

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

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A b s t r a c t. Bones were obtained from three fish species (brown trout *Salmo trutta m. fario*, grayling *Thymallus thymallus* and Carpathian sculpin *Cottus poecilopus*) for regression analysis. Bones used were chosen based upon frequency of occurrence in spraint samples and diagnostic value. Relationships between the length of diagnostic bones and fish length, fish length and weight, and standard length to total length, were assessed for the three fish species. Polynomial regression was deemed most suitable for the relationship between bone length and fish standard length, multiplicative between fish standard length and fish weight, and linear (brown trout) or polynomial (grayling and Carpathian sculpin) for standard length against total length. All calculated regressions were highly significant and displayed high coefficients of determination, ranging between 93.9 and 99.8 %. The uses of the bones examined, and the equations produced, are discussed in the light of their future use in estimating prey numbers, length and biomass in otter diet analysis.

Key words: prey size estimation, biomass, regression, spraint analysis

Introduction

Identification of undigested remains in faeces or the gut is the most common method of analysing the diet of piscivorous predators, however, prey species identification alone can give only basic information on diet composition. More detailed studies on feeding ecology and energy expenditure require additional information, such as size of prey taken and the quantitative proportion of different species or sizes in the diet. Of the various methods developed for estimating the proportion of prey, that expressed as biomass is usually considered as most closely quantifying actual diet composition (e.g. Bekker & Nole 1990, Pierce & Boyle 1991, Prenda & Granada-Lorenzo 1992). This requires an estimation of the number of individual prey items taken, as well as their length and weight; both usually back-calculated from regressions based on the measurements of species-specific (diagnostic) bones found in the faeces or gut. Such data not only helps identify possible species or size preferences within the diet, it could also help identify preferred foraging sites or habitats, important when the fish taken are of economic value. The relationship between measurements of diagnostic bones and fish length, as well as the

relationship between fish length and weight, has been calculated for various fish prey species as regards the diet of Eurasian otters *Lutra lutra* (e.g. Wise 1980, Libois et al. 1987, Libois & Hallet-Libois 1988, Prenda & Granada-Lorencio 1992, Carss & Elston 1996, Carss et al. 1998, Kloskowski et al. 2000, Copp & Kováč 2003).

However, whilst studying the diet of otters in mountainous and sub-mountainous streams of the upper Hornád River catchment, eastern Slovakia (Hájková 2001, Hájková & Hájek 2002), it was found that no bone regressions existed for grayling *Thymallus thymallus* or Carpathian sculpin *Cottus poecilopus*; and only for vertebrae or dentary of brown trout *Salmo trutta* m. *fario* (Wise 1980, Hallet 1982, Feltham & Marquiss 1989, Carss et al. 1998). All three species are typical for mountain streams of Central Europe, and were found in high numbers in the spraints (otter faeces) examined, with 95 % of all spraints analysed containing remains of brown trout, 23 % grayling remains, and 22 % sculpin remains (unpublished data). It was therefore decided to produce regressions for all three species based on the most common diagnostic bones found, including additional bones to those previously published for brown trout.

Material and Methods

Fishes for analysis were obtained between May and October 1999 and 2000 by DC electrofishing from the upper reaches of the River Hornád, including the tributaries Vernársky potok and Veľká biela voda (20°17'–31'E, 48°55'–60'N). The fish were measured to the nearest 1 mm from the mouth to the base of the caudal fin (standard length, SL) and from the mouth to the tip of the flattened caudal fin (total length, TL). Fish were then weighed to the nearest 1 g or 0.1 g (depending on size) and frozen pending further analysis. Bones to calculate regression equations were subsequently obtained from 36 brown trout (SL = 29–336 mm), 22 grayling (SL = 79–256 mm), and 20 Carpathian sculpin (SL = 30–104 mm).

Skeletons were prepared either by boiling and careful cleaning away the flesh or through the use of beetles *Dermestes* sp. A range of diagnostic bones were chosen from the cranial and post-cranial skeleton, based upon their regular occurrence in 320 spraints collected from the River Hornád and its tributaries (see Table 1), their diagnostic value, and their apparent resistance to damage through digestion. In all, 10 bones were selected for brown trout, 4 for grayling, and 7 for Carpathian sculpin. The bones chosen were measured along their longest axis (dentaries of brown trout and grayling along 2 axes) to the nearest 0.02 mm, using vernier callipers. The dentary (*os dentale*) was measured from the edge of the mandibular symphysis to the posterior tip of the ventral process (dimension 1) and from the edge of the mandibular symphysis to the posterior tip of the dorsal process (dimension 2). The maxillary (*os maxillare*) was measured from the anterior edge to the posterior process, the premaxillary (*os praemaxillare*) from the ventral edge of the premaxillary symphysis to the posterior process, and the articular (*os articulare*) from the anterior process to the posterior tip. Measurement of the palatine (*os palatinum*) was taken from the anterior tip to the posterior tip, from the anterior process to the posterior tip for the vomer (*os vomere*), and from the anterior to the posterior tip for the parashenoid (*os parashenoideum*). The preopercular bone (*os praeoperculare*) was measured from the dorsal to ventral tip for brown trout and from the dorsal tip to the ventral spine for Carpathian sculpin, and the opercular bone (*os operculare*) from the dorsal anterior tip to the ventral anterior tip. Three

classes of bones were measured from the spinal column: the maximum width of the first vertebra (*atlas*), and the antero-posterior length of the vertebra body for both precaudal and caudal vertebrae (*vertebrae praecaudales* and *caudales*). All of the bones used, and the appropriate dimensions measured, are illustrated in Figs 1 to 3.

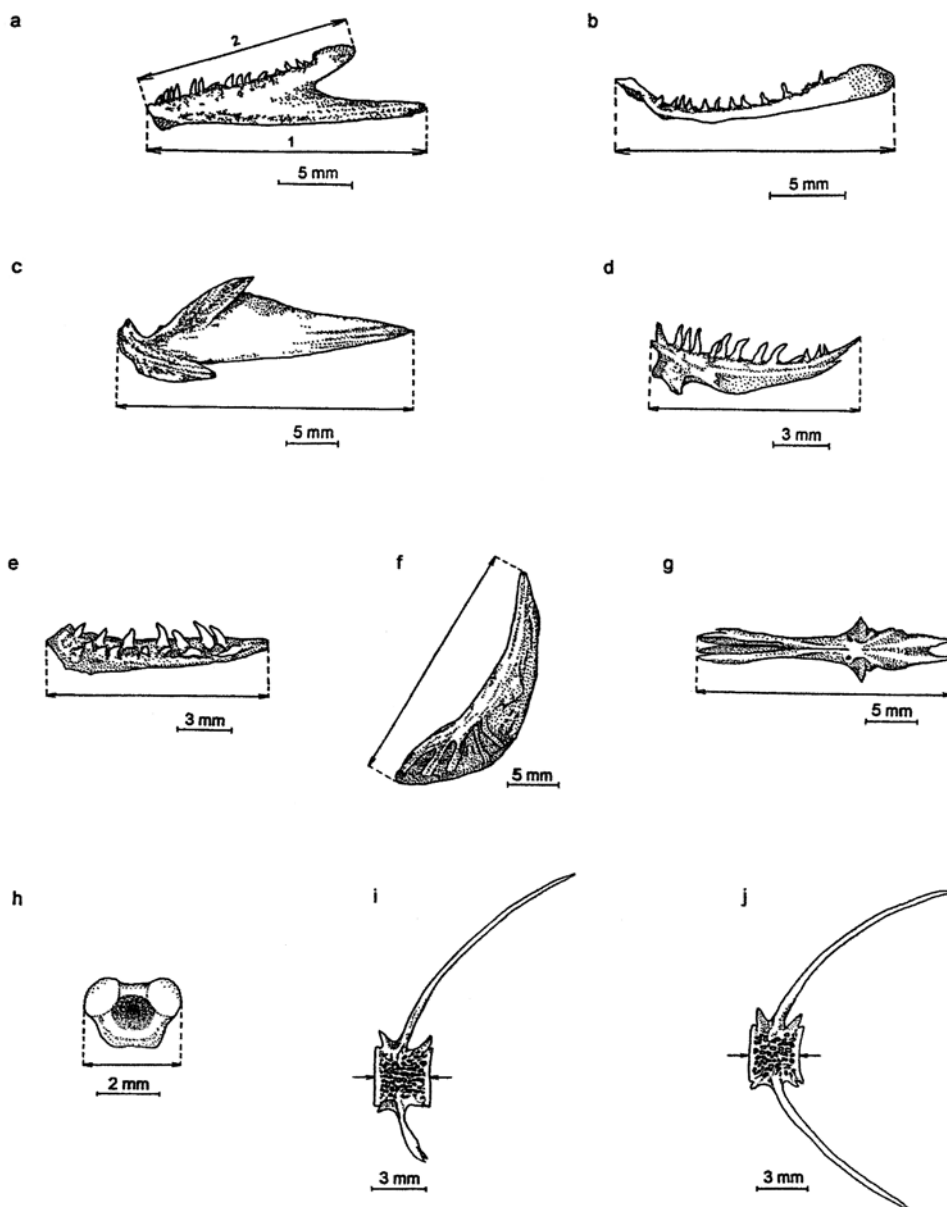


Fig. 1. Dimensions measured for bones of brown trout *Salmo trutta* m. *fario*: a – dentary (dimensions 1 and 2), b – maxillary, c – articular, d – palatine, e – vomer, f – preopercular bone, g – parashenoid, h – atlas, i – precaudal vertebra, j – caudal vertebra.

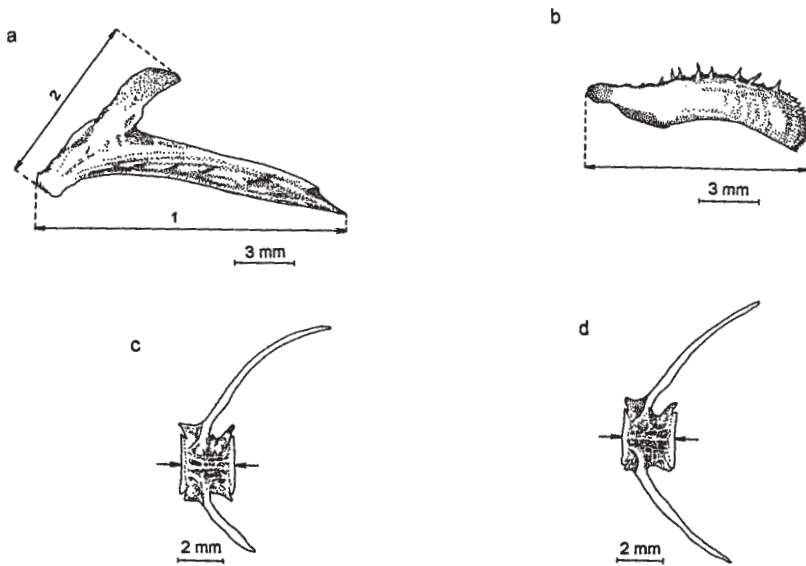


Fig. 2. Dimensions measured for bones of grayling *Thymallus thymallus*: a – dentary (dimensions 1 and 2), b – maxillary, c – precaudal vertebra, d – caudal vertebra.

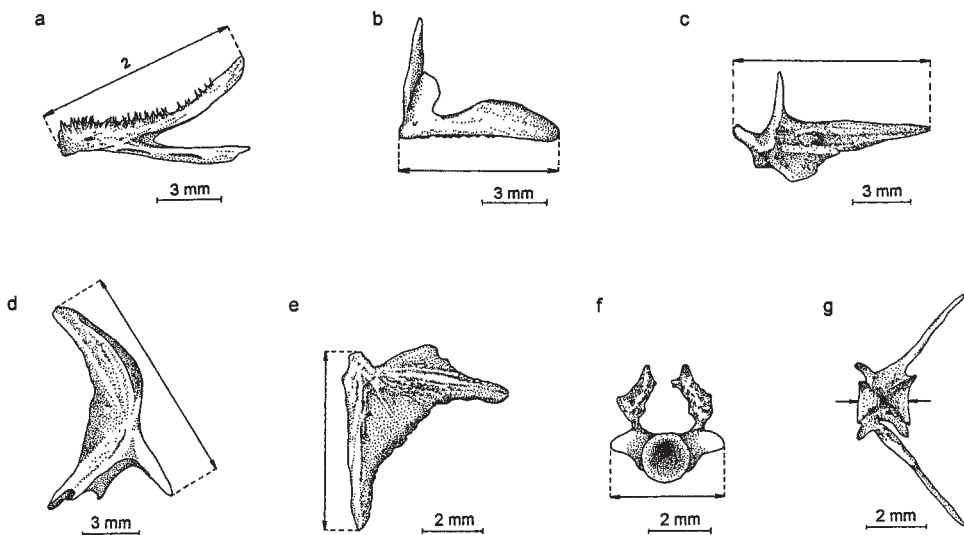


Fig. 3. Dimensions measured for bones of Carpathian sculpin *Cottus poecilopus*: a – dentary (dimension 2), b – premaxillary, c – articular, d – preopercular bone, e – opercular bone, f – atlas, g – caudal vertebra.

Both right and left bones of paired structures (e.g. dentaries, maxillaries, etc.) were measured separately and tested for significant differences using the t-test for paired comparisons. A relatively high number of caudal and precaudal vertebrae from each individual were measured, and the mean used for the calculation of equations. For trout, 13–16 caudal vertebrae (preural vertebrae from 9 to 21–24) and 5–6 precaudal vertebrae

were measured; for grayling, 10–11 caudal vertebrae (preural vertebrae from 11 to 20–21) and 4–5 precaudal vertebrae; and for sculpin, 10–11 caudal vertebrae (preural vertebrae from 6–7 to 15–17). Coefficients of variation were calculated for both the caudal and precaudal vertebrae of each individual and the mean and range used.

Three types of regression equation were calculated: 1) between bone dimension and standard length of fish, 2) between standard length of fish and fish weight, and 3) between standard length and total length of fish. Simple linear, two types of transformed linear (exponential and multiplicative), and non-linear polynomial models were tested for all relationships, and only that with the highest coefficient of determination (r^2) was finally selected for use. The STATGRAPHICS (M a n u g i s t i c s 1993) computer programme was used throughout.

Results

Conspicuous differences were noted between species in the number of diagnostic bones that were apparently able to survive the digestion process intact. Trout bones appeared to be most resistant, with 10 diagnostic bone types regularly identified in the 320 spraints examined, whilst 7 diagnostic bone types were found for sculpin, and only 4 for grayling (Table 1). In the case of grayling, very few undamaged bones were found, and only vertebrae, dentaries and maxillaries were regularly usable. Indeed, only scales usually confirmed the presence of grayling.

Perhaps unsurprisingly, with the high number in each fish, vertebrae, and especially caudal vertebrae, occurred with the highest frequency in spraints. In general, for both trout and sculpin, paired bones tended to be found at higher frequencies than single bones, although within species, the atlas tended to be found at relatively high frequencies compared with some paired bones, especially that for trout (Table 1). Both the vomer and parashenoid were only rarely usable for trout, and equivalent bones were not usable at all for grayling or sculpin.

No significant differences (t-test for paired comparisons, all $P > 0.25$) were noted between any of the paired bone structures, therefore the mean of the right and left measurements were used for the calculation of equations.

A total of 23 regression equations were obtained for the relationships between diagnostic bones and standard length and, based upon highest coefficient of determination (r^2), the

Table 1. Frequency of occurrence (%) of undamaged diagnostic bones examined in a sample of 320 otter spraints from a contemporary diet study (H á j k o v á 2001, H á j k o v á & H á j e k 2002); — = bone either rarely found, lacking diagnostic value, or frequently damaged.

Bone / Species	Brown trout	Grayling	Carpathian sculpin
Dentary	24.9	9.7	31.9
Maxillary	34.1	9.7	—
Premaxillary	—	—	42.0
Articulary	21.0	—	34.8
Palatine	12.8	—	—
Vomer	5.3	—	—
Preopercular bone	14.4	—	49.3
Opercular bone	—	—	31.9
Parashenoid	1.6	—	—
Atlas	27.2	—	20.3
Caudal vertebrae	79.3	11.1	56.5
Precaudal vertebrae	45.3	5.6	—

Table 2. Polynomial regression relationships ($y = a + b.x + c.x^2$) between fish bone measurements (mm) and standard length of fish (mm). n = number of individuals analysed.

Brown trout – *Salmo trutta m. fario*

Bone	n	a	b	c	r ² (%)	F
Dentary 1	36	5.299	9.647	- 0.065	97.90	2907.5
Dentary 2	36	11.788	11.682	- 0.106	97.30	2258.0
Maxillary	36	4.785	10.309	- 0.077	98.18	3340.2
Articulary	35	- 3.391	11.605	- 0.096	98.29	3775.7
Preopercular bone	36	- 11.830	12.066	- 0.062	98.72	4776.5
Palatine	34	2.648	16.358	- 0.166	98.12	3530.6
Vomer	34	1.846	15.724	- 0.156	97.51	2673.8
Parashenoid	35	- 27.351	8.670	- 0.032	98.91	5947.8
Atlas	33	- 6.039	62.209	- 1.833	98.54	4466.2
Caudal vertebrae	35	5.013	77.778	0.104	99.61	16 783.5
Precaudal vertebrae	35	13.326	71.317	0.142	99.54	14 129.5

Grayling – *Thymallus thymallus*

Dentary 1	22	- 86.709	23.942	- 0.319	95.62	2238.0
Dentary 2	22	- 116.683	48.667	- 1.408	96.23	2602.3
Maxillary	22	- 94.795	31.567	- 0.526	97.68	4230.7
Caudal vertebrae	21	7.713	67.373	0.698	97.76	3970.0
Precaudal vertebrae	21	14.649	62.719	0.466	97.60	3711.4

Carpathian sculpin – *Cottus poecilopus*

Dentary 2	19	- 5.545	14.226	- 0.430	93.90	1149.2
Premaxillary	16	3.274	18.858	- 0.850	95.80	1253.9
Articulary	19	- 0.092	12.989	- 0.306	95.52	1566.9
Preopercular bone	20	- 5.945	11.226	- 0.167	97.37	2951.6
Opercular bone	19	6.224	15.692	- 0.408	97.23	2583.0
Atlas	16	23.045	9.471	2.451	95.69	1867.8
Caudal vertebrae	19	2.722	49.616	- 0.416	97.36	2644.2

polynomial regression model was deemed to give the best fit to the data (Table 2). The multiplicative model, however, was found to produce the highest r² values for the relationship between standard length and weight (Table 3) and, for the relationship between standard length and total length, linear regression gave the best fit for trout, and polynomial for both grayling and Carpathian sculpin (Table 4). All regressions were highly significant ($P < 0.001$) and all gave coefficients of determination between 93.9 and 99.8 % (see Tables 2–4).

For trout and grayling, the mean coefficient of variation for the length of vertebrae was lower for precaudal than for caudal vertebrae, however, in both cases, the range was higher. For sculpin, both the mean coefficient of variation and the range were relatively high (Table 5).

Table 3. Multiplicative regression relationship ($y = a.x^b$) between standard length of fish (mm) and fish weight (g). n = number of individuals analysed.

Species	n	a	b	r ² (%)	F
Brown trout	48	1.114 E ⁻⁵	3.081	99.55	10158.2
Grayling	27	8.372 E ⁻⁶	3.110	98.71	1909.6
Carpathian sculpin	20	4.352 E ⁻⁶	3.382	97.96	863.9

Table 4. Linear[#] and polynomial* regression relationships ([#] $y = a + b.x$; * $y = a + b.x + c.x^2$) between standard length (mm) and total length of fish (mm). n = number of individuals analysed.

Species	n	a	b	c	r ² (%)	F
# Brown trout	48	4.804 E ⁻³	1.152	—	99.78	20721.9
* Grayling	27	6.426	1.121	7.37 E ⁻⁵	99.43	26535.8
* Carpathian sculpin	20	-1.316	1.262	-7.96 E ⁻⁴	99.70	26863.9

Table 5. Coefficient of variation for vertebrae measured for regression analysis. CV = caudal vertebrae, PCV = precaudal vertebrae.

	mean (%)	minimum (%)	maximum (%)
Trout CV	4.988	2.715	8.980
Trout PCV	2.569	0.603	7.710
Grayling CV	3.293	1.853	5.625
Grayling PCV	1.860	0	5.497
Carpathian sculpin CV	3.888	0.806	8.151

Discussion

Bones for analysis were chosen under the assumption that a high frequency of occurrence of undamaged bones in spraints represented an increased resistance to digestion. It was also assumed that all fish were eaten whole as uneaten remains were never found in the field and the fish taken were all of small to medium size (Hájková 2001, Hájková & Hájek 2002) and, therefore, that there was an equal probability of finding usable diagnostic bones at the same frequency. It cannot be excluded, however, that some bones may not have been consumed, particularly from larger fish.

The results indicated that some bones were found more frequently than others, both between and within species (Table 1). Seven bone types were found for sculpin at a relatively similar frequency, suggesting that sculpin bones may be equally resistant to digestion; conversely, very few bone types were found for grayling, implying that they may be least able to survive the digestion process intact. Care should be taken with spraint analysis, therefore, if grayling are known to be present in the sample area. For trout, though many usable bone types were regularly found, their frequency of occurrence varied widely, implying that some bones may survive better than others. In general, vertebrae, atlas, and jaw bones occurred with the highest frequency for all species, however, the apparent recovery frequencies of the same bones differed markedly between species (Table 1). Bones chosen for regressions, or for estimating the minimum number of individuals in a collection, should, therefore, not be based upon diagnostic value alone but also on their frequency of occurrence in spraints, the most suitable bone(s) possibly differing between species (see also Feltham & Marquiss 1989).

Prenda & Granado-Lorencio (1992) have suggested that simultaneous use of several bones notably increases the probability of prey identification in predator faeces. This is equally true whether presence/absence or minimum number of individuals is being calculated; however, the use of multiple bones can raise the problem of overestimation of individuals in a spraint. Ideally, bones that occur only once in the body should be preferred, e.g. the atlas bone. The atlas has high diagnostic value in salmonids (though much less so for cyprinids), is

relatively resistant to digestion, and both Feltham & Marquiss (1990) and Carss & Elston (1996) have found that it gives the highest estimate of minimum numbers for salmonids. Our results showed that the atlas might also be a useful bone for assessing numbers of sculpin, but not for grayling due to low occurrence and a lack of distinguishable features.

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 and shape can be paired, and single occurrences may be treated as separate individuals, it can never be completely ruled out that the paired bones come from two individuals of the same size or that the missing bone of the pair will appear in another spraint. Due to both their high occurrence in spraints and their high diagnostic value, however, such bones are increasingly used in otter diet studies (e.g. Prenda & Granada-Lorenzo 1992, Kloskowski et al. 2000). No significant differences were found in size between the left and right bones of a pair, therefore, there appears to be no need to specify which side of the fish was used when back-calculating length from bone size (see also Copp & Kováč 2003).

Vertebrae have often been used in the past in diet analysis (e.g. Wise 1980, Kemenes & Nechay 1990, Sulikava 1996, etc.), partly due to their high frequency of occurrence in spraints (see also Table 1). However, there are a number of problems with their use, including low taxonomic value in fish such as cyprinids (Prenda & Granada-Lorenzo 1992, Conroy et al. 1993), and the large size range in vertebrae length in each individual, causing a possible overlap in vertebrae size of similar sized individuals (Prenda & Granada-Lorenzo 1992, Carss & Elston 1996). To produce regressions for caudal and precaudal vertebrae, only those vertebrae that could also be identified in spraints were used, based upon slope and length of neural and haemal spines. With precaudal vertebrae, one might expect size variation to be lower as there are fewer bones. Variation coefficients for this collection (Table 5), however, indicated that though the mean length variability for precaudal vertebrae was lower than that for caudal in trout and grayling, the ranges were higher, suggesting that caudal vertebrae may be better used for length estimation, at least for long-bodied fish.

Recent research has highlighted other important factors influencing the estimate of minimum number of individuals and prey-size distribution of fish, such as size-related recovery of fish bones in spraints (Carss & Elston 1996, Carss & Nelson 1998) and the influence of otter activity on digestion rate, and hence on bone resistance and recovery (Carss et al. 1998). However, as stated by the authors, replications of feeding trials with captive otters are needed to confirm the results of these studies (Carss et al. 1998). Until such research is integrated into present diet analysis techniques it is likely that back-calculation from bone length will remain the most appropriate method, as long as the drawbacks in its use are fully appreciated.

Though the Carpathian sculpin is a rare or missing species in the west of Europe, it is relatively common in some parts of north and central Europe and northern Asia (Baruš & Oliva 1995, Matland 2000). Except for the preopercular bone, the skeletal morphology of the Carpathian sculpin and the better-known common sculpin (*Cottus gobio*) is very similar. In the absence of suitable calculations for the latter species (for the preopercular bone see Libois et al. 1987), we suggest that our regressions may be used for both, at least for approximate size estimations.

All regressions between bone size and standard length of fish had high r^2 values thus, theoretically, all are suitable for use. However, some regressions, despite high r^2 values,

underestimated the SL of fish, i.e. those for the dentary (dimension 1), palatine, preopercular and parashenoid bones of brown trout (Figs 1 a, d, f, g), and the premaxillary in Carpathian sculpin (Fig. 3 b). All of these bones were prone to damage at the extremities and shortening of the bone by several millimetres could reduce the length estimate by several centimetres. Based upon frequency of occurrence in spraint, resistance to breakage and diagnostic value, we propose that the dentaries (dimension 2), maxillae, articularies and first vertebrae (atlas) should be used in preference for length estimation of trout; and dentaries, articularies, preopercular bones and first vertebrae for sculpin. Other bones, including vertebrae, should only be used when these are not available. For grayling, only the dentary, maxillary and caudal vertebrae were regularly found in spraints, and it is suggested that careful investigation of scales may be required to confirm their presence.

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