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Signature information and individual recognition in the isolation calls of Amazonian manatees, *Trichechus inunguis* (Mammalia: Sirenia)

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Acoustic signals are assumed to form the basis of manatee communication. Empirical evidence of individual vocal recognition has been reported. If manatees can recognize one another by acoustical means, it should be possible to identify individual vocal patterns. We recorded vocalizations of 14 individually housed Amazonian manatees and then digitized selected vocalizations, allowing seven variables to be measured and subjected to multivariate statistical treatment. Discriminant function analysis indicated that individuals can be separated on the basis of variables related to the fundamental frequency and signal duration. We observed significant differences in the vocal patterns between sexes and age classes. Females tended to have greater fundamental frequency and shorter note duration than males. Calves had shorter note durations and greater values for the fundamental frequency range than subadults and adults. An inverse relationship between total body length and fundamental frequency range suggests that the fundamental frequency becomes more defined as the animal ages. The similar individual patterns in the vocalizations of a mother and calf pair are discussed. Individual recognition by Amazonian manatees according to their vocal patterns is suggested through a preliminary playback experiment.

Many animals communicate specific messages accompanied by additional information about their motivation, sex, age, or even their identity (Halliday & Slater 1983). Recognizing and maintaining contact with specific individuals is more challenging for animals that are often separated in such a way that visual contact is no longer efficient. Selective forces to identify and locate distant individuals could promote the evolution of an acoustic basis for individual recognition (Rendall et al. 1996). Thus, in contexts or periods where the transmission of nonacoustic signals is constrained, individuals can still recognize each other by their vocalizations (Falls 1982). Individual recognition is especially important for social species and in the association between parents and offspring (Halliday 1983).

Theoretically, the development of vocal signatures occurs when there is increased probability of misdirecting parental care, most likely when individuals form breeding colonies or in species where mother and offspring are often separated (Jones et al. 1987). In mammals that have low reproductive rates and extended parental care and use sound-based communication, such as sirenians, one might expect vocal recognition to be important for mothers to maintain close contact with their offspring and for identifying and locating their calves after unintentional separations.

Nursing lasts 25 months in Amazonian manatees (V. M. F. Da Silva, J. A. D'Affonseca Neto, G. E. Mattos & R. S. Sousa-Lima, unpublished data). Given this long-term investment in parental care, it is reasonable to assume that there is considerable selective pressure on a mother to nurse her own, rather than another individual's calf, and that some means of reliable identification is

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necessary. Individual recognition by vocal signals also implies the capacity to learn and to become familiar with individual differences (Halliday 1983). Although the majority of studies concerning individual recognition have focused on parent–offspring relationships, it has been shown that monogamous royal penguins, *Aptenodytes patagonicus*, can recognize their mates by individually distinctive vocal patterns (Robisson 1992).

Loesche et al. (1991) suggest that the recognition process is comprised of four main components: the vocal signature (production of unique signals by the sender); perception (ability to perceive signal differences); decision (ability to choose the right individual); and reaction (response according to the decision). Natural selection can affect any of the four components by increasing interindividual variation or decreasing intraindividual variation to improve individual discrimination, by enhancing the attention or the perception of the receiver through improved receiver sensitivity, or by modifying the decision rule and/or the response after recognition. In the current study we focus on the first two components: the presence of vocal signatures and the perception of individual differences.

Caldwell & Caldwell (1965, 1968) observed a great interindividual variation and intraindividual consistency in the spectrogram contours of bottlenose dolphin whistles, *Tursiops truncatus*. Since these pioneering works, vocal signatures have been investigated in many other species. Falls (1982) even suggested that the occurrence of vocal signatures is universal. However, this does not mean that the level of variation is necessarily enough to achieve effective individual recognition in all cases.

Individual recognition has been verified using playback experiments in birds (McArthur 1982; Jones et al. 1987; Abs & Jeismann 1988; Lessells et al. 1991; Loesche et al. 1991; P. Jouventin & T. Aubin, unpublished data; T. Lengagne & J. Lauga, unpublished data), primates (Cheney & Seyfarth 1982; Rendall et al. 1996) and bats (Balcombe 1990). Abs & Jeismann (1988) and Janik et al. (1994) used a different approach to identify vocal signatures by examining the specific parameters responsible for the individualization of acoustic signals in birds and cetaceans, respectively. Multivariate analyses have been widely used for this kind of investigation in birds (McArthur 1982; Jones et al. 1987; McGregor & Byle 1992), bats (Gelfand & McCracken 1986), primates (Hammerschmidt & Todt 1995), wolves (Tooze et al. 1990), foxes (Frommolt et al. 1997), pinnipeds (Insley 1992; Sanvito & Galimberti 2000) and dolphins (McCowan et al. 1998).

West Indian manatees, *Trichechus manatus*, are known to vocalize while playing and eating. They also produce alarm signals, vocalize as a means of maintaining contact between mothers and calves, and often during intra- and interspecific contact (Hartman 1979; Reynolds 1981; Bengston & Fitzgerald 1985). Evans & Herald (1970) and Sonoda & Takemura (1973) successfully recorded the underwater vocalizations of Amazonian manatees, *T. inunguis*. These authors suggest that the primary function of vocalization in this species is for communication rather than as a navigational tool. Thus far, no study has adequately proven the occurrence of vocal signatures in sirenians. Empirical evidence of individual vocal recognition in the West Indian manatee (Reynolds 1981) and the great variability in vocalizations among individual dugongs, *Dugong dugong* (Anderson & Barclay 1995) could be considered evidence for the existence of vocal identity. Our goal in this paper is to test the following hypotheses: (1) that there is some difference in vocal parameters among individual Amazonian manatees; (2) that there is a difference in vocal parameters between sexes and age classes in this species; and (3) that there is an effect of body size on the vocal parameters.

METHODS

Study Animals

We recorded eight male and six female Amazonian manatees in the facilities of three research institutions that maintain this species in captivity in Brazil: the Laboratório de Mamíferos Aquáticos of the Instituto Nacional de Pesquisas da Amazônia (LMA-INPA), Manaus, state of Amazonas; the Centro de Preservação e Pesquisa de Mamíferos Aquáticos of Manaus Energia (CPPMA), Balbina, state of Amazonas; and the Parque Zoobotânico of the Museu Paraense Emílio Goeldi (MPEG), Belém, state of Pará (Table 1).

The animals are kept either in groups or isolated depending on the current management objectives. There are three round pools at LMA (10 m in diameter, 4 m in depth, with a capacity for 200 m^3 of water each) connected by two smaller ones (3.5 × 2.5 × 1.5 m). At CPPMA there are three octagonal pools (10 m in diameter, 3 m in depth, with a capacity for 200 m^3 of water each) connected by two smaller ones (3.5 × 2.5 × 1.5 m), and at MPEG the housing resembles a natural ‘igapó’ (typical Amazonian flooded forest) and has an area of 198 m^2 with depth varying from 1.2–2 m. Note that all of these facilities were constructed according to the federal laws of animal welfare and the advice of the Brazilian Society of ZOOs.

Recordings

We made the recordings between 11 August and 9 September 1998 on TDK SA60 cassette tapes (type II) using a Sony Walkman Pro (WM-D6C; flat audio frequency response 40 Hz–15 kHz ± 3 dB) and an omnidirectional hydrophone (model 50Ca of the Cetacean Research Technology) with a sensitivity of -161 dB re 1 μPa and a frequency range response of 0.01–310 kHz.

Ethical note

It was necessary to isolate individuals during each recording session to ensure that each vocalization could be identified to individual and that all vocalizations were produced in a similar context.

We isolated adult and subadult individuals either in pools with the sides and bottom covered with rubber tarpaulins (to avoid sound contamination from the connecting or nearby pools) or placed them in totally isolated

Table 1. Identification of the animals studied

Individual	Institution*	Sex	Age class†	Origin (location/state)‡	Body length (cm)
Açaí	CPPMA	M	C	Codajás/AM	103.0
Anamã	INPA	M	C	Anamã Lake/AM	112.5
Ariá	INPA	F	C	Ariaú's Lake/AM	113.0
Boo	INPA	F	A	Unknown	211.0
Carimbó	CPPMA	M	S	Vigia/AM	199.0
Erê	INPA	M	C	INPA (Boo's calf)	112.0
Guarani	INPA	M	S	Cuieiras River/AM	174.0
Macuxi	INPA	F	C	Tacutú River/RR	112.0
Manacá	INPA	F	S	Manacapurú/AM	176.0
Marcelo	CPPMA	M	C	Barcelos/AM	135.0
Santinha	CPPMA	F	C	Santarém/PA	100.0
Preto	CPPMA	M	C	Rio Preto da Erva/AM	130.0
Purú	INPA	M	S	Manacapurú/AM	160.0
Uiara	MPEG	F	C	Breves/PA	100.0

*CPPMA: Centro de Preservação e Pesquisa de Mamíferos Aquaticos; INPA: Instituto Nacional de Pesquisas da Amazônia; MPEG: Museu Paraense Emílio Goeldi.
†M: male; F: female; C: calf; S: subadult; A: adult.
‡AM: Amazonas; RR: Roraima; PA: Pará.

pools. We transferred the calves on a stretcher to smaller fibreglass or rubber pools (2.8 × 1.8 × 0.8 m) that were completely isolated. To ensure the animals' well being and to avoid disruption of normal nursing bouts (nursing bout interval=1 h), we limited the separation period between the mother and calf pair (Boo and Erê) to 30 min (15 min for recording each animal). We conducted 25-min recording sessions for all individuals (except Boo and Erê) and registered their behaviour continuously during the entire session.

Sound Analysis

We selected the recordings to be analysed following the criteria of high signal-to-noise ratio and their occurrence in a similar behavioural context. We chose a maximum of 10 sounds at random for each individual, digitized the samples (sample rate 44100 Hz; sample size 16 bits), and then analysed them using the program Canary 1.2.1 (Cornell Laboratory of Ornithology, Ithaca, New York, U.S.A.; filter bandwidth 699.40 Hz; frame length 256 points). To minimize measurement error, we sampled time and frequency from the most intense harmonic that was clearly visible along the length of the signal. We then divided these measurements by the appropriate factor (number of the harmonic) to yield the value of the fundamental frequency.

Statistical Analysis

We considered only two age classes (calves and others) because the distinction between adults and subadults is arbitrary (Hartman 1979). We performed two discriminant analyses, one for each age class, to test for differences in individual vocal patterns. In both cases we treated each individual as a group (Manly 1994). We excluded the variable 'mean duration of the intervals between notes' from these analyses because it presented no variation between some individuals. We performed two separate multiway analyses of variance (MANOVAs) using the seven measured variables (Table 3) to test for differences in the vocal pattern between sexes and between age classes (Everitt & Dunn 1992). We used the mean values of the variables from each individual in a linear regression to examine the correlation between total body length and each of the seven vocal variables (Sokal & Rohlf 1995).

RESULTS

Overall Characteristics of the Vocalizations

Amazonian manatee vocalizations consist of one to four notes (Fig. 1). The vocalizations are mostly harmonic, consisting of 1–12 frequency bands. Some vocalizations present non-harmonic frequency components resulting in a harsh sound quality. We also recorded broadband click-like sounds. However, due to the relative ease of analysis of the harmonic signals, those were the ones investigated for this study. The mean signal duration (duration of the notes plus duration of the intervals between them) was 242 ± 107 ms (range 50–500 ms). The mean fundamental frequency was 4 ± 1.2 kHz. In many cases, the harmonics were more intense than the fundamental frequency band (Fig. 1).

Each individual has a single type of harmonic isolation call. The intraindividual variation is exemplified in Fig. 1. The number of notes may vary in the same individual, but the overall characteristics of the notes remain the same. There is intra- and interindividual variation on the duration of the signals, while the fundamental frequency values are more conservative within individuals.

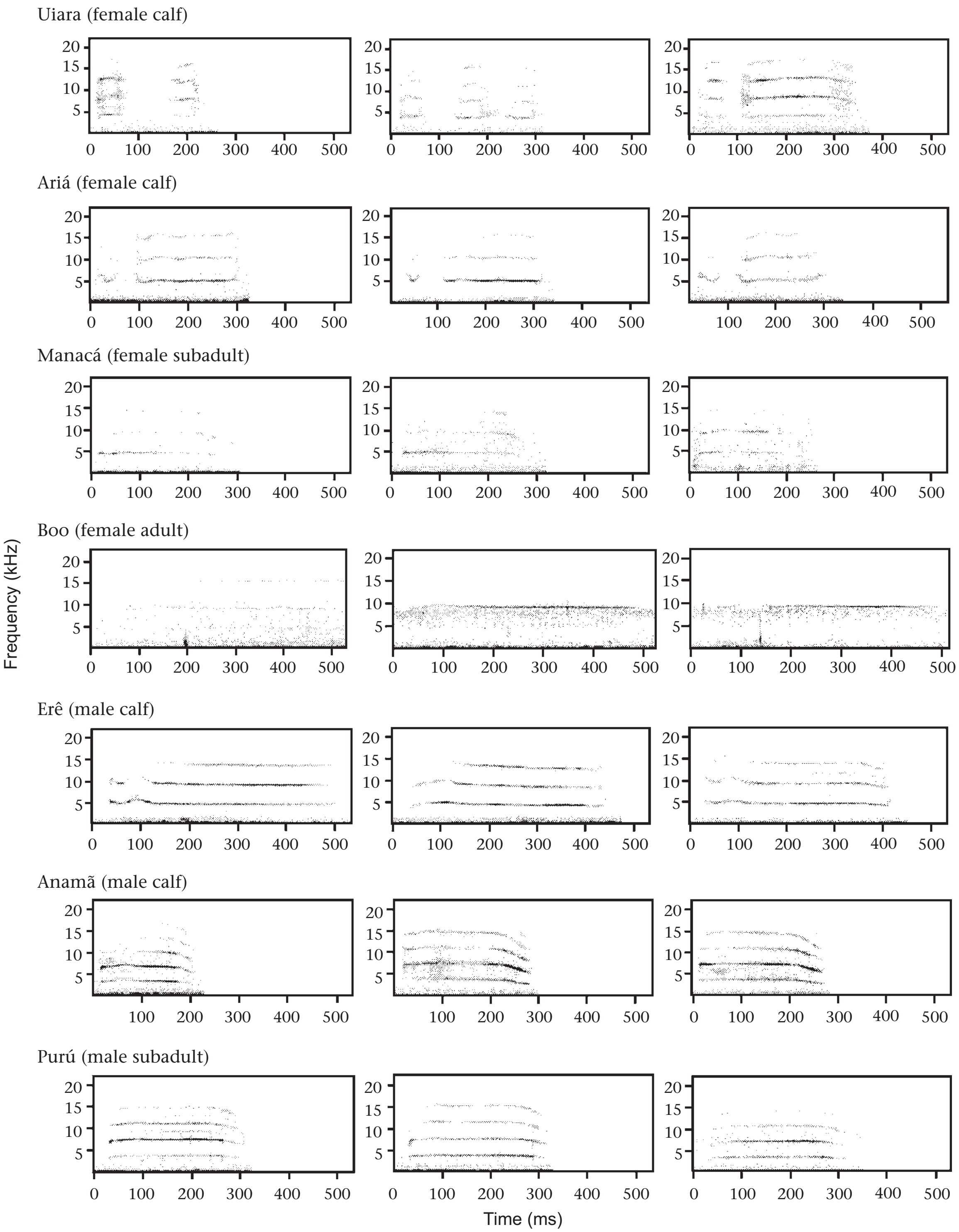

Figure 1. Three examples of spectrograms of vocalizations from each animal recorded. Note the similarity between the calls of Boo and Erê (mother and calf pair).

Data Discrimination

Discriminant function analysis using only calves grouped the vocal variables by individual and sex (Fig. 2). The first axis of the discriminant function showed a strong negative correlation with the mean, minimum and maximum fundamental frequencies (Table 2). Therefore, female calves had higher fundamental frequency values than males. The second axis was inversely correlated with the 'mean note duration' and with 'signal duration' (Table 2), separating the male calf Erê (with longer vocalizations) from the other calves.

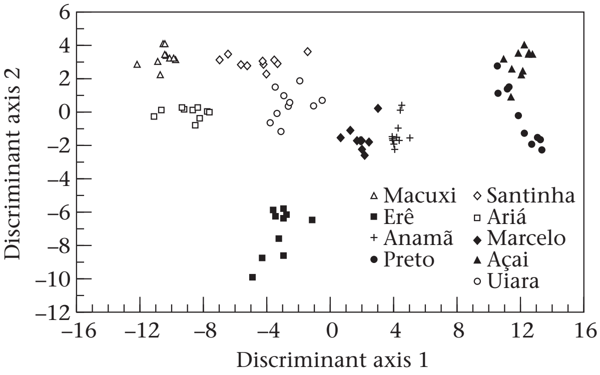

Figure 2. Plot of the two first discriminant functions using calves only. Open symbols indicate females.

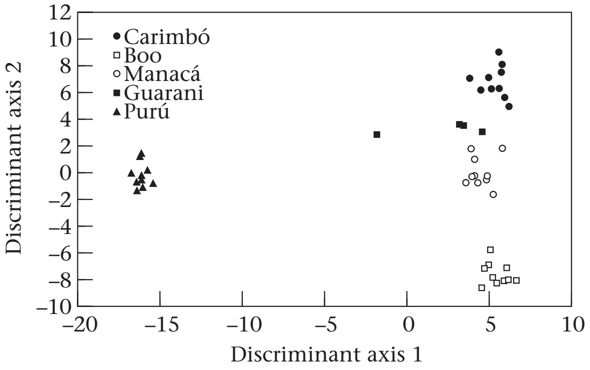

Figure 3. Plot of the two first discriminant functions using subadults and adults only. Open symbols indicate females.

Discriminant function analysis also indicated individual vocal patterns within the considered age class that grouped subadults and adults (Fig. 3), while no sex discrimination was evident. The first axis of the discriminant function showed a strong positive correlation with the mean, minimum and maximum fundamental frequencies. This axis can be considered a function of frequency, isolating the subadult male Purú, with lower-pitched vocalizations, from the rest of the animals. The second axis was inversely correlated with 'mean note duration' and 'signal duration' (Table 2). Note that Boo and her calf (Erê) had the longest signal duration of their respective groups (Figs 2, 3).

Sex and Age Differences

The MANOVAs showed significant differences in the vocal patterns between sexes and age classes. Significant differences between males and females for almost all the variables, except 'signal duration', were found. Females had greater values of mean, maximum and minimum fundamental frequencies, 'duration of the intervals between notes', and 'fundamental range', and smaller values only in 'mean note duration'.

Significant differences between age classes were found in 'signal duration' and 'fundamental range' (Table 3). Calves had smaller values of 'mean note duration' and greater values of 'fundamental range'. The variable 'maximum fundamental frequency' was greater in calves, although it was only marginally significant (level of significance of 5%).

Body Size Effect

There was no sex difference in body size. The only inverse relationship found among all of the variables measured was between the range of the fundamental frequency and total body length ($r = -0.54$; $F_{1,12}=4.9$, $P=0.046$; Fig. 4). However, the regression explained only 30% of the variation, giving it relatively weak predictive power.

DISCUSSION

The data gathered for this study can be easily compared to data on the characteristics of Amazonian manatee vocalizations available in the literature. The duration limits for these kinds of vocalizations have been previously

Table 2. Factor loading of each variable on the two canonical functions of the discriminant function analysis

	Calves		Subadults/adults	
Variables	Function 1	Function 2	Function 1	Function 2
Mean note duration	0.09	−**0.88**	−0.06	−**0.75**
Signal duration	−0.05	−**0.63**	−0.001	−**0.45**
Mean fundamental frequency	−**0.79**	−0.14	**0.86**	−0.11
Maximum fundamental frequency	−**0.41**	−0.23	**0.58**	−0.03
Minimum fundamental frequency	−**0.62**	−0.03	**0.76**	−0.19
Fundamental range	−0.08	−0.18	0.07	0.10
Eigenvalue	66.04	10.38	87.07	27.90
Cumulative proportion of variance	81%	94%	71%	94%
Wilks' λ	0.0001	0.0076	0.00002	0.002
χ^2	740.6	397.8	401.9	234.1
df	48	35	24	15
P value	<0.001	<0.001	<0.001	<0.001

The variables with the highest loading are highlighted in bold.

Table 3. Mean±SD of each variable used in the MANOVA and univariate *F* test between age classes and sexes

	Age class ($\lambda_{7,126}$=0.732, *P*<0.001)				Sex ($\lambda_{7,126}$=0.437, *P*<0.001)			
Variable	Calves (*N*=90)	Others (*N*=44)	$F_{1,132}$	*P* level	Males (*N*=74)	Females (*N*=60)	$F_{1,132}$	*P* level
Mean note duration	172.6±119.7	224.4±140.8	4.91	0.03	215.5±118.7	157.6±134.4	7.0	0.009
Mean interval duration*	11.9±18.7	11.5±21.8	0.02	0.899	7.3±17.6	17.3±20.8	9.2	0.002
Signal duration†	234.6±103.6	255.7±113.6	1.16	0.281	241.6±100.0	241.5±115.9	0.0	0.99
Mean fundamental frequency‡	3.9±1.18	3.9±1.19	0.01	0.901	3.2±1.11	4.8±0.43	109.2	<0.001
Maximum fundamental frequency	4.9±1.71	4.3±1.32	3.99	0.05	3.8±1.48	5.9±0.81	95.2	<0.001
Minimum fundamental frequency	3.3±1.25	3.4±1.15	0.30	0.583	2.6±1.10	4.2±0.60	103.1	<0.001
Fundamental range§	1.7±0.90	0.9±0.40	24.5	<0.001	1.2±0.67	1.7±0.95	11.9	<0.001

*Mean duration of the intervals between notes.
†Duration of the notes plus duration of the intervals.
‡Calculated as the mean value of three intervals of 0.008–0.01 s over the duration.
§Difference between maximum and minimum frequencies.

reported as 0.15–0.22 s (Evans & Herald 1970) and 0.30–0.40 s (Sonoda & Takemura 1973). In the present study we found limits of 0.05–0.50 s. Similarly, the fundamental frequency range in the present study was increased from 6–8 kHz (Evans & Herald 1970) and 2–4 kHz (Sonoda & Takemura 1973) to 1.07–8 kHz. The larger sample size of 14 individuals used in this investigation, compared with only one or two in the previous studies, is probably responsible for the significant improvement in the accuracy of describing the basic characteristics of Amazonian manatee vocalizations.

Intraindividual Variation

The vocalization of one individual varied more in duration than in fundamental frequency (Fig. 1). Although discriminant axis 2 (related to duration, Figs 2, 3) also contributed to the isolation of individual vocal patterns, the fundamental frequency appears to be a more conservative parameter than signal duration.

Insley (1992) verified that the temporal characteristics of two species of pinnipeds are also more variable within an individual than those variables related to the frequency domain. Abs & Jeismann (1988) showed that the state of arousal influences the temporal pattern in courtship songs of birds. Note duration can codify the emotional state in fish (Davis 1988 cited in Lengagne et al. 1997). Bottlenose dolphins produce longer signature whistles when stressed (Caldwell et al. 1990). Contextual information can be related to the alteration of some vocal parameter that would not affect the individual information present in the contour of the whistle (Janik et al. 1994), such as duration.

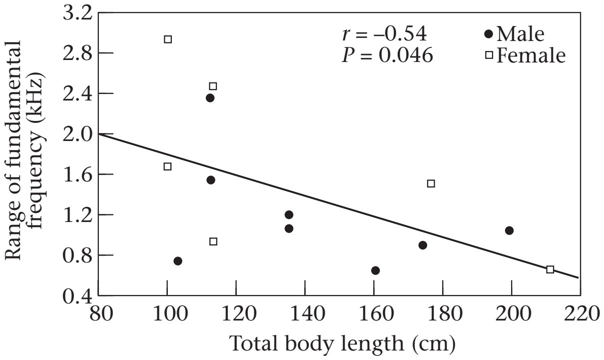

Figure 4. Regression between total body length and range of fundamental frequency.

The evidence that other kinds of information can be related to signal duration lead us to conclude that, because signal duration is a more variable vocal parameter, it can be related either to individuality, or to the motivation of the sender and the context in which the signal is produced.

Taking into account that the main function of manatee vocalization is the maintenance of proximity between mother and calf (Hartman 1979), if signal duration is in fact related to stress and the intensity of the encoded message, then the physical isolation of the mother and calf pair (Boo and Erê) during the recording sessions (the first time they had been separated) might have caused enough distress to result in the production of longer signals. However, the similarity between mother and calf call duration (see Fig. 1) could also be evidence of genetic relatedness or the result of a learning process.

Individual Recognition

The successful grouping of the data by individual in the discriminant function analysis indicates the presence of individually stereotyped vocalizations, a prerequisite for individual recognition by sound (Falls 1982; Jones et al. 1987; Insley 1992). Vocalizations were discriminated based on five characteristics of the signal: maximum, mean and minimum fundamental frequencies, mean note duration and signal duration (Table 2). McArthur (1982) was unable to identify the vocal parameter that would allow individual recognition in piñon jays, *Gymnorhinus cyanocephalus*, and suggested that recognition might be based on 'gestalt' perception of calls, incorporating simultaneous variation in many parameters. As we have found for the manatees, characteristics of the fundamental frequency are important in many species of birds (Robisson 1992) and mammals (Gelfand & McCracken 1986; Tooze et al. 1990; Insley 1992; Frommolt et al. 1997) in which vocal signatures have been verified.

Barbary macaque, *Macaca sylvanus*, mothers are able to recognize their offspring by more than one signal cue, which may improve the robustness of the recognition system against possible distortion caused by the environment (Hammerschmidt & Todt 1995). The recognition system proposed by these authors includes several advantages; for example: differences between individuals do not need to be extreme if they are distributed amongst a number of parameters; one particular parameter does not need to be very distinct in order to guarantee fair discrimination; and, learning of individual characteristics is facilitated by the fact that individuality in vocalizations is not restricted to specific parameters, but can involve any of them. An example of this principle is illustrated by the mother and calf pair (Boo and Erê), which differed from the others predominantly in the parameters 'mean note duration' and 'signal duration'. A potential receiver may easily learn this individual specificity because these parameter values contrast strongly with those of the other manatees.

In timber wolves, *Canis lupus*, the fundamental frequency and the richness of harmonics in the vocalizations is determined primarily by individual characteristics of the vocal apparatus (Tooze et al. 1990). Morphological differences in the structures responsible for phonation or in the neural control of sound production affect the production of calls (Janik 1999). It is likely that in manatees these traits cause sufficient alterations in vocal characteristics such that individual discrimination can occur even by measuring only a few parameters.

Individual information encoded in the variables measured from the isolation calls and in other distinctive features, such as frequency and amplitude modulations observed on the spectrograms (Fig. 1), should be enough evidence to suggest the existence of vocal signatures in Amazonian manatees. Recent recordings reveal stable individual vocal patterns over 4 years (R. S. Sousa Lima, unpublished data). None the less, the analysis of only one call type and the context in which the recordings were made may be considered a limitation on the strength of our evidence. Further investigations of the entire vocal repertoire during intraspecific interactions are required before these calls can be defined as vocal signatures (sensu Caldwell et al. 1990).

The occurrence of vocal identity is suggestive, but does not demonstrate individual recognition. The variation in individual vocal patterns may not be sufficient to always allow accurate discrimination (Falls 1982). The specific parameters measured in this study could be different from those actually used by the animal for individual vocal recognition.

We performed one preliminary playback trial by presenting two acoustic stimuli to the adult female (Boo) to test her ability to perceive and recognize her own calf's vocalizations. Prior to presenting the playback, we conducted a 5-min control period to record Boo's activity, defining a behavioural standard from which any response could be identified. The silent control period was followed by a 5-min playback of vocalizations either from Boo's own calf (Erê), or from a male Amazonian manatee calf (Anamã) that was approximately the same size and age of Boo's calf. The stimuli were played from an Oceaneer underwater speaker (DRS-8, frequency range 100 Hz–16 kHz with peak resonance frequency at 4.5 kHz, 158 dB re 1 μPa) connected to the same recorder used in the acquisition of the original signals. Another 5-min period of silence was allowed following the playback to further monitor the animals' behaviour and ensure that any response detected during the playback was due to the stimulus presented. A 5-h interval separated the two playback sessions. The calf was separated from the mother during the playbacks in order to record its vocalizations.

Boo showed only a mild response to the playback of the unrelated male calf's vocalization. The response consisted of Boo turning her head towards the speaker the first time the stimulus was presented and then going back to her previous activity of swimming around the pool. Boo's response to the stimulus of her calf's vocalization playback was striking and immediate. Boo moved rapidly towards the speaker and touched it with her lips before going to the other side of the pool. Every time the stimulus was presented, she approached the speaker and then went back to the opposite side of the pool, until the 5 min of the playback were over. During the control period, Boo swam around the pool and showed no special interest in the speaker.

Although further trials are needed for absolute verification, our preliminary playback experiments suggest the existence of acoustically based individual recognition in Amazonian manatees.

Mother and Calf Vocal Similarities: Learning or Inheritance?

The similarity between the vocalization patterns of Boo and Erê (Fig. 1) is intriguing. Such an observation could be the result of a learning process and/or of genetic inheritance.

Janik & Slater (2000) defined different forms of social learning in animal communication. They suggest two main forms of social learning, contextual (comprised of usage and comprehension learning) and production learning. Production learning refers to cases in which an individual modifies the form of its signal as a result of experience with those of another individual. It can result either in signals becoming matched, or in distinct differences arising between individuals (Janik 1999).

Vocal learning is considered as production learning in the vocal domain. The similar vocal pattern between Boo and Erê could be a result of production learning by Erê, controlling the respiratory, phonatory and/or filter systems to match the mother's template signal. Janik & Slater (2000) state that the duration and amplitude of a signal can be changed by the respiratory system alone, although the control over the fundamental frequency and its modulations are done by the phonatory system (sound-producing apparatus). Changes in the energy distribution in the vocalization are caused by the filter system (filtering or resonance structures). Bat calls used in mother–infant recognition are frequency-matched (Jones & Ransome 1993). Janik & Slater (2000) suggest that these bat calls are learned. Figure 1 shows the same phenomenon in the vocalizations of Boo and Erê.

Caldwell & Caldwell (1979) suggest that the acoustical environment can alter vocal patterns if these signals are learned. A young captive bottlenose dolphin studied by these authors also developed a vocal signature very similar to that of its mother. Right after Erê's birth, he and the mother (Boo) were isolated from the others and have not been separated from one another since then. Besides the obvious genetic proximity, Boo was the only vocal reference available to Erê. The infant bottlenose dolphin studied by Caldwell & Caldwell (1979) was also exposed almost exclusively to its mother's signature whistle.

The structures responsible for phonation may be more morphologically similar in closely related individuals and thus produce sounds more alike than sounds produced by unrelated animals. Therefore, the influence of genetic relatedness and learning on the observed vocal similarity between parent and offspring cannot be separated yet.

Vocal Differences between Males and Females

Steel (1982) found that male Florida manatees, *T. manatus latirostris*, vocalize at a higher pitch than females. Our results show that females have higher fundamental frequency values than male Amazonian manatees. The Amazonian manatee breeds seasonally (Best 1982) and gender-specific vocal cues could be useful in mate recognition and attraction.

Relationship Between Vocal Parameters and Body Size

The inverse relationship between total body length and the range of the fundamental frequency in Amazonian manatees has low predictive power, but could indicate that maturity has some influence on the decrease in the fundamental frequency range. Gelfand & McCracken (1986) observed significant relationships between all of the vocal parameters and age in bats. Robisson (1992) also verified that some species of penguins show vocal alterations that are related to maturity. He observed that individual vocal identity is related to parameters of the fundamental frequency, which become better defined (more individually distinct) as the young prepare to leave the nest.

Frequency modulation in bottlenose dolphin signature whistles increases with age (Caldwell et al. 1990). Individual identity in bottlenose dolphins has been related to the whistle contour (frequency modulation), rather than to fundamental frequency values. The higher frequency modulation in adult bottlenose dolphins suggests an increment in individual specificity. In Amazonian manatees, this specificity is achieved through well-defined fundamental frequency values, along with other signal characteristics. The greater values of the 'fundamental range' variable in Amazonian manatee calves and the inverse correlation between body size and fundamental range suggest that the fundamental frequency becomes more defined as the animal ages.

The 'mean note duration' in the vocalizations of subadult and adult Amazonian manatees is greater than in calves. Caldwell et al. (1990) suggest that some factor that scales with size may limit the duration (primarily a function of the number of loops per whistle) of sounds that a bottlenose dolphin can produce. Longer signals may require more energy, which is more limited in calves. To call conspecific attention or elicit parental care, Amazonian manatee calves may use a strategy other than the production of long signals, such as increasing the vocalization rate. In fact, the average production rate in Amazonian manatee calves is six vocalizations/min, while subadults and adults vocalize once/min (R. S. Sousa-Lima, unpublished data).

Potential Functions of Vocal Signatures in Sirenians

The production of acoustic signals involves a significant energetic cost (Gerhardt 1983 and must, therefore, have some adaptive value to the sender. What might the functions of the production of individually distinct vocalizations be?

The presence of stereotyped individual signals in Amazonian manatees conforms to the assumption that recognition between conspecifics is important for both parent and offspring, due to the high risk of confusion, as observed in colonial species of birds (Jones et al. 1987). Intuitively, the absence of a well-developed social organization (Reynolds 1981), occurrence in turbid water environment, and the presence of nocturnal activity in manatees (Best & Da Silva 1979) should select for the development of individual vocal recognition capabilities in this species. Dozens of animals are found in feeding aggregations, called 'comidia' by Brazilian hunters. The lack of any strong social organization and the mother's feeding strategies would tend to facilitate the occasional separation of calves from their mothers (Janik 1999). The evolution of individually distinct vocal signals would provide a means for the maintenance of proximity between mother and calf, and the recognition and location of lost calves. The probability of correctly assigning parental care would be greater, thereby increasing an individual's fitness.

Animals that associate with each other early in their lifetime will have plenty of opportunities to learn the vocal characteristics of the individuals in their group (Halliday 1983). The longest and most important bond in manatees is between mother and calf. Other associations are casual, occurring temporarily, for feeding or reproduction (Hartman 1979). Kin recognition, made efficient through individual recognition, can also have adaptive value by providing a way to avoid inbreeding and improve fitness through altruistic behaviour (Halliday 1983).

The signature whistles of bottlenose dolphins are important in establishing vocal or physical contact between individuals (Caldwell et al. 1990) and maintaining group cohesion (Janik & Slater 1998). Mother and calf vocalizations in Amazonian manatees also function as means for maintaining close proximity between individuals (Hartman 1979). Any species in which an association with particular individuals is advantageous should possess some mechanism for group cohesion (Janik & Slater 1998). The individual information encoded in manatee isolation calls improves the efficiency of the cohesion/proximity mechanism. This would be especially important for mothers and calves that are frequently separated (i.e. during foraging). Therefore, the primary function of individually distinct vocalizations in manatees would be identification, location and attraction (cohesion) between specific individuals, markedly between mother and calf.

Halliday & Slater (1983) state that long-lived animals that can recognize each other as individuals can also develop relationships with one another, depending on their past experience. Individual recognition may be important in the maintenance of mating pairs (Halliday 1983) and in male–male competition. A clear social function of interindividual vocal variation has been verified in males of several species of pinnipeds (Stirling 1971; Shipley et al. 1981; Stirling et al. 1987). Reby et al. (1998) indicate that individual vocal characteristics in polygenous fallow deer, *Dama dama*, probably facilitate the establishment of relationships between specific males and females. Females may exercise some preference to mate with particular males that are recognized by their individual vocal pattern. The presence of preferential associations between male and female Antillean manatees, *T. manatus manatus*, in captivity (V. M. Rosa, unpublished data) could also be based on the recognition of individual vocal differences.

The results presented in this study provide evidence for the potential use of vocal parameters, especially those related to the fundamental frequency, in the individual recognition of Amazonian manatees. Further research is needed to demonstrate conclusively whether individual recognition capabilities exist. The best approach will be to perform playback experiments of natural and modified sounds, isolating the most significant parameters, and verifying how much it can be modified until recognition is no longer possible.

Given the precarious status of most Amazonian manatee populations (considered vulnerable by the IUCN; Baille & Groombridge 1996), acoustic surveys can prove to be especially useful in determining population trends in habitats where other methods are difficult or even impossible to use due to poor visibility, dense vegetation, or cryptic habits of the subjects (Baptista & Gaunt 1997). In addition to their shy behaviour on the surface, Amazonian manatees occur in areas with characteristics that make it impossible to observe the animals directly (turbid waters, difficult access, thick vegetation).

By achieving a better understanding of the characteristics and functions of manatee vocalizations, it will be possible to determine the applicability of bioacoustics in the management and preservation of these sirenians throughout the Amazon basin.

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References

Abs, M. & Jeismann, R. 1988. Do courtship songs differ individually in the domestic pigeon *Columbia livia domestica*? *Bioacoustics*, **1,** 151–157.

Anderson, P. K. & Barclay, R. M. R. 1995. Acoustic signals of solitary dugongs: physical characteristics and behavioral correlates. *Journal of Mammalogy*, **76,** 1226–1237.

Baille, J. & Groombridge, B. (Eds) 1996. *Red List of Threatened Animals*. Gland, Switzerland: IUCN.

Baptista, L. F. & Gaunt, S. L. L. 1997. Bioacustics as a tool in conservation studies. In: *Behavioral Approaches to Conservation in the Wild* (Ed. by J. R. Clemmons & R. Buchholtz), pp. 212–242. Cambridge: Cambridge University Press.

Balcombe, J. P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Animal Behaviour*, **39**, 960–966.

Bengston, J. L. & Fitzgerald, S. M. 1985. Potential role of vocalizations in West Indian manatees. *Journal of Mammalogy*, **66,** 816–819.

Best, R. C. 1982. Seasonal breeding in the Amazonian manatee, *Trichechus inunguis* (Mammalia: Sirenia). *Biotropica*, **14,** 76–78.

Best, R. C. & Da Silva, V. M. 1979. Peixe-Boi. Uma Sereia na represa? *Cespaulista*, **16,** 26–29.

Caldwell, M. C. & Caldwell, D. K. 1965. Individualized whistle contours in bottlenose dolphins (*Tursiops truncatus*). *Nature*, **207,** 434–435.

Caldwell, M. C. & Caldwell, D. K. 1968. Vocalization of naive captive dolphins in small groups. *Science*, **159,** 1121–1123.

Caldwell, M. C. & Caldwell, D. K. 1979. The whistle of the Atlantic bottlenosed dolphin (*Tursiops truncatus*): ontogeny. In: *Behavior of Marine Animals: Current Pespectives in Research. Vol. 3* (Ed. by H. E. Winn & B. L. Olla), pp. 369–401. New York: Plenum.

Caldwell, M. C., Caldwell, D. K. & Tyack, P. L. 1990. Review of the signature-whistle hypothesis for the Atlantic bottlenose dolphin. In: *The Bottlenose Dolphin* (Ed. by S. Leatherwood & R. R. Reeves), pp. 199–234. San Diego, California: Academic Press.

Cheney, D. L. & Seyfarth, R. M. 1982. How vervet monkeys perceive their grunts: field playback experiments. *Animal Behaviour*, **30**, 739–751.

Evans, W. E. & Herald, E. S. 1970. Underwater calls of a captive amazon manatee, *Trichechus inunguis*. *Journal of Mammalogy*, **51,** 820–823.

Everitt, B. S. & Dunn, G. 1992. *Applied Multivariate Data Analysis*. New York: Oxford University Press.

Falls, J. B. 1982. Individual recognition by sounds in birds. In: *Acoustic Communication in Birds. Vol. 2* (Ed. by D. H. Kroodsma & E. H. Miller), pp. 237–278. New York: Academic Press.

Frommolt, K.-H., Kruchenkova, E. P. & Russig, H. 1997. Individuality of territorial barking in Arctic foxes, *Alopex lagopus* (L., 1758). *Zeitschrift für Säugetierkunde, suppl. II*, **62,** 66–70.

Gerhardt, H. C. 1983. Communication and the environment. In: *Animal Behaviour Communication. Vol. 2* (Ed. by T. R. Halliday & P. J. B. Slater), pp. 82–113. Oxford: Blackwell Scientific.

Gelfand, D. L. & McCracken, G. F. 1986. Individual variation in the isolation calls of Mexican free-tailed bat pups (*Tadarida brasiliensis mexicana*). *Animal Behaviour*, **34,** 1078–1086.

Halliday, T. 1983. Information and communication. In: *Animal Behaviour Communication. Vol. 2* (Ed. by T. R. Halliday & P. J. B. Slater), pp. 43–81. Oxford: Blackwell Scientific.

Halliday, T. R. & Slater, P. J. B. 1983. Introduction. In: *Animal Behaviour Communication. Vol. 2* (Ed. by T. R. Halliday & P. J. B. Slater), pp. 1–8. Oxford: Blackwell Scientific.

Hammerschmidt, K. & Todt, D. 1995. Individual differences in vocalizations of young barbary macaques (*Macaca sylvanus*): a multi-parametric analysis to identify critical cues in acoustic signalling. *Behaviour*, **132,** 381–399.

Hartman, D. S. 1979. Ecology and behavior of the manatee, *Trichechus manatus*, in Florida. *Special Publication of the American Society of Mammalogy*, **5,** 1–153.

Insley, S. J. 1992. Mother–offspring separation and acoustic stereotypy: a comparison of call morphology in two species of pinnipeds. *Behaviour*, **120,** 103–122.

Janik, V. M. 1999. Origins and implications of vocal learning in bottlenose dolphins. In: *Mammalian Social Learning: Comparative and Ecological Perspectives* (Ed. by H. O. Box & K. R. Gibson), pp. 308–326. Cambridge: Cambridge University Press.

Janik, V. M. & Slater, P. J. B. 1998. Context-specific use suggests that bottlenose dolphin signature whistles are cohesion calls. *Animal Behaviour*, **56,** 829–838.

Janik, V. M. & Slater, P. J. B. 2000. The different roles of social learning in vocal communication. *Animal Behaviour*, **60,** 1–11. doi: 10.1006/anbe.2000.1410.

Janik, V. M., Dehnhardt, G. & Todt, D. 1994. Signature whistle variations in a bottlenose dolphin, *Tursiops truncatus*. *Behaviour Ecology and Sociobiology*, **35,** 243–248.

Jones, G. & Ransome, R. D. 1993. Echolocation calls of bats are influenced by maternal effects and change over a lifetime. *Proceedings of the Royal Society of London, Series B*, **252,** 125–128.

Jones, I. L., Falls, J. B. & Gaston, A. J. 1987. Vocal recognition between parents and young of ancient merrelets, *Synthliboramphus antiquus* (Aves: Alcidae). *Animal Behaviour*, **35,** 1405–1415.

Lengagne, T., Lauga, J. & Jouventin, P. 1997. A method of independent time and frequency decomposition of bioacoustic signals: inter-individual recognition in four species of penguins. *Comptes rendus de l'Académie des sciences, Serie III, Sciences de la Vie/Life Sciences*, **320,** 885–891.

Lessells, C. M., Coulthard, N. D., Hodgson, P. J. & Krebs, J. R. 1991. Chick recognition in European bee-eaters: acoustic playback experiments. *Animal Behaviour*, **42,** 1031–1033.

Loesche, P., Stoddard, P. K., Higgins, B. J. & Beecher, M. D. 1991. Signature versus perceptual adaptations for individual vocal recognition in swallows. *Behaviour*, **118,** 15–25.

McArthur, P. D. 1982. Mechanisms and development of parent–young vocal recognition in the piñon jay (*Gymnorhinus cyanocephalus*). *Animal Behaviour*, **30,** 62–74.

McCowan, B., Reiss, D. & Gubbins, C. 1998. Social familiarity influences whistle acoustic structure in adult female bottlenose dolphins (*Tursiops truncatus*). *Aquatic Mammals*, **24,** 27–40.

McGregor, P. K. & Byle, P. 1992. Individually distinctive bittern booms: potential as a census tool. *Bioacoustics*, **4,** 93–109.

Manly, B. J. F. 1994. *Multivariate Statistical Methods: a Primer*. 2nd edn. London: Chapman & Hall.

Reby, D., Joaquim, J., Lauga, J., Lek, S. & Aulagnier, S. 1998. Individuality in the groams of fallow deer (*Dama dama*) bucks. *Journal of Zoology*, **245,** 79–84.

Rendall, D., Rodman, P. S. & Emond, R. E. 1996. Vocal recognition of individuals and kin in free-ranging rhesus monkeys. *Animal Behaviour*, **51,** 1007–1015.

Reynolds III, J. E. 1981. Aspects of the social behaviour and herd structure of a semi-isolated colony of West Indian manatees *Trichechus manatus*. *Mammalia*, **45,** 431–451.

Robisson, P. 1992. Roles of pitch and duration in the discrimination of the mate's call in the king penguin *Aptenodytes patagonicus*. *Bioacoustics*, **4,** 25–36.

Sanvito, S. & Galimberti, F. 2000. Bioacoustics of southern elephant seals. II. Individual and geographical variation in male aggressive vocalisations. *Bioacoustics*, **10,** 287–307.

Shipley, C., Hines, M. & Buchwald, J. S. 1981. Individual differences in threat calls of northern elephant seal bulls. *Animal Behaviour*, **29,** 12–19.

Sokal, R. R. & Rohlf, F. J. 1995. *Biometry*. 3rd edn. New York: W. H. Freeman.

Sonoda, S. & Takemura, A. 1973. Underwater sounds of the manatees, *Trichechus manatus manatus* and *Trichechus inunguis* (Trichechidae). *Reports of the Institute for Breeding Research, Tokyo University of Agriculture*, **4,** 19–24.

Steel, C. 1982. Vocalization patterns and corresponding behaviour of the West Indian manatee (*Trichechus manatus*). Ph.D. thesis, Florida Institute of Technology.

Stirling, I. 1971. Studies on the behaviour of the South Australian fur seal, *Arctocephalus forsteri* (Lesson) I. Annual cycle, posture and calls, and adult males during the breeding season. *Australian Journal of Zoology*, **19,** 243–266.

Stirling, I., Calvert, W. & Spencer, C. 1987. Evidence of stereotyped underwater vocalisations of male Atlantic walruses (*Odobenus rosmarus rosmarus*). *Canadian Journal of Zoology*, **65,** 2311–2321.

Tooze, Z. J., Harrington, F. H. & Fentress, J. C. 1990. Individually distinct vocalizations in timber wolves, *Canis lupus*. *Animal Behaviour*, **40,** 723–730.