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Upcall production by southern right whale (*Eubalaena australis*) mother-calf pairs may be independent of diel period in a nursery area

JULIA R. G. DOMBROSKI,[1] Laboratory of Bioacoustics, Biosciences Center, Federal University of Rio Grande do Norte, Natal 59078-970, Rio Grande do Norte, C.P.1511, Brazil; **SUSAN E. PARKS,** Department of Biology, Syracuse University, 107 College Place, Syracuse, New York 13244, U.S.A; **KARINA R. GROCH,** Projeto Baleia Franca, Av. Atlântica, Imbituba 88780-000, Santa Catarina, C.P.201, Brazil; **PAULO A. C. FLORES,** Centro Mamíferos Aquáticos, Instituto Chico Mendes para Conservação da Biodiversidade, Rod. Maurício Sirosky Sobrinho, km 02, Florianópolis 88.053-700, Santa Catarina, Brazil; **RENATA S. SOUSA-LIMA,** Laboratory of Bioacoustics, Biosciences Center, Federal University of Rio Grande do Norte, Natal 59078-970, Rio Grande do Norte, C.P.1511, Brazil and Bioacoustics Research Program, Laboratory of Ornithology, Cornell University, 159 Sapsucker Woods Road, Ithaca, New York 14850, U.S.A.

Finding patterns in signal production may provide important insights into different aspects of a species' behavior (Bradbury and Vehrencamp 1998). For instance, temporal patterns in vocal behavior of baleen whales have been reported for some populations (*e.g.*, humpback whales (*Megaptera novaeangliae)* off Maui in Au *et al.* 2000; minke whales (*Balaenoptera acutorostrata*) from the Stellwagen Bank National Marine Sanctuary in Risch *et al.* 2013; and North Atlantic right whales (NARW, *Eubalaena glacialis*) off the Scotian Shelf in Mellinger *et al.* 2007) and have provided important clues to determine the factors influencing the evolution of communicative behavior of whale species.

Right whales (RW, *Eubalaena* spp.) are known for using low-frequency vocalizations (usually under 1,000 Hz) for communication (Clark 1983, Parks and Tyack 2005). Within the collection of vocal sounds produced by RW, there is a stereotyped upsweep, the upcall, thought to be used as contact signal (Clark 1983, Parks and Tyack 2005). Due to its potential function as a "contact call" and because it is produced by both females and males of all age classes, upcalls are frequently used as a detection target in passive acoustic monitoring studies (Van Parijs *et al.* 2009).

[1]Corresponding author (e-mail: dombroski.julia@gmail.com).

Upcall monitoring efforts have been useful to estimate population size (Marques *et al.* 2011), address changes in abundance (Morano *et al.* 2012), and determine diel and seasonal patterns of occurrence of right whales (Mussoline *et al.* 2012). Temporal patterns of upcall emission have been described for different RW populations and species (Mellinger *et al.* 2007, Munger *et al.* 2008, Morano *et al.* 2012, Mussoline *et al.* 2012, Soldevilla *et al.* 2014, Bort *et al.* 2015). However, to our knowledge, this is the first information about the temporal pattern of calling behavior from the southern right whale (SRW, *Eubalaena australis*) groups off Brazil.

From July to November, part of a population of SRW concentrates in the southern part of Brazil, mainly off the state of Santa Catarina (Groch *et al.* 2005), where this study was conducted. This wintering area provides exceptional conditions for the study of the behavior and communication of mother-calf pairs, particularly in the last 2 mo of the "whale season." Land-based visual surveys conducted between 2002 and 2008 in the area indicate that 95% and 99% of the overall sightings in October and November respectively, are of mother-calf pairs (Seyboth *et al.* 2015). Additionally, aerial surveys conducted in October and November from 2002 to 2015, using the same methodology as described in Groch *et al.* (2005), resulted in only two sightings of whale groups that were not mother-calf pairs in 14 yr (Table 1). The historical occurrence pattern of whale groups in Santa Catarina indicate that this area can be considered primarily a nursery ground between October and November. Therefore, studies conducted during this period at Santa Catarina can address specific questions about the biology and behavior of mother-calf pairs with negligible influence of juveniles and other adult whales.

The objective of the present study was to investigate diel trends in SWR mother-calf calling behavior at Santa Catarina's nursery ground. Based on previously described behavior of SRW mother-calf pairs in wintering areas and on the potential function of upcalls as contact calls, our initial hypothesis predicted invariable

Table 1. Summary of aerial survey sightings of whale groups (mother-calf pairs and others) in October and November by year, from 2002 to 2015. Note that historically the occurrence of other whale groups in the area is very rare in the presented months. Other whale groups were sighted only twice: once in October 2007 and once in November 2002. Both sightings were identified as adult whales unaccompanied by calves.

	Mother-calf pair		Other	
Year	October	November	October	November
2015	0	—	0	—
2014	—	7	0	—
2013	—	7	—	0
2012	—	2	—	0
2011	—	14	—	0
2010	—	9	—	0
2009	—	9	—	0
2008	15	0	0	0
2007	28	7	1	0
2006	11	—	0	—
2005	9	—	0	—
2004	12	0	0	0
2003	19	0	0	0
2002	—	1	—	1

upcalling activity of pairs. Calves and their respective mothers tend to actively maintain closeness as they near the departure date from the wintering grounds (Taber and Thomas 1982, Thomas and Taber 1984). Actually, in this phase of imminent migration, physical closeness between mother and calf may be greater than in any other phase of the calf's development (Taber and Thomas 1982, Thomas and Taber 1984). Thus, as upcalls are thought to be used for contact maintenance (Clark 1983, Parks and Tyack 2005), we could expect the individuals within pairs to be emitting upcalls with no regard to light regime during the final months of the whale season when sounds were recorded for this study.

Gamboa beach (27°57′S, 48°37′W) (Fig. 1) was chosen as a recording site because the area is considered relatively pristine (with low commercial ship traffic due to its distance from the Imbituba harbor) and because it had adequate conditions for the

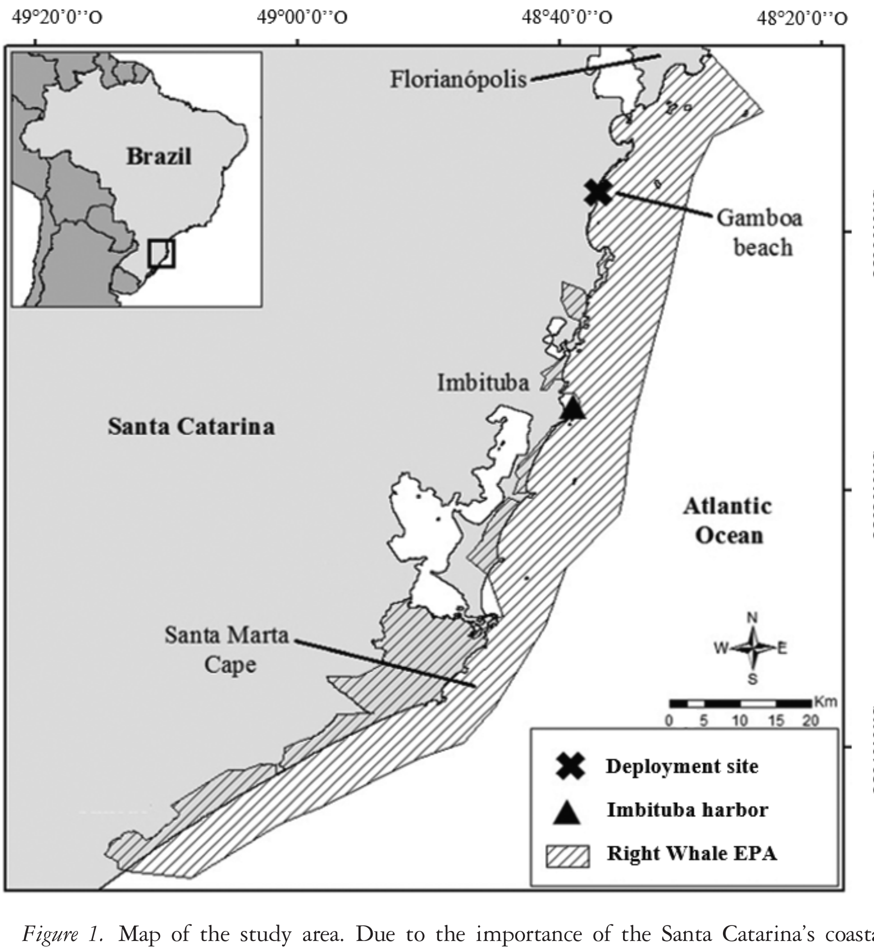

Figure 1. Map of the study area. Due to the importance of the Santa Catarina's coastal waters for SRW, the Brazilian government created the Right Whale Environment Protection Area (Right Whale EPA, Brasil 2000). The Imbituba harbor, located within the Right Whale EPA, concentrates commercial shipping traffic in the area. Archival recording devices were deployed at Gamboa beach, a location considered pristine in terms of underwater noise.

recorder's operation and deployment. One fixed archival autonomous recording unit (DSG Ocean, Loggerhead Instruments, Sarasota, FL) was deployed on 14 October and replaced by a second identical device on 21 October. The second unit remained recording until 28 October, totaling 14 d of recordings in 2011.

Devices were set to continuously sample at a rate of 8 kHz and 16-bit resolution. Each DSG unit was equipped with HTI-96 MIN hydrophones with typical sensitivity of –201 dBV μPa^{-1} between 2 Hz and 30 kHz. Gain level was set to 33 dB (~168 dB re 1μPa clip level). As sampling was interrupted, data from days on which devices were manipulated (deployed, substituted, and/or recovered: 3 d) were excluded from analysis.

In order to elect the most effective method to detect upcalls, trial data consisting of 43 h of recordings were subjected to different detection methods: (1) manual inspection; (2) an automated detection tool developed for NARW upcalls (Urazghildiiev and Clark 2006, Urazghildiiev *et al.* 2009); and (3) a combined technique using the NARW automated tool associated with manual browsing of 1 min segments before and after each detection event using XBAT (Figueroa 2007) as described in Rocha *et al*. 2015.

The combined technique resulted in 635 upcall detections in the trial data set. It had the best performance among the methods tested and the resulting number of detections was considered the number of true positives (635 = 100%). Manual inspection resulted in 464 upcall detections (73%). The automated NARW detection tool detected 505 events and included 386 (61%) true positives and 119 false positives, which were manually verified. False positives were identified as noise, fish sounds, and duplicated detections of the same upcall. The automated detection tool failed to detect all calls in a sequence of closely spaced upcalls (<10 s between calls). However, it was more effective in detecting masked upcalls in comparison to the manual method. Thus, the entire data set was subjected to the combined detection method.

Days with uninterrupted 24 h sampling were divided into four diel periods according to the sun altitude angle in relation to the horizon: Dawn, Day, Dusk, and Night (Munger *et al.* 2008). Dawn was defined by sun altitude between –12° and 0°, which corresponds to the beginning of nautical twilight until sunrise. Day was the period between sunrise and sunset when sun altitude was >0°. Dusk was defined by the period where sun altitude was between –12° and 0° followed by sunset. Night periods were defined as hours of darkness in which sun altitude was <–12° to the horizon. Hourly altitude angle of the sun for Gamboa (27°57′S, 48°37′W) was obtained at the United States Naval Observatory website (http://aa.usno.navy.mil/data). Calling rates (upcalls/h) were calculated within diel periods by dividing the total number of detected calls in a given period by the period duration, as described in Munger *et al.* (2008).

To correct for variation in the number of detected calls in each day, mean-adjusted calling rates were computed by subtracting the daily calling rate from the calling rate of each diel period of that same day (Munger *et al*. 2008). As data were divided into heteroscedastic groups (Levene's test = 11.6, df1 = 3, df2 = 40, $P < 0.001$), the Kruskal-Wallis test was used to verify if the mean ranks of adjusted calling rates across periods were the same. Statistical tests were done using SPSS software (IBM Statistics).

Overall, 3,712 SWR upcalls were detected in 264 h of continuous recordings made off Santa Catarina's nursery area in Brazil. We found no significant variability in adjusted calling rates in relation to diel periods (Kruskal-Wallis test chi-square =

5.8, df = 3, $P = 0.1$). Mean rank of adjusted calling rates for Dawn, Day, Dusk, and Night were 14.5, 23.9, 26.5, and 25.0, respectively. Median and mean values for adjusted calling rates in each diel period are shown in Table 2. Distribution of calling rates by diel period is shown in Figure 2.

To test the influence of outliers on our analysis, the same statistical tests were conducted on our data set after the exclusion of outliers in adjusted calling rates. This additional analysis also did not detect differences of calling rate throughout diel periods ($P > 0.05$). Thus, as we believe that all calling rates accurately correspond to the vocal behavior of whales, we reported here results considering the complete data set, including outliers.

Our study provides evidence which suggests that the upcall emission by SRW mother-calf pairs may be independent of diel period in the nursery area off Brazil, whereas previous studies reported significant trends in upcalling behavior of the North Atlantic right whale. Mellinger *et al*. (2007) and Mussoline *et al*. (2012) conducted research in NARW foraging grounds and reported higher calling rates during daytime and twilight periods. In contrast, Morano *et al*. (2012) and Bort *et al.* (2015) reported greater calling activity at night. Soldevilla *et al*. (2014) also found higher calling activity during the night on NARW winter nursery grounds. For North Pacific right whales (*Eubalaena japonica*), Munger *et al*. (2008) reported a significant diel trend with increased calling rates at night. Finally, in SRW wintering grounds off Argentina, Clark (1983) suggested greater calling activity during the night.

As behavior varies between feeding and wintering areas, differences in temporal patterns in calling behavior may be expected. In feeding areas, calling patterns may be explained by a complex series of factors including the correlation between foraging behavior and vocal activity (Parks *et al.* 2011, Morano *et al*. 2012, Matthews *et al*. 2014). In winter nursery areas, foraging behavior is infrequent making it unlikely that diel trends are linked to feeding behavior in such areas. A more reasonable explanation for increased calling rates during dark periods would be the greater use of acoustic communicative signals when visual cues are less effective (Soldevilla *et al*. 2014). Visual monitoring of whale groups in periods of darkness is impossible. Therefore, increased calling rates at night could be a result of an increased number of vocally active but visually undetectable whales under the hydrophones' detection range, in both wintering and feeding areas (Mellinger *et al*. 2007, Munger *et al*. 2008).

Temporal patterns in calling behavior may be related to site-specific characteristics (Mellinger *et al*. 2007). Thus, our results differ from Clark's study (1983), conducted in a SRW wintering area off Argentina, and from other research conducted in NARW wintering areas (Soldevilla *et al*. 2014 and Bort *et al.* 2015), possibly due to the unique demographic conditions of our study site when this study was conducted: the area is utilized nearly exclusively by mother-calf pairs (see Table 1). In contrast,

Table 2. Mean ($\bar{x} \pm$ SD) and median of adjusted calling rates (upcall/h) for each diel period.

Diel period	Mean	Median
Dawn ($n = 11$)	7.4 ± 12.6	−7.8
Day ($n = 11$)	0.6 ± 4.5	−1.3
Dusk ($n = 11$)	16.0 ± 31.2	9.9
Night ($n = 11$)	0.02 ± 6.5	1.4

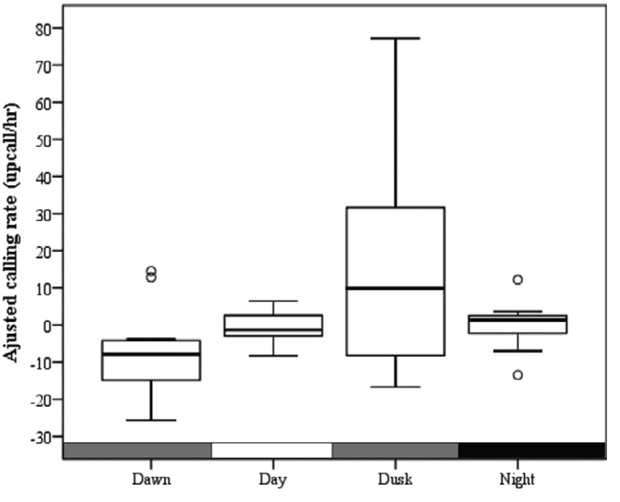

Figure 2. Adjusted calling rate (upcall/h) was not statistically different throughout diel periods. Color bar corresponds to light regime within each diel period: Dawn and Dusk (gray), Day (white), and Night (black).

in other RW wintering areas, the occurrence of lone animals and SAGs were observed (*e.g.*, Clark 1983). Therefore, the temporal pattern reported here—invariable calling rate throughout a 24 h period—may likely refer to the mother-calf pair's vocal dynamics alone while at other wintering grounds, vocalizations of other whale groups present during recordings could have affected the reported temporal pattern of calling behavior.

Greater variability in calling rates as observed in the dusk period in Santa Catarina may indicate encounter events between pairs or other unknown situations that may favor greater or lower vocal activity of pairs. Variable upcall detection might also indicate changes in the number of vocally active animals within the detection range of our recording system, lower vocal activity of animals within the detection range, or changes in detection conditions (Mellinger *et al*. 2007, Munger *et al*. 2008, Soldevilla *et al.* 2014).

Although statistical results support our initial hypothesis of invariable contact signaling during final weeks of the wintering season at the Santa Catarina nursery area, given the short duration of our recorders deployment, the present study was not able to provide evidence about variations in the temporal pattern of calling behavior during early and mid-season, when different spatial relations between mother and calf are expected (Thomas and Talber 1984). Therefore, increasing the sample size may change the homogeneous vocal communication pattern of mother-calf pairs detected in this study. This caveat warrants the collection of longer recordings to further explore the hypothesis that increased acoustic activity occurs shortly before departure from the wintering grounds and an increased sample size to provide further evidence of the presence or absence of a diel pattern throughout the entire season.

Conversely, statistical results reported here may indeed reflect the calling behavior of mother-calf pairs in this wintering ground throughout the season. In this scenario, one can speculate that constant need of contact maintenance between mother-calf could be related to parental care and calf dependence on lactation to acquire energy to migrate to feeding areas (Whitehead and Mann 2000). Furthermore, McCordic *et al.* (2016) suggested that upcalls contain cues for individual identification. If vocal recognition is important for mother right whales and their calves, constant vocal activity could be associated with the individuals' process of learning how to recognize each other by using properties of upcalls before migration (Charrier *et al*. 2009).

Our results suggest that the vocal activity of southern right whale mother-calf pairs may not vary significantly in 24 h in the nursery area off Brazil, and we hypothesize that mother-calf pairs may require constant vocal communication in wintering grounds in the last weeks before migration. Other environmental influences such as tidal variation, moon phase, and boat traffic noise are among factors that may influence vocal activity of whales (Sousa-Lima and Clark 2008), and could be related to temporal patterns of calling behavior of RW off Santa Catarina. Long-term monitoring of the wintering area off Brazil is required to determine whether calling rates change throughout the season and to comprehend the influence of such uninvestigated factors on the vocal behavior of the species. Additionally, it is advisable to investigate the spatial behavior and acoustic communication of RW mother-calf pairs at wintering grounds, taking into consideration the ontogeny of acoustic signals and behavior to expand knowledge about the mother-calf group that is key to the survivorship of all right whale populations.

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