

Maternal investment in reproduction and its consequences in leatherback turtles

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Abstract Maternal investment in reproduction by oviparous non-avian reptiles is usually limited to pre-ovipositional allocations to the number and size of eggs and clutches, thus making these species good subjects for testing hypotheses of reproductive optimality models. Because leatherback turtles (*Dermochelys coriacea*) stand out among oviparous amniotes by having the highest clutch frequency and producing the largest mass of eggs per reproductive season, we quantified maternal investment of 146 female leatherbacks over four nesting seasons (2001–2004) and found high inter- and intra-female variation in several reproductive characteristics. Estimated clutch frequency [coefficient of variation (CV) = 31%] and clutch size (CV = 26%) varied more among females than did egg mass

(CV = 9%) and hatchling mass (CV = 7%). Moreover, clutch size had an approximately threefold higher effect on clutch mass than did egg mass. These results generally support predictions of reproductive optimality models in which species that lay several, large clutches per reproductive season should exhibit low variation in egg size and instead maximize egg number (clutch frequency and/or size). The number of hatchlings emerging per nest was positively correlated with clutch size, but fraction of eggs in a clutch yielding hatchlings (emergence success) was not correlated with clutch size and varied highly among females. In addition, seasonal fecundity and seasonal hatchling production increased with the frequency and the size of clutches (in order of effect size). Our results demonstrate that female leatherbacks exhibit high phenotypic variation in reproductive traits, possibly in response to environmental variability and/or resulting from genotypic variability within the population. Furthermore, high seasonal and lifetime fecundity of leatherbacks probably reflect compensation for high and unpredictable mortality during early life history stages in this species.

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Introduction

Maternal investment in reproduction is shaped by trade-offs between competing functions, such as time allocations to mating behaviors and resource allocations to number, size, and post-hatching parental care

of offspring (Congdon 1989; Roff 1992). Resources available to an individual female animal are finite, and thus constrain reproductive output such that an increase in maternal investment in individual offspring necessarily results in decreased allocation to other components of the overall energy budget (Congdon and Gibbons 1987; Congdon 1989) and the decreased ability of the mother to invest in additional offspring (Trivers 1972). Therefore, optimality models of reproductive output highlight trade-offs between allocations to competing compartments that enhance reproductive success within the constraints of finite energy resources. For example, optimal egg size theory (OES) (Lack 1947, 1948; Williams 1966; Smith and Fretwell 1974; Roff 1992) predicts that natural selection favors trade-offs between egg/neonate size and number of eggs in a clutch within energetic (Congdon 1989), anatomical (Congdon and Gibbons 1987; Shine 1992), and physiological (Bowden et al. 2004) constraints.

Reproductive investment of most oviparous ectothermic vertebrates is limited to pre-ovipositional allocation to number and size of eggs and number of clutches per reproductive season. This is because there is rarely post-hatching parental care, aside from yolk reserves utilized by hatchlings after nest emergence (Congdon 1989). Therefore, allocation of energy and nutrients (e.g., lipids, protein and water) to eggs used for embryogenesis and post-hatching survival of offspring directly represent maternal investment in reproduction (Congdon 1989). For this reason, oviparous non-avian reptiles have been excellent subjects for testing hypotheses of OES models (Congdon and Gibbons 1987). Egg size varies little in some populations and species (Tinkle and Ballinger 1972; Rowe 1994), whereas variation in egg size within and among some species indicates that trade-offs and counter-selected constraints might exist that obscure empirical determination of an optimal egg size (Congdon and Gibbons 1987; Roff 1992; Van Buskirk and Crowder 1994). For species that produce several offspring per reproductive bout, OES predicts that variation in egg size should be minimal and total variation in clutch mass associated with resource availability and physiological constraints (Dunham et al. 1989) should be determined by clutch size rather than by egg size (Smith and Fretwell 1974; Congdon and Gibbons 1987).

Among oviparous non-avian reptiles, sea turtles exhibit the highest absolute reproductive outputs, laying many clutches per reproductive season that contain between 50 and 130 eggs of masses between 27 and 80 g each, depending on species (Miller 1997). Although each clutch of sea turtle eggs represents a smaller proportion of female body mass (~1–10%)

than clutches of many birds and other non-avian reptiles, a sea turtle's relative total egg biomass of all clutches produced in a season (~10–20% of female body mass) is only slightly lower than that of birds and other oviparous reptiles (~10–40%) (Congdon and Gibbons 1985; Roff 1992; Shine 1992).

Investigations of relationships between egg size and other features of maternal investment in sea turtles, compared to other non-avian reptile species and especially other turtle taxa, have been limited to relatively few studies (Bjorndal and Carr 1989; Hays and Speakman 1991; Van Buskirk and Crowder 1994; Tiwari and Bjorndal 2000), but have revealed interesting patterns within and among species. For example, Van Buskirk and Crowder (1994) reported that across sea turtle species, a general pattern exists of maternal investment in either clutches of many small or few large eggs. Inter-population analyses of loggerhead turtles (*Caretta caretta*) revealed that egg sizes varied little whereas clutch sizes varied widely across populations (Tiwari and Bjorndal 2000). Because of their high seasonal fecundity, sea turtles should exhibit little variation in egg size and instead maximize clutch size (Smith and Fretwell 1974; Congdon and Gibbons 1987; Hays and Speakman 1991).

The leatherback turtle (*Dermochelys coriacea*, Vandelli 1761), sole extant member of Dermochelyidae, displays the highest number of clutches (~seven clutches per season) of all sea turtle species, and its combination of large clutch size (~65 eggs) and massive eggs (~80 g) results in the heaviest absolute biomass of eggs per season (~35 kg) of all reptile species (Miller 1997; Reina et al. 2002). Further, leatherbacks exhibit high lifetime fecundity because they repeat this large seasonal reproductive investment every 2–4 years throughout their reproductive lifespan, which could last >20 years (Spotila et al. 1996). In addition to these distinctive egg and clutch characteristics, leatherbacks lay their eggs in the deepest nests (70–100 cm) of all sea turtles. Unlike other oviparous amniotes, leatherbacks also deposit varying masses of shelled albumen gobs (SAGs; Wallace et al. 2004) in each clutch. These SAGs, erroneously referred to as “yolkless eggs” in previous studies, vary greatly in number (~from ten to 100) as well as shape (~10–50 mm diameter) within and among clutches of leatherback females. Possible functions of SAGs have been investigated (Frazier and Salas 1984; Wallace et al. 2004; Caut et al. 2006), but are unknown. Given the distinctive reproductive traits and egg/clutch characteristics of this species, leatherbacks could serve as a useful “Kroghian” organism (Bennett 2003) for testing hypotheses of OES and for investigating patterns of maternal investment in reproduction.

Objectives for this study were: (1) to quantify variation in reproductive traits within and among leatherback females; (2) to test hypotheses of OES by examining the relationships between egg size, neonate size, and clutch size in leatherbacks; (3) to examine patterns of maternal investment in reproduction that leatherbacks exhibit to maximize seasonal fecundity. We analyzed leatherback maternal investment in reproduction in the context of reproductive optimality models (Smith and Fretwell 1974; Roff 1992) and in relation to maternal investment patterns of other oviparous vertebrates.

Materials and methods

We conducted this study in Parque Nacional Marino Las Baulas (PNMB), located on the north Pacific coast of Costa Rica. We collected data on eggs and clutches laid and hatchlings produced by nesting female leatherbacks on Playa Grande and Playa Ventanas, two of the three nesting beaches that comprise PNMB. This population of nesting leatherbacks has been studied extensively for the past 15 years through a saturation tagging program, using passive integrated transponder (PIT) tags that allow for identification and monitoring of individual turtles within and across nesting seasons (Steyermark et al. 1996; Reina et al. 2002).

Reproductive output: leatherback eggs, clutches, and hatchlings

To examine patterns of reproductive output in leatherbacks at PNMB, we collected data on eggs, clutches, and hatchlings from 334 clutches, laid by 146 different individual females (one to seven clutches per female in a season), relocated to hatchery nests over four nesting seasons from 2001 to 2004. The female leatherbacks included in our analyses constituted a sub-sample (25–80%) of the total nesting population in each season. We collected clutches of eggs by hand or in plastic bags placed under the ovipositing female's cloaca during oviposition and relocated them to a beach hatchery on Playa Grande. All eggs used in this study were collected from “doomed” natural nesting locations at or below the natural high tide line, or in areas of high human activity. Advantages to using data collected only from clutches in the beach hatchery include using nests with standardized dimensions, obtaining accurate clutch size counts, and minimizing effects of variation in nest conditions (e.g., sand grain size, moisture and salinity) on embryonic development that could be due to differences in oviposition location on the beach.

For descriptions of hatchery and nest construction methodology at PNMB see Wallace et al. (2004).

Before reburying each clutch in a human-constructed hatchery nest (within 6 h of oviposition), we recorded masses of 20 eggs individually, as well as all SAGs collectively (Ohaus Scout field balance, 200 g capacity, ± 0.01 g; Ohaus, Pine Brook, N.J.), and counted the total number of eggs in the clutch. When hatchlings emerged from nests about 60 days later, we recorded masses of up to 20 individual hatchlings per nest (as above) and measured straight carapace length and carapace width to the nearest 0.1 mm using digital calipers. To characterize intra-clutch variation in hatchling size accurately, we accepted hatchling measurements only from nests for which ten or more hatchlings were processed. Hatchlings were released on the beach and allowed to crawl to the ocean immediately after measurements were made.

We calculated emergence success of hatchlings from each nest using the following formula: $\text{EH/CS} \times 100$, where EH is the total emerged hatchlings (within 2 nights of initial emergence) and CS is the clutch size (original number of eggs deposited). Although hatching success is the metric more often used in sea turtle studies, it can be inflated by including embryos that successfully developed to hatchling stage, but probably would not have emerged from the nest without human intervention. For this reason, we used emergence success in our analyses instead of hatching success because emergence success provides an ecologically relevant measure of hatchling production per nest for individual females. To facilitate comparison between the two metrics, emergence success was $7.4 \pm 5.7\%$ (mean \pm SD) lower than hatching success within clutches in this study.

Body size and estimated clutch frequency of nesting female leatherbacks

Each nesting female was identified by her PIT tags and measured [standard curved carapace length (SCCL) and standard curved carapace width (SCCW)] to the nearest 0.1 cm using a flexible measuring tape. Because PNMB has >90% beach coverage during the nesting season (Reina et al. 2002), multiple measurements for each turtle were obtained and included in our analyses. We calculated mean SCCL and SCCW values for each female per nesting season used in our analyses following the protocol of Price et al. (2004).

We calculated estimated clutch frequencies (ECFs) following Reina et al. (2002) for each individual female that was first observed nesting between October and December in each nesting season. This method assured

that we accurately estimated each turtle's total nests per season, because beach coverage is almost complete from October until and including March (Steyermark et al. 1996; Reina et al. 2002). We estimated the total eggs laid in a season per female by multiplying individual mean clutch size for an individual turtle by the ECF for that individual, and we estimated the total hatchlings produced in a season per female by multiplying individual mean number of emerged hatchlings per clutch by ECF. These calculations do not take into account possible within-season changes in intra-female reproductive output or nest environment conditions, but nonetheless are useful estimates of seasonal fecundity among individual leatherbacks.

Data analyses

We determined effects of female identity on reproductive variables using linear mixed-models (LMM) that treated female as the random effect and year (2001–2004) as the fixed effect on a given response variable (e.g., clutch size). This procedure permitted quantification of the amount of variation in a given response variable explained by variation in among-female effects, while the residual error provided by these models comprised other unobserved sources of variation, such as within-female variation, measurement error, etc. Data from three individual females (of 146 total females) were collected in more than one season, and because excluding data from these females did not have a significant effect on our analyses (data not shown), we included all data from all females in our analyses.

Next, we used LMMs that incorporated other fixed effects to account for the effects of female identity (female random effect) and nesting season (year fixed effect) while assessing the average response of a given variable (e.g., hatchling mass) to a predictor variable (e.g., egg mass) across individual females and across different nesting seasons (Nussey et al. 2005). For purposes of interpreting variation explained in a given analysis, we also report coefficients of determination from linear regressions in addition to results from the LMMs.

Covariation between female body size and reproductive variables has been well established for a wide variety of taxa (Roff 1992). However, the female random effect in our LMM design included the covariate of female size, thus accounting for variation in response variables due to variation in female body size, as well as other sources of variation within and among females. Nonetheless, we determined the magnitude of effects of female size on reproductive variables by comparing the variance components of the female random effect from two models: one with year as the fixed effect

and one which included SCCL and SCCW as additional fixed effects. Thus, we were able to identify differences in the variance components of female effects between the two models as the contribution of female body size to the overall random effect of female identity on a given response variable.

When multiple variables were used as predictors (e.g., clutch size and egg mass) of a given response variable (e.g., clutch mass), we used standardized values (z -scores) for each predictor variable. This procedure allows for more direct interpretation of slope coefficients for each predictor. Specifically, β_i represents the amount of change expected in the dependent variable given a 1 SD change in the predictor variable z_i (Zar 1999).

To standardize effects of time of year, such as changes in nest environments due to changes in climatic conditions, we included in our analyses only nests that were buried in the hatchery on or before 15 December of each season. Data are presented as means \pm 1 SD and we conducted statistical analyses using R 2.2.1 (R Development Core Team 2005).

Results

We used intra-individual means of 334 clutches (consisting of 20,108 total eggs and 7,983 total hatchlings) from 146 individual adult female leatherback turtles (45 in 2001–2002, 40 in 2002–2003, 45 in 2003–2004, and 19 in 2004–2005; two turtles in both 2002 and 2004, one in both 2001 and 2003) in analyses of relationships between egg, clutch, and hatchling characteristics among females (Table 1). Within-female clutch sizes varied by (minimum) six to (maximum) 68 eggs, whereas within-female egg mass varied by 0.8–15.8 g. Among females, mean egg mass varied by only about 9% of the population mean, whereas mean clutch size and mean ECF varied by about 26 and 31% among females, respectively (Table 1).

Egg and hatchling size varied markedly among female leatherbacks. Egg mass increased with clutch size among female leatherbacks ($r^2 = 0.016$; $t_{136} = 2.78$; $P = 0.006$) (Fig. 1a), but was not correlated with total eggs oviposited per season ($r^2 = 0.023$; $t_{123} = 1.65$; $P = 0.102$) (Fig. 1b). Hatchling mass varied among females by ~ 12 g (7% of population mean), and within females by 0.2–6.4 g (Table 1). Among females, hatchling mass ($r^2 = 0.191$; $t_{99} = 5.46$; $P < 0.001$) (Fig. 2), carapace length ($t_{98} = 2.93$; $P = 0.004$), and carapace width ($t_{99} = 2.38$; $P = 0.019$) increased with egg mass.

Maternal investment in reproduction depended more on number of eggs laid than on size of eggs. Clutch mass increased significantly with both egg mass

Table 1 Reproductive variables of a total of 334 clutches from 146 individual leatherback females nesting at Parque Nacional Marino Las Baulas, on the Pacific coast of Costa Rica, from 2001 to 2004. *SSCL* Standard curved carapace length, *SCCW* standard curved carapace width, *SAG* shelled albumen gob, *SCL* straight carapace length, *SCW* straight carapace width

Variable	Population mean (± 1 SD)	Coefficient of variation (%)	Range of individual means	Variance component ^a		Variance component ^b	
				Female	Residual	Female	Residual
SCCL (cm)	145.2 (6.4)	4.4	125.4–161.7	–	–	–	–
SCCW (cm)	104.4 (4.3)	4.1	93.3–115.6	–	–	–	–
Estimated clutch frequency	8.2 (2.5)	30.5	1–12.2	–	–	–	–
Clutch size (no. eggs per nest)	61.8 (16.3)	26.4	21–101	11.3	11.3	11.0	11.4
Total eggs laid per season	500.3 (194.9)	39.0	34–1116	–	–	–	–
Egg mass (g)	80.9 (7.0)	8.7	58.3–97.3	5.9	4.2	5.6	4.2
Clutch mass (g)	5,040.7 (1439.0)	28.5	1,516.2–9,170.8	1,099.2	885.0	1,045.5	887.6
Total SAG mass (g)	947.3 (393.8)	41.6	71.8–1,978.4	242.2	302.5	220.7	303.5
No. hatchlings emerged per nest	23.9 (16.4)	68.6	0–69	7.4	14.3	7.1	14.4
Total hatchlings produced per season	192.3 (147.3)	76.6	0–674	–	–	–	–
Emergence success (%)	41.0 (25.2)	61.0	0–100	11.1	22.3	11.4	22.2
Hatchling mass (g)	40.1 (2.7)	6.7	32.5–46.7	2.0	1.8	2.0	1.8
Hatchling SCL (mm)	56.9 (2.1)	3.7	50.9–63.4	1.6	1.4	1.6	1.4
Hatchling SCW (mm)	38.8 (1.8)	4.6	31.4–42.1	1.1	1.3	1.1	1.3

^a Results from linear mixed-effects models (LMMs) with female as the random effect are included for LMMs with fixed effect of year only

^b Results LMMs with female as the random effect are included for LMMs with fixed effects of year + SCCL + SCCW

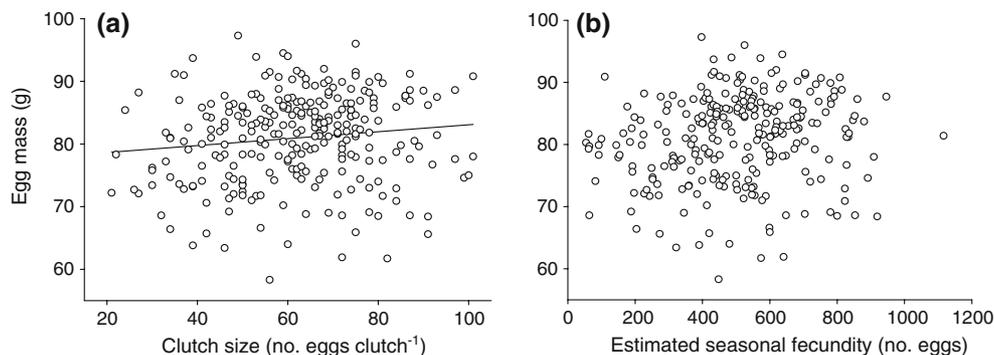


Fig. 1 Egg mass increased with **a** clutch size ($r^2 = 0.016$; $P = 0.006$) but not with **b** estimated seasonal fecundity (total eggs laid) ($r^2 = 0.023$; $P = 0.102$) among leatherback turtles (*Dermochelys coriacea*) nesting at Parque Nacional Marino Las Baulas

(PNMB), Costa Rica. Each point in **a** represents data from an individual clutch, while each point in **b** represents data from an individual female

($r^2 = 0.178$; $t_{135} = 53.69$; $P < 0.0001$) (Fig. 3a) and clutch size ($r^2 = 0.833$; $t_{135} = 158.58$; $P < 0.0001$) (Fig. 3b), but the effect of clutch size ($\beta_{\text{clutch size}} = 1290.2$) was approximately threefold larger than the effect of egg mass ($\beta_{\text{egg mass}} = 434.3$) on clutch mass. There was no relationship between total SAG mass and clutch mass ($t_{131} = 1.03$; $P = 0.306$). However, there was a significant negative relationship between total SAG mass and clutch size ($r^2 = 0.038$; $t_{131} = -2.00$; $P = 0.048$) (Fig. 4a), and a significant positive relationship between SAG mass and egg mass ($r^2 = 0.311$; $t_{136} = 11.72$; $P < 0.0001$) (Fig. 4b).

Leatherbacks that oviposited larger clutches of eggs tended to produce more hatchlings. Among females, there was no relationship between emergence success and clutch size ($t_{162} = -1.54$; $P = 0.126$) (Fig. 5a). However, there was a significant positive relationship between number of emerged hatchlings per nest and clutch size ($r^2 = 0.065$; $t_{177} = 4.78$; $P < 0.001$) (Fig. 5b). Number of emerged hatchlings per nest varied from zero to 69 hatchlings within females. Hatchling emergence success ranged from 0 to 67% among females (Table 1). Within-female variation in emergence success ranged from 9% for one female (63.0 to 72.4%) up

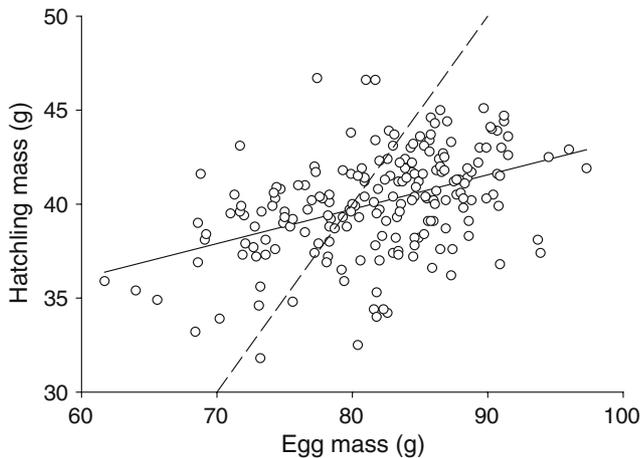


Fig. 2 Hatchling mass increased with egg mass ($r^2 = 0.191$; $P < 0.001$) among leatherbacks nesting at PNMB, Costa Rica. The *dashed line* indicates where hatchling mass varies directly with egg mass, slope = 1. *Each point* represents data from an individual clutch

to 70% for another (1.3–71.0%). One female displayed 0% emergence success over four clutches.

Leatherbacks increased seasonal fecundity and hatchling production primarily through increased clutch frequency and secondarily through increased clutch size. Among leatherback females, estimated total eggs laid per season increased with mean clutch size ($r^2 = 0.261$; $t_{137} = 30.80$; $P < 0.0001$) (Fig. 6a) and ECF ($r^2 = 0.667$; $t_{137} = 46.03$; $P < 0.0001$) (Fig. 6b). Likewise, estimated total hatchlings produced per season per individual female increased with mean clutch size ($r^2 = 0.044$; $t_{137} = 3.27$; $P = 0.0014$) (Fig. 6c) and ECF ($r^2 = 0.242$; $t_{137} = 6.25$; $P < 0.0001$) (Fig. 6d). However, the effect of ECF ($\beta_{\text{ECF}} = 165.0$) was about 1.5-fold larger than the effect of clutch size ($\beta_{\text{clutch size}} = 107.6$) on total eggs laid per season, and was almost twofold larger ($\beta_{\text{ECF}} = 61.8$; $\beta_{\text{clutch size}} = 31.5$) on seasonal hatchling production among leatherback

females. Estimated total hatchlings increased with estimated seasonal fecundity ($r^2 = 0.289$; $t_{138} = 7.35$; $P < 0.0001$). We recognize that interpretation of our analyses using clutch size and ECF as independent variables might have been complicated by an upper bound to the clutch size and ECF data (see Figs. 5b, 6a–d). However, these analyses were unlikely to have suffered appreciably from this artifact.

Estimated variance components of female random effects did not change appreciably with the addition of SCCL and SCCW to LMMs (Table 1), indicating that the effect of female body size on reproductive output variables was minimal. Thus, we did not include female size as a fixed effect in LMMs when determining the effects of other predictor variables on a given response variable because the female random effect accounted for any effect of female body size and sufficiently characterized the effects of variation due to female identity.

Discussion

Female leatherback turtles nesting at PNMB exhibited high inter- and intra-individual variation in reproductive traits such as clutch size, egg size, hatchling size, and emergence success (Table 1). Inter-female variation in clutch frequency and size was much higher than variation in egg mass in this population, supporting hypotheses of OES as well as empirical results that species producing multiple, large clutches per season should maximize the number of their eggs (via increased number and/or size of their clutches) rather than the size of their eggs (Smith and Fretwell 1974; Congdon and Gibbons 1987; Roff 1992; Tiwari and Bjørndal 2000). In addition, increased maternal investment in, first, the number, and then, the size, of clutches—without a concomitant increase in emergence success—resulted in increased total seasonal

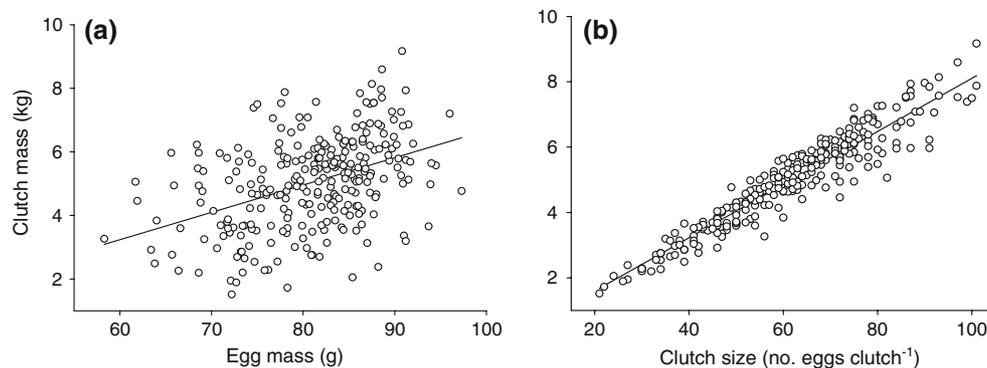


Fig. 3 Clutch mass was positively correlated with both **a** egg mass ($r^2 = 0.178$; $P < 0.0001$) and **b** clutch size ($r^2 = 0.833$; $P < 0.0001$) in leatherback turtles nesting at PNMB, Costa Rica. *Each point* represents data from an individual clutch

Fig. 4 Total shelled albumen gob (*SAG*) mass was negatively correlated to **a** clutch size ($r^2 = 0.038$; $P = 0.048$) but positively correlated to **b** egg mass ($r^2 = 0.311$; $P < 0.0001$) within clutches of leatherback turtles at PNMB, Costa Rica. Each point represents data from an individual clutch

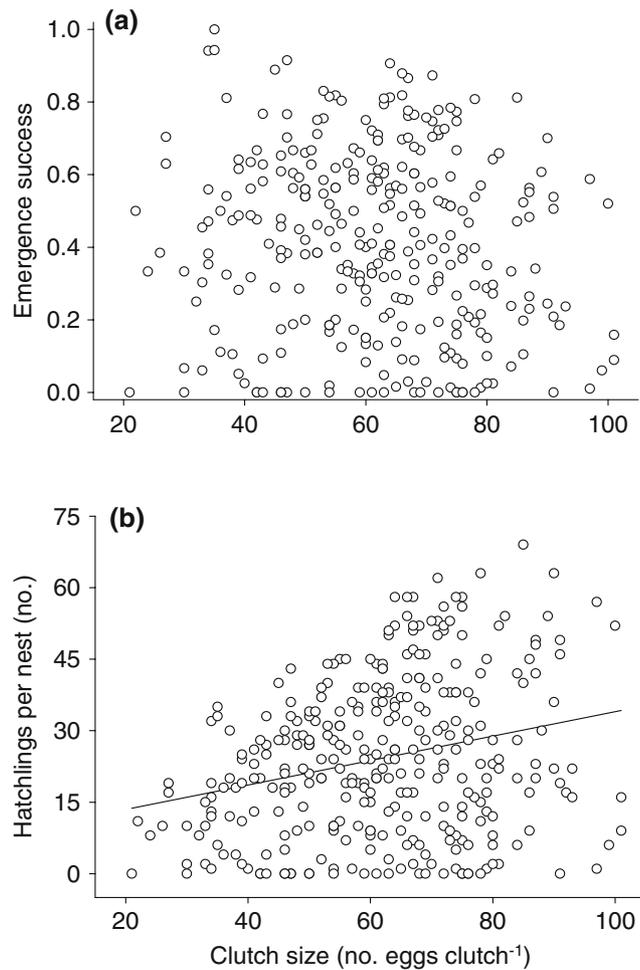
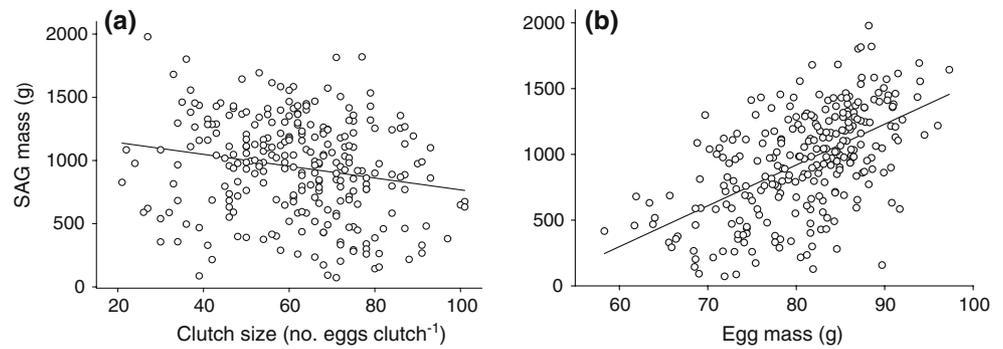


Fig. 5 **a** Emergence success (see text for definition) did not vary significantly with clutch size ($P = 0.126$) among leatherback females nesting at PNMB, Costa Rica, but **b** the total number of emerged hatchlings per nest was positively related to clutch size ($r^2 = 0.065$; $P < 0.001$). Each point represents data from an individual clutch

fecundity and hatchling production. Taken together, these results suggest that leatherbacks manifest a reproductive investment strategy characterized as “more rather than bigger eggs,” probably in response to high and unpredictable mortality at early life stages.

Egg mass showed a significantly positive, but very weak, correlation with clutch size among leatherback females (Fig. 1); clutch size explained only ~2% of the variation in egg mass. The apparent lack of a trade-off between egg size and clutch size suggests that factors governing production of hatchlings of sufficient size and vigor somehow regulate egg size, such that females produce as many eggs, of a sufficient size, as possible, given available resources during egg production. However, clutch size varied more among females than egg mass and hatchling mass (Table 1), as expected for species with large, multiple clutches per reproductive season (Smith and Fretwell 1974; Congdon and Gibbons 1987) such as sea turtles (Hays and Speakman 1991; Tiwari and Bjorndal 2000). Thus, our results indicate that leatherbacks maximize the number, and not necessarily the size, of eggs and neonates.

Leatherback hatchling mass increased with egg mass (Fig. 2), providing further support for the egg mass–neonate mass relationship in turtles (Van Buskirk and Crowder 1994; Finkler and Claussen 1997; Steyermark and Spotila 2001) and a diverse range of other oviparous taxa (see Roff 1992 for review). Increased egg size can confer various advantages to larger neonates related to growth, predator avoidance, and developmental time (Roff 1992). In contrast, Congdon et al. (1993) found no support for the “bigger is better” hypothesis in freshwater turtles. For species that experience high and unpredictable hatchling and juvenile mortality rates, increased neonate size results in decreased fecundity, and the advantages conferred to large neonates are superseded by selection for increased hatchling production (Roff 1992). In the present study, hatchling mass increased only 2 g for each 10-g increase in egg mass. Analyses of egg components from the PNMB leatherback population have demonstrated that variation in leatherback egg mass is chiefly due to variation in albumen mass, and that because hatchling mass is up to 100% greater than yolk mass at a given egg mass, albumen likely contributes significant proportions of water and solids to hatchling

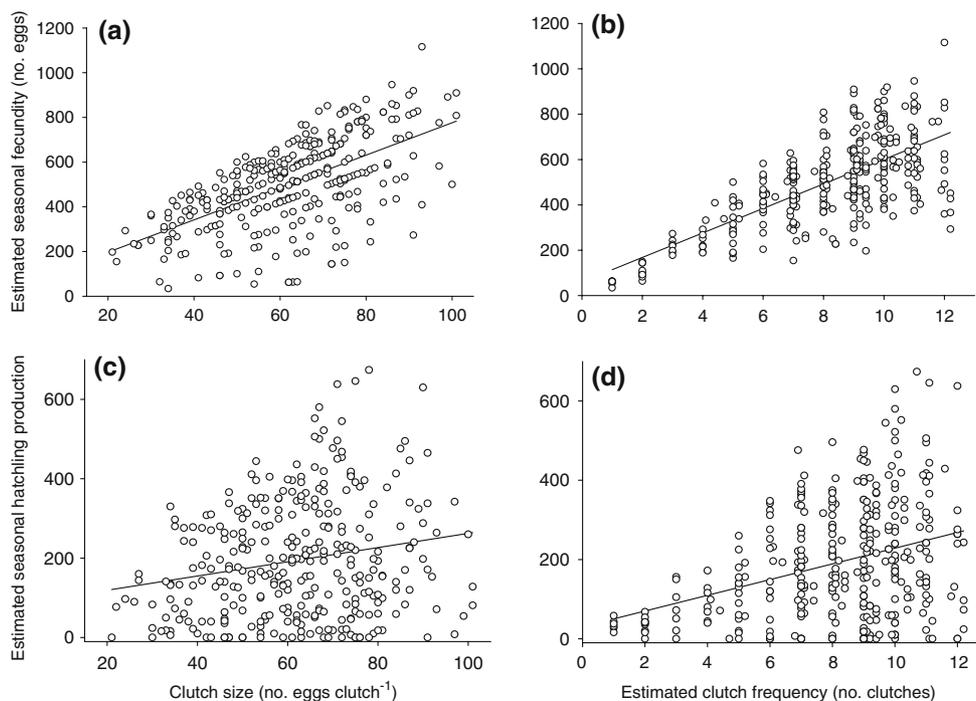


Fig. 6 Estimated seasonal fecundity (total eggs laid) increased with **a** mean clutch size ($r^2 = 0.261$; $P < 0.0001$) and **b** estimated clutch frequency ($r^2 = 0.667$; $P < 0.0001$) among leatherback females at PNMB, Costa Rica. Likewise, estimated seasonal hatch-

ling production increased with **c** mean clutch size ($r^2 = 0.044$; $P = 0.0014$) and **d** ECF ($r^2 = 0.242$; $P < 0.0001$). Each point represents data from an individual female

development (Wallace et al. 2006a). While the influence of egg components on embryonic development in leatherbacks merits further research, considering the large fraction of albumen present in leatherback eggs, it is not surprising that hatchling mass did not increase in direct proportion to egg mass in this study (Fig. 2).

Clutch size had a threefold higher effect than egg mass on clutch mass (Fig. 3). This is not surprising given that clutch size varied much more [coefficient of variation (CV) = 26%] than egg mass (CV = 9%) among females (Table 1). These results support the prediction that species producing many offspring per reproductive bout should manifest minimal variation in egg size and variation in total clutch mass should be determined by clutch size rather than by egg size (Smith and Fretwell 1974; Congdon and Gibbons 1987; Dunham et al. 1989).

Total SAG mass varied in opposite ways with clutch size (negatively) and egg mass (positively) (Fig. 4). Whether SAGs serve a function in leatherback nests remains undetermined (Frazier and Salas 1984; Wallace et al. 2004; Caut et al. 2006), but given the correlations we found between total SAG mass, clutch size, and egg mass, we hypothesize that SAGs are simply “production over-runs,” where the amount of albumen and shell material available for a given clutch of ova (yolks) exceeds the quantity of yolks available.

Accordingly, larger clutches, with more yolks available, have more of the albumen and shell material allocated to eggs, with less remaining for SAGs. On the other hand, because variation in egg mass is attributable mainly to variation in albumen mass (Wallace et al. 2006a), oviducts of female leatherbacks that tend to produce large eggs might be more likely to have surplus albumen available to produce a greater mass of SAGs. In this vein, SAGs might be a manifestation of selection on leatherback reproductive physiology for enhanced albumen production and allocation to individual eggs. The underlying assumption of this hypothesis is that benefits of albumen allocated to individual eggs outweigh the costs of resources deposited in seemingly functionless SAGs when “surplus” albumen is allocated to a clutch. Clearly, detailed investigations of leatherback egg production and developmental physiology are necessary to test the “production over-run” hypothesis.

Emergence success varied widely within and among leatherback females in this study (Table 1; Fig. 5a). Hatching (and emergence) success of leatherback clutches is about 50% across populations (Van Buskirk and Crowder 1994; Bell et al. 2003), but the causes of high embryonic mortality are unknown (Bell et al. 2003). Whereas there was no relationship between emergence success and clutch size (probably due to

high variation in emergence success), increased clutch size resulted in increased number of emerged hatchlings per nest (Fig. 5b). Furthermore, increased seasonal fecundity and seasonal hatchling production among females resulted from increases in both the frequency and size of clutches, with the effect of ECF approximately 1.5-fold larger than the effect of mean clutch size on seasonal fecundity and about twofold larger on seasonal hatchling production (Fig. 5). Not surprisingly, total hatchlings produced increased with seasonal fecundity. Our results demonstrate that leatherbacks increase seasonal reproductive output primarily by increasing the number, and secondarily the size, of their clutches.

Despite wide support for the well-established female size-clutch size/egg size relationships across diverse taxonomic lines (Congdon and Gibbons 1985; Hays and Speakman 1991; Roff 1992; Van Buskirk and Crowder 1994), female body size (as measured by SCCL and SCCW) accounted for little to none of the variation of the overall female random effect on variation in reproductive traits in leatherbacks at PNMB (Table 1). Likewise, female body size explained little variation in clutch size in green sea turtles (*Chelonia mydas*) (Bjorndal and Carr 1989). Other features of female body size (e.g., body volume, body mass) might have greater effects on reproductive variables in leatherbacks. Nonetheless, our results indicate that factors other than SCCL and SCCW, account for the variation in reproductive traits among females.

Phenotypic variation in reproductive investment and allocation patterns among individuals within populations and species can occur because of factors including physical constraints of body size (Congdon and Gibbons 1985; Shine 1992; Olsson and Shine 1997), spatio-temporal environmental variation (e.g., fluctuating resource availability) (Jordan and Snell 2002; Roff 2002), and genotypic variation in reproductive traits (Roff 1992; 2002). Female body size explained extremely little (if any) of the total variation in reproductive variables associated with female identity (Table 1), so physical constraints (e.g., available body cavity space) do not appear to influence patterns of maternal investment in reproduction in this leatherback population. Differential resource availability has been implicated in morphometric and reproductive output differences between leatherback populations, and presumably could explain phenotypic variation in reproductive output within this population if leatherback females nesting at PNMB acquired resources for reproduction from foraging grounds of different quality (Wallace et al. 2006b). Thorough analysis of this hypothesis is warranted.

In temporally variable environments, genetic variation in reproductive traits could be maintained if such variation allowed for different phenotypic reproductive investment patterns that were associated with relative fitness benefits depending on environmental circumstances (Reznick et al. 2000; Roff 2002). Therefore, the large phenotypic variation that we observed in several reproductive traits might reflect different patterns of genotype–environment interactions among individual leatherbacks (Reznick et al. 2000; Roff 2002). To investigate this hypothesis, it would be necessary to assess quantitatively the effects of genotypic variation and their interaction with environmental variability on variation of reproductive trait phenotypes in the population. Unfortunately, because the ideal situation in which to estimate effects of genetic and environmental variation on life history phenotypes typically is a controlled system where genotypes and environments are known and changes in such a system are followed through time (e.g., clonal lines in a laboratory setting) (Spitze 1991; Spitze et al. 1991; Reznick et al. 2000), this prospect would be prohibitively difficult in free-ranging, sexually reproducing leatherbacks. In any case, we speculate that it is more likely that the variation in reproductive traits that we observed among leatherback females during a given reproductive bout reflects variable proximate responses of individual turtles to environmental stochasticity, specifically resource availability (Olsson and Shine 1997; Jordan and Snell 2002; Roff 2002; Wallace et al. 2006b). Empirically linking spatio-temporal environmental fluctuations and proxies of resource availability with reproductive frequency is necessary to address this issue (Saba et al., *in press*). Furthermore, longitudinal analyses of intra-individual reproductive investment (e.g., emergence success, egg and hatchling production) will be useful to distinguish among more or less consistently “successful” or “productive” individual leatherbacks, and to elucidate patterns of reproductive investment within and among individuals as possible responses to variability in environmental conditions and/or in survival during early life stages.

Leatherback females in this population enhanced seasonal fecundity and hatchling production through increased maternal investment in both number and size of clutches, but without a concomitant increase in emergence success of clutches. Moreover, we found high variation in clutch size and emergence success of clutches, but less variation in egg and hatchling size among females. Thus, our results illustrate a “more eggs rather than bigger eggs” maternal investment strategy in leatherbacks, which might reflect compensation for low emergence success of clutches and

presumably high and unpredictable mortality rates of early life history stages with the highest absolute reproductive output [i.e., several large clutches per season, at relatively frequent remigration intervals (~2–4 years)] of any reptile (Mrosovsky 1983; Van Buskirk and Crowder 1994; Miller 1997; Reina et al. 2002). Tracking these traits within individual leatherback females across nesting seasons would provide insight into whether particular strategies of resource allocation exist among and vary within individuals according to differences in reproductive histories and experience.

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