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The use of premaxillary bones of six fish species in giant otter (*Pteronura brasiliensis*) diet analysis

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Abstract: The premaxillary bones of fish are considered key bones due to their highly differentiated features, allowing the identification to species level. When these bones are present in the latrines of giant otter (*Pteronura brasiliensis*), they can be used to identify fish species and estimate the size of prey consumed. We briefly describe the differentiable morphological characteristics of premaxillary bones, useful for the successful identification of six fish species which are important prey for giant otter in the Paraguá river (Bolivian Amazon): *Hoplias malabaricus* (family Erithrynidae), *Serrasalmus rhombeus*, *Pygocentrus nattereri* (family Characidae), *Chaetobranchus flavescens*, *Satanoperca pappaterra* and *Astronotus crassipinnis* (family Cichlidae). We also present the linear regression equations which can be used to estimate the standard length of these fish species based on their bone measurements. Overall, 19 latrine samples were analyzed, which contained 109 premaxillary bones of these six species. Of the bones collected, 53% allowed successful estimation of the standard length of the fish consumed. The use of premaxillary bones in diet studies has a high potential as it permits the identification and estimation of prey size for the majority of species consumed. This method is important for studying the size of the giant otter's fish prey.

Resumen: Los premaxilares de peces son considerados huesos claves por sus características altamente diferenciables, permitiendo la identificación a nivel de especie. Se presentan las aplicaciones de los huesos premaxilares en la identificación y estimación del tamaño de los peces presas de *Pteronura brasiliensis*. Se describen brevemente las características morfológicas diferenciables útiles en la identificación y se presentan las ecuaciones de regresión lineal calculadas para estimar el tamaño estándar de seis especies de peces: *Hoplias malabaricus* (familia Erithrynidae), *Serrasalmus rhombeus, Pygocentrus nattereri* (familia Characidae), *Chaetobranchus flavescens, Satanoperca pappaterra* y *Astronotus crassipinnis* (familia Cichlidae), presas frecuentes en la dieta de londra en el río Paraguá. En 19 muestras de letrinas analizadas se identificaron 109 restos de premaxilares de estas seis especies; el 53% de estas premaxilas permitieron estimar la longitud estándar de los peces consumidos. El uso de estos huesos en estudios de dieta tiene un alto potencial ya que permite la identificación y estimación de tamaño consumido de los peces presas por la londra.

Introduction

The giant otter (*Pteronura brasiliesis*) is a semi-aquatic mammal restricted to the South American continent. Together with the Neotropical river otter (*Lontra longicaudis*) and freshwater dolphins (*Inia geoffrensis, I. boliviensis*) it is considered an important mammalian predator on fish in Amazonian aquatic habitats. The giant otter's diet consists almost exclusively of fish, though it occasionally ingests crabs and shrimps (Carter and Rosas, 1997). Due to its large size and the formation of family groups, it is believed to exert high pressure on fish resources. Duplaix (1980) estimated that one adult giant otter of 20kg consumes 10% of its weight daily. However, there is no information available regarding how giant otters affect local fish population size or fish community structure.

The use of the remains of bones present in feces is one of the most common methods to determine the diet of otters, mainly in the otter species from the genus *Lutra* in Europe (Kruuk and Moorhouse, 1990; Conroy *et al.,* 1993). In this species, the remains of prey bones have been also used to identify and to predict the original size of the prey (Wood, 2005). Reconstructing the original size of the fish prey from undigested remains can constitute a tool to estimate the biomass of the prey consumed, while also determining prey size classes (Hansel *et al.*, 1988).

In the case of giant otters, the correct identification and measurement of fish remains in latrines can also provide information on prey selection and size. Most of the studies conducted so far aimed at identifying prey species have made use of scales, cranial and post-cranial skeleton, otoliths and vertebrae retrieved from latrines (Rosas *et al.*, 1999; Cabral *et al.*, 2010; Mallea Cardenas and Becerra Cardona, 2012).

The information about consumed prey must be reconstructed from fragmentary parts. Even when the digestive process is advanced, the slower digestion of bony material and the linear relation between bone length and fish size allow for reliable identification and size reconstruction for most fish species (Hansel *et al.*, 1988). The identification and estimation of the original length of fish prey ingested by mammalian predators has frequently involved the use of diagnostic bones, mostly cleithra, dentaries, pharyngeal arches, opercles, premaxillae and maxillae (Hansel *et al.*, 1988; Wood, 2005), otoliths and vertebrae (Granadeiro and Silva, 2000). Although all these structures can be used to accurately identify fish remains, premaxilla may be the most easily identifiable and most likely resistant to digestion (Wood, 2005).

There are many advantages of using premaxilla as a diagnostic bony structure. Premaxilla can be used not only to identify multiple fishes to the species level, but also to estimate the original length of partially digested fish (Hansel *et al.*, 1988; Scharf *et al.*, 1998). Premaxillary bones are also effective structures for diet analysis because they resist digestion and they are paired structures, which makes it possible to accurately quantify the number of prey items (Mallea Cardenas and Becerra Cardona, 2012).

The status of the giant otter as a species in danger of extinction[1] (Zambrana *et al.*, 2009) and its importance as top predator increase the interest in carrying out detailed studies about its food ecology. Using bones to identify and estimate prey sizes is a method that can provide reliable data to clarify certain aspects of possible competition between giant otters and fishermen. This type of research can also contribute to the analysis of efficient species conservation measures.

One objective of this research was to describe the use of the differentiated characteristics of premaxilla bones to identify prey fish from giant otter latrines. The other aim was to estimate original prey size from measurements of premaxilla for six prey species which are common in the diet of giant otters in the Paraguá River (Bolivia). Using premaxilla bones, we also calculated the minimum number of prey fish consumed, as a way to demonstrate the wide application of the method.

[1]IUCN Red List of Threatened Species. Version 2014. Available online at www.iucnredlist.org. Consulted on 02 February 2014.

Table 1. Order, family, species, total number of collected individuals (N), number of dissected individuals (n) and size range (standard length in millimeters) of prey used to construct predictive lineal regressions.

Order	Family	Species	Local name	N	n	Size range (mm)
Characiformes	Erythrinidae	*Hoplias malabaricus*	bentón	57	8	140-380
	Characidae	*Serrasalmus rhombeus*	piraña	64	7	138-260
		Pygocentrus nattereri	piraña roja	32	8	120-247
Perciformes	Cichlidae	*Astronotus crassipinnis*	palometa	15	5	120-205
		Chaetobranchus flavescens	kupaká	27	6	115-200
		Satanoperca pappaterra	kupaká	25	4	130-175

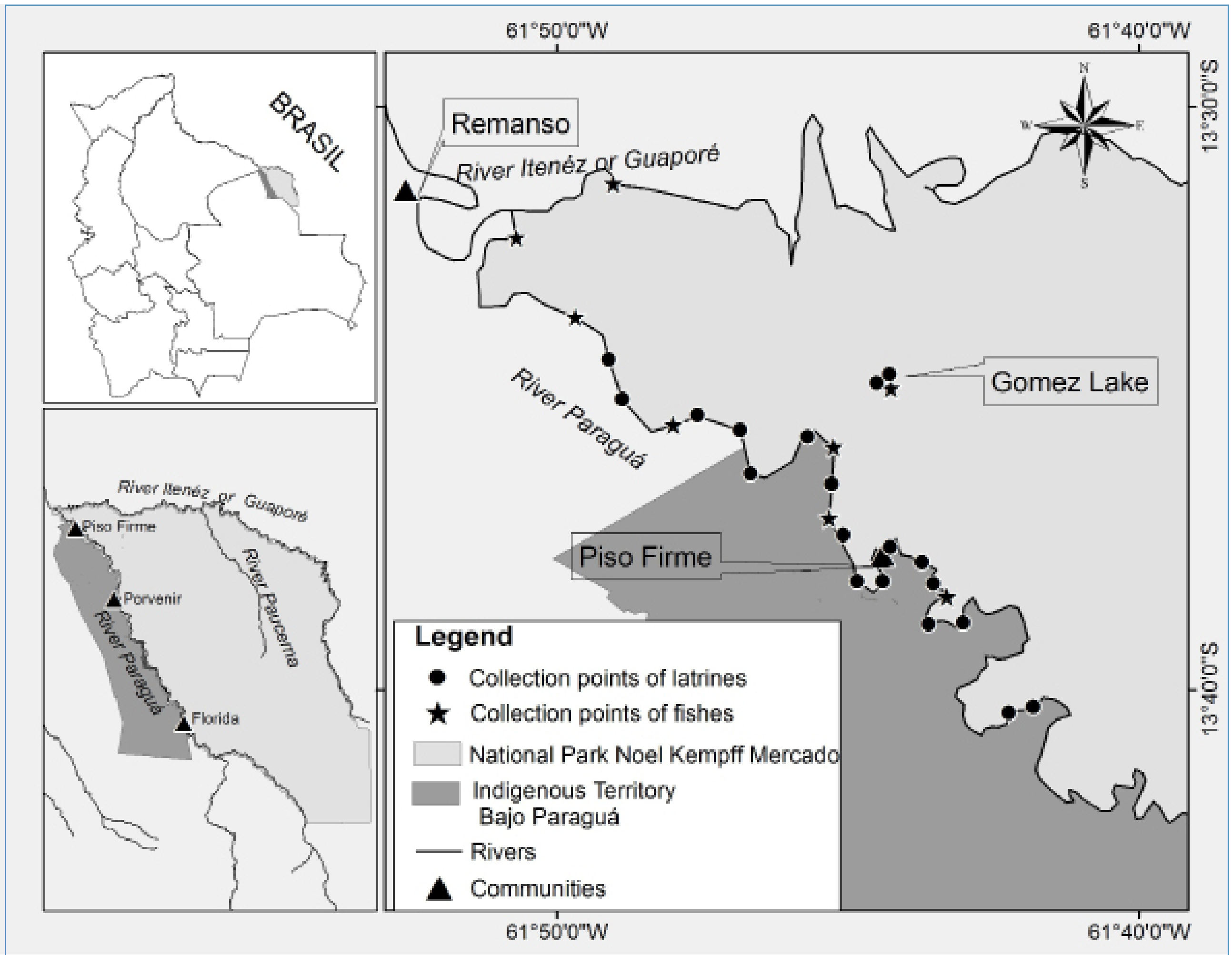

Figure 1. Map of the Noel Kempff Mercado National Park and Indigenous Territory Bajo Paraguá.

Material and methods

Fish and latrine samples

The fish and bone samples from giant otter latrines were collected in the lower basin of the Paraguá River during the low water period, in August 2003. The Paraguá River is a major tributary of the Iténez River, and is the natural boundary between the Noel Kempff Mercado protected area and the Indigenous Territory Bajo Paraguá. The work area corresponded approximately to a river stretch of 60km (Figure 1).

Bones of six fish species (*Hoplias malabaricus*, *Serrasalmus rhombeus*, *Pygocentrus nattereri*, *Chaetobranchus flavescesns*, *Satanoperca pappaterra* and *Astronotus crassipinnis*) were extracted. These species regularly form part of the diet of giant otter in the Paraguá River (Mallea Cardenas and Becerra Cardona, 2012). The fish were caught using a variety of methods, but mainly trawl nets. Fish samples were preserved in formalin 10% and transported to the laboratory, where standard length of each individual fish was measured. The individuals of each species were grouped in size classes of two centimeters and one individual of each size class was chosen for dissection (Table 1).

Scales, skin and most of the muscular tissue of the fish were removed from selected individuals, and the remaining tissue and bones were then put in boiling water with 20g caustic soda for five or eight minutes (depending on the sample size), until the flesh could be easily removed from the skeleton. The bone was then rinsed with abundant water. Sodium hypochlorite (NaClO) and hydrogen peroxide ($H2O^2$) were used to lighten the structures collected, and facilitate the separation of the bones; after each of these procedures the bone was again rinsed with water. The premaxillary bones were dried to room temperature and stored.

Giant otters defecate in localized communal latrines ('spraint areas'), thus each latrine contains the spraints of various giant otter individuals (Groenendijk *et al.*, 2005). Giant otter feces were collected both in fresh and old latrines. The samples were taken at random over the entire area of each latrine. Nineteen samples with similar volume were selected, collected in different points along the study area (see Figure 1).

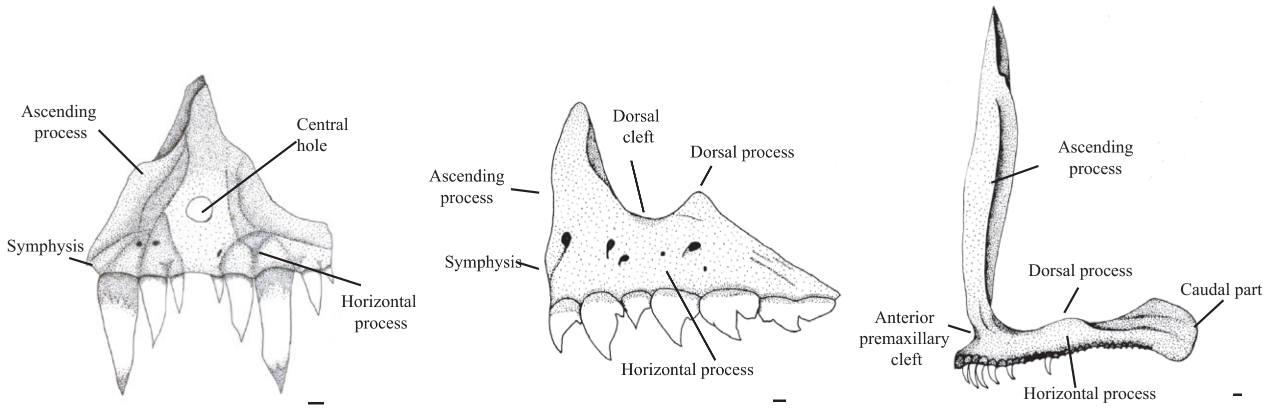

Figure 2. Main morphological characteristics of the premaxillary bones. External view. Scale bars: 1.0mm.

The samples were soaked for 24 hours in water with detergent to eliminate soil remains, and then rinsed with abundant water on two sieves, one with a mesh diameter of 1.25mm and the other of 1.75mm. The samples were dried to the room temperature. Once dried, the premaxillary bones were selected for their identification and subsequent measurement (Mallea Cardenas and Becerra Cardona, 2012).

Identification of fish species and estimation of prey size

In a general way, the premaxilla bone has a vertical or ascending process which is the part of the body that unites to its pair bone, the area of union being denominated symphysis, whereas the horizontal process or lateral branch is the arm or sector of the premaxillary bone where the teeth are located (Machado–Allison, 1986) (Figure 2). However, there exist many variations on this general scheme, and the species-specific characteristics of premaxilla bones enable us to distinguish fish species.

The bones were carefully examined to allow us to identify distinctive characteristics that could be potentially useful. Several bone features were examined for differences among species such as the general shape, size and shape of ascending processes and horizontal processes, and ornamentation such as holes, pores, etc. (Figure 2). The terms used for the description of the premaxilla follow standards set by Machado–Allison (1986) and Conroy *et al.* (1993).

The measurements carried out on the premaxillary bones (see Figure 3) were based mainly on the works of Van Neer (1984), Desse *et al.* (1987; 1990), Rosello and Sancho (1994) and Desse and Desse-Berset (1996). Additionally, the following characteristics were considered: 1) general shape of the bone; 2) key areas of the bone that persist even when it is broken into fragments; 3) characteristic areas of premaxilla (Mallea Cardenas and Becerra Cardona, 2012). The number of measurements taken depended on the general shape of the premaxilla bones and was maximized to increase the probability that standard length of fish from remains could be used based on fragmented bones (Mallea Cardenas and Becerra Cardona, 2012). The measurments were taken with vernier calipers.

Simple linear regression equations were calculated to estimate original standard lengths of six species of fishes based on their bone measurements. Standard lengths were regressed on the average measurements from both left and right premaxillary bones. The equations of lineal regression were calculated using the STATISTICA 6.0 program.

The premaxilla bones collected from the 19 latrines were identified based on a morphological comparison with the dissected premaxilla. The number of bones for each species was assessed and the standard length of the consumed fishes was calculated replacing the values of the measurement in the corresponding linear regression equations. All standard lengths calculated in this way were averaged and standard deviations were calculated.

Estimation of the minimum number of individual fish in the latrine samples

The number of individual fish present in the 19 latrine samples was estimated based on the number of premaxillary bones found. All premaxilla identified by species in each sample were differentiated between right and left. Size differences between left and right premaxillary were taken into account in order to explore whether bones belonged possibly to one individual or to more individuals within the same sample. Left and right premaxillary with noticeable size differences, detectable with a stereomicroscope, were considered to be from different individuals. As a rule, when the difference in fish length calculated from right and left premaxilla was greater than 2mm in *H. malabaricus* and greater than 1mm for the other species, the bones were considered to belong to different individuals. Left and right bones with similar sizes were considered to be from the same individual.

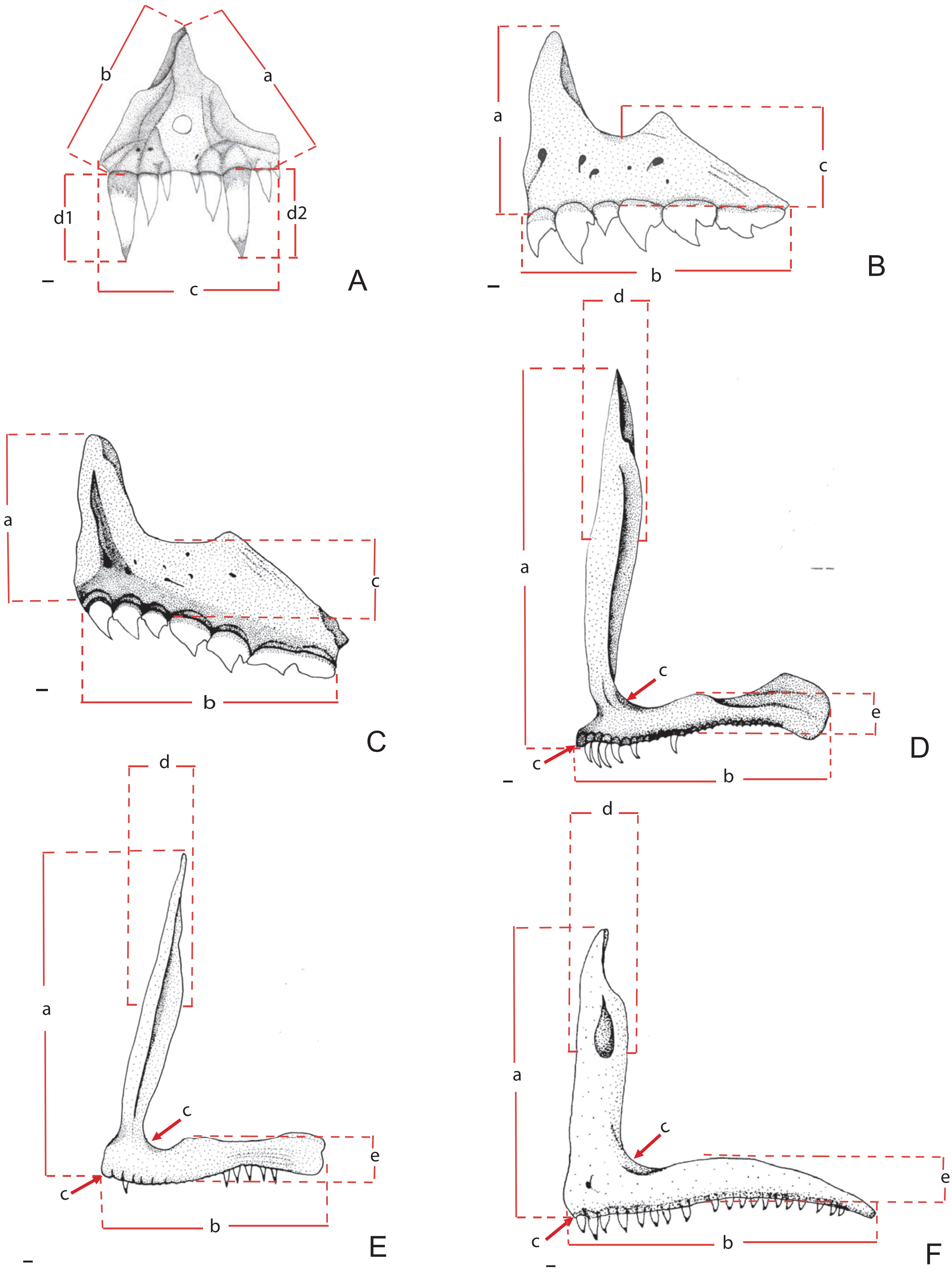

Figure 3. Measurements done on premaxillary bones of: A) *H. malabaricus,* B) *S. rhombeus,* C) *P. nattereri,* D) *C. flavescens,* E) *S. pappaterra,* F) *A. crassipinnis.* External view of left premaxillary. Scale bars: 1.0mm. See regression equations for the measurements in Table 3.

Table 2. Diagnostic characteristics of premaxillary bones and frequency of occurrence in the samples of latrines for six species commonly found in the giant otter diet.

Species	Identification	Degree of fragmentation	Frequency in the sample	Morphological features
Hoplias malabaricus	(++++)	*	♦♦♦	Shape body triangular, central hole, teeth
Serrasalmus rhombeus	(+++)	**	♦♦♦	Shape and ascending process length and dorsal process
Pygocentrus nattereri	(+++)	**	♦♦♦	Wide space between ascending and dorsal processes
Chaetobranchus flavescens	(++++)	**	♦♦♦	Deep anterior premaxillary cleft. Horizontal process pars caudal wide
Satanoperca pappaterra	(+++)	***	♦♦	Soft anterior premaxillary cleft margin Without a clear anterior premaxillary cleft
Astronotus crassipinnis	(+++)	***	♦♦	Horizontal process curved, caudal pars thin

Easiness of differentiation and identification: (+) bad, (++) regular, (+++) good and (++++) very good

Level of fragmentation of premaxillary bones encountered in the samples: (*) low, (**) intermediate, (***) high

Frequency in the sample: (♦) rare, (♦♦) common, (♦♦♦) very common

Results

Identification of prey fish species

The premaxillary bones were easily recognizable among the fish remains. There were also clear differences in the diagnostic characteristics at the family level. The premaxillary bones of *H. malabaricus* have a triangular shape, cichlids have a distinctively thin 'L' shape, and serrasalmids have a solid 'L' shape (Figure 3).

The remains of the premaxillary bones of *H. malabaricus*, even when they were broken into fragments, could be identified easily, due to the presence of teeth and the characteristic hole in the body of the premaxillary. Identifying broken fragments of the premaxillary bones of serrasalmids was more difficult, because the remains need complete sectors of the premaxilla, such as the ascending or horizontal process (ramus). If these sectors are also broken into fragments, the identification at family level is possible but becomes difficult at species level, though the teeth can aid in species identification. The premaxillary bones of the three species of cichlids, even when broken into fragments, were relatively easy to identify.

Table 2 presents a summary of the characteristic features of premaxillary bones encountered in the latrine samples. The easiness of identification, the observed grade of fragmentation, the frequency within the samples and the morphological characteristics most useful in the identification are indicated.

Estimation of standard length of prey fish

All regressions for *H. malabaricus* were highly significant and displayed high coefficients (R^2), between 0.88 and 0.98. Regression equations for *S. rhombeus*, *P. nattereri* and *A. crassipinnis* ranged from highly significant to significant, with R^2 values from 0.53 to 0.99 (Table 3). Regression of the measurements 'a' and 'b' (see Figure 3) were significant for *C. flavescens,* with R^2 values ranging from 0.77 to 0.83. Finally, regression of the measures 'a', 'b' and 'e' were also significant, with R^2 values ranging from 0.95 to 0.99 for *S. pappaterra* (Table 3).

A total of 109 premaxillary remains were identified to species level. Of these, 34% and 36% were from *H. malabaricus* and *C. flavescens* respectively (Table 4). Using the regression equations, the standard length of 58 individual fish (53%) could be estimated. This percentage was relatively low because some remains did not contain the necessary areas to apply the measurements for the regressions, or the regressions results were not significant. For example, 39 bones of *C. flavescens* were encountered, of which 25 were fragmented and only the non-significant measure 'c' could be taken. For this reason, the standard length could only be calculated from four bones (10%).

The standard length estimated for *H. malabaricus* was between 121 to 340mm and frequent size classes ranged from 181 to 240mm. Size classes of *S. rhombeus* ranged between 21 and 200mm and for *P. nattereri* they ranged between 101 and 200mm. The range sizes estimated for the cichlids *C. flavescens*, *S. pappaterra* and *A. crassipinnis* were between 61 and 160mm (Figure 4).

Table 3. Regression equations relating measurements (mm) of premaxillary bones to standard length (SL) for six species of giant otter prey. Ranges of estimated standard lengths are shown for each species. Measurements of premaxillary bones (expressed as 'prmx a', 'prmx b', etc.) are as indicated in Figure 3.

Equations	R^2	p	Standard error	n
Hoplias malabaricus (SL 140-380)				
SL = 35.20+17.79*prmx a	0.98	<0.001	10.06	8
SL = 45.37+19.50*prmx b	0.90	<0.001	25.54	8
SL = 35.64+16.96*prmx c	0.98	<0.001	11.60	8
SL = 44.15+33.81*prmx d1	0.89	<0.001	27.26	8
SL = 50.04+35.13*prmx d2	0.88	<0.001	28.59	8
Serrasalmus rhombeus (SL 138-260)				
SL = 40.08+13.12*prmx a	0.94	<0.001	11.70	7
SL = -15.11+10.27*prmx b	0.98	<0.001	5.85	7
SL = 40.55+31.51*prmx c	0.85	<0.01	18.52	7
Pygocentrus nattereri (SL 120-147)				
SL = 61.08+11.45*prmx a	0.53	<0,05	30.63	8
SL = -3.67+9.18*prmx b	0.94	<0,001	10.74	8
SL = 0.92+46.91*prmx c	0.93	<0,001	11.75	8
Satanoperca pappaterra (SL 130-175)				
SL = -5.28+7.31*prmx a	0.98	<0.01	3.30	4
SL = 27.24+9.68*prmx b	0.95	<0.05	5.69	4
SL = -4.83+36.26*prmx c	0.77	0.12	12.01	4
SL = 22.57+57.40*prmx d	0.89	0.06	8.27	4
SL = -9.40+73.11*prmx e	0.99	<0.001	1.60	4
Chaetobranchus flavescens (SL 115-200)				
SL = -101.04+8.74*prmx a	0.83	<0.05	13.52*	6
SL = -63.25+10.98*prmx b	0.77	<0.05	15.76	6
SL = -33.01+32.97*prmx c	0.39	0.18	25.71	6
SL = 21.03+52.36*prmx d	0.57	0.08	21.52	6
SL = -18.87+71.90*prmx e	0.55	0.09	22.00	6
Astronotus crassipinnis (SL 120-205)				
SL = -15.80+11.12*prmx a	0.96	<0.01	7.91	5
SL = 20.55+7.76*prmx b	0.99	<0.001	1.79	5
SL = -18.50+35.01*prmx c	0.95	<0.01	8.69	5
SL = 17.10+44.33*prmx d	0.99	<0.001	4.02	5

Table 4. Minimum number of individuals belonging to six fish species consumed by giant otter in 19 samples analyzed. N = number of samples in which premaxillary bones of the species were found; Ni = number of premaxillary bones encountered in the 19 samples; Ns = number of premaxillary bones for which the fish standard length could be calculated; Nimin = minimum number of individuals estimated based on identified premaxillary bones, Nsmin = minimum number of individuals for which standard length could be estimated through measurement of premaxillary bones.

Species	N	Ni	Ns	Nimin	Nsmin	Estimated fish size range (mm)
H. malabaricus	13	37	27	33	27	121-340
S. rhombeus	6	10	10	10	10	21-200
P. nattereri	7	13	10	11	10	101-200
C. flavescens	8	39	4	26	4	121-160
S. pappaterra	5	6	3	6	3	101-160
A. crassipinnis	3	4	4	4	4	61-120
Total		109	58	90	58	

Table 5. Minimum number of individuals of six fish species identified and measured successfully in 19 latrine samples from the Paraguá river basin.

	Number of latrine samples																			
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**Total/species**
H. malabaricus	2	11	2	1	2	1	1	3	4											27
S. rhombeus			1							1	1	2	1	2	2					10
P. nattereri		1		2	4	1										1			1	10
C. flavescens													2				1		1	4
S. pappaterra		1			1			1												3
A. crassipinnis		2													1			1		4
Total/sample	**2**	**15**	**3**	**3**	**7**	**2**	**1**	**4**	**4**	**1**	**1**	**2**	**3**	**2**	**3**	**1**	**1**	**1**	**2**	**58**

Minimum number of individual fish in the latrine samples

Based on the identified premaxillary bones, the minimum number of individual fish present in the latrine samples was estimated to be 90 (Table 4). *H. malabaricus* and *C. flavescens* were the species most often encountered in the latrines, whereas the smaller cichlids *S. pappaterra* and *S. crassipinnis* were the least frequent.

Standard size could not be estimated for all identified premaxillary bones. The minimum number of individuals in the latrine samples, estimated by taking into account measured size differences, was 58 (Table 4). The minimum number of individuals in the sample was calculated only for the bones which could be measured successfully (Table 5).

Discussion

The diet of the giant otters is based almost exclusively on fish (Rosas *et al*., 1999). However, with the exception of Cabral *et al*. (2010) and Mallea Cardenas and Becerra Cardona (2012), little published information is available on their diet composition. The present study shows how research on the premaxillary bones of fish collected from communal latrines may help to fill this information gap.

Various authors have shown that premaxillary bones have unique characteristics for different fish species. A description of premaxillae of *H. malabaricus* was presented by Miquelarena (1986) and Gayet *et al.* (2003). These authors highlighted the character of the triangular body and the central hole, besides the caniniform and lanceolate form of the teeth. Miquelarena (1986) and Machado-Allison and Fink (1996) provided descriptions of the unique morphological characteristics of the premaxillary bones of *S. rhombeus* and *P. nattereri*, respectively. Within the cichlid family, Kullander (1987) showed that different characteristics exist among species of the genus *Satanoperca*. Based on findings from this study, we confirm that differences exist between *S. pappaterra*

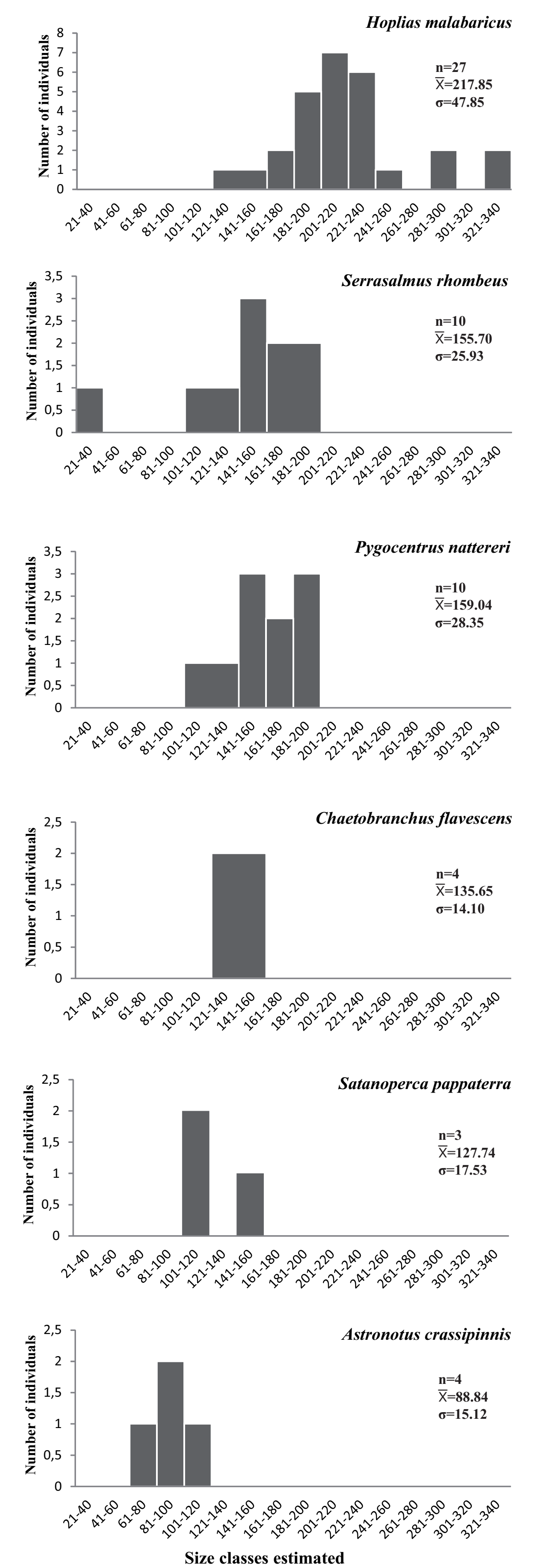

Figure 4. Estimated size of fish in giant otter diet based on premaxillary bones found in latrines in the Paraguá River basin.

and *S. jurupari*. Descriptions of premaxillary bones for other cichlid species were not found in the literature. The premaxillary bones presented in this paper can be considered key bones for identifying fish species consumed, due to their distinguishable characteristics and common presence in otter latrines. The bones of the six fish species were easily identifiable and resistant to digestion. Based on the bone fragments, 100% of the prey species were identified.

The diagrams presented in this paper for *H. malabaricus* and cichlid species do not show the complete set of teeth, which are easily lost during the dissection process and which are rarely present in the latrine samples. Teeth of Serrasalmidae species are better fixed in the bone, and thus readily found in latrine samples. However, the absence of teeth from some species does not diminish the possibilities of identification through premaxillary bones.

Wood (2005), who used only one bone measurement on *Pomatomus saltatriz* (total length of horizontal process) to calculate linear regression equations, recognized that a high number of significant measures should be obtained, in order to improve the use of bone fragments for measurements. In the present study, equations for all the measurements were significant for three species, allowing for the use of fragmented bones. On the other hand, some of the measurements proposed for the premaxillary bones of *S. pappaterra* and *C. flavescens* (particularly 'c') did not yield significant linear regression equations. The anterior parts of the premaxillary, which are very useful for species identification, at the same time are cleft and therefore susceptible to errors in measurement. In the present study, the premaxillary bones of 25 individuals of *C. flavescens* possessed only this diagnostic sector and consequently only measure 'c' could be taken. Considering the importance of this measurement for the estimation of prey size, we recommend an increase in the number of fish dissected and individuals measured, in addition to an increase in the size range. Granadeiro and Silva (2000) showed that regressions obtained along a broad range of sizes have a higher probability to produce significant equations.

Some limitations should be considered when using bones to estimate the original length of prey fish. Scharf *et al.* (1998) suggested that the fixing liquids, such as those used during the present study, may affect bone dimensions. Chemicals were also used during the dissection process, possibly affecting the bone size. However, the same authors mentioned that prey bone size could also be affected by the digestion process (erosion due to digestive juices), leading to errors or bias in the posterior measurements. Despite this bias, fish bones and other hard parts were shown to be excellent predictors of original prey size (Hansel *et al.*, 1988; Sharf *et al.*, 1998).

One additional complication which could limit the use of fish remains in latrines is distortions that may occur during the drying process of the substrate. This corrosive effect may increase in time, meaning that remains from older giant otters latrines that were subjected to harsh weather conditions for a longer period, possibly suffer a loss of bone dimensions.

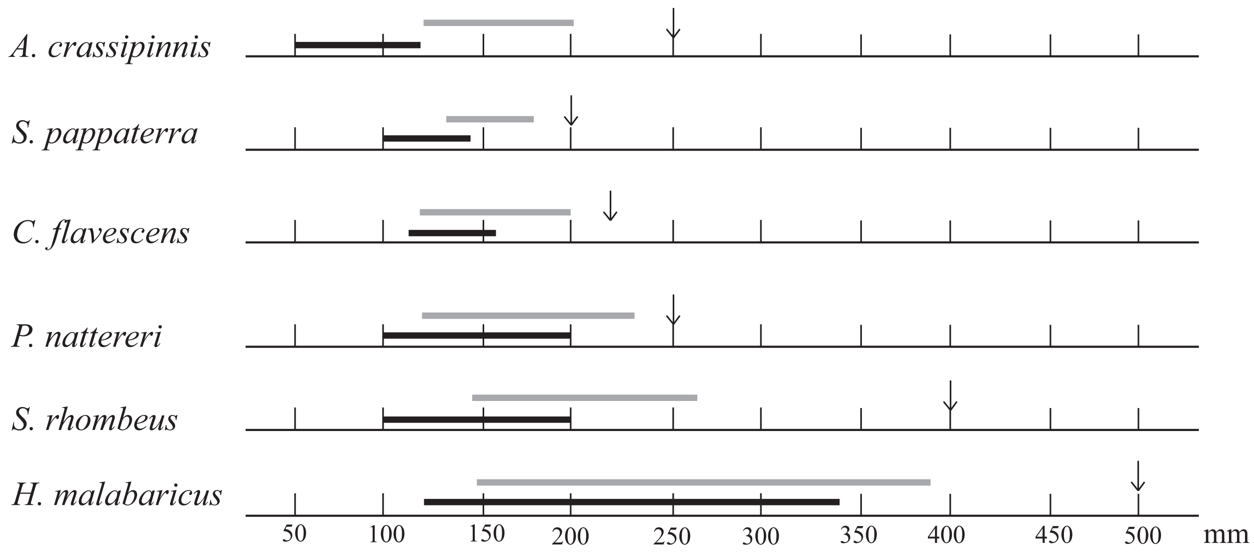

Figure 5. Comparison of size range of fish size estimated from diet analysis (black lines) and size range of collected fish (grey lines). Arrows indicate the maximum size reported in literature.

It is important to indicate that the relative number of individuals of different species provided in Table 4 was estimated for a sample consisting of pooled spraints. Thus, it provides insight in the relative importance of different fish species as a prey for a population of giant otters in a specified area, in this case the Paraguá River basin. However, the data presented does not provide specific information on the number of prey consumed or the prey preference of individual giant otters, which would instead require the collection of individual spraints.

Hájková *et al.* (2003) did not find significant differences between the paired premaxillary bones of three cold water species, which is why they used the mean of the right and left measurements. The same approach was adopted in the present study. Hájková *et al.* (2003) suggested that the use of paired bones (*e.g.* jaw bones) increases the probability of assessing the minimum numbers of a species. However, whereas right and left bones of the same size can be paired, and single occurrences may be treated as separate individuals, it is not possible to completely rule out that the paired bones come from two individuals of the same size or that the missing bone will appear in another spraint. Nevertheless, for giant otters the calculation of the minimum number of individuals may be more exact, due to the fact that latrines of giant otters do contain mixed spraints of all group members. Using the combination of right and left bones of the premaxilla to determine if they correspond to the same individual within the same sample, thus helps avoid the overestimation of prey.

In the past, giant otter diet studies have produced some estimates of prey fish species and size. Duplaix (1980), through direct observation of giant otters, estimated that the majority of fish caught and consumed by giant otters belonged to the order Characiformes. Individuals of the most common prey species, *Hoplias malabaricus,* ranged between 17 and 22cm of length, whereas the size of cichlids (order Perciformes) was between 10 and 15cm of length. Staib (2005), in the Manu Reserve in the southeast of Peru, estimated the size range of preferred prey fish to be between 7 and 30cm total length, and did not mention the consumption of very small or very large prey.

Giant otters in the Paraguá River were found to consume a variety of fish species of variable sizes. The six fish species mentioned in the present study ranged from 61 to 340mm in size. Generally, giant otters in other regions of the Amazon have shown a tendency to catch species and/or individuals of intermediate size. Bentón (*Hoplias malabaricus*), one of the common species in the giant otter diet, can reach up to 500mm in total length (TL) (Ferreira *et al.,* 1998; Dos Santos *et al.,* 2004). The most frequent size range found in the present study for this species was between 201 and 220mm, corresponding to medium-sized individuals, whereas the largest individual observed had a length of 340mm. On the other hand, *S. rhombeus* reaches maximum longitudes of 400mm standard length (SL) and *P. nattereri* of 500mm (SL) (Britski *et al.,* 2007). For these species estimated size of prey in the diet was between 141 and 160mm. *C. flavescens* were found in a size range between 141 and 160mm, whereas the species can reach a maximum growth of 220mm (SL) (Ferreira *et al.*, 1998; Dos Santos *et al.*, 2004). The observed size range of *S. pappaterra* was between 100 and 140mm, whereas this species can reach maximum sizes of 192mm (SL) (Britski *et al,* 2007). Finally, the observed size range for *A. crassipinnis* was between 61 and 120mm, which is an intermediate size for a species that reaches a maximum standard length of 240mm (Britski *et al.*, 2007). Importantly, the population structure for all these species in the Paraguá River is not known, making it difficult to assess whether there is prey selectivity of small, medium and/or large-sized individuals. The only available information is from the (non-random) collection of individuals used for fish extraction in the present study. A comparison (Figure 5) shows that estimated prey size was generally smaller than the range of fish sizes collected. This difference can be explained by the size-selective fishing gear used. However, we assume that the equations obtained can also be used for fish size which is outside the size range collected.

It is probable that smaller individuals of these different species are not consumed by giant otter due to energetic reasons or as a result of problems encountered in detection, capture and/or manipulation. The remains of very small fish found in the samples (25mm, 61mm) could eventually be product of a secondary process, denominated in the literature as 'secondary prey'. This occurs when a fish ingested by the giant otter contains remains of undigested smaller fish in its own stomach. This may occur for large-sized carnivorous species, such as the bentón. The same was observed by Pascual (2000) in her study of sizes of prey consumed by the otter species *Lutra lutra*.

For the same reason, giant otters may not prefer very large individuals, which may be detected, but not easily captured and/or manipulated. The absence or rare occurrence of these large-sized individuals in the latrines may be also due to their rare presence in the area or may be due to the fact that giant otters do not consume the head of these individuals. Studies on prey size selection by the giant otter should be complemented with data on the availability of prey species of different sizes.

One of the major threats to the giant otter populations in the Iténez River basin is human-induced mortality motivated by a perceived and supposed impact of the giant otter on fish populations which are important in subsistence and commercial fisheries (Van Damme *et al.*, 2003; Pickles, 2012; Zambrana Rojas *et al.*, 2012). In showing the range of fish species and sizes consumed by the giant otter, the present study presents key information which represents a first step towards better understanding and responding to this human-predator conflict.

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References

Britski, H.A., de Silimon, K.Z. de S. and Lopes, B.S. (2007) *Peixes do Pantanal: manual de identificação*. 2.ed., Embrapa, Brasília. 227 pp.

Cabral, M.M.M., Zuanon J., De Mattos G.E. and Rosas, F.C.W. (2010) Feeding habits of giant otters *Pteronura brasiliensis* (Carnivora: Mustelidae) in the Balbina hydroelectric reservoir, Central Brazilian Amazon. *Zoologia* 27 (1): 47-53. http://dx.doi.org/10.1590/S1984-46702010000100008

Carter, S.K. and Rosas, F.C.W. (1997) Biology and conservation of the giant otter, *Pteronura brasiliensis*. *Mammal Review* 27(1): 1-26. http://dx.doi.org/10.1111/j.1365-2907.1997.tb00370.x

Conroy, J.W.H., Watt, J., Webb, J.B. and Jones, A. (1993) A guide to the identification of prey remains in otter spraint. *The Mammal Society*, London 16: 3-52.

Desse, J., Desse-Berset, N. and Rocheteau, M. (1987) Contribution à l'ostéométrie du mulet (*Mugil capito* RISSO). *Fiches d'ostéologie animale pour l'Archéologie* (A), n° 2, APDCA, Juan les Pins. 27 pp.

Desse, J., Desse-Berset, N. and Rocheteau, M. (1990) Ostéométrie de la lote d'eau douce (*Lota lota* LINNE). *Fiches d'ostéologie animale pour l'Archéologie* (A), n° 6, APDCA, Juan les Pins. 22 pp.

Desse, J. and Desse-Berset, N. (1996) On the boundaries of osteometry applied to fish. *Archaeofauna* 5: 171-179.

Dos Santos, M.G., De Merona, B., Juras, A.A. and Jegu, M. (2004) *Peixes do baixo Rio Tocantins. 20 anos depois da Usina Hidrelétrica Tucuruí*. Eletronorte, Brasília. 215 pp.

Duplaix, N. (1980) Observations on the ecology and behavior of the giant river otter *Pteronura brasiliensis* in Suriname. *Révue Écologique (Terre et Vie)* 34: 495-620.

Ferreira, E.J.G., Zuanon, J.A.S. and Dos Santos, G.M. (1998) *Peixes Comerciais do Médio Amazonas*. IBAMA, Brasília, Brazil. 211 pp.

Gayet, M., Jégu, M., Bocquentin, J. and Negri, F. (2003) New characoids from the Upper Cretaceous and Paleocene of Bolivia and the Mio-Pliocene of Brazil: Phylogenetic position and paleobiogeographic implications. *Journal of Vertebrate Paleontology* 23(1): 28-46. http://dx.doi.org/10.1671/0272-4634(2003)23%5B28:NCFTUC%5D2.0.CO;2

Granadeiro, J. and Silva, M. (2000) The use of otoliths and vertebrae in the identification and size-estimation of fish in predator-prey studies. *Cybium* 24: 383-393.

Groenendijk, J., Hajek, F., Duplaix, N., Reuther, C., Van Damme, P., Schenck, C., Staib, E., Wallace, R., Waldemarin, H., Notin, R., Marmontel, M., Rosas, F., De Mattos, G.E., Evangelista, E., Utreras, V., Lasso, G., Jacques, H., Matos, K., Roopsind, I. and Botello, J.C. (2005) Surveying and Monitoring Distribution and Population trends of the Giant Otter (*Pteronura brasiliensis*) – Guidelines for a Standardization of Survey Methods as Recommended by the Giant Otter Section of the IUCN/SSC Otter Specialist Group. *Habitat* 16: 1-100.

Hájková, P., Roche, K. and Kocian, L. (2003) On the use of diagnostic bones of brown trout, *Salmo trutta* m. *fario,* grayling, *Thymallus thymallus* and Carpathian sculpin, *Cottus poecilopus* in Eurasian otter, *Lutra lutra* diet analysis. *Folia Zoologica* 52(4): 389-398.

Hansel H.C., Dyke, S.D., Lofy, P.T. and Gray, G.A. (1988) Use of diagnostic bones to identify and estimate original lengths of ingested prey fishes. *Transactions of the American Fisheries Society* 117: 55-62.

Kruuk, H. and Moorhouse, A. (1990) Seasonal and spatial differences in food selection by otters (*Lutra lutra*) in Shetland. *Journal of Zoology* (London) 221: 621-637. http://dx.doi.org/10.1111/j.1469-7998.1990.tb04021.x

Kullander, S.O. (1987) *Cichlid fishes of the Amazon River drainage of Perú*. Swedish Museum of Natural History Department of Vertebrate, Zoology. Stockholm, Sweden. 431 pp.

Machado-Allison, A. (1986) Osteología comparada del neurocráneo y branquiocráneo en los géneros de la subfamilia Serrasalminae (Teleostei – Characidae). *Acta Biologica Venezolana* 12(2): 1-73.

Machado-Allison, A. and Fink, N. (1996) *Los peces caribes de Venezuela: Diagnosis, claves, aspectos ecológicos y evolutivos*. Universidad Central de Venezuela, Caracas, Venezuela. 149 pp.

Mallea Cardenas, H.A. and Becerra Cardona, M.P. (2012) El uso de huesos en la identificación y estimación del tamaño de presas de la londra (*Pteronura brasiliensis*) en el río Paraguá (Bolivia): un estudio de caso. Pages 219-232 *in* Van Damme, P.A., Maldonado, M., Pouilly, M. and Doria, C.R.C. (Eds) *Aguas del río Iténez-Guaporé: recursos hidrobiológicos de un patrimonio binacional (Bolivia y Brasil)*. Editora INIA, Cochabamba, Bolivia. 430 pp.

Miquelarena, A.M. (1986) Estudio de la dentición en peces Caracoideos de la Republica Argentina. *Biología Acuática*, La Plata 8: 1-60.

Pascual, M. (2000) Variaciones estacionales en la dieta de la nutria (*Lutra lutra*) en la cuenca del río Esva (Asturias). Memoria de Licenciatura. Universidad de Oviedo, Portugal. 21 pp.

Pickles, R. (2012) La importancia de la cuenca Iténez para la conservación de la londra (*Pteronura brasiliensis*). Pages 207-215 *in* Van Damme, P.A., Maldonado, M., Pouilly, M. and Doria, C.R.C. (Eds) *Aguas del río Iténez-Guaporé: recursos hidrobiológicos de un patrimonio binacional (Bolivia y Brasil)*. Editora INIA, Cochabamba, Bolivia. 430 pp.

Rosas, F.C., Zuanon, J.A.S. and Carter, S.K. (1999) Feeding ecology of the giant otter *Pteronura brasiliensis*. *Biotropica* 31(3): 502-506.
http://dx.doi.org/10.1111/j.1744-7429.1999.tb00393.x

Rosello, E. and Sancho, G. (1994) Osteology of the chinchard *Trachurus trachurus* (Linneaus, 1758). *Fiches D'Osteologie Animale Pour L'Arqueologie* 8: 1-25.

Scharf, F.S., Yetter, R.M., Summers, A.P. and Juanes, F. (1998) Enhancing diet analyses of piscivorous fishes in the northwest Atlantic through identification and reconstruction of original prey sizes from ingested remains. *Fishery Bulletin* 96: 575–588.

Staib, E. (2005) *Eco-etología del Lobo de Río* (Pteronura brasiliensis) *en el Sureste del Perú.* Sociedad Zoológica de Francfort, Lima, Peru. 195 pp.

Van Damme, P.A., Ten, S., Wallace, R., Painter, L., Taber, A., Gonzáles Jiménez, R., Fraser, A., Tapia, C., Michels, H., Delaunoy, Y., Saravia, J.L. and Vargas, J. (2003) Distribution and population status of the giant otter, *Pteronura brasiliensis*, in Bolivia. *IUCN Otter Specialist Group Bulletin* 19: 87-95.

Van Neer, W. (1984) The use of fish remains in African archaeozoology. Pages 155-167 *in* Desse-Berset, N. (Ed.) *2èmes Rencontres d'Archéo-lchthyologie*. Editions du CNRS, Paris, France.

Wood, A. (2005) Using bone measurements to estimate the original sizes of bluefish (*Pomatomus saltatriz*) from digested remains. *Fishery Bulletin* 103: 461-466.

Zambrana, V., Van Damme, P.A., Becerra, P. and Gónzales-Jimenez, R. (2009) *Pteronura brasiliensis.* Pages 475-476 *in* Ministerio de Medio Ambiente y Agua 2009. *Libro Rojo de la fauna silvestre de vertebrados de Bolivia*. La Paz, Bolivia. 561 pp.

Zambrana Rojas, V., Pickles, R.S., and Van Damme, P.A. (2012) Abundancia relativa de la londra (*Pteronura brasiliensis*) en los ríos Blanco y San Martín (cuenca del río Iténez, Beni-Bolivia). Pages 185-193 *in* Van Damme, P.A., Maldonado, M., Pouilly, M. and Doria, C.R.C. (Eds) *Aguas del río Iténez-Guaporé: recursos hidrobiológicos de un patrimonio binacional (Bolivia y Brasil)*. Editora INIA, Cochabamba, Bolivia. 430 pp.