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TECHNICAL REPORT

Indirect, physiological assessment of reproductive state and breeding chronology in free-living birds: an example in the Marbled Murrelet (*Brachyramphus marmoratus*)

B. A. VANDERKIST,* T. D. WILLIAMS,† D. F. BERTRAM,†‡
L. W. LOUGHEED† and J. L. RYDER†

**Department of Molecular Biology and Biochemistry, 8888 University Drive, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6, †Department of Biological Sciences, 8888 University Drive, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6, and ‡Canadian Wildlife Service, Pacific and Yukon Region, 5421 Robertson Road, RR # 1, Delta, British Columbia, Canada V4K 3N2*

Summary

1. An indirect, physiological method to assess reproductive state in individuals of unknown status is described. The plasma levels of two main yolk precursors, vitellogenin (VTG) and very-low density lipoproteins (VLDL), are focused on as indices of egg production, for the characterization of fecund females.
2. Data for a species where breeding chronology could be directly assessed, at the population level (Cassin's Auklet, *Ptychoramphus aleuticus*), confirmed the validity of this approach: plasma VTG levels were highest during the defined egg-laying period, and the highest proportion of females were defined as 'egg-producing' in this period.
3. Analysis of samples for Marbled Murrelets (*Brachyramphus marmoratus*) caught off-nest (i.e. where all individuals were of unknown status), clearly identified a putative egg-laying phase, with a single, protracted laying period (cf. multiple-broodiness).
4. Analysis of body mass confirmed our characterization of 'egg-producing' females: birds with elevated plasma VTG were on average 40 g heavier than other females, equivalent to the mass of the single egg (36–41 g).
5. Indirect, physiological assessment of reproductive state provided valuable information on the breeding biology of Marbled Murrelets which would have been difficult to obtain in any other way (e.g. proportion of fecund females, breeding phenology, single *vs* multiple-clutch breeding pattern). Despite some limitations, this technique should be applicable to any oviparous vertebrate population where essential information on breeding biology cannot be obtained by more traditional methods.

Key-words: Physiological methods, *Ptychoramphus*, vitellogenin

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Introduction

Many life-history traits and behavioural decisions are state-dependent (e.g. McNamara & Houston 1992; Houston 1993), and consequently it is important to know the state of individuals in any particular study (this might be their physiological state or 'condition', migratory state or reproductive state). In the case of reproductive state or breeding stage this process is relatively straightforward for most species: investigators can simply locate large numbers of breeding individuals and identify breeding state by direct observation (see Clutton-Brock, Guiness & Albon 1982; Cooke, Rockwell & Lank 1995, for excellent examples). This also allows direct assessment of other key reproductive parameters such as breeding phenology (onset and duration of the reproductive phase) and, potentially, population structure (sex ratio, non-breeders *vs* breeders, or proportion of fecund females). Some species cannot be studied in this way, however, either because they are too cryptic, because they use remote breeding habitats, or because disturbance at breeding sites must be minimized (as in the case of rare or endangered species).

Recently, there has been much interest in the use of physiological methods to assess physiological state (namely 'condition', reviewed in Brown 1996; immune function, Ots, Muramagi & Horak 1998), or migratory state (Jenni-Eiermann & Jenni 1994; Williams *et al*. 1999) indirectly in birds of unknown status. In this

paper we extend this approach to the characterization of reproductive state and breeding phenology in a population of Marbled Murrelet (*Brachyramphus marmoratus*, Gmelin 1789) where individuals can only be caught in large numbers off the nest (and thus where these birds are all of unknown breeding status). The Marbled Murrelet is extremely difficult to study because it nests in relatively inaccessible habitats: large trees in coastal old growth forest. Concern for the Marbled Murrelet has grown in recent years due to observed population declines over much of the species range (near-shore waters of Alaska to central California) associated with fragmentation and harvesting of nesting habitats through forestry development (Nelson 1997). However, a lack of basic information on population and breeding biology has hampered the development of management and conservation protocols for this species (Ralph *et al*. 1995).

Hormonal and physiological changes associated with the avian reproductive cycle have been well characterized in a wide range of both domestic and free-living species (Wingfield & Farner 1978; Dawson 1983; Mays, Vleck & Dawson 1991; Cockrem & Seddon 1994; Christians & Williams 1999). This allows the identification of stage-specific hormone(s) or metabolite profiles that might be used to characterize the reproductive state of individuals in a mixed population (e.g. in most vertebrates, androgens and gonadotropins are elevated during early stages of breeding, such as courtship and oogenesis, whereas prolactin is elevated during later periods coincident with lactation or brood-rearing; Goodman 1999; Williams 1999). In this paper we focus on the two main egg yolk precursors of oviparous (non-mammalian) vertebrates: vitellogenin (VTG) and very low-density lipoprotein (VLDL). Both precursors are synthesized and secreted by the liver in response to oestradiol (E2), and are greatly elevated in the circulation during egg production (Griffin & Hermier 1988; Burley & Vadehra 1989; Mitchell & Carlisle 1991). We tested whether measurement of plasma levels of VTG and VLDL could be used to detect evidence of egg production in individual birds (distinguishing egg-producing, or fecund; females from non-egg-producing individuals), and to provide information on breeding phenology at the population level in free-living Marbled Murrelets captured off the nest. We validated this methodology using plasma samples from a related species, Cassin's Auklets (*Ptychoramphus aleuticus*, Pallas 1811), where breeding chronology could be determined (at least at the population level) by directly monitoring large numbers of nest burrows at the colony.

Materials and methods

CAPTURE, BLOOD SAMPLING AND SEXING OF BIRDS

Cassin's Auklets were captured on Triangle Island (British Columbia, Canada; 50°52′ N 129°05′ W) in March–June 1997 and 1998. Birds were intercepted as they left nesting burrows in the colony using soft plastic 'pheasant' nets at the base of nesting slopes (all captures between 0200 and 0530 h). Although the reproductive status of individual Cassin's Auklets was unknown, timing of egg-laying, incubation and chick-rearing in the colony was monitored directly by researchers checking large numbers (~400) of active burrows, allowing accurate breeding chronologies to be determined (Triangle Island Research Station, unpublished data). Marbled Murrelets were captured at Desolation Sound, British Columbia (50°05′ N, 124°40′ W) between May–August 1996 and 1997. In 1996, a floating mist-net system (Kaiser *et al*. 1995) was used to capture adults as they flew between inshore marine foraging areas and forest breeding areas, through Theodosia Inlet, Desolation Sound around dawn (0400–0700 h) and dusk (2000–2300 h). In 1997, in addition to mist-netting, a 'night-lighting' technique (Whitworth *et al*. 1997) was used to capture birds on the water at night (2300–0500 h) in Desolation Sound. Both Marbled Murrelets and Cassin's Auklets were blood sampled and sexed following the methods described in Vanderkist *et al*. (1999), and body mass was recorded for all captured individuals.

BREEDING CHRONOLOGY OF CASSIN'S AUKLETS

Descriptive statistics were used to construct an interval (mean ±2 SD) around mean laying and hatching dates (Triangle Island Research Station, unpublished data); laying, mean 9 April (range 18 March to 30 April); hatching, mean 12 May (range 24 April to 30 May). The intervals for laying and hatching periods overlapped by about 7 days, so the mid-point of the overlap (27 April) was used as the boundary between the stages of laying and incubation. For the chick-rearing stage, an average fledging period of 41–50 days was used (Manuwal & Thoresen 1993). In 1998, plasma samples of presumed prelaying Cassin's Auklets were also obtained, collected as early in the season as possible (24–28 March) before any eggs were detected in nest burrows.

PLASMA ANALYSES AND CLASSIFICATION OF EGG-PRODUCING AND NON-EGG-PRODUCING BIRDS

Plasma levels of VTG and VLDL were assayed with diagnostic kits (Zn, Cat no. 435–14909; Triglyceride E Kit, Code no. 432–40201, Wako Pure Chemical Industries Ltd, Richmond, VA, USA) following the methods of (Mitchell & Carlisle 1991). Vitellogenic zinc was used as an index for VTG, and total triglyceride as a measure of VLDL (see also Williams & Christians 1997; Williams & Martyniuk 2000). Inter-assay coefficients of variation for VLDL and VTG were 4·3% and 8·6%, respectively, and intra-assay coefficients

of variation were 3·1% and 5·9%, respectively. In some cases, both VTG and VLDL could not be measured due to small plasma sample volume.

Plasma levels of VTG and VLDL in male birds were used to assign an upper limit for classification of 'non-egg-producing' individuals, since males normally do not produce VTG and they have basal VLDL levels (Mitchell & Carlisle 1991; Williams & Christians 1997). The highest values of VTG and VLDL observed in male Cassin's Auklets were 0·11 μg ml^{-1} Zn and 1·36 mM triglyceride, respectively. For male Marbled Murrelets, the highest values of VTG and VLDL were 0·26 μg ml^{-1} Zn and 6·62 mM triglyceride, respectively. To be conservative, the maximum yolk precursor values obtained for males were doubled and used as limits to classify females as either egg-producing or non-egg-producing birds.

STATISTICAL ANALYSIS

All statistical analyses were performed with SAS (version 6·11) or Minitab (version 11) statistical software. Both parametric and non-parametric statistical analyses were used depending upon the results of normality tests for the data being examined. Values are presented as the mean ± standard deviation unless otherwise stated.

Results

CASSIN'S AUKLETS

Cassin's Auklets classified as egg-producing birds were detected from 24 March to 30 April, and capture dates for these birds closely matched the range of laying dates obtained by direct observation of nest burrows (18 March to 30 April). Plasma VTG levels were on average two- and three-fold higher, respectively, during the defined egg-laying period compared to the prelaying and incubation periods (Fig. 1). The proportion of females classified as egg-producers using VTG differed significantly between breeding stages (Fisher's exact test, $\chi^2_2 = 11{\cdot}5$, $P < 0{\cdot}01$), with the highest proportion recorded during the laying period (Table 1).

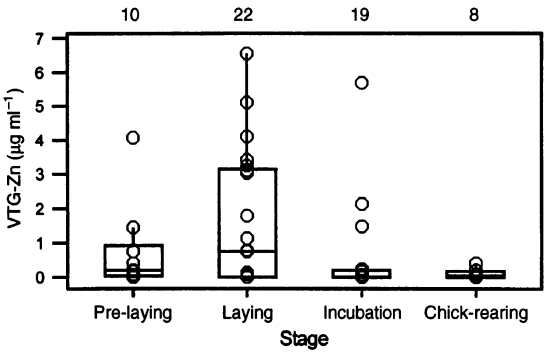

Fig. 1. Variation in plasma vitellogenin levels by defined reproductive stage in Cassin's auklets. Sample sizes for each stage are given at the top of each box plot.

The proportion of females classified as egg-producers using VLDL also differed significantly between reproductive stages (Fisher's exact test; $\chi^2_2 = 6{\cdot}9$ $P < 0{\cdot}05$; Table 1). The absolute number of female Cassin's Auklets classified as egg-producing was less using VLDL than using VTG (Table 1). However, the proportion of females classified as egg-producing were only marginally significantly different between the two methods ($\chi^2_1 = 3{\cdot}85$, $P = 0{\cdot}05$).

MARBLED MURRELETS

No Marbled Murrelet females were classified as egg-producing birds using either VTG or VLDL in 1996. Birds were only captured by mist-netting in 1996 and, more importantly, the range of sample dates was much later in 1996 (June–August) than in 1997 (May–August; Fig. 2). In contrast, in 1997 there was much greater interindividual variation in plasma VTG levels in female Murrelets (Table 2) when the night-lighting technique permitted the capture of birds earlier in the season. In 1997, Marbled Murrelet females classified as egg-producing birds were detected between 14 May and 3 July based on plasma VTG levels, clearly indicating the putative egg-laying period for this species. Only 2 out of a total of 23 females classified as egg-producing using VTG were captured by mist-netting.

As with Cassin's Auklets, use of plasma VTG detected a greater absolute number of egg-producing

Table 1. Levels of VTG-Zn and VLDL and the proportion of egg-producing female Cassin's Auklets within each reproductive stage. Limits for classifying individuals as egg-producing using VTG-Zn and triglyceride were 0·22 μg ml^{-1} Zn and 2·7 mM triglyceride, respectively

Reproductive stage	Yolk precursor					
	VTG (μg ml^{-1} Zn)			VLDL (mM TTRIG)		
	n	Mean ± SD (range)	% elevated VTG-Zn	*n*	Mean ± SD (range)	% elevated VLDL
Prelaying	10	0·73 ± 1·26 (0·00–4·07)	30	9	1·54 ± 0·73 (0·75–2·74)	11
Laying	22	1·66 ± 1·97 (0·00–6·56)	55	24	7·40 ± 11·53 (0·54–42·21)	33
Incubation	19	0·55 ± 1·37 (0·00–5·70)	16	18	3·15 ± 7·91 (0·59–34·64)	11
Chick-rearing	8	0·10 ± 0·15 (0·00–0·43)	0	8	0·98 ± 0·36 (0·423–1·453)	0

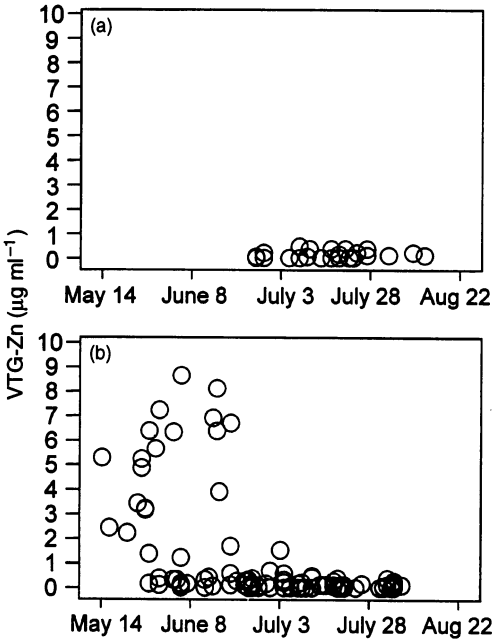

Fig. 2. Variation in plasma vitellogenin levels in relation to capture date in Marbled Murrelets, in (a) 1996 (n = 24) and (b) 1997 (n = 107).

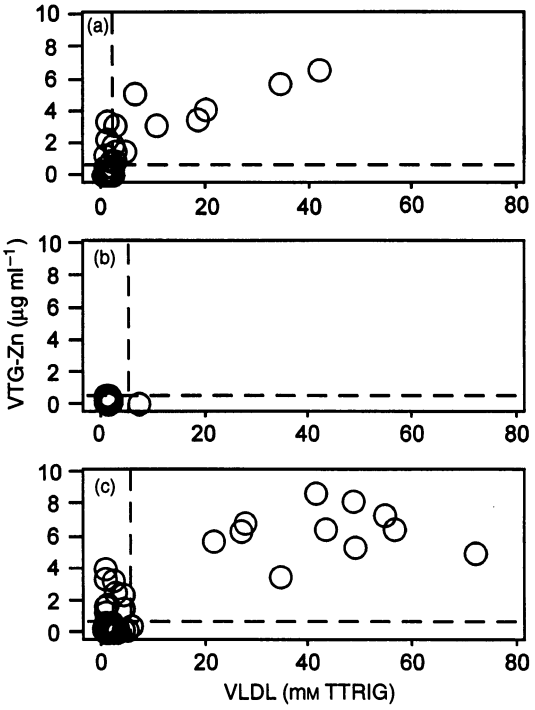

Fig. 3. Relationship between plasma vitellogenin and plasma VLDL in individual birds, for (a) Cassin's Auklets, (b) Marbled Murrelets in 1996 and (c) Marbled Murrelets in 1997. Dashed lines are the egg-producing limits for each yolk precursor as described in the text.

females than did VLDL, but there was no significant difference in the proportion of egg-producing females detected using either yolk precursor ($\chi^2_1 = 2{\cdot}44$, $P > 0{\cdot}1$).

RELATIONSHIP BETWEEN PLASMA VTG AND VLDL WITHIN INDIVIDUALS

Plasma levels of VTG and VLDL were positively correlated in female Cassin's Auklets ($F_{1,53} = 118{\cdot}3$, $P < 0{\cdot}001$, $r^2 = 0{\cdot}69$), in female Marbled Murrelets captured in 1997 ($F_{1,101} = 256{\cdot}42$, $P < 0{\cdot}001$, $r^2 = 0{\cdot}72$), but not in female Marbled Murrelets captured in 1996 ($F_{1,22} = 1{\cdot}59$, $P > 0{\cdot}2$, $r^2 = 0{\cdot}067$; Fig. 3). Some individuals of both species would have been classified as egg-producers using VTG as an index, but not if using VLDL (Fig. 3). Three patterns of VTG–VLDL levels were defined: (a) elevated VTG and VLDL; (b) elevated VTG only; and (c) neither VTG or VLDL elevated (no females from either species displayed elevated levels of VLDL only). Two female Marbled Murrelets with high VTG but not VLDL (pattern b) were known to be carrying eggs at their time of capture, since an egg was physically detected in the oviduct during banding.

There was a significant difference in body mass between individuals with the defined patterns of egg-precursor levels for female Cassin's Auklets ($F_{2,44} = 9{\cdot}63$, $P < 0{\cdot}001$; Fig. 4). Females with elevated levels of both VTG and VLDL (pattern a) and females with elevated levels of VTG only (pattern b) were significantly heavier than females with basal levels of both VTG and VLDL (pattern c; Bonferonni-adjusted comparison, df = 44, $t_{crit} = 2{\cdot}49$). The 95% confidence intervals for the body mass of egg-producing and

Table 2. Levels of VTG-Zn and VLDL for 1997 Marbled Murrelets by 30-day intervals and proportions of egg-producing females within each capture method using either VTG-Zn or VLDL as an index

	Yolk precursor					
	VTG-Zn (µg ml^{-1})			VLDL (mM TTRIG)		
		% elevated VTG-Zn			% elevated VTG-Zn	
	Mean ± SD (range)	Night-lighting	Mist-netting	Mean ± SD (range)	Night-lighting	Mist-netting
14 May to 12 June	2·58 ± 2·7 (0·00–8·67)	60 (15/25)	0 (0/2)	17·3 ± 24·5 (0·7–80·4)	38 (10/26)	0 (0/2)
13 June to 12 July	0·83 ± 1·94 (0·00–8·13)	100 (6/6)	4 (2/45)	5·2 ± 14·4 (0·5–81·2)	57 (4/7)	0 (0/44)
13 July to 11 August	0·11 ± 0·11 (0·00–0·38)	0 (0/24)	0 (0/5)	1·1 ± 0·4 (0·5–2·9)	0 (0/32)	0 (0/6)
Total	1·08 ± 2·10 (0·00–8·67)	38 (21/55)	4·0 (2/52)	6·7 ± 16·3 (0·5–81·2)	22 (14/65)	0 (0/52)

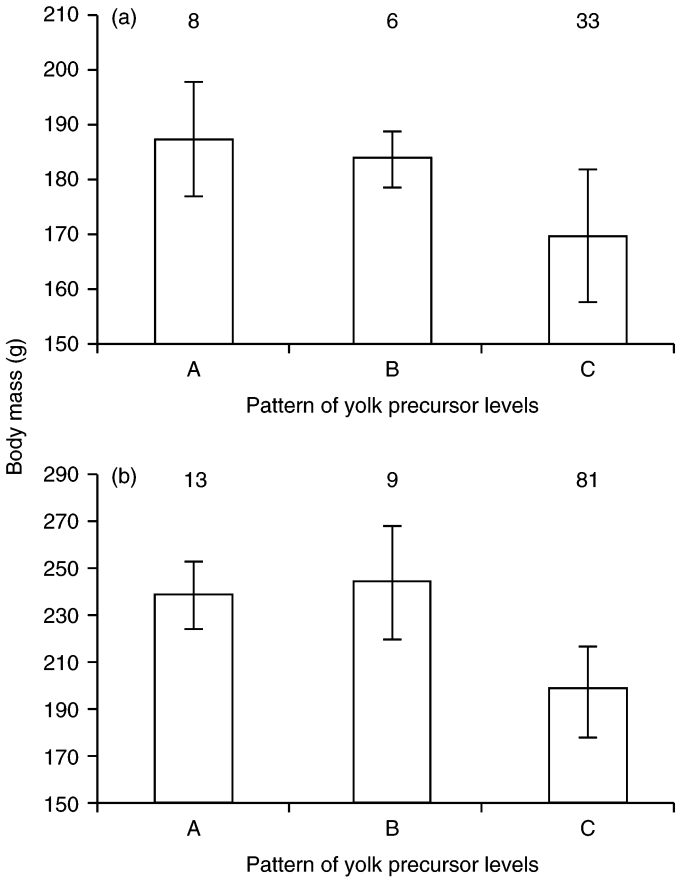

Fig. 4. Variation in body mass for (a) female Cassin's Auklets and (b) Marbled Murrelets in relation to the patterns of yolk precursor levels: (A) both VTG-Zn and VLDL levels elevated, (B) only VTG-Zn levels elevated and (C) neither VTG-Zn or VLDL levels elevated. Numbers at top of graphs are sample sizes, error bars are standard deviations.

non-egg-producing female Cassin's Auklets were 179–190 g, and 166–174 g, respectively. Similarly, female Marbled Murrelets captured in 1997 showed a significant difference in body mass between patterns of yolk-precursor levels ($F_{2,100} = 35{\cdot}30$, $P < 0{\cdot}001$; Fig. 4). Again, females with elevated levels of VTG and VLDL (pattern a) and females with elevated levels of only VTG (pattern b) were significantly heavier than those with low levels of both VTG and VLDL (pattern c; df = 101, $t_{crit} = 2{\cdot}43$). The 95% confidence intervals for the body mass of egg-producing, non-egg-producing and male Marbled Murrelets were 225–245 g, 195–202 g and 198–202 g, respectively.

Discussion

In this study we tested the validity of using indirect, physiological, measures to provide information on reproductive state and breeding phenology, in a situation where these data could not be obtained through more traditional approaches (direct observation of nests). We focused on plasma levels of the two main yolk precursors, vitellogenin and very low-density lipoprotein because we predicted that these would provide highly specific indicators of a single reproductive stage: elevated levels should occur in egg-producing females, but not in non-breeders, males or females at other breeding stages. Our data for Cassin's Auklets, where breeding could be directly monitored, support this assumption and confirm the validity of this technique. In this species, the highest levels of VTG and VLDL were observed during the defined laying period (constructed from direct burrow observations), and the highest proportion of birds were identified as 'egg-producing' during this period (see Fig. 1). In Marbled Murrelets, where the status of individuals and timing of breeding were completely unknown, measurement of plasma VTG and VLDL clearly identified the putative egg-laying period (from mid-May to early July, see Fig. 2). These data also indicate that Marbled Murrelets have a single, protracted breeding season, with no evidence of a bimodal distribution which would be associated with multiple broods (second clutches). In addition, since we obtained data on VTG and VLDL for *individual* birds, this technique can accurately assign reproductive status to some individuals (egg-laying females). This may be valuable where studies need to target fecund females (e.g. for radio-transmitter attachment in behavioural studies).

Both yolk precursors, VTG and VLDL, were measured to assess whether these were equally reliable as indices of egg production. In fact, VTG detected greater absolute numbers of egg-producing females than did VLDL, in both Cassin's Auklets and Marbled Murrelets. Some evidence was therefore obtained that VTG and VLDL were elevated in plasma for different lengths of time in relation to egg formation, because there was not 100% agreement when classifying a female as an egg-producing bird. For example, the two Marbled Murrelets carrying eggs at their time of capture had elevated levels of VTG, but not VLDL; these birds would have been misclassified as 'non-egg-producing' females had we only considered VLDL. This result is somewhat surprising given that both yolk precursors are essential for yolk formation. In birds, triglyceride-rich lipoproteins (VLDL) are a significant component of egg-yolk (66% of hen's egg yolk, Griffin & Hermier 1988; Williams 1999). Furthermore, both yolk-precursors are under the same oestrogenic control (Wiskocil *et al*. 1980; Wahli *et al*. 1981; Burley & Vadehra 1989; Mitchell & Carlisle 1991), and are also believed to share the same oocyte receptor in birds (Stifani *et al*. 1990; Elkin *et al*. 1995). However, very little is known about yolk precursor dynamics during the rapid yolk development period, or about the relative half-life of each precursor, and this may explain the discrepancies found. An alternative explanation is that VLDL provides a 'noisier' signal for egg-production than does VTG. In contrast to VTG, non-yolk VLDL does occur in non-egg-laying birds, i.e. the baseline value for non-breeders is not zero (as it is for VTG). Plasma levels of this non-yolk VLDL can vary with diet and physiological state (e.g. fasting *vs* fattening). However, diurnal variation in

plasma triglycerides or variation due to feeding/fasting is typically in the range 0·5–3·0 mg ml^{-1} (e.g. Jenni-Eierman & Jenni 1996, 1997). This is similar to the range of variation we report for birds during chick-rearing (see, for example, Fig. 3) when no egg formation was occurring but when there was most likely natural variability in whether birds had fed recently or not. Variation in VLDL levels in non-breeders therefore occurs at an order of magnitude lower than VLDL levels in laying birds (10–40 mg ml^{-1}). Thus, although there were almost certainly differences in the physiological state of the birds we caught (fed *vs* fasted), as well as between Cassin's Auklets and Marbled Murrelets, we doubt that this confounds interpretation of VLDL in relation to egg-laying to any great extent. Plasma VTG levels are independent of diet (T. D. Williams unpublished data for Zebra Finches), and do not appear to show marked diurnal variation (Redshaw & Follett 1976; T. D. Williams, unpublished data), reducing the potential confounding effects of time of capture and physiological state (fed *vs* fasted) for this precursor. Our data, and those for other studies, therefore suggest that VTG provides the more accurate and reliable index of egg production. Nevertheless, there are two advantages of using VLDL: (a) VLDL is easier to measure than VTG, and (b) the VLDL assay requires smaller plasma sample volumes (so this yolk precursor may have value in certain studies).

Few previous studies have analysed plasma levels of VTG and VLDL in relation to egg formation in free-living or domesticated birds. In chickens (Mitchell & Carlisle 1991) and captive-breeding Zebra Finches (*Taeniopygia guttata*; Williams & Christians 1997) plasma yolk precursors are elevated during laying, but are undetectable in immatures, non-breeding females and males. Similarly, Berry, Millar & Louw (1979) reported elevated levels of phosvitin (a precursor of VTG) and triglyceride in breeding but not non-breeding female Cape Cormorants (*Phalacrocorax capensis*). In free-living European Starlings (*Sturnus vulgaris*), plasma VTG was significantly higher in laying birds compared with prebreeders (sampled 1–4 days before egg-laying), and had decreased to less than 10% of peak values only 2 days after clutch completion (Christians & Williams 1999). These studies confirm that elevated plasma VTG levels are highly specific to egg formation and laying. Marbled Murrelets and Cassin's Auklets lay a single egg clutch, and rapid yolk development, during which yolk precursors should be elevated, takes about 14 days in Cassin's Auklets (Astheimer & Grau 1990; no data available for Murrelets).

In our study, body mass varied significantly with plasma VTG levels, confirming our characterization of 'egg-producing' females. For Marbled Murrelets, the difference in mean body mass between birds with low levels of VTG (pattern C) and those with high levels of VTG (patterns A and B) was in the range of 40–46 g, consistent with the expected egg mass of this species (36–41 g, Nelson 1997). Body mass itself therefore proved to be a somewhat useful indicator of egg-production in Marbled Murrelets, with egg-producing females being significantly heavier than both males and non-egg-producing females. This finding might be useful for researchers who wish to maximize the probability of marking, sampling or manipulating females within a capture sample, e.g. for the attachment of radio-transmitters on breeding individuals which could then be tracked back to a nest-site.

In one year of our study (1996), when Marbled Murrelets were captured late in the season, from June to August, using floating mist-nets, we detected no egg-producing females. Night-lighting allowed us to capture birds much earlier in the season (in 1997), and consequently many more birds were identified as 'egg-producing'. Of 77 female Marbled Murrelets captured by mist-netting at Theodosia Inlet, Desolation Sound during the 2 years of this study, only two were classified as egg-producing using VTG. This is unlikely to represent a bias due to different capture techniques, rather it simply is due to the fact that mist-netting could not be employed as early in the season as night-lighting (when egg-production would be more probable). In 1997, egg-producing Marbled Murrelets were detected from 14 May to 3 July. These dates agree reasonably well with Marbled Murrelet chronology estimates in British Columbia (Hamer & Nelson 1995), that predicted incubation to last from 2 May to 4 July, assuming a 14-day rapid yolk development period in Marbled Murrelets as in Cassin's Auklets (Astheimer 1986). However, because our night-lighting sampling effort from 23 June to 12 July was greatly reduced (associated with a switch to mist-netting), it is possible that during this time there were still significant numbers of individuals producing eggs that would have been detected had we maintained our night-lighting effort. Similarly, juvenile Marbled Murrelets were first observed at sea on 27 June in 1997 (C. Lougheed, personal communication). Assuming 58 days between laying and fledging (Hamer & Nelson 1995), this indicates that some birds started laying before our first captures. Further sampling, with earlier capture of birds, will hopefully refine our estimates of breeding chronology of Marbled Murrelets using this technique, as well as addressing the issue of inter-annual variation in breeding phenology.

In summary, measurement of plasma VTG and VLDL provided useful indicators of reproductive state (egg production), although our data indicate that VTG provides the more robust, reliable index. Use of yolk precursors for this type of analysis has the additional advantage that these involve relatively simple diagnostic assays (cf. more involved ELISA (enzyme-linked inmmunosorbent assay) or RIA (radioimmunoassay) techniques). A limitation of this general approach is that single measures of hormone or metabolite profiles only provide a static picture of dynamic physiological systems. We could only assess whether

females were or were not producing an egg at the time of capture. Owing to interindividual variation in the timing of breeding among our samples of birds, there were probably females that had already laid eggs and were well into later stages of reproduction, along with females that may have been preparing to initiate egg formation. Both of these groups of females would be classified as non-egg-producing birds, but were not necessarily non-breeding adults. Circulating levels of yolk precursors therefore provide a *minimum* estimate of the proportion of breeding females in a given capture sample. Nevertheless, in our study, indirect, physiological assessment of reproductive state provided valuable information on the breeding biology of Marbled Murrelets which would have been difficult to obtain in any other way (e.g. breeding phenology, single *vs* multiple-clutch breeding pattern, as well as reproductive status for *some* individuals at the time of capture). Combining measurement of yolk precursors with that of other hormones (e.g. prolactin) might resolve some of the problems of underestimating the number of fecund females. Individuals sampled during the defined egg-laying period with low VTG but high prolactin (indicating ongoing parental care) are likely to have previously laid an egg (i.e. to have completed egg formation), whereas individuals with a low VTG: low prolactin profile are more likely to be true non-breeders. Finally, given the widespread occurrence and sex-specific nature of vitellogenin this approach should be applicable to all oviparous vertebrates (see, e.g., Takemura & Oka 1998; Heppell & Sullivan 2000).

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