

**Standardized Criteria for the Recognition of Embryonic Nutritional Patterns  
in Squamate Reptiles**



Daniel G. Blackburn

*Copeia*, Vol. 1994, No. 4. (Dec. 19, 1994), pp. 925-935.

Stable URL:

<http://links.jstor.org/sici?sici=0045-8511%2819941219%293%3A1994%3A4%3C925%3ASCFTRO%3E2.0.CO%3B2-Y>

*Copeia* is currently published by American Society of Ichthyologists and Herpetologists.

---

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/about/terms.html>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/asih.html>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

---

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact [support@jstor.org](mailto:support@jstor.org).

- and N. B. Davies (eds.). Blackwell Scientific Publications. Oxford, England.
- ROMERO, H. 1965. Catálogo sistemático de los peces del Alto Lerma con descripción de una nueva especie. *An. Esc. nac. Cienc. Biol., Méx.* 14:47–80.
- SUTHERLAND, W., AND M. C. M. DE JONG. 1991. The evolutionarily stable strategy for secondary sexual characters. *Behav. Ecol.* 2:16–20.
- TRIVERS, R. L. 1972. Parental investment and sexual selection, p. 136–179. *In: Sexual selection and the descent of man.* B. Campbell (ed). Aldine, Chicago, Illinois.
- UYENO, T., R. R. MILLER, AND J. M. FITZSIMONS. 1983. Karyology of the Cyprinodontoid fishes of the Mexican family Goodeidae. *Copeia* 1982:497–510.
- SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EAST ANGLIA, NORWICH NR4 7TJ, ENGLAND. PRESENT ADDRESS: CENTRO DE ECOLOGÍA, UNAM, A. P. 70-275, C.P. 04510, MÉXICO D. F., MÉXICO. Submitted: 13 June 1992. Accepted: 18 Nov. 1993. Section editor: W. J. Matthews.

*Copeia*, 1994(4), pp. 925–935

## Standardized Criteria for the Recognition of Embryonic Nutritional Patterns in Squamate Reptiles

DANIEL G. BLACKBURN

**In squamates, nutrients for embryonic development can be derived from the egg itself or from the placental membranes. This paper critically evaluates criteria that have been used to distinguish developmental nutritional patterns, with an emphasis on viviparous squamates. Composition analysis of eggs and neonates is the most reliable means of quantifying sources of nutrients for development but tends to underestimate placental nutrient provision. Data on gestational changes in dry mass of the viviparous conceptus indicate only the predominant source of nutrients for development. Specialized allantoic morphology offers moderate evidence for placentotrophy, but the functional correlates of several placental features remain unknown. Radiotracer studies reveal the capacity for placental transport but not its importance to embryonic nutrition. Gestational changes in wet mass of the conceptus reveal little about nutrient sources in relatively lecithotrophic forms, as do comparisons of the relative size of the ovum with the fetus or the mother. Future reports should consider the nature and quality of evidence for inferences about developmental nutritional modes.**

For more than a century, researchers have characterized squamate reptiles according to how nutrients are supplied to the developing embryos (Giacomini, 1891; Yaron, 1985). Two patterns that represent extremes of a continuum are distinguished: "lecithotrophy," in which the yolk provides the nutrients for development; and "matrotrophy," in which substantial quantities of nutrients are supplied by extra-ovelline means (Wourms, 1981; Blackburn, 1992, 1994), such as placental membranes. The conceptual division of squamates into categories based on these two developmental patterns is central to concepts of reproductive diversity. Moreover, the distinction is the basis for quantitative evolutionary analyses (Blackburn et al., 1985; Blackburn, 1992), formal tests of life-history theory (Stewart, 1989), and comparisons

between reptiles and other vertebrates (Guillette, 1987; Jones and Baxter, 1991; Blackburn, 1992).

The criteria used to recognize embryonic nutritional modes have varied extensively among researchers, historical periods, and taxa. Despite growing interest in reptilian viviparity and fetal nutrition, these criteria have not been subjected to critical review or examined in the light of new information that challenges traditional assumptions. Consequently, similar bodies of evidence have led researchers to discrepant conclusions, and no clear standards have been established for recognition of common patterns in diverse taxa. A similar situation exists with respect to the distinction between reptilian oviparity (egg-laying reproduction) and viviparity (live-bearing reproduction), with the added

complication that the observations underlying recognition of these patterns in particular species are seldom identified (Blackburn, 1993a).

This paper has two major goals: (1) to evaluate the criteria used to distinguish embryonic nutritional modes in reptiles, along with their underlying assumptions; and (2) to identify a hierarchy of lines of evidence for recognition of particular patterns, based on reliability. Emphasis is placed on nutritional patterns in viviparous (live-bearing) squamates, whose embryos can derive nutrients from both vitelline and placental sources. However, the principles involved are broadly applicable to reptiles, amphibians, and fishes. This paper is the second in a series; a companion paper has proposed standardized criteria for the distinction between oviparity and viviparity (Blackburn, 1993a).

#### AVAILABLE CRITERIA

By virtue of the structure of the squamate uterus, as well as the thinness of the shell membrane in typical viviparous forms, the uterine lining lies in close proximity to the extraembryonic membranes, forming any of several types of placental arrangements (Stewart and Blackburn, 1988). Maternal-fetal nutrient transfer in viviparous squamates is necessarily placental (Blackburn, 1993b), and matrotrophy, therefore, can be termed "placentotrophy." Strict lecithotrophy is usually assumed to characterize both oviparous and many viviparous squamates. However, lecithotrophy and placentotrophy represent extremes of a continuum, and viviparous females can provide nutrients via both yolk and placenta. Consequently, squamates can be characterized according to the predominant source of nutrients for development (e.g., Stewart, 1989; Stewart et al., 1990).

Ideally, criteria for recognition of these embryonic nutritional patterns should be reliable, consistent with available evidence, and easy to apply. One of these features, ease of application, is not possible in distinguishing nutrient sources with precision, because of the need for sophisticated analysis and experimentation (see below). However, the remaining characteristics represent practical standards for criteria that are to be used in recognizing embryonic nutritional patterns. Each of the criteria available for the determination of developmental nutritional patterns in squamates is discussed below.

*Chemical composition analysis.*—A precise method for determining sources of nutrients for development in viviparous squamates is a com-

parison of the chemical composition of the early zygote and the offspring near the time of parturition. Initiated early in this century (Giersberg, 1923), this method has been refined considerably (Thompson, 1981; Stewart and Castillo, 1984; Stewart, 1989). The rationale is that, for any component, an increase that cannot have resulted from metabolic conversion must reflect uptake by the conceptus. Thus, comparisons of viviparous forms can allow quantification of components supplied by the yolk and the placenta and, if analyzed separately, the shell membrane. Likewise, in oviparous forms, composition analysis allows quantification of nutrients derived from the yolk, the eggshell, and albumen.

In those few viviparous squamates where fetal nutrition has been quantified by composition analysis, ovulated yolk typically supplies most of the organic nutrients for development, with placental sources providing significant inorganic contributions (e.g., Thompson, 1982; Stewart and Castillo, 1984; Stewart et al., 1990). In contrast, in the viviparous Australian skink *Pseudemoia entrecasteauxii*, placental transfer is estimated to account for nearly half of the organic material for growth and development (Stewart and Thompson, 1993). As expected, analyses of eggs and hatchlings of oviparous species have revealed a developmental decrease in total lipid and protein (Florian, 1990; Stewart and Thompson, 1993). Squamate analyses also have revealed that the eggshell can provide significant quantities of calcium to the oviparous embryo (e.g., M. J. Packard et al., 1985; M. J. Packard and G. C. Packard, 1988; Stewart and Thompson, 1993).

Chemical composition analysis has not always been used to optimal advantage. Some studies have drawn conclusions from data that were not analyzed statistically (Clark et al., 1955; Jenkins and Simkiss, 1968; Thompson, 1981), leading to at least one invalid conclusion. Whereas a study on *Thamnophis sirtalis* concluded that ash content of the conceptus increased during gestation (Clark and Sissen, 1956), Shine's (1977) reanalysis of the data indicated that the purported trend was not statistically significant. Recent demonstration of intraspecific variation among eggs and among females, and the subtlety of gestational changes in relatively lecithotrophic, viviparous species (e.g., Stewart and Castillo, 1984; Stewart, 1989), illustrate the need for careful statistical analyses that take into account individual variation and interactions among variables.

Past analyses of organic composition of eggs and offspring have been based on several im-

placit, but sometimes untested, assumptions. One assumption is that maternal carbohydrates are not a significant means of nutrient provision; carbohydrates have not been quantified in past studies. A related assumption is that carbohydrates synthesized by the fetus do not constitute a significant component of the neonate. That carbohydrates comprise but a small proportion of yolk stores is indicated by a few early studies (Needham 1931; Fukada, 1939), and quantification of protein and lipid has revealed little residual organic content that could represent carbohydrate (Stewart and Castillo, 1984; Florian, 1990). However, despite the fact that catabolism of yolk lipid may be sufficient to account for fetal metabolism in one viviparous species (Thompson, 1982), placental provision of simple carbohydrates cannot be ruled out in live-bearing forms, in view of information on viviparous mammals (Barcroft, 1952; Huggett, 1954).

Another assumption of past composition analyses is that the amount of nonprotein nitrogen is small in both eggs and neonates, because protein typically has been estimated from total nitrogen content following Kjeldahl digestion. Unfortunately, data on yolk composition and nitrogen excretion in squamates are scanty (e.g., Athavale and Mulherkar, 1967; G. C. Packard et al., 1977; G. C. Packard and M. J. Packard, 1987); whether these data are typical for squamates is not known. Quantitative data on nitrogen excretion and loss across the placenta in viviparous species would permit more precise estimates of protein catabolism and might reveal an unanticipated amount of placental transfer of amino acids.

Although chemical composition analysis remains a most effective way to quantify nutrient provision, at least two additional complications affect its application to viviparous species. First, such analyses tend to underestimate extra-vitelline provision of nutrients. They indicate net gain or loss of nutrients by the developing conceptus during gestation and, consequently, do not reveal nutrients of oviductal origin that are metabolized or otherwise lost during development. In the viviparous lizard *Eulamprus quoyii*, for example, chemical composition analysis suggests no net gain of nitrogen by the conceptus (Thompson, 1981), although radiotracer studies reveal maternal-fetal transfer of amino acids (Thompson, 1977a). Either the extent of maternal-fetal transfer of nitrogen in this species is negligible or the amount of nitrogen lost from the egg (e.g., as wastes) is roughly equivalent to that gained via the placenta.

A second complication is that the amount of

nutrients supplied via the placenta is not necessarily constant for a species or even for an individual female over her reproductive lifetime. Recent analyses on thamnophiine snakes suggest that placentotrophy includes a facultative component and that nutrient provision is altered according to nutrient availability or female size (Stewart, 1989; Stewart et al., 1990). Moreover, variation among females necessitates comparison of sibling eggs and neonates, involving surgical removal of some of the eggs during gestation (Stewart and Castillo, 1984). However, whether removal of eggs early in gestation alters the level of nutrient provision to the remaining conceptuses (Clark et al., 1955), as occurs in certain mammals (van Marthens et al., 1972), is unknown. In view of such difficulties, even the most precise data on sources of nutrients for viviparous development may provide only general information on nutrient provision in a species.

Two other factors complicate analyses of egg-laying squamates. First, in oviparous squamates, substantial egg development can precede oviposition (Shine, 1983). Whether any organic nutrients are absorbed from the oviduct during this period has not been determined (Blackburn, 1992), nor has metabolic loss prior to oviposition been quantified. Although little embryonic growth is apparent before egg deposition in many oviparous squamates, the yolk + embryo of freshly oviposited eggs probably ought not be assumed to be identical in composition to that of the newly fertilized ovum. An additional issue is that the eggshell itself is a significant source of inorganic nutrients such as calcium in oviparous squamates (M. J. Packard et al., 1984, 1992; Stewart and Thompson, 1993). Thus, analyses of oviparous forms ideally should compare ova at ovulation, eggs following deposition of the eggshell, and newly emergent hatchlings.

*Developmental changes in dry mass of the conceptus.*—Comparisons of the mass of the newly ovulated ovum and neonate often have been used to estimate sources of nutrients for development in viviparous species. Oviparous reptilian eggs, with their lecithotrophic nutrition, typically decrease in dry mass during development (Table 1), presumably reflecting catabolism and metabolic loss. Consequently, a substantial decrease in mass of the conceptus of viviparous forms usually is assumed to indicate lecithotrophy as well (Guillette, 1981; Thompson, 1981). By the same token, placentotrophy has been assumed when dry mass of the neonate greatly exceeds (Blackburn et al., 1984; Stewart

TABLE 1. DEVELOPMENTAL CHANGES IN MASS OF THE EGG IN SELECTED OVIPAROUS SQUAMATES. Numbers represent change in mass as a percentage of initial (oviposited) egg mass [i.e., (final mass - initial mass)/initial mass]. n.s. = no significant change.

Species	Change in mass		Source
	Dry mass	Wet mass	
<i>Anolis auratus</i>	—	+82 to 204% <sup>ab</sup>	Andrews and Sexton, 1981
<i>Bassiana duferreyi</i>	-15% <sup>cd</sup>	+10% <sup>d</sup>	Stewart and Thompson, 1993
<i>Callisaurus draconoides</i>	—	+53 to 100% <sup>ab</sup>	M. J. Packard et al., 1982
<i>Coluber constrictor</i>	-14% <sup>a</sup>	+53% <sup>a</sup>	M. J. Packard et al., 1984
<i>Crotaphytus collaris</i>	—	+82% <sup>ab</sup>	Tracy and Snell, 1985
<i>Dipsosaurus dorsalis</i>	—	+33%	Muth, 1981
<i>Eumeces fasciatus</i>	-33%	+14% <sup>d</sup>	Florian, 1990
<i>Iguana iguana</i>	-27 to -29% <sup>ce</sup>	-3 to +52% <sup>ab</sup>	M. J. Packard et al., 1992
<i>Liolaemus tenuis</i>	—	+125%	Lemus et al., 1984
<i>Pituophis melanoleucus</i>	—	-14 to +51% <sup>ab</sup>	Gutzke and Packard, 1987
<i>Pogona barbata</i>	-29.6% <sup>cd</sup>	+66%	M. J. Packard et al., 1985
<i>Sceloporus aeneus</i>	-59% <sup>ce</sup>	+17% <sup>c</sup>	Guillette, 1981
<i>Sceloporus undulatus</i>	—	+106 to 170% <sup>ab</sup>	Tracy, 1980

<sup>a</sup> Numbers estimated from graph.

<sup>b</sup> Depending on incubation conditions (e.g., soil matric potential).

<sup>c</sup> Numbers calculated from reported data.

<sup>d</sup> Shell mass excluded throughout.

<sup>e</sup> Comparison of hatching with developing egg soon after oviposition (*I. iguana*) or ovulation (*S. aeneus*); final shell mass excluded.

and Thompson, 1993) or even is equivalent to (Shine, 1977; Guillette and Casas-Andreu, 1987) that of the egg early in development.

A substantial increase in dry mass of the viviparous conceptus during gestation (Table 2), as occurs in the skinks *Pseudemoia entrecasteauxii* (Stewart and Thompson, 1993) and *Mabuya bis-triata* (Blackburn and Vitt, 1992) clearly indicates a substantial reliance on placentotrophic nutrition; any increase in mass over that of the newly fertilized ovum must reflect uptake from maternal tissues. However, although such data

allow estimates of the contribution of placental sources to neonatal mass, a disadvantage of this approach is that quantification of specific nutrients is not possible, because losses resulting from catabolism are not calculated.

Somewhat less information can be extracted from data on viviparous forms in which dry mass of the conceptus does not increase markedly during gestation (Table 2). Rather than indicating strict lecithotrophy, a dry mass decrease may simply indicate that any placentotrophic nutrition is insufficient to compensate for met-

TABLE 2. DEVELOPMENTAL CHANGES IN MASS OF THE OVIDUCTAL CONCEPTUS IN SELECTED VIVIPAROUS SQUAMATES. Numbers represent change in mass as a percent of initial (postovulatory) mass. Among forms known to have some degree of placentotrophy,<sup>a</sup> note the degree of overlap with values on oviparous eggs (Table 1). n.s. = no significant change.

Species	Change in mass		Source
	Dry mass	Wet mass	
<i>Sceloporus bicanthalis</i>	-54%	+23%	Guillette, 1981
<i>Vipera berus</i>	—	+51% <sup>b</sup>	Jenkins and Simkiss, 1968
<i>Notechis scutatus</i>	-35%	+96%	Shine, 1977
<i>Virginia striatula</i> <sup>a</sup>	-31%	+48%	Stewart, 1989
<i>Thamnophis ordinoides</i> <sup>a</sup>	-25%	+41%	Stewart et al., 1990
<i>Nerodia rhombifera</i> <sup>a</sup>	-22%	+59%	Stewart and Castillo, 1984
<i>Elgaria coerulea</i> <sup>a</sup>	-18%	+142%	Stewart and Castillo, 1984
<i>Austrelaps superbus</i>	—	+143%	Shine, 1977
<i>Pseudechis porphyriacus</i>	n.s.	+207%	Shine, 1977
<i>Eulamprus quoyii</i> <sup>a</sup>	-10% <sup>b</sup>	+96% <sup>b</sup>	Thompson, 1977b
<i>Pseudemoia entrecasteauxii</i> <sup>a</sup>	+68%	+296%	Stewart and Thompson, 1993
<i>Chalcides chalcides</i> <sup>a</sup>	—	+600-800% <sup>b</sup>	Ghiara et al., 1987

<sup>a</sup> Animals in which evidence exists for some maternal-fetal transfer of nutrients (see text).

<sup>b</sup> Not tested for statistical significance.

abolic loss and waste loss by the developing egg. In fact, in several viviparous squamates, the conceptus exhibits a substantial dry mass decrease during gestation, despite the placentotrophic supply of at least small amounts of certain nutrients (Table 2). Likewise, in viviparous *Lacerta vivipara*, which have the capacity for placental transfer of Na, I, and P (Panigel, 1956), the oviductal clutch shows no gestational change in dry mass (Avery, 1975). In any case, changes in dry mass ideally should be demonstrated through statistical analyses that control for differences among eggs and among females. Claims for increases in dry mass of the conceptus in *Regina grahami* (Hall, 1969) and *Thamnophis sirtalis* (Clark and Sissen, 1956) have been challenged by subsequent statistical analyses of reported data (Shine, 1977). As for viviparous species where dry mass of the conceptus remains nearly constant during development, extra-vitelline sources probably make some contribution to fetal nutrition; however, the magnitude of that contribution cannot be estimated accurately from mass data alone.

Developmental changes in egg dry mass in oviparous species have been suggested as a standard for inferring degree of lecithotrophy in viviparous forms (Hogarth, 1976; Thompson, 1981). However, given the amount of interspecific variation in relatively lecithotrophic squamates (Table 1–2), inferences from mass data in such forms are tenuous. Moreover, mass of the newly hatched eggshell is usually overlooked in analysis of oviparous eggs, with consequent overestimation of developmental decrease in dry mass. Furthermore, although caloric content per mg of egg dry mass may be fairly constant in oviparous squamates (Vitt, 1978), little reason exists to suppose that efficiency of conversion of yolk to embryo is consistent across taxa and under different conditions of temperature and substrate water potential.

*Developmental changes in wet mass.*—A large increase in wet mass of the developing conceptus sometimes is used to infer placentotrophic supply of nutrients in viviparous squamates (Branson and Baker, 1974; Ghiara et al., 1987). The rationale is that substantial nutrient provision would be accompanied by a larger increase in water than would occur if the species were lecithotrophic. An underlying assumption is that water content of neonates is unrelated to nutritional mode or site of development.

A substantial increase in wet mass of the developing egg is typical of oviparous as well as viviparous squamates (Table 1–2). Consequent-

ly, for most viviparous squamates, data on changes in wet mass of the developing conceptus are much less useful than dry mass data in distinguishing fetal nutritional patterns. Although a very large increase in wet mass of the conceptus (Ghiara et al., 1987; Vitt and Blackburn, 1991) indicates placentotrophy, no restricted range for wet mass increases of eggs can be cited as indicating strict lecithotrophy. Another problem is that wet mass does not correlate with energy content in relatively lecithotrophic forms, because fetuses have a higher water content than yolks (Shine, 1977). For such reasons, inferences of the extent of placentotrophy from wet mass data in viviparous forms are questionable (see Shine, 1977; Jenkins and Simkiss, 1968).

Comparisons to lecithotrophic eggs of oviparous squamates do little to clarify the situation, because oviposited eggs can double or triple in wet mass through water uptake (Andrews and Sexton, 1981; Tracy, 1980). An additional difficulty is that developmental increases in wet mass of oviparous eggs can depend on substrate water potential (M. J. Packard et al., 1980, 1992; Gutzke and Packard, 1987) and other incubation conditions (M. J. Packard et al., 1982; G. C. Packard and M. J. Packard, 1993). Thus, a broad range of values may characterize any given species. Finally, even comparisons between oviparous and viviparous congeners can be misleading. Jacobi (1936) found that eggs of the oviparous *Lacerta agilis* underwent a far greater increase in wet mass than those of the viviparous *L. vivipara*. If values for oviparous and viviparous *Lacerta* in Jacobi's (1936) study had been reversed, one easily might have inferred that the viviparous form was highly placentotrophic.

In sum, data on gestational changes in wet mass of the conceptus of viviparous forms can be broadly indicative of extremes of lecithotrophy and placentotrophy. However, such data do not allow estimates of relative contributions of vitelline and extra-vitelline sources in species occupying intermediate positions in the nutritional continuum, and, for all species, data tend to be imprecise measures of nutrient sources.

*Relative size of ovum and neonate.*—Fetal nutritional sources in viviparous squamates often are estimated by qualitative comparisons of size of the ovulated ovum with that of the neonate. For example, in *Chalcides chalcides*, females ovulate ova of about 3 mm in diameter and, after a three-month gestation, give birth to neonates of about 94 mm in total length (Giacomini, 1891). In Brazilian *Mabuya heathi*, the ovum is 1 mm in diameter, and the neonate is about 30

mm in SVL (Blackburn et al., 1984). The large size difference between the ovum and the neonate in these species demonstrates that extra-vitelline sources account for most of neonatal content. The large gestational increase in mass of the conceptus (Blackburn et al., 1984; Ghiara et al., 1987) confirms the important role of extra-vitelline nutrition.

In most known viviparous species, the ovum and near-term fetus are comparable in size, and lecithotrophy is often inferred. However, estimates based on relative size are imprecise, not only because of catabolism of vitelline nutrients but also because the neonate contains a much higher percentage of water than the ovulated yolk (Shine, 1977; Stewart et al., 1990). Moreover, although very general conclusions about the role of lecithotrophic nutrition are possible when the ovum and neonate are similar in size, some degree of placentotrophy cannot be ruled out. Eggs and young are of comparable size in most or all of the relatively lecithotrophic, viviparous squamates where some placental transfer of nutrients has been demonstrated (Blackburn, 1993b). Therefore, although a size comparison of the neonate and ovum may allow tentative inferences about probable sources of nutrients for development, such inferences are less reliable and much less precise than those based on dry mass and chemical composition.

*Relative size of the ovum and female.*—Weekes (1930) inferred the presence of placentotrophy in the viviparous skink *Pseudemoia* ("*Leiolopisma*") *entrecaesteauxii*, based partly on her observation that ova of this lizard were "barely two-thirds the size of oviparous species of the same adult size" (p. 570). Although this nominal species does include placentotrophic representatives (Stewart and Thompson, 1993; supra vide), Weekes' (1930) criterion was insufficient, because it assumed a direct relationship between egg size and adult female size in interspecific comparisons. Notwithstanding the presence of such a relationship in a few taxa with restricted clutch sizes (Andrews and Rand, 1974; Vitt, 1986), no such relationship has been demonstrated for squamates in general (see Frankenberg and Werner, 1992) nor is one to be expected, given the likely trade-off between offspring size and number. In particular cases, relative contributions of nutritional sources can be hypothesized from comparisons of egg size in closely related species, especially when data are corrected for differences in maternal body size (Stewart and Thompson, 1993). However, in general, such comparisons are based on untested assumptions and are less reliable than quan-

titative comparisons of eggs and neonates from the same species.

*Timing of yolk depletion.*—Placentotrophy occasionally has been inferred in viviparous squamates based on the timing of yolk depletion vs parturition. For example, in the snake *Enhydrys dussumieri*, Parameswaran (1962) indicated that fetuses grew from a total length of 17 cm to 23 cm following yolk depletion, from which he inferred placental provision of nutrients late in development. Likewise, in *Shinisaurus crocodilurus*, embryos reportedly continue to develop and grow following yolk depletion (Huang, 1983).

Such tantalizing reports should be interpreted with caution for at least two reasons. First, when reports are based on samples taken during development rather than on the history of individual embryos, statistical analyses that take individual variation and sample sizes into account are particularly important. Second, before inferences on placentotrophy are warranted from apparent depletion of yolk before birth, fetuses must be demonstrated to contain neither abdominal yolk nor lipid deposits sufficient to fuel any observed growth. The yolk sac typically is withdrawn into the abdomen late in development in squamates and can supply nutrients after birth or hatching (e.g., Fitch, 1960; Troyer, 1983; M. J. Packard et al., 1992). In addition, embryos of some species can store energy as abdominal fat bodies (Snell and Tracy, 1985; Christian et al., 1991).

*Placental structure.*—Studies of placental morphology have revealed considerable diversity in the chorioallantoic placenta (allantoplacenta) as well as in the various placentae that develop from the yolk sac (Yaron, 1985; Blackburn, 1993b; Stewart, 1993). Functional correlates of this structural diversity remain largely unknown; however, a few patterns have become evident with respect to the allantoplacenta. In viviparous squamates that are thought from composition analyses to be lecithotrophic (e.g., Stewart and Castillo, 1984; Stewart, 1989; Stewart et al., 1990), this placenta forms through simple apposition of the chorioallantois to the uterine lining (Stewart, 1985, 1990; Blackburn, 1993b). Epithelial layers overlying fetal and maternal placental capillaries are thinned, as is the shell membrane, presumably enhancing gas exchange; however, specializations for nutrient transfer have not been observed. Similar allantoplacentae have been observed in several other squamates with large-yolked eggs (e.g., Guil-

lette and Jones, 1985; Yaron, 1985; Blackburn, 1993b).

In contrast, in three saurian lineages, the chorioallantoic placenta exhibits such specializations as placentomes, chorionic areolae, hypertrophied and microvilliated chorionic epithelia, uterine epithelial syncytia, and interdigitated fetal and maternal tissues (Blackburn, 1993b, 1993c). These specializations also are found among mammals (Blackburn, 1992). Squamates with such features have been judged from other criteria to be highly placentotrophic, e.g., *Pseudemoia entrecasteauxii* (Stewart and Thompson, 1993), *Chalcides chalcides* (Ghiara et al., 1987), and New World *Mabuia* (Blackburn and Vitt, 1992). Correlation of such elaborate chorioallantoic specializations with other evidence for placentotrophy suggests such features to be good evidence for nutrient transfer.

On the other hand, placental provision of nutrients has been inferred from less obvious anatomical features in squamates for which other evidence of placentotrophy is unavailable. Among these species are *Vipera berus* (Bellairs et al., 1955), *Enhydrina schistosa* (Kasturirangan, 1951a), *Hydrophis cyanocinctus* (Kasturirangan, 1951b), *Enhydris dussumieri* (Parameswaran, 1962), and *Chalcides ocellatus* (Giacomini, 1906). Purported evidence for nutrient transfer in these species include "secretory" uterine epithelium, "absorptive" chorionic epithelium, and interdigitated chorioallantoic and uterine tissues. In at least one of these species, *V. berus*, little reason exists to suspect substantial placentotrophy; oviductal conceptuses exhibit a gestational wet mass increase of approximately 52%, placing them toward the lower end of the range for oviposited eggs (Table 2). In addition, although detailed analyses have not been done in *V. berus*, preliminary data suggest no substantial placental provision of certain inorganic ions (Jenkins and Simkiss, 1968). An added complication is that, for *V. berus* and the other species listed, no micrographs of sufficient quality to document the suggested specializations have been published, exemplifying a common problem in squamate anatomical studies. Thus, whether specialized allantoic placental morphology always correlates with substantial nutrient transfer is difficult to judge.

Despite the correlation of structural and functional specializations in highly matrotrophic forms, the possibility of substantial maternal-fetal nutrient transfer in simple allantoic placentae cannot be ruled out. Apposition of fetal and uterine vessels in such placentae would seem to offer a potential site for haematotrophic transfer (Blackburn, 1993b). As for features of the squa-

mate yolk sac placenta, such as different arrangements of extraembryonic tissues, and enlarged maternal and fetal epithelia of omphaloplacenta and omphalallantoic placenta (Stewart, 1992, 1993), functional attributes remain elusive. Likewise, the functional significance of enzymes that have been demonstrated histochemically in placental membranes (Hoffman, 1970; Baxter, 1987) is unclear. In sum, comparisons based on placental morphology may be useful for members of a placentotrophic clade (Blackburn and Vitt, 1992) but, at present, mainly provide information for formulating functional hypotheses to be tested by other means.

*Transfer experiments.*—Placental physiology has been studied in several viviparous lizards and snakes by injecting radiolabeled nutrients into pregnant females and subsequently assaying fetal tissues. Using this approach, maternal-fetal transfer of amino acids or their metabolites has been demonstrated in *Thamnophis sirtalis* (Hoffman, 1970), *Xantusia vigilis* (Yaron, 1985), *Eulamprus quoyii* (Thompson, 1977a), and *Chamaeleo pumilus* (Veith, 1974). Placental transfer of inorganic ions such as sodium and iodine has been demonstrated by such means in a few other species (Panigel, 1956; Conaway and Fleming, 1960; Hoffman, 1970).

Use of radiolabeled substances does reveal qualitative placental transfer of specific nutrients. However, such a procedure has not yet proven useful as a means of quantifying nutrient provision via the placenta vs the yolk. The difficulty is that all squamates that have been appropriately examined have the capacity for placental transfer of at least some labeled nutrients, and incipient placentotrophy may be common among viviparous squamates (Blackburn, 1992, 1993b). Use of transfer experiments only indicates the capacity for placental transfer of specific substances; it does not demonstrate an embryonic requirement for extra-vitelline nutrients or allow quantification of nutrient provision. Consequently, such experiments do not permit judgments about where in the lecithotrophy-placentotrophy continuum a particular species may lie.

#### APPLICATION OF CRITERIA

In concert with the companion publication to this paper (Blackburn, 1993a), criteria for inferring sources of nutrients for development in squamates are listed in Table 3, along with estimates of their reliability and practicality. The latter represent subjective judgments, justifi-

TABLE 3. CRITERIA FOR THE RECOGNITION OF DEVELOPMENTAL NUTRITIONAL PATTERNS IN SQUAMATE REPTILES. Reliability is estimated on a relative scale (5 = highly reliable, 0 = unreliable), as is ease of use (5 = highly practical, 1 = difficult to apply).

Criterion	Reliability	Practicality
Composition analysis	5	2
Dry mass changes	2 (5 <sup>a</sup> )	3
Wet mass changes	1 (4 <sup>a</sup> )	4
Size of ovum vs neonate <sup>b</sup>	1 (4 <sup>a</sup> )	4
Size of ovum vs female <sup>b</sup>	0	—
Timing of yolk sac depletion <sup>b</sup>	0–3 <sup>c</sup>	2
Placental morphology <sup>b</sup>	0–3 <sup>d</sup>	3
Transfer experiments <sup>b</sup>	1	2

<sup>a</sup> Only in cases of extreme placentotrophy.

<sup>b</sup> Does not yield quantitative data.

<sup>c</sup> Depending on whether fetal lipid or abdominal yolk stores are determined, and on the timing of yolk depletion relative to birth.

<sup>d</sup> Depending on the degree of specialization observed.

cation for which is provided above; other workers are, of course, free to modify these assessments. The most reliable method for quantifying sources of nutrients for development seems to be chemical composition analysis, cautionary statements above notwithstanding. Morphology offers a means of forming testable functional hypotheses, but further study is required to clarify structural-functional relationships. Experiments with radiolabeled substances indicate the capacity for placental transfer, but neither allow quantification nor indicate an embryonic reliance on placental provision. Data on dry mass can indicate the primary source of nutrients in viviparous forms but permit quantification only in cases of extreme placentotrophy. Comparisons based on wet mass or size of the viviparous neonate vs the ovum are much less useful and, like the remaining criteria, tend to be unreliable except in cases of extreme placentotrophy.

None of the criteria listed is reliable and easy to apply to squamates in general, and several criteria involve elaborate techniques or experiments. Analysis of chemical composition can entail difficulties in lecithotrophic forms, because sibling yolks and neonates may need to be analyzed to reveal facultative placentotrophy and variation that is the result of female size (see Stewart, 1989). The superior reliability of this approach nevertheless renders it the criterion of choice when quantitative information is needed. However, radiotracer studies may reveal placental transfer of substances for which the fetus shows no net gain during gestation, and studies of placental morphology continue to be useful in revealing potential sites and mechanisms of nutrient transfer.

Our knowledge of several aspects of developmental physiology in squamates is fragmentary, and we lack a complete picture for even a single species. Criteria for recognition of reproductive patterns undoubtedly will be refined as information continues to accumulate. However, as with standards for recognition of oviparity and viviparity (Blackburn, 1993a, 1994), adoption of accurate, broadly applicable criteria for recognition of developmental nutritional patterns is vital for documentation of interspecific diversity and analyses of evolutionary trends.

#### ACKNOWLEDGMENTS

I am grateful to M. J. Packard, J. R. Stewart, and R. Shine for carefully evaluating the manuscript.

#### LITERATURE CITED

- ANDREWS, R. M., AND A. S. RAND. 1974. Reproductive effort in anoline lizards. *Ecology* 55:1317–1327.
- , AND O. J. SEXTON. 1981. Water relations of the eggs of *Anolis auratus* and *Anolis limifrons*. *Ibid.* 62:556–562.
- ATHAVALE, M. V., AND L. MULHERKAR. 1967. Studies in nitrogen excretion during the development of *Calotes versicolor* Daud. *J. Anim. Morphol. Physiol.* 14:89–97.
- AVERY, R. A. 1975. Clutch size and reproductive effort in the lizard *Lacerta vivipara* Jacquin. *Ecologia* 19:165–170.
- BARCROFT, J. 1952. Foetal respiration and circulation, p. 398–441. *In*: Marshall's physiology of reproduction. 3d ed. A. S. Parkes (ed.). Longman's Green, London, England.
- BAXTER, D. C. 1987. Placentation in the viviparous lined snake, *Tropidoclonion lineatum*: ontogeny of the extraembryonic membranes and histochemistry of placental tissues. Unpubl. master's thesis, Univ. of Tulsa, Tulsa, Oklahoma.
- BELLAIRS, R., I. GRIFFITHS, AND A. D'A. BELLAIRS. 1995. Placentation in the adder *Vipera berus*. *Nature* 176:657–658.
- BLACKBURN, D. G. 1992. Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptiles and other vertebrates. *Amer. Zool.* 32:313–321.
- . 1993a. Standardized criteria for the recognition of reproductive modes in squamate reptiles. *Herpetologica* 49:118–132.
- . 1993b. Chorioallantoic placentation in squamate reptiles: structure, function, development, and evolution. *J. Exp. Zool.* 266:414–430.
- . 1993c. Histology of the late-stage placentae in the matrotrophic skink *Chalcides chalcides* (Lacertilia; Scincidae). *J. Morph.* 216:179–195.
- . 1994. Discrepant usage of the term "ovoviviparity" in the herpetological literature. *Herpetol. Jour.* In press.

- , AND L. J. VITT. 1992. Reproduction in South American lizards of the genus *Mabuya*, p. 150–164. *In: Reproductive biology of South American vertebrates: aquatic and terrestrial*. W. Hamlett (ed.). Springer-Verlag Press, New York, New York.
- , H. E. EVANS, AND L. J. VITT. 1985. Evolution of fetal nutritional adaptations, p. 437–439. *In: Functional morphology in vertebrates*. H.-R. Duncker and G. Fleischer (eds.). Gustav Fischer, Stuttgart, Germany.
- , L. J. VITT, AND C. A. BEUCHAT. 1984. Eutherian-like reproductive specializations in a viviparous reptile. *Proc. Natl. Acad. Sci. (Washington)* 81:4860–4863.
- BRANSON, B. A., AND E. C. BAKER. 1974. An ecological study of the queen snake, *Regina septemvittata* (Say) in Kentucky. *Tulane Stud. Zool. Bot.* 18:153–171.
- CHRISTIAN, K. A., W. T. LAWRENCE, AND H. L. SNELL. 1991. Effect of soil moisture on yolk and fat distribution in hatchling lizards from natural nests. *Comp. Biochem. Physiol.* 99A:13–19.
- CLARK, H. AND B. SISKEN. 1956. Nitrogenous excretion by embryos of the viviparous snake *Thamnophis s. sirtalis* (L.). *Ibid.* 33:384–393.
- , B. FLORIO, AND R. HUROWITZ. 1955. Embryonic growth of *Thamnophis s. sirtalis* in relation to fertilization date and placental function. *Copeia* 1955:9–13.
- CONAWAY, C. H., AND W. R. FLEMING. 1960. Placental transmission of Na<sup>22</sup> and I<sup>131</sup> in *Natrix*. *Copeia* 1960:360–366.
- FITCH, H. S. 1960. Autecology of the copperhead. *Univ. Kansas Publ. Mus. Nat. Hist.* 13:85–288.
- FLORIAN, D. 1990. Development of extraembryonic membranes and chemical composition of eggs and hatchlings of the oviparous lizard, *Eumeces fasciatus* (Reptilia, Squamata). Unpubl. masters's thesis, Univ. of Tulsa, Tulsa, Oklahoma.
- FRANKENBERG, E., AND Y. L. WERNER. 1992. Egg, clutch and maternal sizes in lizards: intra- and interspecific relations in near-Eastern Agamidae and Lacertidae. *Herpetol. Jour.* 2:7–18.
- FUKADA, S. 1939. Beitrage zur Embryochemie der Schlangen. *J. Biochem. (Tokyo)* 30:125–134.
- GHIARA, G., F. ANGELINI, M. ZERANI, A. GOBBETTI, G. CAFIERO, AND V. CAPUTO. 1987. Evolution of viviparity in Scincidae (Reptilia, Lacertilia). *Acta Embryol. Morphol. Exper. new series*, 8:187–201.
- GIACOMINI, E. 1891. Matériaux pour l'étude du développement du *Seps chalcides*. *Arch. Ital. Biol.* 16:332–359.
- . 1906. Sulla maniera di gestazione e sugli annessi embrionali del *Gongylus ocellatus* Forsk. *Mem. R. Accad. Sci. Inst. Bologna (VI)* 3:401–445 (1906).
- GIEBERSBERG, H. 1923. Untersuchungen über Physiologie und Histologie des Eileiters der Reptilien und Vögel: nebst einem Beitrag zur Fasergene Zeitchr. f. wissensch. Zoologie 70:1–97.
- GUILLETTE, L. J., JR. 1981. Reproductive strategies and the evolution of viviparity in two allopatric populations of the Mexican lizard *Sceloporus aeneus*. Unpubl. Ph.D. diss., Univ. of Colorado, Boulder.
- . 1987. The evolution of viviparity in fishes, amphibians, and reptiles, p. 523–562. *In: Hormones and reproduction in fishes, amphibians, and reptiles*. D. O. Norris and R. E. Jones (eds.). Plenum Press, New York, New York.
- , AND G. CASAS-ANDREU. 1987. The reproductive biology of the high elevation Mexican lizard *Barisia imbricata*. *Herpetologica* 43:29–38.
- , AND R. E. JONES. 1985. Ovarian, oviductal, and placental morphology of the reproductively bimodal lizard, *Sceloporus anenus*. *J. Morph.* 184:85–98.
- GUTZKE, W. H. N., AND G. C. PACKARD. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of bullsnakes (*Pituophis melanoleucus*). *Physiol. Zool.* 60:9–17.
- HALL, R. J. 1969. Ecological observations on Graham's watersnake (*Regina grahami* Baird and Girard). *Am. Midl. Nat.* 81:156–163.
- HOFFMAN, L. H. 1970. Placentation in the garter snake, *Thamnophis sirtalis*. *J. Morph.* 131:57–88.
- HOGARTH, P. J. 1976. Viviparity. *Studies in Biology* #75, p. 1–66. Arnold, London, England.
- HUANG, Q. 1983. A preliminary observation on ovoviviparous habit of *Shinisaurus crocodilurus* in captivity. *Acta Herpetol. Sinica* 2:77–79.
- HUGGETT, A. S. G. 1954. Problems of sugar transport in the placenta of the ungulate, p. 534–618. *In: Gestation*. L. B. Flexner (ed.). Corlies, Macy and Company, New York, New York.
- JACOBI, L. 1936. Ovoviviparie bei einheimischen Eidechsen. *Z. Wiss. Zool.* 148:401–464.
- JENKINS, N. K., AND K. SIMKISS. 1968. The calcium and phosphate metabolism of reproducing reptiles with particular reference to the adder (*Vipera berus*). *Comp. Biochem. Physiol.* 26:865–876.
- JONES, R. E., AND D. C. BAXTER. 1991. Gestation, with emphasis on corpus luteum biology, placentation, and parturition, Vol. 4, Part A, p. 205–302. *In: Vertebrate endocrinology: fundamentals and biomedical implications*, Academic Press, New York, New York.
- KASTURIRANGAN, L. R. 1951a. Placentation in the sea-snake, *Enhydrina schistosa* (Daudin). *Proc. Indian Acad. Sci.* 34:1–32.
- . 1951b. The allantoplacenta of the sea snake *Hydrophis cyanocinctus* Daudin. *J. Zool. Soc. India* 8:277–289.
- LEMUS, D., R. MARTIN, M. J. BLANQUEZ, M. FUENZALIDA, AND J. ILLANES. 1984. Estudio comparado del desarrollo embrionario de especies ovíparas y una ovovivípara (*Gallus gallus*, *Corturnix c. japonica* y *Liolaemus tenuis* t.). *Zbl. Vet. Med. C. Anat. Histol. Embryol.* 13:252–260.
- MUTH, A. 1981. Water relations of desert iguana (*Dipsosaurus dorsalis*) eggs. *Physiol. Zool.* 54:441–451.
- NEEDHAM, J. 1931. *Chemical embryology*. Cambridge Univ. Press, Cambridge, England.
- PACKARD, G. C., AND M. J. PACKARD. 1987. Water relations and nitrogen excretion in embryos of the oviparous snake *Coluber constrictor*. *Copeia* 1987:395–406.
- , AND ———. 1988. The physiological ecology of reptilian eggs and embryos, p. 523–605. *In: Bi-*

- ology of the Reptilia. Vol. 16. C. Gans and R. B. Huey (eds.). Liss, New York, New York.
- , AND ———. 1993. Sources of variation in laboratory measurements of water relations of reptilian eggs and embryos. *Physiol. Zool.* 66:115–127.
- , C. R. TRACY, AND J. J. ROTH. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the Class Reptilia. *Biol. Rev.* 52:71–105.
- PACKARD, M. J., AND G. C. PACKARD. 1988. Sources of calcium and phosphorus during embryogenesis in bullsnakes (*Pituophis melanoleucus*). *J. Exp. Zool.* 246:132–138.
- , ———, AND T. J. BOARDMAN. 1980. Water balance of the eggs of a desert lizard (*Callisaurus draconoides*). *Can. J. Zool.* 58:2051–2058.
- , ———, AND ———. 1982. Structure of eggshells and water relations of reptilian eggs. *Herpetologica* 38:136–155.
- , ———, AND W. H. N. GUTZKE. 1984. Calcium metabolism in embryos of the oviparous snake *Coluber constrictor*. *J. Exp. Biol.* 110:99–112.
- , ———, J. D. MILLER, M. E. JONES, AND W. H. N. GUTZKE. 1985. Calcium mobilization, water balance, and growth in embryos of the agamid lizard *Amphibolurus barbatus*. *J. Exp. Zool.* 235:349–357.
- , J. A. PHILLIPS, AND G. C. PACKARD. 1992. Sources of mineral for green iguanas (*Iguana iguana*) developing in eggs exposed to different hydric environments. *Copeia* 1992:851–858.
- PANIGEL, M. 1956. Contribution a l'étude de l'ovoviviparité chez les reptiles: gestation et parturition chez le lézard vivipare *Zootoca vivipara*. *Ann. S. Nat. Zool.* 11:571–665.
- PARAMESWARAN, K. N. 1962. The foetal membranes and placentation of *Enhydryis dussumieri* (Smith). *Proc. Indian Acad. Sci. (B)* 56:302–326.
- SHINE, R. 1977. Reproduction in Australian elapid snakes. II. Female reproductive cycles. *Aust. J. Zool.* 25:655–666.
- . 1983. Reptilian reproductive modes: the oviparity-viviparity continuum. *Herpetologica* 39:1–8.
- SNELL, H. L., AND C. R. TRACY. 1985. Behavioral and morphological adaptations by Galapagos land iguanas (*Conolophus subcristatus*) to water and energy requirements of eggs and neonates. *Amer. Zool.* 25:1009–1018.
- STEWART, J. R. 1985. Placentation in the lizard *Gerrhonotus coeruleus* with a comparison to the extraembryonic membranes of the oviparous *Gerrhonotus multicarinatus* (Sauria, Anguillidae). *J. Morph.* 185:101–114.
- . 1989. Facultative placentotrophy and the evolution of squamate placentation: quality of eggs and neonates in *Virginia striatula*. *Amer. Nat.* 133:111–137.
- . 1990. Development of the extraembryonic membranes and histology of the placenta in *Virginia striatula* (Squamata: Serpentes). *J. Morph.* 205:33–43.
- . 1992. Placental structure and nutritional provision to embryos in predominantly lecithotrophic viviparous reptiles. *Amer. Zool.* 32:303–312.
- . 1993. Yolk sac placentation in reptiles: structural innovation in a fundamental vertebrate fetal nutritional system. *J. Exp. Zool.* 266:431–449.
- , AND D. G. BLACKBURN. 1988. Reptilian placentation: structural diversity and terminology. *Copeia* 1988:838–851.
- , AND R. E. CASTILLO. 1984. Nutritional provision of the yolk of two species of viviparous reptiles. *Physiol. Zool.* 57:377–383.
- , AND M. B. THOMPSON. 1993. A novel pattern of embryonic nutrition in a viviparous reptile. *J. Exp. Biol.* 174:97–108.
- , D. G. BLACKBURN, D. C. BAXTER, AND L. H. HOFFMAN. 1990. Nutritional provision to the embryos in *Thamnophis ordinoides* (Squamata: Colubridae), a predominantly lecithotrophic placental reptile. *Physiol. Zool.* 63:722–734.
- THOMPSON, J. 1977a. The transfer of amino acids across the placenta of a viviparous lizard, *Sphenomorphus quoyi*. *Theriogenology* 8:158.
- . 1977b. Embryo-maternal relationships in a viviparous skink *Sphenomorphus quoyi* (Lacertilia: Scincidae), p. 279–280. *In: Reproduction and evolution.* J. H. Calaby and C. H. Tyndale-Biscoe (eds.). Australian Academy of Sciences, Canberra City, New South Wales, Australia.
- . 1981. A study of the sources of nutrients for embryonic development in a viviparous lizard, *Sphenomorphus quoyii*. *Comp. Biochem. Physiol. Ser. A, Comp. Physiol.* 70:509–518.
- . 1982. Uptake of inorganic ions from the maternal circulation during development of the embryo of a viviparous lizard, *Sphenomorphus quoyii*. *Ibid.* 71:107–112.
- TRACY, C. R. 1980. Water-relations of parchment-shelled lizard (*Sceloporus undulatus*) eggs. *Copeia* 1980:478–482.
- , AND H. L. SNELL. 1985. Interrelations among water and energy relations of reptilian eggs, embryos, and hatchlings. *Amer. Zool.* 25:999–1008.
- TROYER, K. 1983. Posthatching yolk energy in a lizard: utilization pattern and interclutch variation. *Oecologia* 58:340–343.
- VAN MARTHENS, E., L. GRAUIL, AND S. ZAMENHOF. 1972. Enhancement of prenatal development by operative restriction of litter size in the rabbit. *Life Sci.* 11:1031–1035.
- VEITH, W. J. 1974. Reproductive biology of *Chamaeleo pumilus pumilus* with special reference to the role of the corpus luteum and progesterone. *Zool. Afr.* 9:161–183.
- VITT, L. J. 1978. Caloric content of lizard and snake (Reptilia) eggs and bodies and the conversion of weight to caloric data. *J. Herpetol.* 12:65–72.
- . 1986. Reproductive tactics of sympatric gekkonid lizards with a comment on the evolutionary and ecological consequences of invariant clutch size. *Copeia* 1986:773–786.
- , AND D. G. BLACKBURN. 1991. The ecology and life history of the viviparous lizard *Mabuya bis-triata* (Scincidae) in the Brazilian Amazon. *Copeia* 1991:916–927.
- WEEKES, C. H. 1930. On placentation in reptiles. II. *Proc. Linn. Soc. New South Wales* 55:550–576.

WOURMS, J. P. 1981. Viviparity: the maternal-fetal relationship in fishes. *Amer. Zool.* 21:473–515.

YARON, Z. 1985. Reptile placentation and gestation: structure, function, and endocrine control, p. 527–603. *In: Biology of the Reptilia*. Vol. 15. C. Gans and F. Billet (eds.). John Wiley and Sons, New York, New York.

DEPARTMENT OF BIOLOGY, LIFE SCIENCES CENTER, TRINITY COLLEGE, HARTFORD, CONNECTICUT 06106. Submitted: 20 Aug. 1993. Accepted: 23 Nov. 1993. Section editor: J. R. Gold.

*Copeia*, 1994(4), pp. 935–943

## Pathology in Eggs, Embryos, and Hatchlings of the Australian Lungfish, *Neoceratodus forsteri* (Osteichthyes: Dipnoi)

A. KEMP

**Eggs of the Australian lungfish, *Neoceratodus forsteri*, found in natural spawning localities in the Brisbane River, southeast Queensland, are sometimes infected or damaged at the time of collection. Others develop infection after transfer to the laboratory. An oomycete fungus, probably a species of *Saprolegnia*, as well as the ubiquitous freshwater bacteria *Aeromonas hydrophila* and *Pseudomonas* sp., have been isolated from infected eggs sampled in the river and are also found in embryos that develop infection when reared in the laboratory. Abnormalities may also appear during the development of eggs and embryos in the laboratory and in the environment. Losses of hatchlings in the laboratory can occasionally be traced to extreme developmental abnormalities, but most hatchling deaths are caused by bacterial infection.**

The Australian lungfish, *Neoceratodus forsteri*, spawns in most years in the Brisbane River, in southeast Queensland, between mid-Aug. and mid-Dec. Onset of spawning is apparently associated with increasing photoperiod, provided that suitable aquatic weeds are available for oviposition (Kemp, 1984). Eggs are usually found adhering to water weeds within one meter of the surface, in flowing or still water, under the shade of overhanging trees, or in full sunlight. They are normally laid singly, occasionally two together, or in clusters. During the breeding season, eggs are relatively easy to locate in known spawning areas.

Eggs and embryos of the lungfish are exposed to a number of environmental influences. Water quality in the Brisbane River during the spawning season is usually affected by the lack of rain in late winter and early spring. Water levels are also erratic because the Brisbane River is used as a source of water for the city of Brisbane. This causes variation in the temperature of the water in the spawning grounds as water is released from deep cold reservoirs further upstream to replace water used (Kemp, 1984). Eggs may be laid close to the surface of the water, and this exposes them to further fluctuations in temperature and light. Eggs may also

be deposited in parts of the river that are stagnant, turbid, or shallow; and a number of the eggs laid in natural surroundings do not survive to hatch. Conditions in Enoggera Reservoir also reflect lack of rain in the preceding months, but the reservoir is deep and water is not removed regularly, so the environment is more stable.

This paper summarizes information on developmental abnormalities in lungfish eggs and embryos, and the effects of disease, in lungfish collections in two areas of the Brisbane River from 1984–1991 and from localities in Enoggera Reservoir near Brisbane in 1969–1973. An analysis of possible pathogens in Brisbane River eggs in 1990 is provided. Causes of death in laboratory-reared hatchlings are also described and discussed.

### METHODS

Eggs were collected from two localities in the Brisbane River, southeast Queensland, one at Fernvale and the other several kilometers downstream at Hill End, and from several areas in Enoggera Reservoir. Eggs were reared in the laboratory by the methods described in Kemp (1981). Water used for rearing the eggs was as close to the composition of the natural river or