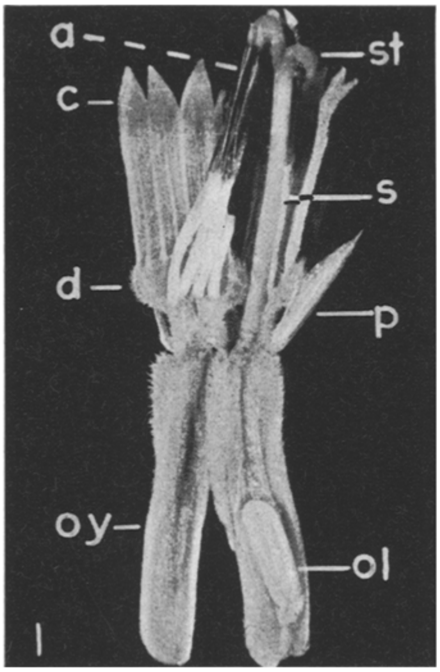

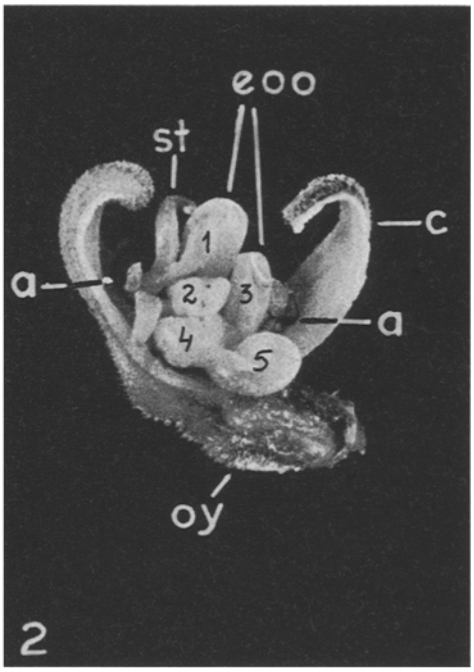

Fig. 1. Vertical halves of a normal disc floret showing a single basally attached intra-ovarian ovule.

Fig. 2. Disc floret from chlorflurenol-treated plant showing the boat-shaped corolla lobes and the 5 extra-ovarian ovules. a, anther; c, corolla; d, disc; eoo, extra-ovarian ovule; ol, ovule; oy, ovary; p, pappus; s, style; st, stigma.

fined to the ovary, another emerging laterally through the ovary wall. The florets in which the ovules had come out of the corolla were of 2 kinds: a) those in which the length of the ovary was the same as in controls and the loculus was filled with ovules, b) those in which the ovary was short and solid; the ovules being borne at the base of the corolla or on it. The extra-ovarian ovules differed from the normal ones in their size and shape, but showed the endothelium and the embryo sac.

There are many papers dealing with the effects of morphactins on flower development. To our knowledge, the only previous report on the occurrence of exposed ovules is by UMA[7,8] in the treated linseed plants. The ovules were found to protrude from the top of the ovary or occasionally attached to the base of a stamen. The exposed ovules of linseed contained smaller embryos and their ultimate fate was not known. In the sunflower also mature exposed seeds have not been observed. The formation of extra-ovarian ovules was recorded in plants treated at all concentrations of chlorflurenol. However, the maximum incidence of this feature was noted at the lowest concentration. The increase in the number of ovules suggests an accentuation of female sex expression. The details of ontogeny and embryology of the exposed ovules are being studied.

Summary. Foliar spray of aqueous chlorflurenol solution induced the development of 1–7 (rarely up to 14) extra-ovarian ovules in the disc florets of sunflower in contrast with a single intra-ovarian ovule found in the controls. The incidence was highest at 3×10^{-5} *M* concentration.

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[1] G. SCHNEIDER, A. Rev. Plant Physiol. *21*, 499 (1970).
[2] N. SANKHLA, Z. Pfl. Physiol. *41*, 350 (1969).
[3] H. Y. MOHAN RAM and V. S. JAISWAL, Naturwissenschaften *58*, 149 (1971).
[4] N. SANKHLA and S. P. VYAS, Biochem. Physiol. Pflanzen *164*, 22 (1973).
[5] D. VON DENFFER, G. FRICKE and F. RINGE, Ber. dt bot. *3*, 61 (1969).
[6] M. ILYAS and B. BARMA, Biologia pl. *15*, 155 (1973).
[7] M. C. UMA, Curr. Sci. *41*, 114 (1972).
[8] M. C. UMA, Ph. D. Thesis. University of Delhi, India (1973).
[9] Acknowledgments. We are indebted to M/S Celamerck, Ingelheim, Germany for the supply of chlorflurenol and to the Govt. of India, Department of Atomic Energy, for a research fellowship to one of us (M.I).

Relationship Between Size of Muscle Fibres and Body Dimensions in a Number of Teleosts

In 1956, JOUBERT[1] reviewed some of the literature which dealt with the effect of species on muscle fibre size. He quoted BOWMAN[2], who in 1840, must have been one of the first to suggest some sort of genetic control over muscle fibre size. He stated that within each class of animals there is an extensive range of body size and probably also muscle fibre size. Since then, several studies have been made to investigate the effect of species on muscle fibre size within the mammalian class. WARRINGSHOLZ[3], and later JOUBERT[1], measured muscle fibre diameter in 4 domestic mammals, but they found no relationship with body size. However, GAUTHIER and PADYKULA[4], in a more recent extensive survey, did

[1] D. M. JOUBERT, J. agric. Sci. *47*, 59 (1956).
[2] W. BOWMAN, Phil. Trans. *130*, 457 (1840).
[3] C. W. WARRINGSHOLZ, Arch. wiss. prakt. Tierheilk. *29*, 5 (1903).
[4] G. F. GAUTHIER and H. A. PADYKULA, J. Cell Biol. *28*, 333 (1966).

establish a direct relationship between fibre size in the diaphragm of 13 mammalian species and body size. GEORGE and NAIK[5], working on birds, also found that muscle fibre area increased with body weight. This last study seems to be the only one concerned with investigating the relationship between fibre size and body size outside the mammalian class. It was decided, therefore, to investigate this relationship (if any) in fish, which have so far not been studied and which also have an easily measured 'shape' in contrast to mammals.

Materials and methods. 17 different species of teleost (Table I, identified according to SMITH[6]) were caught over a 3-day period from rock pools found in the exposed coral reef off the Kenya coast (4°20'S). The fish were stunned by a blow on the head and then fixed in 5% formalin for 24 h with a small dorsal and ventral skin incision to aid penetration of fixative. After fixation, the length (l) of the fish was measured (from snout to base of tail) as well as its greatest height (h) and width (w) (using microcalipers). The fish were then decalcified, according to SCHAFFER[7], in order to make sectioning easier. The whole fish was sectioned transversely at a position about two-thirds of its length from the head end; 7 µm sections were cut on a Leitz rotary microtome and stained in Haematoxylin and Eosin.

Table I. List of fish used in investigation with mean muscle fibre diameter (D), estimated body volume (V) and length:height ratio (l/h)

No.	Name	D (µm)	V (c.c.)	l/h
1	*Canthigaster margaritatus*	44.3	2.94	2.38
2	*Scorpaena mossambica*	42.3	1.72	2.42
3	*Stethojulis strigiventer*	27.2	0.71	3.18
4	*Halichoeres dianthus*	16.0	0.38	2.96
5	*Halichoeres scapularis*	34.9	1.23	4.27
6	*Caracanthus zeylonicus*	48.0	2.41	2.77
7	*Abudefduf lacrymatus*	25.1	1.31	2.13
8	*Cheilodipterus quinquelineatus*	35.4	0.95	2.94
9	*Branchiostegus japonicus*	48.6	1.70	3.53
10	*Abudefduf sparoides*	51.5	1.31	1.85
11	*Apogon monochrous*	49.5	0.94	2.51
12	*Gobiodon citrinus*	15.1	0.04	4.07
13	*Siganus oramin*	88.7	20.18	2.26
14	*Apogonichthyoides uninotatus*	28.6	0.67	3.32
15	*Petroscirtes mitratus*	21.9	0.11	8.51
16	*Acropoma japonicum*	23.3	0.30	3.16
17	*Grammistes sexlineatus*	47.9	8.44	2.63

Table II. Coefficients of correlation (r) between various parameters[a]

Parameters correlated	n	r	P
D v. V	17	0.814	< 0.001
D v. log (V)	17	0.844	< 0.001
D v. V (13 omitted)	16	0.536	< 0.05
D v. log (V) (13 omitted)	16	0.765	< 0.001
D v. 1/h	17	−0.412	n.s.
D v. log (1/h)	17	−0.482	< 0.05
D v. 1/h (13 omitted)	16	−0.432	n.s.
D v. log (1/h) (13 omitted)	16	−0.493	< 0.1

[a] D, muscle fibre diameter; V, volume of fish; l/h, length: height ratio.

By means of an eye-piece gracticule in a Leitz microscope, 100 muscle fibres were measured[8] for each fish. The approximate 'volume' (V) of each fish was estimated using the following equation:

$$V = \frac{1}{3} \cdot \pi \cdot \frac{h \cdot w \cdot l}{4} \quad \text{(see above for explanation of symbols)}$$

This equation is the volume of 2 elliptical cones (total length, l) with their bases end to end. This will give some indication, at least, of relative volumes of the fish used.

It was found that the lengths of the fish used were on average 29.9% (with a standard error of $\pm$ 4.2) of the maximum length they can attain (according to SMITH[6]). In order to standardize the results, it was decided to adjust all Volume and Fibre diameter measurements to their estimated values at 30% of the maximum length of the fish. These were very small adjustments, which could easily be carried out assuming that, for volume changes, length: height: width ratios are constant for each species. Muscle fibre diameter adjustments were based on the fact that changes in fibre diameters are related to changes in overall muscle girth[9].

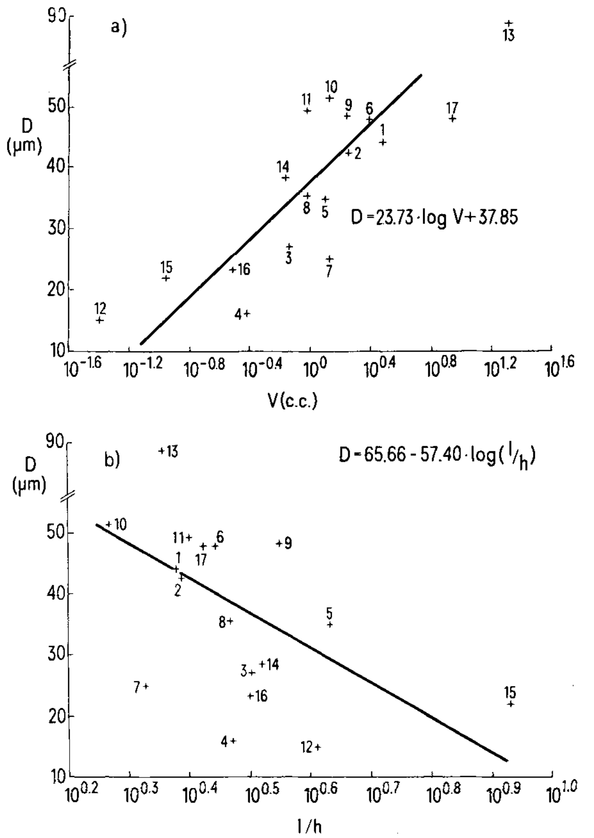
Results. Table I lists all the fish used in this investigation together with the mean muscle fibre diameter (D), 'volume' (V) and length: maximum height ratio (l/h) for each fish. The parameters D and V shown in this table have both been 'adjusted' to 30% of the fish's maximum length. Table II shows the coefficients of correlation between various parameters. All coefficients in this table are based on the 'adjusted' values shown in Table I. In some correlations, fish number 13 was omitted as it appears to be very different in fibre size and volume from the other fish (see Figure). It should be noted that, using original values for Volume and Fibre diameter (with *no* adjustment to 30% of the fish's maximum length), correlation coefficients were significant for D against log(V) ($r = 0.68$, $p < 0.01$) and for D against l/h ($r = 0.57$, $p < 0.02$). Other correlations were of the same order as Table II but not significant. The Figure shows the variation of muscle fibre diameter with volume and length: height ratio of the fish.

Discussion. The results of this investigation appear to show that the diameter of muscle fibres in various species of fish is related in some way to the volume of the fish. Larger species of fish seem to have larger muscle fibres. The fact that the correlation coefficients are highest when log (Volume) is used, seems reasonable when it is realized that the volume parameter is a cubed dimension as compared to the diameter measurement. As already mentioned, this relationship between fibre size and body size has already been demonstrated in several species of birds by GEORGE and NAIK[5]. SMITH[10], working with chickens, also concluded that muscle size is related to body size. In mammals, although this relationship has been demonstrated by GAUTHIER and PADYKULA[4] using 13 species, other workers[1,3] have not been able to show this, even though the later workers used smaller numbers of species. This discrepancy in the mammalian class may be due in part to the much greater range of body sizes and dimen-

[5] J. C. GEORGE and R. M. NAIK, J. Anim. Morph. Physiol. *6*, 90 (1959).
[6] J. L. B. SMITH, *The Sea Fishes of Southern Africa*, 5th edn. (Central News Agency, South Africa 1965).
[7] J. SCHAFFER, Z. wiss. Mikrosk. *19*, 308 and 441 (1902).
[8] N. C. STICKLAND, Ph. D. Thesis, University of Hull (1973).
[9] N. C. STICKLAND and G. GOLDSPINK, Anim. Prod. *16*, 135 (1973).
[10] J. H. SMITH, Poultry Sci. *42*, 283 (1963).

sions than that found in the bird or fish class. In fact, if certain groups of mammals are treated separately, a direct relationship between fibre size and body size is seen. JULIAN and CARDINET[11] found that large dogs had larger muscle fibres than small dogs, and LUFF and GOLDSPINK[12] found a similar stituation in mice.

The results of this investigation also suggest that there is an inverse relationship between muscle fibre size and length:height ratio (*l/h*). This relationship becomes statistically significant when log (*l/h*) is used, suggesting that the relationship is not linear.



Shows the relationship between mean muscle fibre diameter (D) and (a) Volume of fish (V), (b) length: height ratio (*l/h*). Both V and *l/h* axes are written on a log scale. The equations for the plotted lines of regression are shown. The numbers against each fish correspond to the fish numbers shown in Table I.

There is some evidence[13] that faster fish tend to have a higher length:height ratio. It would seem, therefore, that faster fish may have smaller fibre diameters. In mammals, GAUTHIER and PADYKULA[4] reasoned that fibre size is related to body size because body size is inversely related to metabolic activity[14]. Only if metabolic activity in fish is related to relative speed can it be said that the results presented here agree with those found in mammals.

In mammals, the decrease in fibre size with increasing metabolic activity is due to a higher proportion of red muscle fibres, as well as a decrease in size of the white muscle fibres[4]. In the fish studied here, the characteristic lateral line strip of red muscle fibres[15] appeared very small or non-existent, and, in any case, these fibres were not included in the measurements. However, in some of the 'faster' fish, a 'mosaic' arrangement of small and large fibres was seen in the bulk of muscle which might correspond to the mosiac arrangement of red and white muscle fibres seen in some fish[15]. A histochemical study would be required to investigate this idea.

Taken as a whole, the results pose difficult problems. If muscle fibre size is directly related to body size and inversely related to *l/h* then this suggests that smaller fish are more stream-lined with smaller muscle fibres. It is possible that the relationship between fibre size and *l/h* is a result of a relationship in the fish used here between body size and *l/h*. A wider range of teleosts should be examined, preferably in which body size and *l/h* are unrelated, in order to investigate these inter-relationships more fully[16].

Summary. In a survey of 17 species of teleosts, a direct relationship was found between the diameter of muscle fibres and estimated volume of the fish. The results also suggested an inverse relationship between muscle fibre diameter and 'streamlinedness' of the fish (as measured by length: height ratio).

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[11] L. M. JULIAN and G. H. CARDINET, Anat. Rec. *139*, 243 (1961).
[12] A. R. LUFF and G. GOLDSPINK, Life Sci. *6*, 1821 (1967).
[13] P. GREENWAY, Experientia *21*, 489 (1965).
[14] F. G. BENEDICT, *Vital Energetics. A Study in Comparative Basal Metabolism* (Carnegie Inst. Washington Publ. 1938), No. 503.
[15] R. BODDEKE, E. J. SLIJPER and A. VAN DER STELT, Proc. K. ned. Akad. Wet., Series C. *62*, 589 (1959).
[16] Thanks are due to Mr. THEO D'SOUZA for help in identifying the fish used in this investigation.

Isolation and Culture of Mesophyll Protoplasts of *Hyoscyamus niger* L. var. *annuus* Sims[1]

Plants of *Hyoscyamus* were grown in the greenhouse under long day conditions for 1 month. Differentiated leaves were cut off and washed 3 times with tap water, and then surface sterilized by treatment with 4% NaOCl for 3 min. The hypochlorite was removed by 3 successive washes in sterile water. After the lower epidermal layer had been stripped away with forceps, the leaves were treated as described by MEYER[2], 1 h premaceration followed by 1,5 h maceration. The protoplasts were separated from unmacerated material by filtration through a plastic gauze and then sedimented by centrifugation at 50 *g* for 5 min. The sedimented protoplasts were washed 3 times with fresh salt solution and resedimented by centrifugation. All these solutions were adjusted at pH 5.2.

About 1 ml of protoplast suspension (Figure 1) was mixed with about 10 ml of liquid nutrient media described by DURAND et al.[3], Na_2EDTA and $FeSO_4 \times 7\ H_2O$

[1] Supported by a grant of the Bundesministerium für Forschung und Technologie, Bonn.
[2] Y. MEYER, Protoplasma *81*, 363 (1974).
[3] J. DURAND, I. POTRYKUS et G. DONN, Z. Pflanzenphysiol. *69*, 26 (1973).