HERPETOLOGICA

PARENTAL INVESTMENT, EMBRYO GROWTH, AND HATCHLING LIPID RESERVES IN SOFTSHELL TURTLES (*APALONE MUTICA*) FROM ARKANSAS

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ABSTRACT: The smooth softshell *Apalone mutica* is a North American trionychid turtle typically found in moderate to fast moving rivers and streams. Such habitats contrast with more resource rich environments (e.g., marshes and swamps) inhabited by other turtle species for which parental investment and egg and hatchling components have been studied. We measured components of eggs, embryos, and hatchlings and determined levels of pre-ovulatory parental investment in *Apalone mutica* from Arkansas. Eggs averaged 7.34 g wet mass and 28.8% non-polar lipids (NPL) by dry mass. Triacylglycerol, an energy storage lipid, was the major lipid class component of eggs, averaging 82% of total egg lipids. Levels of structural (mostly polar) lipid classes were generally similar to those found in eggs of other turtle species, although proportions of phophatidylethanolamine were higher and increased with egg size. Incubation time averaged 55 ± SE 3.4 d. At 18 and 36 d of development, embryo dry mass represented 11% and 34%, respectively, of embryo dry mass at hatching. Thus, approximately two-thirds of the total increase in embryo dry mass occurred during the final one-third (18 d) of development. Hatchlings averaged 6.60 g wet mass and 25.9% NPL by dry mass. The index of parental investment in care (hatchling NPL/egg NPL) averaged 75.4%, the highest such index reported among turtles. High levels of pre-ovulatory, parental investment in trophic care of neonates appear to be a factor important to hatchling survival during early life stages such as dispersal from the nest, movement to aquatic habitats, and the period prior to which hatchlings attain a positive energy balance in relatively low resource environments.

Key words: *Apalone mutica*; Egg lipids; Embryo growth; Hatchling; Parental investment

AMONG reptiles, hatchling body size is the phenotypic characteristic most commonly examined in relation to variation in offspring fitness (Congdon et al., 1999; Janzen, 1993*a*; Laurie and Brown, 1990; Pianka, 1970; Shine, 1978; Sinervo and Huey, 1990; Stearns, 1992). For oviparous species, such focus on size appears warranted because materials allocated to eggs represent the majority of parental investment and increased egg size typically results in larger neonates (Congdon et al., 1983*a*; Cox and Marion, 1978; Ewert, 1979; Iverson, 1979). Some studies, however, have indicated that energy reserves may also be an important component of hatchling quality (Congdon and Gibbons, 1990; Congdon et al., 1983*a,b*; Gutzke et al., 1987; Kramer and Bennett, 1981; Lance and Morafka, 2001; Marlen and Fischer, 1999; Nagle et al., 1998; Rowe et al., 1995; Troyer, 1983; Wilhoft, 1986). Materials allocated to eggs are used to support two conceptually distinct processes: (1) parental investment in

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embryogenesis (PIE) and (2) parental investment in care (PIC; Congdon, 1989; Congdon and Gibbons, 1990).

In organisms that lack post-ovulatory behavioral parental care, parental investment in trophic care of neonates can be manifested in the form of a yolk sac containing substantial quantities of high energy lipids. For example, at hatching, energy storage lipids often constitute >15% of the dry mass of neonate turtles, and such stores represent between 40% and 60% of the original egg lipids (Congdon, 1989; Congdon and Gibbons, 1990; Congdon et al., 1983*a*; Marlen and Fischer, 1999; Nagle et al., 1998; Thompson et al., 1999). Thus, for many turtle species, lipid stores represent a substantial portion of the energy invested in individual offspring. Hatchlings of some species contain enough stored lipids to support basal maintenance metabolism for time periods ranging from 55 d (Marlen and Fischer, 1999) to >180 d (Nagle et al., 1998). Lipid reserves in hatchling turtles can also provide energy for nest dispersal and subsequent movements to and within aquatic habitats, such as the swimming frenzies of hatchling sea turtles (Kramer and Bennett, 1981).

The index of pre-ovulatory PIC has been defined as the ratio of hatchling non-polar lipids (NPL) to egg NPL (Congdon, 1989). The importance of PIC to the fitness of a female parent is indirect and mediated by the level of energy reserves required by hatchlings (Congdon and Gibbons, 1990; Nagle et al., 1998; Troyer, 1983). Resource availability for hatchling turtles among different environments should require different levels of energy reserves. For example, levels of mean net primary production in lotic ecosystems (500 g/m²/yr) are much lower than those of lentic ecosystems (2500 g/m²/yr; Whittaker and Likens, 1973). Thus, turtle species typically found in riverine environments should produce hatchlings with higher levels of energy reserves compared with species that inhabit lentic ecosystems such as marshes and swamps.

The smooth softshell *Apalone mutica* is a North American trionychid turtle that typically inhabits moderate to fast moving rivers and large streams (Ernst et al., 1994; Ewert, 1979; Fitch and Plummer, 1975; Janzen, 1993*b*; Plummer, 1976, 1977*a*; Plummer and Shirer, 1975; Webb, 1962). Hatchlings of *A. mutica* prefer shallow waters on the lee end of sandbars and the shallow puddles created by highly dissected shorelines (Plummer, 1977*a*). Such sandbar habitats can be low in aquatic resources available to turtles, as evidenced by the preponderance of terrestrial invertebrates found in the diet of male *A. mutica* (Plummer and Farrar, 1981) that inhabit sandbar microhabitats similar to those of hatchlings and juveniles (Plummer and Shirer, 1975). In contrast, adult female *A. mutica* prefer deeper water and feed primarily on aquatic tricopterans (Plummer and Farrar, 1981).

To determine the parental investment strategy of *A. mutica* from Arkansas, we examined the following: (1) lean and lipid components of eggs, including both polar (structural) and non-polar (energy storage) lipids, (2) lean and NPL components of embryos and hatchlings, and (3) the PIC index. Since hatchlings of *A. mutica* often emerge into lotic habitats that are, in general, less productive than lentic habitats typically inhabited by many other neonatal turtles, we tested the following predictions.

Compared to other turtles that occupy more resource rich environments such as marshes and swamps,

(P_1) the eggs of *A. mutica* should contain proportionally greater levels of NPL;

(P_2) the neonates of *A. mutica* should have higher levels of NPL at hatching;

(P_3) the PIC index should be higher in *A. mutica*.

MATERIALS AND METHODS

Between 12–27 June 1990, we collected eggs of *A. mutica* from 15 newly constructed nests on sandbars along the White River, near Georgetown, White County, Arkansas, USA. One hundred forty-four eggs were individually marked with clutch number and egg number, packed in moist sand, and transported to the laboratory at Harding University. All eggs were mea-

sured with dial calipers (width, 0.1 mm) and weighed to the nearest 0.01 g. Subsamples of 3–5 eggs from each clutch (total $n = 51$) were placed in a sealed plastic bag and frozen at −15 C. All other eggs were individually buried in moist vermiculite (1:1 ratio of water:vermiculite by weight) in covered plastic boxes. Eggs were buried at a depth that left about 20% of the egg above the surface of the vermiculite, and substrate moisture levels were maintained at approximately −300 kPa throughout incubation using procedures developed in an earlier study (Plummer and Snell, 1988). Egg boxes were placed in a Hovabator incubator (G.Q.F. Manufacturing Company, Savannah, Georgia, USA) and incubated at constant 30 C, the temperature considered optimal in a study of the thermal limits of *A. mutica* eggs collected from the White River (Plummer et al., 1994). Samples of eggs were removed from incubation and frozen at approximately 33% (18 d; $n = 17$) and 67% (36 d; $n = 24$) of incubation. A final sample of 52 hatchlings was frozen at pipping ($\chi = 55$ d). All samples of eggs, embryos, and hatchlings were frozen on dry ice and transported to the Savannah River Ecology Laboratory (SREL).

At SREL, samples were stored frozen at −55 C until they were thawed for separation into components for analyses. Undeveloped eggs were separated into eggshells and egg yolks. Eggs containing partially developed embryos and pipped hatchlings were separated into eggshells and body and yolk components. All samples were lyophilized for 96 to 120 h and weighed; tissue samples were ground and stored under nitrogen at −55 C.

Subsamples of egg yolk, embryos, and hatchlings were placed in pre-weighed cellulose thimbles, and NPL were extracted for 3 h using petroleum ether in a Soxtec apparatus. The proportion of NPL in each sample was determined gravimetrically (Fischer et al., 1991). The index of PIC was calculated by dividing the amount of hatchling NPL by the average amount of NPL in eggs from the respective clutch (Congdon, 1989).

Lipid class proportions were determined to distinguish maternal allocation to energy storage lipids from allocation to other functions. Total lipids were extracted using a modified micro-Folch extraction technique. Subsamples of egg yolk (approximately 0.1 g) were dissolved in 5 ml of a 2:1 ratio of chloroform:methanol, washed with a 0.9% NaCl, and then centrifuged (see Gardner et al., 1985). Samples containing approximately 10 μg total lipid were drawn into 50 μl capillary minipipettes and flame sealed with a butane torch. Prepared samples were then stored at −20 C for up to 30 d prior to analysis using thin-layer chromatography (TLC) coupled with flame-ionization detection (FID). Lipid components were detected using an Iatroscan MK IV Analyzer (Iatron Laboratories, Tokyo, Japan) following procedures detailed in Nagle et al. (1998).

Samples of eggs, embryos, and hatchlings were represented from 15, 8, and 14 clutches, respectively, in statistical analyses. Because measures of egg and hatchling characteristics were highly correlated, differences among clutches were determined using multivariate analysis of variance (MANOVA). Sample sizes of embryos were considered too small for adequate comparisons among females. To determine whether lipid components varied in relation to mean egg dry mass among clutches, individual lipid class proportions were arcsine, square-root transformed and compared using ANOVA. To determine changes in total NPL during development, we compared total NPL proportions of eggs, hatchling yolks, and hatchling bodies among clutches using ANOVA. Statistical tests were performed using SAS 6.10 for Windows (SAS Institute, Inc., Cary, North Carolina, USA) with levels of significance set at $\alpha = 0.05$.

Results

Eggs and Pre-ovulatory Parental Investment

Egg width ($F_{14,36} = 4.11$; $P = 0.0003$), lipid mass ($F_{14,36} = 2.21$; $P = 0.028$), and lean mass ($F_{14,36} = 3.88$; $P = 0.0005$) all varied significantly among females, although egg wet mass ($F_{14,36} = 1.81$; $P =$

TABLE 1.—Characteristics of *Apalone mutica* eggs; n = 51 eggs from 15 clutches; values are mean, 1 SE (min-max).

Diameter (mm)	Egg wet mass (g)	Egg % water	Shell dry mass (g)	Yolk dry mass (g)	Yolk lean mass (g)	Yolk NPL mass (g)	Yolk % NPL
24.2, 0.44	7.34, 0.15	62.20, 0.86	0.714, 0.016	2.06, 0.07	1.46, 0.05	0.59, 002	28.77, 0.56
(18.0-25.4)	(5.89-8.29)	(53.32-66.08)	(0.645-0.834)	(1.53-2.69)	(1.10-2.01)	(0.43-0.73)	(25.50-33.50)

0.076) did not (Table 1). Eggshell dry mass represented 28% (1 SE = 0.12) of total egg dry mass. Lean and NPL components of eggs increased at rates uniform to increases in total egg yolk mass (Fig. 1).

Triacylglycerol, an energy storage lipid, was the major lipid class component of eggs (Table 2). Structural egg lipids included cholesterol, small fractions of cholesterol esters, and three phospholipids (Table 2). Among all clutches of eggs, a significant positive correlation was found between mean egg size and proportion of phosphatidylethanolamine ($F_{1,11}$ = 4.91; P = 0.049). No other lipid class proportions were found to be related to egg size (all P-values > 0.05).

Embryos

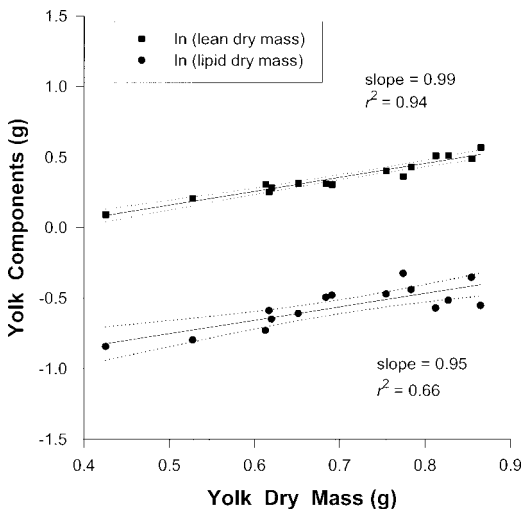Embryo dry mass at 18 d (0.13 g; Table 3) represented 7% of the total dry mass of egg components (yolk + embryo) and 11% of total embryo body mass at hatching. At 36 d, embryo dry mass (0.41 g; Table 3) represented 22% of total egg dry mass and 34% of total embryo body mass at hatching. Approximately two-thirds of the increase in dry mass of hatchlings occurred during the last one-third (18 d) of development (Fig. 2b). Body lipids were below detection limits in 18-d embryos and increased most rapidly during the last one-third of development (Fig. 2b). The rapid increase in body lean and lipid mass during the last one-third of development was concomitant with the most rapid decrease in egg yolk lean and lipid components, but not in the reduction of eggshell mass (Fig. 2).

Hatchling Lipid Reserves and Pre-ovulatory Parental Investment in Care

Incubation time averaged 55 ± SE 3.4 d. Hatchling wet mass ranged from 5.34–7.48 g, and hatchling lipid stores (proportion of NPL per unit dry mass) averaged 25.9% (1 SE = 0.77; Table 4). Total NPL and lean dry masses of hatchlings averaged 0.14 g and 0.10 g, respectively, less than similar components of eggs (Tables 1, 4). Clutch effects were substantial among hatchlings, with hatchling total mass ($F_{13,38}$

FIG. 1.—Relationships of clutch lean and lipid component means to a range of total egg dry masses in *Apalone mutica*.

TABLE 2.—Lipid components of *Apalone mutica* eggs. Results are expressed as wt% of total lipid. Detection limit for triacylglycerol fatty acids (0/0 for all eggs) was approximately 0.05% of total egg lipid composition.

Lipid class	Mean % + 1 SE	Min %–Max %
Triacylglycerols	81.6 + 0.6	78.6-84.5
Triacylglycerol fatty acids	0.0	N/A
Cholesterol	1.2 + 0.03	1.0-1.3
Cholesterol ester	0.6 + 0.1	0.1-1.5
Phosphatidylethanol-amine	4.7 + 0.5	2.6-8.4
Phosphatidylcholine	9.1 + 0.3	7.6-11.1
Sphingomyelin	1.3 + 0.2	0.2-2.3

TABLE 3.—Characteristics of *Apalone mutica* eggs and embryos. Values are mean, 1 SE (min-max).

Stage	Soma dry mass (g)	Yolk dry mass (g)	Yolk NPL (g)	Total NPL (g)
Embyros at 18 d	0.127, 0.023 (0.063-0.235)	1.706, 0.128 (1.152-1.706)	0.457, 0.038 (0.304-0.596)	0.463, 0.035 (0.318-0.597)
Embryos at 36 d	0.412, 0.110 (0.176-0.989)	1.432, 0.169 (0.392-1.877)	0.410, 0.052 (0.110-0.410)	0.440, 0.048 (0.156-0.622)

= 8.39; P = 0.0001), body wet mass ($F_{13,38}$ = 2.73; P = 0.008), yolk wet mass ($F_{13,38}$ = 4.30; P = 0.0002), and yolk lipid mass ($F_{13,38}$ = 5.30; P = 0.0001) all varying significantly among females. Conversion efficiency calculated as hatchling dry mass/ egg yolk dry mass averaged 83%. Eggshell dry mass decreased on average 0.23 g (33%) during development (Fig. 2a). Hatchling body dry mass represented 71% of the remaining egg components (hatchling body + yolk dry mass) at hatching (Table 4). The index of PIC (hatchling NPL/ egg NPL) averaged 75.4% (1 SE = 2.4; Table 5).
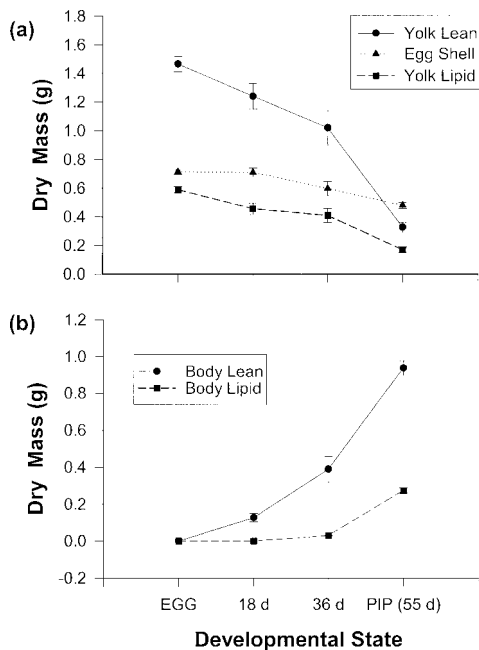

FIG. 2.—Changes in (a) egg components (excluding embryos) and (b) embryo and hatchling components during development in *Apalone mutica*.

DISCUSSION

Egg Composition

Proportions of both water and NPL in eggs of *A. mutica* were intermediate to values reported for other turtle species (Congdon and Gibbons, 1985; Nagle et al., 1998; Rowe et al., 1995). Non-polar lipids increased at rates uniform to increases in egg yolk masses of *A. mutica,* a pattern observed in other turtles (Congdon and Gibbons, 1985), alligators (Fischer et al., 1991), and several amphibian species (Komoroski et al., 1998).

(P_1)*: Eggs of* A. mutica *should have proportionally high levels of NPL.*—Egg NPL proportions for *A. mutica* (28.8%) ranked seventh out of nine species examined (Table 4). However, most of the species that ranked higher were those whose hatchlings exhibited delayed emergence from the nest. For hatchlings that delay nest emergence, the period of time spent in the nest post-hatching may be substantial, often exceeding the incubation period by several-fold (Gibbons and Nelson, 1978). Thus, high levels of energy reserves are required for maintenance metabolism of hatchlings that exhibit delayed nest emergence (Congdon and Gibbons, 1985).

Lipid Components of Eggs

Lipid class proportions of eggs were similar among clutches. Triacylglycerol, an energy storage lipid, represented the major lipid class component (82%) of *A. mutica* eggs (Table 2). Similar values have been reported for eggs of four species of kinosternid turtles (Nagle et al., 1998), desert tortoises (*Gopherus agassizii*: Lance and Morafka, 2001), and tuataras (*Sphenodon punctatus*: Body, 1985). Lower values of approximately 70% triacylglycerol have been reported for eggs

TABLE 4.—Characteristics of hatchling *Apalone mutica*. Values are mean, 1 SE (min-max).

Total wet mass (g)	Soma dry mass (g)	Yolk dry mass (g)	Total dry mass (g)	Yolk NPL (g)	Total NPL (g)	% Total NPL	% PIC
6.60, 0.18 (5.34-7.48)	1.214, 0.042 (0.911-1.516)	0.497, 0.055 (0.193-0.860)	1.711, 0.048 (1.333-2.066)	0.170, 0.021 (0.057-0.346)	0.445, 0.021 (0.303-0.630)	25.88, 0.77 (19.58-33.85)	75.42, 2.40 (55.19-88.85)

of four other turtle species (*Emydura macquarii*: Thompson et al., 1999; *Chrysemys picta, Emydoidea blandingii,* and *Chelydra serpentina*: Rowe et al., 1995), alligators (*Alligator mississippiensis*: Noble et al., 1990), and a lizard species (*Sceloporus undulatus*: Ballinger et al., 1992). High levels of energy storage lipids in *A. mutica* and other reptile eggs provide fuel during embryogenesis and subsequent post-hatching energy reserves for neonates (Congdon and Gibbons, 1985, 1990; Congdon et al., 1983*a,b*; Kramer and Bennett, 1981; Lance and Morafka, 2001; Marlen and Fischer, 1999; Nagle et al., 1998; Rowe et al., 1995; Thompson et al., 1999; Troyer, 1983).

Proportions of total phospholipids, cholesterol, and cholesterol esters were similar to values reported for desert tortoises (Lance and Morafka, 2001) and four species of kinosternid turtles (Nagle et al., 1998). However, proportions of phosphatidylethanolamine increased with egg size among clutches of *A. mutica* and averaged about twice the levels reported in kinosternids. Whether or not this difference represents factors contributing to rapid embryo growth during the short incubation period of *A. mutica* (Ewert, 1985; Janzen, 1993*b*), compared to much longer incubation periods for kinosternid turtles (Ewert, 1985; Nagle et al., 1998), remains unknown.

Embryo Growth Dynamics

Development of embryos falls into three general categories: (1) cell division and tissue differentiation and development, (2) organogenesis, and (3) embryo growth prior to hatching. For most turtle eggs, the timing of the three developmental categories corresponds roughly to three equal portions of the total incubation period (Ewert, 1985). Proportional increases in dry mass of *A. mutica* embryos represented 11%, 23%, and 66% of the total hatchling soma at 18 d, 36 d, and pipping (mean = 55 d), respectively. Embryo mass increased at rates of 0.007, 0.016, and 0.067 g/d during the three periods. Rapid growth of the embryo during the 18 d prior to hatching was associated with the most rapid decline in lean material in the egg yolk

TABLE 5.—Characteristics of turtle eggs and hatchlings with their sampled locations.

Family Species	Egg yolk dry mass (g)	Egg yolk % NPL	Hatch-ling total dry mass (g)	Hatch-ling % NPL	Delayed emer-gence typical?	PIC index (%)	Location (reference)
Chelidae							
Emydura macquarii	1.46	31.4	1.24	21.7	No	59	New South Wales, Australia (Thompson et al., 1999)
Chelydridae							
Chelydra serpentina	2.59	21.5	1.42	20.3	No	52	New Jersey, U.S.A. (Wilhoft, 1986)
Emydidae							
Chrysemys picta	1.32	37.1	0.91	25.4	Yes	55	Nebraska, U.S.A. (Gutzke et al., 1987)
Deirochelys reticularia	1.97	33.8	1.42	27.4	Yes	61	South Carolina, U.S.A. (Congdon et al., 1983*a*)
Trachemys scripta	2.00	32.0	1.36	30.8	Yes	66	Illinois, U.S.A. (Marlen and Fischer, 1999)
Kinosternidae							
Kinosternon subrubrum	0.73	32.9	0.54	15.8	Yes	40	South Carolina, U.S.A. (Nagle et al., 1998)
Sternotherus minor	1.00	30.9	0.84	18.6	No	50	Florida, U.S.A. (Nagle et al., 1998)
S. odoratus	0.74	28.2	0.57	17.5	No	55	South Carolina, U.S.A. (Nagle et al., 1998)
Trionychidae							
Apalone mutica	2.06	28.8	1.71	25.9	No	75	Arkansas, U.S.A. (present study)

(Fig. 2a,b). In contrast, declines in both lipids and eggshell mass were relatively constant during the entire period of development (Fig. 2b). Hatchling total dry mass represented 83% of the average total dry mass of eggs, a value higher than those reported for painted turtles (72%; Ewert, 1979) and chicken turtles (72%; Congdon et al., 1983*a*) but similar to that reported for American alligators (79%; Fischer et al., 1991).

Hatchling Lipid Reserves and Parental Investment in Care

Trivers (1972) considered parental investment as a trade-off in allocation among individual offspring. Allocation of NPL by *A. mutica,* at levels far exceeding those necessary to produce complete offspring, meet the criteria of Trivers for large parental investments because such energy could have been allocated for the production of additional offspring. Allocation of NPL to offspring may be divided into two categories: NPL catabolized during embryogenesis, or parental investment in embryogenesis (PIE); and NPL reserves al-located to fuel the hatchling after it leaves the egg, or parental investment in care (i.e., PIC; Congdon, 1989).

For a given egg size, natural selection on hatchling energy reserves and, indirectly, NPL levels in eggs and the subsequent index of PIC should be strongly influenced by hatchling resource environments (Congdon and Gibbons, 1990; Fischer et al., 1991; Nagle et al., 1998; Troyer, 1983).

(P_2): *Hatchling* A. mutica *should have high proportions of NPL compared to other turtles.*—Lipid reserves in hatchlings of *A. mutica* (mean = 26% of total hatchling dry mass) ranked third among nine species (Table 5). All of the species with hatchling lipid levels similar to *A. mutica* were those whose hatchlings delay emergence from nests, a behavior requiring substantial energy reserves (Congdon and Gibbons, 1985).

Energy storage lipids allocated to hatchling turtles may provide energy necessary for nest emergence and dispersal (Kramer and Bennett, 1981), maintenance metabolism (Congdon and Gibbons, 1985; Nagle

et al., 1998), or a source of metabolic water (Congdon and Gibbons, 1990).

(P_3): *The PIC index should be high in* A. mutica *compared to other turtles.—Apalone mutica* had the highest PIC index (75%) found among turtles, averaging only 25% of egg NPL used during embryogenesis. In comparison, levels of PIC range from 40–66% in other turtle species (Table 5). The level of PIC in *A. mutica* was also somewhat higher than that described for *Alligator americanensis* (approximately 70%; Fischer et al., 1991).

It remains to be determined how local or regional differences in resource availability and other aspects of habitat quality may affect lipid allocation and subsequent PIC indices, both within and among turtle species and in other oviparous organisms. In a study of captive *G. agassizii*, for example, differences in diet may have been responsible for substantial variation among females in lipid class composition of hatchling yolk sacs (Lance and Morafka, 2001). In some bird species, however, diet may affect yolk fatty acid composition but not the relative proportions of lipid classes (Speake et al., 1998). Future studies within and among reptile species are required to address this issue.

CONCLUSIONS

Parental investment in *A. mutica* appears to function by promoting the quality of hatchlings rather than strictly the size of hatchlings. Females of *A. mutica* invest high levels of energy in hatchling reserves, yet compared with other turtle species, often produce large or multiple clutches of small hatchlings relative to female body size (Goldsmith, 1944; Muller, 1921; Plummer, 1977*b*). Hatchlings of *A. mutica* emerge into environments in which high energy reserves may be required to obtain a positive energy balance among relatively scarce and patchy resources.

Apalone mutica eggs from Arkansas contained NPL proportions that were intermediate to those found in other freshwater turtles. Several factors, however, ultimately determine the proportion of egg NPL subsequently available to hatchling turtles (PIC). In most species, the amount of NPL catabolized during embryogenesis represents the majority of lipid allocated to offspring production (PIE). Among species, however, higher levels of energy storage lipids may be required to sustain: (1) embryos that reach developmental maturity earlier during incubation or (2) embryos that require longer developmental periods. Indeed, developmental rate and the duration of incubation can sometimes obscure the relationships between NPL levels in eggs and those available to hatchling turtles (Nagle et al., 1998).

In *A. mutica,* rapid embryo growth during the final one-third of incubation coupled with a relatively short incubation period (Ewert, 1985; Janzen, 1993*b*; our data) result in hatchlings that contain comparatively high levels of NPL reserves. Although such short incubation periods are uncommon among turtle species with rigid-shelled eggs (Ewert, 1985), accelerated incubation periods are typical for species that nest in the uncertain environment of sandbars (Ewert, 1979). Consequently, the uncertain resource environments that neonates of *A. mutica* enter into may be the ultimate factor influencing the high levels of parental investment in care provided by adult females.

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