NOTE

EFFECT OF LARVAL DENSITY ON DEVELOPMENT AND METAMORPHOSIS IN TWO ANURAN SPECIES (BUFONIDAE AND LEPTODACTYLIDAE) OF THE PATAGONIAN STEPPE

FABIÁN GASTÓN JARA [1-2]
CARMEN ADRIA ÚBEDA [1]
MARIANA LOZADA [1-2]

[1] Centro Regional Bariloche, Universidad Nacional del Comahue, Unidad Postal Universidad, (R8400FRF) Bariloche, Río Negro, Argentina

[2] Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

fjara@crub.uncoma.edu.ar
cubeda@bariloche.com.ar
mlozada@crub.uncoma.edu.ar

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Various studies have shown that the survival and development of amphibian larvae are controlled, among other factors, by their density (*e.g.* Rose, 1960; Wilbur, 1977; Dash and Hota, 1980; Semlitsch and Caldwell, 1982). In many anuran species, an increase in density inhibits larval development (*e.g.* Rose, 1960; Licht, 1967). Within a population, this inhibitory phenomenon produces large and small larvae, a reduction in survivorship, lengthening of the larval period and smaller metamorphs (*e.g.* Licht, 1967; Dash and Hota, 1980).

In Patagonia, there are some anuran species with seasonal, relatively short larval development occurring in typically temporary environments. The two most widely distributed species in the arid extra-Andean Patagonia are *Pleurodema bufoninum* Bell 1843 (Leptodactylidae) and *Bufo spinulosus papillosus* Philippi 1902 (Bufonidae). The larvae of both species develop during spring and the beginning of summer, and may do so in the same bodies of water (temporary or semi-permanent). The traits of these two species and the environments they inhabit are appropriate for conducting a comparative study of the effects of larval density.

The aim of this study was to assess the effect of larval density on larval development in these two species through an experimental study to investigate the effect of larval density, during larval development, on developmental stages, size of larvae and size of newly-metamorphosed juveniles and, if either of the species show inhibition, explore whether it is reversible.

Eggs were collected from bodies of water located in the valley of the Ñireco stream (41°10'52.3" S; 71°19'13.6" W), 906 m elev., near the town of San Carlos de Bariloche (Río Negro, Argentina). One clutch for each species was employed in order to reduce genetic variability (e.g. Kehr, 1987). The clutches were transferred to the laboratory and were placed in containers of tap water until hatching. Larval stages were determined following Gosner (1960).

To determine the effect of density on larval development the following experiment was carried out. Larvae at stage 22 were placed in trays (experimental units), at three different densities (treatments), with four replicates each, using a random design. Three treatments were established: 1) low density with 10 larvae per tray, 2) medium density with 20, and 3) high density

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Species	Treatment = individual per tray	CV of total length (larvae)	Survivorship percentage (larvae)	Total length (larvae)	Mass (larvae)	Length (juvenile)	Time to complete metamorphosis
Pleurodema bufoninum	10	11.03	100 a	38.40 a (31.12-44.94)	522.50 a (125-1261)	16.87 a (14.47-19.94)	92.75 a (83-99)
	20	23.85	91.25 b (94.15-100)	35.56 a (15.53-51.98)	505.00 a (27-1099)	15.46 b (13.19-18.55)	102.42 a (102-125)
	50	28.78	78.50 c (78-90)	30.57 b (12.41-50.88)	312.25 b (17-1412)	15.17 b (9.57-20.18)	162.88 b (144-176)
Bufo spinulosus papillosus	10	5.70	100 d	32.98 c (28.06-36.57)	362.50 c (304-432)	10.91 c (9.31-12.43)	88.75 c (88-90)
	20	8.39	86.25 e (85-90)	29.71 d (23.8-33.63)	245.00 d (175-321)	10.57 c (8.69-12.73)	137.25 d (122-149)
	50	9.12	82.00 e (78-86)	26.63 e (20.57-33.28)	237.50 d (104-482)	10.37 d (8.17-10.56)	212.75 e (193-226)

Table 1. Mean coefficients of variation (CV) per treatment of larval total length, mean larval survivorship (percentage), mean total length (mm) and mean mass (mg) when four-limb stage appear in each treatment, juvenile mean length (mm) and time (days) to complete metamorphosis from the beginning of the experiment up to the moment the last individual ended metamorphosis in each trail. Minimum and maximum rank values in brackets. N = 4 replicates per treatment. The different letters indicate significant differences (Scheffé test, $P < 0.05$).

with 50. Due to the differential size of the two larval species, two tray sizes were selected: 12 x 17 x 5 cm for *B. spinulosus papillosus*, containing 600 ml of water and 24 x 17 x 5 cm for *P. bufoninum*, containing 1200 ml. All larvae were fed *ad libitum* on boiled lettuce and kept in the laboratory under homogeneous lighting and temperature conditions (19-22 °C).

Every 14 days we recorded larval length and developmental stage. We recorded total length, with a digital caliper, and survivorship per tray. For each species, when in any tray, the first larval stage with four limbs developed (stage 42) all larvae were measured, weighed and their developmental stage recorded. Mass was recorded using an electronic analytical scale.

To determine whether density affects size of metamorphosed individuals, all larvae were allowed to complete larval development (stage 46). Each newly metamorph was measured (SVL) and days to reach metamorphosis were recorded.

Two-way ANOVA (species x density) was performed to determine the effect of density on each variable (length, mass, developmental stage, and time to metamorphosis). Scheffé Test was performed (Steel and Torrie, 1988) Larvae length coefficients of variation (CV) were calculated to compare density effect on both species.

To explore whether the inhibitory effect on larval development is reversible, a second experiment was conducted. From the high density trays, the smallest individuals and the least developed were subsequently reared individually (*i.e.* one larva per tray, N = 6 for *P. bufoninum*, mean length 18.92 mm and N = 5 for *B. spinulosus papillosus* mean length 22.48 mm) under

the same conditions as in the original experiments. To obtain the percentage of reversibility of inhibition, all larvae were allowed to complete their larval development (stage 46). Total length of each juvenile was measured and survivorship was recorded.

For *P. bufoninum*, after 56 days of development (density experiment), individuals with four limbs appeared in low and high density treatments (Fig. 1a). For *B. spinulosus papillosus*, after 81 days, the first stages with four limbs appeared in the low density treatment (Fig. 1b). Density increase significantly reduced larval survivorship for both species ($F_{2,18}$ = 78.29, P < 0.001, N = 24), but no difference was observed between species ($F_{1,18}$ = 0.15, P > 0.05, N = 24) and the interaction between factors was significant ($F_{2,18}$ = 3.64, P < 0.05, N =24; Table 1). For both species, density increase reduced larval length ($F_{2,18}$ = 68.49, P < 0.001, N = 24). Density affected differently mean length of both species ($F_{1,18}$ = 104.47, P < 0.001, N = 24) and interaction between factors was not significant ($F_{2,18}$ = 0.28, P > 0.05, N = 24). *B. spinulosus papillosus* length was significantly different in all treatments, while for *P. bufoninum* only the high density treatment differed significantly (Table 1).

Density increase reduced mean mass ($F_{2,18}$ = 29.6, P < 0.001, N = 24), and affected both species differently ($F_{1,18}$ = 84.88, P < 0.001, N = 24). Moreover, interaction among factors was significant ($F_{2,18}$ = 8.94, P < 0.01, N = 24). For *P. bufoninum*, high density treatment differed from the other two, while for *B. spinulosus papillosus* low density treatment differed significantly (Table 1).

The percentage composition of developmental stages was affected by density (Fig. 2) ($F_{2,18}$ = 34.07, P < 0.001, N = 24), and affected both species differently ($F_{1,18}$ = 65.20, P < 0.001, N = 24). For *P. bufoninum*, density increase widened developmental stage range, reducing the proportion of larvae in advanced stages. Conversely, for *B. spinulosus papillosus*, a major synchronic delay in development was observed (Fig. 2b). Interaction between factors was significant ($F_{2,18}$ = 10.53, P < 0.001, N = 24).

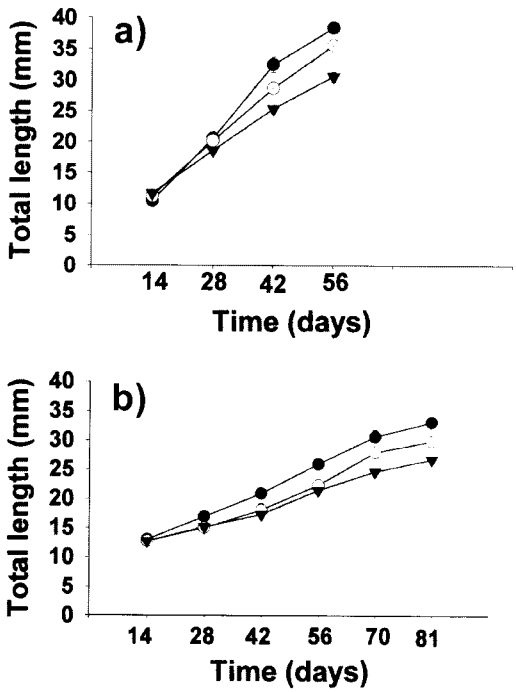

Figure 1. Mean total length (± SE) in larvae of a) *Pleurodema bufoninum* and b) *Bufo spinulosus papillosus* in three density treatments: 10 (dark circles), 20 (empty circles) and 50 (dark triangles) individuals per tray.

Density significantly affected time to complete metamorphosis ($F_{2,18}$ = 168.27, P < 0.001, N = 24), and did not affect both species differently ($F_{1,18}$ = 0.3, P > 0.05, N = 24). Additionally, interaction between factors was significant ($F_{2,18}$ = 12.11, P < 0.001, N = 24). For *P. bufoninum,* time was significantly lower in the low and medium density treatments (Table 1). For *B. spinulosus papillosus* time was different in all treatments (Table 1), being greater when larvae were reared at greater densities.

Density affected juvenile body length ($F_{2,18}$ = 21.21, P < 0.05, N = 24), affec-

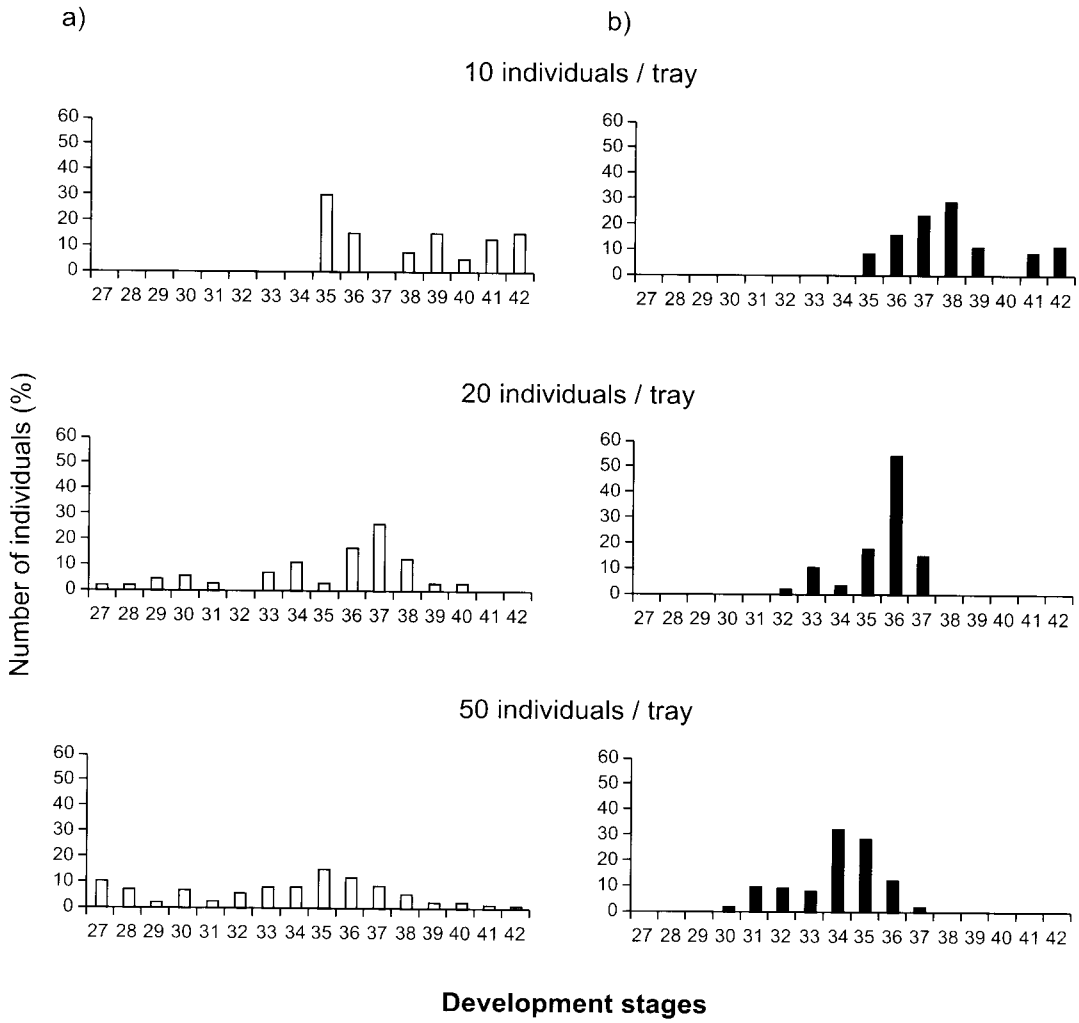

Figure 2. Distribution of larval developmental stages for *Pleurodema bufoninum* (a) and *Bufo spinulosus papillosus* (b) at the end of the density experiments. Developmental stages are according to Gosner (1960).

ting both species differently ($F_{1,18}$ = 1243.93, $P < 0.05$, N = 24). Interaction between factors was significant ($F_{2,18}$ = 6.32, $P < 0.05$, N = 24). For *P. bufoninum,* juvenile body length was greater in the low density treatment (Scheffé Test, $P < 0.01$) (Table 1). For *B. spinulosus papillosus* length was greater in low and medium density treatments (Scheffé Test, $P < 0.05$) (Table 1).

For both species, 100% of inhibited larvae were found to recover when reared alone and completed metamorphosis. For *B. spinulosus papillosus,* first stages with four limbs developed at 85 days after isolation began, while for *P. bufoninum* they developed at 62 days. Growth-inhibited *P. bufoninum* larvae reared alone, attained a greater length than those reared at the low density treatment (Kolmogorov-Smirnov Test, $P < 0.05$). Newly metamorphosed juveniles of both species reared alone did not differ in size from juveniles reared at

low density treatments (Kolmogorov-Smirnov Test, $P > 0.05$). Juveniles of both species fed normally and remained alive during three weeks of observation.

The increase in population density affects larval development of *Pleurodema bufoninum* and *Bufo spinulosus papillosus*, revealing similarities and differences in their responses. For both species, it was found that density increase lengthened duration of larval period, reduced total length and mass of larvae and increased time to complete metamorphosis, results which agree with other studies (Dash and Hota, 1980; Semlitsch and Caldwell, 1982, among others).

Both species survived under extremely high density conditions during several months. Similar survivorship has been mentioned for other anurans, which also inhabit temporary bodies of water, lay large numbers of eggs and, like *B. spinulosus papillosus,* are explosive breeders. Density increase also affected metamorph size of both species. Metamorphs' size in the highest density treatment was lower than in the others. Metamorph body size influence adult survivorship and reproductive potential (Berven, 1990; Altwegg and Reyer, 2003).

Growth-inhibited larvae are able to recover, once they are freed from the crowding effect. Response similarities to density increase could be related to the fact that both species can share the same habitats during development. However, a greater synchronism was observed in *B. spinulosus papillosus*. This synchronism persisted when the larvae were reared at high densities. Wassersug (1973) proposed that synchronism in development may have evolved as an anti-predatory mechanism.

The effect of the increase in density was more marked in *P. bufoninum*. The larval development of *P. bufoninum* was less synchronous than in *B. spinulosus papillosus*, as shown by the composition of stages at the end of the experiment. A greater inhibition in *P. bufoninum* was also observed, since growth-inhibited larvae attained greater length after being reared in isolation, compared to the larvae in the low density treatment, while in *B. spinulosus papillosus*, no such difference was found.

The different responses of the two species to increased density may correspond to their different behavioral characteristics. In their natural environments, *B. spinulosus papillosus* larvae aggregate during the afternoon in the shallow zones, attaining high densities. These aggregations occur until the final larval stages and a conspicuous synchronism in development is observed. Other studies of inhibition of development of anuran larvae by Wassersug (1973) showed that larvae of the genus *Bufo* were always less inhibited than the larvae of other genera. These data agree with what we observed in our study and could be considered as further support in predicting that population density will affect the development of gregarious larvae of *Bufo* species to a lesser degree. In contrast, *P. bufoninum* larvae are evenly distributed, showing no pattern of aggregation. Conditions of high density could have a more negative effect on *P. bufoninum* larvae than on *B. spinulosus papillosus* larvae, and may be linked to the solitary behavior of *P. bufoninum*. To conclude, this study shows how two anuran species that develop in the same kind of environment have certain similar patterns in their response to an increase in population density. Nevertheless, the differences found between the two species suggest adaptive peculiarities of each one.

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