

## **The use of first vertebrae in separating, and estimating the size of, trout (*Salmo trutta*) and salmon (*Salmo salar*) in bone remains**

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(With 3 plates and 3 figures in the text)

A method is described in which a single bone, the first vertebra, is used to distinguish bone remains of juvenile salmon (*Salmo salar*) and trout (*Salmo trutta*) with 90% certainty. A single regression of salmon and trout first vertebra-width versus fish-length of fish predicted the latter with an accuracy of  $\pm 10$  mm (95% confidence limits) for salmonids of 45–150 mm fish-length. First vertebrae were assigned to salmon or trout based on three visual characters with 89%–90% certainty. Salmon are more variable than trout for the three characters. Of first vertebrae, 6.5% were not readily identifiable as either salmon or trout, and a further 3.0% were misclassified using our criteria.

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### **Introduction**

It is often difficult to assess directly the diet of piscivores by field observations. Studies of diet have been forced to rely upon the examination of undigested remains in stomach contents or faeces (e.g. mergansers, Munro & Clemens, 1937; penguins, Offredo & Ridoux, 1986; pike, Mann & Beaumont, 1980; trout, Trippel & Beamish, 1987; seals, Prime & Hammond, 1985; otters, Wise, 1980). To get the most useful sample of prey items from such material requires the use of remains that are resistant to digestion, and diagnostic of prey species. Pharyngeal arches have been particularly useful in identifying specific cyprinid fishes as they are robust bones, and bear teeth of characteristic shape and number for each species (e.g. Wheeler, 1978), and their size can be used to estimate the lengths of fishes consumed (Raven, 1986). Vertebrae can be used in a similar way, to distinguish the remains of some fishes (Watson, 1978; Wise, 1980), including (using X-ray

photographs of individual vertebrae) adult salmonids (Desse & Desse, 1976; Meunier & Desse, 1978).

Commercially valued juvenile salmon are predated by red-breasted mergansers (*Mergus serrator*) and goosanders (*M. merganser*) in Scotland, and such predation might reduce the numbers of adult salmon available for harvest (Shearer *et al.*, 1987). We could, however, find no published method of distinguishing bones of juvenile salmon (*Salmo salar*) from those of juvenile trout (*S. trutta*). Examination of red-breasted merganser gut contents showed that the first vertebra produced the highest minimal numbers estimate of salmonids consumed, due to its prolonged resistance to digestion (Feltham, pers. obs.). In this paper, we described a method of identifying juvenile salmon and trout from first vertebrae and estimating the original sizes of these fish.

## Materials and methods

### *Preparation of skeletal material*

Juvenile salmon and trout from 45–150 mm fish-length were obtained from several Scottish rivers. Fish-lengths were measured to the nearest 1.0 mm from tip of snout to fork of tail, and each fish was incubated in a saturated solution of a biological washing powder. Each was then rinsed in a 250- $\mu$ m sieve to remove excess detergent and filtered. Bones were dried at room temperature and first vertebrae extracted from the disarticulated skeletons under a binocular microscope.

### *Estimating the size of salmonids*

The maximum width of the first vertebrae (Fig. 1a and b) was measured to the nearest 0.02 mm from process edge to process edge on the anterior surface (Fig. 1a) using a microscope with an eye-piece graticule. The relationship between first vertebra width and fish-length was calculated from measurements of 76 trout, 88 wild salmon and 36 hatchery salmon.

### *Distinguishing juvenile salmon and trout*

A number of characters were considered as possible for distinguishing first vertebrae of salmon and trout, for example, surface pores, ratios of bone measurements, and overall bone shape. Three features were finally identified as being of potentially most use, based on a small sample of first vertebrae from fish of equal fish-length.

(a) In the trout the first vertebra appears 'tall' relative to vertebral width and is notably 'waisted' (Plate I a). The dorsal part (above pecked line; Plate I a) has typically parallel sides; in many small fish (< 60 mm) even sloping outwards ventro-dorsally. The 'waist' may be sharply angled outwards or in larger fish sharply curved outwards.

Salmon vertebrae appear 'short' relative to vertebral width (Plate I b) and have no obvious 'waist'. The dorsal half of the ventral view slopes outwards dorso-ventrally. Though some specimens appear quite 'tall' (cf. trout, e.g. Plate I c), or 'waisted' (Plate I d), the general squat appearance and sloping outwards dorso-ventrally is still apparent. Salmon vertebrae are more variable than trout.

(b) Trout vertebrae have typically round or broadly ovate pores (Plate II a) extending over the complete area. Pores are often large and sometimes few in number.

Salmon pores are typically slit-like (Plate II b) or narrowly ovate (Plate II d) and are often arranged in columns (Plate II b). This applies particularly to the pores in the dorsal region of the 'bridge' which shows the above characteristics even though the pores in the ventral region may appear round. The presence of slit-like

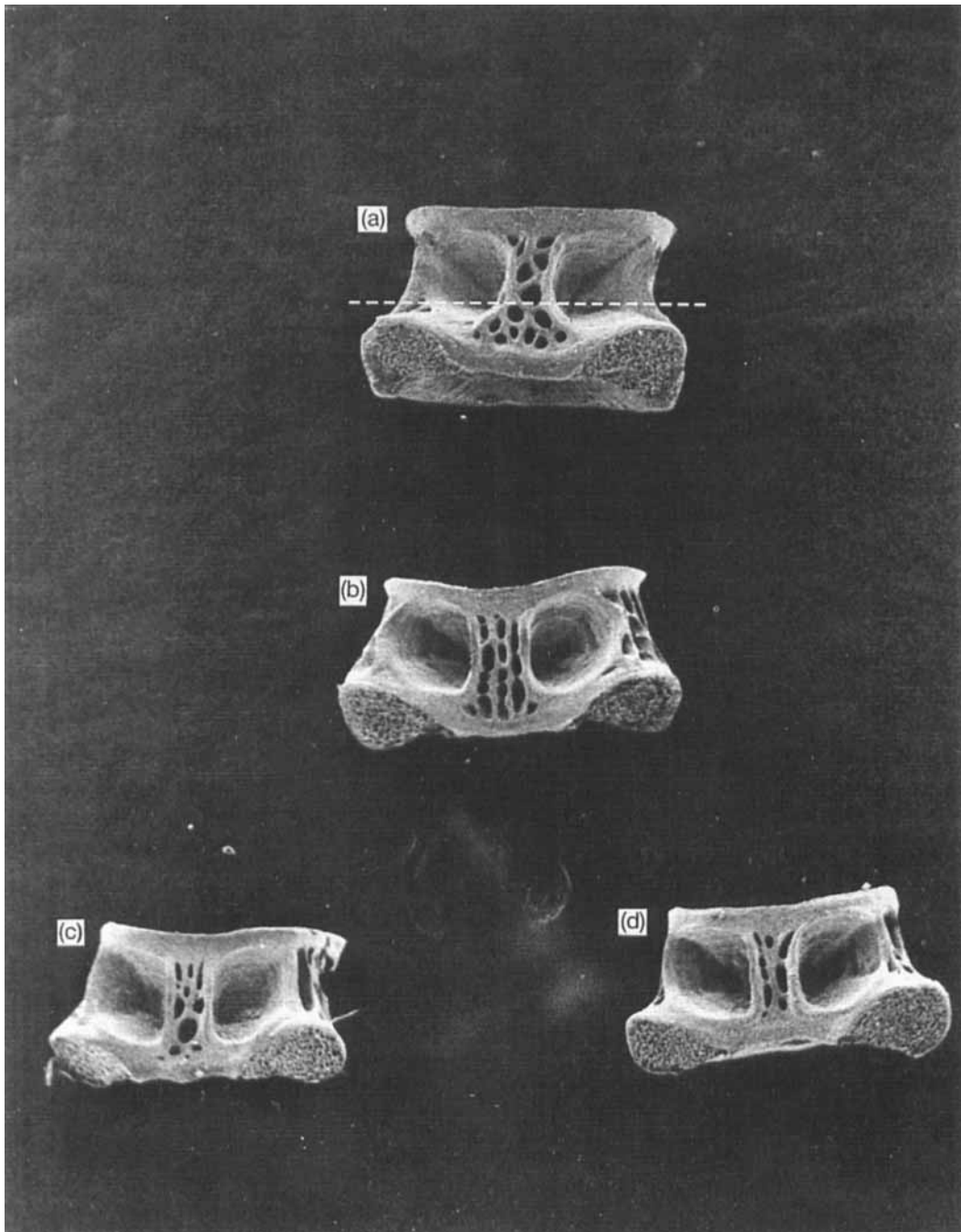


PLATE I. The shapes of the ventral profile of salmonid first vertebrae ( $\times 45$ ). (a) Trout; (b-d) salmon.

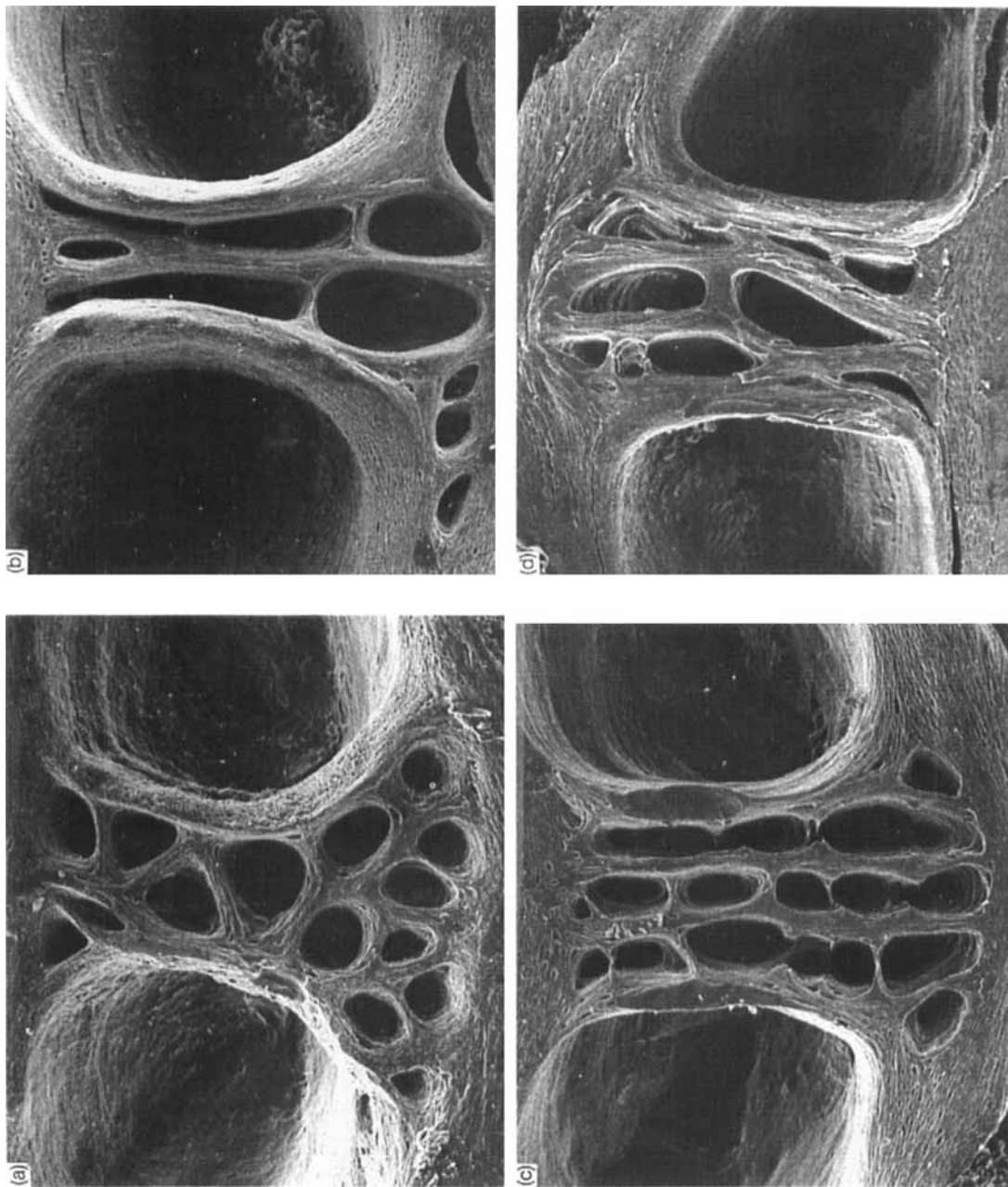


PLATE II. The shapes of the pores on the ventral surface of salmonid first vertebrae ( $\times 120$ ). (a) Trout; (b-d) salmon.

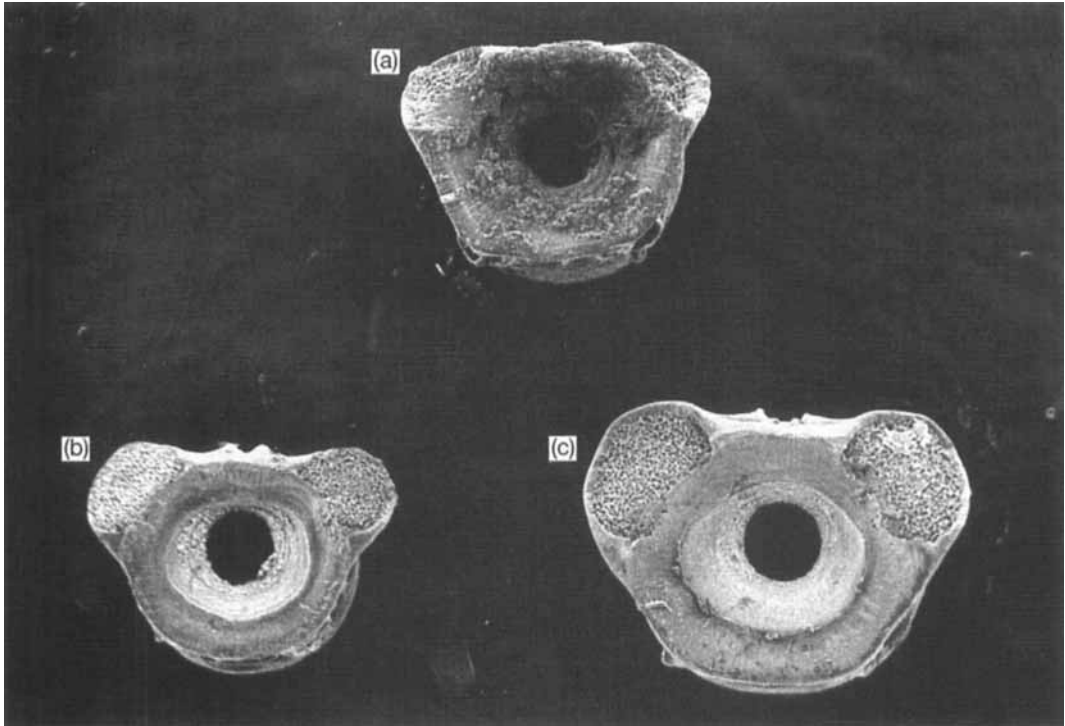


PLATE II. The shapes of the pores on the ventral surface of salmonid first vertebrae ( $\times 120$ ). (a) Trout; (b-d) salmon.

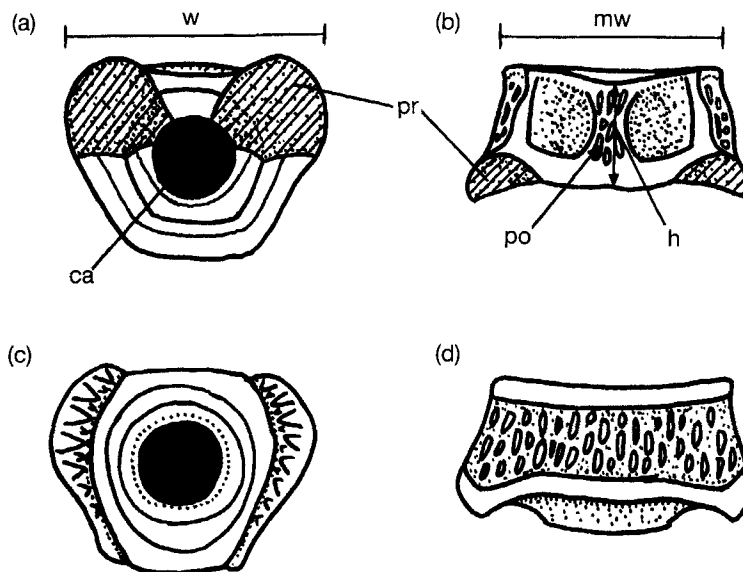


FIG. 1. Diagrams of salmonid first vertebrae showing (a) anterior, (b) ventral, (c) posterior and (d) dorsal profiles. (ca = central canal; po = pores; pr = processes; h = height; mw = minimum width, this may occur near the top of the processes in some vertebrae; w = maximum width).

pores even in the presence of broadly ovate/round pores is generally diagnostic of salmon. Salmon vertebrae have more variably shaped pores.

(c) Trout processes (Fig. 1a; hatched areas) are angular or flattened (Plate II a) as opposed to rounded (Plates III b–c) and do not protrude as 'ears' from the sides of the vertebra (e.g. Plate III b). In fish of length > 100 mm, flattened processes occur more frequently than angular ones.

In the salmon, the processes give a distinctly 'eared' impression, particularly in smaller fish. The processes are usually very rounded and only occasionally angular. In fish of length > 100 mm, the processes appear less 'eared' but are smoothly rounded (Plate III c) as opposed to flattened (cf. trout).

### *Estimating the accuracy of diagnostic features*

Two inexperienced observers examined a mixed sample of salmon and trout first vertebrae ( $n = 117$ ) in a series of 10 tests. Vertebrae were numbered only and observers did not know the ratio of salmon to trout in the sample. Observers used the three criteria presented above to classify first vertebrae as salmon (S), trout (T) or 'unknown' (S/T). The final identification was decided on a two-thirds majority (e.g. Pores = S, Shape = S, Processes = T, final classification = S). Where characters were 'split' between salmon and trout (e.g. S, S/T, T or S/T, S/T, S) the vertebra was recorded as 'unknown'. Preliminary trials suggested that some observers

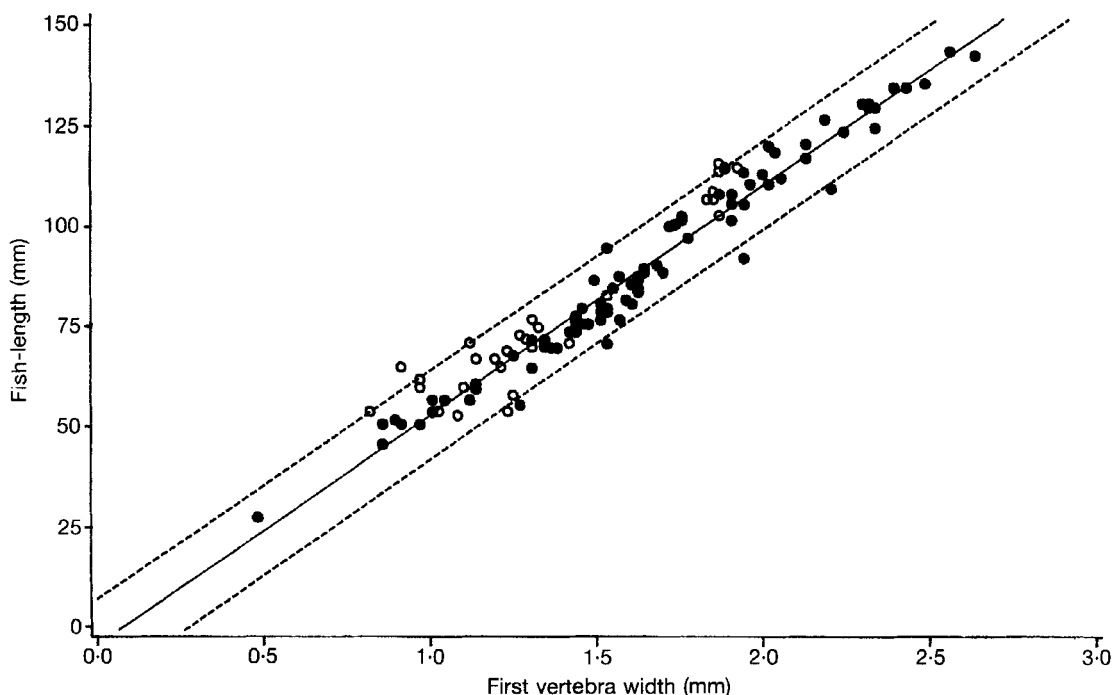


FIG. 2. The relationship between fish-length (mm) of juvenile salmon and the width of their first vertebrae: (●) = wild; (○) = hatchery-reared. The regression line ( $FL = 63.0 \text{ FVW} - 11.7$ ) is plotted with 95% confidence limits for individual points.

might have difficulty in deciding whether some vertebrae were relatively 'tall' or 'short', with respect to the shape character, resulting in a lowered proportion of correctly classified bones (Inexperienced 82%, Experienced 92%,  $n = 137$ ). For vertebrae recorded as 'unknown' using the visual characters, the 'height/minimum width ratio' of the first vertebra was measured (Fig. 1) and used to decide the final classification; i.e.  $1.49:1 = 76\%$  trout,  $1.50-1.69:1 =$  'unknown' 50% trout, 50% salmon,  $1.70:1 = 82\%$  salmon.

## Results

### *Estimating fish size from vertebral measurement*

The relationships of first vertebra width (FVW) to fish-length of hatchery-reared salmon, wild salmon and trout are linear (Figs 2 and 3). Measurements of hatchery-reared salmon fall within the 95% confidence limits of the regression line of first vertebra width vs. fish-length for wild salmon.

The regression of salmonid fish-length on first vertebra width may be described by a single equation:

$$FL = 60.5 \text{ FVW} - 8.95$$

$$(r^2 = 0.961, F_{161,1} = 726, P < 0.01).$$

The confidence limits about the regression line suggest that measurements of first vertebrae can predict fish-length of fish with an accuracy of  $\pm 10$  mm.

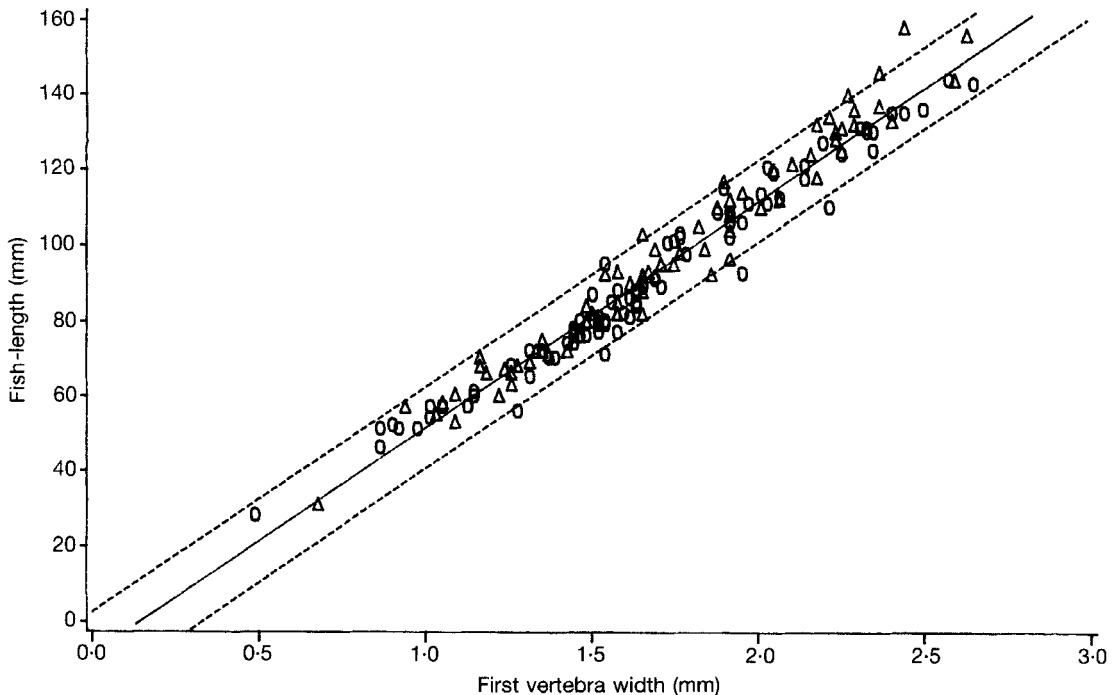


FIG. 3. The relationship between fish-length (mm) of juvenile salmonids and the width of their first vertebrae: (O) = trout; ( $\Delta$ ) = salmon. The regression line ( $FL = 60.5 \text{ FVW} - 8.95$ ) is plotted with 95% confidence limits for individual points.

*Distinguishing juvenile salmon and trout*

Pores and shape appeared to be more useful single characters for species separation than processes. The proportion of vertebrae correctly classified as salmon or trout, however, was greater, and the variation about the mean smaller, using the three characters combined (Table I). Measuring the 'height/minimum width ratio' of vertebrae that could not be classified as salmon or trout using the three visual characters improved further the proportion of vertebrae correctly classified (Table I). There was no significant difference in the numbers of vertebrae assigned to each group (salmon, trout, unknown and incorrect) for the 10 repeat tests, suggesting little between- and within-observer variation (Friedman two-way analysis of variance;  $F_1=2.24$ , *d.f.* 9, NS). Furthermore, the number of vertebrae assigned to each group by the inexperienced observers did not differ significantly from those of an experienced observer who did not measure the 'height/minimum width ratio' of 'unknown' vertebrae (Friedman two-way analysis of variance;  $F_1=0.10$ , *d.f.* 3, NS). This could suggest that with experience, naive observers may improve their ability to visualize the relative height of the first vertebra, without the need for time consuming measurement. The sample of vertebrae contained 77 salmon and 40 trout;  $89.0 \pm 2.7\%$  of trout and  $90.4 \pm 1.2\%$  of salmon vertebrae (95% C.I.) were classified correctly. Of the vertebrae,  $6.7 \pm 0.5\%$  were classified as 'unknown', whilst only  $3.3 \pm 0.5\%$  were incorrectly classified. There was no tendency to classify vertebrae incorrectly or as unknown with respect to fish species (paired *t*-test =  $t_a=0.458$ , NS).

From a paired *t*-test conducted on vertebrae from fish  $\leq$  and  $>$  the median fish-length of our sample (82.5 mm), however, we found no tendency to classify vertebrae incorrectly or as 'unknown' with respect to vertebral size ( $t_9=0.727$ , NS).

**Discussion**

The salmonid first vertebra is very robust; resistance to mechanical breakdown within the gizzard of mergansers presumably being afforded by the two hard processes on the antero-ventral surface. Breakdown of the first vertebra in this bird appears to follow a pattern of gradual erosion

TABLE I

*The proportion of first vertebrae ( $\pm$  S.D.) classified correctly, incorrectly and as 'unknown' in a mixed sample of salmon and trout vertebrae ( $n=117$ ). Data are for three visual characters individually, together, and combined with the measured 'height/minimum width' ratio*

Characters	Proportion of first vertebrae in each class (%)		
	Correct	Incorrect	Unknown
Pores	$79.5 \pm 4.9$	$8.4 \pm 1.8$	$10.1 \pm 3.2$
Shape	$78.5 \pm 5.9$	$10.4 \pm 2.8$	$9.6 \pm 2.4$
Processes	$70.6 \pm 6.3$	$11.2 \pm 2.3$	$18.1 \pm 2.9$
All characters	$81.9 \pm 1.4$	$6.8 \pm 1.5$	$10.9 \pm 1.5$
* All characters + 'height/minimum width' ratio	$89.9 \pm 1.1$	$3.2 \pm 0.9$	$6.5 \pm 0.7$

\* Used to aid identification of 'unknown' and incorrectly classified vertebrae, found using the three visual characters combined (see **Methods**)



of the bone 'bridge' between the two processes (Fig. 1 a), which finally breaks to leave horseshoe-shaped bones, although such fragmented first vertebrae are uncommon. Chemical erosion of the first vertebra is unlikely to produce large errors in estimating fish-length, since there is little visual evidence of erosion (e.g. pitting) of the two antero-ventral processes (Feltham, pers. obs. on the contents of 45 merganser stomachs).

The ease of identification of this bone in salmon and trout confers advantages over the more rapidly eroding otoliths, and the use of such a single identifier bone overcomes problems of size variations of thoracic and caudal vertebrae within fish, that are encountered when trying to estimate fork-length from measurements of these vertebrae (Casteel, 1976; Wise, 1980).

The first vertebra is, however, unlikely to be a useful identifier bone in all fish species. Few sandeel (*Ammodytes* sp.) 'firsts' were found in merganser guts, for example, though many otoliths were present. Eel (*Anguilla anguilla*) 'firsts' have very small antero-ventral processes and are difficult to distinguish from some thoracic vertebrae.

The methods we describe use criteria which will distinguish salmon and trout in only 90% of instances. Since, however, there are no apparent biases in either species or size class of fish within the 'unknown' group of vertebrae, observers could assign members of this group to either salmon or trout in the proportions found in the correctly classified groups, without much error. Similarly, since a single regression line is used to estimate fish size for both salmon and trout, size estimates of the 'unknown' group would be little biased by not knowing the species.

Though not infallible, the method has the additional advantage of estimating the minimal numbers of fish consumed and their original sizes.

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