

THE USE OF OTOLITHS AND VERTEBRAE IN THE IDENTIFICATION AND SIZE-ESTIMATION OF FISH IN PREDATOR-PREY STUDIES

by

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ABSTRACT. Relationships between the size of otoliths and vertebrae, and body length are presented for eleven teleost species of the north-east Atlantic. Relationships between teleost size and weight are also presented for these species. These equations can be used to reconstruct the original dimensions of prey from the size of hard structures found in food samples of marine piscivores. For each fish species we selected otoliths and vertebrae which possessed unique features enabling diagnosis of specimens to the level of genus or species. Linear and non-linear functions provided the best fit for relations between hard structure size and fish length, and exponential functions yielded the best predictors for fish weight. For some species, the relationships obtained in this study differed from similar functions obtained by other authors at different locations. This calls for caution when using equations derived from specimens on other locations, especially if precise quantitative data are required.

RÉSUMÉ. L'utilisation d'otolithes et de vertèbres pour l'identification et l'évaluation de la taille des poissons dans les études prédateur-proie.

Les relations entre la taille des otolithes et des vertèbres, et la longueur totale, d'une part, et les relations entre la taille et le poids, d'autre part, sont présentées pour onze espèces de téléostéens de l'Atlantique nord-est. Ces équations peuvent être utilisées pour évaluer la taille des proies à partir de la taille des structures dures trouvées dans les échantillons de nourriture des piscivores marins. Pour chaque espèce de poisson on a choisi des structures dures possédant des caractères uniques qui permettent l'identification de l'exemplaire jusqu'au niveau du genre ou de l'espèce. Des fonctions linéaires et non-linéaires ont fourni le meilleur ajustement pour les relations entre la taille des structures dures et la longueur totale des poissons. Les fonctions exponentielles ont permis d'obtenir de meilleures estimations du poids des poissons. Pour certaines espèces, les relations obtenues dans cette étude sont différentes des fonctions similaires obtenues par d'autres auteurs dans différents endroits, ce qui pose un problème de précaution lorsque l'on utilise des équations obtenues à partir d'exemplaires recueillis à des endroits différents, en particulier si on désire obtenir des données quantitatives précises.

Keywords. Teleostei - ANE - Diet - Otoliths - Vertebrae - Identification - Regression analysis.

Many dietary studies of piscivorous animals require the analysis of hard remains found in food samples, in stomachs or in faeces (e.g., otoliths, scales, bones), for the identification of prey species (Recchia and Read, 1989; Prime and Hammond, 1990; Pierce and Boyle, 1991). Hyslop (1980), Duffy and Jackson (1986) and Pierce and Boyle

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(1991) extensively reviewed the methods used in the analysis of the diet of predatory fishes, seabirds and marine mammals, respectively. These authors provided a comprehensive evaluation of the bias and limitations associated with each method. They acknowledged that otoliths are among the most widely used structures for identification of fish prey (Pierce and Boyle, 1991). Indeed, fish otoliths exhibit a high interspecific variability, which encouraged the production of keys and identification guides for a variety of species (e.g., Nolf, 1985; Härkönen, 1986; Smale *et al.*, 1995).

Otoliths can also be used to estimate the size of the prey. Fish size and/or weight can be functionally related to an appropriate otolith measurement (length, width, thickness or weight) and the resulting relationships can subsequently be used for size estimation (e.g., Nolf, 1985; Pierce and Boyle, 1991; Brown and Pierce, 1997; Tollit *et al.*, 1997).

Despite being the densest structures in teleost fish (Treacy and Crawford, 1981), otoliths can be damaged during the digestive process, both by mechanical (e.g., chewing) and chemical actions on calcium carbonate (Pierce and Boyle, 1991; Pierce *et al.*, 1993), which limits their use in prey identification and size estimation. Alternative hard pieces have increasingly been used as a complement to validate the information provided by the otolith analysis. Fish vertebrae are numerous and exhibit a strong interspecific variation in morphological traits (Desse and Du Buit, 1970, 1971; Casteel, 1976; Desse *et al.*, 1989; Rojo, 1991). Among others, these traits include size, shape and orientation of dorsal and ventral zygapophysis, size and shape of neural and haemal spines, waisting and sculpturing of the centrum and location and size of foramina (Desse and Du Buit, 1970, 1971; Casteel, 1976; Watson, 1978; Pierce and Boyle, 1991; Pierce *et al.*, 1991a; Watt *et al.*, 1997). In addition, vertebrae being mineralised (calcium phosphate), they are expected to be less easily digestible than otoliths, and thus will remain longer in the stomach of piscivores (Pierce *et al.*, 1993). Hence, vertebrae represent a valuable instrument to aid fish identification, to produce more accurate estimates of the minimum number of prey and also to back-calculate their size (Desse *et al.*, 1989; Feltham and Marquiss, 1989; Feltham, 1990; Desse and Desse-Berset, 1996a, 1996b).

Although several studies have reported on relationships between the size of otoliths and vertebrae and body dimensions (e.g., Casteel, 1976; Wise, 1980; Härkönen, 1986; Desse *et al.*, 1989; Feltham and Marquiss, 1989; Rechia and Read, 1989; Gamboa, 1991; Plötz *et al.*, 1991; Hammond *et al.*, 1994a, 1994b; Desse and Desse-Berset, 1996a, 1996b; Brown and Pierce, 1997; Tollit *et al.*, 1997; Watt *et al.*, 1997), such information is still lacking for many fish species.

In this paper, we present relationships between otolith size and fish dimensions for some common species occurring in the North-eastern Atlantic Ocean. We also describe diagnosing traits of the vertebrae of some fish prey species, which can aid prey identification in studies of diet. Finally, we provide relationships between vertebrae size and fish dimension, which enable back-calculation of their size.

MATERIAL AND METHODS

We selected eleven fish species, which regularly occur in the diet of seabirds and marine mammals in the subtropical north-east Atlantic Ocean (Granadeiro *et al.*, 1998; Silva, 1999). The selected species and corresponding sample sizes were: sardine *Sardina pilchardus* (Walbaum, 1792), n=36, snipe fish *Macroramphosus* spp. (L., 1758),

n=240, (L., 1758), n=34, blue whiting *Micromesistius poutassou* (Risso, 1826), n=40, pouting *Trisopterus luscus* (L., 1758), n=30, boar fish *Capros aper* (L., 1758), n=30, horse mackerel *Trachurus trachurus* (L., 1758), n=39, Mediterranean horse mackerel *T. mediterraneus* (Steindachner, 1869), n=11, blue jack mackerel *T. picturatus* (Bowdich, 1825), n=10, chub mackerel *Scomber japonicus* (Houttuyn, 1782), n=2 and Atlantic mackerel *Scombrus* (L., 1758), n=8. There is still some debate about the taxonomic separation of *Macroramphosus scolopax* and *M. gracilis* (e.g., Ehrich, 1975; Brêthes, 1979) so in this study this group will be referred to as *Macroramphosus* species.

This study focuses on some non-target species for fisheries. Hence, most fish used in this study were obtained in pelagic trawling operations, carried out off the Portuguese continental shelf by the R/V “Noruega” of Instituto de Investigação das Pescas e do Mar. This situation limited the size of some of our samples, and made it impossible to work with fully balanced samples. We tried to compensate this situation by supplementing the samples with specimens available at the local market, and caught off the Portuguese coast. Most fish were frozen immediately after collection and some specimens (<5%) were analysed fresh.

We measured total length (to the nearest millimetre) for all species, except *Capros aper* and *Macroramphosus* spp., for which we measured standard length, because the specimens frequently had their caudal fins damaged. All specimens were weighed to the nearest 0.1g (*Capros aper* and *Macroramphosus* spp.) or 0.01g (all other species), and subsequently cooked in a microwave oven. The microwave oven was preferred to boiling because it does not cause distortion of the bone shape (Pierce *et al.*, 1993). The otoliths were extracted and the flesh was carefully removed from the vertebrae with forceps and a soft brush. *Sardina pilchardus*, *Capros aper*, *Macroramphosus* spp. and *Scomber* spp. have small and fragile otoliths, which are frequently broken or strongly eroded in food samples. Therefore, we did not calculate otolith-size/body-size relationships for these species. Furthermore, we carefully examined all vertebrae of each species to select those that could be individually identified among all others, even when the fish skeleton was entirely disarticulated. We just used these vertebrae to develop functions to enable back-calculating fish size. Hence, we were able to calculate relationships between vertebra size and fish dimensions for *S. pilchardus*, *C. aper*, *Macroramphosus* spp., *Trachurus* spp. and scombrids. Otoliths (maximum length) and diagnostic vertebrae (centrum length or centrum width) were measured under a binocular microscope fitted with an eye-piece graticule, to the nearest 0.02mm, and preserved dry.

Table 1. Relationships between otolith length (OL) and fish total length (TL) (both in mm). Coefficient of determination (r²) and sample sizes (n) are indicated. The regression equations presented are those with the highest r². All regressions significant at p<0.0001.

Species	Regression	r ²	n	Range of TL (mm)
<i>Micromesistius poutassou</i>	TL = 14.49 * OL ^{1.13}	0.95	240	120-249
<i>Trisopterus luscus</i>	TL = 33.73 * OL - 94.54	0.94	50	102-340
<i>Merluccius merluccius</i>	TL = 19.06 * OL ^{1.05}	0.99	54	109-512
<i>Trachurus trachurus</i>	TL = 34.01 * OL - 28.99	0.95	39	114-353
<i>Trachurus picturatus</i>	TL = 40.09 * OL - 37.15	0.97	10	120-353
<i>Trachurus mediterraneus</i>	TL = 43.05 * OL - 71.28	0.98	11	120-335
<i>Trachurus species combined</i>	TL = 35.49 * OL - 30.99	0.92	60	114-353

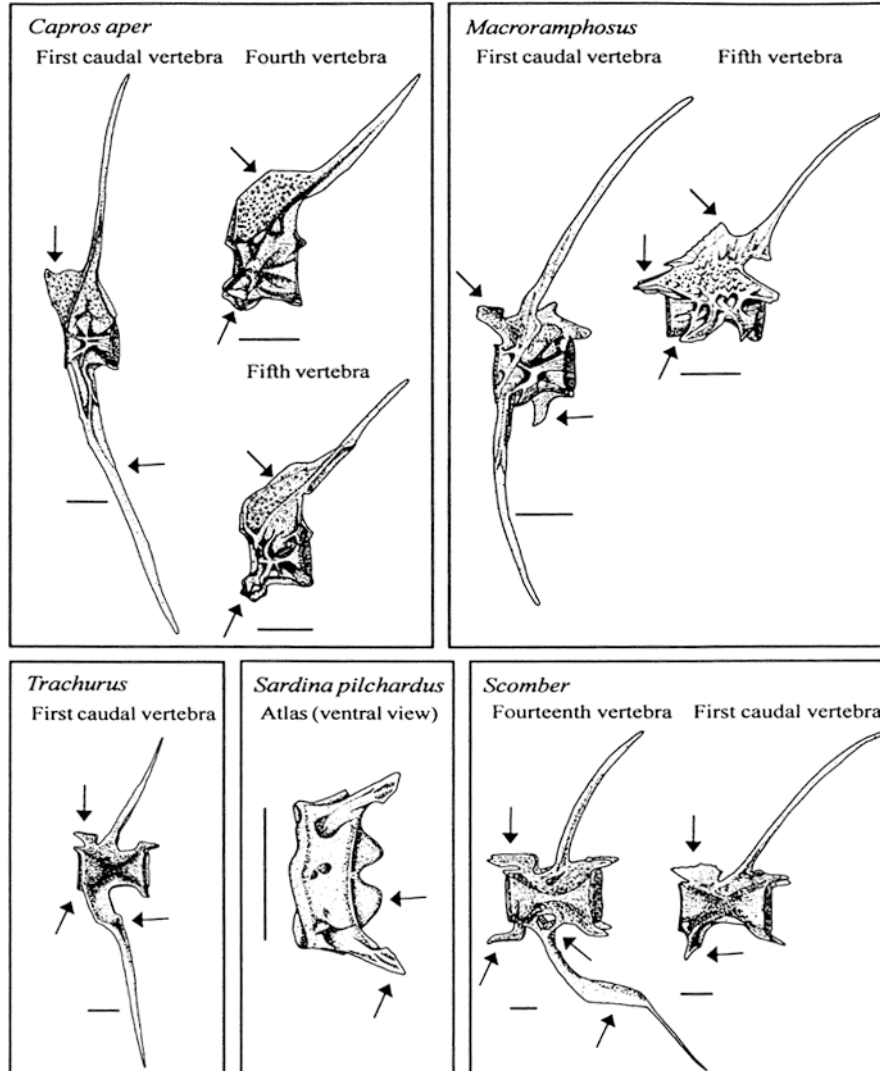


Fig. 1. Lateral views of vertebrae of *Capros aper*, *Macroramphosus* spp., *Trachurus* species and *Scomber* species and ventral view of first vertebrae of *Sardina pilchardus*. Main diagnostic features (arrows) and the location of each vertebra within the vertebral column are indicated. Scale bars = 1 mm.

We tested both linear and non-linear functions to describe the relationship between fish size and otolith/vertebra dimensions (length and width), using the former as the dependent variable. We applied the same procedure to fish-size/fish-weight relationships in all species. In each case, we adopted those relationships which explained the highest proportion of the variance (highest coefficient of determination, r^2). Statistical procedures followed Zar (1996).

RESULTS

Otolith size/body size relationships

There was no significant difference in fish size/fish weight relationship obtained for frozen and fresh specimens of similar size range *Trisopterus luscus* (Analysis of Covariance, ANCOVA: for slope, $F_{1,46} = 0.01$, ns; for intercept, $F_{1,47} = 0.03$, ns). Based on these findings, and given that all samples were kept frozen for approximately similar (and

Table 1. Some diagnostic features of the vertebrae used to calculate size relationships

Species	Vertebrae	Diagnostic characters
<i>Capros aper</i>	4th vertebra	Prominent ventral prezygapophysis, pointing forward Broad dorsal prezygapophysis, forming a lamellar plate Centrum with one horizontal rib, sometimes with supporting septa Strong neural spine, bend posteriorly
	5th vertebra	Prominent ventral prezygapophysis, pointing downwards Broad dorsal prezygapophyses, forming a lamellar plate Centrum with one horizontal rib, sometimes with supporting septa Strong neural spine, bend posteriorly
	1st caudal	Well developed dorsal prezygapophysis, projecting in front of the centrum Long haemal and neural spine Centrum with one horizontal rib, sometimes with supporting septa Lamellar bone plate between the dorsal margin of centrum and the neural spine
<i>Macroramphosus</i> spp.	5th vertebrae	Dorsal region strongly sculptured, forming a crest in the upper portion Well developed dorsal pre- and postzygapophysis Well developed and prominent lateral apophysis, slightly pointing forward Fragile haemal spine, in posterior position
	1st caudal	Strongly sculptured centrum, with a horizontal rib and some supporting septa Flattened and well developed dorsal prezygapophysis, point upwards Prominent lateral apophysis, slightly pointing forward
<i>Trachurus</i> spp.	1st caudal	Prominent right-angled dorsal prezygapophysis Anterior border of haemal arch curves steeply backwards to form a strong haemal spine, creating a distinctive angle Ventral prezygapophysis absent
<i>Sardina pilchardus</i>	1st	Strongly antero-posterior compression Well developed and prominent parapophysis Two dorsal bone plates, projecting above the 2 nd vertebrae
<i>Scomber</i> spp.	1st caudal	Short haemal spine Prominent crest-shaped dorsal prezygapophysis Well developed postzygapophyses
	14th vertebrae	Very prominent and right-angled prezygapophysis Strongly angled haemal spine, with a laterally flattened region Supporting strut behind the neural spine, delimiting a large aperture

relatively short) periods, we assumed that freezing did not affect these relationships in the other study species.

Table 1 presents the relationships between otolith length and total length for *Micromesistius poutassou*, *Trisopterus luscus*, *Merluccius merluccius*, and *Trachurus* species. All calculated regressions were highly significant (all $r^2 \geq 0.92$, $p \leq 0.0001$). Non-linear functions provided the best fit for *Micromesistius poutassou* and *Merluccius merluccius*, whilst relationships for *Trisopterus luscus* and *Trachurus* species were best described by linear functions (Table 1).

There were significant differences among the three *Trachurus* species in the slope regression lines relating the otolith length to the total fish length (ANCOVA: for slope, $F_{2,54} = 11.11$, $p \leq 0.01$). However, in many cases it proves impossible to identify the otoliths to the species level. For these circumstances (and despite the differences in slope mentioned above), we calculated a relationship using data pooled from these three species, which was also highly significant (Table 1).

Distinction among the three *Trachurus* species on the basis of otolith shape is very difficult. Therefore, in addition to individual regressions, we calculated a relationship using data pooled from the three species. This regression was also highly significant (Table 1), despite the existence of significant differences among species in the corresponding regression lines (ANCOVA: for slope, $F_{2,54} = 11.11$; $p \leq 0.01$).

Vertebrae size/body size relationships

Figure 1 presents the vertebrae which were used to compute relationships and the

Table 2. Relationships between dimension of vertebrae and fish length (both in mm). All equations used vertebra length as the independent variable, except for *Sardina pilchardus* where vertebra width was chosen. Coefficient of determination (r^2) and sample sizes (n) are indicated. The regression equations presented are those with the highest r^2 . TL: total length; SL: standard length; VW: vertebra width; VL: vertebra length. All regressions significant at $p \leq 0.0001$.

Species	Vertebrae	Regression	r^2	n	Range of fish (mm)
<i>Sardina pilchardus</i>	1st	TL = 50.06 * VW + 23.31	0.93	86	79-219
<i>Capros aper</i>	4th	SL = 34.36 * VL + 10.48	0.91	30	65-94
	5th	SL = 32.64 * VL + 9.37	0.90	30	65-94
	1st caudal	SL = 33.06 * VL + 11.97	0.92	30	65-94
<i>Macroramphosus</i> spp.	5th	SL = 31.41 * VL + 15.92	0.97	40	48-146
	1st caudal	SL = 55.16 * VL - 2.37	0.97	40	48-146
<i>Trachurus trachurus</i>	1st caudal	TL = 36.58 * VL + 6.17	0.93	32	114-268
<i>Trachurus picturatus</i>	1st caudal	TL = 32.23 * VL + 19.37	0.96	10	129-270
<i>Trachurus mediterraneus</i>	1st caudal	TL = 33.60 * VL + 13.70	0.99	11	120-335
<i>Trachurus</i> species combined	1st caudal	TL = 33.21 * VL + 18.91	0.97	53	114-335
<i>Scomber scombrus</i>	2nd with closed haemal arch	TL = 34.79 * VL + 36.51	0.97	21	178-365
	1st caudal	TL = 34.49 * VL + 38.53	0.97	21	178-365
<i>Scomber japonicus</i>	2nd with closed haemal arch	TL = 34.34 * VL + 42.00	0.97	32	201-385
	1st caudal	TL = 33.22 * VL + 47.79	0.97	32	201-385
<i>Scomber</i> spp. combined	2nd with closed haemal arch	TL = 34.24 * VL + 41.55	0.97	53	178-385
	1st caudal	TL = 33.70 * VL + 44.47	0.98	53	178-385

relative position in the vertebral column of the fish. Some of the diagnosing characters of these vertebrae are listed in table III, and are also indicated in figure 4. Vertebrae length provided the best predictor of fish size for all species except *Sardina pilchardus*, for which we selected vertebra width. All regressions were highly significant (Table III).

The vertebrae of *Trachurus* did not allow identification to the level of species, so we pooled the data from these species. The regression obtained from the pooled data was highly significant ($p < 0.001$), despite the existence of significant differences among species in the intercept (but not slope) of the regression lines (ANCOVA: for elevation, $F_{2,49} = 2.30$, $p < 0.05$; for slope, $F_{2,47} = 0.57$, ns).

No such interspecific differences were found in the genus *Scomber* (ANCOVA for second vertebra with closed haemal arch: for elevation, $F_{1,50} = 0.15$, ns; for slope $F_{1,49} = 0.07$, ns; ANCOVA for first caudal vertebra: for elevation, $F_{1,50} = 0.46$, ns; for slope, $F_{1,49} = 0.66$, ns). The regression line for data from both species was highly significant (Table III).

Fish size/weight relationships

Table IV presents the relationships between fish (standard or total) length and fish weight for the species studied. All regressions were highly significant ($p < 0.001$).

DISCUSSION

All equations relating otolith length with fish size explained a very large proportion of the variance in the data and are thus appropriate to estimate the size of prey fish. Linear functions are usually adequate to describe these relationships (e.g., Casteel, 1976; Härkönen, 1986; Hammond *et al.*, 1994a, 1994b; Brown and Pierce, 1997; Tollit *et al.*,

Table IV. Relationships between fish length (in mm) and fish fresh weight (W, in g). All equations used fish total length (TL) as the independent variable, except for *Capros aper* and *Macroramphosus* spp. where standard length (SL) was used instead. Coefficient of determination (r^2) and sample sizes (n) are indicated. The regression equations presented are those with the highest r^2 . All regressions significant at $p < 0.0001$.

Species	Regression	r^2	n	Range of weight (g)
<i>Sardina pilchardus</i>	$W = 6.92 \cdot 10^{-6} \cdot TL^{3.03}$	0.92	81	17.0-89.3
<i>Capros aper</i>	$W = 7.64 \cdot 10^{-5} \cdot SL^{2.83}$	0.95	30	10.3-27.8
<i>Macroramphosus</i> spp.	$W = 2.98 \cdot 10^{-5} \cdot SL^{2.67}$	0.98	40	0.9-17.9
<i>Micromesistius poutassou</i>	$W = 3.40 \cdot 10^{-6} \cdot TL^{3.09}$	0.99	240	9.6-80.6
<i>Trisopterus luscus</i>	$W = 4.33 \cdot 10^{-6} \cdot TL^{3.19}$	0.99	50	10.5-532.4
<i>Merluccius merluccius</i>	$W = 2.30 \cdot 10^{-6} \cdot TL^{3.18}$	0.99	54	7.3-921.4
<i>Trachurus trachurus</i>	$W = 1.77 \cdot 10^{-5} \cdot TL^{2.84}$	0.99	39	11.5-333.2
<i>Trachurus picturatus</i>	$W = 1.53 \cdot 10^{-5} \cdot TL^{2.87}$	0.99	10	17.7-146.5
<i>Trachurus mediterraneus</i>	$W = 8.18 \cdot 10^{-7} \cdot TL^{3.44}$	0.99	11	14.7-431.7
<i>Trachurus</i> species combined	$W = 6.73 \cdot 10^{-6} \cdot TL^{3.03}$	0.98	60	11.5-431.7
<i>Scomber scombrus</i>	$W = 1.12 \cdot 10^{-7} \cdot TL^{3.75}$	0.97	28	33.4-421.1
<i>Scomber japonicus</i>	$W = 4.73 \cdot 10^{-8} \cdot TL^{3.90}$	0.93	32	44.1-520.4
<i>Scomber</i> species combined	$W = 8.00 \cdot 10^{-8} \cdot TL^{3.81}$	0.95	60	33.4-520.4

1997), but in this study curvilinear functions provided the best fit for *Micromesistius poutassou* and *Merluccius merluccius*. Härkönen (1986) presented linear functions to describe the relation between otolith size and fish size in these two species. Within the common range of otoliths size, the equations presented in this study for *Micromesistius poutassou* and *Merluccius merluccius* yielded length estimates which were 1%-14% lower than those obtained using Härkönen's (1986) equations. Indeed, the equations derived by Härkönen (1986) for the two species showed a positive difference in intercept, but apparently not in slope, but a statistical analysis of these differences was not possible. These allometric differences indicate a geographical variation in the relationships between otolith size and fish size in populations of widely distributed fish species, as suggested by Härkönen (1986). This calls for caution to ensure the use of appropriate local relationships when estimating fish size from otolith measurements.

Otoliths are currently recognised as one of the most useful tools to identify fish remains in food samples of piscivores. However, before being available for identification, otoliths are exposed to a variable degree of chemical and mechanical abrasion in the digestive track of predators. Hence, small otoliths are likely to be totally dissolved and thus some species may fail to be detected. On the other hand, partial digestion will bias estimates of prey size (da Silva and Neilson, 1985; Jobling and Breiby, 1986; Pierce and Boyle, 1991; Pierce *et al.*, 1993) and can sometimes prevent identification. Feeding experiments with captive animals (mainly pinnipeds), have been used to derive correction factors to minimise errors due to otolith digestion (e.g., Prime and Hammond, 1990; Hammond *et al.*, 1994a, 1994b; Tollit *et al.*, 1997). These procedures do increase the accuracy of fish size estimates, but they require complex calibration work to control for differences in predator species and condition, and size and type of prey (e.g., Tollit *et al.*, 1997 and references therein), which is unfeasible for many fish predators. In addition to acid digestion, otoliths can become physically damaged during the process of ingestion by predators or during sample collection and processing, in which case reconstructing fish size and even identifying the prey can become impossible.

These facts have led to an increasing number of studies using alternative hard pieces as a means to provide an adequate description and quantification of the diets of piscivores. Fish vertebrae possess diagnosing characters allowing identification to the level of species or genus, and this study emphasises their potential use for identification in dietary studies of piscivorous animals. The vertebrae used in this study showed no major variation in form and shape with fish size, which made identification reliable and relatively simple, as reported by Hansel *et al.* (1988). There are a few guides for the identification of fish bones (Newsome, 1977; Watson, 1978; Borodulina, 1984; Hansel *et al.*, 1988; Watt *et al.*, 1997), but construction of a reference collection is strongly recommended to assess inter- and intraspecific variability in bone shape. After some familiarisation, it is possible to identify fragments of vertebrae, as long as some details of their structure are not destroyed.

Our results showed that vertebrae of the study species can be used to accurately estimate the size of prey, enabling cross-validation of estimates obtained from otolith measurements. Indeed, all relationships between fish size and vertebrae dimensions explained a large proportion of the variance in the data, even when information from related species were pooled (*Trachurus* species and scombrids). Furthermore, *in vitro* (Pierce *et al.*, 1993) and *in vivo* (Pierce *et al.*, 1993; Carss and Elston, 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. If positive identi-

fication of the vertebrae used to derive the regressions is not possible, the closest vertebrae should be used, since there seems to be little variation in the centrum size between adjacent vertebrae (Watt *et al.*, 1997).

To our knowledge there are no published relationships between vertebra size and fish length for the species presented in this study, so we can not assess whether a geographic variation similar to that recorded in otoliths exists. However, the relationships between fish length and vertebra length are probably much more constant than those involving otoliths (at least if different populations fail to exhibit geographical variation in the number of vertebrae), as they represent a "structural" component of fish total length (see Ford, 1937).

Fish weight can be estimated directly by regression on a given measurement (length or width), or through a two-step procedure, first using a relationship between the structure size and fish size and then applying a fish size/fish weight equation (Casteel, 1976). The use of two regressions instead of a single one may introduce additional errors (Casteel, 1976; Pierce and Boyle, 1991). However, there is generally considerable seasonal and geographical variation in fish size/fish weight relationships (e.g., Coull *et al.*, 1989; Pierce *et al.*, 1991b). Therefore, the use of two functions is recommended, when one intends to estimate fish weight based on regressions derived for other areas.

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