

Lizards combine stored energy and recently acquired nutrients flexibly to fuel reproduction

Daniel A. Warner^{1*}, Xavier Bonnet², Keith A. Hobson³ and Richard Shine¹

¹School of Biological Sciences, University of Sydney, New South Wales 2006, Australia; ²Centre d'Etudes Biologiques de Chizé, CNRS, F-79360 Villiers en Bois, France; and ³Environment Canada, 11 Innovation Boulevard, Saskatoon, Saskatchewan, Canada, S7N 3H5

Summary

1. Energy allocation strategies for reproduction are viewed typically as a continuum between reliance on ‘income’ (recently acquired energy) vs. ‘capital’ (stored reserves) for fuelling reproduction. Because ectothermy facilitates long-term energy storage and often involves low feeding rates, traditional views suggest that many ectotherms rely heavily on stored reserves for egg production.
2. We explored the temporal relationship between energy intake and expenditure in a multi-clutching lizard (*Amphibolurus muricatus*) by evaluating the effect of maternal nutrition on reproductive output and by contrasting $\delta^{13}\text{C}$ measurements of the maternal diet and endogenous energy stores with that of the eggs produced.
3. Our experiment revealed that females utilize both endogenous energy stores and recently acquired food to fuel reproduction; this pattern did not shift seasonally from first to second clutches produced. Importantly, however, egg lipid was derived primarily from capital, whereas egg protein was derived about equally from both income and capital.
4. Overall, these results suggest that the energy allocation strategy used for reproduction differs among egg components, and that the use of recently acquired energy for reproduction may be more widespread in ectotherms than thought previously.

Key-words: *Amphibolurus muricatus*, capital breeding, carbon-13, income breeding, stable isotopes

Introduction

The temporal relationship between the acquisition of energy and its expenditure for reproduction is an important axis of life history variation. Although energy acquisition and allocation tactics cover a continuum, they can be viewed usefully in terms of two end-points: reliance on ‘capital’ vs. ‘income’ to fuel reproductive expenditure (Bonnet, Bradshaw & Shine 1998; Meijer & Drent 1999). ‘Income’ breeders expend energy for reproduction soon after that energy is acquired, whereas ‘capital’ breeders gather energy over long periods prior to utilizing these stored reserves for reproduction (reviewed by Jönsson 1997). These two strategies of energy allocation can influence selection on life-history attributes (e.g. the number and quality of offspring produced, or the frequency of reproduction: Jönsson 1997; Bonnet *et al.* 2001; Brown & Shine 2002; Lourdais *et al.* 2002), as well as the sensitivity of reproductive output to local conditions. For example, local resource availability should influence immediate

reproductive output strongly in income breeders, but not in capital breeders (due to the temporal separation of energy acquisition and expenditure in the latter group). Clearly, both types of energy allocation strategies have associated costs and benefits; long-term energy storage may allow capital breeders to cope with unpredictable environments or low food availability (Calow 1979; Santos & Llorente 2004), whereas income breeders can increase their reproductive output more rapidly in response to an increase in local food supply (Jönsson 1997).

Most research on these issues has focused upon avian and mammalian species (Drent & Daan 1980; Festa-Bianchet, Gaillard & Jorgenson 1998; Kunkele 2000; Veloso & Bozinovic 2000; Voigt 2003), and detailed studies have revealed that these high-energy systems rely on income breeding to fuel some aspect of reproductive expenditure (Bronson & Manning 1991; Klaassen *et al.* 2001; Gauthier, Béty & Hobson 2003; Wheatley *et al.* 2008). On the other hand, features associated with ectothermy may facilitate long-term energy storage, such that capital breeding may be more energetically efficient than income breeding in ectotherms (Bonnet *et al.* 1998). Consistent with this idea, stored energy reserves (i.e. fat bodies) are used to fuel reproduction in many ectothermic animals (Schultz, Clifton & Warner 1991;

*Correspondence author and present address: Daniel A. Warner, Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50011, USA. E-mail: dwarner@iastate.edu

Doughty & Shine 1997; Madsen & Shine 1999; Girish & Saidapur 2000; Glazier 2000; Arrington *et al.* 2006), and females of some species can produce offspring despite not feeding over the entire reproductive season (Bonnet *et al.* 1998). Nevertheless, some ectothermic species rely, at least partially, on income to fuel reproduction (Hahn & Tinkle 1965; Shanbhag & Prasad 1992).

Unfortunately, there is no simple way to assess strategies of maternal energy allocation. The most obvious measures involve comparisons of maternal condition prior to vitellogenesis with that after oviposition; a capital breeder should show a significant decrease (due to depletion of energy reserves), whereas an income breeder should not change between these two time-periods (Ankney & MacInnes 1978; Astheimer & Grau 1985). Unfortunately, maternal condition might also fall due to inadequate nutrition, so that a decrease in mass might occur even if reproduction was fuelled entirely by income. Directly measuring changes in body reserves rather than simply overall maternal mass (Guillette & Sullivan 1985; Méndez de la Cruz *et al.* 1988) can help to discriminate between these two interpretations, but requires assumptions about the degree to which a female's maintenance metabolic needs are fuelled by energy stores. Faced with such problems in interpreting descriptive data, experiments in which we manipulate either food supply or endogenous energy stores may offer the most promise. Thus, removing fat bodies experimentally provides direct evidence of their use in egg production (Hahn & Tinkle 1965; Shanbhag & Prasad 1992), albeit with all the possible confounding factors due to surgical intervention. An alternative approach is to manipulate the nutritional quality of the prey provided to reproducing females. By manipulating diet quality, we can distinguish between maternal mass changes due to nutrition vs. to reproductive allocation. Also, we can use naturally occurring stable isotopes (particularly ^{13}C) to track the flow of specific nutrients in such a system (Peterson & Fry 1987; Hobson 1995, 2006; Kelly 2000). That is, we can assess directly the isotopic contents of the food source and the endogenous reserves responsible for the nutrients eventually allocated to eggs or offspring (O'Brien, Schrag & Del Rio 2000; Hobson *et al.* 2004; Cherel, Hobson & Weimerskirch 2005), thereby distinguishing between income vs. capital breeding strategies (Hobson, Hughs & Ewins 1997; Klaassen *et al.* 2001; Gauthier *et al.* 2003; Morrison & Hobson 2004).

We used stable isotopes to explore how reproduction is fuelled in a multi-clutching ectotherm, the jacky dragon (*Amphibolurus muricatus*), a common agamid lizard of coastal heathlands in south-eastern Australia. Jacky dragons make an excellent model system for addressing the above issues for several reasons. First, jacky dragons begin reproductive activities relatively soon (about 1 month) after emerging from overwinter inactivity, suggesting that they have limited time to acquire sufficient energy to fuel reproduction (that is, if they rely upon recently ingested energy as opposed to fat reserves). Secondly, jacky dragons lay eggs throughout much of the summer, from mid-October to early February (Harlow & Taylor 2000). This extended reproductive season suggests that their energy

allocation strategy might shift throughout the course of the season as maternal energy stores are depleted. Thirdly, jacky dragons produce up to four clutches of eggs in a single reproductive season, with a 3–4-week interval between successive clutches (Warner *et al.* 2008). These aspects of their reproduction allow us to evaluate how (or if) individual females shift energy allocation strategies in successive clutches within a season.

In the present study, we manipulated the quality, as well as the isotopic content, of the maternal diet as soon as the lizards emerged from their overwinter inactive period and this manipulation was maintained throughout the subsequent reproductive season. We then evaluated changes in maternal mass and reproductive output of the females maintained on our two diet regimens. In addition to these analyses, we measured the isotopic content (^{13}C) of egg components (protein and lipid) produced by females, as well as the isotopic content of the endogenous reserves (muscle, fat body and liver) of females prior to the reproductive season. The resulting data allowed us to examine critically three plausible alternative scenarios in terms of energy allocation tactics: (1) female jacky dragons are 'pure' capital breeders (the energy used for egg production is derived from food eaten and energy stored within the female's body prior to winter); (2) female jacky dragons are 'pure' income breeders (the energy used for egg production is derived from food eaten immediately prior to breeding); and (3) female jacky dragons use a mixture of tactics; for example, they might act as capital breeders for the first clutch of the season but then switch to income breeding for subsequent clutches (Shanbhag & Prasad 1992).

Materials and methods

EXPERIMENTAL DESIGN

Adult female jacky dragons ($n = 19$) were collected from natural areas surrounding Sydney, Australia in spring 2003. Dragons were housed in large ($2 \times 2 \text{ m}$) seminatural field enclosures located at Macquarie University (within the natural range of this species). The enclosures contained several branches for perching/basking, and shelters/vegetation were provided for hiding. Each enclosure contained two females with one male. Lizards were fed a combination of roaches and crickets (dusted with vitamin/calcium supplement), and water was provided *ad libitum*. All lizards were kept under these conditions for 1 year prior to the initiation of this experiment. Because all lizards were fed the same diet, we assumed that the stable isotope values of the diet and dragon tissues did not differ among individuals.

In spring 2004, immediately after emergence from the inactive winter period, lizards were divided into two experimental groups. One group of lizards ($n = 7$) was fed captive-bred crickets (control diet) throughout the entire reproductive season (September 2004–February 2005). These crickets were raised on a high-quality primarily C3 diet (endive leaves, carrots, apples and cat food). The second group of lizards ($n = 12$) was fed captive-bred, corn-raised crickets (experimental diet) throughout the same period as the lizards on the control diet. The corn-raised crickets were fed only corn, a poor-quality diet that lacks essential amino acids (Benton, Harper & Elvehjem 1955; Contreras, Elías & Bressani 1980; Webb, Hedges & Simpson 1998), for at least 1 month prior to being eaten by the lizards. We

Table 1. Mean stable isotope values for diet, endogenous tissues and egg components (first and second clutches pooled). The percentage of energy derived from the endogenous source was calculated by using values from fat and muscle tissues to represent the endogenous end-point, and using values from the experimental diet as the exogenous end-point. That is, the 100% exogenous end-point was based on the $\delta^{13}\text{C}$ value of the corn-raised crickets, and the 100% endogenous end-point was based on that for the fat and muscle tissues. This method enabled us to use the isotopic value from egg yolk to calculate the percentage of energy derived from the endogenous source. For example, the $-17.0\text{\textperthousand}$ value for $\delta^{13}\text{C}$ in protein of egg yolk lies 46.7% of the way between the values for the endogenous tissue ($-21.9\text{\textperthousand}$) and experimental diet ($-12.7\text{\textperthousand}$)

Isotope	Control diet	Experimental diet (corn-raised crickets)	Endogenous tissues (mean \pm 1 SD)	Egg yolk	% from endogenous source (mean \pm 1 SD)
$\delta^{13}\text{C}$ from protein (%)	-22.5	-12.7	-21.9 ± 1.25 (muscle tissue)	-17.0 ± 0.13	46.7%
$\delta^{13}\text{C}$ from lipid (%)	-24.8	-15.4	-23.6 ± 0.37 (fat tissue)	-21.8 ± 0.10	78.0%

SD: standard deviation.

chose corn as a cricket diet because this C4 plant has higher relative abundance of ^{13}C compared to the C3 plants forming the diet of the control group (Bender 1968) (see Table 1 for isotope values for the diet treatments). Our 1-month period probably provided enough time for isotope levels to stabilize in the cricket tissues; evidence from other invertebrates suggests that the turnover time for ^{13}C is approximately 15 days (Gratton & Forbes 2006), and was also confirmed by the much more enriched ^{13}C content of the experimental crickets (Table 1). By feeding isotopically different diets to two groups of lizards that emerged from the inactive winter period with the same endogenous tissue isotopic signatures, we could evaluate how isotopic values of the eggs produced by these two groups reflected recently acquired vs. long-term stored dietary isotopic differences. If egg macronutrients reflected recently ingested dietary isotopic values, we reasoned that this would be good evidence for income breeding. Moreover, comparisons of body condition between females from each diet treatment enabled us to evaluate whether declines in body condition were due to dietary quality, rather than reproductive investment strategies.

Females were weighed and measured [snout–vent length (SVL)] soon after their emergence in spring (well prior to ovulation) and then again after they nested in their enclosures. Female dragons were fed crickets raised on the diets specified above three times per week. Although the number of crickets consumed by each lizard was not quantified, dragons typically ate all the food as soon as it was provided. Thus, because the same quantity of food was provided in each enclosure, we reasoned that all lizards ate similar amounts of food during this study. All eggs that were produced were removed from the nests, then taken to the laboratory and weighed. One or two eggs per clutch, selected randomly, were frozen for later isotopic analysis.

Immediately after emergence from the inactive winter period in 2006, six female jacky dragons were killed by decapitation and their fat bodies, liver and muscle tissues were removed immediately for isotopic analysis. Although these animals were obtained 2 years after our diet manipulations, the diet given to these individuals was the same (crickets and roaches) as that preceding our experiment in 2004. Thus, the isotopic signatures in the endogenous tissues of these animals should be similar to those of the experimental animals from 2004 after emergence. Measurements of isotopes in these endogenous tissues provided data on the stable carbon isotope values of stored reserves prior to food uptake during the reproductive season.

STABLE ISOTOPE ANALYSES

Egg yolk was separated into lipid and lipid-free (i.e. protein) constituents prior to isotopic analysis. Whole yolk was first freeze-dried and powdered in an analytical mill. For egg and crickets, we extracted

lipids using a 2 : 1 chloroform : methanol mixture. Lipid-free tissues were weighed into tin cups and analysed for $^{13}\text{C}/^{12}\text{C}$ relative abundance using a Carlo Erba elemental analyser interfaced in continuous-flow mode with a Europa 20 : 20 isotope ratio mass spectrometer. Lipid values were analysed similarly.

Isotope values were expressed in the standard ‘ δ ’ notation in parts per thousand relative to the PeeDee Bellemnite standard for $\delta^{13}\text{C}$ values. The working laboratory standard was chicken egg albumen, which was placed in sequence between every five unknowns. We estimated within-run measurement error to be $\pm 0.1\text{\textperthousand}$ for $\delta^{13}\text{C}$ values.

Modelling of the relative contributions of endogenous and exogenous sources to eggs was based on a simple two-end-point, one-isotope (i.e. ^{13}C) mixing model. As carnivores, jacky dragons were assumed to show no isotopic discrimination in ^{13}C between exogenous protein and egg proteins or between exogenous lipids and egg lipids under an income model that assumes metabolic routing of these macromolecules (Hobson 1995). Similarly, the mobilization of endogenous proteins and lipids to corresponding protein and lipid fractions of eggs under the capital strategy was also not expected to show discrimination for $\delta^{13}\text{C}$ values (Hobson 1995).

STATISTICAL ANALYSES

Statistical analyses were performed with SAS software (SAS Institute 1997). Repeated-measures analysis of covariance (ANCOVA) was used to compare changes in maternal body condition between the two diet treatments. As an index of body condition, we evaluated ln body mass (dependent variable) adjusted for ln body length (SVL; covariate) (Hayes & Shonkwiler 2001) both before vitellogenesis and after oviposition of the first clutch. Such an index of body condition is likely to be correlated with body reserves (Naulleau & Bonnet 1996; Santos & Llorente 2004). Residual scores of the regression of mass on body length, a similar analysis to that employed in the current study, provide reliable estimates of body condition (Hayes & Shonkwiler 2001; Schulte-Hostedde *et al.* 2005). The effect of maternal diet on reproductive output (number of clutches, egg mass, clutch mass and clutch size) was evaluated with ANCOVA using diet as the independent variable, reproductive output as the dependent variable and maternal mass as the covariate.

We evaluated the effects of diet treatment and clutch number on stable isotope values in eggs with a two-factor analysis of variance (ANOVA), using diet treatment and clutch number as fixed factors (and their interaction) and isotopic value as the dependent variable; overall results did not change when egg mass was used as a covariate. We used only the first two clutches in our comparative analyses of stable isotopes because none of the corn-raised diet animals produced more than two clutches. Because treating successive clutches from

the same females as independent units may introduce pseudoreplication, we performed an additional analysis in which each clutch was nested within female identity in the model. The overall results remained unchanged.

In an additional analysis, we used data from all clutches produced (including two third clutches from females in the control treatment). The considerable overlap between oviposition dates for the first, second and third clutches (first clutches were laid from 18 October to 26 December; second clutches from 26 November to 28 December; the third clutches were laid on 15 and 16 December) allowed us to evaluate the relationship between oviposition date and stable isotope values within clutches using regression analysis, regardless of clutch number.

To evaluate the relative contributions of endogenous vs. exogenous reserves toward reproduction, we assumed that the jacky dragon followed a carnivore model for exogenous routing to eggs (Hobson 1995). Because the $\delta^{13}\text{C}$ content of endogenous tissues was, as expected, similar to that of the control diet (Table 1), we used only clutches from the experimental diet to evaluate the proportion of exogenous vs. endogenous sources of energy allocated towards egg production. Based on the $\delta^{13}\text{C}$ value of the corn-raised crickets (exogenous energy source) and that of the fat and muscle tissue (endogenous energy sources), we were able to determine egg component isotopic end-points of a continuum ranging from those formed from 100% exogenous to those formed from 100% endogenous sources of energy. However, we used different endogenous end-points for the protein vs. lipid analyses; for the protein end-point, we used the mean $\delta^{13}\text{C}$ value from muscle tissue and for the lipid end-point, we used the mean $\delta^{13}\text{C}$ value from the fat tissue. This approach assumes metabolic routing of lipid stores to lipids in eggs and metabolic routing of body protein stores to protein components of eggs (e.g. Gauthier *et al.* 2003). In practice, our calculations revealed similar results regardless of which tissue (liver, muscle or fat body) was used to represent the endogenous end-point. Our measurements of $\delta^{13}\text{C}$ values in the protein and lipid of the egg yolks allowed us to evaluate the proportion of resources that originated from income vs. capital. Analyses of variance were used to evaluate the effect of clutch number on the proportion of egg materials derived from endogenous sources.

Results

MATERNAL BODY CONDITION AND REPRODUCTIVE OUTPUT

Females maintained on the corn-raised cricket diet declined substantially in body condition after oviposition of the first clutch, whereas females on the control diet changed less (Fig. 1; interaction between diet treatment and timing of reproductive events; $F_{1,14} = 3.4, P = 0.048$). Female condition prior to vitellogenesis did not differ between diet treatments ($P = 0.426$), but females maintained on the corn-raised cricket diet had a significantly lower body condition after oviposition of the first clutch ($P = 0.040$).

Only four females (33%) maintained on the corn-raised cricket diet produced a second clutch, whereas half the females fed the control diet produced two or more clutches. Accordingly, females fed corn-raised crickets produced significantly fewer clutches than did those on the control diet (mean number of clutches produced on corn-raised cricket

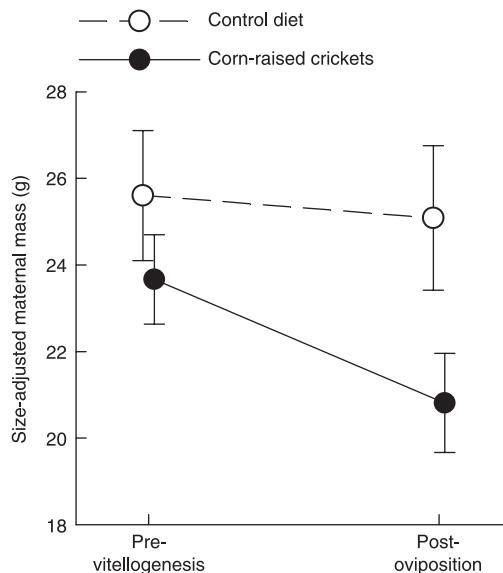


Fig. 1. Effect of diet treatment on maternal mass of jacky dragons at two time periods: (i) prior to vitellogenesis and (ii) post-oviposition of the first clutch. Least-squares means are reported from a repeated-measures analysis of covariance (snout–vent length = covariate). Error bars represent 1 standard error.

diet = 1.25; mean number of clutches produced on control diet = 1.83; $F_{1,15} = 10.3, P = 0.006$), but the timing of oviposition of the first clutch was not affected ($F_{1,14} = 1.9, P = 0.195$). Mean egg mass and total clutch mass were also influenced by the diet treatments. Females fed corn-raised crickets produced larger eggs than those on the control diet (mean egg mass: 1.34 g vs. 1.00 g, respectively; $F_{1,14} = 10.5, P = 0.006$), resulting in greater clutch mass for females maintained on corn-raised crickets (mean clutch mass: 6.65 g vs. 4.82 g; $F_{1,14} = 5.5, P = 0.035$). Clutch size did not differ between females raised on the two diet treatments ($F_{1,15} = 0.02, P = 0.881$; mean clutch size for females in the control and experimental diet treatments were 5.1 and 5.2 eggs per clutch, respectively).

ISOTOPIC COMPOSITION OF EGGS

Eggs produced by females fed corn-raised crickets had greater $\delta^{13}\text{C}$ values than eggs produced by females on the control diet (Fig. 2; egg protein: $F_{1,21} = 35.7, P < 0.001$; egg lipid: $F_{1,20} = 25.8, P < 0.001$) and $\delta^{13}\text{C}$ values of the endogenous tissues (from females immediately after winter emergence) were similar to those of the control diet (Table 1). The $\delta^{13}\text{C}$ values of both egg protein and lipid did not differ between the first and second clutches produced by females (Fig. 2; egg protein: $F_{1,21} = 0.6, P = 0.454$; egg lipid: $F_{1,20} = 0.3, P = 0.608$). The interactive effect of diet and clutch number on $\delta^{13}\text{C}$ was not significant for proteins ($F_{1,21} = 0.3, P = 0.599$) or lipids ($F_{1,20} = 0.5, P = 0.499$). Isotopic values of eggs produced by females on the control diet increased over the season (egg protein: $r^2 = 0.66, P = 0.020$; egg lipid: $r^2 = 0.69, P = 0.019$),

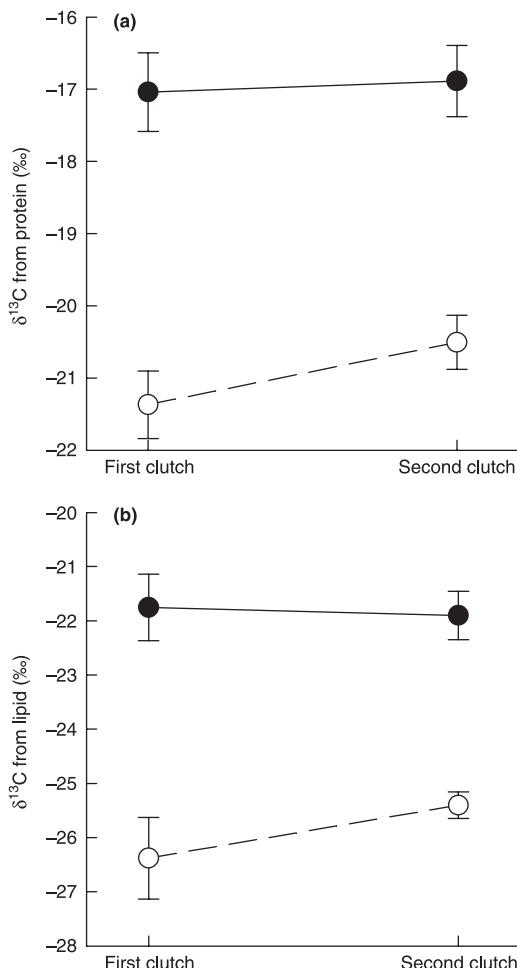


Fig. 2. Mean stable isotope values for first and second clutches produced by female jacky dragons in outdoor enclosures. Solid circles and lines represent lizards fed corn-raised crickets, open circles and dashed lines represent lizards on the control diet. (a) $\delta^{13}\text{C}$ values from egg protein. (b) $\delta^{13}\text{C}$ values from egg lipid. Mean values are reported and error bars represent 1 standard error.

but this pattern was not evident in eggs produced by females on the experimental diet (Fig. 3; egg protein: $r^2 = 0.21$, $P = 0.449$; egg lipid: $r^2 = 0.31$, $P = 0.268$).

Exogenous income and endogenous reserves were both involved with egg formation, but the relative contributions of these two energy sources differed between egg components (Table 1). Endogenous sources (stored reserves) fuelled 78% of lipid allocation towards eggs, whereas a mixed strategy (46.7% endogenous) was used for allocation of proteins towards eggs. These patterns did not differ between the first and second clutches produced by females for $\delta^{13}\text{C}$ in egg protein (47.2% vs. 45.5% endogenous source in first and second clutches, respectively; $F_{1,13} = 0.03$, $P = 0.875$) or in egg lipid (77.5% vs. 79.3% endogenous source for first and second clutches, respectively; $F_{1,13} = 0.02$, $P = 0.893$); nor did these patterns shift throughout the reproductive season ($\delta^{13}\text{C}$ in protein $r^2 = 0.05$, $P = 0.448$; $\delta^{13}\text{C}$ in lipid: $r^2 = 0.09$, $P = 0.269$).

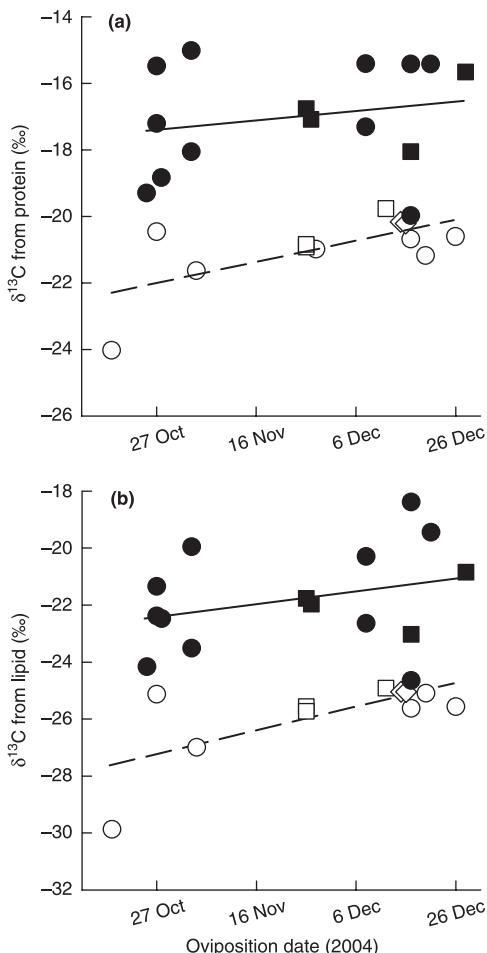


Fig. 3. Relationship between date of oviposition and stable isotope values. Solid symbols and lines represent eggs from lizards that were fed corn-raised crickets, open symbols and dashed lines represent eggs from animals on the control diet. Circles represent first clutches, squares represent second clutches and diamonds represent third clutches. (a) $\delta^{13}\text{C}$ values from egg protein. (b) $\delta^{13}\text{C}$ values from egg lipid.

Discussion

BODY CONDITION AND REPRODUCTIVE OUTPUT

The decline in body condition of females maintained on the poor-quality diet (i.e. corn-raised crickets), and the stability of body condition of females fed the control diet, hints at income breeding in jacky dragons. However, the effect of maternal diet on reproductive output can be interpreted as either income breeding or a mixture of strategies. For example, the decline in total reproductive output due to the poor-quality diet is indicative of income breeding (Gregory 2006). Upon closer examination, however, the main reason for this decline in total reproductive output was that most females fed the poor-quality diet failed to produce a second clutch of the season. Thus, this pattern suggests that the first clutch of the season relies, at least partially, on stored reserves, and the second clutch is fuelled primarily by recently acquired prey (Shanbhag & Prasad 1992; Brown & Shine 2002). Our

analyses of the stable isotope data enabled us to address these interpretations further (see more discussion below).

We also found that dietary quality influenced maternal investment into eggs. That is, females fed the poor-quality diet produced larger eggs and a greater clutch mass, despite the overall negative effect on reproductive output. This allocation strategy has been shown previously (Warner, Lovren & Shine 2007), and could potentially counterbalance the negative effect of poor maternal nutrition on total reproductive output. By producing larger eggs (which produce large offspring) when poor resources are available, females may be able to enhance offspring quality under suboptimal reproductive conditions. Similar patterns have been observed in other taxa (Semlitsch & Gibbons 1990).

Although our dietary manipulations had dramatic effects on maternal reproductive output, it is difficult to know how these manipulations relate to variation in diet quality in nature. Indeed, variation in diet quality has never been quantified for wild populations of jacky dragons. Nevertheless, the plant diet of herbivorous insects can vary dramatically in nutritive value, particularly in protein quality (Boulter & Derbyshire 1978). The consequent variation in protein quality of host plants can influence the chemistry (including amino acid composition) of the insects that ingest those plants (Felton 1996). Thus, our dietary manipulations to the insect prey (crickets) used in this study may reflect the variation in diet quality available to jacky dragons in the field. More detailed research is needed to understand the full range of diet quality that jacky dragons ingest in nature.

ISOTOPIC EVIDENCE FOR A MIXED CAPITAL/INCOME STRATEGY

Overall, stable isotope analyses provide evidence that jacky dragons use a mixture of income and capital to fuel reproduction. Patterns of isotopic changes in eggs with oviposition date would suggest temporal shifts in allocation of endogenous and exogenous nutrients to eggs (as has been suggested for other multiple-clutching lizard species, based on other types of data: Hahn & Tinkle 1965; Shanbhag & Prasad 1992). Indeed, seasonal shifts in isotopic signatures were evident in eggs from the control treatment; however, the lack of temporal change in the percentage of endogenous input into eggs contradicts this interpretation, and rather suggests that the source of energy for reproduction does not change seasonally in jacky dragons. That is, females consistently utilize both endogenous and exogenous sources of energy for the production of first and second clutches.

Although females used a mixture of capital and income for reproduction, diet quality may influence the relative contributions of endogenous vs. exogenous input into egg production. For both protein and lipid $\delta^{13}\text{C}$ values, the difference in eggs from females maintained on the different diets was less than half the difference between dietary isotopic values. Because both experimental and control animals started off with the same endogenous isotopic signatures, this lack of a 1 : 1 relationship between dietary isotopic differences

and egg differences is best explained by the experimental group mobilizing more isotopically depleted endogenous reserves to eggs thereby dampening the dietary difference in $\delta^{13}\text{C}$ values. This hypothesis is strengthened by the fact that the control animals showed $\delta^{13}\text{C}$ isotopic discrimination factors between diet and eggs that resemble closely those for carnivorous birds using a purely income strategy (Hobson 1995). These patterns suggest that female jacky dragons may combine the relative quantities of exogenous vs. endogenous input flexibly depending upon the quality of the diet.

EGG COMPOSITION

Although reproduction is fuelled by both income and capital in jacky dragons, the sources of energy used for channelling proteins vs. lipids into eggs differ. Isotopic analyses indicated that egg protein is derived approximately equally from exogenous (53.3%) and endogenous (46.7%) sources, whereas egg lipids are derived primarily from stored energy (78%). This pattern resembles that found in an oviparous water snake (*Natrix maura*), although this species tends to rely mainly upon income for proteins (Santos *et al.* 2007). Because proteins and lipids are the primary nutrients within eggs, and are critical for the maintenance of embryonic development, these patterns of endogenous and exogenous sources of energy allocation have important implications for the survival of embryos. Generally, yolk proteins make up nearly 60% of the dry mass of lizard eggs, whereas lipids make up about 30% (Thompson & Speake 2004). Although proteins support some of the development and growth of embryos (Palmer & Guillette 1991), lipids act as the primary source of energy in the yolk to support embryonic development in birds (Speake, Murray & Noble 1998) and oviparous lizards (Thompson *et al.* 2001).

These different sources of egg lipid and protein have important ecological and evolutionary implications for utilization of overwinter energy stores, as well as foraging behaviours. Our results suggest a high demand for stored lipids at the onset of the reproductive season, soon after winter (Derickson 1976; Taylor 1986). Such a demand may place strong selection on prewinter foraging efficiency and fat storage ability because lipid stores influence female reproductive success in the following spring (Zuffi, Giudici & Ioalè 1999). However, our results also suggest that reproductive success depends on the mobilization of amino acids, and hence on prey abundance both before and during the reproductive season. Indeed, female jacky dragons adjust their reproductive tactics based upon the availability of these nutrients, either drawn on protein stores or on income. Such divergence in the mother's reliance on lipids vs. proteins for reproduction may reflect the greater physiological costs of protein storage compared to lipid storage (Schmidt-Nielsen 1990). Indeed, because proteins come primarily from muscle, protein mobilization can compromise locomotion and performance after reproduction (Lourdais *et al.* 2004; Santos *et al.* 2007); lipid mobilization would have little effect because the lipids are derived primarily from fat bodies.

Conclusions

Although theory suggests that most ectotherms rely primarily upon stored energy for reproduction (and hence are capital breeders: Bonnet *et al.* 1998), this generality has been challenged by several recent findings. For example, the snake *Vipera aspis*, long considered a ‘typical’ capital breeder, also relies to some degree on recently acquired energy for reproduction (Bonnet *et al.* 1999, 2001; Lourdais *et al.* 2002). In the snake *Natrix maura*, females facultatively adjust their reliance on stored reserves in response to food availability (Santos & Llorente 2004). Similarly, multi-clutching tropical lizards (*Calotes versicolour*) and snakes (*Tropidonophis mairii*) rely on capital for early clutches, but depend on income for producing late clutches (Shanbhag & Prasad 1992; Brown & Shine 2002). It is likely that both income and capital breeding strategies occur in single-clutch reptile species as well (Doughty & Shine 1997), but detailed information for such species is currently lacking.

In the present study, we add to this diversity of tactics by showing that another multi-clutching ectotherm (the jacky dragon) relies on both recently acquired and stored energy for reproduction, but the relative contributions of these energy sources differ between egg components and the pattern does not change across successive clutches. Importantly, interpretations of our results based solely upon dietary effects on body condition and reproductive output could lead to incorrect interpretations, but coupled with data from stable isotopes, we were able to characterize more accurately the reproductive allocation strategy used by the jacky dragon. It seems likely that further research on energy-allocation tactics in ectotherms will reveal a considerable diversity in patterns, reinforcing the value of these organisms as model systems in addressing ecological and evolutionary questions.

Acknowledgements

Funding was provided from the Australian Research Council. We thank J. Thomas for maintaining the corn-raised cricket colony and for help with lizard husbandry. Thanks to W. Du, T. Langkilde, R. Peters, B. Phillips, T. Schwartz, J. Thomas, D. Van Dyk, M. Wall and T. Wilson for help collecting lizards, and to R. Radder and T. Schwartz for comments on an early draft of this paper. B. M. Alvarez assisted with isotope measurements that were performed at the Department of Soil Science stable-isotope facility, University of Saskatchewan. All lizards were collected under permit S10658 of the New South Wales National Parks Service. All protocols for this research were approved by the University of Sydney Animal Care and Ethics Committee (protocol number L04/12–2004/1/4018) and by the Macquarie University Animal Care and Ethics Committee (protocol number 2004/014).

References

- Ankney, C.D. & MacInnes, C.D. (1978) Nutrient reserves and reproductive performance of female lesser snow geese. *Auk*, **95**, 459–471.
- Arrington, D.A., Davidson, B.K., Winemiller, K.O. & Layman, C.A. (2006) Influence of life history and seasonal hydrology on lipid storage in three neotropical fish species. *Journal of Fish Biology*, **68**, 1347–1361.
- Astheimer, L.B. & Grau, C.R. (1985) The timing and energetic consequences of egg formation in the Adelie penguin. *Condor*, **87**, 256–268.
- Bender, M.M. (1968) Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon*, **10**, 468–472.
- Benton, D.A., Harper, A.E. & Elvehjem, C.A. (1955) Effect of isoleucine supplementation on the growth of rats fed zein or corn diets. *Archives of Biochemistry and Biophysics*, **57**, 13–19.
- Bonnet, X., Bradshaw, D. & Shine, R. (1998) Capital versus income breeding: an ectothermic perspective. *Oikos*, **83**, 333–342.
- Bonnet, X., Naulleau, G., Shine, R. & Lourdais, O. (1999) What is the appropriate timescale for measuring costs of reproduction in a ‘capital breeder’ such as the aspic viper? *Evolutionary Ecology*, **13**, 485–497.
- Bonnet, X., Naulleau, G., Shine, R. & Lourdais, O. (2001) Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. *Oikos*, **92**, 297–308.
- Boulter, D. & Derbyshire, E. (1978) The general properties, classification and distribution of plant proteins. *Plant Proteins* (ed. G. Norton), pp. 3–24. Butterworths, London, UK.
- Bronson, F.H. & Manning, J.M. (1991) The energetic regulation of ovulation: a realistic role of body fat. *Biology of Reproduction*, **44**, 945–950.
- Brown, G.P. & Shine, R. (2002) Reproductive ecology of a tropical naticine snake, *Tropidonophis mairii* (Colubridae). *Journal of Zoology, London*, **258**, 63–72.
- Calow, P. (1979) The cost of reproduction – a physiological approach. *Biological Reviews*, **54**, 23–40.
- Cherel, Y., Hobson, K.A. & Weimerskirch, H. (2005) Using stable isotopes to study resource acquisition and allocation in procellariiform seabirds. *Oecologia*, **145**, 533–540.
- Contreras, G., Elías, L.G. & Bressani, R. (1980) Limitations of corn (*Zea mays*) and common bean (*Phaseolus vulgaris*) diets as protein and calorie sources. *Plant Foods for Human Nutrition*, **30**, 145–153.
- Derickson, W.K. (1976) Lipid storage and utilization in reptiles. *American Zoologist*, **16**, 711–723.
- Doughty, P. & Shine, R. (1997) Detecting life history tradeoffs: measuring energy stores in ‘capital’ breeders reveals costs of reproduction. *Oecologia*, **110**, 508–513.
- Drent, R.H. & Daan, S. (1980) The prudent parent: energetic adjustments in avian breeding. *Ardea*, **68**, 225–252.
- Felton, G.W. (1996) Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Archives of Insect Biochemistry and Physiology*, **32**, 107–130.
- Festa-Bianchet, M., Gaillard, J.M. & Jorgenson, J.T. (1998) Mass- and density-dependent reproductive success and reproductive costs in a capital breeder. *American Naturalist*, **152**, 367–379.
- Gauthier, G., Béty, J. & Hobson, K.A. (2003) Are greater snow geese capital breeders? New evidence from stable isotope model. *Ecology*, **84**, 3250–3264.
- Girish, S. & Saidapur, S.K. (2000) Interrelationship between food availability, fat body, and ovarian cycles in the frog, *Rana tigrina*, with a discussion on the role of fat body in anuran reproduction. *Journal of Experimental Zoology*, **286**, 487–493.
- Glazier, D.S. (2000) Is fatter fitter? Body storage and reproduction in ten populations of the freshwater amphipod *Gammarus minus*. *Oecologia*, **122**, 335–345.
- Gratton, C. & Forbes, A.E. (2006) Changes in $\delta^{13}\text{C}$ stable isotopes in multiple tissues of insect predators fed isotopically distinct prey. *Oecologia*, **147**, 615–624.
- Gregory, P.T. (2006) Influence of income and capital on reproduction in a viviparous snake: direct and indirect effects. *Journal of Zoology*, **270**, 414–419.
- Guillette, L.J. & Sullivan, W.P. (1985) The reproductive and fat body cycles of the lizard, *Sceloporus formosus*. *Journal of Herpetology*, **19**, 474–480.
- Hahn, W.E. & Tinkle, D.W. (1965) Fat body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard *Uta stansburiana*. *Journal of Experimental Zoology*, **158**, 79–86.
- Harlow, P.S. & Taylor, J.E. (2000) Reproductive ecology of the jacky dragon (*Amphibolurus muricatus*): an agamid lizard with temperature-dependent sex determination. *Austral Ecology*, **25**, 640–652.
- Hayes, J.P. & Shonkwiler, J.S. (2001) Morphometric indicators of body condition: worthwhile or wishful thinking? *Body Composition Analysis of Animals: a Handbook of Non-Destructive Methods* (ed. J.R. Speakman), pp. 8–38. Cambridge University Press, Cambridge, UK.
- Hobson, K.A. (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: patterns of isotopic fractionation and turnover. *Condor*, **97**, 752–762.
- Hobson, K.A. (2006) Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea*, **94**, 359–369.
- Hobson, K.A., Atwell, L., Wassenaar, L.I. & Yerkes, T. (2004) Estimating endogenous nutrient allocation to reproduction in redhead ducks: a dual isotope approach using δD and $\delta^{13}\text{C}$ measurements of female and egg tissues. *Functional Ecology*, **18**, 737–745.

- Hobson, K.A., Hughes, K. & Ewins, P.J. (1997) Using stable isotopes analysis to identify endogenous and exogenous sources of nutrients in eggs of migratory birds: applications to Great Lakes contaminants research. *Auk*, **114**, 467–478.
- Jönsson, K.I. (1997) Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos*, **78**, 57–66.
- Kelly, J.F. (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, **78**, 1–27.
- Klaassen, M., Lindstrom, A., Meltofte, H. & Piersma, T. (2001) Arctic waders are not capital breeders. *Nature*, **413**, 794.
- Kunkel, J. (2000) Does primiparity affect the efficiency of converting energy to offspring production in the guinea-pig? *Canadian Journal of Zoology*, **78**, 300–306.
- Lourdais, O., Bonnet, X., Shine, R., Denardo, D., Naulleau, G. & Guillon, M. (2002) Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. *Journal of Animal Ecology*, **71**, 470–479.
- Lourdais, O., Brischoux, F., DeNardo, D. & Shine, R. (2004) Protein catabolism in pregnant snakes (*Epicrates cenchria maurus* Boidae) compromises musculature and performance after reproduction. *Journal of Comparative Physiology B*, **174**, 383–391.
- Madsen, T. & Shine, R. (1999) The adjustment of reproductive threshold to prey abundance in a capital breeder. *Journal of Animal Ecology*, **68**, 571–580.
- Meijer, T. & Drent, R. (1999) Re-examination of the capital and income dichotomy in breeding birds. *Ibis*, **141**, 399–414.
- Méndez de la Cruz, F.R., Guillette, L.J., Santa Cruz, M.V. & Casas-Andreu, G. (1988) Reproductive and fat body cycles of the viviparous lizard *Sceloporus mucronatus* (Sauria, Iguanidae). *Journal of Herpetology*, **22**, 1–12.
- Morrison, R.I.G. & Hobson, K.A. (2004) Use of body stores in shorebirds after arrival on high-arctic breeding grounds. *Auk*, **121**, 333–344.
- Naulleau, G. & Bonnet, X. (1996) Body condition threshold for breeding in a viviparous snake. *Oecologia*, **107**, 301–306.
- O'Brien, D.M., Schrag, D.P. & Del Rio, C.M. (2000) Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. *Ecology*, **81**, 2822–2831.
- Palmer, B.D. & Guillette, L.J. Jr (1991) Oviductal proteins and their influence on embryonic development in birds and reptiles. *Egg Incubation: its Effects on Embryonic Development in Birds and Reptiles* (eds D.C. Deeming & M.W.J. Ferguson), pp. 29–46. Cambridge University Press, Cambridge, UK.
- Peterson, B.J. & Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, **18**, 293–320.
- Santos, X., Arenas, C., Llorente, G.A. & Ruiz, X. (2007) Exploring the origin of egg protein in an oviparous water snake (*Natrix maura*). *Comparative Biochemistry and Physiology A*, **147**, 165–172.
- Santos, X. & Llorente, G.A. (2004) Lipid dynamics in the viperine snake, *Natrix maura*, from the Ebro Delta (NE Spain). *Oikos*, **105**, 132–140.
- SAS Institute. (1997) *SAS/STAT User's Guide*. SAS Institute, Inc., Cary, NC.
- Schmidt-Nielsen, K. (1990) *Animal Physiology: Adaptation and Environment*. Cambridge University Press, Cambridge.
- Schulte-Hostedde, A.I., Zinner, B., Millar, J.S. & Hickling, G.J. (2005) Restitution of mass-size residuals: validating body condition indices. *Ecology*, **86**, 155–163.
- Schultz, E.T., Clifton, L.M. & Warner, R.R. (1991) Energetic constraints and size-based tactics: the adaptive significance of breeding schedule variation in a marine fish (Embiotocidae: *Micrometres minimus*). *American Naturalist*, **138**, 1408–1430.
- Semlitsch, R.D. & Gibbons, J.W. (1990) Effects of egg size on success of larval salamanders in complex aquatic environments. *Ecology*, **71**, 1789–1795.
- Shanbhag, B.A. & Prasad, B.S.K. (1992) Fat body ovarian relationship in the garden lizard, *Calotes versicolor* (Daud.). *Journal of Experimental Zoology*, **264**, 454–460.
- Speake, B.K., Murray, A.M.B. & Noble, R.C. (1998) Transport and transformations of yolk lipids during development of the avian embryo. *Progress in Lipid Research*, **37**, 1–32.
- Taylor, J.A. (1986) Seasonal energy storage in the Australian lizard, *Ctenotus taeniatus*. *Copeia*, **1986**, 445–453.
- Thompson, M.B. & Speake, B.K. (2004) Egg morphology and composition. *Reptilian Incubation: Environment, Evolution, and Behaviour* (ed. D.C. Deeming), pp. 45–74. Nottingham University Press, Nottingham, UK.
- Thompson, M.B., Speake, B.K., Russell, K.J. & McCartney, R.J. (2001) Utilisation of lipids, protein, ions and energy during embryonic development of Australian skinks in the genus *Lampropholis*. *Comparative Biochemistry and Physiology*, **129**, 313–326.
- Veloso, C. & Bozinovic, F. (2000) Effect of food quality on energetics of reproduction in a precocial rodent, *Octodon degus*. *Journal of Mammalogy*, **81**, 971–978.
- Voigt, C.C. (2003) Reproductive energetics of the nectar-feeding bat *Glossophaga soricina* (phylllostomidae). *Journal of Comparative Physiology B*, **173**, 79–85.
- Warner, D.A., Lovern, M.B. & Shine, R. (2007) Maternal nutrition affects reproductive output and sex allocation in a lizard with environmental sex determination. *Proceedings of the Royal Society of London, Series B*, **274**, 883–890.
- Warner, D.A., Lovern, M.B. & Shine, R. (2008) Maternal influences on offspring phenotypes and sex ratios in a multi-clutching lizard with environmental sex determination. *Biological Journal of the Linnean Society*, in press.
- Webb, S.C., Hedges, R.M. & Simpson, S.J. (1998) Diet quality influences the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of locusts and their biochemical components. *Journal of Experimental Biology*, **201**, 2903–2911.
- Wheatley, K.E., Bradshaw, C.J.A., Harcourt, R.G. & Hindell, M.A. (2008) Feast or famine: evidence for mixed capital-income breeding strategies in Weddell seals. *Oecologia*, **155**, 11–20.
- Zuffi, M.A.L., Giudici, F. & Ioalet, P. (1999) Frequency and effort of reproduction in female *Vipera aspis* from a southern population. *Acta Oecologica*, **20**, 633–638.

Received 25 February 2007; accepted 25 May 2008

Handling Editor: Murray Humphries