



Instituto Nacional de Pesquisas da Amazônia

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Investimento maternal diferenciado e uma nova abordagem de fotometria em filhotes de boto-vermelho (*Inia geoffrensis*) em uma área de várzea na Amazônia central.

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Manaus, Amazonas

Abril, 2018

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Sinopse:

Validou-se uma nova abordagem de fotometria, neste estudo foi usada pra estimar o tamanho dos filhotes do boto-vermelho (*Inia geoffrensis*) mediante o comprimento total conhecido da mãe. Dados de fotometria foram usados junto com dados longitudinais para estudar o investimento maternal diferenciado entre machos e fêmeas no período de dependência em relação à ontogenia do desenvolvimento do dimorfismo sexual na espécie. Evidencias de um maior investimento para machos foram registradas nas taxas e num comprimento maior ao final do cuidado parental quando comparado às fêmeas.

Palavras-chave: Amazônia, cuidado maternal, dimorfismo sexual, odontoceto, comprimento.

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Resumo

Em espécies poligínicas, a forte competição entre machos favorece o desenvolvimento de características que dão vantagens a eles no acesso às fêmeas, considerando que indivíduos maiores provavelmente terão mais sucesso reprodutivo. A evolução do dimorfismo sexual mostra diferenças entre os sexos, tanto na duração quanto nas taxas de crescimento. De acordo com a teoria de investimento diferenciado, durante o período parental, pode haver um investimento diferenciado entre os sexos, não somente nas taxas de crescimento, mas também no tamanho das crias e na duração do cuidado maternal, sendo o investimento maior para machos. Como os cetáceos passam a maior parte do tempo submersos, torna-se extremamente difícil obter os tamanhos de filhotes na água sem capturas. Esses dados são importantes para a classificação por classe de idade, permitindo entender a biologia e ecologia da espécie, assim técnicas não invasivas como a fotometria se torna eficaz. A fotometria permite a coleta de dados ao longo do tempo em animais identificados individualmente e, conseqüentemente, possibilitam gerar estimativas sobre o tamanho e crescimento dos filhotes. No primeiro capítulo deste estudo, descreve-se uma nova abordagem do uso da fotometria, que possibilita estimar o tamanho total de indivíduos (*objetivo*) quando parte do corpo de outro indivíduo (*referência*), de comprimento total conhecido ou estimado, estiver próximo. A proporção entre a distância do espiráculo e a ponta da nadadeira dorsal na imagem foi usada para estimar o comprimento total do filhote com base no comprimento conhecido da mãe. A acurácia dessa técnica foi testada em 33 imagens de 12 pares de mães com filhotes de boto-vermelho (*Inia geoffrensis*), houve uma média de erro de 3.8%. No segundo capítulo, um total de 221 observações, em 74 indivíduos de idade e tamanho conhecido (43 fêmeas e 31 machos), foram utilizadas para analisar o investimento maternal entre os sexos e a influência na evolução do dimorfismo sexual na espécie. Para isso, foram usados dados de capturas, fotometria e observações diárias, ao longo de 25 anos do Projeto Boto. A idade e o tamanho, no final do período de dependência do filhote, foram analisados usando Modelos Lineares Generalizados Mistos (GLMM) com distribuição binomial. Para testar a diferença das taxas de crescimento entre os sexos, durante o período de dependência e juvenil, foram utilizados Modelos Lineares Mistos (LME). Não foram encontradas diferenças no tempo de cuidado parental entre filhotes machos e fêmeas (3.07 anos, $p > 0.5$), porém, houve diferença no tamanho corporal no final do período de dependência maternal (172.21 cm machos e 165.58 fêmeas, $p < 0.05$). Em comparação, as taxas de crescimento durante o período juvenil não foram significativas. Machos apresentam um período juvenil mais longo do que fêmeas, pois continuam a crescer após as fêmeas terem 200 cm. Os resultados indicam que, durante o mesmo período de cuidado maternal entre filhotes machos e fêmeas, os filhotes machos se tornarão independentes com tamanho corporal maior do que o das fêmeas o que é consequência das diferenças nas taxas de crescimento achadas, durante o cuidado maternal, maiores para os machos. Visto que as taxas de crescimento no período juvenil não foram significativas, assume-se que a teoria do investimento diferenciado para o boto-vermelho pode ser aceita e que afetará o desenvolvimento do dimorfismo sexual existente nessa espécie, assim como um período maior de crescimento nos machos.

Abstract

Polygynous species usually have strong competition between males. This fact favours the development of certain characteristics that will give them advantages in order to have access to females, since bigger males may have higher reproductive success. The evolution of sexual dimorphism may show differences between sexes during growth period and also in relation to growth rates, which can be affected by maternal behaviour through parental care period. According to sex-biased investment theory, across that period it may be a sex-biased investment toward males, not just in growth rates but also may be reflected in duration of parental care and offspring size at the end of it. Odontocetes spent most of their time below the water surface making difficult to gather population and reproductive parameters. This challenge has enhance the development of non-invasive research tools, as photogrammetry, which in addition to a long term research with marked animals may allow access to the estimation of those parameters. Besides, it may allow retrieving size data from calves when the parental care period is long. The first chapter describes a new photogrammetry approach in order to estimate the body length of a *target* dolphin in situations where part of the body of another animal of known (or estimated) length - the *reference* animal - is in close proximity. The distance from blowhole to tip of dorsal fin was used as proportion to estimate the total length of the calf in the image. The accuracy of the technique was tested using 33 images of mother and calf pairs of Amazon River dolphin (*Inia geoffrensis*), given a mean error of 3.8%. In the second chapter, the sex-biased maternal investment theory and its influence in the ontogeny process of sexual dimorphism was tested. A total of 211 (172 capture and 39 photogrammetry) observations from 74 individuals (43 females and 31 males) of known length, age and sex were used. Age and length at the end of maternal period was analysed using Generalized Linear mixed models (GLMM) with binomial distribution. In order to test for slope differences between sexes across dependent period and independence period Mixed Linear Models (LME) were used. No differences were found in age at the end of maternal care between sexes (3.07 years, $p > 0.5$); however, differences at length were found (172.21 cm males and 165.58 females, $p < 0.05$). These length differences are the result of the growth rate differences observed within a maternal care period ($p < 0.05$), on the other hand, no differences within juvenile period were found between sexes. Males still growing after females reach their asymptote, having a longer juvenile period than females. Therefore, the results indicate that during the same period of maternal care for male and females, males will become independents with a bigger size as a consequence of the different growth rates found within dependent period. Also, as growth rates in juvenile period were the same for both sexes, we can assume that sex-biased investment is applied in botos. Thus, differences in growth rates by additional maternal investment and different periods of growing are the processes from which Amazon River dolphin reached sexual dimorphism.

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Introdução geral

Nos mamíferos, diferentes estratégias reprodutivas têm levado através da seleção natural, a diferenças morfológicas entre os sexos, sendo o macho geralmente maior por competir com outros pelo acesso às fêmeas. Por outro lado, as fêmeas adotam uma trajetória de crescimento mais conservativa, atingindo a maturidade sexual primeiro e investindo energia na reprodução (Riedman 1990, Watts & Pusey 2002). Essas diferenças geralmente são observadas em indivíduos adultos. Contudo, durante a ontogenia do organismo, as taxas de crescimento ou a duração do crescimento podem ser selecionadas como indicadores do dimorfismo sexual (Badyaev 2002), tendo uma estreita relação com as condições sociais e ecológicas da espécie (Leigh 1992, Joffe 1997, Isaac 2005).

Em espécies poligínicas a fêmea é exclusivamente responsável pelo cuidado do filhote. Quando o sexo da prole é um fator importante para o sucesso reprodutivo, o investimento pode ser diferenciado. Deste modo, de acordo com a teoria de investimento diferencial entre os sexos, as mães vão investir mais nos filhotes machos (Trivers & Willard 1973, Maynard-Smith 1980, Clutton-Brock *et al.* 1981). Em contrapartida, o maior gasto energético pode afetar a sobrevivência e reprodução futura da mãe (Trivers 1972).

Os cetáceos odontocetos geralmente são poligínicos, apresentando longo cuidado parental devido à necessidade de aprendizado de habilidades sociais e de forrageio que vão garantir a sobrevivência dos filhotes até se tornarem independentes (Brodie 1969, Mann & Smuts 1999, Guo *et al.* 2010, Colbeck 2013). O investimento diferenciado entre sexos pode ser medido pela frequência da lactação (Ortiz *et al.* 1984, Lee & Moss 1986) e, pela duração do cuidado parental e sua influência nas taxas de crescimento (Lee *et al.* 1991), uma vez que o tamanho do filhote vai determinar a sua sobrevivência (Clutton-Brock *et al.* 1987, Huang *et al.* 2011), o sucesso reprodutivo, entre outros aspectos da história de vida do animal (Albon *et al.* 1987, Bérubé *et al.* 1996, Dmitriew 2011). Portanto, o conhecimento da idade e do tamanho dos indivíduos é essencial para entendermos como o investimento maternal vai afetar as diferentes estratégias reprodutivas e o desenvolvimento do dimorfismo sexual, uma vez que os mecanismos entre o crescimento rápido e lento vão ser refletidos nas taxas de crescimento (Dmitriew 2011), as quais podem ser suscetíveis a efeitos maternos (Bernardo 1996, Mousseau and Fox 1998).

O estudo de odontocetos é extremamente difícil, devido passarem a maior parte do tempo submersos. Pesquisas de longa duração com animais individualmente identificáveis são recomendadas não somente para analisar a variação no crescimento entre os indivíduos

mediante o uso de dados longitudinais de tamanho e idade (McFee 2010), mas também para entender aspectos da estratégia reprodutiva no acompanhamento de mães com filhotes ao longo do tempo (Mann & Karniski 2017). Em estudos de longa duração, as medidas de tamanho são obtidas, geralmente, por técnicas invasivas, tais como captura e contenção dos indivíduos (Read *et al.* 1993, da Siva & Martin 2000). No entanto, esse método pode impedir, em algumas espécies, a captura de filhotes (Read *et al.* 1993). Com base nessas informações, ferramentas não invasivas, como a fotometria, está sendo, nesse trabalho, a principal ferramenta para coleta de dados de tamanho de filhotes, que junto com acompanhamento ao longo prazo dos indivíduos da população fornecem também informação sobre a idade dos indivíduos.

O boto-vermelho (*Inia geoffrensis*) é um cetáceo odontoceto amplamente distribuído na bacia Amazônica e restrito à água doce. Os machos são 55% maiores e 16% mais pesados do que as fêmeas, sendo um dos odontocetos apresentando maior dimorfismo sexual depois do cachalote (*Physeter macrocephalus*) (Martin & da Silva 2006). Os botos machos apresentam intensa competitividade pelo acesso às fêmeas e também segregação sexual, ocorrendo em maior proporção no rio principal enquanto que as fêmeas ocorrem em áreas de lago, provavelmente, por serem locais que ofereçam maior proteção para criar seus filhotes (Martin & da Silva 2004, Mintzer *et al.* 2016). Fêmeas de boto-vermelho tem alto investimento reprodutivo, com gestação de mais de 12 meses e uma longa associação entre mãe e filhote, podendo ficar juntos por cerca de três anos (Martin & da Silva 2018).

O Projeto Boto vem coletando informações longitudinais sobre o boto-vermelho há 24 anos, na Reserva de Desenvolvimento Sustentável Mamirauá. A população em estudo é composta por animais marcados criogenicamente, permitindo a identificação individual. O processo de captura e marcação ocorre anualmente, geralmente no mês de novembro. Nessa ocasião ainda são coletadas as medidas de comprimento e peso, amostras de sangue, tecido, leite e, determinado o sexo dos indivíduos, Observações diárias são posteriormente realizadas, com o intuito de obtenção dos parâmetros biológicos e ecológicos específicos da espécie (da Silva & Martin 2000).

Esse trabalho foi dividido em dois capítulos. O primeiro capítulo tem como objetivo validar uma nova ferramenta de fotometria usando simplesmente uma câmera digital, possibilitando estimar o tamanho de indivíduos que estão associados a outros de tamanho conhecido ou estimado medindo, nas fotografias, a proporção do espiráculo até a ponta da dorsal; nesse estudo utilizamos as mães com seu filhote, devido a sua próxima associação. O

segundo capítulo tem como objetivo testar a teoria de investimento maternal diferenciado no boto-vermelho e avaliar como esse investimento pode afetar, ao longo da ontogenia, o desenvolvimento do dimorfismo sexual, utilizando dados de animais marcados da base dados do Projeto Boto de tamanho, idade e sexo conhecidos. Para aumentar o tamanho amostral de filhotes de tamanho conhecido, incluíram-se os dados de tamanho de filhotes obtidos pela técnica de fotometria, descrita no primeiro capítulo desse trabalho.

Objetivos

1. Validar uma nova técnica de fotometria que permite estimar o comprimento do indivíduo (*objetivo*) quando associados a outros de tamanho conhecido ou estimado (*referência*).
2. Testar a teoria de investimento maternal diferenciado no boto-vermelho e avaliar como esse investimento pode afetar, ao longo da ontogenia, o desenvolvimento do dimorfismo sexual.

Marina Gaona Calderón, Vera M.F. da Silva, Adrian A. Barnett² and Anthony R. Martin. **Estimating the length of dolphins using photographs where another animal of known or estimated length is in close proximity.** Manuscrito aceito publicado na revista Marine Mammal Science 23 Março 2018. DOI: 10.1111/mms.12503.

Estimating the body length of wild cetaceans is notoriously difficult, and consequently a long-standing source of frustration for biologists for whom this information would be of considerable value. The many problems to be overcome include the fact that some, or all, of the target animal is usually underwater, and is unavailable to the observer. In addition, cetaceans have flexible bodies. A variety of techniques have been developed to overcome these difficulties, ranging from aerial photogrammetry, to paired lasers and underwater photography (*e.g.*, Whitehead and Payne 1981, Glockner and Venus, 1983, Perryman and Lynn 1993, Ratnaswamy and Winn 1993, Dawson *et al.* 1995, Brägar and Chong 1999, Jaquet 2006, Webster *et al.* 2010, Dittmann and Slooten 2016, Cheney *et al.* 2017). However, for reasons of logistical complexity, cost or time required for data processing, few are feasible for routine day-to-day fieldwork from small boats. In this note we describe a very simple, but apparently novel, technique for estimating the body length of dolphins using just a digital camera, in situations where part of the body of another animal of known (or estimated) length is in close proximity. The technique was developed in circumstances where the body length of a substantial proportion of a study population is known by direct measurement. However, it would be equally appropriate for use under circumstances where, for example, typical adult length and the relative length of parts of the animal visible to field observers can be estimated from measurements of stranded or bycaught animals.

Digital imaging technology has transformed field studies of cetaceans in recent years (Hammond *et al.* 1990, Markowitz *et al.* 2003). It has become the main tool for recognizing marked botos (Amazon river dolphins, *Inia geoffrensis*) in a population that has been studied for 25 yr in and near the Mamirauá Reserve, Amazonas State, Brazil (da Silva and Martin 2000; Martin and da Silva 2004; Mintzer *et al.* 2013, 2016). Up to the end of 2016, more than 650 animals had been uniquely marked, and daily observations typically yield photographs identifying 20-50 individuals. Photographs of lactating females at the water surface may include their dependent calf, and so can often allow a subjective assessment of the relative size of the calf, even though only part of the body is ever visible when the dolphins surface to breathe. This information is particularly valuable when the mother is rarely seen and her reproductive status is otherwise uncertain. During the fieldwork it became apparent that the length of the calf (the *target animal*) could potentially be estimated quite accurately when the dorsal surface of both mother and calf were visible in the same image, especially if the body length of the mother (the *reference animal*) was known or could itself be estimated independently by other photogrammetry techniques or using the mean size from the literature.

Every year, some 50-80 botos are captured, marked, measured and released by Projeto Boto researchers. Consequently, the total body length of many animals is known with an accuracy of within 5 cm. The mean asymptotic body length is 200 ± 1 cm for females, and 232 ± 2 cm for males (Martin and da Silva 2006). Equally importantly for the purposes of this paper, the ratio between total body length and a part of the body most frequently visible to a field observer (the distance between the blowhole and the tip of the dorsal fin - the *BDF*) can also be measured. In principle, therefore, a photograph that shows *BDF* for both target and reference animals should allow an estimate of the target animal's total body length (target TL) using the following equation:

$$\text{target TL} = \left(\frac{\text{target BDF}_i}{\text{ref BDF}_i} \right) * \text{ref TL} * F \quad (\text{Equation 1})$$

where:

target *BDF*_{*i*} is the measurement of the target animal *BDF* from the image (mm) (Fig. 1),

ref *BDF*_{*i*} is the measurement of the reference animal *BDF* from the image (mm) (Fig. 1),

ref TL is the known or estimated total body length of the reference animal, from the tip of the rostrum to the fluke notch (cm),

F is (mean ref *BDF*/TL) / (mean target *BDF*/TL) as measured on live (or dead/stranded) animals, the mean calculated from a pool of animals of the relevant sex and size class, on the assumption that ratios may differ between animals of different classes. If the ratio is constant across all animals, then *F* = 1 and can be ignored.

The perfect image for this technique is one in which the upper surface of both animals can be clearly seen (albeit mostly in profile), and the animals are the same distance from, and lying perpendicularly to, the lens. Deviations from this ideal scenario are, of course, commonplace in the field and will increase error unless they can be accounted for (Table 1).

| Cause of error | Impact (I) and means of correction (C) |
|----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Target animal nearer or further from lens than the reference animal. | (I) Image of the nearer animal will be magnified more than that of the more distant one. (C) Estimate the range to each animal and correct the calculation using Equation 2. |
| One or both animals not at 90° to the lens axis. | (I) If both animals are at the same angle to the lens (<i>i.e.</i> , parallel to each other), then the technique will be valid but measurements will be less precise. If one animal is at a greater angle than the other, the estimate of its length will be negatively biased. (C) Estimate the angle (θ) of each animal from the perpendicular and multiply the apparent BDF (<i>i</i>) by $1/\cosine(\theta)$. |
| Body is curved in the image due to surfacing “rollout” behaviour. | (I) The estimated of body length will be negatively biased. (C) If both animals are curved to the same degree, then no correction is necessary. If one is more curved than the other, the image is not valid for this technique and should be discarded. |

Table 1. The most common causes of error when analysing field photographs, their impact and the means of correcting them.

If the target animal is nearer or further from the camera than the reference animal, then it will appear to be artificially smaller or larger, respectively, but this effect is linearly related to distance and can be simply corrected as follows:

$$\text{target TL (corr)} = \text{target TL (uncorr)} * \frac{\text{range to target animal (m)}}{\text{range to reference animal (m)}} \quad (\text{Equation 2})$$

where:

TL (corr) is the corrected target TL, and TL (uncorr) is the uncorrected target TL.

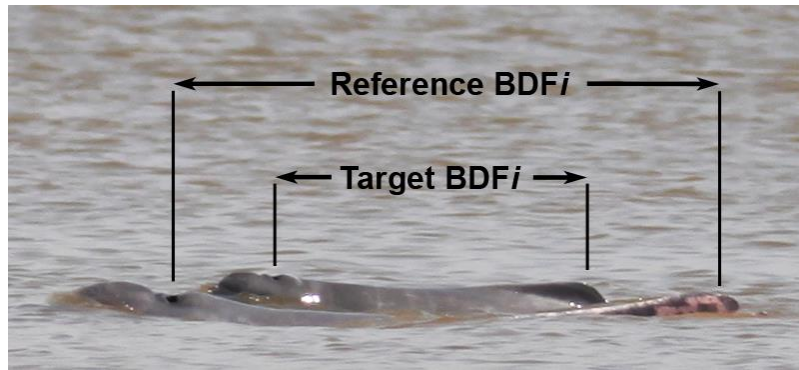


Figure 1. Image dated 25 September 2014 of a 21 yr-old adult female boto ('F9' - the *reference* animal) with her calf (the *target* animal), which was known from independent sightings to be approximately 3 mo old at the time. From this image, the calf was estimated to be 117 cm in total length. In this case, the calf was further from the camera than was the mother, so the estimate of the distance from blowhole to tip of the dorsal fin (BDF) for the calf would have been under-estimated had it not been corrected. At a range of 30 m, and with a distance of 1 m between the center line of the target animal and that of the reference animal, the correction is +3% (see Equation 2).

If the difference in range to the animals (target and reference) is small, then so would be the potential error if the difference was ignored or inaccurately estimated. For example, at ranges of 30 m and 31 m, the error would be just 3%. If both the target animal and reference animal are adequately in focus in an image, such that their blowholes can be seen, the narrow depth of field of the lenses normally used in the field implies that they are at similar distances from the photographer. In practice, it is always apparent if one or other animal is closer to the photographer, and a simple correction in the calculation will reduce the error from this to an inconsequential level.

Image quality, in terms of focus and resolution, is only of relevance insofar as it must be adequate to allow unambiguous identification of the measuring points - in the case of this study the blowhole and tip of the dorsal fin of both animals.

The image can be magnified as much as necessary (on a computer screen, for example) to allow target BDF i and ref BDF i to be measured, because it is the relative, not absolute, measurement of these characters that is subsequently used. Neither the focal length of the lens or the distance from lens to subjects is normally relevant, except when the animals are not equidistant and an estimate of range will assist in correcting the error that this produces, as mentioned above (Table 1, Equation 2).

In the event that target and reference animals are photographed in separate images within the same rapid shooting sequence and with the same lens setting, with one surfacing

immediately after the other but in close proximity, it may still be possible to obtain a reliable length estimate because the essential conditions for the technique to work may have been met.

Body measurements were taken from 42 adult female *I. geoffrensis* and 41 growing animals of less than approximately 3 yr of age. The relationship between BDF and TL was not quite linear across all ages in this species, but a linear regression adequately described the relationship (Fig. 2):

$$\ln(\text{BDF}) = 0.9723 * \ln(\text{TL}) - 0.6291 \quad (R^2 = 0.938)$$

Consequently, for *I. geoffrensis* Equation 1 above becomes

$$\text{target TL} = \left(\frac{\ln(\text{target BDF}_i)}{\ln(\text{ref BDF}_i)} \right) * \text{ref TL} \quad (\text{Equation 3})$$

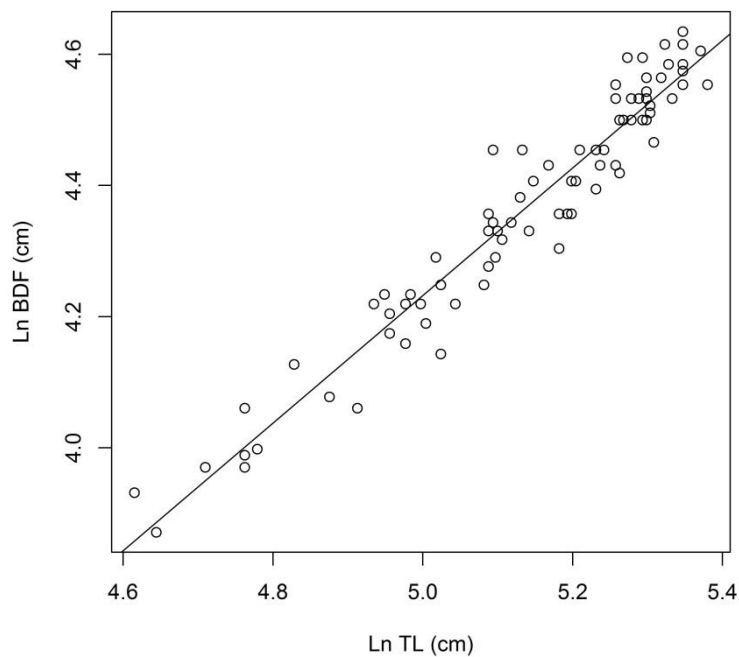


Figure 2. Plot of the natural logarithm of the measured distance from center of the blowhole to tip of dorsal fin (BDF) against natural logarithm of measured total body length (TL) for 83 live captured botos. The regression equation is $\ln(\text{BDF}) = 0.9723 * \ln(\text{TL}) - 0.6291$. $R^2 = 0.938$.

The accuracy of this technique was tested using 33 images from 12 mother-calf pairs taken shortly before or after both target and reference animals were captured and measured in

2016. All images showed the dolphins perpendicular to the lens and were taken within a one-month period, thereby minimizing error that would otherwise have arisen due to natural growth. In this population, calves grow at rates of up to 45 cm/yr and reproductive females grow at no more than 5 cm/yr (authors' unpublished data), so estimated lengths would be expected to vary from measured lengths by up to a cumulative 1% per week (and half of this rate for older calves). Error in accuracy was defined as the difference between the real and estimated length of the target animal, divided by the real length and expressed as a percentage. The mean error of the 33 estimates was 3.8% (range: 0-12.5%, SD = 3.3%). For further information see Table 2.

| Calf ID | Nº pict | Real size (cm) | Mean estimated size (cm) | ± SD |
|---------|---------|----------------|--------------------------|------|
| 633 | 3 | 119 | 123.33 | 2.08 |
| 635 | 2 | 142 | 149.00 | 5.65 |
| 636 | 1 | 101 | 111 | - |
| 638 | 6 | 117 | 118.33 | 7.06 |
| 639 | 1 | 154 | 154 | - |
| 640 | 1 | 165 | 155 | - |
| 643 | 1 | 151 | 147 | - |
| 649 | 1 | 148 | 150 | - |
| 658 | 2 | 104 | 111 | 8.48 |
| 659 | 6 | 117 | 118.33 | 3.66 |
| 665 | 4 | 111 | 111.25 | 3.59 |
| 666 | 5 | 141 | 144.4 | 7.23 |

Table 2. Summary of measurements data from all the photographed individuals.

If the body axis of one or both animals is not perpendicular to the lens, the apparent BDFi measurement will be an under-estimate of the true BDFi. At small angles, the difference will be minor (e.g., at 80° or 100° the under-estimate is 1.5%), but at angles of 30° from the perpendicular, the error increases to 15%. If no better image can be used, and if an estimate of the angle (the difference from 90°) can be made, then the apparent BDFi can be corrected as follows:

$$\text{true BDFi} = \frac{\text{apparent BDFi}}{\cosine \theta} \quad (\text{Equation 4})$$

where:

apparent BDF_i is the measurement of BDF from the image,

true BDF_i is the measurement of BDF that would have been realized had the animal been perpendicular to the lens at the same location,

θ is the angular difference between 90° and that of the animal's body axis (as would be seen from above, not as perceived from the boat).

The degree of error involved in using this technique will vary depending on circumstances, but even a 10% error may be acceptable when no other measurement is available and when, for example, the aim is to classify animals into age- or maturity-classes, in support of an understanding of life history (Vincenzi *et al.* 2014), survival rates in early ages (Brough *et al.* 2016) or habitat preferences, (Dawson *et al.* 1995, Pack *et al.* 2017).

In our study, the main objective was to determine when a female was accompanied by a new calf, having normally weaned the previous calf a short time earlier. Lactating botos and their calves usually show very little of themselves to the observer, and even experienced fieldworkers may have difficulty in visually estimating the length of a calf (as a proxy for age) from a distance. The technique described here dramatically improved our ability to determine when a mother was accompanied by a calf different to the one with which she was previously seen, and therefore to accurately estimate reproductive rates, age at weaning and related measures. Repeated length estimates of calves throughout their period of dependence also yielded data of sufficient quality to allow the construction of a growth curve. Although the technique was used in this case only to estimate the size of individuals smaller than the reference animal, it would be equally applicable for larger animals.

Some earlier studies extrapolated from measurement of part of the body, *e.g.*, the base of the dorsal fin (Webster *et al.* 2010, Dittmann and Slooten 2016), to estimate the length of the entire body. Others have estimated the length of a juvenile whale by comparing it with the length of its mother in an aerial image and using measurements from captured or stranded adults to estimate the length of the mother (Whitehead and Payne 1981, Glockner and Venus 1983). The technique described in this paper is novel because it requires no equipment other than a normal SLR camera body and lens, and no task-specific fieldwork; the photographs used are simply a subset of those taken during daily surveys.

All estimation techniques are subject to error, and in this case the scale of error was known because of the opportunity to measure some target animals directly and independently

within a short time of them being photographed. Only one other published study of cetaceans had access to known-length animals, and it produced similarly low levels of error (mean error = 2.3%) using paired lasers to estimate the BDF measurement (Cheney *et al.* 2017).

We mention above that the technique is equally applicable whether the length of the reference animal is known or estimated from the literature, though of course the estimated length of the target animal is accordingly less precise. Similarly, Glockner and Venus (1983) used the mean body length of mature female humpback whales (*Megaptera novaeangliae*) to estimate the size of calves. In the case of botos, using the mean length of an adult female reference animal, rather than a known length, would add an error of <3% in over half the cases, and <7% in over 90% ($n = 151$ adult female lengths, mean = 199 cm, SD = 8.2 cm).

This simple and relatively inexpensive field technique has yielded valuable information in our dolphin work and should be a useful tool elsewhere. It would be appropriate whenever information from live or dead animals is available to provide estimates of size and body proportions of reference animals. Although the size of live animals is only known in a limited number of studies, the length of adults can be estimated from measurements of dead animals with known error in many cetaceans. As such, this technique could be widely applicable.

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Marina Gaona Calderón, Vera M.F. da Silva, Tiago Pires, Adrian A. Barnett and Anthony R. Martin.
Sex-specific maternal investment and early growth rates explain sexual dimorphism in a freshwater cetacean (*Inia geoffrensis*).

Summary

1. Polygynous mammals are commonly sexual dimorphic, males are bigger than females because they will compete for access to females, and therefore show greater variation in reproductive success. The evolution of sexual dimorphism may show differences between sexes in growth duration period and growth rates, which can be affected by maternal effects under maternal care period. The theory of differential sex-specific energy allocation predicts that mothers should invest more in sons than daughters. Such investment may be reflected in different growth rates, size at the end of maternal care, as well as different time spent under maternal care, since a longer period is required to reach that larger size, that is, itself, an indicator of enhanced future reproduction success.
2. The Amazon River dolphin (*Inia geoffrensis*) is highly size-dimorphic, with males up to 16% longer and 55% heavier than females. As they can mate all year around, the competition between males is very intense. Using longitudinal data from Amazon River dolphins, we explore whether maternal investment is reflected in parental care of sons and how this might be related to processes of selection for sexual dimorphism in males.
3. Applying GLMM, between-sex differences in calf size at the end of maternal care were found (males 172.21 cm and females 165.58 cm, $p < 0.05$), while no differences in duration time were observed ($p > 0.05$). LME were used to identify differences in growth rates between sexes within calf stage and juvenile stage. Differences in slope were found within calf stage ($p < 0.05$), however no differences were recorded during the juvenile period ($p > 0.05$), which suggests a higher investment in sons under maternal care and then a longer juvenile period.
4. Amazon River dolphin or boto has no predators and no resources limitations so that there is little risk associated with the higher energetic investment associated with the production of sons. However, in order to obtain a fully rounded picture, information on how maternal condition might differentially affect male or female offspring and how the higher cost of male offspring might affect maternal condition and production of future offspring are needed.

Introduction

Parental care probably is the most important period of a mammal's life; it is a period of high parental investment, where allocated resources must be partitioned between both current and future reproduction and between offspring quality and quantity. Such investment will affect the survival of the reproductive female concerned, as well as influencing the capacity for survival and reproduction success of their offspring after the independence (Trivers 1974, Stearns 1992).

In mammals with a polygynous mating system, only the mother invests directly in the offspring (Clutton-Brock, Albon & Guinness 1981, Clutton-Brock, Guinness & Albon 1982). As males do not have to invest in parental care they can invest energy in both, growth faster and have more extended growth periods, and so reach the sizes that, in exceeding those of females, lead to sexual dimorphism (Alexander *et al.* 1979, Badyaev 2002). The degree of sexual dimorphism may affect between male for access to females, the limitation factor, and therefore, variability in reproductive success (Clutton-Brock, Albon & Guinness 1988, Trivers 1972, Emlen & Oring 1977, Isaac 2005). Under these assumptions, the theory of differential allocation predicts that mothers will invest more in sons than daughters (Trivers & Willard 1973, Maynard-Smith 1980).

Offspring sex-dependant variability in maternal care investment can be reflected in variety parameters, including differential growth rates (Andersson 1994, Ono & Boness 1996) which results in offspring of different sexes being different sizes at the end of the period of maternal care (Ono & Boness 1996). Extended maternal care period may affect maternal condition and therefore reduce mothers' lifetime reproductive success (Reiter, Stinson & LeBoeuf 1978; Maynard Smith 1980; Clutton-Brock, Albon & Guinness 1981; Clutton-Brock, Guinness & Albon 1982; Best, Canham & MacLeod 1984, Nichols, Fullard & Amos 2014). Male survival may be affected if the mother dies early in lactation since the risk of mortality following such forced separation is age dependent (Festa-Bianchet, Jorgenson & Wishart 1994, Noren & Edwards 2007). Nevertheless, while some studies have found such sex-biased differences, as for example in ungulates (Clutton-Brock, Albon & Guinness 1981), elephants (Lee & Moss 1986), and pinnipeds (Reiter, Stinson & LeBoeuf 1978, Kovacs & Lavigne 1986*a,b*) others have not (ungulates: Byers & Moodie 1990; pinnipeds: Kovacs 1987, Cappozzo, Campagna & Monserrat 1991). Maternal investment has been also measured as suckling frequency and suckling bout duration (Reiter, Stinson & LeBoeuf 1978, Clutton-

Brock, Guinness & Albon 1982, Lee & Moss 1986, Trillmich 1986); and differences in birth size and weight to estimate prenatal investment (Kovacs & Lavigne 1986b).

Growth rates and growth duration have been described as the two processes during ontogeny, which lead to selection of sexual dimorphism (Badyaev 2002). The degree of competition, availability of resources, and maternal effects, among others, may affect how these processes modulate the life history of a species. In complex societies with high degrees of competition, in primates, for example, both mechanisms may act together, where, after becoming independent from their mothers, immature males have a longer juvenile period than do immature females (Leigh 1995).

Studies of maternal investment in marine mammals have mostly focused on pinnipeds (Reiter, Stinson & LeBoeuf 1978, Kovacs & Levinge 1986a,b, Cappozzo, Campagna & Monserrat 1991, Guinet, Goldsworthy & Robinson 1999, Boltnev & York 2001), since their presence on land during the breeding season facilitates data collection. In contrast, the permanently aquatic nature of cetaceans complexifies the collection of similar data. Among odontocetes, in general, males are bigger or more robust than females (Tolley *et al.* 1995), with the sperm whale being the most sexually dimorphic, males 60% larger than females (Best, Canham & MacLeod 1984). However, in some species sexual dimorphism is inversed (*e.g.*, *Phocoena phocoena*: Hohn *et al.* 1996; *Phocoena sinus*: Read & Tolley 1997). In general, odontocetes have long periods of parental care, followed by a long juvenile period before reaching sexual maturity (Krzyszczuk *et al.* 2017), allowing them to develop the skills to chase highly mobile prey, as well as learn the social skills necessary for life in complex groups (Brodie 1969, Mann & Smuts 1999, Colbeck *et al.* 2013), and live in a variety of different social structures depending of their life history strategy (Connor *et al.* 1998).

In this study, we use the duration of the parental period as evidence of variation in the extent of maternal investment between sexes in accordance with parental care theory, and test how this relates to ontogenic selection for sexual dimorphism in the Amazon river dolphin or boto (*Inia geoffrensis*) a freshwater odontocete endemic to (Best & da Silva 1989; Gravena *et al.* 2014, Hrbek *et al.* 2014), but widely distributed in, the Amazon and the Orinoco river basins (Best & da Silva 1989, 1993; Martin & da Silva 2004). Unlike other species of river dolphins, which show reverse sexual dimorphism (Ralls 1976), the boto is highly dimorphic, with males are 16% longer than females and 55% heavier, and with a more pronounced pink colouration. They are also more scarred than females, indicative of intense inter-male aggression (Martin & da Silva 2006). In addition, Martin and da Silva (2004) have described

a strong spatial segregation between sexes. While this is common in other odontocetes with sexual dimorphism (e.g., *Physeter macrocephalus*: Whitehead 2003), botos have not been described as a social species, and group association may be something temporary associated with areas of high-quality feeding or reproduction (Martin and da Silva 2004). Historically reproductive parameters had been derived from the study of a small number of carcasses producing scant information on the life history of the species (Harrison & Brownell 1971, Best & da Silva 1984). However, Martin & da Silva (2018) described extensive variation in the lactation period duration (from 1.5 to 5.8 yrs.); more than 12 months of pregnancy; a mean inter-birth-interval of 4.6 years and females first gave birth with a mean 9.7 years, which indicates extended parental care in this species and long juvenile period. However, nothing is known about maternal investment and how this relates to the role of ontogeny in the evolution of sexual dimorphism.

Accordingly, in the current study we: 1) compared age and length between males and females calves at the end of the parental care period, and 2) compare the growth rates between sexes during the two key growth periods: the maternal care and the juvenile phase and so, estimate the extent of differential maternal investment. We predict that (i) males calves will have a longer maternal care periods than females since they reach a bigger size than females; (ii) males calves will also have faster growth rates within the maternal period because of additional maternal investment, and (iii) no differences in growth rate between sexes are expected in juvenile period as males will have extended juvenile periods, so growing for longer than females.

Materials and methods

Study site and population

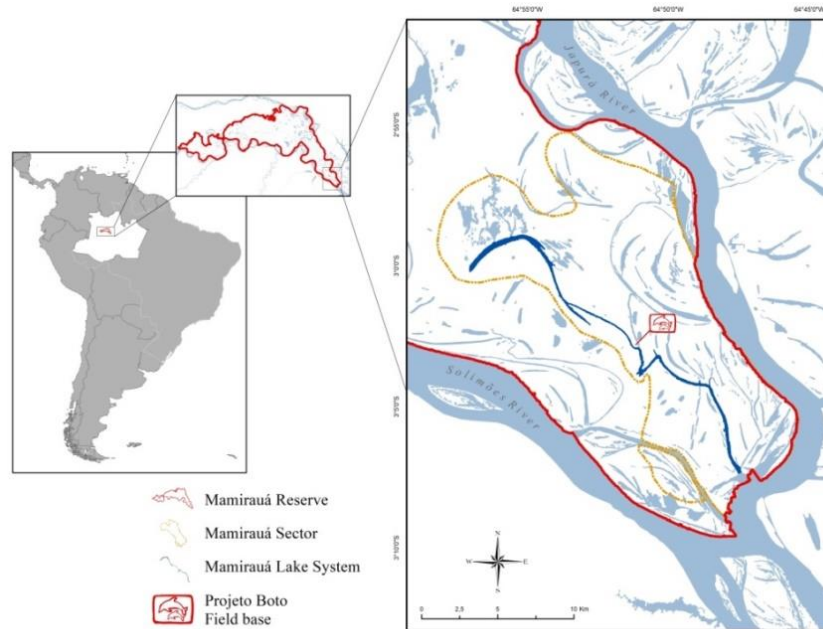


Figure 1. Map of the study area showing the whole Mamirauá reserve and more specific Mamirauá sector between Japurá and Solimões rivers, where is found the Projeto Boto field base.

Projeto Boto has been collecting demographic, biological and ecological data on Amazon River dolphin since 1994. The study is located in Mamirauá Sustainable and Development Reserve (MSDR) at 500 km southwest from Manaus, Amazonia, Brazil and is constricted by the confluence of the Solimões and Japurá rivers (Martin & da Silva, 2004). The research is focused within Mamirauá sector and surrounding area, covering approximately 225 km² of 11.240 km² of flooded forest of the overall reserve. Focal area, as the whole reserve, experiences seasonal floods by the regional white-water rivers (Junk *et al.* 2014). These rivers are characterised by the high amount of sediments (Sioli 1984). The annual flood pulses are synchronized with the falling water, which results in marked dry (September to November) and the rising water seasons (end of November to July), when the waters flood the forest, so restricting terrestrial habitat and expanding aquatic (Junk *et al.* 1989). The mean level annual water fluctuation at MSDR is 10,6 meters (Ramalho *et al.* 2009).

To date, over 680 individual botos have been monitored using photo-identification of unique freezing brands. Each year, usually in the month of November, Projeto Boto has its

annual capture campaigns. The animals are captured, branded and biological information, length (between the tip of the rostrum and the tail notch), weight and sex are recorded; after the procedure the animals are released. For more information about Projeto Boto methodology see the detailed description in da Silva & Martin (2000). Since 2014 calves' length measurements have also been estimated by photogrammetry using a single digital camera. Since the mother length is known by capture data or may be estimated by mean total length for females (mean = 200 ± 1.22 cm, Martin & da Silva 2006) it is possible to estimate the calf total length when they are in close proximity and parallel to the lens using the distance from blowhole to the tip of dorsal fin in the image. The description of the methodology can be found in Gaona *et al.* (2018). However, just length estimations from branded calves across time were used since the sex is known. Therefore, the individuals used in this analysis were of known sex, age, and size from capture and photogrammetry data. Besides, only perfect pictures were used for the analyses in order to avoid additional biases (Gaona *et al.*, 2018).

The age used in the models was estimated inferring the day of birth (in days) using information from daily observation from the Projeto Boto data set. It was assigned to a calf under the following conditions: (1) Only calves, with an interval between the last sighting of the mother without a calf and the first observation of the mother with calf must be 3 months or less, were select; (2) fulfilling the first condition the birth date was establish as the half of the interval; (3) data records of females without continuity through time with the calf were discarded. In order to estimate age and size at independence, for each known age-size individual was recorded as 0 if the dolphin was captured with the mother (dependent) or 1 if was captured alone (independent). Finally, for the purpose of growth rates comparison between sexes and periods, calf stage is defined from birth until the end of maternal care period. The age of the calf at the end of maternal care obtained in the first part of the analysis was used to define the end of maternal care period. Juvenile stage is the period between the end of parental care and 9.7 years and 200 cm, since is when females reached sexual maturity (Martin & da Silva 2018), therefore, years before this time are related with the juvenile period. All the measures over 9.7 years were excluded from the analysis in order to avoid biases of mature females and be able to compare exclusively juvenile and growing animals (Figure 1).

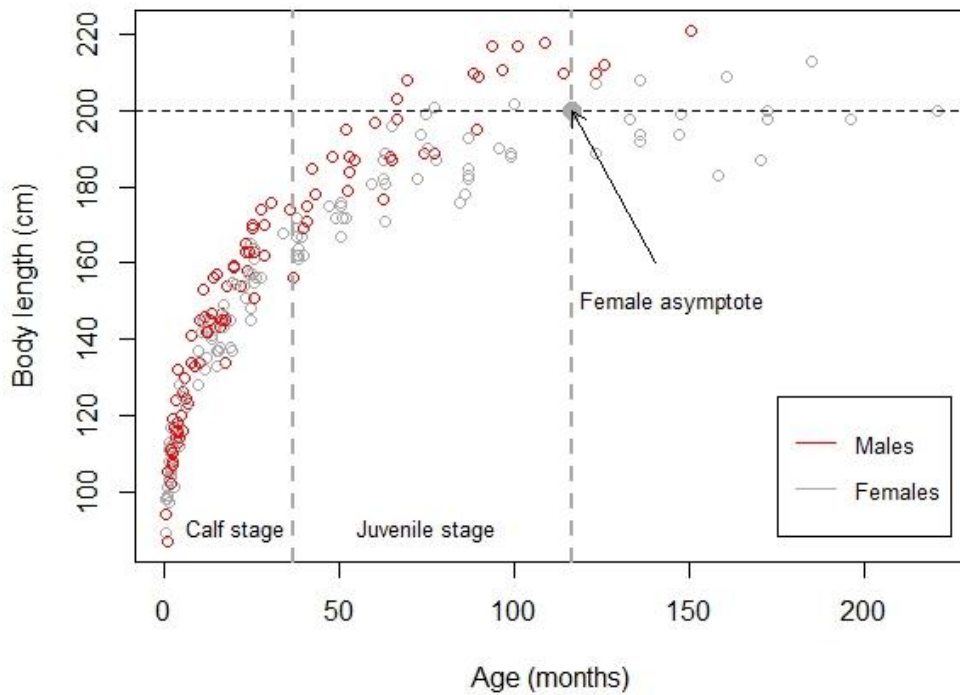


Figure 2. Representation of different stages analysed for differences in growth rates between sexes.

Statistical Analysis

All statistical analyses were performed in the R statistical package 3.4.3 (R Development Core Team, Vienna, Austria). To investigate whether male and female offspring are likely to become independent at different ages and length, dependence condition was defined as the variable response with binomial values (0= dependent and 1= independent). Different models were performed, one model for age (days) and a second for length (cm), both as continuous variables. Sex was used as fixed effect in both models in order to check for differences between sexes. The model was analysed using binomial generalized linear mixed models (GLMM) using the package lme4 (Bates *et al.* 2015), and because of the binomial data, binomial family has been used in the GLMM. Since the data included repeated measurements from each individual, the identity of the calf was fitted as a random factor to avoid pseudo replication. Then, the length and age at which 50% of individuals are predicted to be independent was estimated in the GLMM:

$$\text{Dependence} \sim \text{Size (cm)} + \text{Sex} + (1 | \text{ID})$$

$$\text{Dependence} \sim \text{Age (days)} + \text{Sex} + (1 | \text{ID})$$

The data was linearized applying logarithms in order to explore the differences in growth rate between sexes in calf stage and juvenile stage. Then, the data was fitted using

Linear Mixed Effects (LME) explicitly using maximum likelihood (ML) not the default Restricted Maximum Likelihood (RMEL) with nlme R package (Pinheiro *et al.* 2017) to each period (dependent, independent) using the interaction with sex as a fixed factor in order to test slope differences between sexes. Again, because of repeated measurements from the same individual, calf ID was used as random variable:

Calf stage: Size (cm) ~Sex*Age (days) + (1| ID)

Juvenile stage: Size (cm) ~Sex*Age (days) + (1| ID)

The relationship length and age within dependent period was compared between data sets (173 capture data and 51 photogrammetry data) to identify outliers or possible errors in measurements.

Results

Repeated measurements of known age-length individuals' subset was comprised by 74 individuals (43 females and 31 males) with 211 total observations including repeated measurements from the same individuals (Table 1). 19 measurements were excluded since were overestimating the size. From those 74 individuals, 11 had photogrammetry measurements only within dependence period and no photogrammetry data for independence period.

| Age class | N | Female | | | N | Males | | |
|-----------|----------|--------------|------------|----------------|----|-------|------|---------|
| | | mean | SD | range | | mean | SD | range |
| 1 | 29 | 109 | 9.1 | 89-128 | 24 | 113.3 | 10.5 | 87-132 |
| 2 | 16 | 139.1 | 5.6 | 128-149 | 21 | 142.4 | 9.3 | 123-157 |
| 3 | 17 | 152.9 | 8 | 137-165 | 16 | 163.6 | 6.9 | 151-176 |
| 4 | 9 | 165 | 3.7 | 161-172 | 6 | 171.6 | 9.5 | 156-185 |
| 5 | 7 | 172.7 | 3.03 | 167-176 | 7 | 185.6 | 5.9 | 178-195 |
| 6 | 7 | 183.9 | 7.8 | 171-196 | 6 | 191.7 | 9.5 | 177-203 |
| 7 | 6 | 192.2 | 7.2 | 182-201 | 3 | 195.3 | 11 | 189-208 |
| 8 | 6 | 182.8 | 6 | 176-193 | 3 | 204.7 | 8.4 | 195-210 |
| 9* | 4 | 192.2 | 6.5 | 188-202 | 3 | 215 | 3.5 | 211-217 |
| 10 | - | - | - | - | 2 | 214 | 5.7 | 210-218 |
| 11 | 2 | 198 | 12.7 | 189-207 | 2 | 211 | 1.4 | 210-218 |
| 12 | 4 | 198 | 7.1 | 192-208 | - | - | - | - |
| 13 | 2 | 196.5 | 3.5 | 194-199 | 1 | 221 | - | 221 |
| 14 | 2 | 196 | 18.4 | 183-209 | - | - | - | - |

| | | | | | | | | |
|----|---|-----|------|---------|---|---|---|---|
| 15 | 3 | 195 | 7 | 187-200 | - | - | - | - |
| 16 | 1 | 213 | - | 213 | - | - | - | - |
| 17 | 2 | 190 | 11.1 | 182-198 | - | - | - | - |
| 18 | - | - | - | - | - | - | - | - |
| 19 | 1 | 200 | - | - | - | - | - | - |
| 20 | - | - | - | - | - | - | - | - |
| 21 | - | - | - | - | - | - | - | - |

*Approximated mean age at first give birth (Martin and da Silva 2018).

Table 1. Summary of size distribution by age class, that is, the mean value± the standard deviation (SD) for Amazon River dolphins using in the analysis.

No differences at independent age were found between sexes ($p>0.05$), the age at which the 50% calves became independent is 3.07 years. However, differences at size were statistically significant ($p<0.05$), the 50% of males' calves became independent with 172.21 cm, while females with 165.58 cm (Figure 3). Therefore, males and females spend the same time with their mother but they reach independence with different sizes, suggesting additional maternal investment in sons in growth, though not in nursing duration.

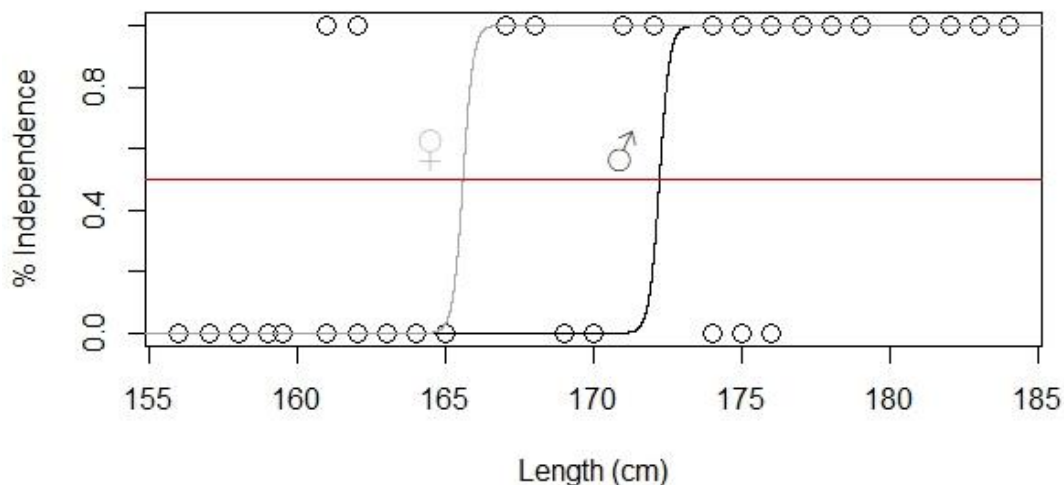


Figure 4. Binomial GLMM regression fitted to length and independence status of boto calves. For a better data visualization only length between 155 cm – 185 cm were selected for the plot. The length at which 50% of specimens are predicted to be independent is 165.58 cm for females and 172.21 for males. Each point represents one measurement, even measurements of repeated individuals.

The slopes for male and female within calf stage were statistically different ($p<0.05$) while the duration of maternal care was the same time between sexes (3.07 years); thus,

different sizes founded at the end of nursing suggested that male calves require greater maternal investment than females as they have faster growth rates (Figure 5). On the other hand, the slope between males and females has not differences in the juvenile period, which also support the early investment by the mother (Figure 6) and indicate a longer juvenile period for males as they still growing to reach a maximum size of 250 cm (Martin & da Silva 2006). The summary data from the different stages can be found in Table 2.

| | MALE | | | | FEMALE | | | |
|-----------------------|------|-----------|------|------------|--------|----------|-------|------------|
| | N | MEAN | SD | RANGE | N | MEAN | SD | RANGE |
| CALF STAGE | 63 | 137.4 cm | 22.8 | 87-176 cm | 63 | 129.4 cm | 21.3 | 89-168 cm |
| JUVENILE STAGE | 28 | 193.64 cm | 14.5 | 169-218 cm | 38 | 179.8 cm | 11.62 | 161-202 cm |

Table 2. Summary of length data (in cm) from different stages (Calf Stage and Juvenile Stage) for male and female boto.

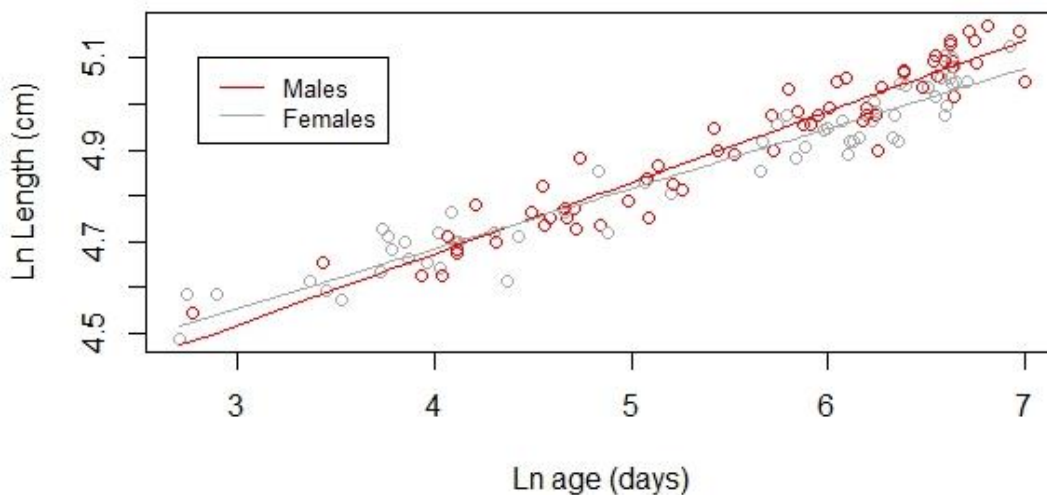


Figure 5. Linear relationship between Ln size (cm) in function of Ln Age (days) in boto within calf stage. The linear equation for males is $\text{Ln}(\text{Length}) = 4.06 + 0.55 * \text{Ln}(\text{age})$, while for females $\text{Ln}(\text{Length}) = 4.16 + 0.13 * \text{Ln}(\text{age})$.

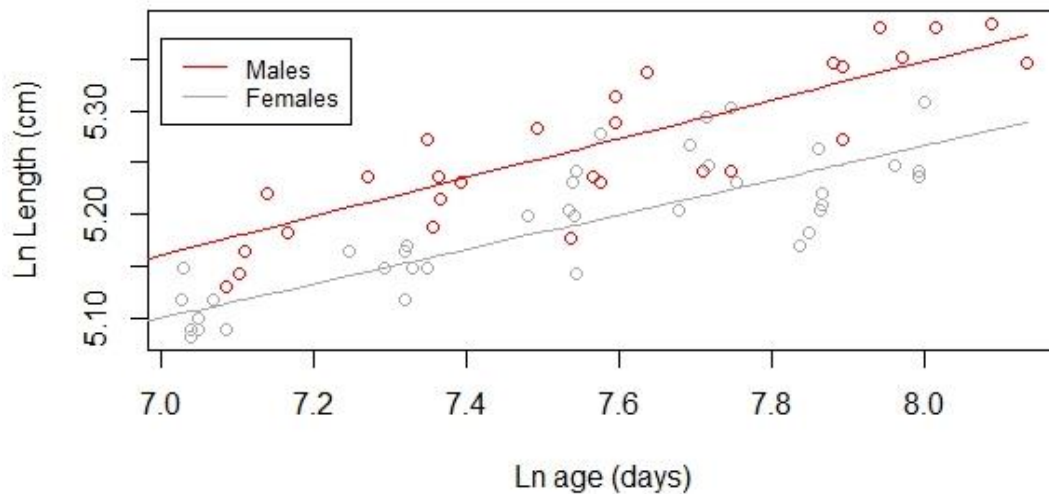


Figure 6. Linear relationship between Ln size (cm) in function of Ln Age (days) in Amazon River dolphin juvenile stage (range: 3 years - 9.7 years). The linear equation for males is $\text{Ln}(\text{Length}) = 3.8 + 0.19 \cdot \text{Ln}(\text{age})$, while for females $\text{Ln}(\text{Length}) = 3.9 + 0.17 \cdot \text{Ln}(\text{age})$.

Discussion

The three main results of the study are 1) no differences in duration of maternal care period, 2) sex differences in length at the end of maternal care period, and 3) sex differences in growth during the maternal care phase, but no differences during the juvenile phase (i.e., after independence from mother, but before sexual maturity).

Parental care

Amazon River dolphin calves became independent about 3 years old. This finding agrees with the results of Martin & da Silva (2018) who obtained a mean value for the same population of 2.8 years (range = 1.5 years – 5.8 years), though with no differences in duration between sexes. This long association between mother and calf is common in complex animals since, in addition to the extended period required for nutrition, they also have a learning period in which they develop social and foraging skills (Brodie 1969, Joffe 1997, Mann & Smuts 1999, Colbeck *et al.* 2013) while they still under the protection of their mother.

Most studies of odontocetes have estimated the age or length at weaning rather than that at which calves start swimming alone. However, in the literature both terms have been used near-interchangeably, even though they imply very different meanings (Clapham *et al.* 1999, Chivers *et al.* 2016). This makes it difficult to compare the results obtained in the current work with those from other studies. On the other hand, some studies defined when calves start

swimming alone, such Archer & Robertson (2004) described a period of 2 years (142 cm) for *Stenella attenuata*, Chivers *et al.* (2016) got 11.1 months (145.1 cm) and 14 months (140.1 cm) in *Delphinus capensis* and *Delphinus delphis* respectively and up to 8 years in *Tursiops truncatus* (Scott, Wells & Irvine 1990, Mann *et al.* 2000). However, none of these studies compared males and females. A number of other studies have investigated differential maternal investment by sex: Nichols *et al.* (2014) found that greater number of dependent sons in long-fin pilot whales (*Globicephala melas*) resulted in a lower probability of the mother being pregnant with further offspring, as males' calves had a longer dependent period. Longer suckling periods by male pilot whales, *Globicephala macrorhynchus* (Kasuya & Marsh 1984) and sperm whale, *Physeter macrocephalus* (up to 13 years in males: Best, Canham & MacLeod 1984) had been also found by analysis of stomach contents of dead young animals. Both species have matrilineal groups, in which sons remain with their mother until they move to other pods to mate. In addition, both species are deep divers (Waltwood *et al.* 2006, Aguilar *et al.* 2008), implying in additional energy cost to the mother when is raising her calf since they spent great energy with deep diving (Huang *et al.* 2011). This results in a larger investment by the mother since the duration of maternal care is extended to guarantee survival and eventual reproductive success for the offspring (Huang *et al.* 2011).

Sex-bias in maternal care has also been recorded in other sexually dimorphic species of mammals (northern elephant seal: Reiter, Stinson & LeBoeuf 1978, elephant: Lee & Moss 1986), though not universally (e.g. Harp seals, Kovacs 1987). Based on the theory of sex-based maternal investment (Clutton-Brock, Guinness & Albon 1982, Maynard-Smith 1980, Reiter, Stinson & LeBoeuf 1978, Trivers & Willard 1973), we would expect a longer parental care for offspring males than for females. However, no significance difference was recorded for *Inia geoffrensis* this in the current study, suggesting that mothers spend equal time investing in both sexes. Therefore, raising a son may not affect the future reproduction, at least as a consequence of parental care duration, any more than does raising a daughter. This result could explain the fact that in botos it is common to encounter the mother swimming with both, the previous and the new calf (Martin & da Silva 2018). An additional explanation concerning the lack of differences in maternal care duration may be relate to the lack of natural predators for botos (Best & da Silva, 1984, 1989; Gomez-Salazar, Trujillo & Whitehead 2012, Martin & da Silva 2018), and the absence of food resource limitation since the food base is very broad (43–45 species: da Silva, 1983, Best & da Silva 1984, da Silva & Martin 2000).

Length at the end of parental care and growth rates

Differential growth rates between male and female botos at the calf stage explains their different sizes when maternal care ends, with males being bigger than females (172.21 cm males, 165.58 cm females). Higher frequency of suckling by males has been used to explain their higher growth, as they therefore have greater milk intakes than females, since accelerated growth rates implies additional energy (Dmitriew 2011, Lavigueur & Barrete 1992, Ono & Boness 1996). Also, both milk quality and quantity has been proposed to explain different growth rates between sexes (Duncan, Harvey & Wells 1984; Hinde 2009; Ono & Boness 1996; Robert & Braun 2012). Milk in odontocetes is very rich in protein, which is important for tissue growth (West *et al.* 2007). Since boto calves did not show differences in maternal care duration, this suggests that male botos may have a higher suckling frequency to maintain the accelerated growth rate we recorded. This would increase the cost for the mother to raise a son, since growth rates are susceptible to maternal effects (Bernardo 1996, Mousseau & Fox 1998). However, measuring suckling frequencies is extremely difficult with any wild dolphin, including at the current boto study site as the high silt content of the water gives near-zero visibility; on the other hand, analyses of milk composition is currently underway that will provide an understanding of the changes in milk composition that occur as the calves develop, as well as if there are variations in milk served to calves of different sexes (Projeto Boto *unpublished data*).

An additional reason to believe maternal investment is higher for males calves is that no between-sex differences in growth rates were detected across the juvenile stage after maternal care, but before female sexual maturation. In the juvenile stage botos males are not growing faster because they are going to grow for longer; however, in order to maintain linear differences in growth, males need to gain mass and therefore they need to feed more than females.

Faster growth rates in offspring males in early life-stages have been described in pilot whales (*Globicephala melas*) during the first 5 years of life (Nichols *et al.* 2014), which was related to a higher cost for mothers. However, studies of early growth have given widely variable results both within, and between, species. For example, in bottlenose dolphin some authors found faster early growth rates in females (Read *et al.* 1993, McFee 2010), while others (Fernandez & Hohn 1998, Stolen, Odell & Barros 2002, Neuenhoff *et. al* 2011) found similar trajectories for both sexes in the first years of life. On the other hand, Sergeant (1973)

found the same growth rates for both sexes of beluga whale calves (*Delphinapterus leucas*) during the lactation period. However, these studies had reported a longer period of growth for males after the females reached sexual maturity, so that females mature faster than males do, and males continue to grow after they reach sexual maturity, while females do not.

Amazon River dolphin males also show extended growth periods, reaching sexual maturity later than females. Females have their first ovulation about 8.7 years and become pregnant at 9.7 years when they are in a body length range of 180-200 cm, with being the 200 cm mean asymptotic length (Martin & da Silva 2006, Martin & da Silva 2018). Although no information is available about the age or length at which male botos reach sexual maturity, they still growing, reaching their asymptote at 231.5 ± 1.53 cm (Martin & da Silva 2006), however males of 250 have been registered by the Projeto Boto.

These differences in maturation time have been describe as one of the processes by which natural selection favours the evolution of sexual dimorphism during the juvenile stage, which is beneficial as small size reduces competition with bigger males in polygynous species (Jarman 1983, Leigh 1995, Badyaev 2002). This process known as bimaturism is strongly linked with the degree of competition between males in species with multimale/multifemale breeding systems (Leigh 1995). Selection of the processes of growth trajectories leading to sexual dimorphism, have been widely studied in primates and have been found to be strongly related to their social and ecological conditions (Leigh 1995, Joffe 1997).

Boto can mate all year-round; in consequence females have a low degree of birth synchrony, increasing the competition between males during the period of dolphin aggregation in the dry season, where most conceptions also occurs (Martin & da Silva 2018). The degree of aggression between males is indicated by the high level of scarring and even the development of cobblestone-like skin lesions, in males longer than 219 cm, which may function as a weapon or shield (Martin & da Silva 2006). Emlen & Oring (1977) describe for polygynous species a male dominance polygyny where males exhibit communal displaying; this behaviour is common in systems where males don't have to defend resources or females directly, and females select males on the basis of status. Male dominance polygyny had been reported in botos, as has socio-sexual display behaviour (Martin, da Silva & Rothery 2008). In addition, socially-dominant mature males are bright pink (Martin & da Silva 2006), which could also play a role in sexual advertisement.

The extent of sexual size dimorphism in Amazon River dolphins suggests that male size is important in determining mating success and natural selection has been selected both growing

trajectories. However, late maturation appears to be the most likely prime cause of sexual dimorphism in this species, even when sexual differences are already visible in calves' growth rates and size because of maternal investment. Higher growth rates are related to higher mortality in males since they need more energy for growth and are, therefore, highly vulnerable to environmental changes or resources limitation (Pontier *et al.* 1989) requiring good physical conditions in order to survive (Mann & Watson-Capps 2005). In consequence, in addition to enhancing her own long-term reproductive success, the higher investment by the mother in males may also function to reduce male mortality during the maternal care phase (Trivers & Willard 1973, Janson & van Schaik 1993), as well as during the critical juvenile period, where males mortality exceeds that for females in many species of mammals (Reiter, Stinson & LeBoeuf 1978, Clutton-Brock, Albon & Guinness 1985).

That differential investment has been found requires further studies to investigate how maternal condition and age are affected along with other reproductive parameters, since having a son is costly, females in better condition may produce more males (Trivers & Willard 1973, Clutton-Brock, Albon & Guinness 1981, Anderson & Fedak 1987, Ono & Boness 1996, Setchell *et al.* 2001). In addition, a more experienced mother will be able to sustain costly offspring by having larger calves (Eckardt *et al.* 2016, Cheney *et al.* 2017). Other studies of maternal investment indicate that having sons might affect maternal fitness, via the survival and/or health of the future offspring, as well as via birth interval (Trivers 1972, Clutton-Brock, Albon & Guinness 1981, Bérubé, Festa-Bianchet & Jorgenson 1996, Lee & Moss 1996, Eckardt *et al.* 2016).

On the other hand, the environment in which the species lives may allow the female boto to adopt this high cost strategy, since they are limited by neither resources nor the risk of predation. In addition, annual water level variation may provide an additional promoting factor: the peak of boto calving coincides with lowest water levels in the study area. At this time, fish are more concentrated and females can be more selective in their diet (Best & da Silva 1989) which is likely to greatly assist with bearing the higher cost of lactation. Later, once calves have developed, water season creates protected, shallow water, areas for mother and calves at the margin of the riverine floodplain that are free of currents and in which they can raise their calves, and which have both a high density and diversity of fish species for feeding (Martin & da Silva 2004, Mintzer *et al.* 2006).

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Conclusão geral

Estudos sobre análise de investimento maternal oferecem informações sobre as necessidades dos indivíduos em desenvolvimento para atingirem o tamanho e as condições físicas de que precisam para sobreviverem e, por conseguinte, contribuir na manutenção da população no futuro. As mudanças no ambiente ou nos recursos alimentares podem afetar o ótimo desenvolvimento dos indivíduos, podendo impactar negativamente, ainda mais quando um dos sexos requer maior investimento.

Neste trabalho, registrou-se maior investimento nos machos por apresentarem maiores taxas de crescimento durante o cuidado parental, sendo, portanto, mais custosos para as mães. Além disso, observou-se período de crescimento mais longo em machos, fato que indica precisarem eles de um alto aporte energético para manterem o custo de crescimento e atingirem grandes tamanhos. Mudanças nos recursos alimentares também podem influenciar a condição física da mãe, não podendo ela prover os filhotes da energia suficiente para que sobrevivam.

Hodiernamente, a bacia Amazônica afronta vários problemas ambientais como a massiva construção de barragens que consegue alterar a disponibilidade de peixe, o alimento principal do boto-vermelho; a poluição dos rios; as drenagens dos lagos em prol da agricultura e a pesca intensiva. Tais fatores constituem-se causas da extinção do golfinho de rio na China (*Lipotes vexillifer*), da crítica situação do golfinho de rio da Índia, no Paquistão e no Irã (*Platanista sp.*). O boto-vermelho atualmente classifica-se com dados insuficientes pela IUCN. Futuros estudos sobre como o investimento maternal pode afetar a condição física da mãe, a sobrevivência dos filhotes e a futura reprodução são essenciais para entender-se como a população pode ser afetada ante essas mudanças. Portanto, há que se estabeleçam planos de conservação, garantindo assim, a permanência do boto-vermelho no ecossistema.

No primeiro capítulo, avaliou-se como uma simples ferramenta de fotometria possibilita estimar o tamanho dos filhotes sem precisar de tecnologias mais complexas para ambientes difíceis ou que precisem de trabalho de campo específico pra isso. A mesma ferramenta pode ser aplicada para outras espécies sempre que o tamanho do indivíduo próximo seja conhecido ou estimado. A aplicação de tal ferramenta em outros estudos de boto-vermelho poderá ajudar acrescentando a informação sobre parâmetros reprodutivos e populacionais, isso facilitará a classificação dos indivíduos por classe de tamanho.

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