. The lag was considered here as an inverse measure of synchrony, which means that: “the smaller the lag, the greater the synchrony”.

In order to avoid double counting of the same animal in sequential surfacing, we measured only pairs breathing within an interval of three seconds. This criterion was adopted because surfacing events for these dolphin species usually last about three seconds. This procedure was necessary to avoid the possibility of the same individual being recorded twice. The lag interval was measured from when the first animal of the focal pair put the tip of its rostrum outside the water (for franciscanas), or when a small patch of bubbles on the surface was observed (for Guiana dolphins), to the same for the second animal. A sampling unit was defined as a pair of animals that breathed within an interval between zero and three seconds. If more than two animals surfaced within three seconds, only the two spatially closest to each other were considered in the analysis. No surfacing event of an animal was used to compute more than one lag at a time, which means that if an animal was considered as part of one surfacing pair it was not included in another sampling unit at one moment.



Figure 1. A) Synchronous pair of franciscanas. Only the two animals on the left are considered a surfacing pair, following the criteria described in the text (Picture: Projeto Toninhas/Univille); B) Synchronous pair of Guiana dolphin. (Picture: Karina Actis).

4.3.3 Hypothesis and variables

The main hypothesis to be tested here is that under the influence of complex or stressful factors, dolphins will increase breathing synchrony as a form of protection. Synchrony in breathing is expected to increase with longer periods of exposure to research vessels, larger number of boats, larger group sizes, with research vessels that supposedly cause more impact (i.e. aluminum boats make relatively more noise than wooden and inflatable boats), with the proximity to the harbor and when young animals (calves) are present in the group. The proximity among animals in a pair is also expected to be related to synchrony, as a possible indication of the immediate factors that influence it. Explanatory variables considered in this study are described in table 1.

Variables tested	Definition of the variables	Hypothesis
Time of exposure to research boat	Time from the moment a group was approached the occurrence of each synchronized breathing	The greater the time, the higher will be the breathing synchrony
Number of other boats	Number of other boats than the research vessel, at the time a surfacing pair emerged at the water surface. Boats in a radius of 500 m were considered	Breathing synchrony will increase as the number of boats increase
Group size	Number of animals in the group	Breathing synchrony will increase as group size increases
Research boat	Three different vessels were used: (1) an rigid inflatable boat 6m Ion gflex-boat , (2) an aluminum 4 m long boat and (3) a wooden 6 m long boat	Breathing synchrony will increase with boat that produce more noise (e.g. aluminum boat)
Distance from the harbor	The distance from the encounter starting site to the Sao Francisco do Sul harbour	Breathing synchrony will increase in sites closer to the port
Presence of calves	Dolphin pairs were from one of three categories: 1) a pair of mother-calf; 2) a pair of adults in a group with no identified calf; and 3) a pair of adults were at least one calf was detected as Animals were considered calves if presented 1/2 to 2/3 of the total length of adults	Breathing synchrony will be higher in mother-calf pairs, compared to adult pairs
Distance between animals	A category of the distance between animals in a surfacing pair: animals close together(D0 = animals that surfaced in physical contact with each other; D1 = animals up to one body length apart; D2 = animals that were between one and ten body lengths apart; and D3 = more than ten body lengths apart.	Breathing synchrony will be higher according to how close are the animals

Table 1. Hypotheses proposed as explanations for breathing synchrony in franciscana and Guiana dolphins in Babitonga Bay.

4.3.4 Statistical analysis

All analyses were performed using the free software R (R development core team, 2014) and packages: mgcv (Barton, 2014), MuMIn (Barton, 2014), MRSea (Scott-Hayward, 2015) and car (Fox and Weisberg, 2011).

Exploratory analysis were performed to identify correlation among variables (e.g. pairplots and boxplots; Zuur et al., 2007). The lag between dolphins (ranging from 0.0001–3 seconds) was treated as the response variable in statistical models that included Generalized Linear models and Generalized Additive Models (Wood, 2006). A random effect for the level of group was also tested in a mixed model framework (Zuur et al., 2009). Apart from the possible inclusion of the group random effect, model selection was performed automatically using spatially adaptive smoothing (SALSA; Walker et al., 2011) and GCV score. After a model was automatically selected by the SALSA function, model selection was re-checked using the ‘Anova’ function to test significance of model terms, starting with the full model and dropping one term at a time. Automatic model selection was confirmed for both species.

Results

Thirty-four surveys resulted in a total research effort of 104 hours, with an average duration of 3 hours per survey. Guiana dolphins were registered in 60 (52%) encounters, while franciscanas were registered in 55 (48%), out of 115 encounters.

Group size for Guiana dolphins ranged from 2 to 51 (median=11.2) while for franciscanas ranged from 2 to 20 (median=10.3). Regarding the distance between animals, Guiana dolphins and franciscanas were close together in 29.62% and 9.94%; were up to one body length apart in 47.27% and 29.08%; were between one and ten body lengths apart in 21.56% and 46.68%; and were more than ten body lengths apart in 1.52% and 14.28%.

For both species animals surfaced in less than one second apart in the majority of dolphin pairs considered in this study, representing 81.1% of franciscana pairs and 61.7% of Guiana dolphin pairs (Figure 3).

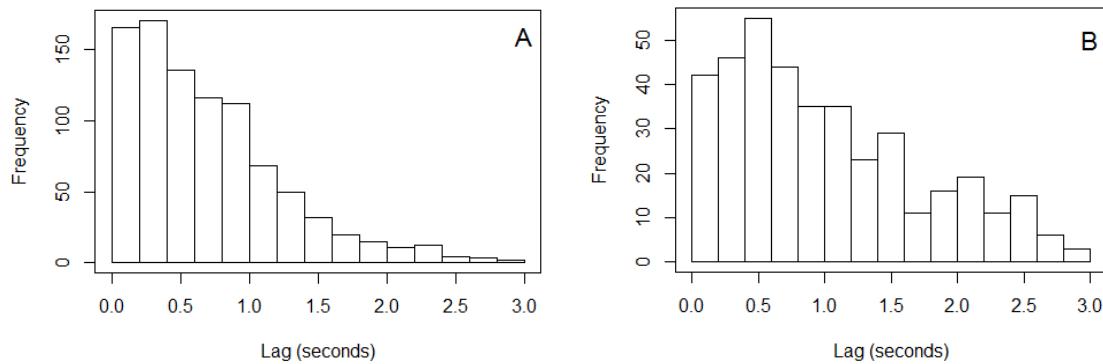


Figure 2. Histogram of lag for Guiana dolphins (A) and for franciscanas (B) pairs.

4.3.5 *Guiana dolphins*

Modeling analysis for Guiana dolphins included 50 encounters and 918 paired surfacing events. The selected model included “distance between animals”, “research boat” and “group size”. Breathing synchrony in Guiana dolphins was higher according to how close animals are from each other (Figure 3) and relatively lower when the aluminum boat was used (Figure 4). A smooth function with four degrees of freedom was selected for variable “group size” (Figure 5). Although breathing synchrony shows a positive relationship (i.e. lag decreases) with group size, there is a deficit in observations for group sizes higher than approximately 20 individuals. The distribution and link function used in the model were the Gamma and inverse link, respectively, and resultant coefficients have the opposite signal (positive/negative) of the biological interpretation.

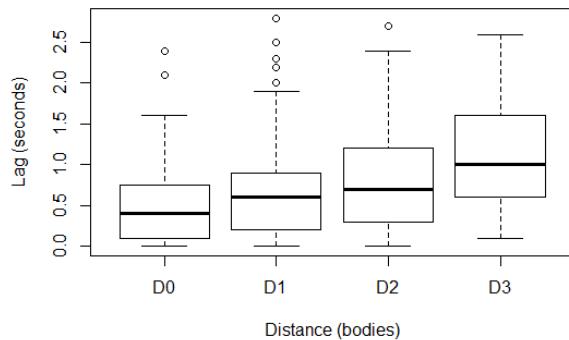


Figure 3. Boxplots of Guiana dolphin lags per category of distance between animals in a pair (D0= animals close together; D1 = animals up to one body length apart; D2= animals that were between one and ten body lengths apart and D3 = more than ten body lengths apart).

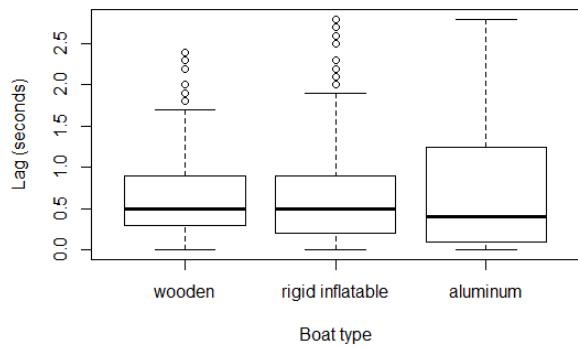


Figure 4. Boxplot of Guiana dolphin lags per research boat used. See text for details.

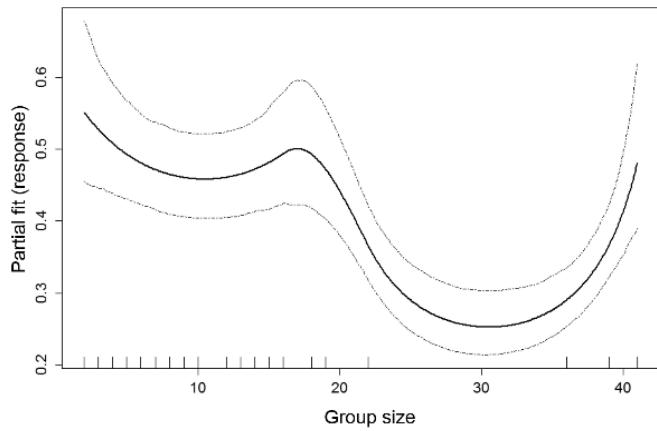


Figure 5. Partial fit (R package MRSea; Scott-Hayward, 2015) for Guiana dolphin group size (4 degrees of freedom).

4.3.6 *Franciscana dolphins*

Analysis for franciscana dolphins included 28 encounters comprising 392 surfacing events. The best model included only variable “distance between animals” (Figure 6) and synchrony was positively correlated (i.e. lag decreased) to how close dolphins are from each other.

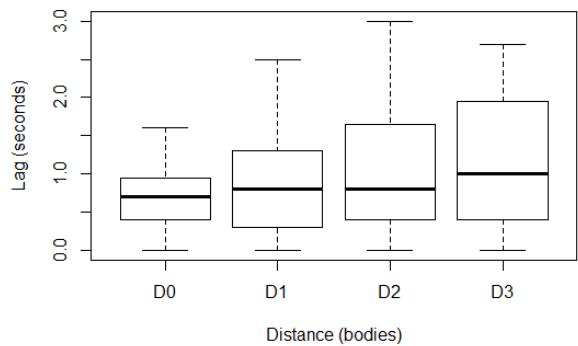


Figure 6. Boxplots of franciscana lags per category of distance between animals in a pair (D0= animals close together; D1 = animals up to one body length apart; D2= animals that were between one and ten body lengths apart and D3 = more than ten body lengths apart).

4.4 Discussion

Synchronous breathing is a frequent event for paired dolphins of both species studied here and seems to reflect a preference of the swimming pairs for breathing synchronously regardless external factors.

The present species differ in their synchronous surfacing frequencies, with Guiana dolphins synchronizing their breath surfacing more frequently than franciscanas. The frequency of synchronous breathing in adult pairs of franciscana dolphins were similar to that found by Bordino et al. (1999) (67%, n=125). For Guiana dolphins, synchronous breathing frequencies found in this study were similar to what was previously presented by Tosi (2007) (95% at rest and 80% in the displacement). Such similarities within each species, regardless of geographical location, show that species-specific characteristics are more determinants for the behavioral aspect than external factors affecting the surrounding habitat.

The modeling results presented here suggest that the distance between animals in a pair, that breath together and in less than 3 seconds apart, plays an important role on the synchronization of breathing for both franciscanas and Guiana dolphins. Animals are clearly more synchronized when closer to each other. The occurrence of synchronous breathing in individuals that are very close was also found during synchronous surfacings between paired male bottlenose dolphins (*Tursiops truncatus*), as described by Connor et al. (2006). In that study, the largest proportion of synchronies was found between 0.5 to 1 meter distance between individuals, and the authors suggest that swimming close together may be a tension-reducing signal, measured from the occurrence of incidental synchronous surfacing. As pointed out in the study of Sakai et al. (2010), bottlenose

dolphins (*Tursiops truncatus*) swimming in close parallel formation did not restrict synchronization to the breathing episodes only, but frequently continued for 6 seconds or more. This highlights that dolphins try to maintain close formation in order to synchronize their movements. Those authors raise the possibility that the synchronization of breathing may be rather a result of social behavior and swimming pair components, and not only for maintaining the proximity between individuals, since bottlenose dolphins do not need breathing exactly synchronous to keep the proximity. The same seems to occur to long-finned pilot whales (*Globicephala melas*). However, because of their remarkable larger size, they inevitably take more time out of water when breathing, than smaller animals and as animals maintaining proximity do not need to breathe <1 s apart (Senigaglia and Whitehead, 2012). In contrast, both species considered in the present study are small cetaceans: up to 1.8 m for Guiana dolphins (Rosas et al. 2003) and 1.5 m for franciscana (Bota et al., 2010). Therefore, the amount of surface time needed for small dolphins to breathe is likely to be lower and the physical proximity between animals is a necessary factor to synchronize their breathing. Nevertheless, even if the relationship between animals' proximity and their synchrony does not necessarily has causality nature, it is clearly correlated and may be linked to other social features not considered here. For example, both physical proximity and the synchrony of breathing could be used to increase hydrodynamic efficiency, resulting in greater energy conservation as proposed by Norris and Schilt (1988).

As previously described for bottlenose dolphins (Hastie et al., 2003) and for pilot whales (Senigaglia and Whitehead, 2012), Guiana dolphins also increased the breathing synchrony when there were more individuals in the group. However, a significant positive relationship between group size and breathing synchrony could be interpreted merely as the consequence of many individuals being at the same place (i.e. probability of clustering) (Hastie et al., 2003). Here we measured the synchrony within pairs of animals (involving two animals only) and assumed that the positive relationship between synchrony and group size is more than a simple fact of chance, given the sample size. According to Senigaglia and Whitehead (2012) larger group sizes increase the possibility of including several "social units" and therefore of a more complex and potentially stressful environment. Indeed, during data collection, small sub groups could be distinguished, especially in larger group sizes.

In contrast to Guiana dolphins, group size does not seem to influence synchronous breathing in franciscanas. This may happen because the franciscana population that inhabits Babitonga bay is smaller and restricted home range than Guiana dolphins (Sartori, 2014). In this sense, there is little variation in group size and the influence of variable on breathing synchrony in franciscanas, was not possible to be detected.

The inclusion of variable "research boat" in the model selected for Guiana dolphins indicates that its effect on the response variable may depend on the boat's characteristics. However, only the aluminum boat was significantly different from the wooden boat and there is no clear visual differentiation in the plot of lags per research boat (Figure 4). The relatively negative influence of the aluminum boat on the synchronous breathing of *S. guianensis* may be explained by a possible acoustic interference from the noise produced by boat on the communication and coordination of dolphins. Also, the variable "research boat" may be correlated to other variables that were not considered in this study, such as individual characteristics of pilots, which varied depending on the boat used. Although the approximation procedures when approaching the animals was always the same, that may also be a source of variation. Previous studies report an inability of acoustic communication due to noise pollution caused by boats for cetacean species (Erbe, 2002; Pirotta et al., 2015). For example Erbe (2002) has found that long and repeated exposure of killer whales (*Orcinus orca*) to the sound produced by ships within a radius of up to 450 meters can reduce by 5 dB or more your hearing range. On the other hand, it is known that the waters of Babitonga Bay present great turbidity (visibility mean = 1.2 m during this study), so acoustic communication may be extremely important for dolphins in the area. The same occurs in other species during periods of limited visibility, such as in sperm whales (*Physeter macrocephalus*) who coordinate deep dives at night using anything more than sounds (Pirotta et al., 2015). Moreover Deconto (2013) found that Guiana dolphins in the Cananéia estuary and Guarapuava bay (in the limit between São Paulo and Paraná states) need higher acoustic communication at night. As the aluminum boat is supposedly the noisiest of the three boats used (by the motor power and building material), it is possible that a greater noise produced by this boat is the cause of the relatively less synchrony found here.

For both *S. guianensis* and *P. blainvilllei*, variables "number of boats", "time of exposure to research boat" and "distance to the harbor" were not included in selected models. This may suggest that these animals are habituated to some immediate human

disturbance in Babitonga bay or do not respond to such potential stressors by adapting their breathing behavior. Other possibly unmeasured reactions may be present in this study, however not directly observed. For example, this would be the case if animals undertook longer dives or abandoned the area as a response to sources of disturbance (Pirotta et al., 2015; Senigaglia et al., 2016). In order to better understand the potential impact that different research vessels may have on Babitonga bay dolphins' behavior, different boat types should be tested (i.e. sailing boats).

We recommend that future studies include variables related to the social nature of the dolphins, such as behavior and animal identification. It would be desirable to include more than two animals in the sampling unit in order to analyze synchrony of breath in social units. In the same way, it would be important to study the behavioral reactions in general and the synchrony of breathing in particular by a standardized methodology so stressing factors can be reliably identified. Once we can identify potential anthropogenic stressors, such information may be used to inform management measures within the bay for protecting these dolphin population.

4.5 Conclusion

Social factors and species-specific characteristics are more important in the breathing synchrony of the present franciscana and Guiana dolphins than external factors. Synchrony in breathing varies mainly in response to immediate factors such as the distance between animals, which may be a result of an effort for maintaining proximity, an effect of social behavior and individual features of dolphins in a swimming pair. Through the use of statistical models we identified more factors related to the synchrony of breath in Guiana dolphins than in franciscanas.

4.6 Acknowledgements

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1 Breathing synchrony in franciscana (*Pontoporia blainvillei*) and Guiana dolphins 2 (*Sotalia guianensis*) in southern Brazil

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19 **Abstract**

20 Synchronous breathing may be a useful proxy for studying other, and perhaps more
21 complex, aspects of cetacean behavior. Here we describe a study conducted in Babitonga
22 Bay, southern Brazil, where we investigated the synchrony of breathing in two small
23 populations of franciscana (*Pontoporia blainvillii*) and Guiana dolphins (*Sotalia*
24 *guianensis*). The bay is affected by different sources of anthropogenic disturbances, such
25 as boat activity and point-source pollution. We assumed breathing synchrony to be the
26 inverse of the time between breathing surfacing displays of dolphins within a swimming
27 pair, which we refer to as *lag*. The relationship between lag and anthropogenic and animal-
28 related variables was evaluated with Generalized Additive Models. For franciscana
29 dolphins, breathing synchrony was only positively related to the proximity between
30 animals. Breathing synchrony in Guiana dolphins was positively related to both the
31 proximity between animals and to group size, and varied significantly depending on the
32 research boat used. Proximal characteristics (*i.e.*, of individuals or of the group) of these
33 dolphin species seem to be related to the synchronization in breathing more than to the
34 environmental variables assessed here. Results presented expand the current knowledge
35 of these two dolphin species and provide general insights into the breathing synchrony for
36 cetaceans.

37 Keywords: Brazil; Behavior; Breathing synchrony; Dolphin proximity; *Pontoporia*
38 *blainvillii*; *Sotalia guianensis*.

39

40 **Introduction**

41 Babitonga Bay, in southern Brazil, is inhabited by resident populations of two small
42 cetacean species: the franciscana dolphin (*Pontoporia blainvillei*) and the Guiana dolphin
43 (*Sotalia guianensis*) (Cremer and Simões-Lopes 2008, Hardt *et al.* 2010). In 2003, there
44 were 50 franciscana dolphins (95% CI = 28–89) (Cremer and Simões-Lopes 2008) and
45 179 Guiana dolphins (95% CI = 93–344) (Cremer *et al.* 2011). The franciscana dolphin is
46 currently classified as *Vulnerable* by the IUCN Red List (Reeves 2012). Information about
47 Guiana dolphin populations is scarce and the species is considered as *Data Deficient* by
48 the IUCN (Secchi 2012) and *Vulnerable* by the Brazilian Ministry of the Environment (MMA
49 2014). Because the bay is formed by an estuary characterized by calm and shallow
50 waters, an absence of large predators and resource rich, it represents a high quality
51 natural habitat where dolphins feed, rest, and reproduce, and where both dolphin
52 populations show a high degree of residency (Cremer and Simões-Lopes 2008, Botta *et*
53 *al.* 2010, Hardt *et al.* 2010).

54 Babitonga Bay is listed in the *Priority Areas for the Conservation of Marine*
55 *Mammals Biodiversity*, and is considered to be of *extremely high* biological importance by
56 the Brazilian Institute of Environment and Renewable Natural Resources (MMA 2007).
57 Nevertheless, human pressure in the area is increasing and more than 500,000 people
58 currently live in the surrounding municipalities, resulting in water contamination,
59 deforestation, port and boat activities, overfishing, and degradation of the shoreline,
60 including in the mangrove (Cremer 2006). The ineffective implementation of management
61 strategies has failed to mitigate these negative impacts. The potential impact of such
62 disturbances on the cetacean species inhabiting the bay and how dolphins may adapt
63 remains poorly understood.

64 Because dolphins spend most of the time underwater, behavioral activities are
65 usually restricted to observations at the surface (*e.g.*, Hastie *et al.* 2003, Connor *et al.*
66 2006, Senigaglia and Whitehead 2012) or with underwater recording devices (*e.g.*,
67 submersible cameras and/or acoustic hydrophones) (Aoki *et al.* 2013). For this reason,
68 research on synchronous breathing is a useful proxy for studying cetacean behaviors,
69 since it can be considered a discrete, unambiguous display that is easily observed from
70 above water (Hastie *et al.* 2003). Synchronous breathing itself is not a special or distinctive

71 behavior category, but is usually observed along other coordinated activities, such as
72 cooperative feeding, group resting, inter- and intra-specific encounters, courtship or
73 mating (Perelberg and Schuster 2008). In this sense, measures of synchronicity may be
74 used as an index of cohesion and sociality among individuals, groups or populations.

75 Synchronous behaviors (Connor *et al.* 2006) have been described in a wide variety
76 of animals and across many behaviors and activities, including penguins diving
77 (Takahashi *et al.* 2004), frogs calling in chorus (Grafe 2003), and fish and birds performing
78 complex and coordinated group swimming and flying, respectively (Parrish *et al.* 2002,
79 Ballerini *et al.* 2008). For cetaceans, proximate factors (*e.g.*, individual characteristics) are
80 known to be related to their breathing synchrony (Hastie *et al.* 2003, Connor *et al.* 2006,
81 Senigaglia and Whitehead 2012). Specifically, group size, population stability, type of
82 social behavior, and presence of boats have been shown to be positively related to
83 synchronized breathing. On the other hand, when animals are in groups, the presence of
84 calves has been found to be negatively related to synchronized breathing (Hastie *et al.*
85 2003). When only mother-calf pairs have been studied, substantially higher synchronized
86 breathing was observed (Fellner *et al.* 2006). More generally, the synchronization of
87 breathing may also play a role in strengthening social ties, especially in stressful situations
88 such as in large groups, when calves are accompanying more than one adult, and in the
89 presence of boats (Senigaglia and Whitehead 2012). Synchronized breathing likely has a
90 functional role in maintaining affiliative relationships within swimming pairs and more
91 complex social groups, and can be influenced by the social structure of a population
92 (Senigaglia *et al.* 2012), level of kinship, age, and sex of animals (Sakai *et al.* 2010). In
93 habitats severely disturbed by humans, breathing synchrony may have a key role in social
94 cohesion during periods of inefficient communication caused by boat engine noise (Hastie
95 *et al.* 2003), which may increase animal stress due to acoustic masking. The
96 synchronization of breathing in cetaceans may help maintain close proximity and
97 strengthen alliances, with animals synchronizing their surfacing in order to avoid
98 separating from a companion (Connor *et al.* 2006). Perelberg and Schuster (2008)
99 suggested that synchronized breathing in dolphins is a cooperation effort, influenced not
100 only by immediate individual outcomes (*e.g.*, food, protection), but also by social factors
101 (*e.g.*, strengthening social bonds).

102 To better understand how breathing synchrony in cetaceans responds to external
103 factors, such as human disturbance, it is essential that such adaptations are investigated.
104 Our objective was to characterize the breathing synchrony in franciscana and Guiana
105 dolphins and to evaluate the influence of anthropogenic and biological variables on this
106 behavioral display in the dolphin populations inhabiting Babitonga Bay. We assumed
107 synchronized breathing changes in response to stressful conditions, and that this behavior
108 is linked to intrinsic features of a population, such as group size and distance between
109 individuals within a group. We expect our results will expand the biological knowledge of
110 these two species and provide general insights on the importance of breathing
111 synchronization for cetaceans.

112 **Materials and methods**

113 **Study area**

114 Our study was conducted in Babitonga Bay ($48^{\circ}38'W$; $26^{\circ}14'S$), southern Brazil (Fig. 1).
115 The bay is approximately 160 km^2 and is fed by several rivers: Cubatão, Cachoeira,
116 Palmital, and Parati. The eastern end of the bay is connected to the Atlantic Ocean
117 through a 1.7 km wide channel, with mean depth of 6 m (max = 28 m). The bay is under
118 a micro tidal regime domain, with an average height of 0.84 m (min = 0.2 m, max = 1.9 m)
119 (Truccolo *et al.* 2006). Babitonga Bay shoreline consists of mangroves, rocky shores, and
120 muddy sand beaches. There are 24 small islands in the inner waters of the bay. Six cities
121 surround the bay (Joinville, Araquari, São Francisco do Sul, Garuva, Itapoá, and Barra do
122 Sul), which potentially are the major source of disturbance. There are two large harbors
123 in the bay resulting in intense maritime traffic by commercial, recreational fishing, touristic
124 and ferry boats.

125 **Data collection**

126 Breathing synchrony of franciscana and Guiana dolphin data were collected between
127 October 2013 and September 2014, periodically when weather permitted. Surveys were
128 conducted only during good weather conditions (*i.e.*, Beaufort sea state < 3 and no rain).
129 Thus, a majority of the surveys (92.6%) took place in the morning. Area searches for
130 dolphins were conducted from small boats (up to 6 m long) at constant speed (20 km/h).
131 The research team always included a pilot, a video camera operator and a data recorder.

132 When a dolphin group (*i.e.*, two or more animals) was detected, boat speed was reduced
133 and animals were followed at a distance of not less than 15 m. Three boats were used at
134 different times during the study (Table 1).

135 Data were organized into encounters. An encounter was defined as the period from
136 when a group was first sighted up to 30 min of observation, or until 10 min after animals
137 went out of sight. Surveys (search for dolphins in a day) frequently included more than
138 one encounter. Each encounter was recorded with a digital Panasonic® HC-V100 video
139 recorder (full HD 1920×1080, 42× optical zoom). Video recording focused on dolphin pairs
140 or small subgroups, and when more than one pair of dolphins was included in a video
141 frame, each pair was analyzed separately, starting with the closest pair (to the boat). A
142 lack of identifiable marks (on dorsal fins) and water turbidity prevented tracking individual
143 animals (mean water visibility during the study was 1.2 ± 0.2 m). Sampling units were focal
144 pairs (Altmann 1974) and if more than two animals surfaced within 3 s, only the two
145 animals in closest proximity to each other were considered for that paired surfacing event
146 (Senigaglia and Whitehead 2012) (Fig. S1, Supporting Information). Thus, each surfacing
147 event of an animal was only considered once in the analysis.

148 Video files were visually analyzed frame by frame on Windows Live Movie Maker
149 software (version 2.6) and the time between the start of the surfacing/breathing activity of
150 dolphin pairs was measured. Each interval was measured in seconds (s) and labeled lag
151 (Senigaglia and Whitehead 2012). The lag was defined as the inverse of synchrony, *i.e.*,
152 the shorter the lag, the higher the synchrony (Senigaglia and Whitehead 2012). To
153 minimize double counting animals in sequential surfacing events, we only considered
154 pairs with lag up to 3 s, similar to methods described in Senigaglia *et al.* (2012), and
155 Senigaglia and Whitehead (2012). For franciscana dolphins, lag was measured as the
156 time between the exposure of the tip of the first animal's rostrum surfaced to the moment
157 the rostrum of the second animal in the pair surfaced. For Guiana dolphins, the moment
158 bubbles broke the water surface immediately before the animals surfaced was used to
159 measure lag. The different displays used to measure lag were adopted because of
160 differences in the behavior of the two species and to identify a clear and distinctive sign
161 that the animals were starting to surface.

162 **Factors potentially related to breathing synchrony**

163 The lag in breathing synchrony was expected to vary with time of exposure to the research
164 boat, the number of boats in the surroundings, group sizes, the proximity to the nearest
165 harbor, and when calves were present in the group. Because the distance between
166 animals (Table 1) in a pair was expected to be related to breathing synchrony, the distance
167 between animals was measured as a qualitative categorical variable. Also, because the
168 research boat may influence dolphin breathing synchrony, we considered factors for the
169 different research boats in the analysis. Details on the variables are described in Table 1.

170 **Analysis**

171 All analysis were performed using the free software R (R development core team 2016).
172 Exploratory analyses were performed to identify correlation between explanatory
173 variables (*e.g.*, correlation coefficients, pair plots and boxplots) (Zuur *et al.* 2007). The lag
174 between dolphins (ranging from 0.001–3 s) was treated as the response variable in
175 Generalized Additive Models (Wood 2006) with a Gamma distribution and inverse-link
176 function. Because dolphin pairs within groups were unavoidably sampled more than once,
177 due to our inability to track individuals, the inclusion of a random effect term for the level
178 of group, which was the lowest possible level of identification of animals, was considered
179 in a mixed model framework (Zuur *et al.* 2009) using “mgcv” R package (Wood 2011).
180 Smoothing knot locations were automatically selected using spatially adaptive smoothing
181 (SALSA, Walker *et al.* 2011) in the “MRSea” R package (Scott-Hayward 2017). Model
182 selection was made using backward selection procedures starting with the full models and
183 dropping one term at a time at the $\alpha = 0.05$ level using the “Anova” R function (package
184 “car”; Fox and Weisberg 2011). The random effect term for group size was evaluated by
185 the amount of variation explained.

186 **Results**

187 Thirty-four surveys resulted in 104 h of video recordings. Focal pairs of Guiana dolphins
188 were closer to each other than franciscana dolphins (Fig. S2, *Supporting Information*),
189 probably reflecting their tighter group formations. Group size of Guiana dolphins ranged
190 from 2 to 51 individuals (median = 11.2), whereas franciscana groups were slightly
191 smaller, with 2 to 20 individuals (median = 10.3). Distances from the harbor ranged from
192 1.36 to 12.78 km for franciscana dolphins and from 0.38 to 11.96 km for Guiana dolphin

193 encounters. Both species had a high proportion of relatively short lag times, less than 1 s
194 (81.1% of Guiana dolphin pairs and 61.7% in franciscana pairs) (Fig. S3, *Supporting*
195 *Information*). Mean surface time was 1.42 s (95% CI: 0.91–1.93) for franciscana and
196 1.41 s (95% CI: 0.8–2.02) for Guiana dolphins.

197 Correlation between variables for both species was low (all Pearson correlation
198 coefficients < 0.3), and visual inspection of pair-plots and boxplots revealed acceptable
199 data behavior for GAMs. Model diagnostic plots indicated that residual autocorrelation
200 was absent (acf plots; Fig. S4, *Supporting Information*). Although some variables selected
201 in the models were highly significant, both selected models had low levels of explained
202 deviance. The random effect for group level was not supported in the models, explaining
203 no extra variation in the data.

204 *Franciscana dolphins*

205 For franciscana dolphins 28 encounters comprising 392 paired surfacing events were
206 analyzed. Only distance between animals was positively related with synchrony (Fig. 2).
207 Thus, lag decreased with proximity between animals within pairs. Model coefficients for
208 levels D2 (one to 10 body lengths apart) and D3 (> 10 body lengths apart) differed
209 significantly (at $\alpha = 0.05$) from the intercept (Table S1, *Supporting Information*).

210 *Guiana dolphins*

211 Analysis for Guiana dolphins included 50 encounters and 918 paired surfacing events.
212 Breathing synchrony was positively related to proximity between paired animals and
213 significantly lower when *boat B* was used in data collection (Fig. 3). A smooth function
214 with four degrees of freedom was selected for variable group size (Fig. 3). Although
215 breathing synchrony showed a positive relationship (*i.e.*, lag decreases) with group size,
216 observations of groups larger than approximately 20 individuals were sparse. Model
217 coefficients can be found in the *Supporting Information* (Table S1).

218 *Discussion*

219 The breathing synchrony for both franciscana and Guiana dolphins were related more to
220 proximate (*i.e.*, individual or group characteristics) than external factors, such as the
221 presence of boats, other than the research boat. The two species, however, differed
222 slightly in their synchronous surfacing frequencies with Guiana dolphins presenting

shorter average lag times than franciscana dolphins. Our results support the hypothesis that synchronization in breathing is related to spatial proximity, where individuals that were closer to each other had shorter lags than individuals that were more distant from each other. Similarly, Senigaglia and Whitehead (2012) indicated synchronous breathing to be frequently displayed in long-finned pilot whales (*Globicephala melas*), but concluded that synchronization in breathing for that species was not strongly related with spatial proximity. Pilot whales are much larger animals (adults can be more than 5 m long) compared to franciscana dolphins (around 1.5 m) and Guiana dolphins (up to 1.8 m). Pilot whales inevitably require more time out of water and distance between individuals may be greater than smaller animals (e.g., the present dolphins), even though pilot whales do not need to breathe within 1 s apart to maintain breathing synchronization and proximity (Senigaglia and Whitehead, 2012). Because Guiana dolphins and franciscana dolphins are two of the smallest cetacean species spatial proximity between animals may be relatively more important for synchronization of breathing. This assumes that time out of water (for any single breathing) increases with increasing body size, which to our knowledge has not yet been formally tested.

Even though we found the proximity between animals to be strongly related to breathing synchronization for both species, it is difficult to infer a causal relationship between distance and breathing synchrony. Individuals may be close because they are genetically and socially related (e.g., mother-calf pair, members of the same family group), they may be engaged in a coordinated behavior (e.g., cooperative foraging, courting/mating), or more likely, because of combinations thereof, or even for unknown or random reasons. Similar synchronous surfacing patterns were observed in paired male bottlenose dolphins (*Tursiops aduncus*) by Connor *et al.* (2006). In their study, the largest proportion of synchronies occurred when individuals were between 0.5 and 1 m apart, and the authors suggested that swimming close together may be a tension-reducing signal. Sakai *et al.* (2010) observed that bottlenose dolphins swimming in close parallel formation do not restrict synchronization to the breathing episode itself, but it continued for 6 s or more during most events. They proposed that this behavior display should be considered a *social behavior*, a component of paired swimming, and not only for maintaining the proximity between individuals.

254 The negative relationship between *boat B* (aluminum; Table 1) and synchronous
255 breathing of Guiana dolphins may be explained by acoustic interference (masking) from
256 the noise produced by that boat on the communication and coordination of dolphins. In
257 fact, there were significant differences in the level of noise produced by each research
258 boat used in this study (Median Test, $P < 0.001$; *unpublished data*, M.J. Cremer). Previous
259 studies have reported reduced acoustic communication in other cetacean species due to
260 noise pollution caused by boats (Erbe 2002, Pirotta *et al.* 2015). For example, Erbe (2002)
261 suggested that long and repeated exposure of killer whales (*Orcinus orca*) to the sound
262 produced by ships within a radius of up to 450 m can reduce their hearing capability by 5
263 dB or more. In addition, the high turbidity of Babitonga Bay may be a contributing factor
264 to the importance of acoustic communication for dolphins. *Boat B* was made of aluminum
265 and produced relatively more noise than the other research boats that were made from
266 other materials (Table 1), probably masking acoustic communication. This may explain
267 the lower synchrony found in encounters observed from that boat.

268 Group size has also been shown to be positively related to breathing synchrony in
269 bottlenose dolphins (Hastie *et al.* 2003) and pilot whales (Senigaglia and Whitehead
270 2012). The contrasting results of the influence of group size on breathing synchrony when
271 comparing the two populations studied here may reflect intrinsic differences in their social
272 organization. Within Babitonga Bay, Guiana dolphins were more abundant and frequently
273 occurred in larger and tighter groups than franciscana dolphins. Danilewicz *et al.* (2004)
274 suggested that the franciscana dolphin's social system is unusual for cetacean species
275 being comprised of family groups exhibiting serial monogamy. In contrast, the social
276 organization of the Guiana dolphins has been described as having a *fission-fusion* nature
277 (Lunardi and Ferreira 2014). A significant positive relationship between group size and
278 breathing synchrony may be interpreted merely as the consequence of more individuals
279 being at the same place (*i.e.*, probability of clustering) (Hastie *et al.* 2003). Moreover,
280 according to Senigaglia and Whitehead (2012), larger group sizes increase the possibility
281 of including several *social units*, and therefore a more complex and potentially stressful
282 environment. This may also be an artifact of the data collection methods where only the
283 two closest individuals were considered as a surfacing pair and included in the analysis.
284 Animals closely related might have been at relatively closer proximity.

Potentially stressful factors, such as the number of boats in the surroundings, time of exposure to research boat and distance to the nearest harbor were not found to be related to the breathing synchrony of either species here. Possibly, (1) these animals are habituated to some of the immediate human disturbances in Babitonga Bay, or (2) if they are not habituated to disturbances, they may not respond to such stressors by changing breathing behavior. Another possibility is that (3) variables that really represent such disturbances for these animals were not included here. Franciscana dolphins, for example, are known to avoid engine powered vessels (Bordino *et al.* 2002) and the present study area is the only known site where this species is frequently observed among transiting boats. Dolphins may respond to stressful factors by undertaking longer dives and moving away from the sources of disturbance (Pirotta *et al.* 2015, Senigaglia *et al.* 2016), which was not shown in our results. Moreover, mammal species may present physiological reactions to external stressors that are not visually detectable. For example, heart rate increased in black bears (*Ursus americanus*) in response to unmanned aerial vehicles passing overhead, although no change in behavior was observed (Ditmer *et al.* 2015).

A limitation in the present study was the inability to identify and track individual animals and individual surfacing pairs, inevitably resulting in multiple samples from the same animal or pair. If information on animal or pair identification was available, those might have helped quantify some of the variability in lag by including random effects for these levels of the data. The only random effect available to be tested was for group/encounter, which did not improve the models when included. Because of the potential of resampling pairs and without information to describe such correlation (individual and/or pair identification), the data could be expected to be highly autocorrelated. However, model diagnostics indicated that our approach of considering each surfacing event as an independent sample was valid, with no autocorrelation in the residuals in the selected models (Fig. S4, *Supporting Information*).

Further studies on breathing synchrony of the dolphin species inhabiting Babitonga Bay should include information on animal identification and the activity of the animals (*i.e.*, foraging, transiting, resting). Moreover, it may be very informative to analyze breathing synchrony within social units as suggested by Senigaglia and Whitehead (2012). Finally,

316 controlled exposure experiments (see Tyack *et al.* 2003, Kastelein *et al.* 2005) could be
317 used to directly test behavioral responses to threats in these populations.

318 Conclusion

319 Social factors and species-specific characteristics were more important than external
320 factors (*e.g.*, presence of boats) in describing breathing synchronization of franciscana
321 and Guiana dolphins. Breathing synchrony varied in response to immediate factors such
322 as the distance between animals, which may result in efforts to maintain proximity. For
323 Guiana dolphins, synchronization was likely influenced by the louder noise produced by
324 the aluminum boat, which could interfere with communication between dolphins. Here we
325 identified three factors related to the breathing synchrony of two coastal dolphin species:
326 distance between animals within a pair, for franciscana dolphins; distance between
327 animals in a pair, group size, and the type of research boat used for data collection, for
328 Guiana dolphins. Individual animal identification and other environmental covariates
329 should be tested in statistical models to better understand how they may influence
330 synchronization of breathing for these species.

331

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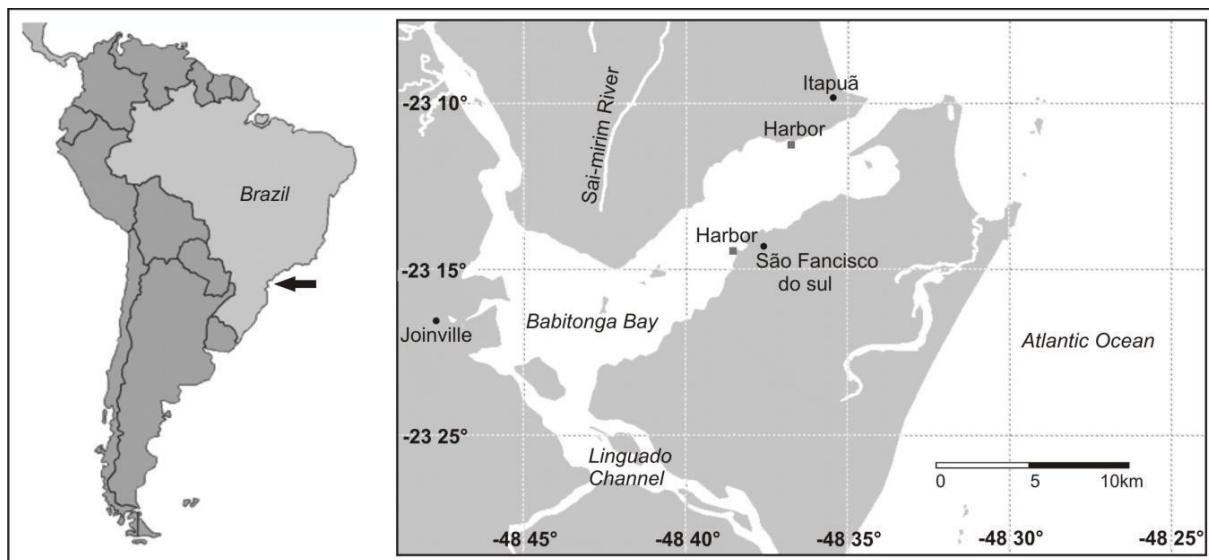
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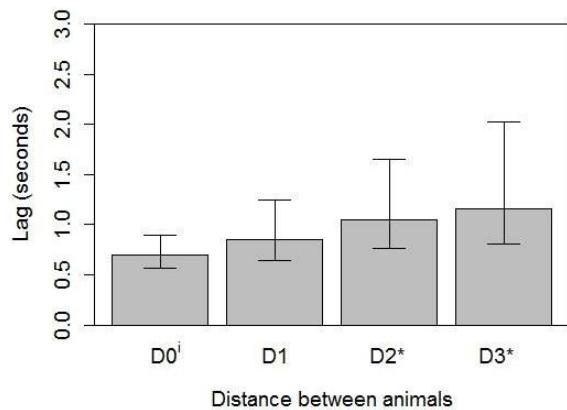
Table

460 *Table 1.* Explanatory variables proposed for modelling breathing synchrony of franciscana
 461 and Guiana dolphins inhabiting Babitonga Bay.

Variable	Definition	Expectation
Distance between animals	A category of the distance between animals in a surfacing pair: D0 = animals close together; D1 = animals up to one body length apart; D2 = animals that were between one and ten body lengths apart; and D3 = more than ten body lengths apart	Breathing synchrony may be positively related to the distance among paired animals
Group size	Number of animals in the group	Breathing synchrony may increase as group size increases
Research boat	3 different boats were used: boat A was a 6.2-meter long rigid inflatable boat with a four-stroke engine outboard motor of 200 HP; boat B was a 5.5-meter long aluminum boat with a four-stroke engine outboard motor of 60 HP; and boat C was a 6-meter long wooden boat with a two-stroke engine outboard motor of 15 HP	Breathing synchrony will vary with boat due to differences in the potential impact caused
Number of boats	Number of boats other than the research boat, at the time a surfacing pair emerged at the water surface. Boats in a radius of 500 m were considered	Breathing synchrony may increase as the number of boats increase
Time of exposure to research boat	Time from the moment a group was approached to the occurrence of each synchronized breathing	The greater the time, the higher will be the breathing synchrony
Distance from the harbor	The distance from the encounter starting site to the São Francisco do Sul harbor	Breathing synchrony may be higher in areas closer to the port
Presence of calves	Dolphin pairs were from one of 3 categories: 1) a pair of mother-calf; 2) a pair of adults in a group with no identified calf; and 3) a pair of adults where at least one calf was detected. Animals were considered calves if presented up to 2/3 of the total length of adults	Breathing synchrony may be higher in mother-calf pairs, compared to other type of pairs

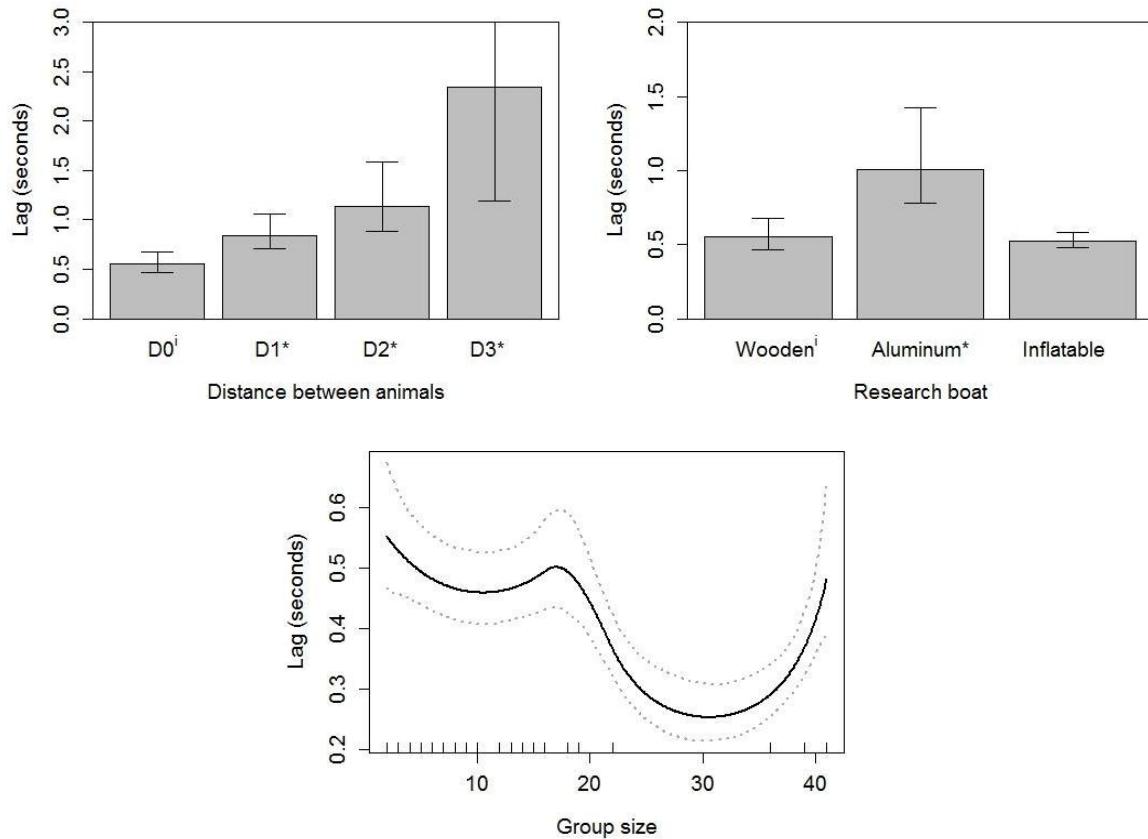
Figures

465 Figure 1. Babitonga Bay and its location (indicated by a black arrow) in the Southern coast
466 of Brazil.



467

468 Figure 2. Partial fit of distance between animals for franciscana dolphins. Bars represent
 469 coefficients and confidence intervals in the response scale, lag. (i = intercept;
 470 * = significantly different from the intercept at $\alpha = 0.01$; D0 = animals close together;
 471 D1 = animals up to one body length apart; D2 = animals between one and ten body
 472 lengths apart; D3 = more than ten body lengths apart).



473

474

475 Figure 3. Partial fit of distance between animals, research boat and group size for Guiana
 476 dolphins. Bars represent coefficients and confidence intervals in the response scale, lag.
 477 The upper limit of the confidence interval for level D3 of distance between animals was
 478 omitted. In the group size plot, the smooth curve (black line) and confidence intervals (grey
 479 dotted lines) in the scale of lag, were created with the “runPartialPlots” function, MRSea
 480 R package. (i^i = intercept; * = significantly different from the intercept at $\alpha = 0.01$;
 481 D0 = animals close together; D1 = animals up to one body length apart; D2 = animals
 482 between one and ten body lengths apart; D3 = more than ten body lengths apart).