

Influence of larval density on the predatory rate of *Gambusia affinis*

	Density of larvae offered/625 cm ²							
	5	10	25	50	75	100	125	150
Male	5.0 ± 0.00	10.0 ± 0.00	12.6 ± 4.08	13.8 ± 2.45	14.5 ± 2.29	14.8 ± 3.02	14.3 ± 1.04	14.0 ± 0.76
Female non-gestating	5.0 ± 0.00	10.0 ± 0.00	20.7 ± 3.25	22.7 ± 3.62	23.5 ± 3.90	23.6 ± 3.60	23.0 ± 1.24	23.3 ± 0.95
Female gestating	5.0 ± 0.00	10.0 ± 0.00	21.5 ± 3.94	22.8 ± 2.54	24.2 ± 1.55	24.5 ± 2.30	25.1 ± 0.78	24.9 ± 1.32

to predate for 10 h/day. All the experiments were conducted at a temperature of $25 \pm 2^\circ\text{C}$. The experiments in each series were repeated on 3 successive days and hence the performance of 5 individuals yielded a total of 15 observations in each series.

Results and discussion. The table represents the influence of density of 4th instar *Culex fatigans* mosquito larvae on the predatory rates of male, non-gestating and gestating female *Gambusia affinis*. From this it is evident that the fish consumed all the larvae when the prey density was 5 or 10. With further increase in density of larvae to 25/625 cm², neither male nor female fish predated all the larvae. This trend remained unaltered at further density levels. However, there was a significant difference between the predatory rates of males to females. Male *G. affinis* consumed a lower number of larvae as compared to the female, and this pattern of feeding remained unaltered at densities from 25 to 150 larvae/625 cm². The lower intake of males may be attributed to the smaller body weight of the male *G. affinis* (Katre⁶). It is also clear from the table that increase in density level beyond 25 larvae/625 cm² did not markedly alter the predatory rates of either male or female fish. Ware³ has also reported a similar predatory behaviour in the rainbow trout (*Salmo gairdneri*) in relation to the density of *Cragonyx* and *Hyaella*.

Figure 1 illustrates the average predatory rate of male, non-gestating and gestating *Gambusia affinis* at the different prey density levels. Larval densities of 5, 10, etc., markedly increase the predatory rate of *G. affinis* till a critical density (which in the present study is 23 larvae) is reached. Beyond this density level, the curve tends to flatten out and reaches an asymptote indicating

that there is no marked change in the predatory rates of fish with further increases in prey density. This type of predatory behaviour of *G. affinis* can be represented by an empirical equation:

$$N = \frac{N_{\max}}{1 + K \left(\frac{D_c}{D} \right)^2}$$

where:

N = Number of larvae predated at any density D ,

N_{\max} = Maximum number of larvae predated,

K = Experimental constant (0.75),

and D_c = 'Critical density' beyond which the predatory pattern of the fish becomes independent of larval density.

Under laboratory conditions, when fed on 4th instar *Culex fatigans* larvae, the dragonfly nymph *Mesogomphus lineatus* was also reported to display a similar relationship with the prey densities⁷. The data obtained for *M. lineatus* by Mathavan⁷ is plotted in figure 2. It may be seen that the present empirical equation obtained for *G. affinis* fits the data of Mathavan⁷ on *M. lineatus* very well, indicating that the predatory behaviour of *M. lineatus* in relation to the prey density remains similar to that of *G. affinis*.

With the help of the proposed model, at known values of N_{\max} and D_c , it is possible to predict the stocking rates of the biological agents for successful control of mosquito larvae in natural systems.

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Difference between frog and toad tadpoles in response of the keratinizing epidermis of the oral region to excess of vitamin A

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Summary. Rearing of the tadpoles of the frog, *Rana breviceps*, in solutions containing 2.5, 5, 7.5, 10 and 15 IU/ml vitamin A palmitate resulted in reduction or complete disappearance of the keratinized epidermal material over the jaws and of the horny labial teeth. The tadpoles of the toad, *Bufo andersonii*, however, were not affected in this way at all by exposure to even 20 and 30 IU/ml vitamin A in the rearing medium.

The role of vitamin A in differentiation and maintenance of epithelia as mucoid or keratinizing type is well known. Deficiency of this vitamin results in mucoid epithelia becoming keratinized, whereas its excess inhibits keratinization and induces mucous metaplasia and hyperplasia in epidermis and other epithelia¹⁻⁴. However, investigations of this nature have been concerned mainly with

avian and mammalian tissues, and the possible role of this vitamin in epithelial differentiation in lower vertebrates has remained neglected. In the amphibian larvae at least, vitamin A excess is reported to promote hyperplasia of the mucosal cells of the intestine. Feeding of large amounts of this vitamin to *Xenopus laevis* tadpoles was found to increase the number of goblet cells in the intes-

tinal epithelium, which secreted large amounts of mucus into the lumen of the alimentary canal^{5,6}. Greatly increased mucus secretion from the alimentary tract was also noticed in *Bufo andersonii* tadpoles reared in vitamin A solutions⁷. The epidermis of frog and toad larvae is generally mucoid, but in the mouth region it produces keratinized structures represented by rows of horny teeth of the labial fringe and the black hard ridges over the larval jaws. This oral armature is a purely larval feature and disappears at metamorphosis. During the larval life, however, as the teeth and ridges wear away due to use, they are continuously replaced by keratinization of epidermal cells. The present communication reports observations on the effect of vitamin A excess on these structures of the oral region in the tadpoles of a frog and a toad species. In the investigations described below, the experimental groups of tadpoles were reared in solutions of vitamin A palmitate (Arovit - Roche, India) and controls in water. The animals were transferred to fresh media every alternate day and maximally fed boiled spinach.

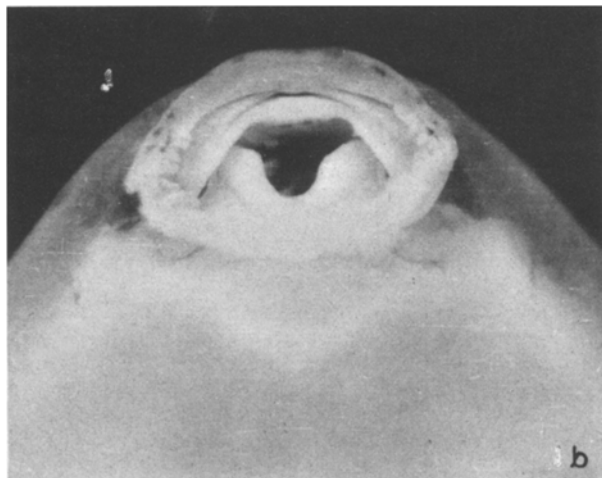
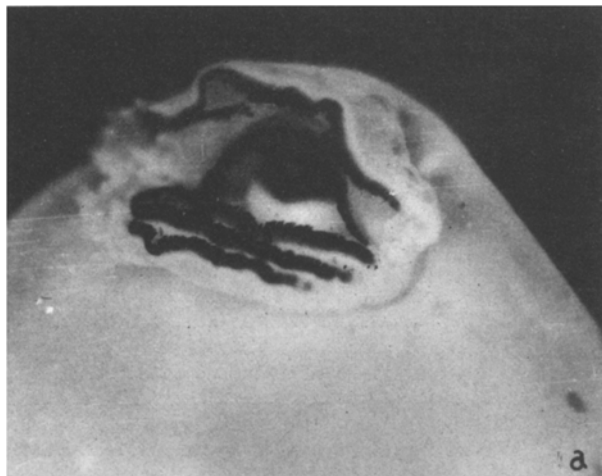
Materials and methods. In the first series of experiments, well-developed tadpoles of the frog, *Rana breviceps* (Schneider), and of the toad, *Bufo andersonii* (Boulenger), were maintained in 15 IU/ml solutions of vitamin A at 25°C for 12 days. Within 3 days it was noticed that the number of horny teeth on the labia in the treated *Rana*

tadpoles was less than in controls; in some cases an entire row of teeth was missing. By the end of 12 days, all the teeth and also the black keratinized epidermal ridges over the larval jaws had disappeared in every one of the 24 treated frog tadpoles (figure). The *Bufo* tadpoles, however, were not affected at all and there was no apparent difference between the oral armature of the control and treated larvae of this species.

In another series of experiments, groups of very young to older tadpoles of the *Bufo* species were exposed to still higher concentrations (20 and 30 IU/ml) of the vitamin continuously for a fortnight at 30–32°C. These concentrations caused much mortality especially among the younger tadpoles but had absolutely no effect on the keratinized epidermal structures of the mouth region in the toad larvae of any age.

In the third series of experiments, 1-day-old tadpoles of *Rana breviceps* were reared in solutions containing 1, 2.5, 5, 7.5, 10 or 15 IU/ml of vitamin A at room temperature (30–32°C) for 24 days. The effect on oral armature was found to be related to the amount of the vitamin in the rearing medium. The tadpoles exposed to 1 IU/ml were not affected at all, and those kept in 2.5, 5 and 7.5 IU/ml concentrations showed varying degrees of reduction in the number of labial teeth, the highest amount causing the most reduction. In the tadpoles exposed to 10 and 15 IU/ml vitamin A, all teeth in both upper and lower lips and also the horny material capping the jaws disappeared altogether.

Discussion. The above observations indicate a sharp difference among the anurans with respect to responsiveness of their epidermis to keratin-inhibiting influence of hypervitaminosis A. The keratinizing epidermis of the oral region of *Rana breviceps* tadpoles appeared to be highly sensitive to this influence, whereas that of *Bufo andersonii* larvae did not respond to even a much higher degree of hypervitaminosis A. It seemed that in the frog larvae excess of this vitamin inhibited replacement of horny teeth by stopping further keratinization of the underlying epidermis. In the adult stage, the skin of toads is tough and warty while that of frogs remains smooth and very mucoid. It would be interesting to investigate whether the adult skins of the 2 species also vary in response to vitamin A. For mammals it is known that the response of foetal skin to excess vitamin A in organ culture varies according to species, age and the region of the body from where the skin is taken^{8–10}.



Ventral views of the mouth region of the tadpoles of *Rana breviceps*. a Reared in water. b Reared in 15 IU/ml vitamin A palmitate solution for 12 days.

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