The paradoxical frog *Pseudis paradoxa*: larval anatomical characteristics, including gonadal maturation

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The genus *Pseudis* is unique amongst anuran amphibians in that body growth occurs mostly or entirely in the larval phase, with huge tadpoles metamorphosing into smaller but adult-sized frogs. Selected organ systems were studied in tadpoles of the paradoxical frog *Pseudis paradoxa* in order to determine whether they have any special features associated with their large size and unique life history. Testes of large tadpoles were well developed with spermatogenesis proceeding before metamorphosis; ovaries were also well developed in later stage tadpoles with large pre-vitellogenic oocytes. In later stage tadpoles, unusually for anuran larvae, the intestine had developed longitudinal internal ridges, increasing the internal surface area. Lungs of later stage tadpoles showed internal septation, again an unusual feature for anuran larvae. Finally, later stage tadpoles had a considerably thickened epidermis compared to earlier stages, though no sign of adult-type glands. Overall, these results suggest that as *Pseudis* tadpoles grow in size beyond the norm for conventional anuran larvae, a suite of features normally associated with adult anurans begins to develop before metamorphosis.

Key words: Anura, amphibians, tadpole, Trinidad

INTRODUCTION

Amongst anuran amphibians the genus *Pseudis*, the paradoxical frogs, is unique in that the larvae grow to an enormous size before metamorphosing into smaller adult-sized frogs. The life history of these species appears contrary to theories such as those of Wassersug (1975) and Werner (1986), which predict limits on larval growth in anurans. In a companion paper (Downie et al., 2009) we report on tadpole growth and metamorphosis and also on overall morphology and ecology, in an attempt to explain the life history of these species. A key finding is that *Pseudis* tadpole growth rate does not appear exceptional for tropical tadpoles in a nutrient-rich habitat – it simply continues for a longer period than in other species.

However, it might be expected that to function effectively at large size, *Pseudis* tadpoles would have to alter aspects of their functional anatomy, especially those where the functional needs of larger size, subject to cubic scaling, cannot be accommodated simply by linearly-scaled structures. For example, the simple tubular intestine found in most anuran tadpoles may not absorb effectively when scaled up in size. Similarly, the sac-like lungs of other tadpole species would increase inadequately in absorptive surface area without sub-division. Another feature worth investigating is the skin: the very thin simple epidermis found in most tadpoles may afford inadequate protection for the giant tadpoles of *Pseudis*, especially during their prolonged larval life.

Finally, since *Pseudis* are at adult size following metamorphosis (Downie et al., 2009), we can ask at what stage their gonads mature. The normal situation is that newly metamorphosed anurans are juveniles that undergo considerable growth before they are ready to mature; their gonads, consequently, are at early developmental stages at metamorphosis (Viertel & Richter, 1999). If *Pseudis* is functionally mature at or soon after metamorphosis, we might expect gonadal maturation to occur in the latter stages of larval life, a unique situation for anurans.

In this paper, we report on aspects of the development and structure of the gonads, alimentary canal, lungs and skin of *Pseudis paradoxa* tadpoles.

MATERIALS AND METHODS

Pseudis paradoxa tadpoles, metamorphs and adults were collected over a 21-year period (12 field seasons) at five main sites in Trinidad, West Indies, with occasional collections made elsewhere. Two sites were sampled at Nariva swamp: first, the canal extending westward to Bush Bush island (10°24.3'N, 61°02'W); at the east end, this passes through mangroves, but opens soon into an extensive freshwater swamp. We accessed the canal by boat, and the collection sites were the canal itself and adjacent open pools fringed by dense aquatic vegetation.

Second, further south, the swamp extends close to the Manzanilla Road, and we were able to access by foot a set of pools just north of the Kernahan Road (10°22.5'N, 61°01'W).

Bamboo Grove, located at Valsayn, just south of the Churchill–Roosevelt Highway (10°38'N, 61°25'W), has been sporadically active as a fish farm during the 21 year sampling period. It contains a set of large ponds on both sides of the access road. The ponds are partly rain-fed, but water is also pumped in to flood them to suitable depth whenever fish are being reared.

Columbus Bay in Icacos (10°05'N, 61°54.3'W) is at the south-western tip of Trinidad. The beach is fringed by coconut palms. Behind the narrow fringe of palms to the

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Table 1. *P. paradoxa* gonad lengths. All specimens field caught except some of the stage 45–46 individuals, which were laboratory-reared (4 males; 1 female). Because small numbers of specimens were available at some stages, specimens at adjacent stages have been grouped where tadpoles sizes were similar.

Gonad type	Stage	*n*	Mean length ±SD
Testis	31–33	3	2.3±0.4
	37	2	2.7±0.1
	38–39	4	3.6±1.5
	45–46	9	4.5±1.3
Ovary	37–38	2	6.9±2.7
	45–46	3	7.0±1.7

right of the access road is a set of seasonal ponds that support populations of several frog species, including *P. paradoxa*.

Bonasse swamp (10° 05.8'N, 61°51'W), also in southwest Trinidad, is 2 km east of the village of Bonasse in Cedros. The Southern Main Road passes through swamps and the area sampled is on the north side of the road.

Tadpoles were captured during daylight hours. At Bamboo Grove and in the Nariva canal, it was possible to use a 3 m long seine net, but at other sites, the density of the aquatic vegetation made seining impracticable. A robust handnet with a long wooden handle was the most effective method. The water was generally too turbid and deep to allow tadpoles to be detected visually. On collection, tadpoles were transferred to a large bucket of swamp water and transported within 3 h to the University of West Indies, St Augustine.

Adults were captured at night and were located either visually (sitting on surface vegetation, e.g. lily pads, or simply with their heads above water) or by call. We experimented with several methods of catching the frogs. They are extremely wary and quickly dive when disturbed. The most successful method was to approach a located frog very slowly, with lights off, until close enough to catch it with both hands. Adults were transported to the laboratory in 2 litre polythene tubs, or polythene bags, containing a shallow depth of swamp water and with small punched air-holes.

Tadpoles and adults used for morphometric anatomical and histological investigations were killed by lethal anaesthesia in MS 222 or Benzocaine, as soon as possible after collection. They were then fixed in buffered neutral formalin (BNF) or Bouin's fluid. For wet weights of whole animals and parts, specimens were dried of surface fluid and weighed using an electronic balance accurate to 0.01 or 0.001 g. Lengths were measured using callipers accurate to 0.1 mm, or for very small specimens, using a calibrated dissecting microscope. In *P. paradoxa*, as tadpoles grow, the vent becomes a large structure, often damaged during storage. Because the vent was not a reliable point to use for the end of the body, we used the base of the hindlimbs as equivalent to the vent in our snout–vent length (SVL) measurements. For organ dry weights, specimens were dried overnight in an oven at 70 °C.

Tadpoles were staged after fixation using Gosner (1960). Since tadpoles range considerably in size over Gosner stages 26–28 (assessed mainly by hind-limb form), their lengths and weights were also noted on staging. When comparing *P. paradoxa* development with other species in the literature where different staging systems have been used, we have used the equivalence table in McDiarmid & Altig (1999).

Tissues to be used for histological investigation were processed for wax histology using conventional methods, sectioned at 6–8 μm and stained using haemalum and eosin with alcian blue.

Tadpoles for laboratory growth experiments were transferred to glass tanks in the laboratory, containing dechlorinated tap water mixed with swamp water. Airstones were located at both ends of the tanks. Laboratory air temperature was approximately 28 °C, and water temperature 26 °C. Details of the feeding regime and growth results are presented in the companion paper (Downie et al., 2009). Gonad and fat body development results from laboratory reared tadpoles are presented here.

For statistical analysis, Minitab Statistical Analysis Software, version 13.3, was used.

RESULTS

Gonads and fat bodies

The gonads develop on the ventral surface of each kidney, with the fat bodies attached at the anterior end of

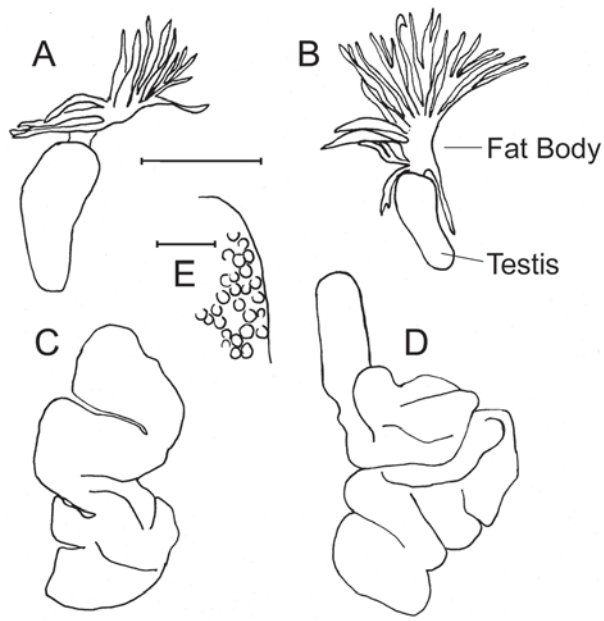

Fig. 1. Camera lucida drawings of *P. paradoxa* tadpole gonads and fat bodies. A) Testis and fat body, stage 37 tadpole; B) testis and fat body, stage 38 tadpole; C) ovary, stage 37 tadpole; D) ovary, stage 37–38 tadpole; E) enlargement of part of specimen D, to show oocytes. Scales: 2 mm line for drawings A–D, 0.5 mm line for drawing E.

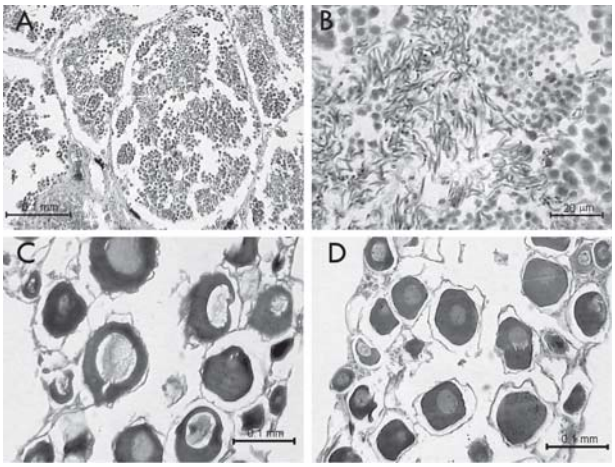

Fig. 2. Developmental state of the gonads in *P. paradoxa* tadpoles and newly metamorphosed individuals: transverse sections, stained haemalum, eosin and alcian blue. A,B) Testis of newly metamorphosed male; A: low magnification to show seminiferous tubule organization: scale bar = 0.1 mm; B: high magnification to show sperm: scale bar = 20 μm. C, D) Tadpole ovaries to show oocyte development; C: stage 37 155 mm long tadpole: scale bar = 0.1 mm; D: stage 37, 180 mm long tadpole: scale bar = 0.1 mm.

each gonad. We examined only one early-stage gonad (stage 26; total tadpole length 67.0 mm) and found it to be undifferentiated. We did not determine the stage at which the gonads became differentiated, but they were clearly male or female by stage 31 (two males, total lengths 102 and 124 mm respectively). From the small sample we were able to measure, gonads of both sexes grew in length up until the end of metamorphosis (Table 1). Testes were elongated, almost bean-shaped; ovaries were wider and sub-divided into lobes (Fig. 1).

Histological examination of the testes showed that they were organized into seminiferous tubules as early as stage 31. Tubule diameter was around 90μm in most of the stage 45–46 metamorphs, though one metamorph had substantially wider tubules at around 330 μm (Fig. 2). Spermatogenesis was evident as early as stage 31, but clearly increased (as shown by the numbers of sperm visible) as tadpoles approached and proceeded through metamorphosis. The number of mature sperm visible was greatest in the metamorph with the widest seminiferous tubules.

We examined histological sections of six ovaries, three from large tadpoles (155–180 mm total length) at stages 37–38, and three from metamorphs (stages 45–46). The lobular structure comprised follicles of various diameters, containing oocytes up to a diameter of around 130 μm (Fig. 2). There was no obvious difference between the ovaries of the metamorphs and the late-stage tadpoles. None of the oocytes showed signs of vitellogenesis.

Fat bodies were attached to the gonads and composed of tiny branching finger-like processes in smaller tadpoles (Fig. 1). By stages 38–39, most tadpoles had huge fat bodies, filling up a large proportion of the abdominal cavity. Fat body wet weights in two stage 38 tadpoles were 1.14 and 1.23 g respectively (1.22 and 1.88% of body mass). Dry weights of fat bodies from seven stage 38–40 tadpoles ranged from 0.117 to 0.861 g (mean 0.34 g ±0.274 SD).

We also measured fat body dry weights in two groups of tadpoles at or near the end of metamorphosis. These were six field-collected specimens from Columbus Bay (range 0.003–0.647 g; mean 0.461 g ±0.245 SD) and five laboratory-reared metamorphs (range 1.633–3.249 g; mean 2.182 g ±0.638 SD).

From these data, the wild-caught stage 38–40 and metamorphic (stage 45–46) tadpoles had similar mean fat body weights, but the laboratory-reared fat bodies were much larger (ANOVA: $F_{2, 18}$=35.201, P=0.000; significant differences between the laboratory-reared group and each of the others; no difference between stage 38–40 tadpoles and field-caught metamorphs).

Mouth and alimentary canal

Morphology of the oral disc was fully developed by stage 26 (Fig. 3). This consisted of serrated beak, two dorsal continuous tooth rows and three ventral tooth rows, one sub-divided, the others continuous. The margin of the oral disc had two rows of papillae except on the medio-dorsal side. Occasional specimens showed slight deviations from this pattern, with one having a condition similar in appearance to cleft palate (a stage 38 tadpole, at

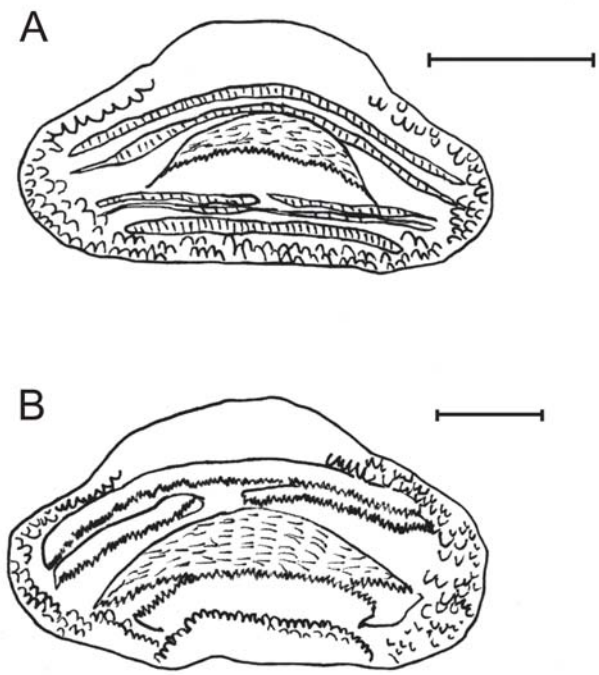

Fig. 3. Camera lucida drawings of *P. paradoxa* tadpole oral discs. A) Small tadpole, total length 30 mm, stage 26–27: scale bar = 1 mm; B) large tadpole, total length 155 mm, stage 38: scale bar = 2 mm. Drawings made as seen in unmanipulated view of disc; they do not show all the tooth rows or full view of jaws.

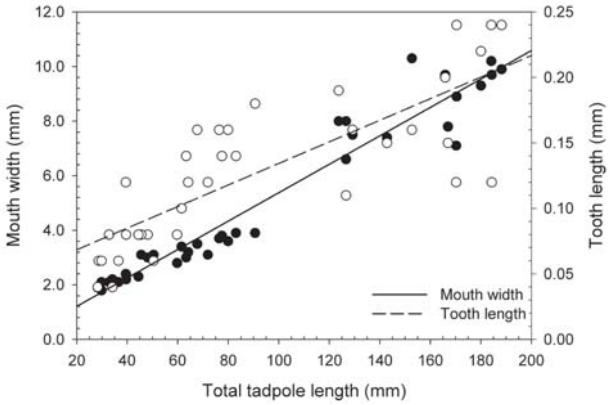

Fig. 4. Relationships between mouth width, tooth length and total body length in *P. paradoxa* tadpoles. Closed circles = mouth width (mm); open circles = tooth length (mm). Regression line statistics: mouth width, y=0.17+0.05x, r^2=0.94, P<0.001; tooth length, y=0.05+0.0008x, r^2=0.64, P<0.0001. Slopes of the two regression lines are significantly different (t=23.7, P<0.0001), with the tooth length slope less than that for mouth width.

full size, suggesting no obvious effect of the abnormal morphology on feeding ability). Oral disc dimensions changed with tadpole growth. Figure 4 shows the relationship between tadpole length, oral disc width and tooth length. Although there is considerable scatter in the data, both oral disc parameters increased significantly as tadpoles grew. However, rate of tooth length increase was less than oral disc width increase.

Table 2 shows the relationship between gut dimensions and body size over a range of stages. As tadpoles grew, both gut length and diameter increased considerably; however, the ratio between gut and body length remained nearly the same (17–18:1) between stages 27 and 38. Using gut length and diameter to calculate an approximate gut capacity gives 2.5 ml at stage 27 and 13.0 ml at stage 38. In relation to body length, this represents an approximate doubling of capacity (capacity/body length ratio stage 27, 1.02; stage 38, 1.97).

Gut length and diameter had reduced considerably in the single stage 42 tadpole (start of metamorphosis) we examined and was reduced further by stage 45 (near the end of metamorphosis).

Figure 5A–C shows the histological appearance of the intestine (ileum) at stages 27, 38 and 45 and Table 3 gives the dimensions and composition of the tissue layers. At stage 27, the ileum was a thin-walled tube with an unfolded columnar epithelial lining lacking goblet cells. By stage 38, the muscle layer and submucosa had thickened noticeably and the epithelial lining was now folded and rich in goblet cells. Dissection of the ileum at this stage showed that the epithelial folds were sectional views of irregular transverse ridges (Fig. 5D). By stage 45 (late in metamorphosis), folds were higher and goblet cells even more frequent. Sections of the hindgut (colon) at the same stages (not shown) were similar to the ileum.

Examination of gut contents in dissected specimens showed that tadpoles at all stages fed mainly on plant material: filamentous algae were predominant, but fragments of larger plants were also common. Some specimens contained abundant unicellular algae. We also found small amounts of animal-based material: legs of small arthropods, dipteran larvae, small annelids and tardigrades, but no nematode worms. The guts of stage 45 metamorphs were essentially empty.

Lungs

The lungs during tadpole stages were elongated sacs narrowing posteriorly. Their length increased approximately in proportion to body length as the tadpoles grew (Fig. 6). During metamorphosis, the lungs shortened and broadened, finishing as rounded sacs (Fig. 7). In fully grown tadpoles (stages 38–40: 7 specimens) lungs were 40.4% (±10.4% SD) of body length (excluding the tail) whereas they were 24.6% (±8.4% SD) of body length by the end of metamorphosis (stages 44–45; 6 specimens). The shape transformation during metamorphosis involved the elimination of the narrow posterior end of the lung, which persisted in recent post-metamorphic specimens as a solid bud of tissue (Fig. 7).

During early stages, the lungs of tadpoles were delicate sacs that collapsed when removed from the body, but as tadpoles grew, the lungs became much more substantial. Dissection of the lung of a later tadpole (stage 37, total length 180.00 mm) showed a substantial cavernous sub-divided wall, not significantly different from the interior of the adult lung, but quite distinct from the simple lungs of a conventional tadpole such as *Phrynohyas venulosa* (Fig. 8). In section, later stage *P. paradoxa* tadpole lungs showed a substantial structure with highly vascularised walls (Fig. 8).

Skin

Data on skin organization are shown in Table 4 and Figure 9. Skin organization in earlier tadpoles (stage 26–27) was typical for anuran larvae: a thin epidermis underlain by a collagenous basal lamella; ventral skin a little thicker than

Table 2. Relationship between gut dimensions, stage and body size.

		Mean size±SD		Mean gut dimensions±SD		
Stage	*n*	Body length (mm)	Mass (g)	Diameter (mm)	Length (mm)	Mass (g)
27	3	24.4±1.0	4.4±1.0	1.5±0.3	450.1±18.0	0.80±0.04 (2)
38	2	65.6±3.3	79.4±19.6	3.8±0.0	1149.0±262.5	9.98±3.38
42	1	37.5	35.2	1.5	253.8	0.40
45	2	45.6±4.2	34.1±3.0	1.1±0.1	72.3±3.3	0.22±0.06

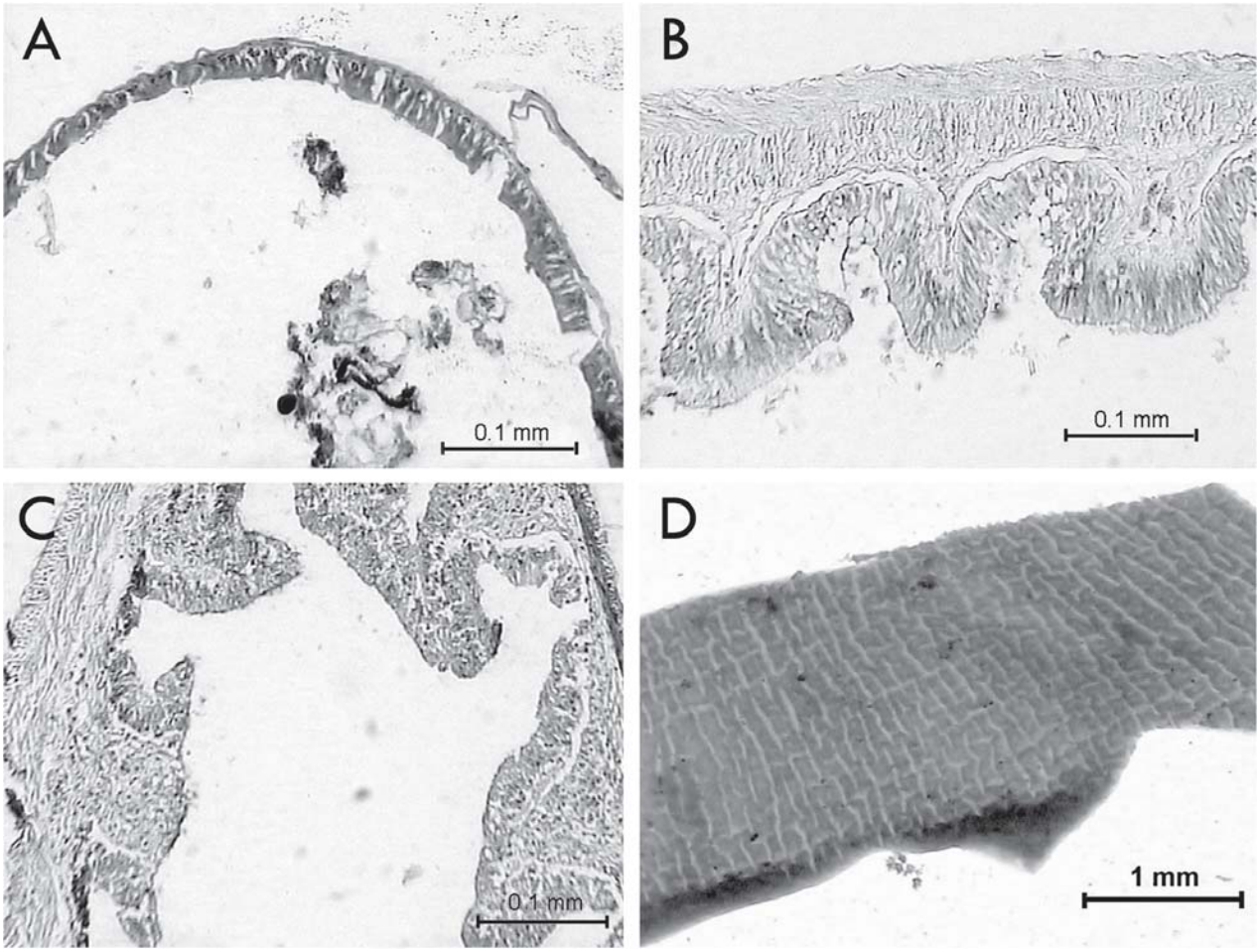

Fig. 5. Morphology of the intestine (ileum) in *P. paradoxa*. A–C) Transverse sections, stained haemalum, eosin and alcian blue; A: stage 27 tadpole; B: stage 38 tadpole; C: newly metamorphosed frog, stage 45. D) stage 38 tadpole, dissected ileum showing morphology of inner lining. Scale bars: A, B, C = 0.1 mm; D = 1 mm.

dorsal. However, the skin was dramatically thicker in later stage (37–38) tadpoles, but still lacked adult glands. By the end of metamorphosis (stages 45–46), the epidermis was thinner, the basal lamella had disappeared and the dermis was densely packed with at least two kinds of glands.

DISCUSSION

Gonadal development and maturation

Viertel & Richter (1999) have reviewed gonadal differentiation and development in tadpoles, and Ogielska & Kotusz (2004) have provided a detailed staging scheme for ovarian development. As is general for vertebrates, the initial gonad is indifferent and it is not possible to determine from its structure whether the individual will develop as a male or a female. Tanimura & Iwasawa (1988, 1989) have provided detailed descriptions of gonadal development in *Rana nigromaculata* and *Rhacophorus arboreus*. *R. nigromaculata* shows sex differentiation by Gosner stages 29–32. After stage 35, testes have cord-like rudimentary seminiferous tubules, but even by five days after metamorphosis, these tubules have yet to develop lumina. Ovaries have small follicles by stage 40; oocytes are still less than 100 μm diameter by stage 43. In *R. arboreus* gonadal sex differentiation was not clear until stage 43. By stage 46, oocytes had enlarged to 70 μm and to about 100 μm by seven days after metamorphosis. Testes retained rudimentary cord-like structures as late as seven days after metamorphosis.

Table 3. Intestine (ileum) tissue dimensions and composition. Two specimens examined for each stage – dimensions similar in each specimen.

Stage	Muscle thickness	Epithelial height	Fold height	Goblet cell presence
27	3–6 μm	28 μm	None	None
38	69–92 μm	28–34 μm	92 μm	Frequent
45	35–46 μm	28 μm	115 μm	Many

Ogielska & Kotusz (2004) found sexual differentiation (their ovary stage IV) at stage 31 in *Rana temporaria* and stage 28 in *Rana lessonae*. Ovarian stage VI was reached at stage 43 in *R. temporaria* and stage 33–35 in *R. lessonae* (characterized by small oocytes, maximum 50–60 μm); stage IX (with oocytes up to 100 μm) by the start of metamorphosis in *R. lessonae* and four weeks after metamorphosis in *R. temporaria*.

Our finding of spermatogenesis and well-formed seminiferous tubules as early as Gosner stage 31 seems to be unique amongst amphibians studied so far. Our sample of

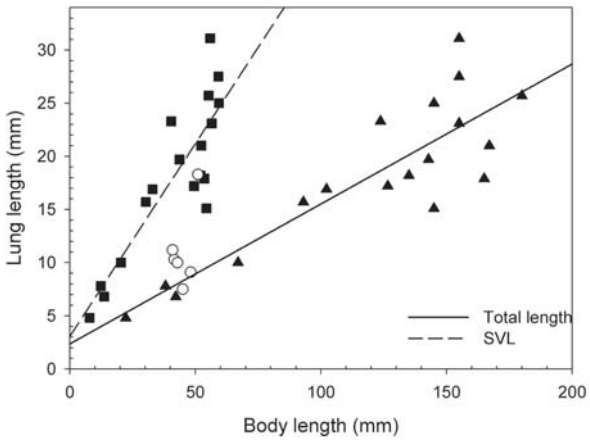

Fig. 6. Relationships between lung length and body length (total and SVL) in *P. paradoxa* tadpoles and late metamorphs (stage 45–46). Black triangles = tadpole total length; black squares = tadpole SVL; open circles = late metamorph SVL. Regression line statistics: tadpole total length, y=2.36+0.132x, r^2=0.75, P<0.0001; tadpole SVL, y=3.046+0.363x, r^2=0.75, P<0.0001. Relationship between body size and lung size clearly different in late metamorphs. Slopes of the two regression lines are significantly different: t=4.143, P<0.01.

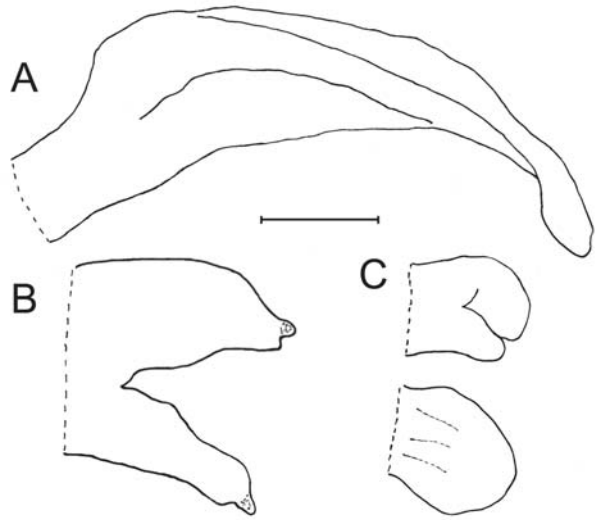

Fig. 7. Camera lucida outline drawings of *P. paradoxa* lungs. A) Tadpole, stage 37–38; B) metamorphic individual with 34 mm tail remaining; C) adult. All to the same scale. Scale bar = 5 mm.

females was smaller, but the size of oocytes in large stage 37–38 tadpoles suggest that the ovaries were already at Ogielska & Kotusz (2004) stage X, i.e. the stage normally found in juvenile frogs, with vitellogenic stages still to come.

There are precedents for such precocious gonadal development, but only in abnormal or experimental conditions. Wakahara (1994) and Kauki & Wakahara (1999) found accelerated testis development in experimental metamorphosis-arrested larvae of the salamander *Hynobius retardatus*: ovarian development was unaffected. They concluded that increased thyroid stimulating hormone (the result of thyroid inhibition) was the causal effect.

Table 4. *P. paradoxa* skin: tissue dimensions and composition.

Stage	Epidermis	Basal lamella/dermis	Glands
a) Dorsal			
26–27	1–2 cell layers plus periderm, 17 μm thick	Basal lamella 3 μm thick. Thin vascular dermis (20 μm)	Absent
37–38	Multiple layers: cuboidal at surface, columnar deeper, 100 μm thick	Basal lamella 30 μm thick. Thick (30 μm) vascular dermis with loose connective tissue	Absent
45–46	4–5 cell layers, cuboidal and squamous, 30 μm thick	Basal lamella absent; dermis 65 μm thick	Dermis packed with granular and acinar glands connecting to surface
b) Ventral			
26–27	2 cell layers plus periderm, 28 μm thick	Basal lamella 6 μm. Thin vascular dermis	Absent
37–38	Multiple layers: cuboidal at surface, columnar deeper, 120 μm thick	Basal lamella 40 μm thick. Loose connective tissue dermis with abundant blood vessels	Absent
45–46	5–6 cell layers, cuboidal and squamous, 50 μm thick	Basal lamella absent; dermis 60 μm thick	Dermis packed with mainly acinar glands connecting to surface

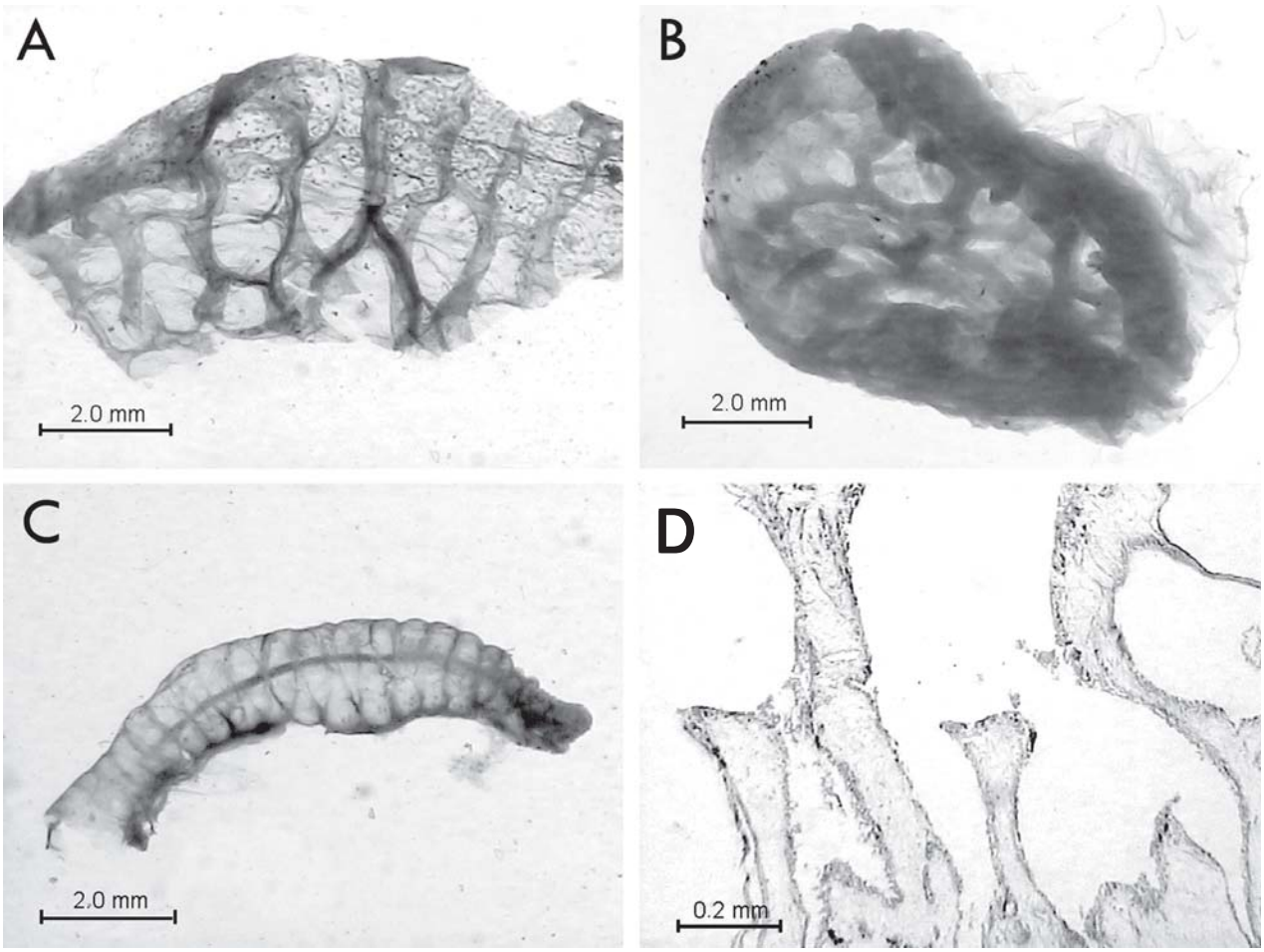

Fig. 8. Morphology of the lung in *P. paradoxa* tadpoles and adults, with a more conventional tadpole lung (*Phrynohyas venulosa*) as comparison. A–C) Whole or dissected specimens. A: *Pseudis* tadpole, stage 37, dissected to show inner wall morphology; B: *Pseudis* adult, dissected to show inner wall morphology; C: *Phrynohyas* tadpole, stage 38, whole lung. D) Transverse section, stained haemalum, eosin and alcian blue, *Pseudis* tadpole stage 37, showing structure of septa. Scale bars: A–C = 2.0 mm; D = 0.2 mm.

Rot-Nikcevic & Wassersug (2004) reported on 25 spontaneously-arising giant *Xenopus laevis* tadpoles. These tadpoles occur occasionally in breeding colonies, lack thyroid glands and grow to a length up to five times normal while remaining at Gosner stages 31–40 for up to several years. Out of the 25 tadpoles, 13 had well developed gonads, 12 females and one male. Ovaries were full of oocytes in vitellogenic stages (around 600 μm). The single male had testes with mature sperm, very similar in appearance to the *P. paradoxa* testes at stages 38–39 that we have described. The giant tadpoles also had large fat bodies, absent in normal *X. laevis* at the equivalent stages.

Our conclusion, based both on body size and gonadal differentiation, is that male *P. paradoxa* are essentially mature at metamorphosis, and that the females may be ready to initiate the vitellogenic phase of oogenesis. However, further studies will be needed to determine how long after metamorphosis males and females are actually able to breed. Vitellogenesis can be prolonged in temperate species, but can occur in a few weeks in tropical species (Davidson & Hough, 1969). In Trinidad, normal seasonality involves a rainy season from May to December, then a dry season from December to May. For a permanent swamp species like *P. paradoxa*, a life cycle that allows the aquatic growth phase to end about the end of the rainy season, with individuals almost in breeding condition, ready to start breeding when the next rains arrive, seems highly adaptive.

In considering evolutionary changes in amphibian life cycles Wassersug (1975) noted that both paedomorphosis and direct development occur in urodeles, but only direct development occurs as a life cycle change in anurans, never paedomorphosis. Although Altig (2006) has proposed that the initial evolution of the anurans involved a paedomorphic developmental change, it remains true that modern anurans show no evidence for full maturation in the larval state. As a possible explanation for this, Wassersug (1975) suggested that anuran tadpoles and adults are much more different from each other than are urodele juveniles and adults in morphology, feeding habitats, etc. A neotenic anuran would have to evolve a complex suite of new characters in order to reproduce. We suggest that *Pseudis* has gone as far in

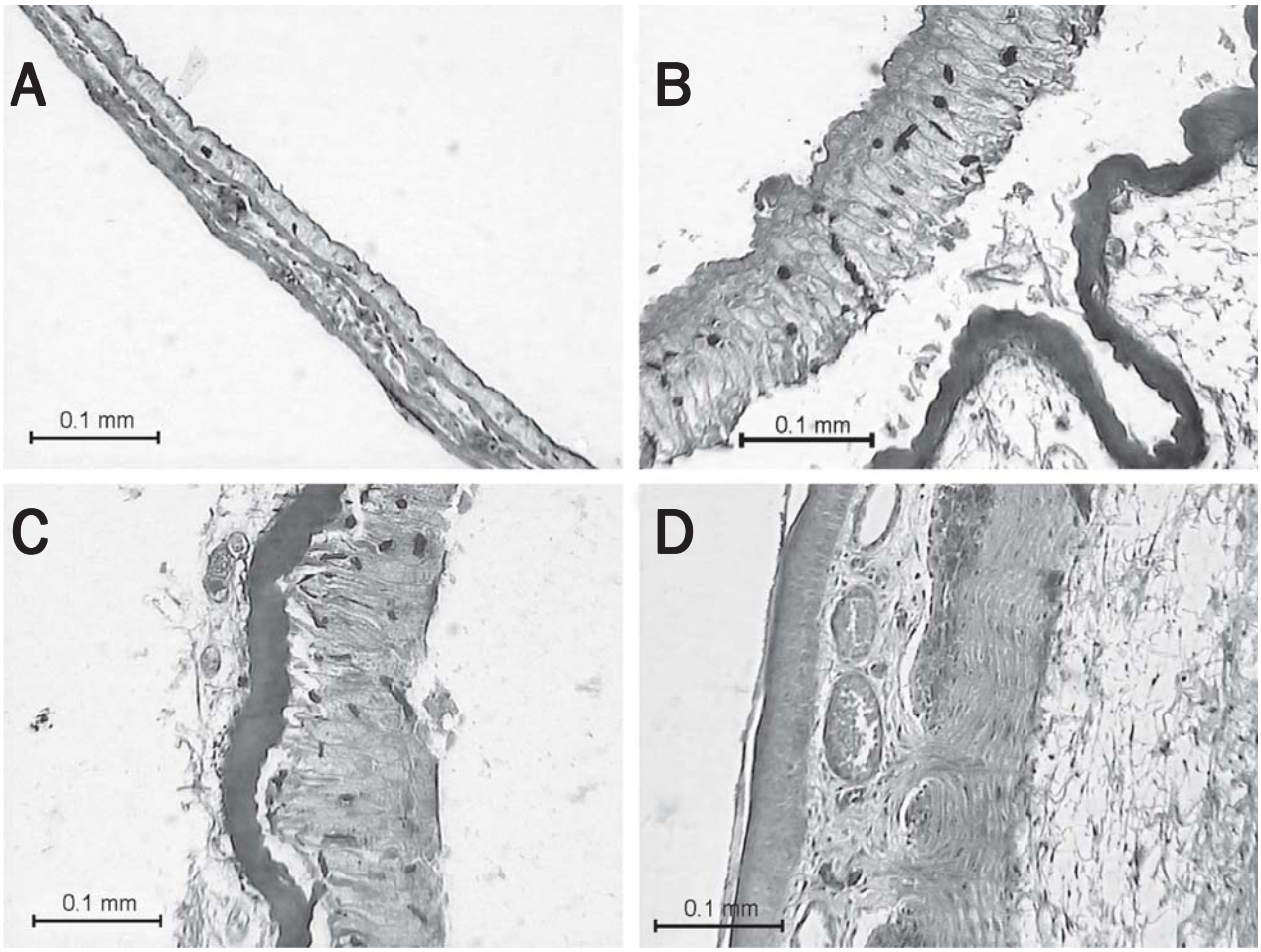

Fig. 9. Morphology of the skin in *P. paradoxa*. A–D: transverse sections, stained haemalum, eosin and alcian blue. A: tadpole stage 27, ventral skin, epidermis upper right layer; B: tadpole stage 38, ventral skin, epidermis upper left layer; densely-stained undulating layer, basal lamella. C: tadpole stage 38, dorsal skin, epidermis right layer. D: newly metamorphosed frog, ventral skin, epidermis left layer; glands embedded in dermis. Scale bars: all = 0.1 mm.

this direction as is possible by subsuming the normal post-metamorphic juvenile growth phase into the tadpole, including gonadal development to near maturity.

Fat bodies

In a laboratory study on *Bufo terrestris*, Beck & Congdon (2003) found that fat body mobilization was not a significant factor in metamorphosis, despite the lack of food intake during this phase of development. Our field-caught *P. paradoxa* had similar mean fat body sizes in late tadpoles (stage 38–40) and metamorphs, although individual variation was very considerable. Our laboratory-reared metamorphs had fat bodies about five times heavier than the field specimens. This suggests that the laboratory specimens were better fed than specimens in the field, or that in the predator-free laboratory environment they were able to spend more time on feeding. Interestingly, this did not lead to significantly different decisions on the size at metamorphosis, though it may mean that our growth rates in the laboratory were faster than those in the field. Given the seasonality and gonadal maturation described above, fat body size at the end of metamorphosis may be important for survival over the dry season and for the initiation of reproduction at the start of the wet season.

Mouth and alimentary canal

Viertel & Richter (1999) have reviewed the organization of the alimentary canal in anuran tadpoles. We have not attempted a full-scale description of the gut in *P. paradoxa*, but have reported simply on two regions, the ileum (from close to the "switchback point" – Pretty et al. (1995) – of the intestinal coil) and the colon. In early *P. paradoxa* (stage 27), the gut structure at these two points was similar to conventional tadpoles: a thin-walled tube with an unfolded columnar epithelial lining and a thin smooth muscle layer. In later stage tadpoles, construction of the gut was considerably more substantial, with irregular folds which presumably increase the inner absorptive surface area. This appears to be a feature unique to *P. paradoxa*.

Otherwise, we found no unusual features in the alimentary system of *P. paradoxa*. Later-stage tadpoles did have traces of animal-based material in their guts, but we suspect that this was ingested incidentally along with the large quantities of plant material that these tadpoles process.

Wassersug (1975) felt that the general limitation on anuran tadpole size at metamorphosis could be explained by reliance on filter-feeding and algal browsing, combined

with poor digestive capacity. He found that small tadpoles cleared an algal suspension faster than large tadpoles and attributed this to isometric rather than allometric growth of the food processing system. He also felt that tadpoles mainly extracted nutrients through cell walls, with no cellulose digestion involved. *Pseudis* (and to a lesser extent *Rana catesbeiana* and a few other species) look like the most obvious exceptions to Wassersug's (1975) argument. We have not attempted a detailed analysis of the alimentary system, but we have found some evidence of morphological complexity in the intestine, possibly related to enhancing digestive/absorptive capacity; our growth rate data (Downie et al., 2009) also suggest that growth does not slow down as tadpoles grow in size. In any case, recent studies on food assimilation and selectivity in anuran tadpoles (for example, see Skelly & Golon, 2003) have emphasized diversity in assimilation efficiency between species and between different food sources, and possible mechanisms for enhancing assimilation, such as cell wall abrasion by ingested mineral particles.

Another possible means of enhancing assimilation efficiency is by fermentation. Pryor & Bjorndal (2005) have produced evidence that the gastrointestinal nematode *Gyrinicola batrachiensis* can speed American bullfrog tadpole growth by generating an intestinal fermentation, producing absorbable fatty acids and other nutrients. *G. batrachiensis* is found in a wide variety of tadpole species (Pryor & Greiner, 2004) and has generally been assumed to be a parasite: the suggestion that it has a mutualistic interaction with its host is intriguing. We did not find this nematode in our *P. paradoxa* sample, but Kerr & Hamann (2003) reported it as the commonest "parasite" in *P. paradoxa* tadpoles sampled from two ponds in Argentina.

It is interesting that both oral disc width and tooth length increased with tadpole growth, oral disc width approximately isometrically, tooth length a little less so. Tooth length may be somewhat constrained by underlying structure, or there may be no advantage in increasing tooth length beyond a particular point.

Lungs, skin and respiration

Respiration in tadpoles has been reviewed by Viertel & Richter (1999), Ultsch et al. (1999) and Hoff et al. (1999). Tadpoles may use three surfaces as gas exchangers: gills, skin and lungs. The relative use of these surfaces varies with tadpole type and to some extent with habitat features, such as oxygen concentration. In some groups, lungs remain as buds until metamorphosis (e.g. bufonids) but in others, lungs develop in early tadpole stages and elongate as the tadpoles grow. Viertel & Richter (1999) claim that tadpole lungs are never more than thin-walled undivided sacs, sparsely supplied with capillaries, and doubt that they have a significant role in respiration. However, Ultsch et al. (1999) note that when oxygen concentration in the water is lowered, some tadpole species respond by increasing their migrations to the surface to ventilate the lungs. Hoff et al. (1999) note that breathing at the surface is an aspect of behaviour in many tadpole species, and that lung ventilation has both respiratory and hydrostatic functions. Rondeau & Gee (2005) found that substrate particle ingestion was used by tadpoles to increase specific gravity, with adjustments in lung volume maintaining neutral buoyancy.

Our results indicate that the lungs of later stage *P. paradoxa* tadpoles are well developed, with highly subdivided interiors and well-vascularized walls. So far, this appears to be a unique feature among tadpole species under normal conditions. Even in the large tadpoles of the American bullfrog, lung septation does not develop till metamorphosis is under way (Atkinson & Just, 1975). However, the lungs of the giant *Xenopus laevis* tadpoles described by Rot-Nikcevic & Wassersug (2004) are not unlike those of *P. paradoxa*. They found two lung morphologies: lungs were either air-filled and essentially normal, but with more septa, or almost solid, with many septa and abundant smooth muscle and connective tissue. The distal ends of *P. paradoxa* lungs are almost solid, and the proximal ends normal but with considerable septation. De Souza & Kuribara (2006) have found that in normoxic conditions, *P. paradoxa* tadpoles obtain 96% of their oxygen from water, but that under hypoxic conditions, this can change dramatically, with tadpoles obtaining as much as 76% of their oxygen needs from air. De Souza & Kuribara (2006) note that hypoxic conditions are common where *P. paradoxa* tadpoles occur in Brazil, and that surfacing behaviour increases in frequency with tadpole growth and development.

Viertel & Richter (1999) doubt the role of the skin of tadpoles in respiration: though large in surface area, the vascular supply is not impressive until after metamorphosis. Ultsch et al. (1999) review studies which show that cutaneous gas exchange is significant in anuran tadpoles, but note that this may partly relate to the high proportion of the body, especially in the tail, that is skin. In later stage *P. paradoxa* tadpoles, the relative skin surface area must be lower than in small tadpoles, and our results show that the skin is considerably thicker than in smaller tadpoles. In particular, the combination of the thickened epidermis and thick avascular basal lamella looks like a substantial barrier to respiratory exchange. We have not examined the skin surface all over the body, but if it is similar to the areas we have looked at we expect cutaneous respiration to be unimportant in later stage *P. paradoxa* tadpoles. The function of skin thickening in later-stage *P. paradoxa* tadpoles is unclear: possibly it has a structural or protective role.

Skeletal development

While this paper was under revision, Fabrezi & Goldberg (2009) reported on skeletal development in tadpoles of *Pseudis platensis*. Their conclusion was that skull and post-cranial ossification are well advanced or complete by the end of metamorphosis in *P. platensis*, compared to other anurans, and that the patterns they observed suggest that individuals have reached full adult size when fully metamorphosed. These conclusions concur with our findings for some of the other organ systems reported here.

ACKNOWLEDGEMENTS

JRD wishes to thank Julian Kenny for introducing him to the paradoxical frog. JRD's field work in Trinidad was supported by the Carnegie Trust, the British Council and the University of Glasgow. Many students on University of Glasgow Trinidad Expeditions helped capture *Pseudis* tadpoles and adults. Thanks to the Zoology Division, University of the West Indies, St Augustine for provision of laboratory space and to the Wildlife Section for permission to collect *Pseudis* in Trinidad. Thanks particularly to Florence McGarrity for turning many versions of this paper into coherent typescript, to Norman Tait for work on the photographs, to Joanna Smith and Suzanne Livingstone for comments on the manuscript and to Naomi Barron for fieldwork assistance.

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Accepted: 31 March 2009