Bone Length of Iberian Freshwater Fish, as a Predictor of Length and Biomass of Prey Consumed by Piscivores

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ABSTRACT

We measured various fish bones from 13 Iberian freshwater fish species and one hybrid species. Original total body lengths were then back-calculated using bone measurements. Bones usually found in prey remains left by piscivorous predators, were usually from the head skeleton and from the vertebral column. The 73 regressions obtained between bone length and fish length were linear for all species examined. Coefficients of determination ranged between 75.6 % and 99.5 %. To estimate fish biomass, length-weight relations were used for each species, too. Bone length vs. fish length relationships found in the literature for seven additional species inhabiting the Iberian Peninsula were also included, totalling 29 regression equations. The amount of dietary information available from fish predator remains can be greatly increased by using these relationships. In this paper, information is provided covering in excess of 37 % of lberian freshwater fish fauna, including the most abundant and widespread species.

Key words: diagnostic bones, fish length back-calculation, fish predators, Spain.

RESUMEN

Se han realizado medidas a 13 tipos de huesos de peces para retrocalcular su longitud total. Estos huesos, que se encuentran normalmente en los restos dejados por depredadores ictiófagos, pertenecen al esqueleto de la cabeza y a la columna vertebral de 13 especies de peces (más un híbrido) de aguas continentales de la Península Ibérica. Las 73 ecuaciones obtenidas entre la longitud del hueso y la del pez fueron lineales para todas las especies estudiadas; los coeficientes de determinación oscilaron entre el 75.6 % y el 99.5 %. Para la estimación de la biomasa de los peces se han calculado las regresiones longitud-peso para cada una de las especies. Además, se incluyen, extraídas de la bibliografía, las relaciones entre la longitud del hueso y la longitud del pez para otras siete especies de peces que habitan en la Península Ibérica. Con este método la cantidad de información extraíble del análisis de la dieta de depredadores ictiófagos se puede incrementar considerablemente. Las ecuaciones que se aportan abarcan a más del 37 % de la ictiofauna Ibérica de aguas continentales, con inclusión de las especies más abundantes y de más amplia distribución.

Palabras clave: huesos diagnóstico, retrocálculo de la talla de los peces, depredadores ictiófagos, España.

INTRODUCTION

Bones have often been med, both by biologists and archaeologists, to identify fish remains and to estimate fish lengths (e. g. Rojo, 1987; Hansel *et al.*, 1988; Roselló, 1989; Prenda & Granado-Lorencio, 1992a; Conroy *et al.*, 1993). The relationship between the length of a fish and the length of some of their bones is constant. Thus, once the length of a given bone is known, it is

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Table 1. Regressivn statistics ($TL = a + bBL$) relating measurements (in mm) of different bones (BL) to total length (TL) for 13 Hsh species and one hybrid from the lberian Peninsula. *Ranges of estimated total length are also shown.* *** $p<0.001$. *Regresiones ($TL = a + bBL$) entre la longitud de diferentes estructuras óseas (BL, en mm) y la longitud total (TL, en mm) para 13 especies de peces y un híbrido presentes en la Peninsula Ibérica. Se incluye el rango de la longitud estimada total.* *** $p<0.001$

Species	bone	N	a	b	r^2 (%)	estimated length (mm)
Anguilla anguilla	maxillae	38	43.24	27.28	87.6***	204-514
	dentary	38	59.75	20.45	84.0***	193-485
	opercle	38	46.73	33.75	89.2***	208-474
	basio-occipital	31	-116.25	111.63	95.0***	200-498
	vertebrae	36	10.43	111.46	98.3***	181-447
Salmo trutta	premaxillae	26	32.06	29.84	81.3***	129-350
	maxillae	31	14.00	8.49	82.1***	100-342
	dentary	31	47.38	8.13	75.6***	113-342
	vomer	29	65.00	10.17	77.1***	143-347
	basihial	30	-4.02	20.38	81.6***	127-335
	paiatine	31	8.02	13.03	82.2***	114-350
	vertebrae	36	-10.31	89.76	95.6***	106-205
Barbus bocagei	premaxillae	40	-10.93	27.01	98.5***	35-567
	maxillae	42	-15.14	20.85	99.3***	27-579
	dentary	42	-15.78	24.05	99.4***	30-578
	pharyngeal bone	42	-21.34	17.16	99.4***	22-572
	vertebrae	42	0.62	76.22	99.5***	35-558
Chondrostoma polylepis	premaxillae	41	-3.01	24.72	98.1***	24-206
	inaxillae	43	-4.65	21.54	98.8***	24-214
	dentary	44	-18.32	24.13	98.7***	25-209
	pharyngeal bone	43	-5.72	19.51	98.8***	25-210
	vertebrae	44	7.88	63.80	99.0***	33-226
Squalius pyrenaicus	premaxillae	54	5.16	18.25	97.6***	29-178
	maxillae	56	2.77	16.77	98.1***	30-176
	dentary	57	-0.70	15.56	97.3***	30-172
	pharyngeal bone	57	3.54	14.21	97.2***	32-165
	vertebrae	54	5.24	60.52	98.5***	34-166
Carassius auratus	premaxillae	27	-6.72	22.46	93.2***	53-173
	maxillae	27	-14.05	20.61	94.4***	53-174
	dentary	27	-22.83	17.75	97.1***	53-176
	pharyngeal bone	26	-22.37	12.40	92.9***	53-174
	vertebrae	27	4.21	57.10	89.4***	50-166
Gobio gobio	premaxillae	31	-2.90	20.47	92.4***	33-99
	inaxillae	31	0.42	16.23	97.6***	35-100
	dentary	31	-3.47	17.89	96.8***	37-102
	pharyngeal bone	30	-1.95	14.87	96.8***	36-106
	vertebrae	32	7.71	53.60	97.3***	36-106
Squalius alburnoides	premaxillae	32	5.00	18.55	97.0***	29-88
	maxillae	32	4.99	16.67	93.4***	32-88
	dentary	34	-2.19	16.33	93.3***	28-88
	pharyngeal bone	34	2.41	15.81	94.7***	32-98
	vertebrae	35	3.13	58.35	98.8***	30-91

Cont.

Table 1. (Cont.)

Species	**bone**	**N**	**a**	**b**	**r^2 (%)**	**estimated length (mm)**
S. pyrenaicus X	premaxillae	28	12.56	15.54	94.8***	53-115
S. alburnoides	maxillae	28	11.99	13.93	96.1***	51-115
	dentary	28	3.06	14.94	91.7***	45-115
	pharyngeal bone	28	7.21	14.14	97.4***	51-120
	vertebrae	26	7.47	55.25	96.4***	52-112
Cobitis paludica	maxillae	28	11.12	23.18	95.0***	45-100
	dentary	27	6.49	26.01	96.8***	46-96
	opercle	25	12.62	17.97	97.6***	45-95
	pharyngeal bone	27	0.30	26.78	97.3***	44-96
	vertebrae	28	1.33	62.1	95.4***	44-94
Gambusia holbrooki	premaxillae	25	-2.55	16.07	96.7***	18-47
	maxillae	25	-3.13	19.53	98.1***	16-46
	dentary	24	0.33	17.84	96.0***	17-47
	vertebrae	25	-1.11	57.35	97.3***	18-46
Liza aurata	premaxillae	27	-16.84	46.10	98.7***	96-396
	maxillae	27	3.65	21.30	99.1***	95-389
	dentary	26	17.42	18.58	98.6***	96-396
	suborbital	26	-18.43	29.29	98.4***	91-384
	vertebrae	28	8.27	41.84	99.1***	97-400
Liza aurata	premaxillae	27	-16.84	46.10	98.7***	96-396
	maxillae	27	3.65	21.30	99.1***	95-389
	dentary	26	17.42	18.58	98.6***	96-396
	suborbital	26	-18.43	29.29	98.4***	91-384
	vertebrae	28	8.27	41.84	99.1***	97-400
Micropterus salmoides	premaxillae	41	50.49	7.69	94.1***	170-342
	maxillae	41	43.39	7.06	96.6***	172-344
	dentary	41	36.04	7.29	96.7***	178-346
	preopercular	38	27.56	6.79	98.1***	171-330
	pterigoides	36	27.26	10.56	98.4***	171-332
	vertebrae	41	3.99	56.38	97.4***	176-328
Lepomis gibbosus	premaxillae	74	11.72	13.71	96.7***	41-125
	maxillae	74	9.85	10.32	97.1***	41-123
	dentary	74	3.52	14.30	97.5***	39-121
	preopercular	62	0.16	7.23	99.1***	38-120
	pterigoides	69	5.16	13.53	98.6***	40-125
	vertebrae	74	2.91	53.29	98.7***	39-116

possible to accurately estimate the fish length using regression equations (Carss & Elston, 1996; Jacobsen & Hansen, 1996). Using these, authors have established the size-distributions of fish populations and the fish biomass ingested by several fish predators (Mann & Beaumont, 1980; Hansel *et al.,* 1988; Carss & Elston, 1996; Jacobsen & Hansen, 1996).

In the Iberian Peninsula, in contrast with other European areas, there are few works in which the fish length vs. bone length relationships has been used (but see Prenda & Granado-Lorencio, 1992a). Here, we provide the regression equations to estimate the body length of 13 freshwater fish species plus one hybrid, based on several, easily identifiable bones found in sprain ts, faeces or stomach contents of fish predators. Also included are equations for seven additional freshwater fish species inhabiting the Iberian Peninsula, obtained from published papers. The potential use of this type of work is broadened by the inclusion of a significant fraction of the most relevant fish species found in the area.

MATERIAL AND METHODS

A total of 530 fish specimens between 16 and 579 mm in total length and from 13 species plus one hybrid were dissected (Table 1). The relationships between the lengths of bones and total body length were determined on all specimens. In addition, more than 1,400 fish specimens were measured and weighed, and a length-weight regression equation drawn. The fish were collected in several streams and reservoirs from the River Tagus basin in Portugal, and in Spain from the River Guadalquivir basin, during 1995 and 1997, respectively. Specimens were immediately transported to the laboratory to be weighed (total wet weight, ± 0.001 g) and measured (total length, ± 1.0 mm) and then frozen for later analysis. To remove bones, the fish were placed in boiling water (1-10', depending on fish size), until the flesh could be easily removed. Bones were then measured with an ocular micrometer at 10x or 20x power (± 0.01 mm) or using a hand calliper (± 0.05 mm) for large bones.

Simple linear regression equations were drawn to estimate total body length from bone measurements for 14 fish species belonging to nine families. Total body lengths were regressed against measurements of each type of bone. In the case of paired bones (i.e. premaxillae, maxillae, dentary, opercle, pharyngeal bone, suborbital and pterigoides), both the left and right bones were measured and the mean of both lengths used in the regressions. The mean length of the first six caudal vertebrae was employed in the regressions. Anterior caudal vertebrae were used because they show a smaller range of variation than the anterior abdominal and thoracic bones. Also, caudal vertebrae are less prone to breakage than other vertebrae, resulting in a greater likelihood of finding well-preserved bones (Conroy *et al.,* 1993).

A total of 13 different bones were analysed, 12 belonging to the head skeleton (i.e. maxillae, premaxillae, dentary, pharyngeal bone, fifth branchial arch, opercle, preopercle, pterigoides, basio-occipital, vomer, basihial, palatine and suborbital) and the caudal vertebrae (Table 1). Most of them can be easily assigned to species level, except vertebrae which can be identified to family level (Prenda *et al.,* 1997). An example of the latter can be found in recent work done on otter spraints. Bones used in the present work are thought to be resistant to digestion (Carss & Elston, 1996). Figure 1 shows the types of bone analysed and the lengths measured on each of them (maximum lengths). A complete description of each bone can be found in Prenda & Granado-Lorencio, (1992b) and in Prenda *et al.* (1997). Dentaries of salmonids, mugilids, poecilids and centrarchids were measured from the anterior edge (symphysis) to the posterodorsal tip (Fig. 1, bones no. 8, 23, 27, 31 and 33) and dentaries of cyprinids and cobitids from the symphysis to the posteroventral tip (Fig. 1, bones no.14 and 20). Dentaries from eels were measured from the anterior edge to the posterior edge (Fig. 1, bone no. 2). Maxillae were measured from the anterior edge to the posterior edge (comisural edge) (Fig. 1, bones no. 1, 7, 15, 18, 29 and 32), except poecilids and mugilids' maxillae, which were mea-

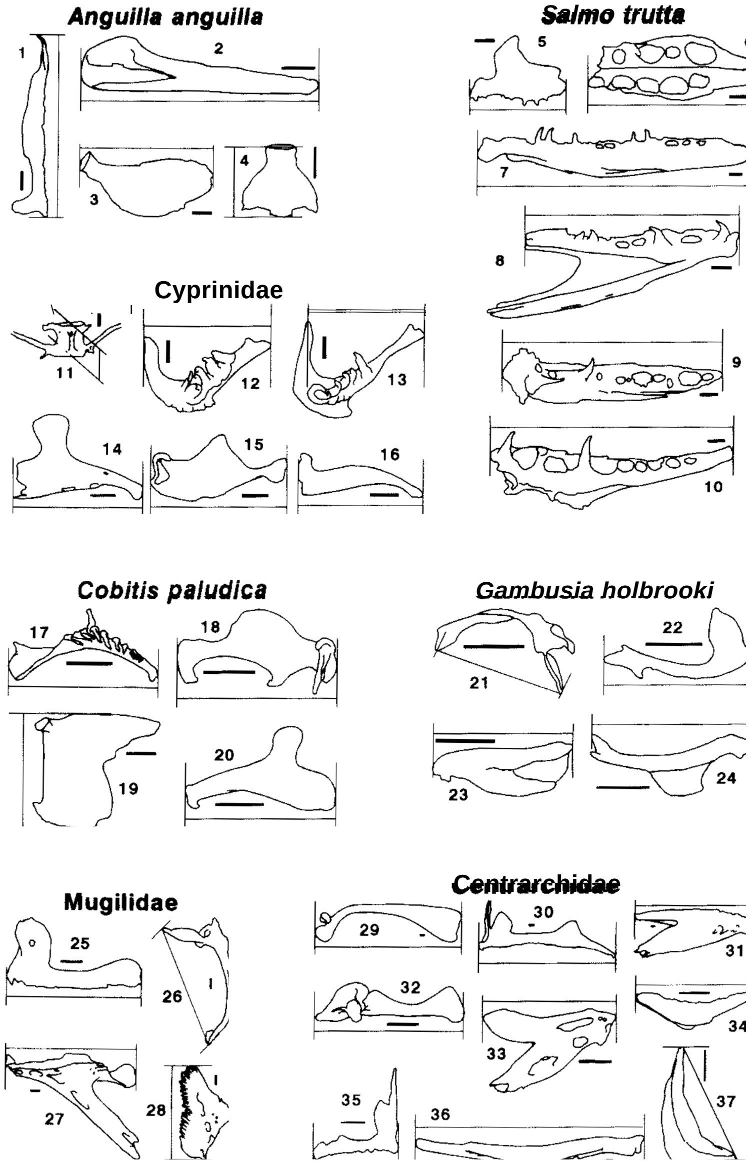

Figure 1. Different views of the types of bone studied in this work, showing the measurements taken of each one. Maximum length was measured of each bone. ***A. unguilla:*** 1=maxillae, 2=dentary, 3=opercle, 4=basio-occipital; *S. trutta:* 5=premaxillae, 6=palatine, 7=maxillae, 8=dentary, 9=vomer, 10=basihial; Cyprinidae: 11=vertebrae, 12=pharyngeal bone, 13=pharyngeal bone, 14=dentary, 15=maxillae, 16=premaxillae; *C. paludica:* 17=pharyngeal bone, 18=maxillae, 19=opercle, 20=dentary; *G. holbrooki:* 21=maxillae, 22=premaxillae, 23=dentary, 24=pterigoides; Mugilidae: 25=premaxillae, 26=maxillae, 27=dentary, 28=suborbital; Centrarchidae: 29-32=maxillae, 31-33=dentary, 30-35=premaxillae, 34-36=pterigoides, 37=preopercle *(M. salmoides:* 29, *30,* 31, 36, 37; *L. gibbosus*: 32, **33,** 34, 35). Scale bars 1 mm. *Distintas vistas* ***de*** *los huesos estudiados* ***en*** *este trabajo.* ***en*** *las que se muestran las medidas tomadas a cada uno. En todos los casos* ***se*** *midió la longitud máxima.* ***A.*** *unguilla: 1=maxilar, 2=dentario, 3–opérculo. 4=basioccipital:* S. trutta: *5=premaxillar, 6=palatino, 7=maxilar: 8=dentario, 9=vómer, 10=basihial;* ***Cyprinidae:*** *11=vértebra, 12 -hueso faríngeo,* **13=** *hueso faríngeo, 14=dentario, 15=maxilar, 16=premaxilar;* C. paludica: *17= hueso faríngeo, 18=maxilar, 19=opérculo, 20=dentario;* G. holbrooki: *21=maxilar, 22=premaxilar, 23=dentario, 24=pterigoides; Mugilidae: 25=premaxilar, 26=maxilar, 27=dentario, 28=suborbital; Centrarchidae: 29 y 32=maxilar, 31 y 33=dentario, 30 y 35=premaxilar, 34 y36=pterigoides, 37=preopérculo (M. salmoides: 29, 30, 31, 36, 37; L. gibbosus: 32, 33, 34, 35). Las barras de escala* ***se*** *corresponden con 1* ***mm.***

Table 2. Regression statistics (*TL = a + bBL*) taken from the literature, relating measurements (in mm) of different bones (*BL*) to total length (*TI,*) for 10 fish species from the lberian Peninsula. Ranges of estimated total length are also shown for *Anguilla anguilla*, *Oncorhyncus mykiss* and *Gasterosteus aculeatus* using as predictor various bones. *** p<0.001. † **fork** length. *Regresiones (*TL = a + bBL*) tomadas de la bibliografía entre la longitud de **diferentes** estructuras **óseas** (*BL, *en mm) y la longitud total (*TL, *en mm) pura 10 especies de peces presentes en la Península Ibérica. Se incluye el **rango** de la longitud estimada total para* Anguilla anguilla, Oncorhyncus mykiss *y* Gasterosteus aculeatus *utilizando algunos huesos **como predictores** Se incluye la **Fig**. en la que aparece la medida **tomada** u cada hueso.* *** *p<0.001.* † longitud furcal.

(1) Libois *et al* (1987), (2) Carss & Elston (1996), (3) Hansel *et al.* (1988), (4) Feltham & Marquiss (1989), (5) Conroy *et al.* (1993), (6) Mann & Beaumoiit (1980), (7) Libois *et al.* (1988), (8) Wise (1980).

Species	bone		fig.	N	a	b	r	estimated length	ref.
Anguilla anguilla	anguloarticular	length	2.1	66	2.51	3.15	0.982	64-585	(1)
	ceratohial	length (a)	2.2	64	3.15	2.82	0.979	68-585	(1)
	ceratohial	length (b)	2.2	64	4.26	4.28	0.973	68-585	(1)
	thoracic vertebrae	length	1.11	40	9.08	113.6	0.950	147-590	(2)
Oncorhynchus mykiss	cleithrum	length	2.5	45	-16.70	10.27	97.0 (r^2)	90-201†	(3)
	dentary	length	1.8	45	1.71	18.18	90.0 (r^2)	92-917†	(3)
	opercle	length	2.4	45	4.44	15.64	94.0 (r^2)	92-192†	(3)
Salmo spp	atlas	width	2.6	200	-8.95	60.50	96.1 (r^2)		(4)
Phoxinus phoxinus	caudal vertebrae	length	1.11		1.27	0.51	0.988		(5)
	thoracic veriebrae	length	1.11		2.06	0.51	0.984		(5)
	pharyngeal bone	tip (c)	2.7	47	3.77	13.71			(6)
	pharyngeal bone	gape (b)	2.7	47	8.57	16.54			(6)
	pharyngeal bone	shank (a)	2.7	47	3.31	17.11			(6)
Gobio gobio	pharyngeal bone	tip (c)	2.7	88	-7.13	18.84			(6)
	pharyngeal bone	gape (b)	2.7	88	-2.92	23.46			(6)
	pharyngeal bone	shank (a)	2.7	88	2.77	28.16			(6)
	pharyngeal bone	(a)	2.8	17	7.82	142.25	94.8 (r^2)		(7)
	dentary	(a)	2.1	15	-1.74	34.77	97.4 (r^2)		(7)
Cyprinus carpio	maxillae	(a)	2.9	25	4.80	66.87	0.974		(7)
	dentary	(a)	2.1	25	2.56	25.02	0.988		(7)
	dentary	(c)	2.1	25	-1.38	66.14	0.987		(7)
	pharyngeal bone	(b)	2.8	25	-10.25	54.32	0.975		(7)
Tinca tinca	maxillae	(a)	2.9	41	10.93	85.65	0.989		(7)
	dentary	(b)	2.1	41	1.98	85.24	0.985		(7)
	pharyngeal bone	(b)	2.8	41	1.38	107.43	0.985		(7)
	pharyngeal bone	(a)	2.8	41	15.21	81 17	0.983		(7)
Esox lucius	caudal veriebrae	length	1.11	26	2.66	0.83	0.987		(8)
Barbatula barbatula	caudal vertebrae	length	1.11		0.51	0.60			(5)
	thoracic vertebrae	length	1.11		0.52	0.49			(5)
Gasterosteus aculeatus	pelvic bone	length	2.3	115	-2.54	7.69	0.975	28-74	(1)

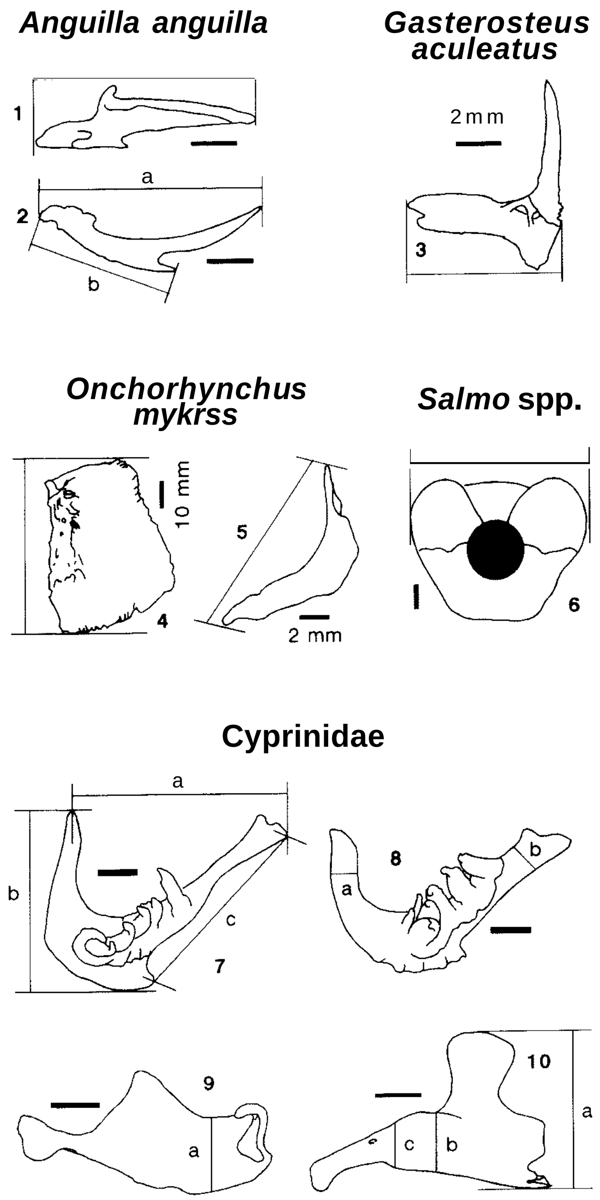

Figure 2. Different views ofthe types of bone whose regressions (bone length vs. fish length) were obtained from the literature. The measurements taken of each bone are represented. In small lettering, additional dimensions measured on bones are indicated. The corresponding equations for these bones are in Table 2. *A. anguilla*: 1=anguloarticular, 2=ceratohial; *G. uculeatus:* 3=pelvic bone; *O. Mykiss*: 4=opercle, 5=cleithrum; *Salmo* spp.: 6=atlas; Cyprinidae: 7=pharyngeal bone (a=shank, b=gape, c=tip), 8=pharyngeal bone (a=superior tip width, b=inferior tip width); 9=maxillae (a=minimum height of maxillae body), 10=dentary (a=maximum height, b=height of bone body at the level of the superior aboral apophysis, c=maximun height of bone body). Scale bars 1 min, unless otherwise stated. *Distintas vistas de los huesos cuyas rectas de regresión longitud del hueso-longitud del pez han sido extraídas de la bibliografía, con indicación de las medidas tomadas a cada uno. Las ecuaciones para estos huesos aparecen en la Tabla 2. En los casos en que se tomó más de una medida se indica con letra minúscula.* A. anguilla: *1=anguloarticular, 2=ceratohial;* G. aculeatus: *3= hueso pélvico; O.* Mykiss: *4= opérculo, 5 cleitro;* Salmo *spp., 6=atlas; Cyprinidae: 7 hueso faríngeo (a=distancia entre los extremos de las ramas superior e inferior: h anchura, c =longituddel brazo inferior), 8= hueso faríngeo (u anchura de la rama superior, b=anchura de la rama inferior); 9= maxilar (a=altura mínima del cuerpo del maxilar), 10– dentario (a=altura máxima del hueso, b=altura del cuerpo del hueso en la base de la apófisis aboral superior, c=altura máxima del cuerpo del dentario). Las barras de escala se corresponden con 1 mm, excepto cuando se indica otra cifra.*

sured diagonally, from the internal apophysis tip to the posterior tip (Fig. 1, bones no. 21 and 26). Premaxillae were measured from the anterior edge (symphysis) to the posteroventral tip (Fig. 1, bones no. 5, 16, 22, 25, 29 and 30). Cyprinids and cobitids pharyngeal bones were measured from the superior tip to the inferior tip (Fig. 1, bones no. 12, 13 and 17). Opercles from eel were measured from the fulcrum to the posterior edge (Fig. 1, bone no. 3) and opercles from cobitids were measured from the anterodorsal edge to the anteroventral margin (Fig. 1, bone no. 19). Preopercles of centrarchids were measured from the dorsal tip to the ventral margin (Fig. 1, bone no. 37). Vomer, basihial, palatine and pterigoides were measured from the anterior edge to the posterior margin (Fig. 1, bones no. 6, 9, 10, 24, 34 and 36). Suborbital bones were measured from the dorsal margin to the ventral margin (Fig. 1, bone no. 28). Basio-occipital bones of eel and all vertebrae were measured from the anterior edge to the posterior edge (Fig. 1, bones no. 4 and 11).

Also, twenty nine bone length vs.fish length regression equations for ten other fish species were obtained from the literature (see Table 2). Figure 2 shows the types of bones used in these equations, except for caudal vertebrae, which are represented in Figure 1. Also shown, in small print are the type of measurements taken on each bone. Nomenclature for fish species follows Doadrio (2001).

RESULTS

Best regressions between bone length and total fish length were linear and all had positive slopes which were significantly different from zero (*F*-test, p<0.001). Coefficients of determination ranged between 75.6 % and 99.5 % (Table 1). Usually, the best fit was obtained with vertebrae ineasurements. In most cases, the coefficients of determination for each species varied little between bones used in regressions. The exception was trout, with coeficients ranging between 75.6 % for dentary and 95.6 % for the veriebrae. Thus, all types of bone used were good predictors of fish length for the species analysed (see Table 1).

Additional bone length vs. fish length regression equations from other published works are shown in Table 2. In total, summing our data to those available in the literature, more than 37 % of Iberian freshwater fish fauna have been considered, including the most abundant and widespread species (Doadrio *et al.,* 1991; Doadrio, 2001). In Table 3, length-weight relationships are shown, using predicted body lengths (Table 1). Both length and biomass of fish prey ingested by aquatic predators can thus be estimated.

DISCUSSION

Once the prey have been identified, several methods can be employed to analyse fish predator diets from prey remains (e.g. Wise, 1980; Hansel *et al.,* 1988; Prenda & Granado-Lorencio, 1992a; Jacobsen & Hansen, 1996; Carss & Elston, 1996). Estimates of original length and biomass of prey fish using bone length vs. fish length relationships usually provide the best approach to analysing predators' diet (Hansel *et al.*, 1988; Prenda & Granado-Lorencio, 1992a).

Table 3. Regression statistics ($TW=a*TL^b$) relating measurements (in mm) of total length *(TL)* to total fresh weight (*TW,* in g) for 13 fish species and one hybrid from the Iberian Peninsula. (1) $a=e^{a'}$. *** P<0.001. *Regresiones ($TW=a*TL^b$) entre la longitud total (TL, en mm) y el peso fresco total (TW, en g) para 13 especies de peces y un híbrido presentes en la Península Ibérica. (1) $a=e^{a}$ *** P<0.001*

Species	N	a' (1)	b	r^2 (%)
A. anguilla	36	-13.3309	3.00203	96.0***
S. trutta	31	-11.2537	2.96703	99.4***
B. bocagei	173	-11.1708	2.89086	99.3***
Ch. polylepis	181	-11.5686	2.95052	97.9***
S. pyrenaicus	158	-12.2008	3.17496	99.1***
C. auratus	27	-11.5396	3.06948	94.7***
G gobio	41	-13.3113	3.38425	98.5***
S. alhurnoides	127	-12.4556	3.16628	97.7***
S. pyrenaicus x S. alburnoides	22	-11,9465	3.05992	98.4***
C. paludica	80	-14.7476	3.64807	96.9***
G holbrooki	304	-12.4559	3.25992	97.1***
L. aurata	73	-11.9295	3.04453	98.7***
M. salmoides	121	-11.2112	2.99031	99.8***
L. gibbosus	77	-12.2832	3.29864	97.3***

In a previous paper we discussed the advantages of the use of different bones to identify the prey of piscivores and back-calculate their body length (Prenda & Granado-Lorencio, 1992a).

A combination of headbones and vertebrae give best results. Headbones (i.e. premaxillae, maxillae, dentary, pharyngeal bones, opercles, preopercles, ...) are easily identified to species level and vertebrae are commonly found in prey remains (Prenda & Granado-Lorencio, 1992a). In the absence of any of the easily-identifiable headbones, the vertebrae can be used. For instance, predators such as otters *(Lutra lutra)* may discard the head of large prey, and then the vertebrae may be the only identifiable and measurable bone. Vertebrae, however, presents several major problems as predictor of body length, i.e. (1) few vertebrae permit to distinguish within families (e.g. cyprinids; Buhan, 1972) and they generally lack any diagnostic value, (2) they present high within-individual variability (Conroy *et al.,* 1993) and, thus, samples based on few prey may produce widely different estimates of fish size, and (3) in large prey, vertebrae usually appear cut in the centrum and deformed (Prenda & Granado-Lorencio, 1992a).

Jacobsen & Hansen (1996) and also Wise (1980), used fish vertebrae to test the size estimation method we have also used here. Jacobsen & Hansen found, using otter *(Lutra lutra)* spraint analysis, that the method provided a similar fish length distribution for Cyprinids and Percids to that obtained from direct diet analysis. Frequency distributions, however, were not comparable for salmonids or eel, or more generally, for large fish specimens (Jacobsen & Hansen, 1996). On the other hand, Carss & Elston (1996) found in experimental trials with captive otters *(Lutra lutra)* that vertebrae length vs. fish length equations consistently underestimated true fish length. Also, the estimation of the minimal number of fish represented in a spraint, based on vertebrae size, could be one fish, or two as a maximum (Carss & Elston, 1996). To overcome these sources of errors, Carss & Elston (1996) proposed two models, specifically designed for salmonids and eel. These models gave accurate estimates of the number and size of prey consumed by otters. However, to apply them, it was necessary to know the recovery probabilities of bones of different-sized fish, and to know these, feeding trials for each fish species considered are needed.

We recommend to quantify the number of ingested prey identifying and singling out single or paired bones, and vertebrae. This method ensures correct species identification and may facilitate the quantification of minimum requirements in the predator's diet in terms of number of fish (Prenda & Granado-Lorencio, 1992a; Carss & Elston, 1996).

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