

THE EFFECTS OF SHELTER AVAILABILITY AND SUBSTRATE QUALITY ON BEHAVIOUR AND POST-METAMORPHIC GROWTH IN THREE SPECIES OF ANURANS: IMPLICATIONS FOR CAPTIVE BREEDING

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Growth rate strongly influences survival and reproductive success in anurans, particularly during larval and juvenile stages. In tadpoles the availability of shelter has been linked to increased growth rates, but work on recently metamorphosed anurans has been limited. Three species (*Physalaemus pustulosus*, *Leptodactylus fuscus* and *Mannophryne trinitatis*) were used to examine the effects that shelters have on growth rates and behaviour in the laboratory. Shelter availability had a strong effect on growth in *M. trinitatis* and a weaker effect on *L. fuscus* and *P. pustulosus*. Shelter provided advantages in the trade-off between predator avoidance and resource gathering and/or osmoregulatory benefits. Osmoregulatory benefits may have had the greater impact on growth rates for three reasons: (1) the ability to burrow in *L. fuscus* and *P. pustulosus* would more likely offset the osmoregulatory benefits of having shelters available, as individuals without shelters spent significantly more time burrowing; (2) individuals with shelter available were more active than those without, but the difference was not significant; and (3) the humidity under the shelters was significantly greater than within the rest of the tank. All frogs spent a large amount of time under shelters, if available. Regardless of the causes for any accelerated growth rate, shelters should therefore be provided in any terrarium for captive breeding colonies. The substrate choice of all three species reflected their natural habitat, with *M. trinitatis* (the stream frog) spending the majority of time in water and the toad-like *P. pustulosus* spending < 5% of its time in water. *L. fuscus* behaviour also reflected its natural habitat preferences. Thus, careful consideration of a species' natural history must be made when selecting the substrates to be used in terraria.

Key words: dendrobatids, juvenile anurans, leptodactylids, osmoregulation, refuge use

INTRODUCTION

Growth rate is an important factor for survival in amphibians (Bardsley & Beebee, 2001) as mortality rates are highest in the very young, predominately due to their small size making them more susceptible to predators. The amount of time it takes individuals to grow to a less vulnerable size has a huge impact on the survival of a clutch (Clarke, 1974). Size has also been shown to have consequences for reproductive success (Ryan, 1980). Female mate choice, in addition to being based on absolute size, has been shown to be influenced by juvenile growth rates in males (Halliday & Verrell, 1988). Consequently, it is important to examine factors that could affect growth rates in anuran life stages. In many animals, the risk of predation is a key factor affecting growth, since foraging activity is reduced in the presence of predators (Babbitt, 2001; Babbitt & Jordan, 1996; Relyea & Werner, 1999; Anholt & Werner, 1995; Skelly & Werner, 1990). This reduction of activity can be advantageous, since prey movement increases the ability of predators to identify and locate them (Lima & Dill, 1990; Werner & Anholt, 1993), but can also result in a trade-

off between growth rate and predation risk. Lima & Dill (1990) reviewed work on many taxa showing the costs of anti-predator activities and attributes such as predator avoidance, predator vigilance, flight response and toxin production. Although there are several strategies and extents to which they are used, an important tactic to avoid predation is the use of refuges or shelters (Sih, 1997). Shelter confers protection by making prey less visible and less accessible to predators. In an open environment a species without a high degree of toxicity may have to remain relatively inactive to avoid detection by predators, which often use movement as a cue. Conversely, if shelters are available the animal has a secure location where it can avoid predators. This can allow the animal to forage more since it can accurately gauge its response to predators, maximising its time spent foraging and minimising wasted energy from fleeing for long distances or unnecessarily (Ydenberg & Dill, 1986). In addition to the advantages conferred by escaping predation costs, shelters could allow most post-metamorphic anurans to increase their ability to capture prey, since most frogs, with the exception of the dendrobatids, are ambush predators. Furthermore, the availability of shelters may reduce osmoregulatory and thermal stresses (Hoffman & Katz, 1989), which can also influence growth rates (Seebacher & Alford, 2002).

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The majority of anuran research is focused on the ecology and biology of the larval and adult life stages, which are typically concentrated around bodies of water and are thus conspicuous and easy to sample. Thus, little emphasis has been placed on the juvenile stage, between metamorphosis and sexual maturity. Like the larval stage, the juvenile stage represents a critical period since individuals are highly vulnerable to predators (Babbitt & Tanner, 1998), and it spans a substantial portion of most anurans' life history, often being substantially longer than the tadpole stage. Therefore, factors affecting growth and survival during this stage will have huge impacts on adult populations. There have been several studies on the effects of shelter availability and predation on growth rates in tadpoles (Babbitt & Tanner, 1998; Relyea & Werner, 1999) and post-metamorphic growth rates in general (Clarke, 1974; Labanick & Schlueter, 1976), but few on the effects of shelter availability or predation on juvenile growth rates.

Reintroductions have recently been utilized to halt the decline of amphibian species and populations, but have had mixed results (Dodd & Seigel, 1991; Bloxam & Tonge, 1995; Burke, 1991), with failures being attributed to poor planning and monitoring. Most key life-history traits of amphibians, such as high fecundity and low maintenance costs, make them prime candidates for relocation, repatriation, and translocation (RRT) programmes. However, one important aspect of any successful amphibian reintroduction programme is establishing a captive-bred population capable of quickly producing surplus individuals for eventual release. Therefore, it is important to find the best method to rear high quality individuals to establish breeding colonies of any potential candidate for RRT programmes. Although none of the three species utilized in this study are endangered or being considered for any current RRT programme, knowledge of their behaviour and growth rates under laboratory conditions will provide valuable insights into general juvenile anuran responses to shelter availability. Such insights could be applied to future amphibian captive-breeding programmes, to establish captive breeding colonies or determine sites with suitable cover to facilitate rapid growth in released individuals.

In this study we examined the growth performance of juvenile individuals of three species with and without shelters provided, to determine if shelter influenced growth rates. Additionally, behavioural responses to the availability of shelters under normal and disturbed conditions were examined to test anti-predator behaviour. These allowed an assessment of whether the availability of shelter facilitates a higher growth rate and determined possible causes related to predator avoidance or osmoregulatory benefits. Finally, the substrate that the frogs spent most of their time on was examined to test whether there was a preference for any one type, to aid in providing an understanding of how the substrates in terraria should be determined.

METHODS AND MATERIALS

SPECIES AND STUDY DESIGN

The three species used were *Physalaemus pustulosus* (Cope), *Mannophryne trinitatis* (Garman) and *Leptodactylus fuscus* (Schneider). All specimens were collected from the wild in Trinidad, West Indies during August 2002, either as spawn or as tadpoles. Tadpoles were reared at Glasgow University at low density and began metamorphosing in early September. Prior to the start of the study the froglets of all species were maintained on *Drosophila* in stock tanks until the experiment began in early October.

P. pustulosus is a small, terrestrial, non-toxic toad-like leptodactylid (males: 28 mm; females: 32 mm) that inhabits savannah areas as well as being a human commensalist (Netting, 1930). Metamorphosis in this species can occur as soon as three weeks after oviposition, depending on rearing conditions (Downie, unpublished observations). *P. pustulosus* is known to be preyed upon by the manicou crab (*Pseudothelphusa garmani*) and fringe-lipped bats (*Trachops cirrhosus*).

L. fuscus is a medium-sized (males: 42 mm; females: 50 mm), terrestrial, non-toxic leptodactylid found in forests and savannahs, but mainly in association with large temporary and/or permanent pools (Kenny, 1969; Hoogmoed & Gorzula, 1979). Metamorphosis occurs as soon as three weeks after oviposition, depending on rearing conditions (Downie, unpublished observations), unless interrupted by dry conditions that can cause the larvae to enter a state of arrested development (Downie, 1984).

M. trinitatis is a small (males: 19-22 mm; females: 22-26 mm), diurnal, terrestrial, non-toxic dendrobatid found in forests or along forest-edges and uses stream and stream-edge habitats to forage among near-by leaf-litter for small insects (Murphy, 1997). Metamorphosis takes place as soon as four weeks after oviposition, depending on rearing conditions (Downie, unpublished observations). Snakes are common predators of *M. trinitatis*, particularly *Liophis reginae zweifleri* (Test *et al.*, 1966; Kenny, 1979). Murphy (1997) lists the history of the taxonomic revisions of this species, which have led to the recent creation of the genus *Mannophryne*.

This study was run under laboratory conditions (constant temperature of 27°C, 65-75% humidity and 12:12 LD schedule) similar to natural conditions in Trinidad's wet season, June to December (Beard, 1946; Granger, 1982). Thirty Perspex tanks (10 cm × 20 cm × 10 cm; ten tanks for each species) were set up in the same manner, each with three separate substrates: gravel, water and sand, and covered in muslin, held in place by elastic bands. Water was held in open Petri dishes in the centre of each tank, with the sand and gravel at either end. Water was added to the sand and gravel to keep them moist. Each of the substrates covered approximately one-third of the tank base, and about 1 cm deep, to allow enough depth for the frogs to burrow. In half of the

tanks (five per species) three PVC shelters, constructed from piping cut into 4 cm arcs of 4 cm height, were added so that a shelter was over a portion of each of the substrates. The shelters were arranged in a way that allowed the frogs to be visible at all times, even when underneath a shelter. Two frogs of similar size were chosen randomly from the stock population of each species and placed together in a tank, so that there were 20 frogs of each species, ten for each treatment (shelter; no shelter) and several individuals remaining in the stock tanks in the event of deaths. Only two individuals were placed in each tank so that individuals could be located and identified easily and to reduce competition for food. For measurement purposes, individuals in each tank were differentiated by slight differences in snout-vent length or pattern differences.

All three species were fed live fruit flies (*Drosophila melanogaster*), obtained from the University's Division of Molecular Genetics and contained in plastic tubes attached to the side of the tanks, so that no additional shelter was provided. All tanks were given tubes with approximately the same quantity of flies, and tubes and water were replenished six days a week during the study, so that there was always an excess of flies and constant moisture levels in the Petri dishes and substrates. The location of the *Drosophila* tubes was changed weekly between five locations, determined randomly by rolling a die (rolls of six were ignored), in order to limit the impact food source position had on any substrate preference. After eight weeks, the diet of *L. fuscus* was changed to silent crickets (*Gryllus assimilis*), obtained from Peregrine Livefoods weekly and split evenly between the ten tanks.

All individual frogs were weighed and measured when initially placed into the experimental tanks and reweighed and measured twice a week for eight weeks, with the exception of *M. trinitatis* (see Results). Additional data were collected from *L. fuscus* for five weeks on the diet of crickets. Mass was measured on a digital balance to 0.001g after the frogs had been quickly surface-dried in a paper towel. Length measurements were taken from the tip of the snout to the vent (SVL) with callipers accurate to 0.1 mm. Although difficult to determine accurately (Turner, 1960), SVL was used in this study since it requires less handling than the measurement of tibia length described by Clarke (1974). Accuracy was increased by holding the dish vertically, forcing the frogs to adopt a position where they could adhere to the side of the dish: this was less variable than the frogs' sitting position. Measurements were repeated two to three times, until repetition or a median measurement was established. Body condition, calculated from the equation proposed by Veith (1987), was assessed, but did not indicate any clear trends and will not be presented.

Behavioural observations, taken on different days from growth measurements, were made at roughly the same time in the afternoon to avoid the effects of any diel variations in behaviour. On the days behavioural

observations were being carried out, feeding was always carried out after the observations were made. Each frog was observed for one minute; the substrate the frog was on, including the walls, and the activity of the frog were noted at three-second intervals, so that twenty readings were taken for each frog each day. The activity of the frogs was categorised as sitting, feeding, turning, sheltering or jumping. The 'turning' category encompassed all movements that were not jumping, such as slight positional changes. Whether the frog was burrowed and/or under a shelter was also noted. The effect of disturbance was also examined by erratically moving the tanks and removing the muslin cover, to elicit an anti-predator response. This was carried out on each tank prior to the second weekly observations, with the exception of the first two weeks.

After all the frogs were removed from the study tanks, two tanks were retained to measure humidity under the shelters and within the tank at large. The relative humidity was measured using an electronic thermo-hygrometer (Oregon Scientific) accurate to 1% RH, on each substrate both under the shelter and as far from the shelter as possible. A total of twelve readings were taken for each substrate in both tanks.

STATISTICAL ANALYSIS

Regression analysis was used to determine the rate of change over time of body weight and SVL for each species of frog. The slopes of the regression equations from the group with shelter available and those without shelter available were compared using a slope *t*-test. For the behaviour data the mean time on each substrate or activity was calculated for each tank for each day of observations. To determine if shelter availability or disturbance influenced substrate preference or activity levels in *P. pustulosus* and *L. fuscus*, multivariate analysis was carried out (on arcsin transformed data in *P. pustulosus*). ANOVA was used, with a post-hoc Tukey test, to determine differences between preferences in substrates. If the two treatments did not influence the substrate preference then a mean was taken for each tank so that the ANOVA was performed with one value for each substrate per tank. Due to the small number of observations, in *M. trinitatis* parametric tests could not be used so all substrate data were combined and analysed using the Kruskal-Wallis test and two chi-squared tests to determine the influence of shelter and disturbance on activity levels. A Mann-Whitney test was used to determine any difference in the time spent in a burrow by frogs when shelters were available or unavailable and if there was a difference between the relative humidity within the tank and under the shelters.

RESULTS

MORTALITY

There were no *P. pustulosus* deaths during the entire study. In *L. fuscus* there was one death and one probable escape. The death occurred in the second half of week 3;

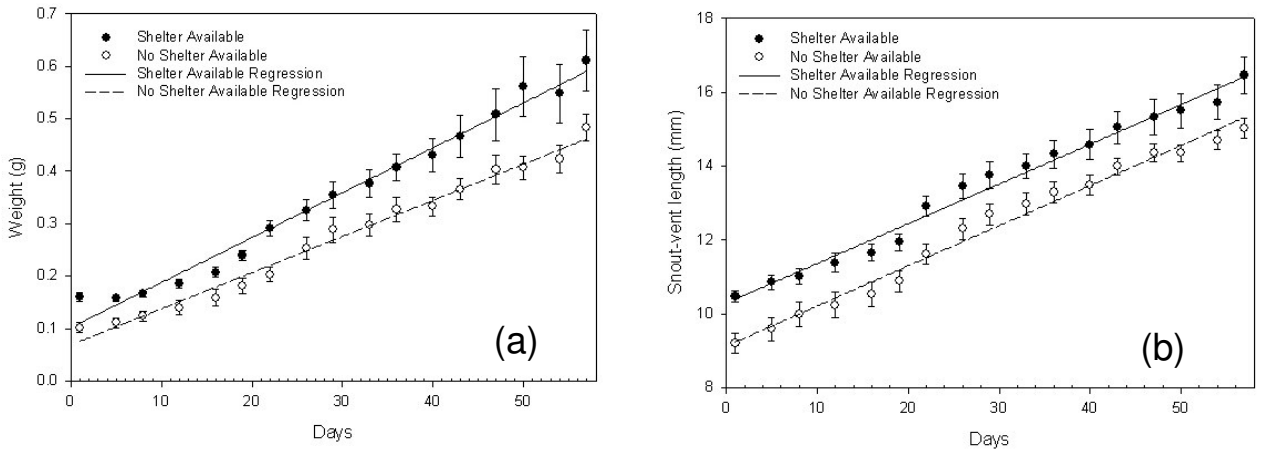


FIG. 1. (a) Mean mass \pm SE of *P. pustulosus* with and without shelter, with regression lines. Shelter: Mass = $0.103 + 0.00854$ days, $r^2 = 98.3\%$. No shelter: Mass = $0.0693 + 0.00687$ days, with $r^2 = 98.6\%$. (b) Mean length \pm SE of *P. pustulosus* with and without shelter, with regression lines. Shelter: SVL = $10.3 + 0.11$ days, $r^2 = 98.3\%$. No shelter: SVL = $9.13 + 0.11$ days, with $r^2 = 98.5\%$.

however, the individual had appeared moribund at the first weekly measurement, so a similar sized individual from the stock tank was measured as a potential replacement. Since the measurements of the replacement individual were taken on the same day as the study individual it was possible to extrapolate measurements for the replacement for previous weeks, by calculating the percentage of change per week in the original individual and subtracting those percentages from the measurements from the new individual taken at the beginning of the week, allowing continuity to be maintained. The probable escape occurred in the final week. The final measurements were obtained by calculating the mean percentage increase in the final week of individuals in the same treatment group and increasing the week 13 measurements of the individual by that percentage.

From the outset of the study *M. trinitatis* had very high mortality rates, with five deaths during the first week and ten in the second week (37.5% weekly mortality rate for the first two weeks). There was no difference

in the rate of mortality between the shelter and non-shelter treatments ($U=29.5$, $P>0.05$). In all instances of death in the first two weeks the dead individuals were removed and replaced with individuals of similar size from the stock tank. After the two weeks all individuals from the stock tank had been used for the study and with four more deaths in week 3 there were not enough individuals remaining to provide sufficient replication. Therefore the remaining individuals were left undisturbed, other than for feeding and watering, in the study tanks. During the final five weeks, there were only eight deaths (5.7% weekly mortality rate). There were still eight individuals alive when the study finished, only six of them frogs that were originally placed in the study tanks in the first week: three in each treatment group. All available data on these individuals were used to give a measure of the growth rates under the two treatments; behavioural data from all individuals were analysed since we were not comparing the behaviour of individual frogs.

TABLE 1. Difference in growth rate between sheltered and non-shelter groups in each of the three species. For *L. fuscus*, Phase 1: 0-26 days; Phase 2: 29-57 days; Phase 3: 61-91 days.

Species			<i>t</i>	df	<i>P</i>
<i>P. pustulosus</i>	Mass		4.69	30	<0.005
	SVL		0.68	30	>0.05
<i>L. fuscus</i>	Mass	Initial 9 weeks	0.96	30	>0.05
		Total 14 weeks	0.49	42	>0.05
		Phase 1	5.85	8	<0.001
		Phase 2	5.57	14	<0.005
		Phase 3	3.88	8	<0.01
	SVL	Initial 9 weeks	3.01	30	<0.005
		Total 14 weeks	4.28	42	<0.001
<i>M. trinitatis</i>	Mass	Initial 9 weeks	4.51	8	<0.005
		Phase 2	0.40	14	>0.05
		Phase 3	4.30	8	<0.005
		Phase 1	4.17	8	<0.005
		SVL	2.21	8	<0.05

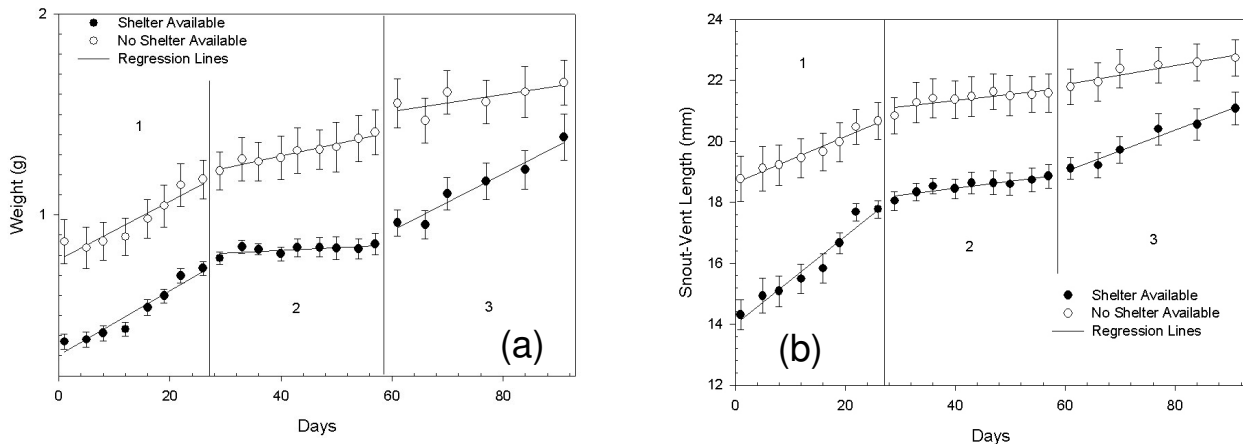


FIG. 2. (a) Mean mass \pm SE of *L. fuscus* with and without shelter, divided into three segments, with regression lines. The slopes of the three segments (ordered from left to right) for the sheltered group are: $b_1=0.016$, $b_2=1.39 \times 10^{-3}$, $b_3=0.014$. The slopes for the non-shelter group are: $b_1=0.0145$, $b_2=6.04 \times 10^{-3}$, $b_3=4.21 \times 10^{-3}$. (b) Mean SVL \pm SE of *L. fuscus* with and without shelter, divided into three segments, with regression lines. The slopes of the three segments (ordered from left to right) for the sheltered group are: $b_1=0.144$, $b_2=0.023$, $b_3=0.068$. The slopes for the non-shelter group are: $b_1=0.076$, $b_2=0.020$, $b_3=0.03$.

TABLE 2. Difference in starting weight and SVL between the two shelter treatments for each of the three species.

Species		<i>t</i>	<i>U</i>	df	<i>P</i>
<i>P. pustulosus</i>	Mass	4.68		17	<0.001
	SVL	4.06		17	<0.005
<i>L. fuscus</i>	Mass	4.30		11	<0.005
	SVL	5.03		15	<0.001
<i>M. trinitatis</i>	Mass		10.0		>0.05
	SVL		9.5		>0.05

GROWTH RATE

P. pustulosus increased in mass faster when shelters were present; however, there was no difference in SVL growth rate between the two treatment groups (Fig 1a, b; Table 1). There was a significant difference in starting mass and SVL of the two treatment groups (Table 2).

During the initial nine-week period the rate of increase in the mass of the *L. fuscus* group with shelters was not significantly different from the rate of increase

in the group without shelters (Fig 2a). However, *L. fuscus* individuals grew faster in SVL when shelters were present (Fig. 2b; Table 1). There was a difference between the groups with and without shelters in starting mass and SVL (Table 2).

When the data collected during the additional five weeks were included the results were similar, with no difference in the increase in mass, but a difference in SVL growth rate. However, it was clear that the growth rate of *L. fuscus* was not constant (Fig 2a,b; Table 1). There were three separate phases of growth for both groups; early growth (days 0-26), a plateau (days 29-57) and finally growth again (days 61-91) when fed on the diet of crickets.

There was a difference in the rate of increase in weight between the two groups during all three growth phases. The group without shelters increased in mass at a faster rate during the second stage, but the reverse was true of the first and third growth phase. When split into the three stages there was a significant difference in the rate of increase in SVL in the first, which was more dramatic than when considering growth as one continuous

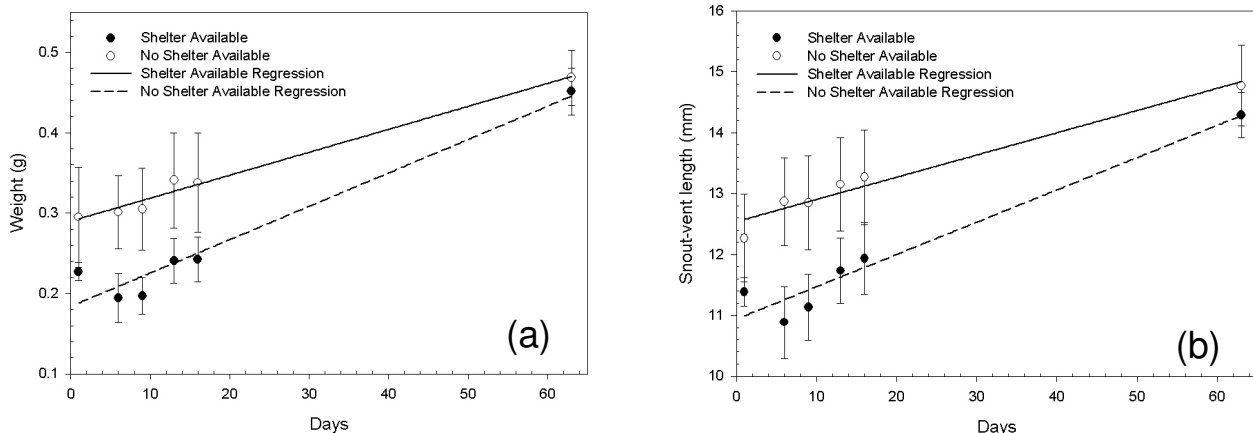


FIG. 3 (a) Mean mass \pm SE of *M. trinitatis* with and without shelter, with regression lines. Shelter: Mass = $0.184 + 0.00415$ days, $r^2 = 94.7\%$. No shelter: Mass = $0.290 + 0.00286$ days, $r^2 = 98.4\%$. (b) Mean SVL \pm SE of *M. trinitatis* with and without shelter, with regression lines. Shelter: SVL = $10.9 + 0.05$ days, $r^2 = 94.3\%$. No shelter: SVL = $12.5 + 0.04$ days, $r^2 = 95.8\%$.

TABLE 3. Percentage time spent burrowed by each species, with and without shelters (means \pm SE). For the results of Mann-Whitney test (U): * $P < 0.05$ ** $P < 0.005$.

	Shelters available	No shelters available	U
<i>P. pustulosus</i>	7.4 \pm 3.9%	29.6 \pm 5.6%	143**
<i>L. fuscus</i>	12.0 \pm 3.6%	38.3 \pm 8.7%	75*
<i>M. trinitatis</i>	3.8 \pm 3.8%	1.9 \pm 1.9%	104.5 NS

process. The second stage did not show any difference, but individuals increased in SVL faster in the third growth phase when shelters were present (Table 1).

M. trinitatis individuals grew significantly faster in mass (Fig 3a) and length (Fig 3b) when shelters were provided (Table 1). There was no significant difference in the starting mass or SVL (Table 2) of the two treatment groups.

BEHAVIOUR

Habitat choice. If shelters were available both *P. pustulosus* and *L. fuscus* spent a majority of the time under them. However, *M. trinitatis* spent only half as much time under the shelters as the other species (mean \pm SE: 37.9 \pm 10.4%). Additionally, in both *P. pustulosus* and *L. fuscus*, individuals spent more time under the shelters when disturbed compared to undisturbed, but the differences were not significant (Fig. 4). When shelters were provided, *P. pustulosus* and *L. fuscus* spent less time burrowed than in the absence of shelters. However, *M. trinitatis* spent very little time burrowed and unlike the other two species, the sheltered group spent marginally, but not significantly, more time burrowed (Table 3).

SUBSTRATE USE

In both *P. pustulosus* and *L. fuscus* disturbance or shelter availability had no influence on which substrate was preferred, and all three species showed significant preferences for particular substrates over others (Fig. 5; *P. pustulosus*: $F_{3,36}=26.16$, $P < 0.01$; *L. fuscus*: $F_{2,27}=6.35$, $P < 0.01$; *M. trinitatis*: $H=29.2$, $df=3$, $P < 0.001$). *P. pustulosus* showed a preference for the ter-

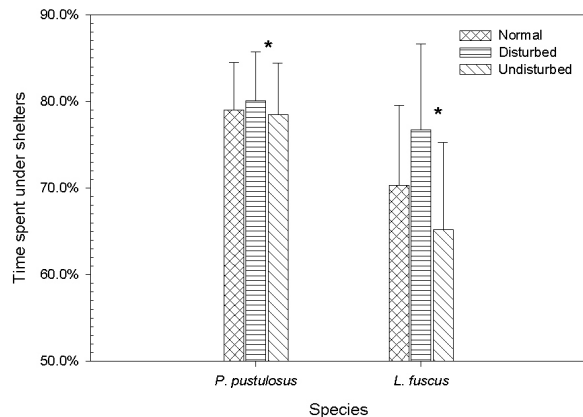


FIG. 4. Mean time spent under shelters in total and under disturbed and undisturbed conditions. Results of Mann-Whitney U -test for differences between disturbed and undisturbed conditions +SE bars (* $P < 0.05$).

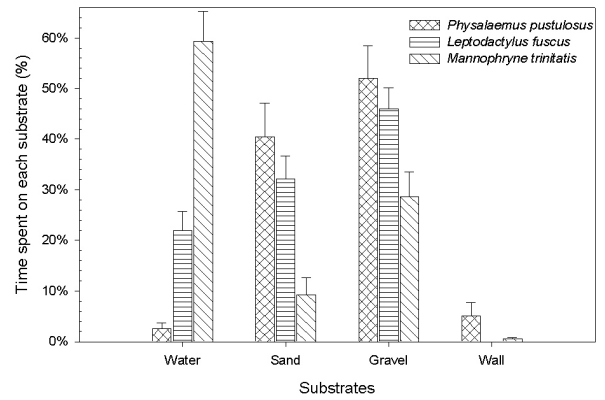


FIG 5. Mean time each species spent on each substrate in total, with SE. The results of ANOVA for each substrate are: water, $F_{2,27}=45.77$, $P < 0.001$; sand, $F_{2,27}=9.37$, $P < 0.005$; gravel, $F_{2,27}=7.83$, $P < 0.005$; tank wall, $F_{2,27}=1.50$, $P > 0.1$

restrial substrates, spending most time on gravel, which was significantly preferred to water or the tank wall. *L. fuscus*, which spent no time on the tank wall (factor omitted from analysis), showed a preference for gravel followed by sand then water. Water was significantly preferred by *M. trinitatis* to the other three remaining substrates, followed by gravel, sand and the tank wall.

The three species had very distinct preferences for the available substrates, with a significant difference in the amount of time spent by the three species on water, sand and gravel, but not the tank wall (Fig. 5). *M. trinitatis* spent the most time in water (almost 60%) compared to about 20% in *L. fuscus* and less than 5% in *P. pustulosus*.

ACTIVITY LEVELS

All three species remained inactive the majority of the time, with *P. pustulosus* being least active (Fig. 6) and *L. fuscus* (Fig. 7) and *M. trinitatis* (Fig. 8) remaining inactive for approximately 98% of the time. In *M. trinitatis*, jumping was a more prevalent activity compared to the other species, with a comparable amount of time spent jumping and turning. No frog of any species, was observed to feed during the timed observation peri-

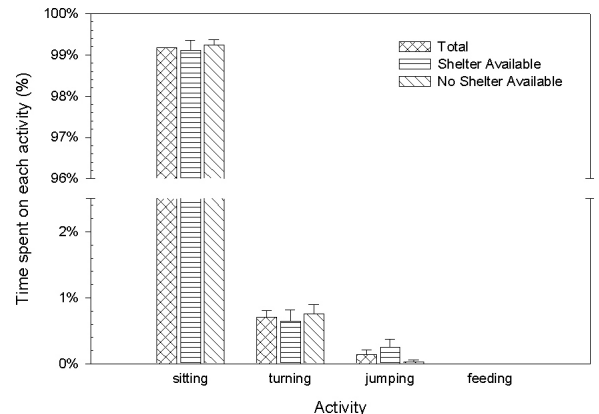


FIG 6. Mean time *P. pustulosus* spent engaged in each behaviour in total, and under both shelter treatments +SE. A break was inserted to allow the very small percentages of turning and jumping to be shown.

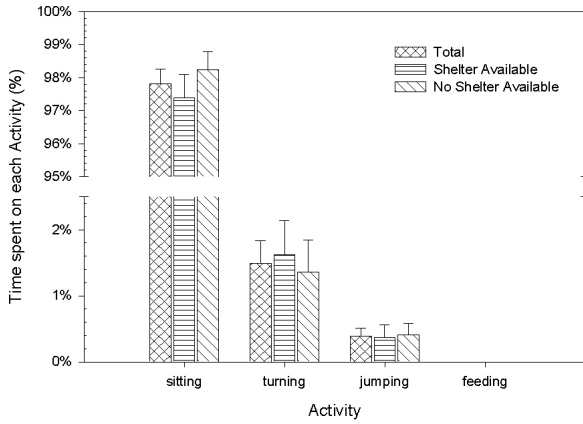


FIG. 7. Mean time *L. fuscus* spent engaged in each behaviour in total and under both shelter treatments \pm SE. A break was inserted to allow the very small percentages of turning and jumping to be shown.

ods, but feeding was witnessed at other times in both *P. pustulosus* and *M. trinitatis*. Although each species was predominantly inactive, there were differences in how activity was influenced by disturbance and shelter treatment.

In *P. pustulosus*, individuals were observed to jump more frequently when shelters were available, but there was no difference in the level of other activities between the different shelter treatments (Table 4). Disturbance had no influence on activity levels in *P. pustulosus*. Conversely, *L. fuscus* exhibited no difference in activity levels between the shelter treatment groups, but when individuals were disturbed they showed an increase in activity (Table 4). There was no significant difference in the activity levels of *M. trinitatis* between the two

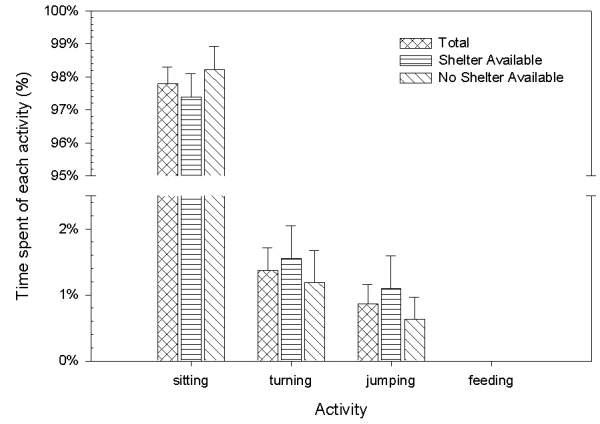


FIG. 8. Mean time *M. trinitatis* spent engaged in each behaviour in total and under both shelter treatments \pm SE. A break was inserted to allow the very small percentages of turning and jumping to be shown.

treatment groups ($\chi^2=1.62$, $df=2$, $P>0.05$) or between the activity levels when disturbed and undisturbed (Table 5, ($\chi^2=5.68$, $df=2$, $P>0.05$).

HUMIDITY

The mean relative humidity under the shelters for all substrates was $91.8\pm 0.5\%$ (mean \pm SE) while elsewhere in the tanks the relative humidity was lower at $86.8\pm 0.6\%$ (mean \pm SE). There was little difference in humidity, for each shelter condition, between the three substrates or between the two tanks. However, there was a significant difference between the relative humidity under the shelters and elsewhere in the tank ($U=152.0$, $P<0.001$).

TABLE 4. Results from multivariate analysis of activity levels for *P. pustulosus* and *L. fuscus*.

	Treatment	Activity	df	F	P
<i>P. pustulosus</i>	Shelter	Sitting	1	0.458	0.508
		Turning	1	0.022	0.883
		Jumping	1	4.965	0.041
	Disturbance	Sitting	1	0.069	0.796
		Turning	1	0.002	0.965
		Jumping	1	2.546	0.130
	Shelter \times Disturbance	Sitting	1	0.052	0.822
		Turning	1	0.003	0.954
		Jumping	1	0.691	0.418
<i>L. fuscus</i>	Shelter	Sitting	1	0.427	0.523
		Turning	1	0.641	0.435
		Jumping	1	0.058	0.812
	Disturbance	Sitting	1	4.730	0.045
		Turning	1	1.690	0.212
		Jumping	1	5.618	0.031
	Shelter \times Disturbance	Sitting	1	0.507	0.487
		Turning	1	0.304	0.589
		Jumping	1	0.234	0.635

TABLE 5. Number of three-second intervals observed for each activity under disturbed and normal conditions in the shelter group for *M. trinitatis*, used in chi-squared analysis.

	Turning	Sitting	Jumping
Disturbed	39	1549	12
Not Disturbed	16	1582	2

DISCUSSION

MORTALITY

M. trinitatis had a high mortality rate compared to the other two species, even though the treatment of all species was the same. We suspect that stress was the cause of the high mortality rate in *M. trinitatis*, since the rate of mortality decreased when disturbance from measuring was removed.

GROWTH RATE

When comparing the rate of increase in mass and SVL between the two shelter treatment groups for each of the three species, there were four cases, representing each species, where frogs grew faster with shelters present. Although there was no significant difference in the rate of growth between the two treatments in the remaining two comparisons – length in *P. pustulosus* and mass in *L. fuscus* – there was no instance where frogs without shelter grew faster than those with shelter.

The differences in growth rate between shelter and non-shelter groups were not large, and may have been affected by two factors: substrate and food suitability. Where shelters were not provided, frogs were able to burrow into the substrate, providing a self-made shelter and thereby reducing the practical difference between our two designs. It is noticeable that the species with the most consistent difference in growth rates (*M. trinitatis*: differences significant for both mass and SVL) spent most of its time in the water rather than on either of the solid substrates, and therefore less often burrowing.

The food available for all species was initially *Drosophila*, but as the frogs grew they may not have been able to consume enough *Drosophila* to maintain growth, and may even have ceased to forage when only *Drosophila* were available. We saw an effect of this sort in two of our species: in *P. pustulosus*, growth rate declined around week nine, with the experiment terminating soon after. In *L. fuscus*, growth on *Drosophila* plateaued after about five weeks, with the frogs seeming to lose interest in the food. Previous work which supports these observations, has shown that anurans change their diet as they grow and that larger individuals are more selective in the size of prey that they consume and may develop negative electivities for prey items that are no longer suitable (Lima, 1998; Newman, 1999). This highlights an important factor in captive rearing, that there may be a threshold age or size at which the diet of anurans changes, which could impact rearing programmes.

Growth resumed in *L. fuscus* when crickets were provided in week nine, and during the final growth phase

individuals grew faster in length and mass when shelters were provided. The diet of crickets may have allowed growth to resume, and at a higher rate for frogs with shelters, for two reasons: each cricket caught provides more food, for less effort, than a fruit fly; and crickets only move along the substrate, rather than being distributed three-dimensionally throughout the tanks, which may make foraging from shelters particularly efficient.

M. trinitatis did not show this effect since this species may continue to forage on small prey as it grows, for three possible reasons. First, adult *M. trinitatis* are the smallest of the three species and there is a relatively small ontogenetic size change from juvenile to adult. Second, the mountain stream habitat used by *M. trinitatis* may have sparse resources of larger prey. Thirdly, *M. trinitatis* may display species-specific foraging activities that do not change with size (Lima & Magnusson, 2000). Gut contents of adults taken from the field (Downie, unpublished observations) include prey items that are no larger than the *Drosophila* used in this study.

It is a possible concern that in two out of the three species used in this study (*P. pustulosus* and *L. fuscus*) the mean initial sizes of frogs in the shelter and non-shelter groups were significantly different. The initial size of frogs varied over a considerable range due to a one to four week post-metamorphic growth period prior to the start of the experiment, not because of inherent differential growth rates between individuals selected for the two treatment groups. We do not believe that these initial differences affected our conclusions because frogs with shelters provided grew faster than those without shelters for all species, whereas the differences in initial size were in the opposite direction in *L. fuscus* and *P. pustulosus*, and there was no such difference in *M. trinitatis*.

BENEFITS OF SHELTER

The obvious benefits of shelter to a juvenile amphibian are in foraging, where shelter may increase the success rate of an ambush predator; and in predator avoidance, where the availability of shelter provides a protected environment during quiescent periods and a convenient place to escape when threatened during active periods. In other taxa (squirrels: Dill & Houtman, 1989; fish: Dill, 1990; McLean & Godin, 1989) individuals have been shown to delay fleeing from threats the nearer they are to refuges, allowing increased foraging times and a reduction in energy expenditure on flight. In fish, Krause *et al.* (1998) have demonstrated that the potential foraging advantages provided by shelter are influenced by body size. Therefore there may be differences in the effects of shelter on growth rate between juvenile and adult individuals.

Less obviously, shelters may have thermoregulatory and osmoregulatory benefits, by providing suitable microclimates. Several studies have demonstrated that shelters can reduce physiological stresses caused by heat, cold weather and drought in terrestrial anurans

(Hoffman & Katz, 1989; Schwarzkopf & Alford, 1996; Parris, 1998; Seebacher & Alford, 2002). Although these factors might seem to be more a threat to survival than to growth, Lillywhite *et al.* (1973) have shown that in addition to metabolic costs involved in restoring water balance after desiccation there are physiological consequences, such as reduced oxygen consumption and metabolic capacity, that can reduce growth rate. In our study, we were able to detect a relative humidity advantage under the shelters, which may have contributed to the growth rate difference. Such benefits might be particularly important in juvenile frogs due to their relatively high surface area/volume ratios.

The aim of this study was to determine whether there was a detectable benefit to growth rate in shelter, rather than to identify the precise nature of the benefits. These could be investigated using different interspecies comparisons: for example, highly toxic species, like some bufonids and dendrobatids may be less influenced by predator avoidance benefits; highly terrestrial species like bufonids might be less reliant on physiological benefits.

Our comparison between shelter and non-shelter groups was complicated by the burrowing behaviour of both *L. fuscus* and *P. pustulosus*. However, Parris's (1998) study on juvenile *Rana* found that individuals that actively dug their own burrows conserved water less efficiently than those that used pre-existing shelters. Therefore, in addition to the energetic cost of active burrowing, there may be an osmotic cost which helps explain growth differences between shelter and non-shelter groups in the two burrowing species. The substrate we provided was rather shallow (about 1 cm) and it would be interesting to determine whether substrate depth had an impact on burrowing behaviour and growth.

SUBSTRATE CHOICE

In each species there were clear but distinct preferences for substrate type: *P. pustulosus* showed the most terrestrial preference, with hardly any time spent in water, and gravel and sand being fairly evenly utilized; *M. trinitatis* was the most aquatic of the three, with most time spent in water, and gravel preferred to sand; *L. fuscus* was intermediate between the other two, but gravel was preferred. These differences reflect well what is known of the natural history of these species. *P. pustulosus* is a toad-like anuran, which spends the majority of its time in terrestrial habitats, returning to water only for reproduction (Marsh *et al.*, 2000); *L. fuscus* is more frog-like, spending its time in damp burrows close to sources of water; *M. trinitatis*, the stream frog, lives permanently close to running streams in the rainforest.

Gravel was the preferred terrestrial substrate in all three species, although this trend was not always significant. Seebacher & Alford (2002) found a substrate preference for rocks over other substrates in *Bufo marinus*, although they did not provide sand as an alternative. Their results indicated that an uneven damp,

rocky surface helped reduce water loss, which could explain why all three species demonstrated a preference for gravel over sand.

In pilot studies on *L. fuscus* and *M. trinitatis* (Downie, unpublished observations), having the food source in a fixed location resulted in preferences that were correlated with the location of the *Drosophila* tubes. This suggests that when a food source is randomly encountered each species will show substrate preferences that reflect their natural habitat, but when food placement is fixed it heavily influences where individuals spend their time. Preliminary work on amphibian habitat enrichment has revealed that feed placement and treatment influences the duration and intensity of foraging behaviour and that amphibians are fully capable of 'learning' the feed-site (Campbell-Palmer, personal communication).

ACTIVITY LEVELS

Measured activity levels were low in all three species, but this is not surprising. *L. fuscus* and *P. pustulosus* are thought to be primarily nocturnal, and our observations were made during the day. However, *M. trinitatis* is diurnally active but showed only marginally higher activity levels than the other two species. In addition, we assessed activity before feeding (in order not to have the data dominated by foraging activity) and frogs were therefore observed at resting activity levels. Although there were differences in activity levels between the shelter groups they were mainly non-significant, so it is unlikely that activity contributed greatly to the differences in growth rates between treatments. Disturbance had an impact on activity level only in *L. fuscus* with a difference between shelter and non-shelter groups. The non-shelter group remained immobile, mainly in their burrows, when disturbed; many of the shelter group were out in the open, and disturbance led them to retreat rapidly under a shelter. For all species there was a similar finding whenever we were attempting to catch the frogs in order to measure them: those with access to shelters immediately jumped under them (personal observations).

IMPLICATIONS FOR CAPTIVE BREEDING PROGRAMMES

None of the species investigated here is currently endangered. However, there are implications in our results for captive management programmes. The provision of shelter was a positive feature in the growth rate of all three species, and should perhaps be universal in the rearing of juvenile frogs.

Handling seemed not to be a problem for two of the species, but seemed to be highly stressful for *M. trinitatis*. Knowledge of the impact of stress should be important in determining how to monitor growth.

The three species had different substrate preferences in the simple three-way choice we offered. Therefore, if knowledge of an endangered species' natural history is limited, then it should be possible to ascertain habitat preferences in many species by means of choice experi-

ments of this kind. Establishing the ideal habitat is likely to be important in achieving fast growth rates in juvenile amphibians and can be used to assess suitable locations for reintroduction or translocation sites.

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