PTILOCHRONOLOGY: A CRITICAL EVALUATION OF ASSUMPTIONS AND UTILITY

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ABSTRACT.—Ptilochronology has been proposed as a method that will open up many new lines of investigation in nutritional ecology, in foraging behavior, and of life-history variables that are affected by (or affect) a bird's nutritional status. The method depends on measurements of a series of daily growth bars to estimate a feather's growth rate, and thereby to quantitate a bird's nutritional status while the feather was growing. The reliability of the method as proposed depends on the fulfillment of several previously unstated assumptions. We identified seven key assumptions and, in experiments with captive White-crowned Sparrows (*Zonotrichia leucophrys gambelii*), examined the likelihood that six of the assumptions could be fulfilled. The seventh assumption, no less crucial than the others, could not be tested empirically. None of the six tested assumptions were consistently fulfilled. Only lethal kinds or levels of nutritional privation consistently slowed feather growth. Moderate or even severe sublethal privation did not produce consistent effects on feather growth. In short, ptilochronology as originally conceived is fraught with uncertainty. It may yield reliable results in very limited, carefully controlled conditions, but this remains to be proved. *Received 23 August 1990, accepted 20 December 1990.*

PTILOCHRONOLOGY (Grubb 1989) is a technique that proposes to use the width of daily growth bars on a tail feather as an index of the feather's growth rate and thereby as an index of a bird's day-by-day nutritional status while the feather was growing. Growth bars are difficult to describe. They are alternating bands of visual contrast across a feather's vane that have been likened "to a pattern such as seen in watered silk" (Lowe 1942). One bar (a pair of contrasting bands) is thought to be produced each day. In practice, a rectrix (the "original" feather) is plucked during a nonmolting season and the replacement ("induced") feather is later also plucked after it has grown to full length. The widths of approximately ten growth bars are measured in a linear phase of feather growth and averaged to yield a mean growth rate. According to Grubb (1989: 319) "Ptilochronology appears to have the potential to reveal new insights into the nutritional ecology of birds. Hypotheses of ultimate causation that predict variation in the nutritional status of birds of different age, sex, dominance status, . . . habitat, migratory pathway, guild [etc.] . . . can be tested directly." An ostensibly simple technique such as this may therefore appeal to a very wide spectrum of avian biologists and might provide a powerful tool for studies in nutritional ecology. Because of this potential it is imperative (1) to specify clearly the assumptions that underlie the method, (2) to identify how nutritional (and other) factors affect the rate of feather elongation, and (3) to establish reliable methods for calibrating the width of growth bars in relation to nutritional state. As subsidiary experiments in studies designed mainly for other purposes, we conducted tests that address these three subjects. The results help to clarify the conditions in which ptilochronology might be serviceable.

MATERIALS AND METHODS

ANIMALS AND HUSBANDRY

All experiments were conducted with White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) captured during their spring or autumn migration through eastern Washington. These birds were initially kept in large outdoor aviaries (W × L × H = 2.7 × 4.6 × 2.4 m), where fresh water and chick-starter mash (20.7% protein, 74.7% carbohydrate, 3.1% fat, 1.5% ash) were available *ad libitum*. During acclimation to conditions indoors, and during the subsequent experiments, the birds were confined singly in small cages (23 × 46 × 26 cm) in constant-condition rooms (air temperature = 20 ± 1°C unless otherwise stated, daily photoperiods as stated later). Food quality and supply differed among experiments. Fresh drinking water was always available.

MEASUREMENTS

In each bird we measured (1) the number of elapsed days between plucking the original feather and first appearance of the induced pinfeather beyond the skin, (2) the feather's total length, and (3) growth increments (±0.25 mm) during linear-phase elongation (≤30–35 mm long) in a single outermost rectrix (R6), a single fifth primary remex (P5), or both. We measured the growing feathers at 3-day intervals. The initial and final measurements of feather length were typically made 6–9 days apart at the same hour. We estimated growth rate by dividing the difference between initial and final lengths by the number of elapsed days. We did not measure the widths of growth bars, which are difficult to see and often invisible in *Z. l. gambelii*. We therefore circumvented the assumption that each growth bar corresponds to precisely 24.0 h of elongation. The birds were weighed (±0.05 g) at appropriate intervals as specified later.

EXPERIMENTS

We undertook ptilochronologic measurements in four sets of experiments in which the amount, quality, or variety of foods were the principal variables. To facilitate reference to these sets we refer to them as "series" designated by acronyms.

Series RA ("restricted adequate" ration).—Winter-phase birds were kept in L:D 8.5:15.5 and their daily *ad libitum* consumption of a high-quality chick-starter mash (see above) was measured for 15 days. On 4 January one R6 and one P5 were plucked from each bird. Control birds continued to eat *ad libitum*. Each morning each restricted bird was given 90% of the average amount of the mash that it had consumed while its body mass was stable during the preceding 15 days. Each midafternoon we confirmed that no food remained in the food cups of restricted birds (i.e. that <100% of *ad libitum* intake was provided). We also continued to measure *ad libitum* intake by control birds to be certain that no large deviations from average food intakes occurred while the feathers were growing.

Series PC ("protein choice" or "protein consumption").—Five experiments were conducted nearly concurrently using autumn-phase birds kept in L:D 11:13 and 20°C. One R6 and one P5 were plucked from each bird, and the growth of the induced feathers and changes of body mass were recorded through appropriate periods of time. All experimental groups were supplied with a semisynthetic maintenance ration that contained 12% protein for at least 1 month before feathers were plucked. *Choice 1 Group*: During the growth of the induced feathers these birds had a simultaneous choice of three diets (1.2%, 12%, or 60% protein) in a 3-cup cafeteria trial. Their mean daily protein consumption was ca. 1.1 g, or ca. 21% of daily food intake. *Choice 2 Group*: Like Choice 1 except that the birds had simultaneous access to 1.2%, 40%, and 60% protein diets during growth of the induced feathers. Their mean daily protein consumption was ca. 1.6 g, or ca. 30% of daily food intake. *Deficient Protein Group*: On the day that R6 and P5 were plucked these birds were switched from the maintenance ration (12% protein) to a 1.2% protein ration. Nine days later, to slow the birds' loss of body mass, the dietary protein content was increased to 2.5%. The birds subsisted on this ration for 15 days more, and they were then given chick-starter mash. *Marginal Protein Group*: Like the Deficient Protein Group except that the birds received a 4.5% protein ration during the growth of the induced feathers. *Disrupted Feeding Group*: We designed this experiment to mimic the intermittent success that a foraging bird may have in finding food. During growth of the induced feathers the birds subsisted on a 12% protein ration. Food was removed from the cages at random times of the day for 3.5 h of each 11-h photophase on days 1 and 2 of successive 3-day cycles.

Series PT ("protein and air temperature").—Winter-phase birds were kept in L:D 9:15, acclimated to a 12% protein maintenance ration for at least 2 weeks, and then divided into four groups (2 groups in 5°C room temperature and 2 in 20°C). One subgroup at each temperature subsisted on a 2.5% protein ration (subadequate), and the other subsisted on a 40% protein ration (superadequate) while a single induced P5 was growing.

Series AA ("amino acids").—Autumn-phase birds were kept on the natural photoperiod for 46.3°N latitude (initially L:D 12:12, decreasing to L:D 10.5:13.5) and 20°C room temperature while they were subsisting on a balanced semisynthetic ration containing 12% protein. Approximately 9 days after we plucked one R6 and one P5 from each bird, we segregated the birds into a control group retained on the initial ration and into four experimental groups given rations that contained either one half (0.50 lys, 0.50 val) or one quarter (0.25 lys, 0.25 val) of the White-crowned Sparrow's dietary requirement for lysine or valine. Actual dietary concentrations of the amino acids were 13.2 mmoles/kg for lysine and 15.0 mmoles/kg for valine in the first set, and 6.6 mmoles/kg for lysine and 7.5 mmoles/kg for valine in the second set (Murphy and King unpubl. data). Birds on the deficient rations lost body mass rapidly. We measured the elongation of R6 and P5 during either 6 days (0.25 restriction) or 9 days (0.50 restriction) of their early growth. We refer to these periods as "Phase 1" in Table 5. The birds were then returned to their earlier maintenance diet ("Phase 2").

In all the experimental series except Series RA we supplied the birds with specially formulated semisynthetic rations (Table 1). The birds in Series RA received chick-starter mash, as already described. Before beginning any of the experiments involving semisynthetic rations we allowed the birds to adjust

TABLE 1. Composition of experimental semisynthetic diets.

Component	% of diet
Protein[a]	variable
Salt mixture[b]	5.5
$NaHCO_3$	0.5–2.5
Choline chloride	0.2
Vegetable oil	7.0
Cod liver oil	1.0
Cellulose[c]	5.0–7.5
Vitamin mixture[d]	2.5
Sand	0.0–3.5
Cornstarch	to 100%

[a] High-nitrogen casein (U.S. Biochemical Corp.) provided all the protein-bound amino acids in the diets. In Series PC and PT, L-cystine, L-arginine·HCl, and glycine supplemented casein as 0.7, 2.5, and 4.3% of protein, respectively. (See text for the total protein contents of the diets.) The diets used in Series AA have been described earlier (Murphy and King 1989). They contained 12.3% crude protein and differing amounts of lysine or valine as indicated in the text. The amino-acid deficient diets were kept isonitrogenous by proportional substitution by glutamic acid.

[b] Fox-Briggs salt mixture (USBC, Cleveland, Ohio).

[c] Celufil-hydrolyzed (USBC).

[d] Specific vitamin supplement (D_3 substituted for D_2, USBC).

to a semisynthetic maintenance ration that contained 12% protein.

RESULTS

Series RA.—This experiment was designed to impose a chronic, moderate shortage of food such as free-living birds might confront. The birds consumed all of their allotted food by midday, which produced a prolonged period of fasting through the rest of the day and the ensuing night, and hence a disruption of their normal feeding rhythm. Restriction to 90% of their voluntary intake of a nutritionally balanced food caused the birds to lose a moderate amount of body mass (7.2% in 18 days), which indicated a moderate plane of nutritional privation (Table 2). The induced P5 grew significantly more slowly in the restricted birds than in the controls. The induced R6 may also have grown more slowly than in the controls, but the difference was not statistically significant. The mean elapsed time between plucking the original feather and emergence of the induced feather did not differ significantly between the two groups for either P5 or R6. With a few exceptions, to be noted later, neither the P5 nor the R6 mean emergence days differed appreciably (none differed significantly by one-way ANOVA) from the corresponding means in any other series or group. Emergence-day data have therefore been omitted from Tables 2–5.

Series PC.—These experiments were designed to examine the abilities of White-crowned Sparrows to select a balanced diet from diets deficient, adequate, and superadequate in protein and to adjust to unsuccessful foraging bouts, as might occur in free-living birds. The Choice 1, Choice 2, and Disrupted Feeding groups did not differ significantly from each other in mean changes of body mass (all less than ca. 3%) or growth rates of P5 and R6. The differences between final lengths of original and induced feathers were small in the three groups and did not differ significantly from each other (Table 3), nor did the mean emergence lag of P5 (7 days after plucking) or R6 (8–10 days). The induced feathers in these three groups were free of defects.

The Marginal Protein Group lost body mass (−11%) during the first 9 days of the experiment, but then essentially stabilized and posted a small gain while the induced feathers were growing. The mean growth rates of P5 and R6 did not differ significantly from the rates in the Choice 1, Choice 2, or Disrupted Feeding groups,

TABLE 2. Change of body mass (g) and rate (mm/day) of feather replacement by winter-phase (January) White-crowned Sparrows (Series RA) fed chick-starter mash either *ad libitum* (Control) or 90% of *ad libitum* intake (Restricted). Values are $\bar{x} \pm$ SE; $n = 6$ birds/group.

		Emergence day[b]		Growth rate[c]	
Group	Body mass change[a]	P5	R6	P5	R6
Control	0.3 ± 0.19	9 ± 1.6	9 ± 1.7	3.66 ± 0.102	2.61 ± 0.090
P[d]	<0.001	0.211	0.976	0.013	0.228
Restricted	−1.9 ± 0.26	8 ± 1.4	9 ± 1.6	3.22 ± 0.129	2.44 ± 0.116

[a] Change of body mass during the 18 days of the experiment. Initial body mass did not differ between Control (26.3 ± 1.90 g) and Restricted (26.9 ± 0.74 g) groups.

[b] Primary 5 (P5) and rectrix 6 (R6) were pulled out following a 15-day acclimation period during which food intake was measured daily and body mass at 3-day intervals. Restricted feeding began on the day that feathers were pulled out (4 January).

[c] Growth rates are averages during the linear phase of feather elongation through approx. 30 mm.

[d] Probabilities (independent pairs t-test) that the two means do not differ from each other.

TABLE 3. Change of body mass (g), rate of feather replacement (mm/day), and relative length (mm) of induced feathers grown by well-nourished and malnourished autumn-phase (October to November) White-crowned Sparrows (Series PC). Values are $\bar{x} \pm$ SE; $n = 6$ birds/group.

Group[b]	Change body mass[a]		Growth		Length of new P5 minus old P5
	≤30 mm	>30 mm	P5	R6	
Choice 1	0.9 ± 0.96A	0.3 ± 0.56A	3.96 ± 0.156A	3.08 ± 0.223A	−0.50 ± 0.342
Choice 2	0.6 ± 0.79A	−0.02 ± 1.36A	4.06 ± 0.225A	3.08 ± 0.178A	−0.40 ± 0.245
Disrupted feeding	−0.5 ± 0.28A	0.8 ± 0.39A	4.08 ± 0.123A	3.05 ± 0.326A	0.67 ± 0.749
Marginal protein	−2.9 ± 0.51C	0.3 ± 1.96A	3.99 ± 0.203A	2.84 ± 0.171A	−2.90 ± 0.731A
Deficient protein	−9.6 ± 2.69B	8.8 ± 1.63B	2.53 ± 0.496B	2.02 ± 0.254B	−9.56 ± 2.337A

[a] Except for the Deficient Protein Group, the means show the changes of body mass during phases of P5 growth from emergent pin to ≤30 mm long and from 30 mm to the end of growth (>30 mm). See text for a description of the dietary regime in the Deficient Protein Group.

[b] Except in the right-hand column, means not followed by the same letter (compare vertically) differ significantly from each other ($P < 0.05$ by Tukey's HSD multiple comparison test). In the right-hand column (feather length) a letter following a mean indicates that the lengths of original and induced feathers differ significantly ($P < 0.05$ by paired t-test); absence of a letter indicates that they do not differ significantly.

but the R6 rate (2.84 mm/day) was appreciably less than the mean rates (3.05–3.08 mm/day) in those groups. The length of the induced P5 was significantly less, by approximately 3 mm, than the length of the original P5 (Table 3). In addition to being slightly shorter than the original feathers, the induced feathers were slightly paler. They did not contain any fault bars. Growth bars, when visible, were erratic and not clearly consistent with growth measures.

The birds of the Deficient Protein Group lost 24.0% (ca. 7.1 g) of their initial body mass during the first 9 days of the experiment, when they were subsisting on a 1.2% protein ration (Table 3). To avert a lethal loss of body mass we changed them to a 2.5% protein ration on day 10, after which they continued to lose body mass slowly and stabilized at a mean (±SE) of 20.0 ± 0.56 g on day 21. After day 24, when the measurements of feather growth were concluded, we fed the birds chick-starter mash for the concluding 9 days of the experiment, during which their mean body mass returned to essentially its initial level. The emergence lag for P5 averaged 9 days (excluding one outlier at 32 days), and the lag for R6 averaged 8 days. Neither differed significantly from the cognate means in any other groups in Series PC. The growth rates of P5 and R6 were only ca. 65% of the mean rates found in well-nourished birds, and were significantly less than in any other group in this series. The induced P5s averaged >9 mm shorter than the original P5s. Both the induced P5s and R6s were dwarfed, frayed, often curved, and paler than normal feathers. It is noteworthy that these feathers, grown by severely malnourished birds, did not contain any complete fault bars, but only occasional fault spots.

Series PT.—These experiments were designed to examine the effect of room temperature on the growth of induced P5s in birds fed either a submarginal (2.5% protein) or a superadequate (40% protein) ration beginning on day 9 after the feathers were plucked. During the 9 preceding days the mean body mass was essentially stable in all four groups; any change ranged from 0 to −0.3 g. During the first few days on the experimental ration, the birds of the two 2.5% protein groups lost nearly 3 g of body mass (Table 4). The group at 5°C continued to lose body mass very slowly until the end of the experiment. In contrast, the group at 20°C continued to lose body mass at a faster rate (significantly more than in the 5°C group). Their mean mass at the end of the experiment, however, was 24.8 g, and hence above a seriously debilitated level. Loss of body mass in the 40% protein groups was initially half or less than half the loss in the 2.5% protein groups, although the apparent difference is not statistically significant. During the second phase of induced feather growth, mean body mass stabilized and recovered moderately in the 5°C group but continued to decrease slightly in the 20°C group, which lacked the thermoregulatory stimulus of the cold-exposed group and therefore ate less and consumed less protein. Mean emergence lag in the induced feathers varied from 7 to 9 days, with no significant differences among the groups. Feather growth rates, although apparently slower at 5°C than at 20°C, likewise did not differ significantly from each other, nor from the P5 growth rates of the corresponding winter-phase controls in Series RA. There was no consistent pattern in the relative lengths of induced and original P5s. In the 2.5%

TABLE 4. Change of body mass (g), rate (mm/day) of feather growth, and relative length (mm) of induced feathers grown by winter-phase (January) White-crowned Sparrows (Series PT) exposed to 5°C or 20°C and fed diets containing 2.5% (n = 12 birds) or 40% protein (n = 6 birds). Values are $\bar{x} \pm$ SE.

Group[b]	Change of body mass[a]		P5 growth rate	Length new P5 minus old P5
	≤30 mm	>30 mm		
2.5% & 5°C	−2.8 ± 0.51A	−0.6 ± 0.30B	3.35 ± 0.179A	0.98 ± 0.601
2.5% & 20°C	−2.8 ± 0.36A	−3.2 ± 0.64A	3.55 ± 0.064A	−2.17 ± 0.431A
40% & 5°C	−1.4 ± 0.95A	0.6 ± 0.22B	3.62 ± 0.111A	2.75 ± 0.418A
40% & 20°C	−1.2 ± 0.29A	−0.4 ± 0.35B	3.69 ± 0.099A	0.60 ± 0.187A

[a] Changes of body mass during phases of P5 growth from emergent pin to ≤30 mm long and from 30 mm to the end of growth (>30 mm).

[b] Except in the right-hand column, means not followed by the same letter (compare vertically) differ significantly from each other ($P < 0.05$ by Tukey's HSD multiple comparison test). In the right-hand column (feather length), a letter following a mean indicates that the lengths of original and induced feathers differ significantly ($P < 0.05$ by paired t-test); absence of a letter indicates that they do not differ significantly.

group at 20°C, the induced P5 was significantly shorter than the original P5. Induced P5s were significantly longer than original P5s in both 40% groups, but the difference was small at 20°C. Induced feathers grown by birds fed 2.5% protein rations and kept at 20°C looked slightly paler and more fragile than original feathers or those grown by other birds in this series. The length of induced and original P5s did not differ significantly in the 2.5% protein group at 5°C.

Series AA.—These experiments examined the effects on feather growth of deficiencies of a single essential amino acid in an otherwise nutritionally adequate ration. Mean body masses in the five groups on the day that P5 and R6 were plucked were essentially alike (28.0–28.8 g). The birds in the four experimental groups maintained a stable mean body mass (2% change or less) through the 12 ensuing days before the beginning of Phase 1. During Phase 1, body mass diminished rapidly while the birds subsisted on the deficient rations. The birds in the 0.25 lys (−14.0% change of body mass) and 0.25 val (−16.2%) groups apparently lost more body mass than those in the 0.50 lys (−6.3%) and 0.50 val (−10.2%) groups, but the differences are not statistically significant (Table 5). At the end of Phase 1 the mean body mass in these groups, in the order just mentioned, was 23.7 g, 23.9 g, 26.6 g, and 25.4 g. When returned to the nutritionally adequate control rations (Phase 2), the birds in all experimental groups except 0.25 val recovered their initial body mass plus a substantial excess.

The mean growth rates of induced P5 and R6 in the 0.50 lys and 0.50 val groups did not differ from the rate in the control group (Table 5), but the growth rates of both feathers were slowed significantly in both the 0.25 lys group (by approx. −30%) and the 0.25 val group (by approx. −22%). The experiments were concluded before many of the induced feathers had reached full length, and so we do not have useful data on the relative lengths of induced and original feathers. Defects in induced feathers were evident in a few cases in birds that subsisted on the 0.50 lys and 0.50 val rations, and defects were pronounced in all birds that subsisted on the 0.25 lys and 0.25 val rations. Severe lysine deficiency produced more marked defects (curvature and fraying) than severe valine defi-

TABLE 5. Change of body mass (g) and rate (mm/day) of feather growth in autumn-phase (October to November) White-crowned Sparrows (Series AA) fed a complete diet (control, n = 6) or diets deficient in lysine (0.25 lys, 0.50 lys; n = 4 each) or valine (0.25 val, 0.50 val; n = 4 each). Values are $\bar{x} \pm$ SE.

Group[a]	Change of body mass[b]		Growth rate	
	Phase 1	Phase 2	P5	R6
Control	1.1 ± 0.20A	0.2 ± 1.18A	3.80 ± 0.091A	2.96 ± 0.143A
0.50 lys	−1.8 ± 0.42B	3.6 ± 1.08AB	3.89 ± 0.074A	2.88 ± 0.083A
0.25 lys	−3.9 ± 1.02B	6.3 ± 1.47B	2.71 ± 0.212B	2.00 ± 0.106B
0.50 val	−2.9 ± 1.13B	4.4 ± 1.53AB	3.50 ± 0.090A	2.64 ± 0.068A
0.25 val	−4.7 ± 0.11B	4.5 ± 0.71AB	3.03 ± 0.159B	2.24 ± 0.142B

[a] Means not followed by the same letter (compare vertically) differ significantly ($P < 0.05$ by Tukey's HSD multiple comparison test).

[b] Changes of body mass while experimental birds were consuming the deficient diets (Phase 1, 9 days for 0.50 lys and 0.50 val, 6 days for 0.25 lys and 0.25 val) and after return to the control diet (Phase 2) until the end of feather growth.

ciency. Lysine deficiency also caused complete depigmentation in up to half the length of P5 and R6 in one bird in the 0.50 lys group and in all birds in the 0.25 lys group. Fault bars were not associated with feather defects.

Failure to induce replacement feathers.—If a new pinfeather did not appear at the surface of a follicle within 32 days (an ample but arbitrary span dictated by experimental schedules) after plucking the original feather, we defined the case as a *failed induction.* Induction failed in 8% of 88 P5s and 3% of 64 R6s. The proportions do not differ significantly from each other (P = 0.239 by Chi-square test), and the grand mean is 6% of 152 feathers. Failure to induce a wing feather was not correlated with failure to induce a tail feather, and vice versa. Plucking failed to induce a new feather in the Series AA control group (2 birds), Series PC Deficient Protein (2 birds) and Marginal Protein (1 bird) groups, Series AA 0.50 lys (2 birds) and 0.25 val (1 bird) groups, and Series PT 2.5% at 5°C group (1 bird). The distribution of failed induction in control groups to that in experimental groups is 1:7. This is approximately the same ratio as the numbers of control and experimental birds (12:88 = 1:7.3), which suggests that failure of feather induction is not affected by nutritional status. If the emergence of a pinfeather required 14 days or more after plucking, we classified induction as "delayed." The normal mean plus 1 SD is 13 days. Four of 152 cases of emergence time were delayed: 1 in Series AA controls, 1 in Series PT 40% at 20°C, and 2 in Series PT 2.5% at 5°C. Thus, neither failure nor delay of feather induction was correlated with nutritional status in captive White-crowned Sparrows. This contrasts with results from free-living Gray Jays (*Perisoreus canadensis*), in which feather induction sometimes failed in birds that lacked food supplementation in their territories, but induction never failed in birds that occupied food-supplemented territories (Waite 1990). The nutritional status of the jays was unknown, however, as they were not weighed, and their food consumption was unknown.

Seasonal variation in feather growth rate.—During the postnuptial molt the linear-phase growth rate of P5 averaged (±SE, n = 12) 4.29 ± 0.037 mm/day in the two Series PC Choice groups combined. In contrast, in autumn-phase (Table 5) and winter-phase (Table 2) controls, the growth rate of induced P5s averaged 3.80 ± 0.091 mm/day in autumn-phase controls and 3.66 ± 0.102 mm/day in winter-phase. The autumn and winter means do not differ significantly from each other (P = 0.389), but both differ from the mean growth rate during postnuptial molt ($P < 0.0001$ in both cases, by Tukey's HSD procedure). The mean growth rate of induced R6s was significantly greater (P = 0.041) in autumn (2.96 ± 0.143 mm/day) than in winter (2.61 ± 0.090 mm/day) (cf. control groups in Table 5 and Table 2, respectively). We did not record the growth of R6 during postnuptial molt because these rectrices are often frayed or broken in caged birds. The mean growth rates of R1-1 (which are about the same length as R6) during postnuptial molt were 3.43 ± 0.060 mm/day in 22 adult *Z. l. gambelii* subsisting on 10% dietary protein, and 3.50 ± 0.050 mm/day in 18 adults subsisting on 20% dietary protein (M. E. Murphy and S. Pearcy unpubl. obs.). During the prenuptial molt in March, the mean growth rate of R1 through the first 10 days after emergence is ca. 3.12 mm/day in free-living *Z. l. gambelii* (Mewaldt and King 1978). In three captives feeding *ad libitum* on chick-starter mash, we found that growth rates of R1 during prenuptial molt ranged from 3.06 to 3.19 mm/day, bracketing the mean found in free-living birds. We believe that seasonal variation in feather growth rates is independent of nutritional status.

DISCUSSION

To fulfill the expectations attributed to ptilochronology (Grubb 1989), the technique must comply with several tacit assumptions:

Assumption 1: Each pair of growth bars results from 24.00 hours of elongation of the feather.—Our own results only indirectly address this assumption, but scattered data in the literature lead us to conclude that it is essentially correct in doves (Riddle 1907, see also Whitman 1907: 14), and sometimes in *Z. l. gambelii* and House Finches (*Carpodacus mexicanus*) (Michener and Michener 1938). Wood (1950: 486), who surveyed the occurrence of growth bars in a wide variety of species, reported that "the visibility of growth bars varies greatly among birds, from nothing to very marked," thus paraphrasing an earlier and much more extensive survey (Glegg 1944) that he had overlooked. Michener and Michener (1938) were ambivalent. Although they concluded that a pair of bars does represent one day's growth, they cautioned (p. 153) against

the use of growth bars to estimate feather growth rate, especially if samples are small. Therefore, a systematic analysis of growth-bar characteristics in the species to be studied should be undertaken to dispel lingering doubts about the reliability of Assumption 1.

Assumption 2: There is a predictable, exclusive, and direct relationship between the growth rate and final length of feathers and the bird's nutritional status while the feathers are growing, regardless of the type of malnutrition.—This assumption is implicit in such statements as these: "Individual growth bars . . . can be counted and measured to establish the number of days taken to grow the feather and which days were more nutritionally constrained than others" (Grubb 1989: 315). Moreover, ". . . feather growth can be divided a posteriori into units (the dark and light portions of the growth bars) that correspond to less than 24-h of growth. Therefore, fine-scale comparisons can be made of feather growth as an index of nutritional status . . ." (p. 317).

Severe malnutrition during the annual molt may indeed shorten rejuvenating feathers, slow their growth, or reduce their mass (Murphy et al. 1988). The growth rates of even single induced feathers appear to be reduced by mild food restriction (90% of control intake of a balanced ration), although the difference of rates was statistically significant only in P5, not in R6 (Table 2). Unlimited access to a protein-deficient ration (1.2% protein) was associated with significant shortening of an induced P5 and reduction of growth rate in P5 and R6 in autumn-phase White-crowned Sparrows, but a marginal-protein ration (4.5% protein) that also induced a significant level of malnutrition (loss of body mass) was associated with only equivocal effects on feather growth (Table 3). In winter-phase birds, in contrast, unlimited access to a submarginal ration (2.5% protein) produced no significant effect on feather growth, compared with birds subsisting on a high-protein ration (Table 4). The growth rates of induced P5 and R6 were significantly reduced in birds subsisting on an otherwise balanced ration that contained only 25% of their lysine or valine requirements, but the growth rates were normal in malnourished birds (significant loss of body mass) subsisting on rations containing 50% of the requirements (Table 5).

This indicates that some *but not all* planes and forms of malnutrition (unseasonal loss of body mass) shorten induced feathers or slow their growth rate, and also notes that the threshold of response may vary seasonally. These data also suggest that the ptilochronological assumption that malnutrition is the *only* cause of slower growth or shorter length in induced feathers needs to be extensively qualified. There is, moreover, direct evidence that factors other than malnutrition affect the growth of induced feathers. We already noted (in Results) that the intrinsic growth rate of induced feathers (both P5 and R6) in well-nourished birds was less in winter than in autumn, and that both were less than during the postnuptial molt. This indicates the existence of an annual cycle of follicular activity or sensitivity. This temporal variability of intrinsic growth rate severely constrains the applications of ptilochronology to vertical studies within brief spans of time, or else makes necessary a calibration of the growth rates of induced feathers in well-nourished birds across seasons.

Young birds growing their postnatal plumage may also undergo a seasonal cycle of growth rate. A seasonal variation of growth rate in the rectrices of nestling Rock Doves (*Columba livia*) was attributed by Janiga (1986) to seasonal variation of food supply. The delivery of food to the squabs was not measured, however, and it is equally plausible that the seasonal variation of growth rate (4.07 mm/day in March to June, 5.06 in July to August, 4.85 in September to October, and 4.05 in November to February) is a manifestation of an annual cycle of intrinsic rate, as we found in adult sparrows. Across seasons, some factor other than nutritional status, and not linked to decreased fitness, affects feather growth rates. The relationship between feather growth rate and nutrition is therefore not an exclusive one.

Air temperature, wind chill, and day length have been nominated as factors that might affect the growth of induced feathers, the first two by reducing follicular temperature and directly slowing growth rate, and the third by affecting foraging time and food intake (Grubb 1989). Results from our Series PT experiments, however, showed no consistent relationships between room temperature and feather growth (Table 4). Growth rates did not differ significantly between the two temperature groups in either dietary regime. In the low-protein group at 20°C, the induced P5 was substantially shorter than the original P5, although the shortening did not differ significantly from that in the high-

protein group at 20°C. This effect in the low-protein group at 20°C was not unexpected, for reasons mentioned in the Results section; but the functional basis of the significantly longer induced P5 in the high-protein group at 5°C is an enigma. In contrast, Honda et al. (1982) reported that air temperature (10°C vs. 23°C) did not significantly affect the final length of induced contour feathers in Japanese Quail (*Coturnix japonica*). In short, air temperature does not have a predictable effect on induced feather growth.

Day length may be not only a permissive factor affecting food intake by constraining foraging time, as Grubb (1989) suggested, but also an inductive signal that affects physiological status. This distinction is crucial conceptually but difficult to sort experimentally. There are only a few data pertinent to the topic. Contrary to Grubb's suggestion, Honda et al. (1982) found that the final length of induced contour feathers in Japanese Quail was greater in feathers grown in L:D 8:16 than in feathers grown in L:D 16:8. Growth rates were the same in the two groups, but growth lasted about a day longer in the short-day group. We showed previously that the growth rates of primary remiges during postnuptial molt in White-crowned Sparrows are statistically indistinguishable in birds kept in L:D 12:12, 16:8, and 20:4, even in subgroups in which food was available for only 12 h of a 16-h or 20-h day (Murphy and King 1986). We found later that mean daily *ad libitum* food intake by molting White-crowned Sparrows did not differ significantly in these three photocycles (Murphy and King 1990).

Assumption 3: Feather growth slows immediately upon the onset of a nutritional shortage (i.e. there is no metabolic latency).—If, as Grubb (1989) proposed, growth bars provide a "day-by-day record of nutritional regime," and the dark and light bands of a growth bar allow "fine-scale comparisons" within a day, then it follows that Assumption 3 must be fulfilled. We can find no evidence, however, that this is so. Riddle (1908: 361) reported that "the rate of linear feather-growth is not affected until the third or fourth day" after the onset of total starvation in doves, and thereafter decreases rapidly. Moreover, we have noted that the mean span of time between plucking and first appearance at the skin of induced feathers was essentially invariant among all our experimental groups. The emergence span was not shorter in well-nourished than in malnourished birds. Finally, we noted that disrupted feeding involving foodless episodes during two of every three days in *Z. l. gambelii* did not slow feather growth (Table 3). These results are difficult to reconcile with Assumption 3, which indicates that its reliability merits further analysis.

Assumption 4: Feather growth slows in direct proportion to the magnitude of a nutritional shortage.—If, as Grubb (1989: 315) proposed, growth bars can show "which days were more nutritionally constrained than others" or that the "extent to which feather growth in nature falls below that of birds living in optimal, artificial conditions [can] be used as an index of nutritional status," then it follows that Assumption 4 must be fulfilled. We find no evidence of a consistent positive correlation between feather growth and nutritional status. Some results suggest a correlation but others do not. For instance, the growth rates of primary remiges in White-crowned Sparrows during postnuptial molt were slower in birds consuming 60% of a control group's intake of a balanced ration than in birds consuming 80% of the control's intake (Murphy et al. 1988). Moreover, the mean growth rate of an induced P5 in Group 2.5% at 20°C (Table 3) is intermediate between the rates in the Marginal Protein and Deficient Protein groups (Table 3), so that the ordination of dietary protein concentration matches the ordination of growth rates: 1.2, 2.5, 4.5% vs. 2.53, 3.55, 3.99 mm/day. The apparent correlation is nonlinear, however, and the comparison is complicated by seasonal differences. Group 2.5% at 20°C consisted of autumn-phase birds and the other two groups consisted of winter-phase birds, in which the intrinsic growth rate of induced feathers is lower than in autumn.

The above results are loosely consistent with Assumption 4, but other results are not. The growth rates of induced P5s did not differ significantly between the low- and high-protein groups at either room temperature (Table 4). Moreover, although rations that contained a quarter of the maintenance requirements for lysine or valine (0.25 lys, 0.25 val) slowed the mean growth rates of induced P5s and R6s compared with the rates in birds subsisting on rations that contained half the requirement (0.50 lys, 0.50 val), the rates in the latter did not differ from rates in well-nourished control birds (Table 5). These two experiments reveal no correlation between induced feather growth and

dietary protein concentration, and an apparent correlation with dietary essential amino acid income that does not manifest itself until a severe level of privation (less than half the maintenance requirement) is imposed. In short, it is not possible at present to predict either the trophic conditions in which Assumption 4 might be valid or the form of a calibration curve (linear, nonlinear, monotonic, polytonic) when a correlation exists.

Assumption 5: The daily growth rate of the original feather (DGO) occurred when birds were well-nourished and, if two or more birds are being compared, in otherwise identical environmental conditions.—If, as Grubb (1989) proposed, "DGI/DGO [is]... taken to be positively correlated with nutritional status" (DGI = daily growth rate of an induced feather), then Assumption 5 must also be valid if reliable conclusions are to be reached. The original feather may be grown during a regular molt or may be a replacement for a feather, or one of a succession of feathers, lost later. The probability that a bird was well-nourished during a DGO period is unknown. The reliability of the DGI/DGO index cannot be evaluated. The uncertainty is compounded by the seasonal variability of feather growth rates, as already mentioned. The conditions in which the DGI/DGO ratio is serviceable appear to be very limited.

Assumption 6: Episodes of nutritional privation always coincide with the standard segment of growth-bar measurement.—The use of ptilochronology in either a natural or an experimental setting implies acceptance of Assumption 6. Standard technique (Grubb 1989) includes measurement of 10 successive growth bars centered at a point two thirds of the feather's length from its proximal end. Malnutrition that occurred at a time not represented in this measured segment would be overlooked. Latency in the response to malnutrition (i.e. nonconformance with Assumption 3) could cause effects on growth rate that fall outside the limits of the measured segment. Field experiments and protocols must be planned and executed with great precision if Assumption 6 is to be fulfilled.

Assumption 7: Any adjustment of a bird's metabolism that can result in a reduced rate of tissue synthesis, as reflected by DGI or DGI/DGO, indicates the occurrence of a nutritional challenge sufficient to affect the bird's life-style and (or) fitness.—This assumption pervades the logic of ptilochronology and is crucial to its integrity. When you accept conclusions based on this method, it means that, regardless of your opinion about the six other assumptions, you are satisfied that Assumption 7 has been fulfilled. Unfortunately, it appears to us that it is impossible at present to verify Assumption 7 empirically, which is why we have addressed it last. It is troublesome that one of the key precepts of ptilochronology is untested, and perhaps untestable. We have discussed elsewhere (King and Murphy 1985) our reservations about interpreting changes of production rates or body mass as uniform indices of nutritional stress and individual fitness.

In the foregoing review we believe we have avoided assaults on straw men, and we think we have concentrated on the major features—pro and con—of ptilochronology. We conclude that the method is burdened with uncertainty, conceivably serviceable only under closely controlled conditions, and then acceptable only to practitioners who have an affirmative opinion about Assumption 7.

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