Crowding effect on growth and metamorphosis of the frog *Microhyla ornata* (Dumeril and Bibron)

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***Microhyla ornata* tadpoles were reared at different densities. Crowding caused interference, leading to competition for food and brought shifts in the duration of metamorphosis, lowering of body weight at metamorphic climax and on survivability. The mortality rate and absolute mortality of tadpoles were a function of the degree of crowding. The degree of crowding affected the transformation size within a range of 52 to 130 mg.**

THE phenotypic plasticity in the growth of conspecific amphibian tadpoles is a function of tadpole density[1–4], absolute size of the habitat[5], food quality, availability of oxygen in water[6,7] and some other environmental factors. Crowding reduces larval survivorship, growth rate and body size at metamorphosis and increases the length of larval period[1–3,8–16]. Pandian and Marian[17–19] showed that the rates of feeding, larval density and food levels can be effectively used to predict growth rates, larval duration and energy cost of metamorphosis. These responses are clearly observed in tadpoles of many frogs than in the tadpoles of toads, which normally exhibit schooling behaviours[16,20].

Microhyla ornata is a tropical frog found in India, Sri Lanka, South-east Asia and South-China. This tiny frog feeds mainly on termites and small insects. In breeding males, the throat is distinctly black. The breeding season of this frog in India commences with the onset of monsoon rains usually in the mid-June and continues throughout the monsoon period of mid-June to mid-October. The mean (± SD) snout–vent length (SVL) of a field population of breeding males and females respectively are 18.8 ± 0.73 mm ($n = 25$) and 21.1 ± 2.05 mm ($n = 25$). The mean body weight (BW) of breeding males and females respectively are 656.6 ± 50.78 mg ($n = 25$) and 993.0 ± 123.9 mg ($n = 25$). The average clutch size is 225.0 ± 89.9 (range 80–361, $n = 12$) with hatching duration of 48 h and hatching success of 98%. This frog spawns in temporary monsoon pools and hence crowding of tadpoles is a common occurrence.

In the present communication, we examine the effect of larval density and crowding on survivorship, growth, metamorphic size and larval duration of *M. ornata* tadpoles.

Fresh spawns of *M. ornata* were collected from temporary rain water pools in the early morning hours and kept in the laboratory (air temperature 27.9 to 29.7°C) for hatching. Larvae hatched from the spawns in 36–50 h ($\bar{X} = 48$ h). Immediately after hatching they were mixed thoroughly in an aquarium and samples from them were taken for the treatment of crowding effect. The staging of larvae was done according to the method of Taylor and Kollros[21]. The emergence of at least one of the forelimbs was taken as an index of onset of metamorphosis, and the little frogs with emergent forelimbs were shifted to containers providing an amphibious environment after recording their individual weights. Village well water conditioned with $Na_2S_2O_3$ at 8 mg/4.5 l and filtered, was used as the culture medium[22].

Populations of six densities (5, 10, 20, 40, 80, 160) were reared with the same food ration (sufficient for higher density populations as there was left-over food at the end of the day in three replicates in culture tray (45 × 30 × 25 cm with 2 l water)). However, higher densities (40, 80 and 160/tray) caused crowding interference which might have led to competition for food. Populations were examined every 48 h interval to determine the survival and growth rate. The body weight of tadpoles in a group of five individuals in triplicate was measured (± 1 mg) using a chainometric balance. The ammonia level in the culture tray was determined spectrophotometrically[23] and the values did not indicate to have caused fouling of water.

A mixed diet was prepared by combining *Amaranthus* sp. leaves, boiled egg yolk and cooked and minced goat meat in the ratio of 5 : 1 : 1 (ref. 2) respectively. From the day 3 of hatching, food was supplied at the rate of 1 g per tray for each population density up to day 15 and the food quantity was increased to 2 g from day 16 onwards till the end of the experiment. Since water of the culture tray was changed at 24 h interval to avoid fouling of the culture medium, water quality was not considered a limiting factor. There was no bad smell or observable fouling of water. All the data were statistically analysed according to the methods of Snedecor and Cochran[24] and Sokal and Rohlf[25]. In low density laboratory populations (5, 10, 20), the larval growth pattern was S-shaped and larvae metamorphosed on the average in 28–36 days with average body weight of 96 to 99 mg and average S–V length of 7.16 to 7.79 mm. On days 10, 17 and 24, during the linear phase of larval growth, the body weight of all the survivors in three replicates of each density was determined (Table 1). A three-way factorial analysis of variance was used to examine the effects of initial density on growth and time of metamorphosis. The analysis shows that initial density has a significant effect ($P < 0.001$) on growth rate and metamorphic time. The S-shaped growth curves (Figure 1) show a distinct right-hand shift with the increase in crowding. This suggests that the growth rate of an individual is inhibited by the remaining members of the population. Keeping food level and space constant, increase in initial population density brought about competition, resulting in slower growth rate at higher densities. Tadpoles at 80

and 160 density/tray respectively tend to metamorphose in a small size (average 86.96 mg, range = 70 to 105 mg and average 84.78 mg, range = 55 to 105 mg) and at a later date (average of 85.84 days and 108.78 days) than the rapidly growing individuals from lower density populations (density 5, 10, 20 and 40). Hence, the effects of initial density are reflected in larval duration and body mass at metamorphosis. This confirms the patterns found in some other anuran species[2,3,16,26].

Body weight at metamorphic climax was analysed by multiple regression analysis with time as the covariate of initial density. The regression line is described by the equation $M = 106.23 + (0.07368)\, N_0 + (-0.31358)\, t$, ($r^2 = 0.95$, $F = 11.638$, d$f = 2, 3$, $p < 0.05$), where M is the body mass at metamorphic climax, N_0 the initial density and t the average time (days) taken for metamorphosis. This indicates that growth is dependent on initial density and is reflected in body weight at metamorphosis.

Table 1. Analysis of variance of body mass of *M. ornata* larvae as a function of initial density (three replicates)

Time in days	Mean body mass (mg) with ± SD					
	Density per tray					
	5	10	20	40	80	160
10	20.58 ± 2.38	28.13 ± 1.64	31.0 ± 2.16	30.0 ± 1.63	21.66 ± 2.35	18.66 ± 1.88
17	71.0 ± 6.48	68.0 ± 5.35	66.7 ± 6.5	60.66 ± 11.32	55.33 ± 7.4	34.0 ± 3.55
24	118.21 ± 5.61	126.0 ± 7.25	124.8 ± 8.82	90.93 ± 13.87	75.63 ± 10.23	49.86 ± 1.63

Analysis of variance

Source	d*f*	SS	MS	F	P
Density	5	10929.75	2185.95	56.48	$p < 0.001$
Time	2	46684.62	23342.3	603.16	$p < 0.001$
Replicate	2	74.9	37.45		
Density × time	18	6037.26	335.4	8.66	$p < 0.001$
Density × replicate	10	1328.88	132.9	3.43	ns
Time × replicate	4	78.41	19.6		
Density × time × replicate	20	773.43	38.7		
Total	61	65907.25			

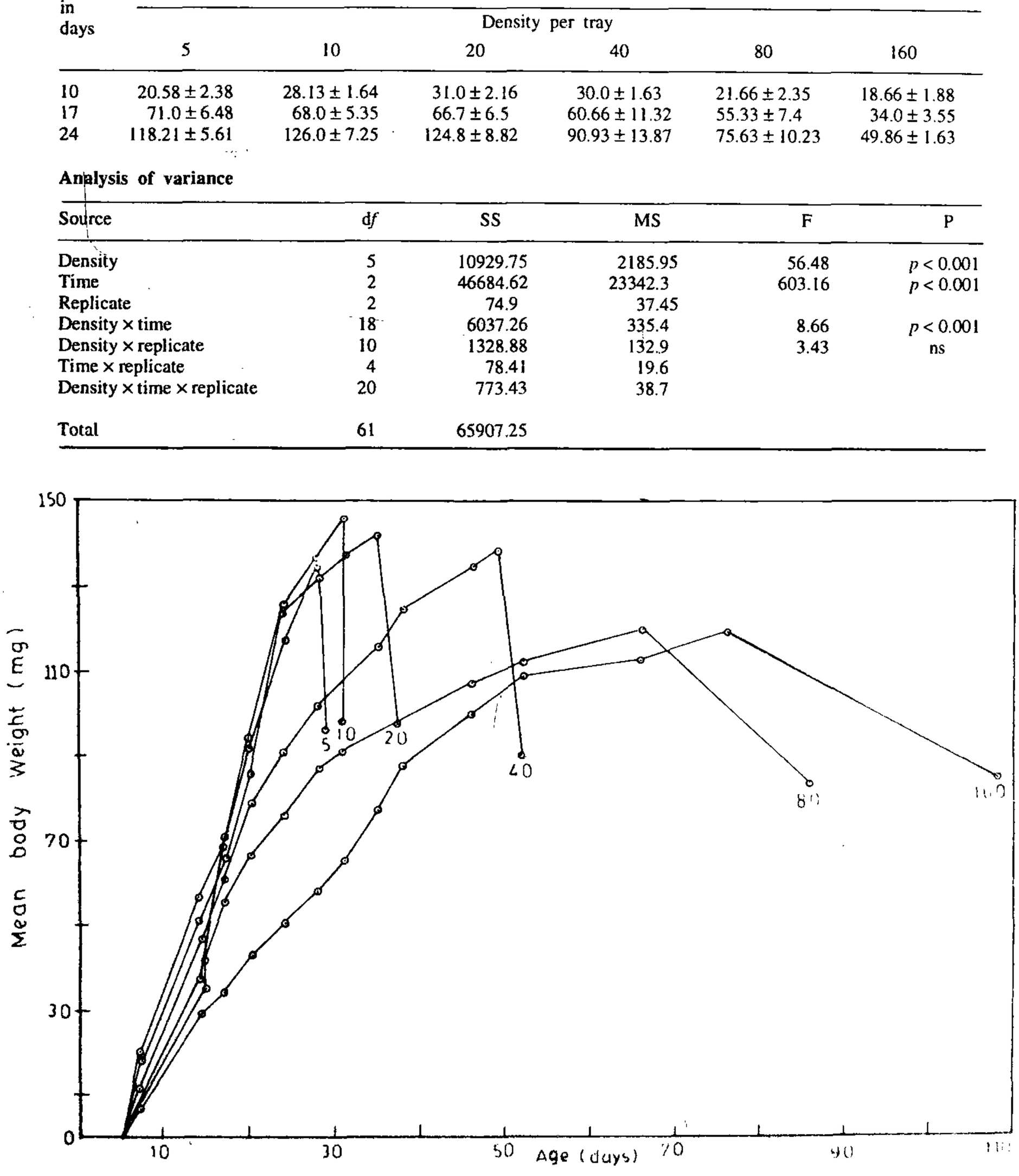

Figure 1. Density-dependent growth rate of *Microhyla ornata* larvae (Numbers indicate no. of tadpoles per tray).

The regression of body weight at metamorphosis on initial density with time as the covariate is highly significant ($F = 11.638$, $p < 0.05$).

The body weight values (mg/larva) at which larvae reached metamorphic climax (MC) stage in different density treatments are 82.0 to 110.0, 95.0 to 107.5, 69.0 to 130.0, 52.5 to 110.0, 70.0 to 105.0, 55.0 to 105.0 in density of 5, 10, 20, 40, 80 and 160 respectively. The minimum and maximum body weight respectively for metamorphosis was 52.5 mg and 130 mg irrespective of density treatments. The larvae did not metamorphose unless they attained ~ 52 mg body weight. The body weight of tadpoles at MC in different density treatments was found to be highly significant ($F = 4.26$, $p < 0.05$). Thus a threshold body size of ~ 52 mg was necessary for metamorphosis to occur.

Mortality rates were computed using the equation $N_t = N_0 e^{-mt}$, where N_t is the number of larvae metamorphosed, N_0 the initial density, m the mortality rate and t the average time (days) taken for metamorphosis. Table 2 gives the mortality rates and standard error of the mean from the data of three replicates. A visual inspection of these mortality rates and their standard errors suggests that mortality rate and absolute mortality are closely related to initial density of the population. This is a deviation from earlier findings[2,22]. Results from Bartlett's test (adjusted $\chi^2 = 1.66$, $df = 5$, p (ns)) showed that variances among replicates at different densities are homogeneous and it favours the hypothesis of homogeneity of slopes. This indicates that mortality rate is dependent on crowding.

Previous workers[2,27] have found that the proportion of anuran larvae that metamorphosed to juvenile frogs was a function of initial density and fitted a semi-logarithmic model: $l_n P_m = -SN_0$, where P_m is the proportion of the population completing metamorphosis and N_0 the initial density. The regression analysis ($F = 33.22$, $p < 0.001$) of $l_n P_m$ with N_0 is significant. Mean absolute mortality per replicate (Table 2) shows that there is high mortality at the highest density population and survivability of larvae is a function of density.

The density treatment has highly significant effect on days to metamorphosis ($F = 68.7$, $p < 0.001$). The metamorphic duration from lower to higher density treatment (28.92, 31.15, 36.92, 52.28, 85.85 and 108.78 days) as the density increased from 5, 10, 20, 40, 80 to 160/tray. A multiple analysis of variance (Table 1) shows that the days required to metamorphose are statistically significant ($F = 603.16$, $p < 0.001$) and is also affected by the interaction of density and length of larval period ($F = 8.66$, $p < 0.01$) which is also statistically significant. Hence, it is interpreted that crowding affects the metamorphic duration in *M. ornata* tadpoles. Metamorphic duration and number of survivors of each density are positively correlated ($r = 0.95$).

Table 2. Mortality rate in laboratory populations of *Microhyla ornata* larvae

Initial density	Mortality rate (regression ± SE)	Mean absolute mortality/replicate
5	0.00514 ± 0.00209	0.67
10	0.00478 ± 0.00195	1.33
20	0.00289 ± 0.00071	2.00
40	0.00448 ± 0.00044	8.33
80	0.00770 ± 0.00028	38.67
160	0.01019 ± 0.00093	106.33

In our investigations we found that food per individual larva and space in the lab culture medium decreased with the increase in larval density, a situation nearer to crowding phenomenon in nature. The result of this study indicates that crowding causes interference competition which has led to competition for food. The size-specific competition in *M. ornata* larvae has resulted in shifts at the time of metamorphosis, affected the mortality rate and the threshold value in body weight at metamorphosis with increasing crowding. Although this finding largely confirms the models discussed for some *Rana* species[1,2], yet it differs with regard to survivability factor and indicates that both mortality rate and absolute mortality are the functions of initial larval density and in this regard, it differs from the model given on *Bufo* species[16,20], which exhibit schooling behaviour.

1. Wilbur, H. M. and Collins, J. P., *Science*, 1973, **82**, 1305–1311.
2. Dash, M. C. and Hota, A. K., *Ecology*, 1980, **61**, 1025–1028.
3. Mishra, P. K. and Dash, M. C., *Alytes*, 1984, **3**, 163–172.
4. Hensley, F. R., *Ecology*, 1993, **74**, 2405–2412.
5. Pearman, P. B., *Ecology*, 1993, **74**, 1982–1991.
6. Mohanty, S. N. and Dash, M. C., *Aquaculture*, 1986, **51**, 89–96.
7. Dash, M. C. and Mahapatro, B. K., *J. Ecobiol.*, 1990, **2**, 67–76.
8. Brockelman, W. Y., *Ecology*, 1969, **50**, 632–644.
9. Shvarts, S. S. and Pyastolva, O. A., *Ekologiya*, 1970, **1**, 77–82.
10. Smith-Gill, S. J. and D. E., *Am. Nat.*, 1978, **112**, 557–570.
11. Collins, J. P., *Ecology*, 1979, **60**, 738–749.
12. Hota, A. K. and Dash, M. C., *Oikos*, 1981, **37**, 349–352.
13. Pyastolva, O. A. and Ivanova, N. L., *Herpetological Investigations in Siberia and the Far East*, Academy of Sciences in the USSR, Zoological Institute, Leningrad, USSR, 1981, pp. 87–91.
14. Travis, J., *Evolution*, 1983, **37**, 496–512.
15. Sokal, A., Litoria, E., *Copeia*, 1984, 868–873.
16. Mahapatro, B. K. and Dash, M. C., *Alytes*, 1987, **6**, 88–89.
17. Pandian, T. J. and Marian, M. P., *Physiol. Zool.*, 1985, **58**, 538–552.
18. Pandian, T. J. and Marian, M. P., *Copeia*, 1985, **5**, 653–662.
19. Pandian, T. J. and Marian, M. P., *Physiol. Behav.*, 1985, **35**, 867–872.
20. Wassersug, R. J. *et al.*, *Behav. Ecol. Sociobiol.*, 1981, **9**, 15–22.
21. Taylor, A. C. and Kollros, J. J., *Anat. Rec.*, 1946, **94**, 7–23.
22. Nace, G. W. and Richards, C. M., Carolina Tips XXXV, No. 12, Burlington, North Carolina, USA, 1972, 45–46.
23. Kaplan, A., in *Methods of Biochemical Analysis* (ed. Glick), John Wiley, New York, 1969.
24. Snedecor, G. W. and Cochran, W. G., *Statistical Methods*, Oxford and IBH, New Delhi, 6th edition.
25. Sokal, R. R. and Rohlf, F. J., *Biometry: The Principles and Practice of Statistics in Biological Research*, W. H. Freeman Co., San Francisco, 1969.
26. Wilbur, H. M., *Ecology*, 1976, **57**, 1289–1296.
27. Wilbur, H. M., *Ecology*, 1977, **58**, 196–200.

Received 23 November 1995; revised accepted 6 February 1996