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Influence of distillery effluent on growth and metamorphosis of *Rana malabarica* (Bibron)

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Abstract. Increase in effluent concentration reduced the period of limb bud emergence and tail resorption; it also produced elevated values for length of limbs, tail and body weight of adult *R. malabarica*. Analysis of variance (Anova test) confirmed that time (week) has pronounced effect on morphological parameters ($P < 0{\cdot}05$) than treatment ($P > 0{\cdot}05$).

Keywords. Distillery effluent; *Rana malabarica*; growth; metamorphosis.

1. Introduction

To assess the effects of various pesticides and industrial effluents on aquatic life, toxicologists generally prefer fish and invertebrates and omit amphibians considerably. A few publications are available on amphibians especially for the effect of pesticides (Cook 1971; Dial 1976; Greenhouse 1976). Except one or two reports (*e.g.* Ghate *et al* 1978) no information is available on the impact of industrial effluents on amphibian tadpoles. The above authors have reported tail abnormalities, eye defects and odema in *Microhyla ornata* exposed to dye factory effluent but omitted the aspects of growth and metamorphosis in their studies. Since amphibians are the components of food webs in both terrestrial as well as aquatic communities, Porter and Hakanson (1976) stressed that preference should be given to amphibians for bioassay studies. The present investigation is a preliminary report dealing with the effect of distillery effluent* on growth and metamorphosis of *Rana malabarica*.

2. Material and methods

The tadpoles of *R. malabarica* (premetamorphic stage**) were collected from their natural habitat (Mundanthurai, Tamil Nadu) fed on boiled leaves of *Amaranthus spinosus* for 3 days and acclimatized to laboratory conditions. Twenty test individuals of equal body length and weight were recruited from the stock and divided into 4 series, each with 5 individuals. The first group of individuals were reared in dechlorinated tap water as control, while the remaining groups were exposed to different sublethal concentrations (0·03, 0·06 and 0·12%) of distillery effluent (Barnabas Xavier 1983). During the experimental period, fresh concentrations of effluent were prepared in 3 l of

* Courtesy Trichy Chemicals and Distilleries Ltd., Tiruchirapalli.

** Before the hind limb bud emergence.

water by mixing the required quantity of the effluent with tap water and supplied to the test individuals daily (Haniffa and Sundaravadhanam 1984). Boiled *A. spinosus* leaves were supplied *ad libitum* to the tadpoles every day. Weekly observations for the change in length of body, tail, hindlimb and forelimb in cm were made. Every week the tadpoles were weighed to 0·001 mg after blotting on a cloth towel (Hota and Dash 1981). All measurements were analysed to standard deviation whereas analysis of variance was attempted after Zar (1974). Calculations were made for correction factor, total sum of squares, summation due to week, summation due to treatment and mean square. F values were separately estimated for time effect (F_1) and treatment effect (F_2) on the length of limbs, tail and body and weight of *R. malabarica*. F value probability was taken from the Anova table (table 3, Snedecor and Cochran 1968).

3. Results and discussion

R. malabarica tadpoles reared in tap water took 84 days to complete metamorphosis. Among the effluent concentrations, individuals exposed to 0·12% took the shortest period of 50 days to complete metamorphosis (table 1). Body length and tail length increased during progressive metamorphic stages and after that the body length remained almost constant whereas tail showed a gradual decrease and was finally resorbed. Peak values of body length were noticed on 29th, 36th, 36th and 15th day in *R. malabarica* reared in tap water, 0·03%, 0·06% and 0·12% effluent respectively. Among all the test individuals, maximum body length of adults (4·8 cm) was noticed for the tadpole exposed to 0·03% followed by those reared in 0·06% (4·7 cm), 0·12% (4·4 cm) and tap water (4·4 cm). During the progressive metamorphic period, the tail length of tadpoles reared in tap water, 0·03%, 0·06% and 0·12% effluent increased from 4·9 to 5·4 cm, 5·2 to 6·2 cm, 5·5 to 5·8 cm and 5·5 to 6·2 cm respectively. Increase in effluent concentration produced a decrease in the period of tail resorption. Tail resorption was noticed much earlier (50 days) for tadpoles exposed to 0·12% followed by those reared in 0·06% (64 days), 0·03% (71 days) and tap water (85 days). Total length (body and tail) rapidly increased and reached the peak during the progressive metamorphic stage and after gradual decrease (retrogressive period) attained a constant value (table 1).

Hind limb bud emergence was much earlier (15th day) for tadpoles exposed to 0·12% followed by those reared in 0·06%, 0·03% (22nd day) and tap water (29th day). Increase in effluent concentration produced elevated values for the final length of hind limb, but the period to attain the maximum length was constant (35 days) at all concentrations except in tap water. Fore limb bud emergence was also quicker (29th day) for tadpoles exposed to 0·12%, when compared with those reared in 0·06% (43rd day), 0·03% (57th day) and tap water (71st day). The difference in fore limb length was rather perceived high as a function of effluent concentration taking almost the same duration (28 days) at the respective concentrations (table 2).

Figure 1 shows the change in body weight of *R. malabarica* reared in tap water and effluent. The control test individuals increased from an initial weight of 7·1 g to 8·5 g on the 36th day and after that slowly decreased to 6·3 g on the 78th day. The corresponding changes during the progressive metamorphic period for those exposed to 0·03, 0·06 and 0·12 effluent were from 7·7 to 9 g, 6·8 to 8·5 g and 7·1 to 7·6 g on the 29th day respectively. The final body weight of *R. malabarica* reared in tap water was much less

Table 1. Influence of distillery effluent on body length and tail length of *R. malabarica*.

	Body length (cm)				Tail length (cm)				Total length (cm)			
Day	Control	0·03%	0·06%	0·12%	Control	0·03%	0·06%	0·12%	Control	0·03%	0·06%	0·12%
1	3·8±	4·1±	4·2±	4·1±	4·9±	5·2±	5·5±	5·5±	8·7±	9·3±	9·7±	9·6±
	0·15	0·17	0·25	0·51	0·15	0·21	0·15	0·51	0·30	0·38	0·40	1·02
8	3·8±	4·2±	4·3±	4·3±	5·0±	5·2±	5·6±	5·7±	8·8±	9·4±	9·9±	10·0±
	0·15	0·27	0·25	0·56	0·15	0·17	0·13	0·15	0·30	0·44	0·38	0·71
15	4·0±	4·3±	4·4±	4·4±	5·2±	5·8±	5·7±	6·2±	9·2±	10·1±	10·1±	10·6±
	0·15	0·21	0·26	0·26	0·12	0·25	0·20	0·21	0·27	0·46	0·46	0·47
22	4·2±	4·4±	4·5±	4·4±	5·4±	6·0±	5·8±	5·7±	9·6±	10·4±	10·3±	10·1±
	0·15	0·21	0·26	0·26	0·12	0·25	0·20	0·23	0·27	0·46	0·46	0·49
29	5·0±	4·6±	4·6±	4·4±	5·2±	6·2±	5·4±	3·7±	10·2±	10·8±	10·0±	8·1±
	0·13	0·25	0·62	0·51	0·30	0·15	0·70	0·15	0·43	0·40	1·32	0·66
36	4·4±	4·8±	4·7±	4·4±	4·4±	5·7±	4·1±	2·0±	8·8±	10·5±	8·8±	6·4±
	0·13	0·30	0·15	0·51	0·58	0·74	1·60	0·21	0·71	1·04	1·75	0·72
43	4·4±	4·8±	4·7±	4·4±	3·8±	4·2±	3·1±	0·9±	8·2±	9·0±	7·8±	5·3±
	0·15	0·39	0·53	0·15	0·31	0·23	1·10	0·02	0·46	0·62	1·63	0·17
50	4·4±	4·8±	4·7±	Adult	3·1±	3·2±	0·8±	Adult	7·5±	8·0±	5·5±	Adult
	0·25	0·39	0·26		0·61	0·20	0·14		0·86	0·59	0·40	
57	4·4±	4·8±	4·7±		2·4±	2·0±	0·6±		6·8±	6·8±	5·3±	
	0·15	0·36	0·15		0·81	0·23	0		0·96	0·59	0·15	
64	4·4±	4·8±	Adult		1·6±	0·9±	Adult		6·0±	5·7±	Adult	
	0·45	0·50			0·25	0·01			0·70	0·51		
71	4·4±	Adult			1·2±	Adult			5·6±	Adult		
	0·51				0·24				0·75			
78	4·4±				0·8±				5·2±			
	0·56				0				0·56			
85	Adult				Adult				Adult			

Each value represents the average performance of 5 individuals and data reported as ± indicate the standard deviation.

Table 2. Influence of distillery effluent on length of hind limb and fore limb of *R. malabarica.*

	Hindlimb length (cm)				Forelimb length (cm)			
Day	Control	0·03%	0·06%	0·12%	Control	0·03%	0·06%	0·12%
1	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
15	0	0	0	0·5 ± 0·06	0	0	0	0
22	0	1·1 ± 0·23	0·8 ± 0·20	1·2 ± 0·32	0	0	0	0
29	1·0 ± 0·14	1·8 ± 0·40	1·3 ± 0·26	1·9 ± 0·35	0	0	0	0·2 ± 0·05
36	1·2 ± 0·15	2·0 ± 0·06	2·1 ± 0·05	2·5 ± 0·26	0	0	0	0·6 ± 0·10
43	1·9 ± 0·12	2·6 ± 0·13	2·4 ± 0·25	2·8 ± 0·05	0	0	0·1 ± 0	1·1 ± 0·06
50	2·5 ± 0·06	2·7 ± 0·05	2·7 ± 0·05	2·8 ± 0·06	0	0	0·6 ± 0·15	1·7 ± 0·10
57	2·5 ± 0·06	2·7 ± 0·06	2·7 ± 0·04		0	0·1 ± 0	1·1 ± 0·12	
64	2·5 ± 0·06	2·7 ± 0·06	2·7 ± 0	0	0	0·6 ± 0·06	1·7 ± 0·10	
71	2·5 ± 0·06	2·7 ± 0·06	0		0·3 ± 0·07	1·1 ± 0·06		
78	2·5 ± 0·22	2·7 ± 0·32			0·8 ± 0·87	1·7 ± 0·05		
85	2·5 ± 0·04				1·8 ± 0·17	1·7 ± 0·06		

Each value represents the average performance of 5 individuals and data reported as ± indicate the standard deviation.

(6·3 g) when compared with those reared at 0·03% (8·2 g), 0·06% (6·8 g) and 0·12% (6·8 g; figure 1). At 0·03% maximum body weight was noticed as two peaks on the 29th and 50th day whereas the same was noticed as only one peak on the 29th day and 22nd day for those exposed to 0·06% and 0·12% effluent respectively. These two peaks correspond to the times of necrosis of old and build up of new tissues in relation to formation of gut and fore and hind limbs and lungs and resorption of gills, old gut and tail. The reason for the elevation of the peak from the control in relation to 0·03% and its depression in the case of 0·06% and 0·12% is under separate investigation.

According to Hota and Dash (1981) body size in poikilotherms is controlled by differences in environmental conditions such as food availability and larval density. The body size and growth rate of *Rana* larvae are functions of amount of available food (Wilbur 1977), density (Brockelman 1969; Wilbur and Collins 1973; De Benedicts 1974) and temperature (Hota and Dash 1981). As already cited, most of the reports on growth and metamorphosis of amphibians deal with influence of food limitation and density. The few reports which are available on the impact of pesticides (*e.g.* Cook 1971; Greenhouse 1976) or industrial effluents on amphibian larvae (*e.g.* Ghate *et al* 1978) mainly deal with teratogenic and embryological properties and do not reveal any information on growth and/or metamorphosis. The toxic agents which inhibit or

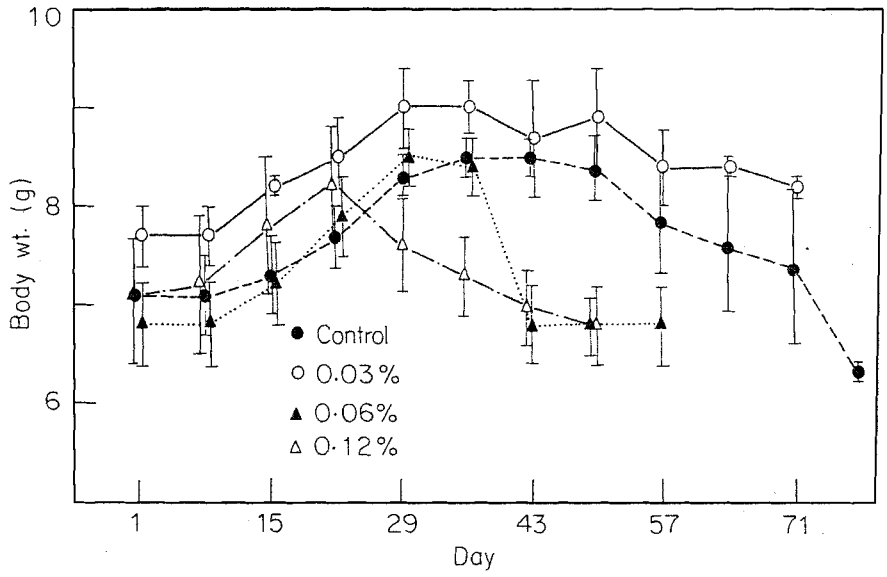

Figure 1. Influence of distillery effluent on body weight of *R. malabarica*. Each value represents the average performance of 5 individuals.

Table 3. Analysis of variance: Influence of time (week) and treatment (effluent concentration) on length of limbs, tail and body and weight of *R. malabarica*

	Time effect		Treatment effect	
Character	*F*1 value	Probability	*F*2 value	Probability
Adult weight	2·917	$P < 0·01$	0·434	$P > 0·05$
Body length	5·857	$P < 0·01$	10·857	$P < 0·01$
Tail length	11·729	$P < 0·01$	0·436	$P > 0·05$
Fore limb	0·857	$P > 0·05$	0·357	$P > 0·05$
Hind limb	2·165	$P < 0·05$	0·410	$P > 0·05$

$P < 0·05$ significant
$P > 0·05$ not significant.

modify the development of the animal, are likely to be detrimental even if the adults of a particular species are apparently unaffected.

Limb bud emergence and tail resorption occurred earlier in tadpoles exposed to 0·12% effluent (tables 1 and 2). According to Haniffa and Sundaravadhanam (1983), *Barbus stigma* exposed to lower concentrations of distillery effluent showed more food consumption and growth than those exposed to tap water. The above authors suggested that the chemical constituents at lower concentrations enhanced the growth through food consumption. Barnabas (1983) also confirmed this by reporting decrease in the duration of metamorphosis and an increase in body weight of *R. malabarica* exposed to lower concentrations (up to 0·12%) and vice versa, at higher concentrations (0·15% and above). Hence it is possible to suggest that the increase in body weight of

R. malabarica at lower concentrations (up to 0·12%) could be due to more food consumption.

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