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Reproductive Investment Compromises Maternal Health in Three Species of Freshwater Turtle

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ABSTRACT

Life-history theory predicts that a trade-off in the allocation of resources between different physiological systems exists because resources are finite. As a result, females investing heavily in reproduction may compromise their future health. We used hematology, serum biochemistry, mass, and morphometric measurements as indicators of physiological health state to investigate whether reproductive investment altered subsequent maternal health in three Australian freshwater turtles: the oblong turtle (*Chelodina oblonga*; $n = 12$), the Macquarie turtle (*Emydura macquarii*; $n = 9$), and the eastern long-necked turtle (*Chelodina longicollis*; $n = 8$). Maternal health was impaired in turtles that produced larger and heavier eggs and clutches. In *C. oblonga* and *E. macquarii*, increased reproductive investment generally resulted in negative changes to the hematology and serum biochemistry profile of maternal blood. Generally, increases in heterophil/lymphocyte ratio, aspartate transaminase, creatine kinase, calcium/phosphorus ratio, and albumin/globulin ratio were observed following reproduction, in addition to a decrease in glucose and total protein. These findings agree with the physiological constraint hypothesis and highlight the connection between life-history evolution and animal physiology by documenting, for the first time, how measures of physiological health state relate to reproductive investment in Australian freshwater turtles. Additionally, our findings suggest that body condition, a readily used morphological biomarker, is a poor predictor of health in turtles. Our results emphasize the need to investigate how maternal health is influenced by the reproductive process in different species.

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Introduction

Reproduction is an energetically expensive process that involves the reallocation of resources that could otherwise be used by competing hormonal, metabolic, and immune physiological systems (Harshman et al. 2007). A trade-off between reproduction and the maintenance of good health may arise as a result and has been documented in several vertebrate species (Uller et al. 2006; Wagner et al. 2008; Norte et al. 2010). Accurate estimations of the physiological condition or health state of an animal can be made by assessing the hematology and plasma or serum biochemistry of the individual, and such evaluations have proven useful tools in ecological studies (Artacho et al. 2007*a*, 2007*b*).

Measures of hematology and biochemistry, along with body mass and morphometrics, are readily applied in avian research and have been used to demonstrate that maternal health is linked to reproductive effort and breeding success in the Nazca booby (*Sula granti*; Apanius et al. 2008), burrowing parrots (*Cyanoliseus patagonus*; Masello et al. 2004), great tits (*Parus major*; Ots et al. 1998; Norte et al. 2010), and Magellanic penguins (*Sphenicus magellanicus*; Moreno et al. 2002). In contrast, although hematology and biochemistry reference ranges for reptiles exist, the literature offers little interpretation beyond establishing these ranges (Dessauer 1970; Stein 1996; Campbell 2006), and the influence that reproduction has on maternal health remains relatively unknown (Perrault et al. 2012). Further, although body condition indices derived from mass and morphometric measurements are widely used to describe the health of an animal, previous research suggests that such indices are poorly correlated with hematological and biochemical measures of health in turtles (Scheelings et al. 2012). Understanding the relationship among these factors is important because maternal reproductive investment by oviparous reptiles is generally restricted to preovipositional allocations to egg size and number, because females do not usually provide parental care after they lay their eggs (Wallace et al. 2007). Therefore, offspring investment in these species is more closely related to the provision of materials and energy toward egg production and retention, contrary to the postovipositional investment provided by avian species (Cockburn 2006).

We used three Australian freshwater species to (1) assess how female physiological health state (referred to hereafter as ma-

ternal health) related to reproductive effort and body condition and (2) examine the relationship between reproductive effort and body condition. We hypothesized that increased reproductive effort would result in measurably compromised maternal health and body condition. To investigate this, we assessed the following physiological and morphometric measures in addition to mass.

Hematology

Hematology is the study of detecting conditions that affect blood (Campbell 2006). Reptilian blood consists of red (erythrocytes) and white (leucocytes) blood cells, and during this study we assessed several measures of both cell types as indicators of maternal health. Total erythrocyte count and hematocrit (the relative volume of red blood cells in total blood volume) reflect the oxygen carrying capacity of the blood. A reduction in both variables is called anemia, which is commonly associated with iron deficiency (Dein 1986; Campbell 1995). Additionally, total leucocyte count (total white cell count [TWCC]) identifies the immune system status and presence of infection or inflammation (Campbell 1995). The primary function of leucocytes is to protect against various pathogenic antigens, and a relative increase in leucocyte concentration is often seen during an immune response (Dein 1986). Heterophils and lymphocytes are the two most abundant white blood cell types in turtles, and the heterophil/lymphocyte ratio (H/L ratio) is readily used as an indicator of stress generally in response to physiological perturbation, limited food availability, and infectious disease (Gross et al. 1983; Maxwell 1993; Stamper et al. 2005). An increase in this ratio is known to occur in individuals exposed to these stressors (Ots et al. 1998). Finally, parasitemia, which is the parasitic infection of blood cells, has also been correlated with reduced reproductive output in female reptiles (Schall 1982; Madsen et al. 2005).

Plasma or Serum Biochemistry

The physiological state of an animal is also reflected in the metabolites present in the blood (Artacho et al. 2007*a*). Metabolites—including total protein and glucose—are used to assess the nutritional state of an individual and generally decrease during periods of starvation or fasting (Ots et al. 1998; Artacho et al. 2007*a*). Furthermore, albumin and globulin are key proteins involved in immune system function, and assessment of the albumin/globulin (A/G) ratio is a useful tool for determining the health status of an animal. Typically, unhealthy individuals will exhibit a lower A/G ratio than healthy individuals (Kawai 1973). Important protein-binding minerals—calcium and phosphorus—are also essential for normal bodily function, and the relative abundance of calcium to phosphorus (calcium/phosphorous [Ca/P] ratio) can have significant effects on egg production and offspring survival in the greater sage grouse (*Centrocercus urophasianus*; Dunbar et al. 2005) and hatching success in the leatherback turtle (*Dermochelys coriacea*; Perrault et al. 2012). Although Ca/P may vary among species, possibly in relation to diet, a balance in both calcium and phosphorous levels is needed (Dunbar et al. 2005; Campbell 2006). To complement plasma or serum protein investigations, an evaluation of the concentration of plasma or serum enzymes—including aspartate transaminase (AST) and creatine kinase (CK)—is used to predict and help differentiate between liver and muscle damage, respectively (Harr 2002). Elevated concentrations of both enzymes are characteristically observed in unhealthy or traumatized individuals.

Mass and Morphology

Body mass and structural size (measured as carapace length) are easily measured in turtles, and a large variation in both variables can be observed at the intraspecific level, resulting from an individual's phenotypic plasticity in response to their surrounding environment (Rowe 1997). The large variation in individual body mass makes this variable difficult to interpret, and for this reason many studies prefer to look at body condition. Body condition is typically calculated from the linear

Table 1: Results of the principle component analysis showing the eigenvalues and individual and cumulative (%) contribution of each principal component for each species

Principal component	Eigenvalue	Individual (%)	Cumulative (%)
Emydura macquarii:			
1	**1.98**	**43.73**	**43.73**
2	**1.53**	**25.93**	**69.65**
3	**1.05**	**12.22**	**81.87**
4	.85	7.97	89.84
5	.75	6.31	96.16
6	.48	2.53	98.69
7	.32	1.16	99.85
8	.12	.15	100.00
Chelodina oblonga:			
1	**1.84**	**33.93**	**33.93**
2	**1.36**	**18.56**	**52.49**
3	**1.28**	**16.32**	**68.81**
4	**1.17**	**13.73**	**82.54**
5	.77	5.96	88.54
6	.75	5.56	94.10
7	.47	2.21	96.30
8	.43	1.86	98.16
9	.37	1.40	99.56
10	.21	.44	100.00
Chelodina longicollis:			
1	**1.74**	**30.34**	**30.34**
2	**1.46**	**21.21**	**51.55**
3	**1.31**	**17.23**	**68.78**
4	**1.17**	**13.66**	**82.44**
5	.97	9.60	92.05
6	.77	5.95	97.99
7	.45	2.00	100.00

Note. Eigenvalues >1 are in bold.

Table 2: Principal component (PC) coefficients, loadings, and final communalities for *Emydura macquarii*

	Standardized coefficients			Loadings after varimax rotation			
Variable	PC1	PC2	PC3	PC1	PC2	PC3	Communality
Hematocrit	.47			**.64**	.47	.48	.86
Aspartate transaminase	.41			**.67**		.47	.69
Creatine kinase		.39	−.40	**.84**	−.33		.84
Calcium/phosphorous ratio	.40		−.48	**.92**			.90
Glucose		−.57			**.88**		.85
Total protein		−.53			**.96**		.95
Albumin/globulin ratio	−.33			−.44	**−.59**		.57
Total leucocyte count		−.39	−.39		.30	**−.76**	.74
Heterophil/lymphocyte ratio	.37		.61			**.94**	.96

Note. Values <0.3 are omitted from the matrix. Values in bold represent the highest existing correlation between variables and the selected components.

regression of body mass against carapace length, and by expressing body condition as a residual, it separates body mass from carapace length. Although using ordinary least squares residuals is likely to result in some loss of accuracy, it is a preferred method because of simple calculation and interpretation (Peig et al. 2010). It is plausible to expect a turtle with a similar carapace length to another—yet with greater mass—to have more resources available for reproduction. Body condition and carapace length have previously been linked to reproductive investment in turtles (Wilkinson et al. 2005; Rasmussen et al. 2010).

Material and Methods

Study Species

Data were collected from three 3 species of freshwater turtle; the western oblong turtle (*Chelodina oblonga*, Gray, 1841; $n = 12$), the Macquarie turtle (*Emydura macquarii*, Gray, 1830; $n = 9$), and the eastern longneck turtle (*Chelodina longicollis*, Shaw, 1794; $n = 8$). *Chelodina oblonga* were trapped between October 1 and October 7, 2010, from Lake Goolelall in Western Australia, using baited, modified funnel traps, while *C. longicollis* and *E. macquarii* were trapped between October 10 and December 15, 2010, from Lake Coranderrk, Victoria, using baited fyke nets. Upon collection, the inguinal fossa of each female was manually palpated to determine if gravid. Females thought to be gravid were later radiographed to confirm presence of calcified eggs. Body mass of the sampled females postoviposition was obtained using an electronic balance (± 0.1 g), and the midline curved carapace length was also recorded (± 0.01 cm).

Reproductive Patterns

Ovulation, calcification, and retention of eggs can extend for several months in freshwater turtles. Therefore, we considered the physiological blood parameters observed at blood sampling just before oviposition to represent the health state of females after investing in the reproductive process. *Chelodina oblonga* mate during late winter and spring, laying multiple clutches between September and January (Kuchling 1988, 1989), whereas *E. macquarii* generally mate between March and April (Cann 1998), laying a single clutch between October and December (Chessman 1986; Spencer 2001). *Chelodina longicollis* typically mate in September and lay up to three clutches between October and January (Kennett et al. 2009).

Hematology and Serum Biochemistry

Methods for blood analyses follow previous published work (Scheelings et al. 2012). Once at the laboratory, females were manually restrained, and 1 mL of blood was collected from the external jugular vein of each animal, using a 22-gauge needle attached to a 3-mL syringe. Blood was immediately transferred to a plain tube (BD Microtainer Tubes, Vacutainer Systems, Franklin Lakes, NJ) for centrifugation, and the resulting serum was analyzed using the avian-reptilian rotar on the Vet Scan analyzer (Abaxis, Union City, CA). Hematocrit was determined manually after standard centrifugation in microhematocrit tubes (Iris Sample Processing, Westwood, MA).

Blood smears were air-dried and stained with Romanowsky stain (Rapid Diff, Australian Biostain, Traralgon, Victoria, Australia) before being examined to identify and assess the prevalence of hemoparasites within erythrocytes. This was determined by counting 1,000 erythrocytes under $\times 1{,}000$ magnification with oil immersion and identifying how many were infected. We considered the intensity of blood parasite infection as an index of parasitemia for each individual. Hemoparasites were not detected in *E. macquarii*, so this variable was disregarded during statistical investigations for this species.

TWCC counts were performed manually, using a hemocytometer. Differential leucocyte counts were obtained by examining 100 leucocytes under oil immersion. Heterophils and lymphocytes were present in the largest quantities and are the

Table 3: Principal component (PC) coefficients, loadings, and final communalities for *Chelodina oblonga*

	Standardized coefficients				Loadings after varimax rotation				
Variable	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	Communality
Total leucocyte count	−.46				**.88**				.85
Hemoparasites	.41			.32	**−.52**			.66	.76
Aspartate transaminase	−.41		−.33		**.84**		.30		.82
Creatine kinase	−.38				**.66**	−.30			.61
Glucose				−.55	**−.63**	−.58			.81
Calcium/phosphorous ratio	−.44				**.68**	.31		−.46	.84
Total protein		.54	.45			**.89**			.87
Hematocrit		.63				.62	**.69**		.87
Albumin/globulin ratio			−.73				**.94**		.93
Heterophil/lymphocyte ratio				.69				**.94**	.88

Note. Values <0.3 are omitted from the matrix. Values in bold represent the highest existing correlation between variables and the selected components.

only immune cells included in this study. The relative abundance of both heterophils and lymphocytes was analyzed using the H/L ratio.

Reproductive Effort

After blood collection, female *C. longicollis* were placed in individual 68-L (60 cm × 38 cm × 40 cm) containers and female *C. oblonga* and *E. macquarii* in individual 150-L (92 cm × 41.5 cm × 32.5 cm) containers so that each female was floating in enough water (28°C) to cover the shell. After allowing 1 h for the turtles to acclimatize to their surroundings, they were given an intramuscular injection of synthetic oxytocin (Butocin, Bumac, New South Wales, Australia) at a dose of 15 international units/kg (Ewert et al. 1978). Successful induction of oviposition generally occurred within 20 min. As eggs were laid, they were immediately patted dry with a paper towel and weighed using an electronic balance (±0.1 g), and length and width were measured using a digital calliper (±0.01 mm).

The following variables were considered to represent the reproductive effort of each female: mean egg mass, mean egg length, mean egg width, mean egg volume, clutch size, and clutch mass. Egg volume was calculated using the formula for an ellipsoid, $V = (\pi/6{,}000) \times LW^2$, where L is egg length and W is egg width.

Statistical Analyses

Statistical analyses were conducted using the R statistical package (ver. 3.0.0; R Development Core Team 2013) and PRIMER (Clarke et al. 2006). Hematology and serum biochemistry variables were normalized before conducting principal component analysis (PCA), a multivariate technique often used to reduce the dimension of a complex data set consisting of interrelated variables (Sharma 1996). PCA yielded several uncorrelated ordered variables known as principle components (PCs; Abdi et al. 2010). PCs with eigenvalues greater than 1 (eigenvalue-one criterion) were considered to explain the greatest amount of variance and were retained for further investigation. Varimax rotation was then applied to the remaining PCs, and the resulting factor loadings were used to determine the contribution of each variable to each PC. A variable was believed to contribute to a PC if its loading was highest for that PC.

Multivariate multiple linear regression analysis was then used to investigate whether (1) maternal health (PCs) was related to reproductive effort or body condition and (2) reproductive effort was related to body condition. Female body condition was calculated from the linear regression of body mass on carapace length, and the resulting residual values represent both above (positive residual value) and below (negative residual value) expected body condition for each female. Further, all reproductive effort variables were log transformed before analysis. To determine the first point above, the measures of either reproductive effort or body condition were regressed on the PCs. To assess the second point above, the measures of reproductive effort were regressed on body condition. Statistical significance was accepted if $P \leq 0.05$, and the correlation coefficient (η^2) is reported for significant relationships.

Ethical Approval

Research was conducted under scientific permits issued by the Victorian Department of Sustainability and the Environment (10005293) and the Western Australian Department of Environment and Conservation (SF007435, CE002893). It was approved by the Biological Sciences Animal Ethics Committee of Monash University (BSCI/2009/28).

Results

Principle Component Analysis

The eigenvalues resulting from the PCA for each species are shown in table 1. Using the eigenvalue-one criterion, three (PC1–PC3), four (PC1–PC4), and four (PC1–PC4) PCs were selected for further analyses for *Emydura macquarii*, *Chelodina oblonga*, and *Chelodina longicollis*, respectively. Collectively, the

Table 4: Principal component (PC) coefficients, loadings, and final communalities for *Chelodina longicollis*

	Standardized coefficients				Loadings after varimax rotation				
Variable	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	Communality
Total leucocyte count		−.54		−.31	**−.61**	.46		−.38	.79
Heterophil/lymphocyte ratio	.38			.38	**.87**				.80
Aspartate transaminase	−.49				**−.62**			.51	.76
Glucose	.39				**.76**				.62
Hematocrit			−.55		.42	**.72**		−.45	.90
Total protein		−.47	−.92	.44		**.83**			.80
Hemoparasites	−.38	.45					**.80**	.48	.94
Calcium/phosphorous ratio		.35	−.52				**.88**	−.30	.88
Albumin/globulin ratio	.40		.48		.37	−.55	**−.68**		.93
Creatine kinase				.59				**.91**	.83

Note. Values <0.3 are omitted from the matrix. Values in bold represent the highest existing correlation between variables and the selected components.

selected PCs explained 81.87%, 82.54%, and 82.44% of the variation in the hematological and biochemical variables for each of the aforementioned species, respectively.

In tables 2–4, the factor loadings highlighted in bold represent the highest correlation between the hematological and biochemical variables and the selected PCs (higher loading scores represent a greater contribution to the PC). For *E. macquarii*, hematocrit, AST, CK, and Ca/P ratio had the highest correlation with PC1; glucose, total protein, and A/G ratio correlated with PC2; and TWCC and H/L ratio correlated with PC3 (table 2). For *C. oblonga*, TWCC, hemoparasites, AST, CK, glucose, and Ca/P ratio correlated with PC1; total protein with PC2; hematocrit and A/G ratio with PC3; and H/L ratio with PC4 (table 3). For *C. longicollis*, TWCC, H/L ratio, AST, and glucose correlated with PC1; hematocrit and total protein with PC2; hemoparasites, Ca/P ratio, and A/G ratio with PC3; and CK with PC4 (table 4).

Multivariate Multiple Linear Regression Analysis

There was no significant relationship between body condition and the selected PCs or the measures of reproductive effort for the three species. However, there was a significant relationship between both PC1 and PC2 and *E. macquarii* total clutch mass ($\eta^2 = 0.41$ and $\eta^2 = 0.41$, respectively) and size ($\eta^2 = 0.38$ and $\eta^2 = 0.41$, respectively; tables 5, 6). Hematocrit, AST, CK, Ca/P, and A/G ratio were all positively related to total clutch mass and size, whereas glucose and total protein were negatively correlated with both variables. There was also a significant relationship between PC3 and *C. oblonga* mean egg mass ($\eta^2 = 0.73$), length (PC3 $\eta^2 = 0.39$), width ($\eta^2 = 0.72$), volume ($\eta^2 = 0.66$), and clutch mass ($\eta^2 = 0.49$; tables 5, 6). Further, PC4 was significantly related to *C. oblonga* egg length ($\eta^2 = 0.10$; tables 5, 6). All aforementioned measures of reproductive effort were positively related to hematocrit, A/G, and H/L ratios.

Discussion

Life-history theory predicts that because resources are finite, there is a trade-off in the allocation of these resources between different physiological systems (Roff 1992). As a result, investment in reproduction may compromise future performance (Gustafsson et al. 1994). Our results support this concept by showing that maternal health is impaired in turtles following production of larger and heavier eggs and clutches. Although different patterns were observed among species in this study,

Table 5: Significant multivariate multiple linear regression analyses results based on measures of reproductive effort and principle components (PCs) of blood variables for each species

Variable and significant PC	Estimates	SE	*T*	*P*	η^2
Emydura macquarii:					
Clutch size:					
PC1	.03	.01	.97	.03	.38
PC2	−.04	.01	−3.11	.02	.41
Clutch mass:					
PC1	.04	.01	3.68	.01	.41
PC2	−.05	.01	−3.68	.01	.41
Chelodina oblonga:					
Clutch mass:					
PC3	.07	.03	2.67	.03	.49
Egg mass:					
PC3	.03	.01	6.28	<.01	.73
Egg length:					
PC3	.01	<.01	3.54	<.01	.39
PC4	.01	<.01	2.50	.04	.10
Egg width:					
PC3	.01	<.01	4.67	<.01	.72
Egg volume:					
PC3	.04	.01	5.16	<.01	.66

Table 6: Female morphometrics and measures of reproductive effort

Measurement	*n*	Mean ± SE	Median	Range
Chelodina oblonga:				
Female body mass (kg)	12	1.63 ± .03	1.63	.86–2.14
Female carapace length (cm)	12	25.45 ± .18	25.00	21.90–28.50
Clutch size	12	13.33 ± .23	13.00	7.00–18.00
Clutch mass (g)	12	137.1 ± 10.14	134.08	58.46–184.85
Egg mass (g)	138	10.78 ± .09	10.77	7.91–13.02
Egg length (mm)	138	34.10 ± .13	34.40	29.70–38.10
Egg width (mm)	138	22.74 ± .08	22.80	19.10–25.00
Egg volume (cm^3)	138	9.23 ± .28	9.30	7.93–10.89
Emydura macquarii:				
Female body mass (kg)	9	2.68 ± .04	2.64	1.94–3.20
Female carapace length (cm)	9	30.73 ± .25	29.10	26.20–34.90
Clutch size	9	22.80 ± .39	23.00	15.00–30.00
Clutch mass (g)	9	208.86 ± 20.41	194.40	137.28–332.70
Egg mass (g)	167	9.86 ± .10	10.05	6.64–12.12
Egg length (mm)	167	35.43 ± .21	35.40	28.60–42.90
Egg width (mm)	167	21.46 ± .09	21.30	19.20–23.90
Egg volume (cm^3)	167	8.55 ± .35	8.33	7.01–10.09
Chelodina longicollis:				
Female body mass (kg)	8	.92 ± .04	.79	.54–1.64
Female carapace length (cm)	8	21.68 ± .37	20.50	18.60–27.80
Clutch size	8	12.30 ± .52	11.00	6.00–18.00
Clutch mass (g)	8	72.28 ± 15.92	54.07	42.42–175.86
Egg mass (g)	80	6.82 ± .18	6.02	5.17–10.26
Egg length (mm)	80	29.75 ± .18	29.60	26.10–32.70
Egg width (mm)	80	18.98 ± .21	18.35	15.80–23.30
Egg volume (cm^3)	80	5.57 ± .41	5.14	4.52–7.92

increased reproductive investment generally resulted in negative changes to the hematology and serum biochemistry profile of maternal blood.

Hematocrit is a measure of the efficiency of oxygen transport in the blood, and a decrease in hematocrit is observed in anemic individuals (Wagner et al. 2008), which can be caused by malnutrition (George 1997). A decrease in hematocrit is regularly observed in female birds during clutch production, suggesting that investing energy in producing eggs compromises the blood-oxygen carrying capacity of the animal and potentially affects future reproductive events (Williams et al. 2004; Wagner et al. 2008). However, unlike birds, hematocrit of *Emydura macquarii* was positively correlated with clutch size and mass and was positively correlated with clutch size and egg mass, length, width, and volume in *Chelodina oblonga* in our study. These findings suggest that healthier female turtles with increased hematocrit levels are capable of producing bigger and heavier clutches consisting of larger eggs without consequently having a negative impact on their hematocrit levels. However, the use of hematocrit as an indicator of maternal health has been questioned because of the instability of this measure in response to age, parasitism, nutritional status and energy expenditure (Fair et al. 2007). Nevertheless, further investigation is needed to verify why clutch production might have opposing effects on hematocrit levels in avian and reptilian groups.

The H/L ratio is a primary indicator of immune system function, and elevated measures suggest possible inflammation due to infection (Diethelm et al. 2006; Van Rijn et al. 2010) or elevated hypothalamus-pituitary-adrenal axis–mediated stress (Davis et al. 2008). In this study, following the production of clutches with longer eggs, female *C. oblonga* had elevated H/L ratios. An increased H/L ratio is often seen in female great tits during the reproductive process (Ots et al. 1998), and females typically have higher H/L ratios than males, presumably related to the stress of brood rearing and their higher level of parental investment (Kilgas et al. 2006; Norte et al. 2010). Furthermore, immune system function is also negatively correlated with reproductive investment in female Mallee dragons (*Ctenophorous fordi*; Uller et al. 2006), further supporting our findings.

Plasma proteins play a role in transport and immune system function and are extremely important indicators of health and nutritional state (Ots et al. 1998; Artacho et al. 2007*a*). Albumin and globulin are the two primary components of plasma protein, and as well as acting as a metabolite carrier, albumin is an amino acid pool for protein synthesis and may also perform

as an energy source. Globulin, on the other hand, functions in assisting individuals to withstand the period immediately following injury (Ots et al. 1998). A decrease in total protein over time is termed "hypoproteinemia" and is associated with many diseases, although it is most prevalent during instances of malnutrition (Ots et al. 1998). In our study, decreased total protein concentrations were considered an indication of poorer health and were evident in *E. macquarii* females that had produced larger and heavier clutches. Additionally, a higher A/G ratio was also associated with these latter variables in *E. macquarii* as well as clutch size and egg mass, length, width, and volume in *C. oblonga*. Although higher A/G ratios are typically seen in healthier individuals (Kawai 1973), they also indicate the underproduction of globulin that may arise from excess production of glucocorticoid. Glucocorticoid—or stress hormone—secretion is one of the most common responses during stress (Johnstone et al. 2012) and has been linked to reduced reproductive investment (Bonier et al. 2009). Therefore, it is plausible that the A/G ratio increased because of a stress-related decrease in globulin generation during reproduction, although this is speculative.

Glucose is the principle metabolite resulting from carbohydrate metabolism, occurring as a direct result of food intake or from the metabolism of glycogen stores (Artacho et al. 2007*a*). Decreased glucose concentration was observed in female *E. macquarii* following increased reproductive effort, presumably because it became depleted as a result of producing larger and heavier clutches. Maintenance of adequate glucose concentrations in blood plasma or serum is pivotal for the maintenance of nervous system function in birds (Rodriguez et al. 2005), and future research should identify how decreased glucose concentrations during reproduction impact the maternal nervous system in turtles.

An increase in AST and CK plasma enzyme concentrations can be indicative of cellular damage (Totzke et al. 1999), although elevated CK can also result from problematic blood collection (Campbell 2006). Plasma AST is an extremely sensitive but nonspecific enzyme indicator of hepatocellular disease, and increased AST is often associated with impaired liver function (Boyd 1988; Campbell 1995). However, because of the presence and activity of AST in many tissues, the assessment of muscle-specific CK is generally also included in biochemical assessments of health in order to distinguish between liver and muscle damage (Dabbert et al. 1993). During our study, both AST and CK were increased in *E. macquarii* females after the production of larger and heavier clutches, suggesting that reproductive investment may have had a negative impact on the maternal hepatic system. This is plausible, given the important role the liver plays during reptilian vitellogenesis, producing egg yolk proteins necessary for egg generation (Ho et al. 1982). Investing in larger or heavier clutches may therefore place a greater burden on the liver to produce more egg yolk protein. Although elevated CK levels were considered accurate and unrelated to blood extraction procedures during this study, a complete analysis of liver function is needed to establish the true effects of reproduction on the hepatic system.

An important finding of this study was that body condition, the biometric indicator of health that is readily used in ecological research, was unrelated to maternal health or reproductive effort in all three species of turtle. Interestingly, studies involving the western pond turtle (*Emys marmorata*) have identified that larger females with higher body condition were actually in poorer physiological health than smaller females with lower body condition (Polo-Cavia et al. 2010), possibly because carapace length and body condition vary intraspecifically as a result of differing levels of phenotypic plasticity to the local environment (Rowe 1997). Collectively, these findings question whether body condition is an accurate representation of maternal health.

In summary, our results demonstrate that female freshwater turtles appear to alter clutch and/or egg size in accordance with maternal health state, agreeing with the physiological constraint hypothesis (Bowden et al. 2004). Moreover, it is evident that different species preferentially invest in different aspects of reproduction, although the consequences of this variation in reproductive investment remain to be elucidated. The findings of this study highlight the need to further investigate how maternal health is impacted by the reproductive process in different species. Focusing on improving the health of mothers in addition to increasing hatching success in waning populations may prove successful for the conservation and management of threatened and endangered species.

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