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OSMOREGULATION IN NESTLING GLAUCOUS-WINGED GULLS

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ABSTRACT.—Blood samples from 32 nestling Glaucous-winged Gulls (*Larus glaucescens*) ranging in age from 1 to 41 days were collected at a nesting site near Vancouver, B.C. The hematocrit was positively correlated, the plasma sodium and chloride concentrations negatively correlated, and the plasma potassium and osmotic concentrations uncorrelated with age. Salt gland secretion was elicited in 23 young gulls by intravenous injection of 1 M NaCl. The concentration and the volume of secretion increased with age. Sodium secretion was positively correlated with the size of the salt gland. The secretion of potassium and minimum maintained concentrations of sodium were positively correlated with age. In the first hour of secretion, hatchlings eliminated 39% of the injected fluid containing 24% of the injected sodium; in contrast, the oldest nestlings removed 77% of the fluid and 55% of the sodium. Cloacal fluid voided by older salt-loaded birds was less concentrated than that of younger ones. Organ weights were determined in 20 nestlings. Kidney weight was 2–3% of the body weight regardless of age, but the relative weights of the Harderian, salt, and adrenal glands decreased with age.

From the time they begin to feed, young marine birds consume foods that are hyperosmotic to their body fluids and must, therefore, eliminate excess solutes (mainly sodium and chloride) in order to remain in osmotic balance. *Adult* birds of marine species eliminate excess sodium chloride by means of the salt glands (Peaker and Linzell 1975), the secretion of which has roughly twice the concentration of sea water. Thus, they can drink sea water or eat salt-rich foods and still maintain osmotic balance. Adult birds also have other osmoregulatory options: e.g., they can select food items and so, theoretically at least, regulate their salt intake; those living along the coast also have access to fresh water. In contrast, nestlings do not have these choices although their parents could select food of low osmotic concentration or extract salts from it before feeding it to them. However, my observations of the food (often whole small fish or mussels) regurgitated by nestlings suggest that the adults do neither of these things. Nestlings do not ordinarily have access to water, although they have been reported to eat grass, which may provide them with free water (Ensor and Phillips 1972). They may also use dew or the parents may bring them fresh water, although neither of these possibilities seems to have been substantiated. Hence, nestling marine birds probably rely heavily on extrarenal secretion to excrete excess salt.

Little has been reported about the rate of maturation of the secretory process or the factors that affect it in such nestlings. Douglas (1968) examined the size and secretory capacity of salt glands from Adélie Penguin chicks (*Pygoscelis adeliae*). He found that the gland's weight and secretory rate were linearly correlated with body size, but that the chloride concentration of the secretion was constant throughout the growth period. He noted secretion in pipping chicks and suggested that even at this age, they were capable of handling the excess salt in their diet of marine invertebrates. Hughes (1968) also found that the salt gland of Common Tern chicks (*Sterna hirundo*) enlarged in linear fashion with body size, even though older birds were less efficient at excreting imposed salt loads than younger ones. This was due to a decrease in the volume secreted by the salt gland, not in the concentration of the secretion. However, some of the birds in that study were salt-loaded more than once during their growth period and this may have influenced their responses. Ensor and Phillips (1972) compared the secretory capacities of salt glands from 4- to 5- and 15- to 16-week-old chicks in two mixed populations of gulls (*Larus argentatus* and *L. fuscus*) that had been raised either on a marine island or an inland freshwater site. The younger birds from the marine environment were smaller than their freshwater counterparts and had larger salt glands, nearly twice as large on a weight-specific basis. The secretion of their glands was twice as concentrated in sodium and was produced at three times the rate of that from freshwater birds. These differences disappeared by the time the birds were 15–16 weeks old.

In the present study, I examined the blood of birds at representative stages of the entire maturation period for age-related changes in hematocrit, electrolyte concentration, and osmotic pressure. I also analyzed extrarenal secretions and cloacal fluid and determined the relative weights of osmoregulatory organs.

MATERIALS AND METHODS

For this study I used Glaucous-winged Gulls (*L. glaucescens*), which I obtained from a nesting site near Vancouver, B.C. (49°30′N, 123°18′W). Sampling was done in the midmorning and, when handled, most birds regurgitated food, which was mainly mussels. Body weight (*BW*, in g) was converted to age (in days) with the equation, $\text{Age} = 0.0389\,BW - 1.2942$ ($r = 0.99$), derived from data of McMannama (1950). One-ml blood samples were obtained on site from 32 nestlings weighing 42–1,086 g, either from a wing vein, or by heart puncture if the bird weighed less than 75 g. These samples were taken to the laboratory within 3 h and transferred to centrifuge tubes, covered with Parafilm, and stirred with an automatic laboratory mixer. Duplicate capillary tubes were filled with mixed blood, sealed, and centrifuged at 2,000 × g for 30 min for determination of hematocrit. The remaining blood was centrifuged at 500 × g to obtain plasma in which the Na, K, and Cl concentrations, and osmotic pressure were measured.

Twenty-two other birds were used in studies of salt gland secretion and to determine organ weights. They were collected as pipping or nestling birds, taken to the laboratory, and held in an incubator or in small cages. They were fed herring (*Clupea palasii*) and given dilute sea water (1:2, v/v) to drink. Most were used within 24 h of capture, although seven were held for several days. In each experiment, the bird was loosely wrapped in cheesecloth, weighed to the nearest 0.1 g, and injected intravenously with 1.0 ml 1 M NaCl/100 g body weight. When the salt gland began to secrete fluid, the bird was hand-held while two 30-min samples of secretion were collected in preweighed vials. The vials were then reweighed and aliquots of the secretion were removed for analysis of Na, K, and Cl. The ion concentrations in samples of spontaneously voided cloacal fluid were also determined. The bird was then killed with an overdose of sodium pentobarbital. Its kidneys and adrenal, Harderian, and salt glands were removed and weighed to the nearest 0.001 g.

Sodium and potassium concentrations were determined with a flame photometer (Instrumentation Laboratory, Inc.); chloride concentration electrimetrically using a chloridometer (Aminco-Cotlove); and osmotic pressure by freezing point determination (Advanced Osmometer).

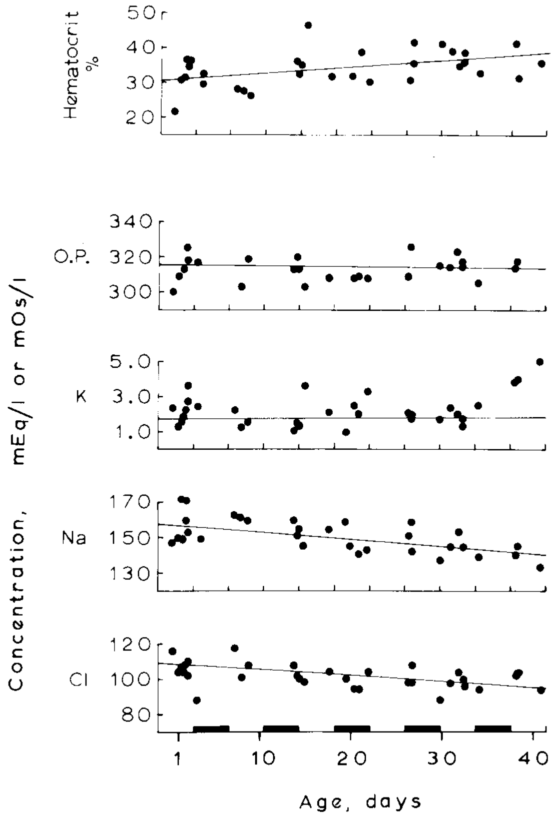

FIGURE 1. Age-related changes in the hematocrit, plasma ionic concentrations, and osmotic pressure (O.P.) of nestling Glaucous-winged Gulls. The alternate black-and-white bars on the abscissa indicate 100 g body weight.

All data are given as means ± 1 SE and were compared using Student's *t*-test.

RESULTS

BLOOD SAMPLES

The mean hematocrit of all the birds was $33.8 \pm 0.9\%$ ($n = 32$). Hematocrit (*H*, %) was positively correlated ($P < 0.025$) with age (in days) in these young gulls: $H = 31.02 + 0.16 \cdot \text{Age}$ (Fig. 1). While the osmotic pressure of the plasma was not correlated with the hematocrit, the concentration (in mEq/l) of both major plasma ions was significantly negatively correlated with the hematocrit: $[\text{Na}^+] = 170.64 - 0.623H$ ($P < 0.05$); $[\text{Cl}^-] = 132.0 - 0.867H$ ($P < 0.001$).

The mean plasma sodium, chloride, and potassium concentrations for all 32 birds were 149.3 ± 1.3, 102.6 ± 1.2, and 2.1 ± 0.2 mEq/l, respectively. Sodium and chloride (but not potassium) concentrations (in mEq/l) decreased with age (in days): $[\text{Na}^+] = 156.0 - 0.38 \cdot \text{Age}$ ($P < 0.001$); $[\text{Cl}^-] = 107.9 - 0.29 \cdot \text{Age}$ ($P < 0.001$). The plasma potassium concentration was constant during maturation, but higher in three birds of fledging size (Fig. 1). The os-

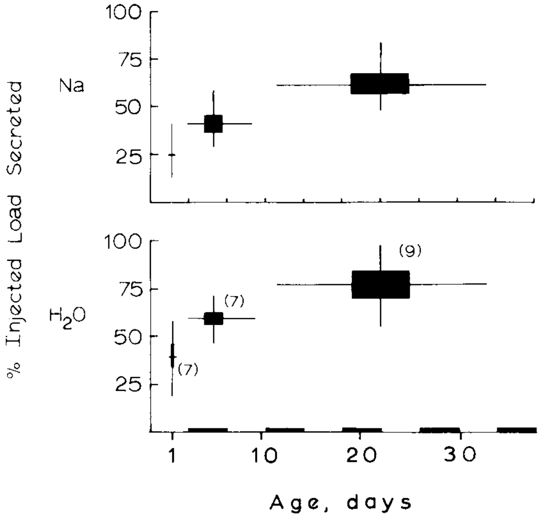

FIGURE 2. Age-related changes in the extrarenal salt excretion of salt-loaded nestling Glaucous-winged Gulls. The secretion was elicited by an intravenous injection of 1.0 ml 1 M NaCl per 100 g body weight and was collected for 1 h. The alternate black-and-white bars on the abscissa indicate 100 g *BW*. Horizontal and vertical lines indicate the ranges; each rectangle represents ±1 SE.

motic pressure of the plasma averaged 315 ± 2 mOsm/l and was not related to age.

SALT GLAND SECRETION

Secretory volume and concentration relative to body size. Pipping birds had dried salt on their nares. Three of the birds included in this study had been taken from nests while pipping and injected with NaCl within hours of emerging from their shells. They secreted 32.5, 52.5, and 54.0% of the injected volume and 12.4, 32.0, and 44.6% of the injected sodium, respectively, during 1 h of salt gland activity. The mean sodium concentrations of their nasal secretions were 380, 359, and 483 mEq/l.

Young gulls improved their ability to eliminate salt loads as they matured (Fig. 2). Seven hatchlings (including the three newly hatched birds described above), having a mean age of 0.98 ± 0.11 days, secreted 39 ± 5% of the injected load, containing 24 ± 4% of the injected sodium, within 60 min after the salt gland became active. The oldest nine chicks, weighing 327–876 g, with a mean age of 26.0 ± 1.8 days, eliminated 77 ± 6% of the load, containing 55 ± 5% of the injected sodium, in the same period of time. These age-related differences are significantly different ($P < 0.001$). The percent of the sodium load secreted was correlated with age (in days) over the growth period, $\% \text{Na} = 29.93 + 0.78 \cdot \text{Age}$ ($P < 0.02$), as was the percent of the injected fluid secreted (F) and age (in days), $\% F = 43.59 + 0.92 \cdot \text{Age}$ ($P < 0.02$). Although the *maximum* sodium concentration of the induced secretion (always present in the first 30-min sample) was not significantly correlated with age ($P < 0.1$), older birds were able to maintain a higher sodium concentration since the *minimum* sodium concentration (Y), which was always present in the second 30-min collection, was significantly related to the birds' ages: $[\text{Na}^+] = 602 + 4.60 \cdot \text{Age}$ ($P < 0.02$); sodium secretion (μEq/min) = 26.70 + 0.23 gland wt. (in g; $P < 0.001$). The potassium concentration of the secretion (in mEq/l) was positively correlated with age: $[\text{K}^+] = 4.28 + 1.47 \cdot \text{Age}$ ($P < 0.001$). Older birds tended to produce secretion with a higher sodium concentration and were able to produce it for a longer period of time than the younger birds.

TABLE 1. Ion concentrations in the cloacal fluid of salt-loaded nestling Glaucous-winged Gulls.[a]

Age (days)	Number of samples	Ion concentration (mEq/l)[b] Na	K	Cl
1–16	14	135 ± 14	33 ± 7	177 ± 12
16–32	6	100 ± 12*	28 ± 8	88 ± 17***

[a] Cloacal fluid was produced during the first hour after intravenous salt loading with 1.0 ml 1 M NaCl/100 g *BW*.

[b] Values are $\bar{x}$ ± SE. Significant differences between pairs of values in each column are indicated by * ($P < 0.05$) and *** ($P < 0.001$).

Time required to activate the salt glands. The one-day-old birds began to produce salt gland secretions 5.7 ± 1.0 min after salt-loading. The time decreased to 3.4 ± 0.6 min in birds 2–12 days old ($P < 0.05$), which was not different from the interval of birds 13–30 days old, which was 3.0 ± 0.6 min.

CLOACAL ION SECRETION

Spontaneously voided cloacal samples were obtained from 19 salt-loaded birds (Table 1). The cloacal fluid of older birds had significantly less sodium and especially chloride than that of younger birds. The potassium concentrations were similar.

ORGAN WEIGHTS

Organ weights were determined in 14 birds at stages within the first half of the growth period (50–445 g) and in six birds at later stages of development (488–876 g). In these groups, the mean % of the *BW* represented by the kidneys was 2.26 ± 0.11 and 2.05 ± 0.19, respectively (the difference is not statistically significant); for salt glands, 0.160 ± 0.013 and 0.071 ± 0.004 ($P < 0.001$); for adrenal glands, 0.037 ± 0.004 and 0.018 ± 0.001 ($P < 0.001$); and for Harderian glands, 0.188 ± 0.011 and 0.120 ± 0.013 ($P < 0.001$).

The growth of the kidneys was linearly related to body growth and that of the Harderian glands was nearly linear; but the growth of the

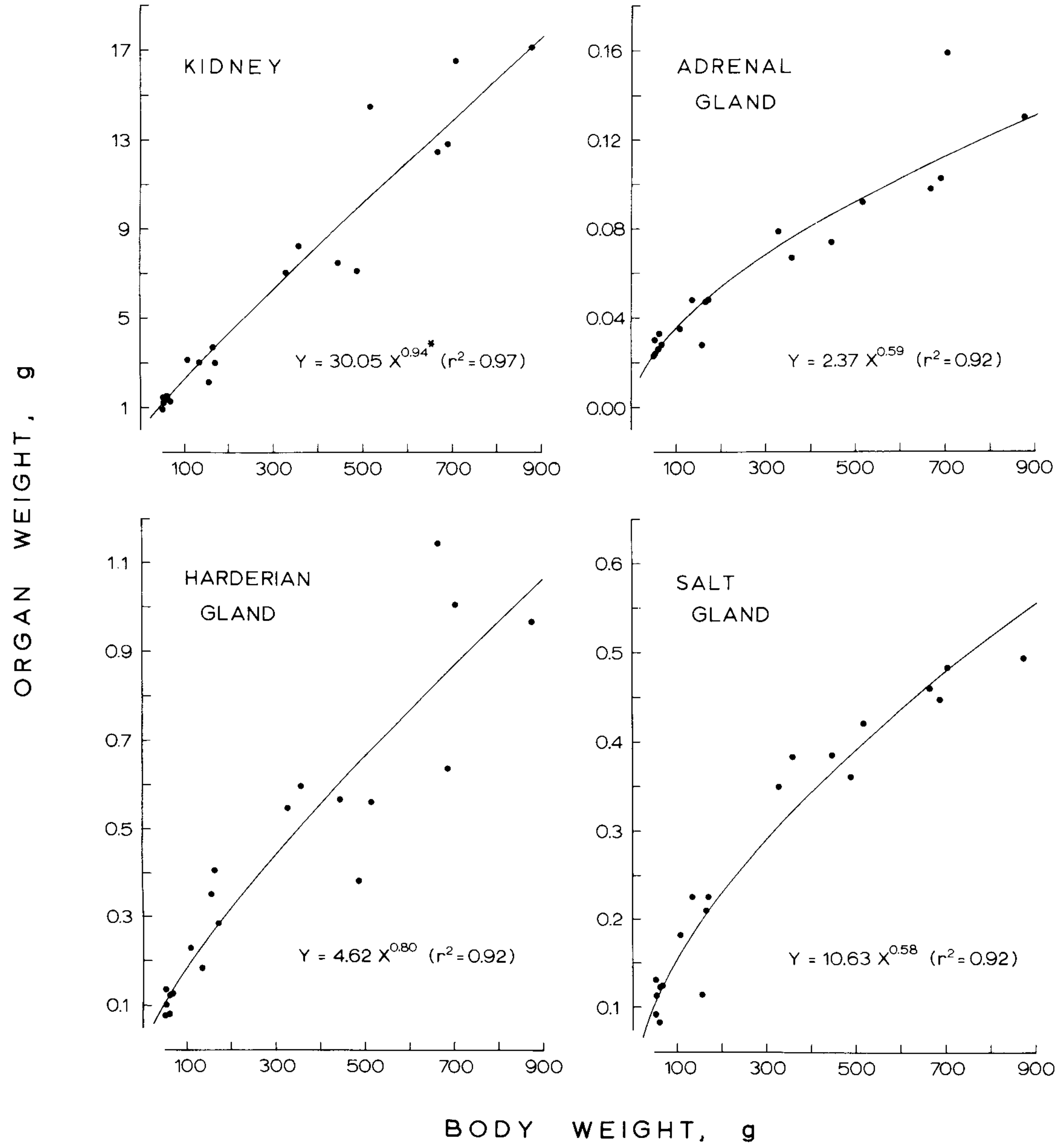

FIGURE 3. Relationship between the weight of osmoregulatory organs and body weight of nestling Glaucous-winged Gulls. * Each line is accompanied by the equation that best relates that organ's weight *in milligrams* to body weight during the maturation period and its coefficient of determination (r^2).

salt and adrenal glands was curvilinearly related to it (Fig. 3). The adrenal and salt glands developed at the same rate, although the salt glands were consistently four times larger than the adrenal glands.

DISCUSSION

Since most nestlings had food in their crops, they were likely under some degree of osmotic stress. Younger chicks had higher plasma sodium and chloride concentrations than older ones (Fig. 1), a finding consistent with the observation that older birds were more efficient at eliminating salt loads extrarenally (Fig. 2). The younger birds also had lower hematocrits. The osmotic concentration of the plasma did not change as the birds grew. Since both major ions, Na and Cl, decreased in concentration, some other constituent(s) apparently accumulated in the plasma with age. These were not identified, but, as growth slowed, excess dietary nitrogen would have to be eliminated and could have contributed to the osmotically active material in the plasma. Also, an increase in activity might have increased the plasma's concentration of bicarbonate ion.

The salt glands of the nestling Glaucous-winged Gulls were larger proportional to their body weight (0.132% *BW*) than those in the only two other species that have been studied during the growth phase, the Common Tern, with a reported value of 0.1% *BW* (Hughes 1968), and the Adélie Penguin, with a value of 0.052% *BW* (Douglas 1968). In terns and penguins, the gland's weight was linearly related to body weight, in contrast to Glaucous-winged Gulls (Fig. 3), in which the relationship was curvilinear.

The concentration of sodium in secretion from newly hatched gull chicks was less than that in sea water, but after one or two days, it exceeded that of sea water. Since the parents begin to feed the chicks hypertonic food soon after they hatch, a hatchling must rely on either stored water or water obtained as its yolk is metabolized to remain in osmotic balance during the first day or two of its life. Younger nestlings have proportionately larger glands than older ones, but, in response to weight-related salt loads, produce secretion at a slower rate (Fig. 2). Hughes (1968) found the opposite pattern during the maturation of terns: older terns were less efficient, secreting 8% of administered salt loads compared to 16% in younger terns. Douglas (1968), however, found little change in the chloride-secreting efficiency of penguins (which was low, 16–20% of administered loads) during growth; the chloride concentration was constant over the growth period and a linear relationship ($r = 0.98$) existed between the salt gland's weight (*GW*, in g) and the maximum rate at which it eliminated chloride (in mEq/min): $Cl = 0.112GW - 0.012$. A similar ($r = 0.90$) relationship was evident for extrarenal sodium elimination in young gulls: $Na = 0.223GW - 0.023$, although the secretory rate in young gulls was twice that of young penguins and their secretory efficiency was up to three times that of terns and penguins.

After salt-loading, the sodium concentration in the cloacal fluid and the plasma was equal, but the chloride concentration was greater in cloacal fluids than the plasma, provided the gulls were in the first half of their maturation period; in older birds, the cloacal fluid sodium and chloride were less than plasma levels (Table 1, Fig. 1). Schmidt-Nielsen et al. (1963) suggested that the cloaca reabsorbs ions and water from the ureteral fluid. Skadhauge (1981) summarized the studies by which he and his co-workers demonstrated that ions are reabsorbed by the lower intestine in several species of land birds. Although marine birds have not been studied, the present data suggest that as their capacity for extrarenal salt secretion improves so does their ability to recover ions from the cloacal fluid. In this regard, Glaucous-winged Gulls differ from Common Terns and Adélie Penguins, which produce hypotonic (Hughes 1968) and very hypertonic cloacal fluid (Douglas 1968), respectively, at all ages.

Osmoregulation in marine birds is accomplished by several organs in addition to the salt glands, including the kidneys, adrenal glands, and possibly the Harderian glands. In growing Glaucous-winged Gulls the kidneys constituted 2–3% *BW* at all ages. In adults of the species, however, they represented only about 1.2% *BW*, a value typical for avian species with salt glands as calculated from the data summarized by Hughes (1970), and higher than the 0.8% *BW* typical of species lacking salt glands ($P < 0.001$).

The relative weights of the adrenal and salt glands decreased at the same rate (Fig. 3) and the adrenal glands were consequently about one-fourth as heavy as the salt glands in gulls of all ages. Like salt glands, the adrenal glands are larger in marine birds (24 mg/100 g *BW*) than in birds associated with fresh water (10 mg/100 g *BW*; Holmes et al. 1961). The adrenal weight of nestling Glaucous-winged Gulls in the first half of the growth period was 37 mg/100 g *BW*, but less than half that value, 18 mg/100 g *BW*, in five older nestlings. The latter value is close to that predicted for the gulls by Holmes et al. (1961).

The Harderian glands may have a role in potassium secretion in ducks (Hughes and Ruch 1969), but probably do not have such a function in adult gulls (Hughes 1969). The Harderian glands of nestling Glaucous-winged Gulls were several times larger (168 mg/100 g *BW*) than those of ducks producing potassium-rich tears (51 mg/100 g *BW*). However, since I did not determine the ion concentrations in the tears of the nestling gulls, I do not know the role that these glands may play in the nestlings' salt and water balance.

The ability of nestling gulls to eliminate salt through the salt glands improved with age. Older chicks produced more secretion (although their glands were proportionately smaller) and it was more concentrated than that of younger birds. Only the smallest birds were unable to produce a secretion hypertonic to sea water. Until the midpoint of their growth period, nestlings eliminated only about 35% of the injected sodium during the first hour after salt loading (they were loaded with 1 M NaCl, which is roughly twice the osmotic load of sea water). Presumably, they would be more efficient in eliminating the less concentrated, natural sea water loads to which they are normally exposed during feeding. (Adults loaded with artificial sea water secreted 57% of the Na in 24 h, whereas birds loaded with 1 M

NaCl secreted 41% [Hughes, unpubl. data].) Nevertheless, especially during the first one or two days after hatching, nestling gulls may be stressed beyond their osmoregulatory capacity, particularly if they must expend water for temperature regulation. If the nest site is cool, shaded by vegetation, and frequently shrouded in fog, growing nestlings probably experience little thermal stress.

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RECENT PUBLICATIONS

Handbook of the birds of India and Pakistan. Vol. 4. Second edition.—Sálim Ali and S. Dillon Ripley. 1983. Oxford University Press, Delhi. 267 p. $26.00. This is a revised edition of a work first published in 1970. (Previous volumes in the series were noted in Condor 84:398 and citations therein.) It covers the Orders Caprimulgiformes through Piciformes and starts into the Passeriformes (Eurylaimidae and Pittidae). New information has been added and a few taxonomic changes have been made. Eleven color plates (including three new ones) depict 88 species, and line drawings of the head or tail are given for many others. Distribution maps, list of references, index. Ali and Ripley must be applauded for their industry, both in compiling the volumes in this series and in bringing them up to date.

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