Table 2. Upper Lethal Temperatures of Incubation in Degrees Centigrade as Function of Salinity and Oxygen (approximations)

Air saturation (per cent)	Fresh water	35‰ S	45‰ S	55‰ S	70‰ S
70	28·5	27·5	24·0	lethal over whole t ° C.-range	lethal over whole t ° C.-range
100	36·1	35·9	34·0	33·2	32·5
300	36·1	36·1	35·5	34·5	34·0

porature of incubation, that is, the lowest of the constant high temperatures at which 100 per cent of the embryos die previous to hatching, shifts considerably under different conditions of salinity and oxygen (Table 2). Lethal temperatures are lowest in all 5 salinities at 70 per cent and highest at 300 per cent air saturation. They decrease with increasing salinity; such a decrease is significantly less pronounced at 300 per cent saturation than at 70 and 100 per cent saturation respectively. A salinity of 85 parts per thousand proved to be lethal under all salinity–oxygen combinations used.

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[1] Baird, S. F., and Girard, C., *Proc. Acad. Nat. Sci. Phila.*, **6**, 387 (1853).
[2] Kinne, O., *Phys. Z.*, **33**, 288 (1960).
[3] Kinne, O., and Kinne, E. M., *Canad. J. Zool.* (in the press).

Regeneration of Limbs in Adult *Hymenochirus boettgeri*

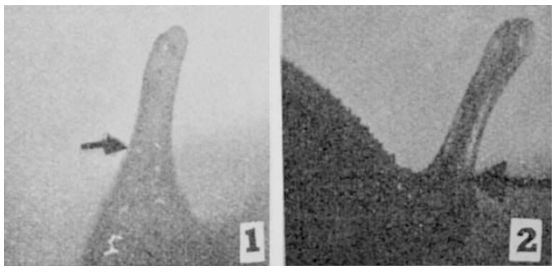
Although many vertebrates have retained to a considerable degree the ability to regenerate organs, adult anurans are generally believed to have lost the power to replace limbs or digits. Isolated cases of regeneration in anurans have been observed, but were considered simply as anomalous occurrences[1–3]. In 1932, Rostand[4] reported that adults of the African 'clawed toad', *Xenopus laevis*, regularly formed heteromorphic regenerates of amputated digits. This has been confirmed for limbs as well as digits by Gallien and Beetschen[5], Beetschen[6], Gitlin[7], and Skowron and Komala[8]. As part of an extensive analysis of regeneration in the anurans, the present communication reports the normal occurrence of regeneration in another African frog.

Limbs of the pipid *Hymenochirus boettgeri* were amputated just distal to the elbow and knee. Digits were also cut at different levels. The wounds were not trimmed, and healing was allowed to proceed naturally. The results are summarized in Table 1.

The limb regenerates took the form of thin rod-like outgrowths not exceeding 5 mm. in length (Figs. 1 and 2). Occasionally, digital outgrowths were observed at the distal end of the regenerate. The course of the regeneration appeared to be similar to that described for *Xenopus laevis*[6,8].

Table 1. Occurrence of Regeneration in Extremities of *Hymenochirus boettgeri*

	Forelimbs	Hind limbs	Fingers	Toes
Regeneration observed	8	6	8	10
No regeneration observed	1	1	2	2



Figs. 1 and 2. (1) 3-mm. fore-limb regenerate of *Hymenochirus boettgeri*. (2) 5-mm. hind-limb regenerate of *Hymenochirus boettgeri*. Arrows indicate point of amputation

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[1] Gadow, H., *The Cambridge Natural History: Amphibia and Reptilia* (1901).
[2] Woodland, W. N. F., *Quart. J. Micro. Sci.*, **65**, 100 (1921).
[3] Thornton, C. S., and Shields, T. W., *Copeia*, 40 (1945).
[4] Rostand, J., *C.R. Soc. Biol.*, **111**, 451 (1932).
[5] Gallien, L., and Beetschen, J. C., *C.R. Soc. Biol.*, **114**, 874 (1951).
[6] Beetschen, J. C., *Bull. Biol. Fr. and Belg.*, **86**, 88 (1952).
[7] Gitlin, G., *Bull. Res. Counc. Israel*, **5**, B, 307 (1956).
[8] Skowron, S., and Komala, Z., *Folia Biologica*, **5**, 53 (1957).

Influence of Tetracycline on Calcification in Normal and Regenerating Teleost Scales

Among the attributes manifested by tetracyclines administered to biological systems are an affinity for calcified tissues[1–4], the concomitant inhibition of calcification[3,4], and fluorescence under ultra-violet illumination. These properties have facilitated the investigation of growth and regeneration of scales in tetracycline-treated teleosts (*Fundulus heteroclitus*).

Adult fish (about 3 in. long) maintained in running sea-water at 21–22° C. were given daily intraperitoneal injections of 0·02 mgm. tetracycline ('Achromycin') per gm. body-weight in 0·1 ml. distilled water. Scales were plucked from the flank at the beginning of the experiment and were allowed to regenerate for 3 weeks. Some scales were then stained for calcium by the von Kossa technique, while others were mounted unstained and examined for tetracycline fluorescence under ultra-violet light. The combination of these two approaches made it possible to correlate the respective patterns of calcium and tetracycline deposition in normal and regenerated scales.

Control fish, injected daily with 0·1 ml. distilled water, regenerated normally calcified scales possessing several concentric growth rings. They stained heavily with silver nitrate (Fig. 1*a*) but exhibited no fluorescence under ultra-violet light. Regenerated scales from experimental fish, in contrast, were considerably less rigid than control regenerates, although their over-all dimensions were normal. The von Kossa stain revealed that such scales were only very lightly calcified in the central ringless areas and that what little calcification had occurred in the concentric rings was largely restricted to depositions immediately peripheral to each of the sculptured lines delineating one ring from the next (Fig. 1*b*). Under ultra-violet light the pattern of fluorescence, indicating the localization of tetracycline in the scale, was found to coincide with that of calcium deposition (Fig. 1*c*). The intensity of both the calcification and the fluorescence diminished progressively in the younger, more peripheral, rings.

Normal (non-regenerated) scales of tetracycline-treated fish were also examined under ultra-violet