Letters

J. Avian Biol. 41: 1–5, 2010
doi: 10.1111/j.1600-048X.2010.05175.x

Received 25 February 2010, accepted 16 September 2010

Feather growth bands and photoperiod

Roger Jovani, Julio Blas, Carlos Navarro and François Mougeot

R. Jovani (jovani@ebd.csic.es), UFZ, Helmholtz Centre for Environmental Research, UFZ. Permoserstr. 15. 04318 Leipzig, Germany. Present address for RJ: Dept. of Evol. Ecol., Doñana Biol. Station (CSIC), Avda. Americo Vespuccio s/n, E-41092 Seville, Spain. – J. Blas, Dept. of Conserv. Biol., Doñana Biol. Station (CSIC), Avda. Americo Vespuccio s/n, E-41092 Seville, Spain. – C. Navarro, Estación Experimental de Zonas Áridas, CSIC, Ctra. de Sacramento s/n La Cañada de San Urbano, E-04120 Almería, Spain. – F. Mougeot, Estación Experimental de Zonas Áridas (EEZA, CSIC) and Instituto de Investigación en Recursos Cinegéticos (IREC, CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain.

Growth bands are alternate dark/light bands perpendicular to the feather rachis. Previous studies indicate that pairs of dark/light bands are grown every 24 h, with light bands being produced at night, and dark ones during the day. Thus, the dark:light width ratio could reflect the photoperiod under which a feather was grown. We tested this hypothesis by inducing feathers to grow under contrasting photoperiods, using red-legged partridges *Alectoris rufa* as a model species. We first validated the assumption that a pair of dark/light band is produced every day. Secondly, we show that dark/light width ratios remain close to 1:1, irrespective of the photoperiod under which feathers were grown. Dark:light width ratios of feathers grown in summer (15 light-hours:9 Darkness-hours) and winter solstices (9l:15d) did not show any consistent pattern of variation within individuals. Thus, the dark/light banding patterns are not simply the product of light regimes and are not indicative of photoperiod. This finding, together with reports of ''aberrant'' growth band patterns (e.g. two growth bands produced over 24 h instead of one) challenges our current knowledge of growth bands. We propose that the normal circadian periodicity of growth bands is primarily driven by circadian rhythms: band formation starts at a point of critically low physiological activity (e.g. during night resting), and thus every 24 h irrespective of photoperiod. Our experiment emphasises that our knowledge of growth bands is weaker than previously appreciated, and that the study of dark/light band patterns on feathers could shed new light on interesting phenomena such as unusual avian biological rhythms and the functioning of internal clocks. Detecting ''aberrant'' banding patterns could therefore allow identifying bird species with unusual activity patterns or physiological rhythms.

Bird feathers show an intriguing banding pattern called ''growth bands'' (also known as ''growth bars''; not to be confounded with ''fault bars'', Jovani and Blas 2004). These are ubiquitous in birds, like for instance the large sandhill crane *Grus canadensis* or the tiny amethyst-throated hummingbird *Lampornis amethystinus* (Fig. 1). Growth bands are pairs of alternate light/dark bands some millimetres wide and perpendicular to the feather rachis (Riddle 1907, 1908, Michener and Michener 1938, Wood 1950). According to previous studies, two key features define growth bands. First, each growth band normally documents 24 h of feather growth (Riddle 1907, 1908, Michener and Michener 1938, Murphy and King 1991, Brodin 1993). Second, experiments have shown that the dark portion of each growth band is produced during the day and the light part during the night (Riddle 1907, 1908, Michener and Michener 1938, Wood 1950).

Early work suggested a link between growth bands and body condition: the better the nutritional status of the bird, the wider the growth bands (Riddle 1908, Wood 1950). In 1989, Grubb introduced the term ''ptilochronology'' as the study of the body condition of birds by measuring the width of growth bands, and this has become a common research tool in avian ecology (reviewed in Grubb 2006). As far as we know, one century after these pioneering studies on growth bands, nobody has proposed or tested whether the ratio of dark/light growth band width could indicate the photoperiod during feather growth, and thus become a potent non-invasive tool with many applications. For instance, knowing the photoperiod during the moult of the innermost and the outermost primary feathers of a migratory passerine (i.e. the first and last to be replaced during complete primary moult) one could infer moult duration without recapturing the bird, and assess the latitude of the moulting grounds. This would not only be relevant for studies on migration, moult and avian physiology, but the use of historical samples (e.g. museum specimens) could also provide far-reaching applications for rapidly evolving research topics such as global change.

Here we report on a test of the hypothesis that the light/dark ratio of growth bands mirrors the photoperiod during feather development (Fig. 2). We used an experimental approach of plucking feathers from a same group of birds to induce re-growth under contrasting photoperiods.

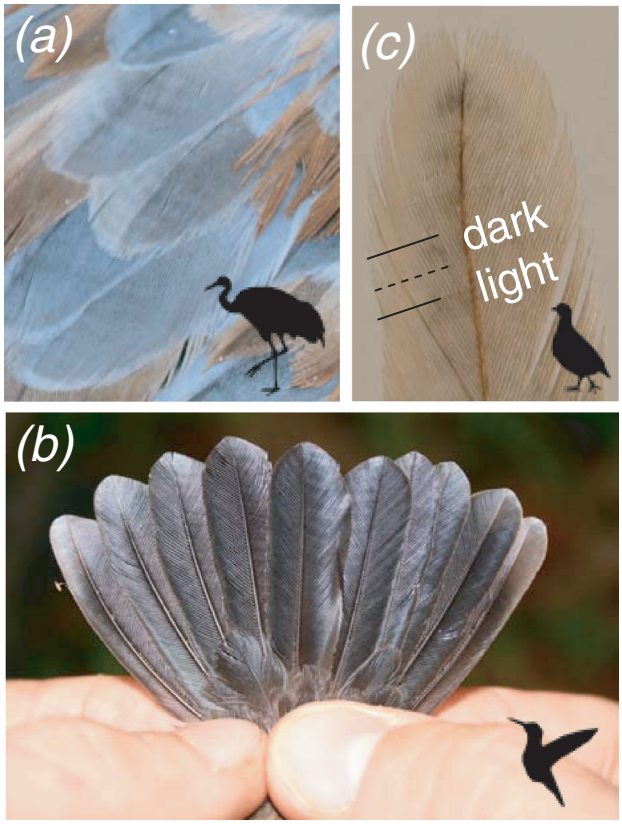

Figure 1. (a) Growth bands on the greater wing coverts of a sandhill crane *Grus canadensis* (photo credit: Janet Hug). (b) Growth bands on the tail feathers of an amethyst-throated hummingbird *Lampornis amethystinus* (photo credit: Santiago Guallar), and (c) A portion of the studied underwing feather of a red-legged partridge. Each pair of dark and light bands constitutes a growth band (photo credit: Julio Blas).

We predicted that the dark/light band ratio would be greater for feathers grown during the summer solstice (longer days) than during the winter solstice (longer nights).

Methods

By plucking a feather one can induce its rapid replacement (Grubb 2006). Our goal was to obtain feathers grown under contrasting photoperiods by the same birds. For the experiment, we used 30 captive red-legged partridges *Alectoris rufa* held in outdoor cages at the Lugar Nuevo breeding facility (Andújar, Jaén, Spain). Throughout the study, the maintenance of the cages and food provisioning were done in the morning, with no subsequent visits after dusk. The isolated nature of the farm (nearest village is ca 30 km away) and the careful work of the animal caretakers ensured that the birds were not disturbed by sound or light at night.

We plucked the two largest underwing feathers (one per wing) three times from June 2004 to February 2005 (Fig. 2). We thereby obtained from the same birds feathers grown during: 1) the longest days of the year (summer solstice; 15 light hours: 9 dark hours), 2) the shortest days of the year (winter solstice; 9l:15d), and 3) during two intermediate photoperiods (in summer and autumn).

From each set of feathers, we selected those showing obvious growth bands (129 feathers from 28 partridges). Each feather was scanned at 300dpi and images analysed using the ImageJ software (http://rsb.info.nih.gov/ij/). We selected the longest section of each feather with continuous growth bands (median = 4, range = 2–8 growth bands), measured the length of each dark portion of the growth band and the total length of the selected feather segment, and calculated the mean% of dark band width in each feather (i.e. a 50% indicates a dark: light ratio of 1:1). We repeated each measure twice and found a high repeatability (intraclass correlation coefficient: $r = 0.91$, ANOVA $F_{61,62} = 21.21$, DF = 61,62, $p < 0.001$; repeatabilities calculated following Lessells and Boag 1987). For subsequent analyses, we used the average % of dark band from the two feathers (whenever available) collected from each bird, reducing the sample to 86. Overall, we obtained data from 16 birds with feathers grown under both 15l:9d and 9l:15d photoperiods.

In order to measure the number of growth bands produced per day, we performed a second experiment, which involved six captive red-legged partridges held at Dehesa de Galiana (IREC, Ciudad Real, central Spain) under the same conditions described above. Underwing feathers (one from each wing) were plucked on April, 25th 2009. On May, 13th 2009, we checked the length of the re-growing feathers and marked their base with a marker pen. Six days later (May 19th) we collected the feathers, which were still growing. Growth bands were easy to visualize at the center of the feathers, and were counted within the section comprised between the ink mark and half the distance towards the base of the feather, i.e. in a section grown during three consecutive days. We were able to do so accurately for 10 out of the 12 feathers collected.

Results

According to our hypothesis, the % of dark band width in each growth band should differ between feathers grown under contrasting photoperiods. However, no significant differences were found (ANOVA $F_{82,3} = 0.667$, $p = 0.575$; overall mean% ($\pm$ SD) 56.97; Fig. 3a). Under the direct influence of photoperiod, the expected% of dark bands on feathers were 62.5% for feather grown during summer solstice (photoperiod = 15l:9d) and 37.5% for feather grown during winter solstice (9l:15d). However, we found 54.7 and 57.1% of dark bands, respectively (Student's t-Test: $t = -1.05$, $p = 0.302$; Fig. 3a). Moreover, we found no consistent patterns of variation within individuals between these two contrasting photoperiods (paired Student's t-Test, $t = -1.19$, $p = 0.251$), rejecting the possibility of a weak but consistent effect of photoperiod on the light:dark ratio at the individual level (note the crossing of lines in Fig. 3b). Finally, in our second experiment, we confirmed that one growth band corresponded to a 24-h period of feather growth: we counted three growth bands in all the feather portions ($n = 10$) that were growing over the course of three days. In three feathers, growth bands could be counted along the entire feather segment, and we also found six growth bands over a six-day feather growth period.

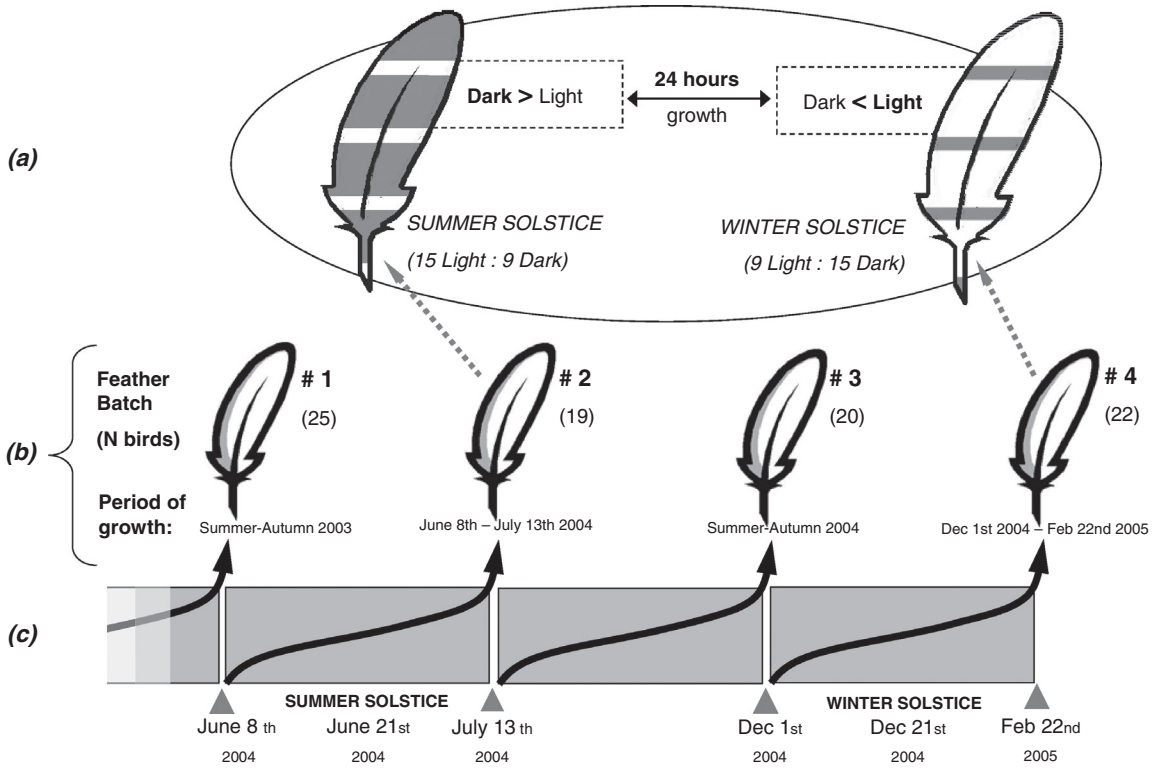

Figure 2. Summary of the hypotheses tested in this study (a) and the experimental design (b) in relation to calendar date (c). (a) Hypotheses: we tested the hypotheses that: (1) one growth band corresponds to a 24 h-period of feather growth, and that (2) the photoperiod exerts an effect on the dark/light ratio of growth bands. Note that the dark portions of the growth bands are grown during the day. (b) Experimental design: four batches of feathers (batches no. 1 to no. 4; codes for Fig. 3) were collected from the same birds throughout the year. The sequential plucking allowed assessing the period of feather growth. (c) Calendar of events: grey arrows indicate dates when feathers were experimentally plucked and collected, and black arrows represent the periods of feather growth between sequential removals.

Discussion

Our study confirms the 24 h = one-growth-band equivalence in red-legged partridges, as already reported in most investigated bird species. Our experiment also allowed us to reject our initial hypothesis that the dark/light width ratio of growth bands depends on the photoperiod during feather development. This rejection challenges our current knowledge about growth bands, as we discuss below.

Recent studies have reported two growth bands (instead of the commonly reported single growth band) every 24 h in Laysan albatross *Phoebastria immutabilis* and nestling pied flycatchers *Ficedula hypoleuca* (Langston and Rohwer 1996, Kern and Cowie 2002). These "aberrant growth band patterns" challenge our traditional view of growth band patterns, and the validity of some assumptions on which the study of ptylochronology is based. For instance, Mauck and Grubb (1995) studied growth bands in breeding Leach's storm petrels *Oceanodroma leucorhoa* but did not mention the chaotic pattern of light and dark bands they found until eleven years later (Grubb 2006).

Our results, together with the reports of unusual growth band patterns indicate that some basic, and until now widely accepted assumptions about growth band formation, should be reconsidered. More specifically, the reported cases of two (instead of one) growth bands every 24 h, and our experimental results that failed to detect a relationship of growth bands with photoperiod suggest that "light" and "dark" bands are not necessarily linked to photoperiod regimes, and that the mechanism producing such growth banding on feathers is not directly influenced by light exposure.

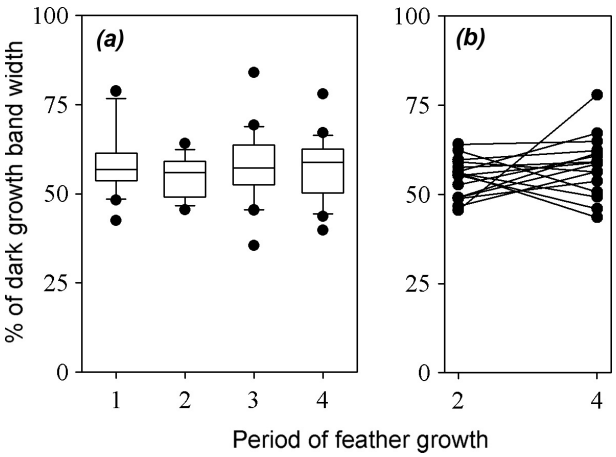

Figure 3. (a) Box-plot of the % of dark bands (i.e. 50% means equal width of dark and light bands) in feathers grown under different photoperiod regimes (see Fig. 2 for the codes; boxes show data within 25th and 75th percentiles, and middle line the median; dots indicate outliers). (b) Changes on the % of dark band width within birds from summer solstice (15 h of light: 9 h of darkness; code 2) to winter solstice (15l:9d; code 4).

We propose an alternative hypothesis, rescuing some ideas formulated a century ago by Oscar Riddle (1907, 1908) to explain these inconsistencies and our unexpected results. Riddle proposed that the shift from dark to light growth bands is triggered by the reduced blood pressure of birds during sleep (he even specified that this would occur between 1 and 5 a.m.). He made this link by suggesting that a change in blood pressure changes the nutrition of the feather follicle during feather growth. If the light portion of the growth band needs about half a day to develop before starting the dark portion, this could explain why one growth band is produced every 24 h regardless of photoperiod, because night sleep occurs every 24 h irrespective of photoperiod. This decouples growth bands from light exposure per se, and instead links growth band patterns to circadian clocks, which control blood pressure rhythms and sleep-wake dynamics in birds. If this is true, growth bands would reflect the periodicity of resting episodes that are strong enough to lower blood pressure (e.g. during deep sleep). Thus, the circadian periodicity of resting events could be producing the normal growth band 24 h-periodicity, and chaotic light-dark banding (such as those reported by Mauck and Grubb 1995) could be revealing a lack of regular diel periodicity.

If the dark/light banding and the number of growth bands produced per day are both related to avian physiology, this opens a new window for research: the study of growth bands could inform on circadian rhythms and biological clocks (a circadian clock within the follicle itself could directly control the light/dark banding during feather growth). This alternative hypothesis could be tested by comparing the growth band patterns of feathers grown from individuals kept in constant darkness or kept under constant light exposure. The study of growth bands patterns in museum specimens could also provide an easy way of identifying species with unusual growth band patterns and therefore possibly atypical circadian rhythms that could be studied in the field afterwards. Comparing growth band width with the expected daily feather growth rates of each species, according to recently developed scaling laws (Rohwer et al. 2009), would quickly reveal bird species with unusual growth banding patterns that do not follow the one-growth-band = one-day equivalence rule.

Acknowledgements – We thank Paloma Prieto, the staff of the Lugar Nuevo experimental facilities and the Consejería de Medio Ambiente de la Junta de Andalucía for their cooperation and assistance. RJ, JB and CN were founded with a postdoctoral fellowship from the Ministerio de Ciencia e Innovación (Spain), a postdoctoral I3P contract (CSIC/European Union) and a predoctoral grant from the Spain's Ministry of Science and Technology (BES-2005-8619) respectively. Michaela Hau, Dale Kennedy and an anonymous reviewer made useful comments on a previous draft.

References

Brodin, A. 1993. Radio-ptilochronology – tracing radioactively labelled food in feathers. – Ornis Scand. 24: 167–173.

Grubb Jr., T. C. 1989. Ptilochronology: feather growth bars as indicators of nutritional status. – Auk 106: 314–320.

Grubb Jr., T. C. 2006. Ptilochronology: feather time and the biology of birds.–Oxford University Press.

Jovani, R. and Blas, J. 2004. Adaptive allocation of stress-induced deformities on bird feathers. – J. Evol. Biol. 17: 294–301.

Kern, M. D. and Cowie, R. J. 2002. Ptilochronology proves unreliable in studies of nestling pied flycatchers. – Ibis 144: 23–29.

Langston, N. E. and Rohwer, S. 1996. Molt-breeding tradeoffs in albatrosses: life history implications for big birds. – Oikos 76: 498–510.

Lessells, C. M. and Boag, P. T. 1987. Unrepeatable repeatabilities: a common mistake. – Auk 104: 116–121.

Mauck, R. A. and Grubb, J. 1995. Petrel parents shunt all experimentally increased reproductive costs to their offspring. – Anim. Behav. 49: 999–1008.

Michener, H. and Michener, J. R. 1938. Bars in flight feathers. – Condor 40: 149–160.

Murphy, M. E. and King, J. R. 1991. Ptilochronology: a critical evaluation of assumptions and utility. – Auk 108: 695–704.

Rohwer, S., Ricklefs, R., Rohwer, V. and Copple, M. 2009. Allometry of the duration of flight feather molt in birds.–PLoS Biology 7 (6) DOI: 10.1371/journal.pbio.1000132 [PLEASE update if possible]

Riddle, O. 1907. A study of fundamental bars in feathers. – Biol. Bull. 14: 165–174.

Riddle, O. 1908. The genesis of fault-bars in feathers and the cause of alternation of light and dark fundamental bars. – Biol. Bull. 14: 328–370.

Wood, H. B. 1950. Growth bars in feathers. – Auk 67: 486–491.

Appendix 1. Table provides data used in Fig. 1a, b

Mass reduction (%) of tissues in birds during active migration (in-flight starvation) or during simulated migration (fasted in laboratory), and the rate of carbon incorporation (k, in days^{-1}) into certain organs or tissues in birds. We included only those data sets with measurements of mass change for at least three different tissues. All available data for isotopic incorporation in birds are shown. Graphs in Fig. 1 comprise all available data listed, apart from the data for the non-migratory barn owl. Data points for rank order in Fig. 1a are based only on citations for which data of all seven tissues (small intestine, liver, gizzard, kidney, heart, flight and leg muscle) are available and aerial feeding can be excluded (great knot; Battley et al. 2000, Battley et al. 2001; garden warbler Hume and Biebach 1996, Schwilch et al. 2002, Bauchinger et al. 2005); pied flycatcher and willow warbler (Schwilch et al. 2002)).

	Small intestine	Liver	Gizzard	Kidney	Heart	Flight muscle	Leg muscle	Sampling	Ref.
Percent reduction during natural migration									
Calidris tenuirostris	64	60	61	56	23	24	14	before and after a flight of 5400 km across the Pacific	Battley et al. 2000 (1)
Sylvia borin	34	35	36	28	30	4	11		Schwilch et al. 2002 (1)
Hirundo rustica#	54	52	20	19	32	35	+15	during migration over continental Europe in autumn and after a flight across the Sahara and the Mediterranean Sea in spring	Schwilch et al. 2002 (1)
Ficedula hypoleuca	36	46	14	22	21	14	5		Schwilch et al. 2002 (1)
Phylloscopus trochilus	37	38	29	20	20	15	8		Schwilch et al. 2002 (1)
Sylvia borin	51	57	34	42	23	25	14	before and after flight across the Sahara during spring migration	Bauchinger et al. 2005 (2)
Percent reduction during simulated migration									
Calidris tenuirostris	42	40	18	31	15	20	11	before and after ca 2 week fast	Battley et al. 2001 (1)
Sylvia borin	63	24	21	30	8	7	7	before and after a 2 day fast	Hume and Biebach 1996 (3)
Sylvia atricapilla	52	54	20					before and after a 2.5–3 day fast	Karasov and Pinshow 1998 (4)
Sylvia atricapilla	45	36	20		21	19		before and after a 1–2 day fast	Karasov et al. 2004 (5)
Tyto alba##	(43)*	61	(43)*		22	34	16	before and after a fast of 2.7–7.7 days	Thouzeau et al. 1999 (6)
Rate of isotopic incorporation (k, in days^{-1})									
Passer domesticus **	0.330	0.200	0.103		0.104	0.082		Sampling over 128 days after diet shift	Carleton et al. 2008
Poephila gutatta	0.330	0.284	0.270	0.248	0.058	0.051	0.038	Sampling over 256 days after diet shift	Bauchinger and McWilliams 2009
Coturnix japonica		0.272				0.056		Sampling over 212 days after diet shift	Hobson and Clark 1992

not included in analysis because aerial feeding during migration can not be excluded.
non-migratory species, not included in analysis.
* data only available for total digestive tract.
** we estimated *k* as $1/\tau$ for tissues best described by one-compartment model and as $k_{\text{two comp}} = pk_1 + (1-p)k_2$ for tissues best described by two-compartment model, where k_1 is the carbon incorporation rate ($1/\tau_1$) for $pool_1$ with pool size p and k_2 is the carbon incorporation ($1/\tau_2$) rate for $pool_2$ with pool size $1-p$.
- Mass changes are based on (1) lean dry tissue, (2) lean dry tissue, with percentage of fat quantified for pooled data of digestive tract (proventriculus, gizzard, small intestine and colon) and subtracted from dry mass of gizzard and small intestine, (3) dry whole tissue, (4) lean wet tissue, (5) wet whole tissue for small intestine and pectoral muscle, dry whole tissue for heart, liver and gizzard, (6) protein estimation.