

Proximate constraints on reproductive output in a northern population of painted turtles: an empirical test of the bet-hedging paradigm

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Abstract: For long-lived, iteroparous organisms whose annual reproductive success is low and unpredictable, the “bet-hedging” life-history paradigm predicts that an increase in resource acquisition should result in an increase in stored lipids and not an increase in reproductive output. We tested whether reproductive patterns in a population of midland painted turtles (*Chrysemys picta marginata* Agassiz, 1857) are consistent with this prediction. Assuming that annual temperature variation is a proxy for variation in resource acquisition, we hypothesized that if harvested resources are generally sequestered for future reproduction, then there should be no relationship between clutch frequency (the number of clutches laid per season) and the temperature experienced when no follicular growth occurred. We hypothesized further that if temperature constrains clutch frequency by limiting the amount of energy that can be allocated to reproduction, then clutch frequency would be related only to the temperature experienced during the period in which follicular growth occurred (fall temperature). We found that clutch frequency was primarily related to fall temperature, which suggests that the amount of thermal energy experienced during periods of follicular development limits the amount of stored energy that can be allocated to developing follicles. This pattern of reproductive allocation is consistent with the bet-hedging paradigm.

Résumé : Chez les organismes itéropares à vie longue dont le succès reproductif annuel est faible et imprévisible, le paradigme du « pari sur l'avenir » des cycles biologiques prédit qu'une augmentation de l'acquisition de ressources devrait entraîner un accroissement des réserves lipidiques plutôt qu'une augmentation du rendement reproductif. Nous avons vérifié si les patrons de reproduction d'une population de tortues peintes du centre (*Chrysemys picta marginata* Agassiz, 1857) s'accordent avec cette prédiction. En assumant que la variation de la température annuelle est une variable de rechange pour la variation de l'acquisition de ressources, nous émettons l'hypothèse qui veut que, si les ressources récoltées sont en général mises en réserve pour des reproductions futures, il n'y ait pas de corrélation entre la fréquence des pontes (le nombre de pontes produites par saison) et la température subie durant la période sans croissance des follicules. Nous formulons une seconde hypothèse selon laquelle, si la température restreint la fréquence des pontes en limitant la quantité d'énergie destinée à la reproduction, la fréquence des pontes est reliée seulement à la température subie durant la période de croissance des follicules (température d'automne). Nous trouvons que la fréquence des pontes est principalement reliée à la température de l'automne, ce qui laisse croire que la quantité d'énergie thermique présente durant les périodes de développement folliculaire limite la quantité d'énergie en réserve qui peut être allouée aux follicules en développement. Ce patron d'allocation reproductive s'accorde avec le paradigme du pari sur l'avenir.

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Introduction

The concept of reproductive effort (RE) is of paramount importance when framing reproductive patterns in a life-history context (Stearns 1992). RE describes the proportion of available energy that is devoted to reproduction (Fisher 1930), and resource availability is one of its two major determinants. In environments with low resource availability, selection may favor late maturity, a long life expectancy, a large energy investment per offspring, low fecundity, and a low RE (Dobzhansky 1950; MacArthur and Wilson 1967; Stearns 1992). The demographic environment is the second important determinant of RE. Williams (1966a, 1966b) pre-

dicted that species with a long life expectancy should have a low RE in each breeding season, whereas species with a short life expectancy should have a large RE, as the risk of mortality is greater.

These concepts have led to the bet-hedging life-history paradigm (Gillespie 1977; Philippi and Seger 1989; Stearns 1992). In environments where offspring survivorship varies unpredictably, selection may favor an increased reproductive life span coupled with a low but constant RE (Murphy 1968; Stearns 1976, 1977). That is, by increasing the number of reproductive events in which an individual participates, the likelihood that a reproductive event will occur when environmental conditions are favorable also increases. Although reproductive output (RO, the total amount of energy invested in reproduction) and RE are not necessarily coupled in any given reproductive bout, the bet-hedging model predicts that RO should be less variable than underlying stored lipids (Congdon and Tinkle 1982). This is because when offspring survivorship is low and unpredictable, there may be little predictable increase in maternal fitness with increased

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RO. Moreover, increasing RO could compromise future reproductive prospects by decreasing lipid reserves. As such, any interannual variation in resource acquisition that occurs should result in synchronous variation in stored lipids, but RO and RE should not vary substantially (Congdon and Tinkle 1982).

Painted turtles (*Chrysemys picta* (Schneider, 1783)) are long-lived, iteroparous organisms with low and unpredictable offspring survivorship (e.g., Congdon and Tinkle 1982; Congdon et al. 2003; Samson 2003), and their abundance and nesting ecology make them ideal organisms with which to test predictions of the bet-hedging paradigm. In northern populations, virtually all mature females produce at least one clutch per season, but about 20%–30% of females produce two clutches (Congdon et al. 2003; Samson 2003). Although RO can presumably increase independent of RE in *C. picta*, anatomical constraints imposed by the calcareous shell and pelvic aperture necessarily restrict the amount of energy that can be invested in a single clutch (Congdon and Gibbons 1987; Iverson and Smith 1993). So within individuals, interannual variation in RO is necessarily associated with increases in clutch frequency (the number of clutches produced per year). Given that the average proportion of total body lipids allocated to the second clutch is estimated to be 28.9% (range 8.81%–50.1%) (Appendix A), we assume that an increase in clutch frequency is an adequate proxy for an increase in RE, and we use long-term data to test whether variation in clutch frequency in a northern population of midland painted turtles (*Chrysemys picta marginata* Agassiz, 1857) is related to environmental variation.

Hypothesis 1

First, clutch frequency may vary synchronously among years at the population level. If this is the case, then it is possible that when synchronous variation in resources acquisition occurs, females devote this energy to reproduction, which necessarily results in an increase in clutch frequency. This would be at odds with the bet-hedging paradigm because it suggests that (i) resource acquisition dictates RO in a given year and, accordingly, (ii) that future reproduction could be compromised because increases in harvested energy are immediately allocated to reproduction. Because body temperature is related to rates of physiological processes including metabolic rates (Gatten 1974), digestion speed (Avery et al. 1993; Koper and Brooks 2000), and energy assimilation efficiency (Kepenik and McManus 1974), large-scale (e.g., annual) temperature variation is likely to result in variations in the rate at which resources are acquired and processed. Ultimately, this should affect the amount of energy that is potentially available for reproduction (e.g., Hennen 1997). We predict that if increases in resource acquisition are translated into increases in RO, then the temperature experienced during periods of follicular development and during periods of follicular quiescence (when no follicular growth is occurring) will be positively related to clutch frequency in *C. picta*.

Hypothesis 2

Second, because a certain amount of heat is required for the completion of anabolic processes (Holt 2000; Obbard and Brooks 1987), the amount of heat experienced during

the period of follicular development may limit the amount of stored energy that can be allocated to developing follicles, even if food energy is not limiting. We predict that if thermal energy constrains RO, then only the temperatures experienced during follicular development will be positively related to clutch frequency. This would not necessarily be inconsistent with the bet-hedging paradigm because it implies that these animals inhabit an environment where resources are not limiting but that other exogenous factors (i.e., temperature and the length of the growing season) limit both RE and RO.

Methods

Site description

This study is part of a larger life-history study that was initiated in Algonquin Provincial Park in 1978 and has been performed in every year since, except 1981, 1982, and from 1986 to 1988. The vast majority of study turtles reside in two ponds. These ponds are about 400 m apart and are similar in depth and vegetative composition; both ponds are bisected by an abandoned railway embankment, and this is the only known nesting location for local females. A detailed description of the Arowhon population can be found in Schwarzkopf and Brooks (1985). All temperature data were provided by an Environment Canada weather station, which was located ~50 km west of our site.

Field procedures

Turtles were captured with dip nets from a canoe between late April and early June in years when sampling occurred. Individuals were brought back to the Wildlife Research Station where maximum plastron length (MPL) was recorded with calipers to the nearest 0.01 cm (for details on other morphometric measurements see Samson 2003; Schwarzkopf and Brooks 1986). Turtles were permanently marked by filing notches in their marginal scutes (Cagle 1939), and each female's unique alphanumeric code was painted on her carapace to facilitate identification during nesting season (animal handling was authorized under protocol No. 04R064, which was approved by the University of Guelph Animal Care Committee). Nesting patrols were initiated when gravid females were detected, which was usually in late May or early June. Patrolling consisted of a minimum of two persons walking back and forth along the abandoned railway embankment (encompassing both ponds) every 45 min from ~1500 to at least 2200. Each researcher records detailed information on all turtle observations. All turtles observed nesting were allowed to nest, but clutches were carefully excavated and brought back to the Wildlife Research Station where various egg measurements were taken.

Clutch frequency observations

Approximately 20%–30% of females in our population lay a second clutch in a given season; this usually occurs 2–3 weeks after the first clutch is deposited, but an inter-nesting interval as short as 8 days has been recorded. Even though the railway embankment is the only known nesting site for individuals in this population, we know that approximately 10%–20% of all nests are not directly observed in each year. We minimized the error associated with clutch

Table 1. Relationship between midland painted turtle (*Chrysemys picta marginata*) clutch frequency, maximum plastron length (MPL), temperature, and sampling intensity for both summer and fall models estimated from generalized estimating equations ($n = 462$ observations from 67 females, data span 1998–2005).

Model	Parameter	β	95% CI	Odds ratio	Type III statistics		
					df	χ^2	P
1*	1 August – 31 October	1.11	0.324–1.90	3.03	1	7.82	0.0052
	MPL	0.343	0.141–0.545	1.41	1	8.90	0.0028
	Sampling intensity	—	—	—	2	15.7	<0.001
2†	1 July – 31 October	1.01	0.132–1.89	2.74	1	5.70	0.017
	MPL	0.329	0.139–0.518	1.39	1	9.37	0.0022
	Sampling intensity	—	—	—	2	16.4	<0.001

Note: Models are ordered based on deviance scores.

*Deviance = 1.234.

†Deviance = 1.243.

uncertainty by meticulously reviewing observations that were recorded by research assistants in the field books. Because turtles often have several failed nest attempts before they successfully deposit a clutch, gravid turtles whose clutches are not located are almost invariably seen roaming and (or) digging on the embankment in the days or hours prior to oviposition. We therefore inferred that an individual laid two clutches in a given season if she was seen twice on the embankment more than 8 days apart. We reviewed the field books for 67 turtles ($n = 462$ observations) that were known to have nested (based on data from the “first” clutch) in at least three consecutive years between 1998 and 2005.

Estimating the periods of follicular development

In the Arowhon population, nesting is usually finished in early July, so follicular quiescence (the period where follicles do not develop) likely occurs between ~1 July and 1 August; follicular recrudescence likely occurs around the beginning of August (Moll 1973; Congdon and Tinkle 1982). Because we were not certain of when the fall period of follicular development is completed (and it may vary from year to year, depending on when hibernation occurs), we tested whether clutch frequency was related to the temperatures experienced between the 213th and the 263rd Julian day (~1 August – 20 September; Rollinson 2006) and between the 213th and the 294th Julian day (~1 August – 31 October) in all models. Although both intervals yielded similar coefficients, the latter interval appeared to be a better predictor of clutch frequency than the former, so only the models that used the 213th–294th Julian interval as the estimated period of follicular development are reported herein. We also tested whether the temperatures experienced just prior to nesting season onset (mean May temperature) were significant predictors of clutch frequency in all final models.

If clutch frequency varies with harvested resources, then the temperatures experienced during both follicular quiescence and follicular development will be related to clutch frequency. Thus, the mean temperatures experienced between the 182nd and the 294th Julian day (~1 July – 31 October) were used to test this hypothesis.

Assumptions and statistical procedures

The primary assumption underlying our analyses is that the vast majority of mature females in a population lay at least one clutch in a season. Samson (2003) reported that the annual probability of oviposition in the Arowhon population is 82%, but this estimate is likely negatively biased owing to sampling intensity (see below). Moreover, 97% of mature females lay at least one clutch annually in a Michigan population (Congdon et al. 2003). If most females already nest once in a season, then any temperature-moderated effect on clutch frequency should be realized primarily or entirely in terms of the number of second clutches being laid.

Variation in sampling intensity occurred over the 1998–2005 period, and this could obviously affect our estimates of clutch frequency. However, if most mature females lay a first clutch in a given year, and assuming there was no large change in the number of mature females in the population during this time (Samson 2003), then the number of first clutches collected by field researchers in a given year should be a good proxy of sampling intensity for that year. Thus, we estimated sampling intensity from the number of first clutches obtained in a given year (Appendix B), and we included sampling intensity as a categorical variable in our analyses.

Our final data set consisted of 462 nesting observations from 67 individuals (MPL range 12.13–17.28 cm) that nested in at least three consecutive years between 1998 and 2005. We used generalized estimating equations with a repeated subjects statement and a logit link function in PROC GENMOD (SAS Institute Inc. 2003) to test our hypotheses. We assumed a priori that, within individuals, reproductive events in successive years would be more likely to be inter-correlated than reproductive events with a greater degree of temporal separation, so we chose an order 1 autoregressive correlation structure as a working correlation matrix. We estimated model fit by dividing model deviance by the degrees of freedom in the model (henceforth referred to as “deviance”). Unfortunately, there are few other methods of assessing model fit in generalized estimating equation analyses (Horton et al. 1999). We tested relevant interaction terms only in our final models.

Table 2. Relationship between midland painted turtle clutch frequency, MPL, temperature, and sampling intensity for a group of small females estimated from generalized estimating equations (MPL range = 12.13–14.98 cm, $n = 188$ observations from 30 turtles, data span 1998–2005).

Model	Parameter	β	95% CI	Odds ratio	Type III statistics		
					df	χ^2	P
3*	1 August – 31 October	1.52	0.453–2.59	4.57	1	5.72	0.017
	MPL	1.03	0.329–1.73	2.80	1	7.58	0.0059
	Sampling intensity	—	—	—	2	6.81	0.033
4†	1 July – 31 October	0.341	–0.714 to 1.40	ns	1	0.42	0.52
	MPL	0.981	0.289–1.67	2.67	1	7.22	0.0072
	Sampling intensity	—	—	—	2	6.48	0.039

Note: Models are ordered based on deviance scores. ns, not significant.

*Deviance = 1.115.

†Deviance = 1.132.

Table 3. Relationship between midland painted turtle clutch frequency, temperature, and sampling intensity for a group of large females estimated from generalized estimating equations (MPL range = 15.02–17.28 cm, $n = 274$ observations from 37 turtles, data span 1998–2005).

Model	Parameter	β	95% CI	Odds ratio	Type III statistics		
					df	χ^2	P
5*	1 August – 31 October	0.978	–0.00520 to 1.96	ns	1	3.81	0.051
	Sampling intensity	—	—	—	2	9.26	0.0098
6†	1 July – 31 October	1.09	0.0933 to 2.08	2.97	1	4.55	0.033
	Sampling intensity	—	—	—	2	9.99	0.0068

Note: Models are ordered based on deviance scores. ns, not significant.

*Deviance = 1.296.

†Deviance = 1.297.

Results

Clutch frequency across all individuals

Across all individuals, model fit was maximized when MPL, sampling intensity, and the mean temperature experienced between 1 August and 31 October (i.e., during the estimated period of follicular development) were used to predict clutch frequency. Including July temperatures (i.e., during follicular quiescence) in the model both increased model deviance and increased the error associated with the estimated relationship between temperature and clutch frequency (Table 1). The addition of mean May temperature (i.e., the temperature that occurred just prior to nesting season in each year) did not yield a significant parameter estimate in model 1 (May temperature, $\beta = -0.0622$, $\chi^2 = -0.96$, $P = 0.338$, deviance = 1.246).

Clutch frequency for different size classes of turtles

Specifying an interaction term between MPL and temperature in model 1 yielded a model deviance similar to (but not lower than) that of model 1. From this, we concluded that the relationship between temperature and clutch frequency might differ for different female body sizes, and we split our final data set into a group of “smaller” and “larger” females to test for such body size specific differences. Because the goal of the segregation was to directly compare the aforementioned relationships among groups of females with different body sizes, we attempted to divide our sample in a manner that resulted in a similar sample size and a similar range of body size in both groups.

Although the actual midpoint MPL of our sample was 14.70 cm (and segregating the sample at this point would have resulted in an equal range of body sizes in both groups), segregating the sample at this point would have resulted in a relatively low sample size in the group of smaller females. We decided a priori to increase the segregation point to an MPL of 15.00 cm to strike a greater balance between the MPL range of each group and the sample size of each group. Segregating the two groups at an MPL of 15.00 cm produced a group of 30 smaller females ($n = 188$ observations) with MPLs between 12.13 and 14.98 cm and a group of 37 larger females ($n = 274$ observations) with MPLs between 15.02 and 17.28 cm.

Small females

For smaller females, clutch frequency was best predicted by MPL, sampling intensity, and the temperature experienced between 1 August and 31 October (i.e., during the estimated fall period of follicular development). Thus, the model that excluded the temperatures experienced during follicular quiescence (i.e., July temperatures) better predicted variation in clutch frequency (model 3, Table 2). Mean May temperature did not yield a significant parameter estimate in model 3 (May temperature, $\beta = 0.0386$, $\chi^2 = 0.10$, $P = 0.75$, deviance = 1.120).

Large females

For larger females, MPL was not a significant predictor of clutch frequency in any of the models. Therefore, this pa-

parameter was omitted from all final models (Table 3). Clutch frequency was significantly related to the temperatures experienced between 1 July and 31 October (i.e., during follicular quiescence and follicular development) and nearly significantly related to the temperatures experienced between 1 August and 31 October (i.e., only during follicular development) (Table 3). However, the latter model had a deviance extremely similar to that of the former, and this indicates that the inclusion of July temperatures in the model did not increase model fit. Mean May temperature did not yield a significant parameter estimate in model 5 (May temperature, $\beta = 0.0012$, $\chi^2 = 0.01$, $P = 0.99$, deviance = 1.302).

Discussion

Relationship between temperature and clutch frequency

The relationship between temperature and clutch frequency differed depending on the size of the females considered in our analyses. For smaller females, the model that incorporated July temperatures had a relatively high deviance, and there was no significant relationship between temperature and clutch frequency. Conversely, the temperatures experienced during the estimated fall period of follicular development were significant predictors of clutch frequency (Table 2). Thus, if large-scale temperature variation acts as a proxy for resource harvesting and processing rates (e.g., Gatten 1974; Kepenis and McManus 1974; Avery et al. 1993; Koper and Brooks 2000), then the energy acquired from harvested resources in the year prior to nesting is not related to clutch frequency. Our data therefore suggest that clutch frequency may be (in part) limited by the amount of stored energy that can be allocated to developing follicles during the relative short growing season experienced at these northern latitudes (Moll 1973; also see below).

For larger females, the temperatures experienced both during follicular quiescence and during the fall period of follicular development were significant predictors of clutch frequency (model 6, Table 3), whereas the β estimate for the relationship between clutch frequency and the fall period of follicular development was marginally insignificant (model 5, Table 3). However, if energy acquisition in July has an appreciable impact on clutch frequency, then one would expect that any model incorporating the July effect would predict clutch frequency better than competing models. It is clear from Table 3 that β estimates for both models (the mean temperature of 1 July – 31 October versus that of 1 August – 31 October) are virtually identical. We can also see that the deviance for the 1 July – 31 October model was almost identical to that of the 1 August – 31 October model (Table 3). Thus, the temperatures experienced during periods of follicular quiescence do not predict variations in clutch frequency any better than temperatures experienced during follicular development. These data suggest that for larger females, resource acquisition may have a minor effect on clutch frequency, but it is concurrently possible that the temperatures experienced during follicular development constrain clutch frequency (in part) by limiting the stored energy that can be allocated to developing follicles.

Energetic considerations

There was no relationship between clutch frequency and MPL at larger body sizes, but this relationship was pronounced in smaller females (Tables 2 and 3). Samson (2003) found that the positive relationship between fecundity (clutch frequency and clutch size) and body size is asymptotic in the Arowhon population, with a maximum fecundity realized at an MPL of 15.5 cm and greater. Although clutch size increased with body size across individuals of all sizes, clutch frequency failed to increase further with body size once an MPL of ~15.5 cm was reached (Samson 2003; also see Congdon et al. 2003). Congdon and Tinkle (1982) showed that stored lipid levels in *C. picta* increase with body size in both absolute and relative terms, and this suggests that the asymptotic relationship between clutch frequency and body size is due to energetic factors. To illustrate, Congdon and Tinkle (1982) reported that for painted turtles, total body lipids are best predicted by the equation $\text{body lipids} = -31.27 + 0.268(\text{carapace length})$, where body lipids is in grams and carapace length is in millimetres ($r^2 = 0.12$, $n = 75$, $P < 0.01$) (Congdon and Tinkle 1982, p. 232). From this equation, we calculated the approximate lipid content of small versus large individuals. We first transformed carapace length into MPL using the equation $\text{carapace length} = -0.118 + 1.122(\text{MPL})$, where carapace length and MPL are in centimetres ($r^2 \sim 0.99$, $n > 150$, $P < 0.01$) (Samson 2003, p. 22). Then, we transformed MPL values into body mass (g) using the equation $\text{body mass} = 0.2626(\text{MPL}^{2.784})$, where MPL is in centimetres ($r^2 \sim 0.97$, $n > 150$, $P < 0.01$) (Samson 2003, p. 86).

According to our calculations, the smallest mature females in the Arowhon population (~300 g body mass) comprise ~6.25 g lipids, or ~2.1% lipids by mass. The largest individuals (~800 g body mass) comprise ~22.3 g lipids, or ~2.8% lipids by mass. Thus, the largest reproductively active females in our population comprise ~25% more total body lipids than do the smallest females. Moreover, an individual of ~500 g, which is the approximate body size at which the relationship between clutch frequency and body size disappears (Samson 2003), is ~2.74% lipids by mass; this is very close to the 2.8% lipids estimated for the largest individuals. Our calculations indicate that, on average, a disparity in total body lipids should exist between smaller and larger individuals in the Arowhon population, but this disparity decreases allometrically so that the difference in energy reserves between intermediate-sized and larger individuals is relatively small.

Energetic considerations imply that, within populations, larger females have more energy for reproduction than do smaller females, and this could drive the observed relationship between body size and clutch frequency. It is therefore interesting that both summer temperature and fall temperature are related to clutch frequency in larger females but that only fall temperature is related to clutch frequency in smaller females. Because smaller females grow more quickly than do larger females (Samson 2003) and because resource acquisition was weakly related to clutch frequency in larger, but not smaller females, it is tempting to suggest that our findings may be indirect evidence for a trade-off between growth and reproduction (e.g., Barnes 1962; Warner 1984; Bowden et al. 2004). In the Arowhon population, a

high frequency of scute-shedding, which is a hallmark of growth in turtles, is observed in late August, particularly in smaller individuals (N. Rollinson, personal observation). Thus, most growth may occur between June and August (also see Congdon and Tinkle 1982). If the resources harvested during this period are used (in part) to support growth, then smaller females would, on average, have less energy for reproduction relative to larger females. However, in this regard, it is somewhat puzzling that fall temperature predicts clutch frequency for smaller females. It could be that growth does not deplete lipid reserves to low levels in these smaller females and that clutch frequency is restricted primarily by heat accumulation during the fall period of follicular development and secondarily by lipid reserves. But this is clearly speculative, and more studies that focus on temporal patterns of lipid usage are needed to understand the reproductive energetics of *C. picta* in a life-history context (e.g., Congdon and Tinkle 1982).

That larger female *C. picta* tend to nest earlier in the season than smaller females (Gibbons and Greene 1990; also see Bowden et al. 2004) could also explain why July temperature was related to clutch frequency for larger, but not smaller, females. If the timing of follicular recrudescence is more tightly coupled with an endogenous cycle than with environmental factors (e.g., Mitchell 1985; Sarkar et al. 1996; Shanbhag et al. 2000), then because larger females oviposit earlier than smaller females, larger females may also begin follicular development earlier than smaller females. If clutch frequency is indeed limited by the amount of stored energy that can be devoted to developing follicles (across individuals of all sizes), then this would explain why July temperatures were not related to clutch frequency in smaller females, and it could explain why both summer temperature and fall temperature models produced similar coefficients for larger females. However, there is evidence that temperature is the primary regulator of the gonadal cycle in *C. picta* (Ganzhorn and Licht 1983) and other turtle species (Mendonca 1987; Sarkar et al. 1996). Thus, the validity of this hypothesis depends crucially on the relative influence of intrinsic versus extrinsic factors on the gonadal cycle of *C. picta*.

Reproductive patterns and life-history theory

For organisms with a long reproductive life span, a reproductive strategy that minimizes the variance in reproductive success should be favored by selection (Philippi and Seger 1989; Stearns 1992). The empirical reasoning is that when the fitness of a certain genotype varies unpredictably over generations, its relative rate of growth should be expressed as its geometric mean fitness and not its arithmetic mean fitness (Philippi and Seger 1989; Stearns 1992). The geometric mean of n numbers is the n th root of their product and it is generally inversely related to the variability of the data set that it describes. Thus, from an ultimate perspective, increases in fecundity that are necessarily coupled with an increase in clutch frequency may be a means by which females increase fecundity without increasing the variance in reproductive success. For example, nest depredation rates often exceed 75% in some species of turtles (Davis and Whiting 1977; Butler et al. 2004; Ahjond and Percival 2005), and these rates can vary stochastically from year to

year (Davis and Whiting 1977; Kolbe and Janzen 2002; Rowe et al. 2005). It is therefore possible that a strategy of laying two or more clutches in a season reduces the variance in reproductive success because it reduces the overall probability of complete reproductive failure in certain years.

Our data do not provide evidence of a substantial relationship between resource acquisition and variation in RO in this northern population of *C. picta*. That clutch frequency appeared to vary primarily with the temperatures experienced during the fall period of follicular development suggests that thermal energy constrains RO by limiting the amount of stored lipids that can be allocated to reproduction. This is interesting because it suggests that temperature is an important proximate determinant of RE and RO in this northern population of *C. picta*. That is, the insensitivity of clutch frequency to the amount of food energy acquired in the year prior to nesting suggests that females are generally able to maintain high lipid reserves (Congdon and Tinkle 1982). This pattern of reproductive allocation appears to be consistent with bet-hedging because it suggests that, in terms of the energy available for reproduction, current and future reproductive events may not trade off. Indeed, there is no evidence that clutch frequency is related to adult mortality in this northern population (Samson 2003), so increases in RO by means of increases in clutch frequency may be favored by selection in *C. picta*.

Seasonal patterns of vitellogenin deposition

It is interesting that May temperature was not a significant predictor of clutch frequency in any model, and it could be that temporal patterns of vitellogenin deposition account for this phenomenon. In freshwater turtles, follicles appear to enlarge in distinct sets over several years, and presumably, each set represents a potential clutch (Callard et al. 1978; Congdon and Tinkle 1982). Roosenburg and Dunham (1997) speculated that vitellogenin is allocated to follicles in general in the summer–fall prior to nesting, whereas vitellogenin is allocated to specific sets of follicles (those that will be ovulated) in the spring in the freshwater turtle *Malaclemys terrapin* (Schoepff, 1793). If this is true, and assuming that there is a threshold size at which follicles have a high probability of being ovulated (Kuchling and Bradshaw 1993), the amount of energy allocated to sets of follicles in the year prior to nesting could determine clutch frequency for the following season. Because we did not detect any relationship between May temperature and clutch frequency, our data support this contention. Furthermore, the observation that egg size is related to spring, but not fall, temperature further corroborates this possibility (Rollinson 2006). The timing of vitellogenin deposition certainly requires further study, but for *C. picta*, there is mounting evidence to support Roosenburg and Dunham's (1997) contention.

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Appendix A. Estimating total body lipid allocation to the second clutch

We estimated the proportion of body lipids used in the second clutch (Plip) for a sample of midland painted turtles (*C. picta marginata*) in the Arowhon population ($n = 133$ females). This sample was taken at random from our database (one data point per female), and it includes only second clutch nest data. Congdon and Tinkle (1982) reported that for painted turtles, total body lipids (dry mass) are best predicted by the equation body lipids = $-31.27 + 0.268(\text{carapace length})$, where body lipids is in grams and carapace length is in millimetres ($r^2 = 0.12$, $n = 75$, $P < 0.01$) (Congdon and Tinkle 1982, p. 232). From this equation, we estimated total body lipids of 133 females in our sample.

Painted turtle eggs are ~67% water (Congdon and Tinkle

Table A1. Estimating variation in sampling intensity among years in the Arowhon population.

Year	Number of first clutches collected	Sampling intensity category (ordinal)
1998	119	1
1999	145	2
2000	178	2
2001	147	2
2002	153	2
2003	171	2
2004	209	3
2005	183	3

1982; also see Rowe (1995) and Harms et al. (2005) who reported similar estimates), and we estimated the mean dry mass of the eggs in our sample from mean egg mass using this value (egg dry mass = mean egg mass $\times$ 0.33). The dry mass of the average egg comprises 28.2% lipids (Congdon and Tinkle 1982), and the proportional lipid content of the egg does not change with female body size (Harms et al. 2005; Rowe 1995). We therefore estimated lipid content of each clutch in our sample by multiplying estimated egg dry mass by the proportional lipid content of the dry egg and then multiplying the estimated mean lipid content of the egg by the number of eggs in the clutch. Finally, we estimated Plip of these females by dividing the estimated total lipids used in the second clutch by total estimated body lipids. Plip of the second clutch averaged (mean $\pm$ SD) $28.9\% \pm 5.89\%$ and ranged from 8.81% to 50.1%.

Appendix B. Estimating variation in sampling intensity among years at our long-term study site

Descriptions of sampling intensity are based primarily on number of first nests collected during the nesting season in a given year (Table A1). However, to a lesser extent, these descriptions involve a combination of personal communications from past researchers and information on the number of researchers present at the nesting site during nesting season. Years 2004 and 2005 were assigned to category 3 because there were more researchers present at the study site (four researchers) during nesting season than in other years (two or three researchers). Three researchers were involved in the study in 1998, but 1998 was nonetheless assigned to category 1 because the researchers usually arrived at the site later in the evening compared with other years. Undoubtedly, many nests and nesting attempts were missed in 1998 because of this later arrival time.