Hyperostosic Bones from the New Zealand Snapper *Chrysophrys auratus* (Sparidae)

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General hyperostoses are well known in fossil fish bone literature (Tiffany et al. 1980). Hyperostosic bones have been described as occurring as nodules on the ventral pterygiophores as well as other bones of Recent fishes (Olsen 1971, Fierstine 1968, Konnerth 1966). Consistent occurrence of hyperostosic bones in many species has been taken to indicate that they are not pathological (Fierstine 1968).

Various roles have been suggested for hyperostosic bones ranging from aids in fin erection (Fierstine 1968) to hydrostatic correction (Breder 1952). The hydrostatic suggestion arose from the observation that hyperostosic bones are "filled with fat" (Breder 1952), and oils in fish bones have been proposed as an aid to neutral buoyancy (Lee et al. 1975). Hyperostoses in sparids of the genus *Chrysophrys* have also been used as characters to separate *Pagrus major* from *Chrysophrys auratus* (Yasuda and Mizuguchi 1969).

Hyperostosic bones occur in the snapper *Chrysophrys auratus* and are usually highly vascularized. We were interested in (1) the possible relationship between size of hyperostosic bones and fish size and (2) the significance of the fatty-acid composition of hyperostoses to the biology of the fish.

Materials and methods

Snapper were measured as fork length, filleted along one side to reveal the hyperostosic bones, and photographed on a measuring board. The hyperostosic bones and a sample of the vertebrae from each fish were dissected out and frozen at −15°C until the fat content was analyzed. The relative sizes of hyperostosic bones were measured from the photographs. Two hyperostosic bones usually occur in the snapper, commonly on the seventh (preural) haemal spine and less commonly on the sixth haemal spine from the tail in specimens of the size range (31–48 cm) used in this study. Both were measured. The eighth and ninth vertebrae from the tail were removed and analyzed as non-hyperostosic comparisons.

One bone was cut through the hyperostosis, mounted in epoxy resin, and ground down to about 50-μm thickness and photographed with a WILD M400 stereomicroscope. Whole fish were x-rayed using a Faxitron Model 804 Radiographic Inspection Unit. Ten of the specimens examined were aged from the otoliths using an annual check-ring method (Paul 1976).

Flesh and connective tissues were scraped off the bones. Prior to chopping, bones bearing the hyperostoses were cut diagnoally. Bones were weighed, chopped into small pieces, and extracted three times with chloroform-methanol (2:1 v/v) in an ultrasonicator bath (Sanophon Ultrasonic Ind.) containing iced-water for 10 minutes and finally extracted once with chloroform. The extracts were combined, filtered, and evaporated at 40°C in a rotary evaporator and weighed. Extracted lipids were stored under nitrogen at −15°C pending analysis.

Fat-free bone residues were dried to constant weight. The lipid extract is expressed as a percentage on a dry matter basis (Table 1). Lipids were separated into classes by column chromatography and preparative thin-layer chromatography. All solvents used were redistilled prior to use. Portions of total lipids dissolved in chloroform were initially separated into fractions on 30 g and 12 g columns (depending upon sample sizes) packed with silica acid (Koch–Light Lab. 5024h, 100–200 mesh) previously activated at 110°C. The elution procedure involved chloroform for neutral lipids and methanol for polar lipids. By combination of silicic-acid column chromatography and preparative thin-layer chromatography, neutral lipids were resolved into their constituent categories (Tables 1, 2).

Preparation of methyl esters and gas-liquid chromatography (GLC) was carried out in the following way. Aliquots of total lipid extracts, which contained fatty acids combined as glycerides and phospholipids, and transesterified with BF_3-methanol (Van Wijngaarden 1967). Fatty-acid methy esters were analyzed by GLC using a Pye Unicam GCV and a Philips PU 4500 capillary chromatograph fitted with flame ionization detectors. Both

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Manuscript accepted 9 August 1989.
Fishery Bulletin, U.S. 88:201–206.

Table 1

Hyperostoses (H) and vertebrae (C), extraction and column chromatography in *Chrysophrys auratus*.

	Fish 1		Fish 2		Fish 3		Fish 4		Fish 5		Fish 6		Fish 7		Fish 8		Average values	
	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	$\bar{H}$	$\bar{C}$
Total lipid extract																		
Weight (mg)	17.4	4.2	58.9	34.7	24.4	12.0	31.4	12.3	16.3	8.6	37.3	12.2	7.3	5.5	51.4	49.4		
% (dry matter basis)	3.11	4.57	16.91	17.73	11.50	10.71	2.04	5.10	6.96	8.14	22.70	16.90	8.00	14.29	29.30	22.93	12.57	12.55
Column chromatography (%)																		
Triglycerides	58.0	57.1	58.4	58.1	58.2	57.2	58.0	57.7	58.3	58.1	58.4	48.2	58.9	58.2	58.6	58.5	58.35	57.89
Sterols and alcohols	29.9	30.9	30.9	30.6	30.6	30.0	30.6	30.9	30.7	30.2	30.0	30.3	30.1	30.9	30.0	29.9	30.35	30.46
Total neutral lipids	87.9	88.0	88.9	88.6	88.6	87.5	88.6	88.6	89.0	88.3	88.4	88.5	89.0	89.1	88.6	88.4	88.63	88.38
Total polar lipids	9.8	9.5	10.7	10.1	10.2	10.0	10.2	10.6	10.4	10.5	9.6	10.6	9.6	10.6	10.1	9.9	10.08	10.23

Table 2

Fatty acid composition of the lipids of hyperostoses (H) and vertebrae (C) (expressed as percentage of GLC peak areas) in *Chrysophrys auratus*.

	Major components as fatty acid methyl esters										
	Fish 1		Fish 2		Fish 3		Fish 4		Fish 5		Snapper fillet**
	H	C	H	C	H	C	H	C	H	C	
Saturated											
14:0	4.1	4.4	3.9	4.2	5.8	6.3	5.1	4.7	4.3	3.8	4.4
15:0	0.8	0.7	1.0	0.8	0.7	0.7	0.8	1.0	0.8	0.9	1.0
16:0	35.9	34.2	31.2	28.0	27.8	26.8	28.5	27.9	35.1	30.8	36.1
17:0	0.9	0.9	0.9	0.9	1.0	1.0	0.9	1.0	0.8	0.8	0.8
18:0	10.9	11.2	11.4	11.0	11.2	12.4	12.0	11.6	11.0	12.6	11.3
Other	0.3	0.7	0.4	1.9	4.4	0.8	1.9	2.8	0.3	1.1	0.9
Total saturated	52.9	52.1	48.8	46.8	50.9	48.0	49.2	49.0	52.3	50.0	54.5
Unsaturated											
16:1 ω_7*	7.9	8.3	8.5	10.4	7.9	6.6	8.4	9.2	8.5	7.9	6.7
16:3 ω_4	0.9	1.0	1.2	1.4	0.9	2.5	1.5	1.2	0.9	1.0	0.8
18:1 ω_9	22.1	21.8	24.9	25.0	21.9	20.9	23.2	21.8	23.0	24.8	22.2
18:2 ω_6	1.0	1.3	1.9	1.0	1.2	2.1	1.6	1.4	2.0	1.7	0.8
18:3 ω_3	2.9	2.6	2.1	1.9	2.9	2.6	2.4	2.2	2.9	2.5	0.6
20:1 ω_9	1.5	1.6	1.0	1.0	2.0	2.2	1.6	1.6	1.0	1.6	1.2
20:4 ω_6	1.7	1.7	1.3	0.5	1.0	1.5	1.1	1.4	1.2	1.1	2.1
20:4 ω_3	1.2	1.9	1.2	1.5	1.3	1.7	1.4	1.6	1.7	1.5	0.2
20:5 ω_3	0.9	1.1	1.9	1.5	2.2	1.9	1.9	2.0	2.1	1.8	0.3
22:5 ω_6	1.0	0.8	1.1	1.1	0.8	1.2	1.1	1.0	0.9	1.0	1.3
22:5 ω_3	0.8	0.7	0.7	1.1	1.1	1.3	1.0	1.2	1.0	1.2	1.8
22:6 ω_3	1.2	1.4	0.3	1.4	0.8	1.1	0.9	0.7	0.4	0.7	1.5
Other	2.1	1.7	3.1	3.6	3.3	4.3	2.8	3.7	0.2	1.4	4.2
Total unsaturated	45.2	45.9	49.2	51.4	47.3	49.9	48.9	49.0	45.8	48.2	43.7
Total branched	1.9	2.0	2.0	1.8	1.8	2.1	1.9	2.0	1.9	1.8	1.8

*ω_1 position of double bond nearest to terminal methyl group.
**Fatty acid composition of snapper fillet reported by Hughes et al. (1980).

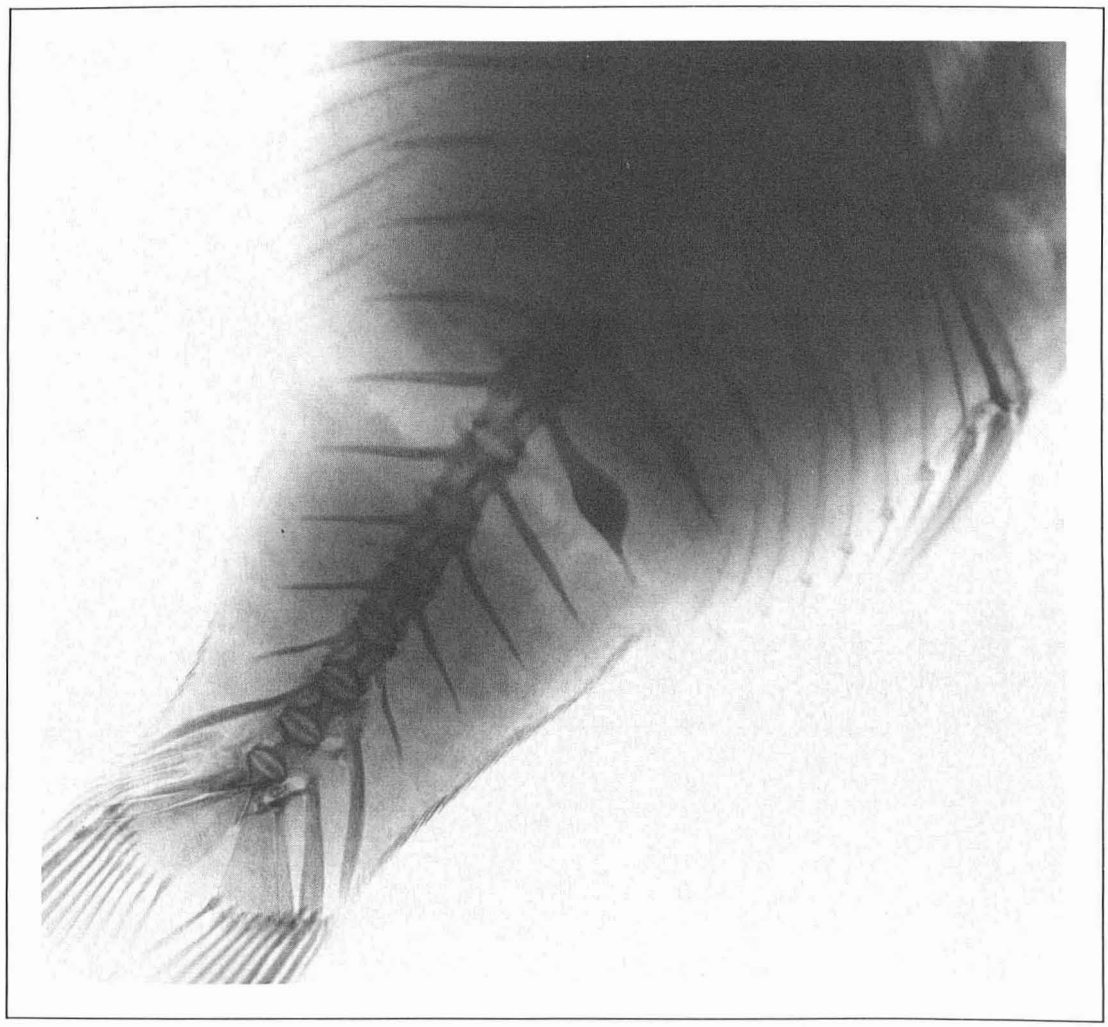

Figure 1
Radiograph of *Chrysophrys auratus* hyperostosic bones showing the enlarged haemal spines.

polar and non-polar columns were employed, the former containing 10% EGSS-X (210 × 0.254 cm i.d.) and the latter a BP-1 capillary column (25 × 0.33 mm i.d.).

Eight pairs of hyperostoses and vertebrae were analyzed (Table 1) for fat content and general fat composition. A subset of pairs of hyperostoses and vertebrae was used in the identifications of fatty-acid methyl esters based upon comparison of retention times with those of authentic reference methyl esters run under identical conditions. Where particular reference standards were not available, equivalent chain-length values reported by Hofstetter et al. (1965) and by Jamieson (1969) were accepted as criteria of identity. Identification was corroborated by hydrogenation of an aliquot of the esterified samples. Saturated and branched chain products were then analyzed by GLC on a polar column. The results of fatty-acid analyses were expressed as the percentage area occupied by each component methyl-ester peak relative to the total peak area. In the fatty-acid composition analyses reported here, the percentages were based on peak areas obtained with the 10% EGSS-X column (Table 2). Fatty-acid composition of snapper fillets (Hughes et al. 1980) is included for comparison in Table 2.

Results

Eight sets of snapper hyperostosic bones and controls were examined. An example of a pair of snapper hyperostosic bones is shown in Figure 1. In life, the hyperostosis has a reddish color and appears to be well vascularized. Commonly, hyperostoses occur both on the sixth and seventh haemal spine from the tail, as has also been described for *Chrysophrys unicolor* (Yasuda and Mizuguchi 1969). Although both hyperostoses may be enlarged, it is more common to find that the one

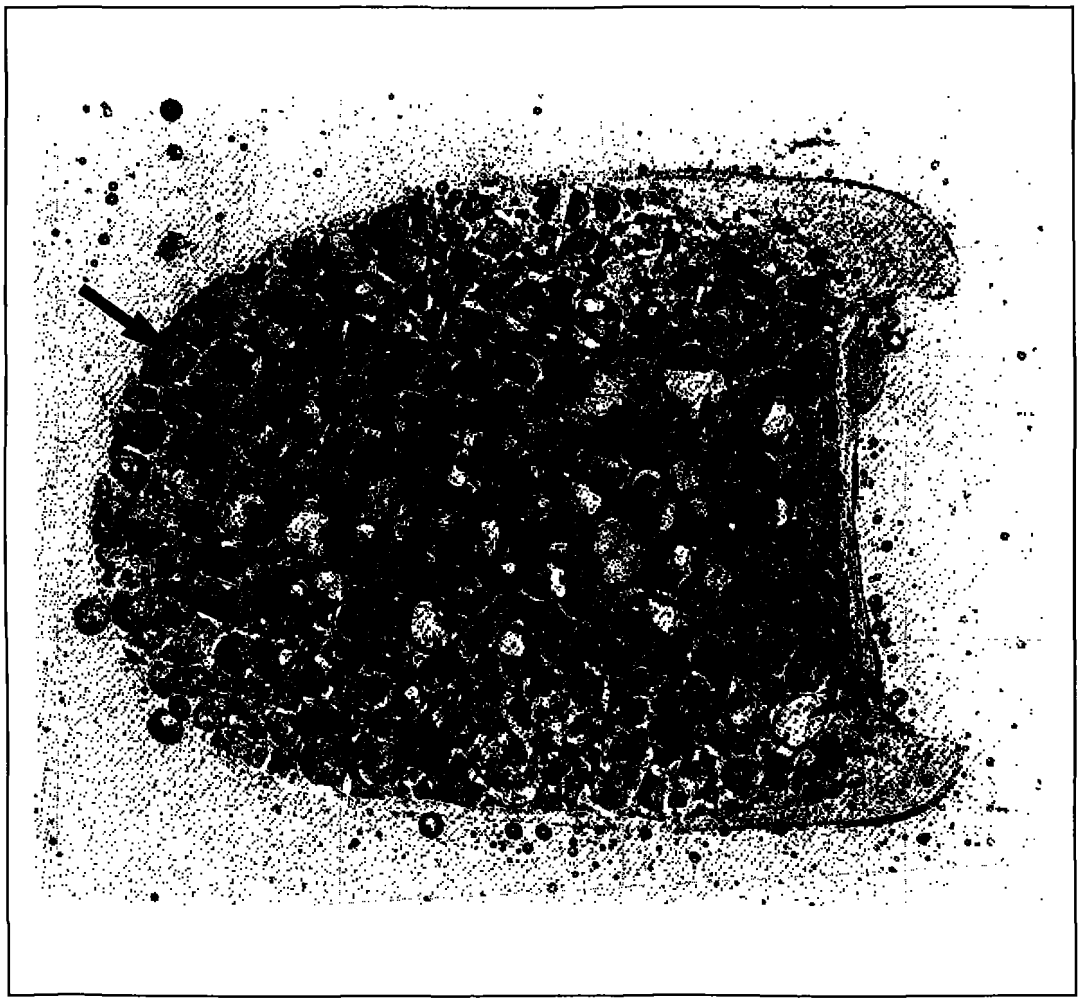

Figure 2
A section of a hyperostosic bone in *Chrysophrys auratus* shows a vacuolated structure (arrows) encased with bone.

furthest from the tail shows less enlargement, as in Figure 1. Section of the snapper hyperostosic bone shows the vacuolated appearance (Fig. 2) characteristic of most fish bones.

The relative size of the most caudal hyperostosis was measured as the ratio of the width of the hyperostosis to the width of the spinal vertebra to which the haemal spine, on which the hyperostosis occurs, was attached. A plot of the ratio of hyperostosis width/vertebra width to fork length is shown in Figure 3. The correlation between relative size of the hyperostosis and fork length was low, $r = 0.58$. The correlation between the ratio of hyperostosis width/vertebra width to annual check-ring age was low, $r = 0.53$.

Eight pairs of hyperostoses/vertebrae were examined for fat content and fat composition (Table 1). The mean fat content of the hyperostoses (% dry matter) was 12.57 ± 19.34, and the mean fat content of vertebrae (% dry matter) was 12.53 ± 13.04. Although there was a certain amount of variation in fat content between hyperostoses and vertebrae, it was unlikely to be significant in any biological sense because fish lipid composition and content have been shown to be affected by diet to a greater extent than is shown in our data (Worthington and Lovell 1973).

The fatty-acid composition was almost identical ($\pm 5\%$) for all samples and very similar to the table presented in Love (1980: 414) for marine fish fatty-acid composition. A typical fatty composition for five pairs of hyperostoses and vertebrae is shown in Table 2. There was no significant difference in fatty-acid composition between the hyperostoses and vertebrae.

Discussion

Hypoerostosic bones of the snapper *Chrysophrys aurata* increased in size with increase in size of the fish,

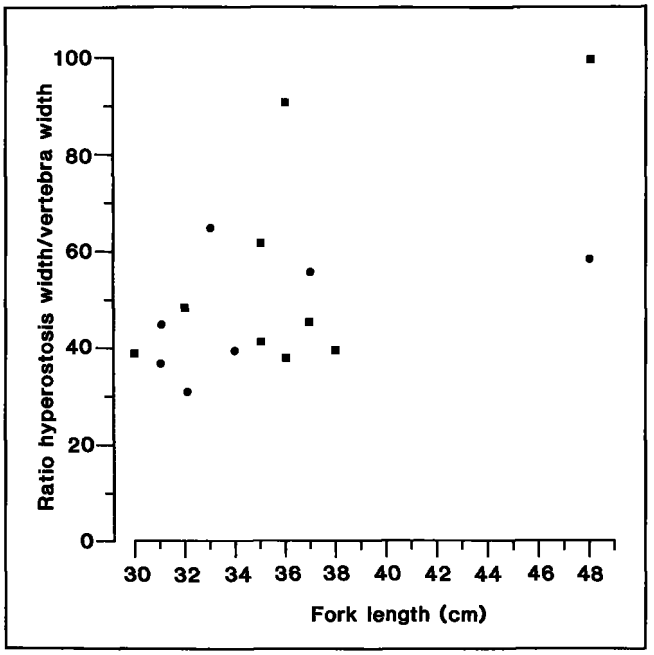

Figure 3
The ratio of hyperostosis/vertebra width in *Chrysophrys auratus* is plotted against fork length.

but the correlation between hyperostosic bone size and fish size was low. The relative size of snapper hyperostosic bones did not appear to be related with any more significance to age than to size.

The fat content of the snapper hyperostosic bones was not significantly different from that of the vertebrae. Nor was the composition of fat in the snapper hyperostosic bones significantly different from either the vertebrae of snapper or fats found in marine fishes generally. The difference in fat content between snapper hyperostosic bones and vertebrae is low (Worthington and Lovell 1973) compared with the potential range of differences in fat content known in fishes.

Snapper hyperostoses are vacuolated, but no more so than other bones in fish. The contribution of fat in hyperostosis to buoyancy is difficult to assess. The amount of lipid as a percent dry weight varies between individuals, but in some individuals is of the same order as the lipid content of the bones of fish species in which bone lipid may have a role in neutral buoyancy (Lee et al. 1975). However, the low correlation with both size and age suggests that if hyperostoses are related to buoyancy correction, then that relationship is tempered by factors other than size.

The use of hyperostoses to discriminate species both in Recent genera, such as *Chrysophrys* (Yasuda and Mizuguchi 1969) and fossil species (Fierstine 1968) points to another possible explanation. Hyperostoses occur with great regularity in certain bones in those species which display hyperostoses. We interpret such regularity as indicating that hyperostoses are under genetic control and are therefore good species characters, as are other genetically determined characters. But the expression of hyperostoses results from an indeterminate kind of ontogenetic process in which there is only a loose correlation between size of the hyperostoses and size or age. Thus the hyperostoses involve the local proliferation of cells (Breder 1952) but in a way that could be seen as physiologically between a normal bony growth and a bony tumor, almost a kind of controlled tumor which contains both fat cells and bony trabeculae. Snapper hyperostoses are readily obtainable and may provide a useful model to study the ultrastructural interactions between proliferating bone and fat cells.

Acknowledgments

The snapper used in this study came from a tagging study conducted by Arthur Hoare of the Fisheries Management Division, MAF, New Zealand. Annual check-ring ages were provided by Larry Paul of the Fisheries Research Division. Expert technical help was provided by Kevin Mulligan.

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