Use of bone and otolith measures for size-estimation of fish in predator-prey studies

Ali Serhan TARKAN[1]*, Çiğdem GÜRSOY GAYGUSUZ[2], Özcan GAYGUSUZ[1] and Hasan ACIPINAR[3]

[1] *Istanbul University, Faculty of Fisheries, Ordu Cad. No. 200, 34470 Laleli, Istanbul, Turkey;*
*e-mail: serhantarkan@yahoo.com, serhan@istanbul.edu.tr

[2] *Çanakkale Onsekiz Mart University, Faculty of Fisheries, 17020 Çanakkale, Turkey*

[3] *Istanbul University, Natural and Applied Sciences Institute, 34850 Avcılar, Istanbul, Turkey*

A b s t r a c t . To estimate the size of fish taken as prey by piscivorous predators, linear or non-linear relationships between bone measures (pharyngeal, opercula, cleithra, anal and dorsal spine bones, otoliths) and body length were elaborated for eleven Eurasian cyprinid fish species captured in three lakes of Turkey: rudd *Scardinius erythrophthalmus*, Baltic vimba *Vimba vimba*, Danube bleak *Chalcalburnus chalcoides*, gibel carp *Carassius gibelio*, roach *Rutilus rutilus*, silver bream *Blicca bjoerkna*, common carp *Cyprinus carpio*, chub *Leuciscus cephalus*, Dnieper chub *Petroleuciscus borysthenicus*, tench *Tinca tinca* and tarek *Alburnus tarichi* (endemic species for Lake Van). All calculated regressions were highly significant, with coefficients of determination >81% in most of cases. The results suggest that the biometric relationships between fish length and some bones (pharyngeal, opercula, cleithra) are well suited for use in prey-predator studies of all the studied species, but otoliths and the dorsal and anal spines can be used for some fish species only (rudd, Baltic vimba, roach, silver bream, gibel carp).

Key words: diet analysis; prey size estimation; piscivorous predators; cyprinids

Introduction

The determination of prey identity and size is a crucial aspect in dietary studies of piscivorous animals (e.g. M a n n & B e a u m o n t 1980, H a n s e l et al. 1988, P i e r c e & B o y l e 1991). In Eurasian fresh waters, cyprinids are often the predominant taxonomic family in the fish assemblages and consequently knowledge of their bone and body biometry is useful in the dietary studies of the piscivorous animals that feed on them (e.g. P r e n d a & G r a n a d o - L o r e n c i o 1992, R a d k e et al. 2000, B e y e r et al. 2006). Although there have been several studies that have used freshwater fish bones to estimate the original lengths and weights of prey consumed by predators (e.g. M a n n & B e a u m o n t 1980, P r e n d a & G r a n a d o - L o r e n c i o 1992, C o p p & K o v á č 2003, M i r a n d a & E s c a l a 2005), there are no known studies for freshwater fishes that have examined the relationships between otolith size and fish length, including different measurements of both the left and right sides. Similarly, few studies have attempted to increase the precision and accuracy of the back-calculated lengths by comparing more than one measurement with both sides of any given bone structures (R a d k e et al. 2000, H á j k o v á et al. 2003). The aim of the present study was to examine these prerequisites for diet analysis. We present the equations for estimating the original lengths and weights of the fishes consumed, from both sides' measurements of diagnostic bones (pharyngeal, opercula, cleithra, dorsal and anal spine and otoliths) of eleven Eurasian cyprinid fish species for which relatively little

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*Corresponding author

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or no biometric information exists. These bones were chosen because of their taxonomic importance (M i r a n d a & E s c a l a 2005), usage in diet analyses of piscivorous animals (e.g. C o p p & R o c h e 2003, B r i t t o n & S h e p h e r d 2005) and in paleontological studies (e.g. Y a s u n o 1997).

Material and Methods

A total of 2256 specimens, representing eleven species, were collected from January 2002 to June 2003 from two water bodies (Ömerli Reservoir and Lake Sapanca) in northwest Turkey and from one lake (Lake Van) in eastern Turkey using various fishing methods (gill-netting, beach-seine netting, fyke netting, electrofishing). The fish were measured for total length (TL) to the nearest mm and weighed to the nearest 0.1 g (Table 1). The otoliths were then extracted and the fish were placed in boiling water (duration depended on fish size) to permit recovery of the pharyngeal bone, opercula, cleithra, dorsal and anal spines. To examine the relationships with fish size, replicate measurements were taken (to the nearest 0.01 mm) of the bones and otoliths using a digital calliper or a micrometer under a binocular microscope from both the left and right sides: five measurements of the pharyngeal bone, six measurements of the opercula, four measurements of the cleithra, three measurements of the otolith and one measurement of the anal and dorsal spines (Fig. 1). Otolith pairs of the lapillus were used because of their relatively bigger size and regular shape as compared to other otolith pairs in cyprinids. However, some species have fragile and small otoliths, which are likely to be broken or strongly eroded in diet samples (e.g. tarek *Alburnus tarichi*, Danube bleak *Chalcalburnus chalcoides*, common carp *Cyprinus carpio*, chub *Leuciscus cephalus*, Dnieper chub *Petroleuciscus borysthenicus* and tench *Tinca tinca*). Therefore, their otolith size vs. body size relationships was not calculated. Linear (y = ax + b) and non-linear (y = ax^b, power model) regression equations and analysis of variance (ANOVA), where y = TL, were fitted to determine what equations best described the relationships between fish size and bone/otolith dimensions. Relationships with the highest coefficient of determination (r^2) were

Table 1. The species, common names, locations, sample sizes and total length (TL) ranges of fish used in the study.

Species	Common name	Location	n	TL range
Alburnus tarichi	Tarek	Lake Van	63	191–246
Blicca bjoerkna	Silver bream	Lake Sapanca	157	120–212
Carassius gibelio	Gibel carp	Ömerli Reservoir	229	86–357
Chalcalburnus chalcoides	Danube bleak	Ömerli Reservoir	36	115–284
"	"	Lake Sapanca	24	180–233
Cyprinus carpio	Common carp	Ömerli Reservoir	7	222–321
"	"	Lake Sapanca	8	168–224
Leuciscus cephalus	European chub	Ömerli Reservoir	12	97–257
Petroleuciscus borysthenicus	Dnieper chub	Ömerli Reservoir	34	98–149
Rutilus rutilus	Roach	Lake Sapanca	563	141–381
Scardinius erythrophthalmus	Rudd	Ömerli Reservoir	509	93–270
"	"	Lake Sapanca	246	85–277
Tinca tinca	Tench	Lake Sapanca	33	135–278
Vimba vimba	Baltic vimba	Ömerli Reservoir	293	99–294
"	"	Lake Sapanca	42	174–262

adopted as the best predictor (Z a r 1999). To test whether left and right side provided similar results, the linear and non-linear regression were calculated for all measures respectively, and the maximum relative deviation between the predicted regression lines was determined. Data of left and right sides of a measure were pooled for further analysis only if maximum relative difference within the range measured was <2.5% (R a d k e et al. 2000). The measures providing the most accurate estimates of the back-calculated lengths were determined by calculating the confidence limits (95%) and then comparing the maximum values of relative error (confidence limit/calculated length) between measures (R a d k e et al. 2000). Analysis of covariance, ANCOVA (Z a r 1999), was used to analyze effect of site on bone and otolith measures of same species (Danube bleak, common carp, rudd *Scardinius erythrophthalmus* and Baltic vimba *Vimba vimba*) occurring in two different locations studied.
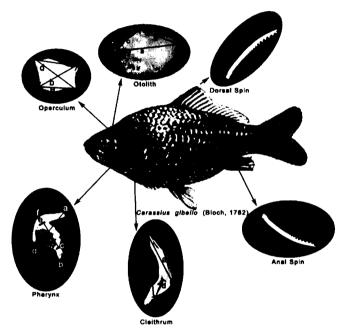

Fig. 1. The bones and otoliths of the fish showing the measures used in the present study.

Results

The relationships between fish TL and bone measurements did not differ between Ömerli Reservoir and Lake Sapanca within the same species (ANCOVA, $P > 0.005$), so data from these locations were combined. The left and right side measurements of the pharyngeal bone were pooled in 69% of all cases. Measurements of tooth width of pharyngeal bone (*ce*) were analyzed separately in all cases except tench and common carp (Appendix 2 and 4). Operculum and cleithrum data from left and right sides were not pooled only for two species (roach *Rutilus rutilus* and silver bream *Blicca bjoerkna*) (Appendices 1 and 3). Non-linear and linear functions provided the best fit for 57% and 43% of all regressions, respectively. All regressions were highly significant $(P<0.001)$ and analysis of bone morphometric parameters vs. TL showed that the regression model explained more than 81% of the variance in all species, with exception of silver bream and tarek in some cases (Appendix 1). The maximum

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values of relative error ranged from 1.2 to 27.6% in pharyngeal, from 1.3 to 24.9% in opercula, from 1.2 to 22.1% in cleithra bones, from 1.3 to 7.0% in otolith, from 5.0 to 13.6% in dorsal spine and from 3.0 to 13.7% in anal spine. Chub was found to give considerably high maximum values of relative error while tarek, silver bream and roach gave low maximum values of relative error for all structures measured. Anal and dorsal spine percent errors were lower for gibel carp *Carassius gibelio* than those in common carp (Appendices 1–4).

Discussion

Linear functions were usually adequate to describe bone size – fish length relationships (M a n n & B e a u m o n t 1980, H a n s e l et al. 1988, P r e n d a & G r a n a d o - L o r e n c i o 1992), but some authors (N e w s o m e 1977, R a d k e et al. 2000) found that curvilinear relationships provided the best fit for some fish as seen in the present study. Left and right measurements of some bone structures do not always provide the same estimate of prey fish length (see R a c z i s k y & S z u b a 1997), so the pooling left and right measurements should be undertaken after adequate statistical analysis only (e.g. R a d k e et al. 2000, C o p p & K o v á č 2003, H á j k o v á et al. 2003). The data for cleithra, opercula, dorsal and anal spine and otolith (Appendix 1–4) could not be compared with those from the literature due to differences in the models and lengths used. However, the observed relationships between pharyngeal bone length and TL for silver bream, gibel carp, common carp, rudd and tench (Appendix 1–4) are similar to those reported by R a d k e et al. (2000). This suggests that the relationship between bone size and body length are relatively constant within species across geographical ranges (e.g. C o p p & K o v á č 2003). However, in the present study the slope and intercept values within Cyprinidae vary greatly, reflecting natural variability in ontogenesis between the species compared. The onset of skeleton formation during early development of fish is species specific, and also strongly depends on environmental conditions, especially temperature and oxygen concentrations (e.g. B a l o n 1981). Both bone measures and otoliths are exposed to a variable degree of chemical and mechanical abrasion in the digestive track of predators. Hence, some small otoliths and bones are likely to be totally dissolved and eroded. Furthermore, partial digestion will bias estimates of prey size (J o b l i n g & B r e i b y 1986, P i e r c e & B o y l e 1991). Use of hard structures may also bias data on food habits by favoring larger over smaller prey fish because their bones may be more resistant to digestion (H a n s e l et al. 1988). Generally, the bones are relatively resistant to digestion and occasionally they are the only recognizable remains (M a n n & B e a u m o n t 1980). Similarly, *in vitro* (P i e r c e et al. 1993) and *in vivo* (P i e r c e et al. 1993, C a r s s & E l s t o n 1996) experiments provided some evidence of a higher resistance of bones to acidic digestion in relation to otoliths, which represents an advantage of the former to back-calculate prey size. Even though otoliths showed relatively low percent errors compared to other structures in the present study (Appendix 1–4), only five of eleven fish species could be suitable for otolith analyses. Fish weight can also be estimated by two-step procedures, first using a relationship between structure length and fish length and then applying a fish length/fish weight equation. Length-weight relationships of fishes studied in the present study were given in T a r k a n et al. (2006). Our results suggested that the biometric relationship between measurements of pharyngeal, opercula, cleithra bones and TL are well suited to the prediction of prey fish lengths from the partly digested remains for all fish species studied. However, dorsal and anal spine bones and otoliths may be used for some fish species only (rudd, Baltic vimba, roach, silver bream, gibel carp). The outputs of this

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study provide a tool for biometric relationships that enable estimation of length and weight using the bones. This information should facilitate the assessment of the diet of piscivorous fauna in Turkey, but potentially wherever these eleven fish species occur.

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Appendix 1. Regression statistics for linear and non-linear relationships relating measurements (mm) of bones (pharyngeal, operculum, cleithrum, dorsal and anal spin) to total length and percent relative errors of each structure for *Alburnus tarichi*, *Blicca bjoerkna* and *Carassius gibelio*. See Figure 1 for measure descriptions. Linear: TL = $ax + b$; non-Linear: TL = ax^b. Coefficient of determination (r^2) and number of data pairs in regression (n) and are indicated.

Species	Structure	Measure	Orientation	Type of regressions	a	b	r^2	n	Relative errror (%)
A. tarichi	Pharyngeal	ab	Pooled	Linear	12.147	84.066	0.61	52	1.42
		bd	Left	Linear	13.443	86.767	0.65	55	1.98
		bd	Right	Linear	14.418	79.687	0.66	53	1.87
		ce	Left	Linear	27.612	80.294	0.65	56	1.86
		ce	Right	Linear	19.798	110.080	0.52	57	1.92
		da	Pooled	Non-linear	63.053	0.620	0.62	54	1.33
		bf	Pooled	Linear	11.884	71.968	0.72	52	1.40
	Operculum	a	Pooled	Linear	10.729	108.120	0.61	58	1.25
		b	Pooled	Linear	6.308	103.120	0.73	59	1.26
		c	Pooled	Non-linear	32.087	0.690	0.78	59	1.31
		d	Pooled	Non-linear	39.736	0.591	0.74	58	1.29
		e	Pooled	Non-linear	60.925	0.514	0.62	58	1.29
		f	Pooled	Non-linear	52.300	0.512	0.75	59	1.28
	Cleithrum	a	Pooled	Non-linear	50.800	0.543	0.65	58	1.24
		b	Pooled	Linear	6.848	82.572	0.69	59	1.28
		c	Pooled	Linear	10.644	91.423	0.69	56	1.26
		d	Pooled	Non-linear	25.296	0.675	0.73	56	1.28
B. bjoerkna	Pharyngeal	ab	Pooled	Linear	15.978	3.195	0.82	121	2.02
		bd	Pooled	Linear	20.967	17.716	0.77	121	2.00
		ce	Left	Non-linear	47.078	0.815	0.67	124	2.83
		ce	Right	Linear	27.213	23.190	0.66	119	3.00
		da	Pooled	Linear	17.892	13.247	0.81	121	2.01
		bf	Pooled	Linear	15.719	6.194	0.81	121	1.99
	Operculum	a	Pooled	Linear	23.373	20.642	0.80	57	2.80
		b	Left	Linear	13.491	0.740	0.91	68	3.67
		b	Right	Linear	13.674	-3.619	0.91	67	3.70
		c	Pooled	Linear	11.002	4.681	0.91	68	2.51
		d	Pooled	Linear	10.772	0.155	0.92	65	2.72
		e	Pooled	Non-linear	14.039	1.069	0.91	57	2.77
		f	Pooled	Linear	14.119	7.679	0.89	65	2.48
	Cleithrum	a	Pooled	Linear	10.031	15.334	0.89	115	2.05
		b	Pooled	Linear	10.831	10.654	0.89	127	1.96
		c	Pooled	Linear	7.143	16.163	0.85	113	1.95
		d	Pooled	Linear	17.880	24.482	0.82	120	2.01
	Otolith	a	Pooled	Linear	97.983	-46.861	0.86	75	2.62
		b	Pooled	Linear	120.940	-14.211	0.83	75	2.71
		c	Pooled	Linear	148.640	-22.899	0.82	75	2.78
C. gibelio	Pharyngeal	ab	Pooled	Non-linear	10.941	0.990	0.94	207	5.09
		bd	Pooled	Non-linear	22.139	0.990	0.92	212	4.58
		ce	Left	Non-linear	26.443	1.151	0.92	207	5.26
		ce	Right	Non-linear	24.220	1.173	0.92	210	5.37
		da	Pooled	Non-linear	14.971	0.973	0.94	209	4.90
		bf	Pooled	Non-linear	11.531	0.983	0.94	207	5.33
	Operculum	a	Pooled	Non-linear	21.499	0.900	0.93	200	5.12
		b	Pooled	Non-linear	11.970	0.914	0.94	200	5.10
		c	Pooled	Non-linear	10.569	0.936	0.95	200	4.94
		d	Pooled	Non-linear	9.797	0.924	0.95	178	5.30
		e	Pooled	Non-linear	13.309	0.937	0.93	133	6.39
		f	Pooled	Non-linear	15.008	0.947	0.94	135	5.74
	Cleithrum	a	Pooled	Non-linear	10.186	0.894	0.89	205	4.91

	b	Pooled	Non-linear	14.847	0.970	0.90	192	4.59
	c	Pooled	Non-linear	7.358	0.970	0.92	188	4.81
	d	Pooled	Non-linear	26.515	0.909	0.91	193	4.78
Dorsal spine	a	Pooled	Non-linear	12.627	0.875	0.89	145	5.04
Anal spine	b	Pooled	Non-linear	16.538	0.810	0.85	146	3.04
Otolith	a	Left	Non-linear	55.895	1.394	0.90	160	6.68
	a	Right	Non-linear	55.525	1.382	0.91	158	6.76
	b	Left	Non-linear	86.571	1.316	0.92	156	6.95
	b	Right	Non-linear	87.125	1.282	0.91	153	6.71
	c	Pooled	Non-linear	136.020	1.358	0.84	154	4.80

Appendix 2. Regression statistics and percent relative errors of each structure for *Chalcalburnus chalcoides*, *Cyprinus carpio* and *Leuciscus cephalus*. See Appendix 1 for symbols, measures.

Species	Structure	Measure	Orientation	Type of regressions	a	b	r^2	n	Relative error (%)
C. chalcoides	Pharyngeal	ab	Pooled	Non-linear	20.865	0.947	0.96	47	8.22
		bd	Pooled	Non-linear	31.757	0.833	0.96	47	8.13
		ce	Left	Non-linear	49.581	0.890	0.93	48	11.30
		ce	Right	Non-linear	45.340	0.895	0.93	48	10.97
		da	Pooled	Non-linear	37.020	0.800	0.93	48	8.23
		bf	Pooled	Non-linear	21.646	0.897	0.95	47	8.24
	Operculum	a	Pooled	Linear	21.941	44.113	0.82	16	8.03
		b	Pooled	Linear	10.221	63.073	0.89	15	9.41
		c	Pooled	Linear	10.565	52.312	0.88	15	8.65
		d	Pooled	Linear	9.724	54.227	0.93	16	8.39
		e	Pooled	Linear	11.947	86.672	0.81	16	8.71
		f	Pooled	Linear	12.853	52.743	0.86	16	8.61
	Cleithrum	a	Pooled	Linear	11.763	44.180	0.82	28	8.96
		b	Pooled	Non-linear	16.914	0.862	0.91	24	9.00
		c	Pooled	Non-linear	11.793	0.917	0.90	24	8.81
		d	Pooled	Non-linear	26.165	0.834	0.88	24	8.99
C. carpio	Pharyngeal	ab	Pooled	Non-linear	7.386	1.103	0.97	15	8.36
		bd	Pooled	Linear	16.998	26.804	0.96	15	8.45
		ce	Pooled	Linear	38.884	-48.468	0.85	15	12.20
		da	Pooled	Non-linear	10.897	1.071	0.96	15	8.85
		bf	Pooled	Non-linear	10.628	0.992	0.97	15	8.61
	Operculum	a	Pooled	Non-linear	19.787	0.899	0.97	13	8.18
		b	Pooled	Non-linear	8.415	1.043	0.96	13	8.30
		c	Pooled	Non-linear	7.237	1.056	0.98	13	8.76
		d	Pooled	Linear	8.589	-20.124	0.98	13	8.85
		e	Pooled	Linear	15.796	-45.813	0.83	13	8.11
		f	Pooled	Non-linear	6.374	1.226	0.88	13	8.55
	Cleithrum	a	Pooled	Non-linear	6.027	1.072	0.97	14	8.98
		b	Pooled	Linear	6.742	53.688	0.96	13	8.68
		c	Pooled	Linear	8.045	-71.063	0.95	13	9.22
		d	Pooled	Non-linear	43.723	0.610	0.94	15	8.08
	Dorsal spine	a	Pooled	Non-linear	29.153	0.641	0.90	4	15.63
	Anal spine	b	Pooled	Linear	5.649	87.095	0.90	4	15.30
L. cephalus	Pharyngeal	ab	Pooled	Non-linear	12.161	1.047	0.97	10	19.24
		bd	Pooled	Linear	18.555	-1.054	0.97	11	17.75
		ce	Left	Linear	23.784	16.716	0.97	11	27.62
		ce	Right	Linear	22.421	13.546	0.95	11	27.40
		da	Pooled	Linear	16.877	4.845	0.99	10	19.17
		bf	Pooled	Non-linear	14.434	0.954	0.98	10	19.08
	Operculum	a	Pooled	Linear	23.929	4.187	0.98	11	24.49
		b	Pooled	Linear	12.113	12.451	0.99	11	23.06

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	c	Pooled	Non-linear	14.441	0.942	0.99	11	24.10
	d	Pooled	Linear	11.604	6.922	0.98	11	23.52
	e	Pooled	Linear	19.444	0.164	0.95	11	23.31
	f	Pooled	Non-linear	15.997	0.990	0.98	11	24.87
Cleithrum	a	Pooled	Non-linear	10.836	1.034	0.98	12	21.19
	b	Pooled	Non-linear	12.348	0.944	0.99	12	21.54
	c	Pooled	Non-linear	8.673	0.983	0.99	12	21.38
	d	Pooled	Non-linear	19.368	0.968	0.98	12	22.09

Appendix 3. Regression statistics and percent relative errors of each structure for *Petroleuciscus borysthenicus, Rutilus rutilus* and *Scardinius erythrophthalmus*. See Appendix 1 for symbols and measures.

Species	Structure	Measure	Orientation	Type of regressions	a	b	r^2	n	Relative errror (%)
P. borysthenicus	Pharyngeal	ab	Pooled	Non-linear	15.931	0.962	0.94	19	4.70
		bd	Pooled	Linear	15.996	19.981	0.92	19	4.73
		ce	Left	Linear	24.234	17.821	0.89	19	7.06
		ce	Right	Linear	18.929	30.655	0.87	19	6.86
		da	Pooled	Non-linear	20.997	0.945	0.91	19	4.74
		bf	Pooled	Non-linear	19.795	0.828	0.92	19	4.83
	Operculum	a	Pooled	Linear	29.648	2.355	0.87	12	6.06
		b	Pooled	Non-linear	14.077	1.009	0.91	15	5.41
		c	Pooled	Non-linear	13.287	0.979	0.92	15	5.60
		d	Pooled	Linear	12.723	-3.194	0.93	13	5.93
		e	Pooled	Non-linear	23.668	0.887	0.86	13	5.90
		f	Pooled	Non-linear	18.038	0.905	0.87	16	5.44
	Cleithrum	a	Pooled	Non-linear	12.390	0.972	0.86	17	5.06
		b	Pooled	Non-linear	13.656	0.892	0.92	19	4.93
		c	Pooled	Non-linear	10.184	0.902	0.93	17	5.05
		d	Pooled	Non-linear	29.825	0.780	0.87	19	4.88
R. rutilus	Pharyngeal	ab	Pooled	Non-linear	17.627	0.943	0.94	636	1.24
		bd	Left	Non-linear	21.982	0.955	0.95	640	1.78
		bd	Right	Non-linear	22.088	0.957	0.95	633	1.76
		ce	Left	Non-linear	37.056	0.859	0.92	655	1.90
		ce	Right	Non-linear	34.055	0.857	0.92	650	1.71
		da	Pooled	Non-linear	22.977	0.880	0.96	1501	1.22
		bf	Pooled	Non-linear	16.453	0.939	0.95	636	1.25
	Operculum	a	Pooled	Non-linear	33.515	0.880	0.94	503	1.76
		b	Pooled	Non-linear	15.878	0.914	0.97	506	1.73
		c	Pooled	Non-linear	14.232	0.925	0.97	511	1.76
		d	Pooled	Non-linear	13.886	0.917	0.97	499	1.92
		e	Pooled	Non-linear	19.184	0.922	0.92	491	1.63
		f	Pooled	Non-linear	17.411	0.925	0.96	502	1.72
	Cleithrum	a	Pooled	Non-linear	16.075	0.854	0.94	575	1.24
		b	Pooled	Non-linear	12.562	0.935	0.97	565	1.26
		c	Pooled	Non-linear	10.230	0.902	0.95	544	1.31
		d	Pooled	Non-linear	23.366	0.882	0.92	566	1.76
	Otolith	a	Left	Non-linear	6.182	1.283	0.89	531	1.82
		a	Right	Non-linear	6.299	1.284	0.88	512	1.83
		b	Pooled	Non-linear	10.757	1.293	0.86	514	1.34
		c	Pooled	Linear	104.310	39.263	0.66	237	1.43
S. erythrophthalmus	Pharyngeal	ab	Pooled	Non-linear	17.627	0.943	0.94	636	4.44
		bd	Left	Non-linear	21.982	0.955	0.95	640	5.18
		bd	Right	Non-linear	22.088	0.957	0.95	633	5.32
		ce	Left	Non-linear	37.056	0.859	0.92	655	5.30
		ce	Right	Non-linear	34.055	0.857	0.92	650	5.79
		da	Pooled	Non-linear	22.977	0.880	0.96	651	4.19

	bf	Pooled	Non-linear	16.453	0.939	0.95	636	4.09
Operculum	a	Pooled	Non-linear	33.515	0.880	0.94	503	6.12
	b	Pooled	Non-linear	15.878	0.914	0.97	506	4.76
	c	Pooled	Non-linear	14.232	0.925	0.97	510	4.70
	d	Pooled	Non-linear	13.886	0.917	0.97	498	4.79
	e	Pooled	Non-linear	19.184	0.922	0.92	491	5.01
	f	Pooled	Non-linear	17.411	0.925	0.96	502	4.83
Cleithrum	a	Pooled	Non-linear	16.075	0.854	0.94	575	3.07
	b	Pooled	Non-linear	12.562	0.935	0.97	565	3.81
	c	Pooled	Non-linear	10.230	0.902	0.95	544	3.25
	d	Pooled	Non-linear	23.366	0.882	0.92	567	3.98
Otolith	a	Left	Non-linear	6.182	1.283	0.89	531	3.62
	a	Right	Non-linear	6.299	1.284	0.88	512	3.84
	b	Pooled	Non-linear	10.757	1.293	0.86	514	2.62
	c	Pooled	Non-linear	13.914	1.212	0.79	495	2.73

Appendix 4. Regression statistics and percent relative errors of each structure for *Tinca tinca* and *Vimba vimba*. See Appendix 1 for symbols and measures.

Species	Structure	Measure	Orientation	Type of regressions	a	b	r^2	n	Relative errror (%)
T. tinca	Pharyngeal	ab	Pooled	Linear	11.935	0.236	0.91	21	7.56
		bd	Pooled	Linear	17.942	42.495	0.93	21	7.02
		ce	Pooled	Non-linear	42.002	0.911	0.82	21	7.54
		da	Pooled	Non-linear	18.337	0.922	0.91	21	7.57
		bf	Pooled	Linear	12.476	11.437	0.93	21	7.54
	Operculum	a	Pooled	Linear	14.282	32.091	0.93	15	7.42
		b	Pooled	Linear	8.859	28.257	0.94	14	8.10
		c	Pooled	Linear	8.414	21.716	0.95	15	7.63
		d	Pooled	Linear	8.211	17.927	0.97	15	7.36
		e	Pooled	Linear	13.039	25.180	0.93	14	7.94
		f	Pooled	Linear	10.484	20.395	0.97	14	8.13
	Cleithrum	a	Pooled	Linear	7.679	17.983	0.93	23	7.25
		b	Pooled	Linear	10.591	4.426	0.93	23	7.14
		c	Pooled	Linear	6.131	11.682	0.93	27	7.37
		d	Pooled	Linear	15.412	50.188	0.86	23	6.97
V. vimba	Pharyngeal	ab	Pooled	Non-linear	18.362	1.007	0.97	399	5.79
		bd	Pooled	Non-linear	36.227	0.857	0.96	300	5.96
		ce	Left	Non-linear	46.501	0.943	0.96	302	9.34
		ce	Right	Non-linear	41.880	0.954	0.96	300	8.59
		da	Pooled	Non-linear	26.313	0.937	0.97	399	5.68
		bf	Pooled	Non-linear	20.930	0.928	0.98	300	5.69
	Operculum	a	Pooled	Non-linear	30.512	0.879	0.97	265	4.10
		b	Pooled	Non-linear	18.523	0.893	0.98	265	4.01
		c	Pooled	Non-linear	17.600	0.884	0.98	265	3.99
		d	Pooled	Non-linear	16.475	0.887	0.98	264	3.93
		e	Pooled	Non-linear	28.417	0.851	0.96	265	4.11
		f	Pooled	Non-linear	21.448	0.914	0.97	266	3.94
	Cleithrum	a	Pooled	Non-linear	16.810	0.892	0.96	303	3.90
		b	Pooled	Non-linear	15.895	0.895	0.98	310	3.98
		c	Pooled	Non-linear	11.224	0.917	0.97	299	4.33
		d	Pooled	Non-linear	28.236	0.849	0.97	311	3.97
	Otolith	a	Pooled	Non-linear	68.769	1.324	0.91	215	4.02
		b	Pooled	Non-linear	124.85	1.279	0.90	199	4.42
		c	Pooled	Non-linear	157.36	1.172	0.86	201	4.15

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