Changes in Plasma Biochemistry and Body Mass During Incubation in the Yellow-legged Gull

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Abstract.—The “Incubatory Reserves Constancy” hypothesis asserts that incubation could be a departure from breeding stress that allows for the maintenance or recovery of body reserves after laying effort (females) or territory defense (males) in those species with bi-parental incubation such as gulls. The plasma composition and body mass of incubating Yellow-legged Gulls (Larus cachinnans) were analyzed and related to the number of days after egg-laying. Female gulls showed an increase in uric acid and cholesterol levels, whereas males showed only an increase in uric acid values throughout this period. Moreover, females increased while males maintained their body masses. These results could reflect a recovery process after the laying effort supporting the Incubatory Reserves Constancy hypothesis in females. Uric acid and urea levels are positively correlated to body condition in Yellow-legged Gulls, which could be the result of a change in diet composition. This disagrees with recent findings on body composition in incubating gulls and could be related to variations in food availability among populations or years, and could reflect flexibility in the investment devoted by each sex. Received 4 December 2001, accepted 18 February 2002.

Key words.—Body-mass change, Incubatory Reserves Constancy Hypothesis, Larus cachinnans, plasma cholesterol, uric acid, urea, Yellow-legged Gull, triglycerides.

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Reproduction requires a high energetic expenditure in birds (Drent and Daan 1980; Blem 1990). The breeding effort commonly results in a decrease of lipid and protein reserves (e.g., Ankney and MacInnes 1978; Alisauskas and Ankeny 1994). However, the incubation period could be an exception to this general pattern in those species with bi-parental care, since the energy expenditure would be distributed between both sexes (Moreno 1989). Thus two opposing hypotheses may be proposed for changes in the energy reserves during incubation: the “Stress Hypothesis” would predict a pattern of gradual loss of reserves, because energetic costs are accumulated throughout the breeding season (Monaghan et al. 1989; Wendeln and Becker 1996), whereas “Incubatory Reserves Constancy Hypothesis” would suggest that the incubation period may be a departure from breeding stress that allows for the maintenance or recovery of body reserves (see Houston et al. 1983; Hario et al. 1991; also suggested by Moreno [1989] for total body mass).

Gulls are monogamous species with bi-parental incubation (Cramp and Simmons 1983) and both sexes incubate for a similar proportion of time (Drent 1970; Burger 1987; Alonso-Alvarez 2001; but see Butler and Janes-Butler 1988). Hario et al. (1991) and Mawhinney et al. (1999) analyzed body composition of Herring Gulls (Larus argentatus) and Great Black-backed Gulls (Larus marinus) respectively, which were culled throughout the breeding season by governmental agencies to control their numbers. Hario et al. (1991) found that both sexes gained mass and fat during incubation, whereas protein reserves remained stable. In contrast, Mawhinney et al. (1999) found that mass and lipid content decreased and protein increased in females, while mass and protein remained stable and fat increased in males. Therefore, the results of these studies suggest different energy expenditure for each sex.

In the present study, we analyzed four plasma biochemical parameters (uric acid, urea, triglycerides and cholesterol) and body mass in incubating Yellow-legged Gulls (Larus cachinnans) to determine changes in reserves of living individuals, thus trying to avoid sacrifice of birds. Plasma levels of uric
acid and urea reflect mobilization of protein reserves during food-shortage periods in birds (Cherel and Le Maho 1985; Boismenu et al. 1992; Alonso-Alvarez and Ferrer 2001). Plasma triglycerides value is related to body fat content (Bacon et al. 1989; Dabbert et al. 1997) and cholesterol level is a good indicator of body-mass loss from an individual's optimum in captive Yellow-legged Gulls (Alonso-Alvarez et al. 2002). However, few studies have analyzed changes in plasma chemistry throughout reproduction in free-living birds (Fairbrother et al. 1990; Cantos et al. 1994; Hollmén et al. 2001). Moreover, as far as we know, the plasma chemistry of breeder individuals has not been previously analyzed in any gull species.

The objectives of the present study were: (1) to determine whether the incubation period represents maintenance or recovery after the pre-laying effort in Yellow-legged Gull, as the Incubatory Reserves Constancy Hypothesis suggests, (2) to evaluate between-sexes differences in the patterns of change in body mass and plasma chemistry, and whether they are consistent with the results provided by Hario et al. (1991) or Mawhinney et al. (1999), and finally, (3) to report gulls' plasma biochemistry during the breeding period.

METHODS

The study was carried out on the Gés Islands (42°15'04"N, 8°53'30"W), which are located in the outer area of the Ría de Vigo (Spain) and are the breeding grounds for over 22,000 pairs of Yellow-legged Gulls. Birds were captured using nest-traps (Weaver and Kadlec 1970) during the incubation period (May 1998). We trapped 33 males and 43 females throughout the 27-31 d incubation period (Gramp and Simmons 1985). The beginning of incubation (following completion of clutch) was determined by visiting the colony every two d. In the last 10 d of incubation, 13 females and four males that had been trapped on the first 10 d were re-captured.

We weighed the birds with a spring balance to the nearest 5 g (Pesola). Two milliliters of blood were taken from each bird with a heparinized syringe from the brachial vein. Blood samples were collected between 11.00 h and 18.00 h to minimize variations caused by circadian rhythms (see Ferrer 1990). The blood was placed in tubes containing lithium heparin, which were kept on ice in cool containers (4°C) for a maximum period of eight h. Then, samples were centrifuged at 3,000 rpm for 10 min and plasma stored at -60°C until analysis.

Biochemical analyses were performed on a spectrophotometer (Hitachi-U2000, Tokyo, Japan). The determinations were made in duplicate on each plasma sample following the methods and authors indicated in parentheses: uric acid (uricase method; Fossati et al. 1980), urea (urease method; Munan et al. 1978), triglycerides (peroxidase-coupled method; McGowan et al. 1983), and cholesterol (cholesterol esterase method; Al-lain et al. 1974). These parameters were analyzed using commercial reagents (Sigma-Aldrich Company, MO, USA). All samples were assayed in the same series. Uric acid: intra-assay CV = 1.9%, accuracy: ± 0.012 mmol/L. Urea: intra-assay Coefficient of Variation (CV) = 1.54%, accuracy: ± 0.33 mmol/L. Triglycerides: intra-assay CV = 1.5%; accuracy: ± 0.05 mmol/L. Cholesterol: intra-assay CV = 0.95%, accuracy: ± 0.08 mmol/L.

Data were tested for normality using the Shapiro-Wilk test. We used parametric tests because all variables were normally distributed. We tested between-sexes differences during first and last ten d of incubation by Student's t-test. Changes in plasma levels in the same individual between the first and the second sampling were tested by paired t-tests. Data of recaptured males were not used due to the small sample size. Relationships among variables were analyzed by Pearson correlation coefficients. In order to avoid pseudo-replication, recaptured males and females were not included in Pearson's correlations. An index of body condition (body mass corrected for size) was obtained as the residuals of the linear regression of body mass on cubed tarsus length of birds (males: R² = 0.18, F_{1,29} = 6.74, P < 0.01; females: R² = 0.35, F_{1,41} = 22.08, P < 0.001).

RESULTS

Female gulls showed lower urea and cholesterol concentrations than males during the first ten days of incubation (Table 1). There were no between-sexes differences in plasma levels during the middle ten days or during the last ten days of incubation (Table 1). Plasma concentration of uric acid in males (t₁₀ = 2.42, P < 0.05) and uric acid and cholesterol in females (t₁₀ = 3.13, P < 0.01; t₁₀ = 2.93, P < 0.01; respectively) showed significant increase between the first and the last 10 d periods (see Table 1). Moreover, the 13 females recaptured in the last days of incubation showed a significant increase in uric acid and cholesterol values (Table 2). Plasma variables and body condition index were not correlated with the laying date (n.s. in all cases and in both sexes).

In males, only uric acid showed a significant correlation with the number of days spent in incubation before blood extraction (Table 3). In females, uric acid, urea, cholesterol and body condition index were positively correlated with the day of incubation (Table 3). In addition, body mass was positively correlated with the day of incubation.
Table 1. Concentrations of four plasma chemicals in the Yellow-legged Gull during the first and last days of incubation period. Values are in mmol/L and are given as the mean ± 1 SE.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First 10 days*</th>
<th>Middle 10 days*</th>
<th>Last 10 days*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.46 ± 0.05</td>
<td>0.57 ± 0.10</td>
<td>1.09 n.s.</td>
</tr>
<tr>
<td>Urea</td>
<td>1.01 ± 0.13</td>
<td>0.57 ± 0.12</td>
<td>2.55 &lt;0.05</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.06 ± 0.17</td>
<td>1.31 ± 0.11</td>
<td>1.26 n.s.</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>7.35 ± 0.52</td>
<td>5.82 ± 0.40</td>
<td>2.81 &lt;0.01</td>
</tr>
</tbody>
</table>

Student's t-test for independent samples.
*11 males and 15 females.
*10 males and 19 females.
*12 males and 9 females.

Plasma concentrations of protein, cholescterol, and triglycerides were significantly different between males and females on the first day of incubation (Table 1). In birds, high levels have been associated with survival and body mass; plasma concentrations in males were lower than in females on the first day of incubation (Table 1). In birds, high levels have been associated with survival and body mass; plasma concentrations in males were lower than in females on the first day of incubation (Table 1). In birds, high levels have been associated with survival and body mass; plasma concentrations in males were lower than in females on the first day of incubation (Table 1). In birds, high levels have been associated with survival and body mass; plasma concentrations in males were lower than in females on the first day of incubation (Table 1).
loss, because muscular protein is used as energy source during the last phase of fasting (Cherel and Le Maho 1985; Boismenu et al. 1992; Castellini and Rea 1992). In the same way, plasma urea has been used as a nutritional condition index (Ferr er 1992, 1993). The protein is a limiting factor during albumen production among bird species (Williams 1996; Ramsay and Houston 1997), including gulls (Herring Gull; Houston et al. 1985). As female gulls differentially suffered the costs of egg production, we should expect high nitrogen values at the start of incubation, decreasing throughout this period. Surprisingly, we found the opposite.

In a recent study, Hollmén et al. (2001) found an increase of uric acid plasma levels in female Common Eiders (Somateria mollissima) during incubation. The Common Eider has uni-parental incubation. Female Eiders rarely leave their nest to feed during this period (26 d), relying energetically on their body reserves to lost approximately 50% of their body masses (Parker and Holm 1990). Hollmén et al. (2001) related the uric acid increase to mobilization of protein reserves and body-mass loss. However, our results suggest that in other circumstances this relationship may be reversed. Both urea and uric acid were positively correlated with body mass corrected for body size in Yellow-legged Gull. Hence, such relationships must be carefully considered. A possible explanation might derive from changes in diet.

A rise in the proportion of protein in the diet increased the plasma level of these nitrogen metabolites in chickens and pigeons (Okumura and Tasaki 1969; Featherston 1969; Luneij and Bruijne 1985). Therefore, our results relate to diet differences, allowing female gulls to increase their protein reserves. The studied birds often fed on refuse tips (rubbish present in the 40% of pellets [Munilla 1997a]), and showed marked modifications in diet throughout the breeding period, as the occurrence of crabs in regurgitations increases significantly during incubation (Munilla 1997b). An increase in the proportion of crustaceans has also found in the diet of Kelp Gulls (Larus dominicanus) in the same period (Bertellotti and Yorio 1999).

On the other hand, triglyceride values did not change during incubation in either sex, indicating stable lipid reserves, since the amount of body fat is positively correlated with plasma triglycerides values (Bacon et al. 1989; Dabbert et al. 1997). Furthermore, cholesterol is lower in females during first ten days, increasing during the rest of incubation period. In Yellow-legged Gulls, the plasma concentration of this lipid is negatively correlated with the individual proportion of body-mass loss, thus providing a reliable index of the body condition (Alonso-Alvarez et al. 2002). Incubating gulls with high cholesterol levels would be relatively heavy birds. The

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males $^a$</th>
<th>Females $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_{12}$</td>
<td>$P$</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.541</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urea</td>
<td>-0.061</td>
<td>n.s.</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.238</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.299</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body condition</td>
<td>0.099</td>
<td>n.s.</td>
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</tbody>
</table>

Table 2. Values of four plasma chemicals (mmol/L) and body mass (g) of 15 female Yellow-legged Gulls captured in the first and last ten days of incubation. Values are given as mean ± 1 SE.

Table 3. Pearson correlation between the day of incubation and four plasma parameters and residuals of body mass on body size (body condition).
cholesterol increase bears out the presence of a recovery pattern in female birds.

In summary, the present results support the hypothesis that body mass and reserves maintain or recover their levels during incubation in those species with bi-parental care. However, whereas females increased body mass, and probably changed diet composition in order to recuperate the protein expenditure during laying effort, males maintained body mass, lipids and protein metabolites throughout incubation. These results are not consistent with the results of Hario et al. (1991) on Herring Gulls or Mawhinney et al. (1999) on Great Black-backed Gulls. The differences between the two studies were explained by differences in the energy expenditure of the sexes during incubation (Mawhinney et al. 1999). Female Great Black-backed Gulls spent more time on the nest than males, thus reducing their feeding time (Butler and Janes-Butler 1983). Alternatively, the disagreement between Hario et al. (1991), Mawhinney et al. (1999) and the present paper might be related to differences in food availability (Kitaysky et al. 1999). Differences in food abundance produced a readjustment of each sex role during incubation in Western Gulls (Larus occidentalis; Pierotti 1981). The plasma chemistry during the incubation period should be analyzed under different food-availability situations, which could be easily manipulated by means of an artificial food supply. Moreover, the relationship between food composition and plasma biochemistry in wild birds should be examined.

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