USING MULTIPLE METHODS TO DESCRIBE BREEDING, STRESS RESPONSE, AND DISTURBANCE OF MARBLED MURRELETS (*BRACHYRAMPHUS MARMORATUS*)

by

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Abstract

I investigated the breeding biology of Marbled Murrelets using (a) vitellogenin (VTG) analyses (b) brood patch (BP) scores (thought to imply incubating adults), and (c) radio telemetry data. VTG analyses allowed description of the 5-month breeding season for Marbled Murrelets, the timing of which did not vary between years (1999-2000). Of the females caught between April to July (the 'egg-production period'), 55% were producing eggs. Using brood patches (BP) to infer reproductive status is an approach that should be used cautiously: 53% Marbled Murrelets caught with fully-developed BP never incubated, and likewise, 50% of fecund, radio-tagged females never incubated (failed incubators?). Of a sample of fecund females, 40% started incubation about 15 days later than expected (delayed incubators?). This suggests large numbers of birds that failed to start incubation, for reasons that were not clear. While investigator disturbance explained some cases, seasonal date also had an effect on breeding success. We detected a seasonal decline in breeding success in Marbled Murrelets, with failed incubators occurring later in the season (by 18 days) than successful incubators, and 'delayed' incubators initiating incubation later (by 24 days) those not delayed. Thus, while capturing murrelets sometimes affected individual breeding status, later breeders were affected more than earlier breeders. This finding suggests that researchers should aim to capture Marbled Murrelets early in the breeding season. My investigation of capture effects also included an analysis of the stress response to capture, using corticosterone. Like other birds, Marbled Murrelets reach maximum corticosterone levels at 30 min. Corticosterone increased with mass in females (but not males), suggesting that females are more sensitive to stress when they are heaviest, during egg-production.

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General Introduction

1. The Study Species: the Marbled Murrelet *Brachyramphus marmoratus.*

The Marbled Murrelet is unique among the Alcidae (Gaston and Jones 1998) because of its preference for nesting solitarily on the mossy limbs of old-growth trees in coastal forests (Nelson 1997). They are secretive around their nests and active at dawn and dusk (DeSanto and Nelson 1995), and as a result, studying Marbled Murrelets has always been a challenge (Cooke 1999). In spite of the considerable attention they have received during the last two decades (DeSanto and Nelson 1995), much of their breeding biology, ecology, and demography remain poorly known (Nelson 1997, Ralph and Long 1995, Cooke 1999, Cam *et al.* in review).

Marbled Murrelets are found along the Pacific Coast, from Alaska to California (Nelson 1997). In spite of the lack of reliable estimates of demographic trends (Cooke 1999, Cam *et al.* in review), population declines have been reported in most of its range (eg., Piatt and Naslund 1995, Burger 1995, Kelson *et al.* 1995) and consequently, Marbled Murrelets are listed as threatened in Canada (Committee on the Status of Endangered Wildlife in Canada), Washington, and Oregon (U.S. Fish and Wildlife Service 1992), and as endangered in California. While oil pollution (Carter and Kuletz 1995) and murrelet bycatch in gill nets (see Carter *et al.* 1995, Fry 1995) contribute to these declines, the loss of murrelet nesting habitat due to forestry practices (Divoky and Horton 1995, Kelson *et al.* 1995) have drawn the most attention, making Marbled

Murrelets central in the controversial issue of forest management and planning (Cam *et al.*, in review).

Until recently (Vanderkist 1999), direct observation at the nest has been the only means of accurately determining the breeding status of individual Marbled Murrelets. Because Marbled Murrelets are noncolonial and their nests are difficult to find, assessment of reproductive status is more easily made when they are caught at sea, where Marbled Murrelets are most easily encountered (see Kaiser *et al.* 1995, Vanderkist *et al.* 2000, Speckman *et al.* 2000). Unfortunately, Marbled Murrelets are sexually monomorphic, age past first year is indeterminable, age of first breeding is unknown, and both pre-breeders and breeders exhibit alternate plumage during the breeding season (Nelson 1997). In addition, using brood patch score to categorize breeding birds may be unreliable (Vanderkist 1999), and in fact, may be misleading if brood patches are assumed to indicate absolute incubation potential (see this study, chapters 2 and 4). Thus, sex, plumage and morphometric measurements offer little assistance in identifying reproductive potential when the bird is captured. However, inclusion of individual reproductive potential would allow more reliable estimates of demographic parameters for this species.

Thus, the objective of my thesis research was to determine reproductive status of individual Marbled Murrelets caught in Desolation Sound, B.C. This study area has been heavily logged for many years, and as a result, nesting habitat for murrelets here is highly fragmented (Cam *et al.* in review, R. Bradley and F. Huettmann, pers. comm). In addition, the Desolation Sound marine area is a popular summer destination for yachts, sailboats, and speedboats, sustains shellfish farms and prawn fishing, and is used as a

transport corridor for log booms (LMT pers obs.). In spite of these major disturbances in their marine and terrestrial habitat, Marbled Murrelets in Desolation Sound remain relatively abundant (see Kaiser *et al.* 1995). However, it is possible that murrelet reproductive status in this study was influenced by the location of the study site. Further, similar studies need to be carried out to determine if the results in this study are widely applicable to other Marbled Murrelet populations. That said, my research presents some results that should be considered in any seabird study: namely, the application of physiological techniques and radio telemetry to seabird research.

2. Methods used to study breeding biology

This work was possible because it was part of a multidisciplinary study of Marbled Murrelets in B.C. (Cooke 1999, Bradley and Cooke 2001), which gave me access to radio telemetry data, used to track radio-tagged adults and find active nests. The collaborative nature of the study enabled me to use radio telemetry data collected by a fellow student (Russ Bradley, Centre for Wildlilfe Ecology, SFU), to confirm breeding status of the Marbled Murrelets. I inferred breeding status for the same individuals independently, using (a) vitellogenin (VTG) analyses, which identifies fecund females (Deeley *et al.* 1975, Bergink *et al.* 1974, Vanderkist *et al.* 2000); and (b) brood patch (BP) scores, thought to indicate incubating adults (Bailey 1952, Gill 1995, Ainley *et al.* 1990).

Accessory to the investigation of breeding status, I investigated the effects of disturbance of Marbled Murrelets by researchers. Evidence that seabirds are detrimentally affected by human observers has been accumulating for years, but is traditionally

measured at the colony (review in Carney and Sydeman 1999). However, there is little or no information on disturbance away from the nest site, or away from the colony. To assess disturbance, I used two techniques: (a) a comparison of the apparent reproductive potential of individuals at capture (using VTG and BP measurements) with the subsequent reproductive behaviour of radio-tagged adults; and (b) an analysis of corticosterone (CORT), a hormone released in response to stressful events (Siegel 1980, Wingfield *et al.* 1995, 1998). Variations in the response to stressful events are known to correlate with a number of factors, including sex (Astheimer *et al.* 1995, Wingfield *et al.* 1995), body condition (Heath & Dufty 1998, Schoech *et al.* 1997), and reproductive stage (Kitaysky *et al.* 1999a). Thus, analysis of the sensitivity to stress can then be compared by measuring CORT release among individuals, between sexes, seasons, years, and study sites (Wingfield *et al.* 1995, Silverin & Wingfield 1998, Holberton & Able 2000).

3. Thesis Objectives and Organization

The chapters in this thesis are all closely related, and explore multiple methods of determining reproductive status in individual Marbled Murrelets captured in Desolation Sound, B.C. Because of its threatened/endangered status, many researchers are working to improve descriptions of this species' biology, to allow more accurate prediction of population sizes and demographic trends. This study does not address demography, but a more basic population assessment: the reproductive potential of individuals contributing to the local population.

In Chapter 1, I extended Vanderkist's (1999) methods to collecting VTG samples across two entire breeding seasons, with the following objectives: (1) assess inter-annual variation in timing of egg-production using VTG analysis; (2) predict incubation and chick-rearing phases by extrapolation (following Lougheed 2000); and (3) use the range and duration of the appearance of egg-producers in the study sample to estimate the proportion of egg-producing females caught during the egg-producing phase. Chapter 2 tests how well BP correlated with nesting events (egg-production and onset of incubation), and whether BP could be used to correctly infer reproductive status of individuals. The results from Chapters 1 and 2 lead into a discussion of disturbance, as described in the following two chapters. Chapter 3 reports the first data on the stress response to capture in Marbled Murrelets, and includes an assessment of (1) whether CORT release increases in Marbled Murrelets after the attachment of radio transmitters, compared to those handled only; and (2) the relationship of CORT with date, mass, year, breeding stage, and sex. In chapter 4, I used physiological and radio telemetry methods to examine the relationships between VTG production, BP development, and subsequent timing of incubation to try to assess the impact of capture, handling, and instrument attachment on a group of Marbled Murrelets carrying radio transmitters.

Chapter 1

Using Physiology To Examine Inter-annual Variation In Breeding Chronology Of Marbled Murrelets In Desolation Sound, B.C.

1.1 Introduction

Reproductive Status in The Marbled Murrelet

Studying the Marbled Murrelet has always been a challenge to biologists because of its secretive habits (Cooke 1999). Reproductive status of Marbled Murrelets is difficult to determine, because unlike other alcids, they nest solitarily and cryptically on mossy limbs of old growth trees (Nelson 1997), making it almost impossible to observe the precise timing of breeding activities such as egg laying or incubation. Consequently, it has not been possible to fully describe the characteristics of their breeding biology (Nelson 1997, Vanderkist 1999, Lougheed 2000), compounding the difficulty to make biological comparisons between Marbled Murrelet populations and with other alcids. Until recently (Vanderkist 1999), direct observation at the nest has been the only means of accurately determining the breeding status of individuals. This approach is ineffective for assessing overall reproductive status of the population, as it depends on observations of individuals already known to be breeding, instead of assessing the relative reproductive contribution made by all individuals to the population.

The classification of the Marbled Murrelet as a threatened species in Canada and the US has made it imperative that researchers make accurate predictions of population sizes and demographic trends. However, the biological framework in which to interpret

demographic trends must include an understanding of reproductive status or reproductive potential of individuals in the population (see Ebert (1999) for details on demographic trends). This study does not address demography, but a more basic population assessment: the number of reproductive females that contribute to the local population.

Often, rare or endangered species have life cycles with stages that are difficult to observe and require innovative studies (Ebert 1999). Because Marbled Murrelets are noncolonial and their nests are difficult to find, assessment of reproductive status is best made at sea, where Marbled Murrelets are most easily encountered (see Kaiser *et al.* 1995, Vanderkist *et al.* 2000, Speckman *et al.* 2000). Beneficially, this strategy makes a random assessment at a population level, instead of assessments made at an individual level as with nest site monitoring. Unfortunately, when captured at sea, away from their nest sites, breeding status is of Marbled Murrelets is almost impossible to determine; they are sexually monomorphic, age past first year is indeterminable by observation, and age of first breeding is unknown (Nelson 1997). Thus, plumage and morphometric measurements offer no assistance in classifying breeding status. In addition, using brood patch score to categorize breeding birds may be unreliable (Vanderkist 1999; see also this study, chapters 2 and 4). Unlike with a physical examination, physiological analysis of plasma taken from birds at the time of capture allows determination of reproductive status in females, using an egg yolk protein called vitellogenin (Vanderkist *et al.* 2000).

Vitellogenin

Vitellogenin (VTG) is a lipophosphoprotein found in the plasma of egg-producing birds (Bergink *et al.* 1974, Deeley *et al.*1975, Mitchell & Carlisle 1991). It is secreted by

the liver in response to estrogenic stimulation, and transported to the ovary via the bloodstream where it is deposited in the developing oocyte (Deeley *et al.* 1975, Wahli *et al.* 1981, Wang & Williams 1982). Plasma levels of VTG increase at onset of yolk deposition (Challenger *et al.* 2001), and are normally undetectable in immature birds, non-egg-producing females, and males (Deeley *et al.* 1975, Mitchell & Carlisle 1991). Thus, measuring plasma levels of VTG accurately identifies fecund females (Mitchell & Carlisle 1991, Vanderkist 1999, Vanderkist *et al.* 2000). Efforts to determine reproductive status using other plasma hormone levels have been inconclusive (eg., testosterone, in Vanderkist 1999). Because the VTG technique could be widely used in avian studies where reproductive activity of females is of interest, we assessed the methodology of VTG analysis, including the effects of freezing and of assaying plasma from birds of unknown reproductive status.

Capture Methods: Dipnetting and Mistnetting

The two main techniques for capturing Marbled Murrelets, dipnetting and mistnetting, catch different groups of birds in our study area. A significant male bias is found in birds caught using mistnets (Vanderkist *et al.* 1999), corresponding to a male bias in chick provisioning late in the season (Bradley *et al.* 2002). It has been suggested that, whereas the dipnets seem to capture pre-incubating, incubating, and chick-feeding murrelets, the mistnets capture primarily chick-feeding birds (Vanderkist 1999, Bradley *et al.* 2002). Because of this, it was expected that the mistnets would not catch egg-producing females. However, until this study, these two capture techniques have never been used concurrently, and could not be directly compared. Here, VTG analyses are used to

examine the proportions of egg-producers caught at the same time with the two capture methods (in both 1999 and 2000).

Using VTG to Describe Egg-production and Reproductive Chronology

Initiation of breeding is highly asynchronous among Marbled Murrelets (Lougheed 2000), but it has been unclear whether this is due to renesting after failure, or to variation in nest initiation (Nelson 1997). Knowledge of breeding chronology is important in understanding the timing and lengths of breeding activities, and what factors affect them (Hamer & Nelson 1995). This knowledge leads to the ability to make decisions regarding the optimum period for monitoring breeding activities (i.e. to count nests or juveniles at sea) (Hamer & Nelson 1995), thereby optimizing research efforts. In addition, understanding breeding chronology should help to evaluate the significance of activity patterns or habitat use (Lougheed 2000). A broader understanding of the relationship between life history patterns and population processes may facilitate the development of general principles to guide wildlife managers (Saether *et al.* 1996).

Vanderkist (1999) was able to use VTG to detect breeding female Marbled Murrelets, but was unable to sample early enough to encompass the entire breeding season. The present study extended Vanderkist's (1999) methods, to compare interannual variation in breeding chronology by (1) using VTG analysis to track asynchrony of egg-production, and describe duration of egg-production in the local population; (2) predict incubation and chick-fledging dates by extrapolation (following Lougheed 2000); and (3) estimate the proportion of females that were egg-producing. (4) This study assessed the effects on VTG assay results when plasma comes from females of unknown reproductive status, and after long-term freezing of plasma. (5)

Finally, VTG analyses were used to examine capture biases between two capture methods used concurrently in the study area (dipnetting and mistnetting).

1.2 Methods

Capturing and Blood Sampling

Marbled Murrelets were captured in Desolation Sound, British Columbia (centre 50° 05'N, 124° 40'W), from 20 April - 4 September 1999, and 19 April - 26 August 2000. 'Dipnetting' (Whitworth *et al.* 1997, Lougheed *et al.* 1998) was used at night (2200–0500 h) to catch birds in Desolation Sound. Searches were conducted from 4.5-m inflatable boats, by scanning the surface of the water with spotlights. Individual birds were captured using a salmon net, placed in dark cloth bags, and transported to the nearest shore (within 1 km of the capture site, and easily reached within 10 min).

Mistnetting caught birds (8 June - 30 July 1999, 14 June - 29 July 2000) at the mouth of Theodosia Inlet (50°04'N, 124° 42W) (Kaiser *et al.* 1995), adjacent to Desolation Sound. Prior to 1 June, it was not possible to catch murrelets in the mistnets because they were not using that inlet as a flyway, (Kaiser *et al.* 1995, L. Lougheed pers. comm.), but after this date, birds were captured concurrently using mistnet and dipnet approaches.

Marbled Murrelets from both capture methods were bled. Blood sampling extended well past the egg-laying and incubation period (early May-mid July, in B.C., Nelson 1997; Lougheed 2000; this study). Birds were bled with no a priori knowledge of their sex; samples were later analyzed using DNA methods of sex determination (Griffths

1996, Vanderkist 1999, Vanderkist *et al.* 1999). Birds were held in a cloth bag during bleeding, their head and eyes covered. Blood samples (1-2 ml) were taken from the brachial vein using a heparinized 1/2" 26 guage hypodermic needle. Blood was dispensed into an Eppendorf tube and kept cold until returning to field camp. Blood was centrifuged at 6,000 rpm for 10 minutes, the plasma was pipetted off, and red blood cells and plasma were frozen at -20°C until they could be transported to laboratory facilities for further analysis.

Vitellogenin Analysis

Vitellogenin (VTG) concentration in the plasma is determined indirectly, with an assay for vitellogenic zinc (following Mitchell & Carlisle 1991), using a diagnostic kit from Wako Chemicals (Zn, Cat. No. 435-14909). Vitellogenic zinc (VTG-Zn) is used as an index for VTG, as described and validated for Marbled Murrelets by Vanderkist (1999) and Vanderkist *et al.* (2000). VTG-Zn is selectively precipitated from the plasma with dextran sulphate (Griffin & Mitchell 1984); the difference between the original (total) plasma zinc and the remaining zinc (albumen-bound) following precipitation provides an index of VTG-Zn (Mitchell & Carlisle 1991, Williams 1999). Hereafter, VTG will be used to refer to both vitellogenin and VTG-Zn.

DNA Sexing

Marbled Murrelets were sexed following the methods described in Vanderkist (1999) (modified from Griffiths 1996), with a few modifications. Blood samples used were either centrifuged red blood cells, or a drop of blood that had been dried on a small piece

of filter paper. Unlike in Griffiths (1996), genomic DNA was not isolated from erythrocytes using a phenol/chloroform extraction. Instead, either 3 ul of red blood cells, or a fragment of the filter paper with dried blood, was rinsed twice with deionized distilled water, centrifuging and decanting the supernatant after each rinse. DNA was extracted using Instagene Matrix (Bio-Rad Laboratories, Hercules, Ca, Cat. No. 732-6030), following the procedures provided by the manufacturer. After extraction, PCR amplification followed the description in Vanderkist (1999). Male birds were identified by the presence of a single 400-base pair band, and females by the presence of both 470 and 400 base pair bands. Success obtaining results was highest using the dried blood samples.

Methodological assessments: negative values and freezing effects.

At the time of capture, neither the reproductive status nor the sex of Marbled Murrelets is known. Thus, it is impossible to classify plasma for future analyses into 'laying' and 'non-laying' categories. Usually, the VTG assay is adjusted according to whether the plasma came from non-laying or laying birds (T. Williams, pers. comm.), by diluting non-laying bird plasma two-fold, and diluting laying bird plasma 4-fold. Due to the unknown nature of the Marbled Murrelet plasma, I carried out the analyses as if all plasma was from laying birds (i.e. diluting four-fold). This would not adversely affect analyses of plasma that actually was from egg-producing Marbled Murrelets, as the assay would have been done correctly. However, for the non-laying birds (males and females alike), doing the assay in this way (i.e. at the 'wrong' dilution) produced a large number of negative values that theoretically were not possible (i.e. it is not possible to have more

zinc bound to VTG molecules (VTG-Zn) than total zinc in the plasma). Therefore, after determination of sex and laying status, I re-assayed a subsample of plasma that had come from males or non-egg-producers, at the correct two-fold dilution. This increased the mean VTG in non-egg-producing birds from negative to positive (see Appendix 1.1). A regression of paired values (two-fold vs. four-fold dilutions; see Appendix 1.2) was then used to correct the remaining VTG values from non-laying plasma, to the theoretical value they would have had, had they been diluted two-fold. Regression equations are included in Appendix 1.2. All male and non-breeding female plasma VTG values were corrected using this equation before further analysis.

In order to quantify the effect of freezing on any conclusions drawn from the analysis of plasma, I plotted the VTG content of plasma after short (5 mo.)- and long (18 mo.)-term freezing. The same plasma samples were used pre- and post-treatment. A significant decrease of VTG with freezing time was found (paired sample t-test $p=0.00$). As with the negative values, a regression of paired values (5 mo. vs 18 mo. freezing) was used to calculate the theoretical effect of long-term freezing on plasma, adjusting all the values for the breeding birds ($n=63$), mimicing an 18-month freezing time (Appendix 1.3).

Classifying Egg-Producers

The term "egg-producers" is distinguished from "egg-layers" in this analysis because it is unclear how many days there are between producing and laying the egg, or if egg-producers always become egg-layers; VTG data can only identify egg-producers. To determine which females were egg-producers, we began by measuring the amount of

VTG in non-egg-producers. The surest non-egg-producers were males. Based on work done by Vanderkist (1999, and Vanderkist *et al.* 1999), we constructed limits around mean VTG + 3 standard deviation (SD) units for males (n=103, 1999 and 2000 pooled data) (Table 1.2). Thus, any VTG values that fall past this limit are outside 99.4% ($\mu + 3\sigma$) of a normal distribution (after Zar 1996), theoretically should be from birds that are producing eggs.

After identifying all egg-producers, mean date of egg-production was determined. To determine dates after which it was unlikely to catch egg-producers, mean egg-producing date plus three SD ($\mu + 3\sigma$) was determined. All females caught past this date were assumed to be not egg-producing. Mean VTG + 3 SD units for non-egg-producing females (n=41, caught after ($\mu + 3\sigma$)), was very similar to that of males (see Appendix Table 2).

Using this approach of ($\mu + 3\sigma$), based on VTG information alone, there is a highly conservative 0.6% chance of mis-classifying a non-egg-producing female or a male as 'egg-producing'. The mean VTG + 3σ in non-egg-producers (females=0.90 ug/ml; males=0.96 ug/ml, Table 1.2) is not intended to describe the normal levels of VTG seen in non-egg-producers, but is used as a conservative lower threshold to classify egg-producing birds: hence, murrelets with VTG $\geq$ 0.96 ug/ml were classified as egg-producers.

Constructing Breeding Chronology

This part of the study used VTG to construct a breeding chronology of Marbled Murrelets in Desolation Sound. Here, mean egg-production date was calculated using only the egg-

producing birds, not all birds captured in the study (as in Figure 1.2, above the hatched line). Marbled Murrelets lay only one egg per breeding attempt (Nelson 1997), therefore plasma VTG in egg-producing females was expected to decrease immediately after completion of this egg. Analyses of VTG can thus be used to predict mean egg-production and fledging periods, and the first juvenile appearances. A 30-day incubation period (Nelson 1997), and a 28-day chick-rearing period (Carter and Sealy 1987) were used to calculate breeding phenology. We estimated that birds with elevated VTG were halfway through egg production. Because elevated VTG is known to be associated with rapid yolk development (Challenger *et al.* 2001) and not the end of egg development, and because our best estimate of the duration of egg-production is 14 days (see Astheimer 1986, Cassin's Auklets), laying date was estimated to occur 7 days following capture (method also used by Lougheed 2000). To achieve predictions that would be closest to natural conditions, we assumed there was no capture effect which might increase the number of days between egg-production and egg-laying beyond the presumed 14-day period. Nighttime detections of juvenile Marbled Murrelets in the study area were used as a comparison with the egg-producing phase, independent of VTG-derived predictions, to gauge the accuracy of predictions based on the egg-producing phase. Dipnetting occurred for 84% of the days we were in the field (Centre for Wildlife Ecology, unpubl. data), and thus, we suggest that the probability of sighting juveniles when they first appeared in the study area during this time was high.

Data Analysis

All statistical analyses were carried out using Minitab (version 13) software. Normality was assessed using Anderson-Darling normality tests, and in most cases, nonparametric test were used: differences in VTG between mistnet and dipnet birds (Mann-Whitney), differences in distribution of egg-producers (Kruskall-Wallis) and mean dates of egg-production (Kruskall-Wallis). Because mean egg-producing date was not significantly different between years, pooled mean egg-producing date was used in comparisons of proportions of egg-producers. Proportions of egg-producing birds (of all females caught) in both years were calculated for each SD away from the mean egg-producing date. Proportions females that were egg-producing were calculated from 17 April (which is 2 SD before the mean) to 15 August (which is 5 SD after the mean). Calculating proportions for more than 2 SD before the mean was impossible, because no birds were caught before then. This would not have caused a biased estimate of mean egg-laying date, because we are confident that early egg-production was captured in the study sample (see results and discussion below).

Proportions of breeders at 20-day intervals were assessed using a test and confidence interval for two proportions (Minitab). Mean date plus one standard deviation (SD) is given for each stage in breeding chronology, to indicate 'mean period' (e.x. mean egg-producing period=mean date $\pm$ 1SD). Significance was assumed at α=0.05. VTG is measured in micrograms per millilitre (ug/ml).

1.3 Results

Inaccuracies resulting from underestimation of VTG in males and non-laying females were avoided by re-assaying a subsample of plasma at the correct dilution, and correcting the remaining non-breeding plasma using a regression equation. Four-fold dilution of nonbreeding plasma seems to interfere with the depletion of VTG, and whenever possible, the correct dilution should be used.

When plasma was stored in a –20°C freezer for up to 18 months, plasma started to degrade and lose moisture. Although the time-dependent decrease in plasma VTG was significant, 100% (63/63) of females would still have been classified as egg-producers. That is, the decrease in VTG was not enough to re-classify egg-producing plasma to non-egg-producing plasma in this study (if VTG >0.96 ug/ml is an egg-producer). This could be a concern for studies in which plasma is not analyzed for some time following collection, but in this study, freezing had no effect on classifying egg-producers.

Comparison of Capture Methods

In both years, mean VTG was significantly higher in dipnetted females than in all males and mistnetted females (Figure 1.1). This clarified that VTG is indeed present only in females (never males), and that mistnetted birds are not producing eggs. In 1999, during the period of overlap between mistnetting and dipnetting (8 Jun through 28 July), 50% (10/20) of females caught with the dipnet method were producing eggs. However, none of the females caught in the mistnets were egg-producers (n=5) (Table 1.1). This was repeated in 2000: during the period of overlap between the two capture methods (14 June

to 29 July), 45% (13/29) of females caught using the dipnet method were producing eggs, while none of the females caught in the mistnets were egg-producers (n=6) (Table 1.1).

Distribution of Egg-Producers

Using VTG, we were able to observe the duration of the egg-producing phase in the population from April to August, for two breeding seasons. We plotted VTG values in females from April-August, for each year separately (1999, 2000) (Figure 1.2). Using egg-producing females only (Figure 1.2, above the hatched line), we calculated the mean and range of egg-production for both years. No males were found with elevated VTG-Zn in either year (1999 mean VTG-Zn=0.28 ± 0.03 ug/ml, n=48; 2000 mean VTG-Zn=0.23 ± 0.05 ug/ml, n=34) (Table 1.1). All birds with VTG-Zn $\geq$ 0.955 ug/ml were females (as determined by DNA sexing).

In 1999, blood samples from 92 females (dipnetted from 20 April to 4 September) were analysed for VTG-Zn (mean=1.37 ± 0.24 ug/ml). Of these, 34% (31/92) had elevated VTG-Zn (≥0.96, mean=4.087 ± 0.36 ug/ml). Egg-producers were not caught during the entire dipnet period, but from 27 April to 6 July (Figures 1.2, 1.3). The mean egg-producing date in 1999 was on May 24 ± 20 (SD) (Julian date 145), with the highest frequency of egg producers (Figure 1.3a) on May 9 (Julian date 130). The duration of detected egg-production was 70 days.

In 2000, blood samples from 66 females (dipnetted from 19 April to 31 July) were analysed for VTG-Zn (mean=2.05 ± 0.35 ug/ml). Of these, 49% (32/66) had elevated VTG-Zn (≥0.96, mean=4.20 ± 0.44 ug/ml). Egg-producers were caught from 20 April through 6 July (Figures 1.2, 1.3). Mean egg-producing date in 2000 was on May 29 ± 21

(SD) (Julian date 150), with the highest frequency of egg-producers captured on May 24 (Julian date 145) (Figure 1.3b). The duration of detected egg-production was 77 days. These data outline the asynchrony in egg-production in both years.

Mean dates of egg-production did not differ between years (Kruskal-Wallis H=0.82, df=1, p=0.37) (Figures 1.2, 1.3). Mean date of egg-production for pooled years was May 27 $\pm$ 2.5 days (SE) (Julian date 148). One standard deviation around the mean ($\pm$ 20 days) ranges from May 7 to June 16, and will be defined as the 'mean egg-producing period'.

Predicting Breeding Chronology

This study used egg-producers to construct a breeding chronology of Marbled Murrelets in Desolation Sound, by predicting successive breeding stages. Table 1.2 outlines the chronology for each breeding season separately, then pooled. Mean periods include central date $\pm$ 1 SD (i.e. each mean period is 40 days), and overlap each other. In 1999, mean incubation period ranged from May 11 – June 20, mean chick-rearing from June 10 – July 20, and mean fledging from July 9 – Aug 16. In 2000, mean incubation period ranged from May 15-June 26, mean chick-rearing from June 14 – July 26, and mean fledging from July 12 – August 23. Based on the mean egg-producing period for pooled years, mean incubation period was estimated to be May 14 – June 23, mean chick-rearing was estimated to occur from June 13 – July 23, and mean fledging was from July 11 – August 20.

Based on the first egg-producers (27 April 1999, 20 April 2000), we predicted that the first juveniles would appear in the study area on 1 July (1999) and 24 June

(2000). First juveniles were actually seen at night (dipnetting) on 30 June and 25 June, respectively (Table 1.2). Calculating from the first (20 April) and last (July 6) egg-producers results in an overall fledging timespan of 77 days, ranging from 24 June to 9 September. Based on first and last egg-producers detected, the entire breeding season ranged from 20 April (start of egg-production) to 9 September (last juvenile fledged), 142 days in total.

Proportions of Egg-producers

Proportions of egg-producers (out of all females) in both years were calculated for each 20-day SD away from the mean egg-producing date (Table 1.3). Proportions of egg-producers were calculated up to 15 August (5 SD after the mean), however, no egg-producers were caught after 6 July in either year. Proportions of egg-producers in each 20-day std. dev. from the mean did not differ significantly between years (Table 1.3). Proportions were highest from 17 April to 6 July, ranging from 0.46 to 0.80 egg-producing females, but did not differ significantly until they dropped (two-sample t-tests, $p=0.001$) after 3 July to 0.008-0.

The proportions of egg-producing females caught during the whole period of egg production (April through July) were 0.54 (n=58, 1999) and 0.56 (n=57, 2000). When including females captured during entire season (April to September), this proportion changes to 0.34 (n=91, 1999), and 0.49 (n=66, 2000).

Figure 1.1: Plasma levels of egg-yolk precursors (VTG-Zn (ug/ml)) found in Marbled Murrelets, by sex (dark=F, light=M) and by net group (hatched = mistnet, open=dipnet), years pooled. Data include birds captured during the *entire* breeding season (April to August). Asterix indicates group that is significantly different (Mann-Whitney, p=0.001).

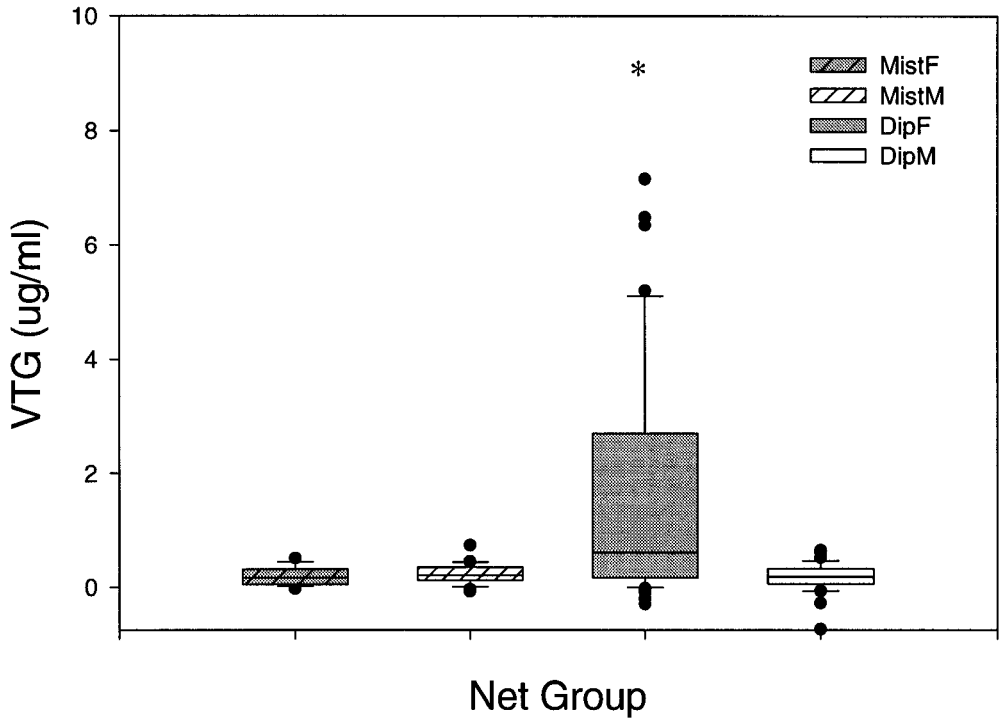


Table 1.1: Plasma VTG-Zn in Marbled Murrelets caught using mistnet and dipnet methods *concurrently* (only during June and July), by sex and year. Data are $\mu \pm$ SE. Tests are done across columns (i.e. within a row, between mistnets and dipnets). No egg-producing females were caught in the mistnets.

		VTG-Zn (ug/ml) (n)		Mann-Whitney	
Year	Sex	Dipnets	Mistnets	W	p
1999	Females	1.85 ± 0.49 (20)	0.14 ± 0.07 (5)	1482.5	0.02
	Males	0.28 ± 0.04 (17)	0.20 ± 0.05 (10)	222.0	0.21
(subset)	Egg-producers	3.45 ± 0.65 (10)	None	-	-
2000	Females	1.75 ± 0.38 (29)	0.23 ± 0.08 (6)	2179.0	0.02
	Males	0.11 ± 0.09 (15)	0.28 ± 0.06 (13)	807.0	0.84
(subset)	Egg-producers	3.64 ± 0.44 (13)	None	-	-
Pooled	Females	1.78 ± 0.30 (49)	0.19 ± 0.05 (11)	7194.5	0.001
	Males	0.18 ± 0.05 (32)	0.24 ± 0.04 (19)	4158.0	0.48
	Egg-producers	3.56 ± 0.37 (23)	None	-	-

Figure 1.2: Vitellogenin levels in females by date. Describes duration of egg-producing period for two breeding seasons (birds captured from April – August/September) in Marbled Murrelets in Desolation Sound, B.C. Values above 0.955 (dotted line) indicate egg-producing birds. Mean egg-producing date shown as '$\mu = (x) \pm SD$' and is calculated using egg-producing birds (i.e. above dotted line).

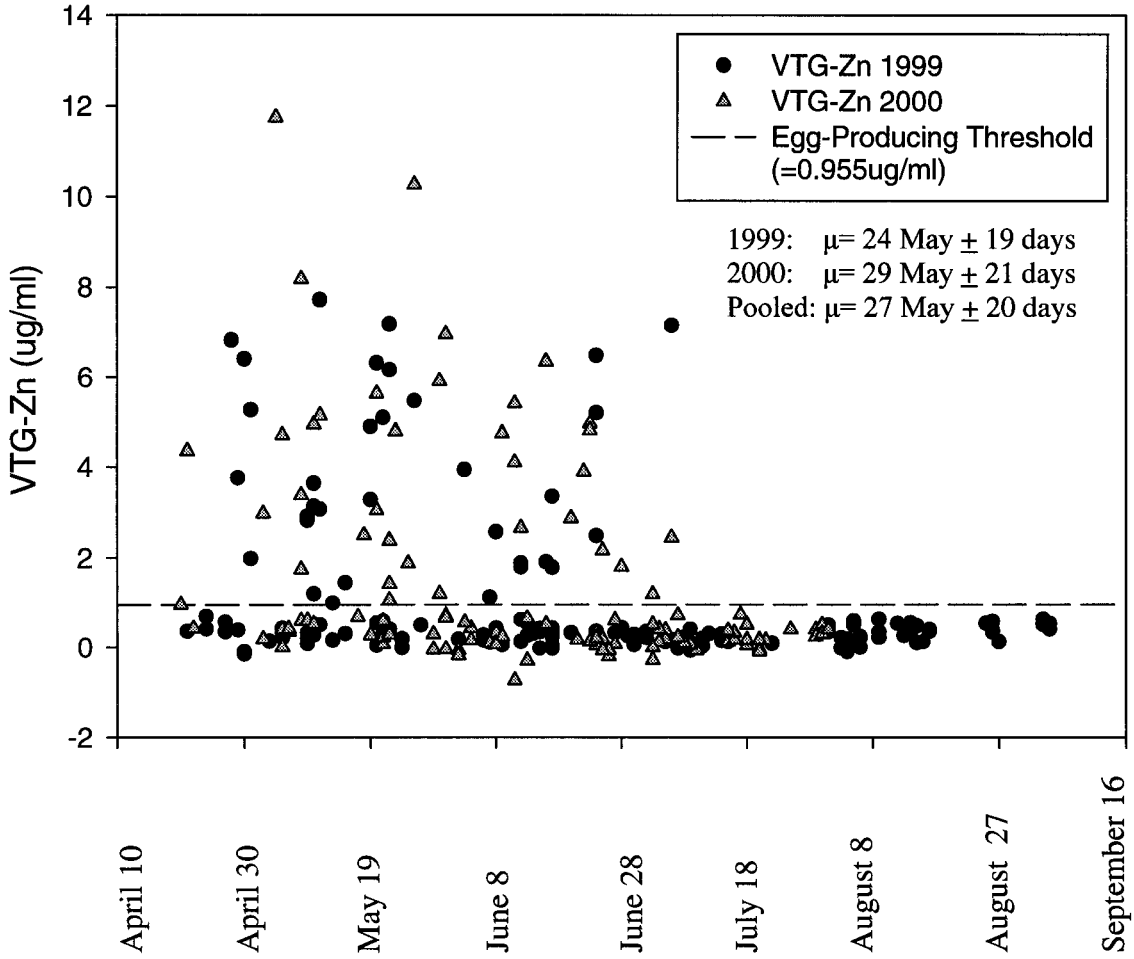


Figure 1.3: Distribution of egg-producers by date in (A) 1999 and (B) 2000 (histogram bars are grouped in 10-day periods).

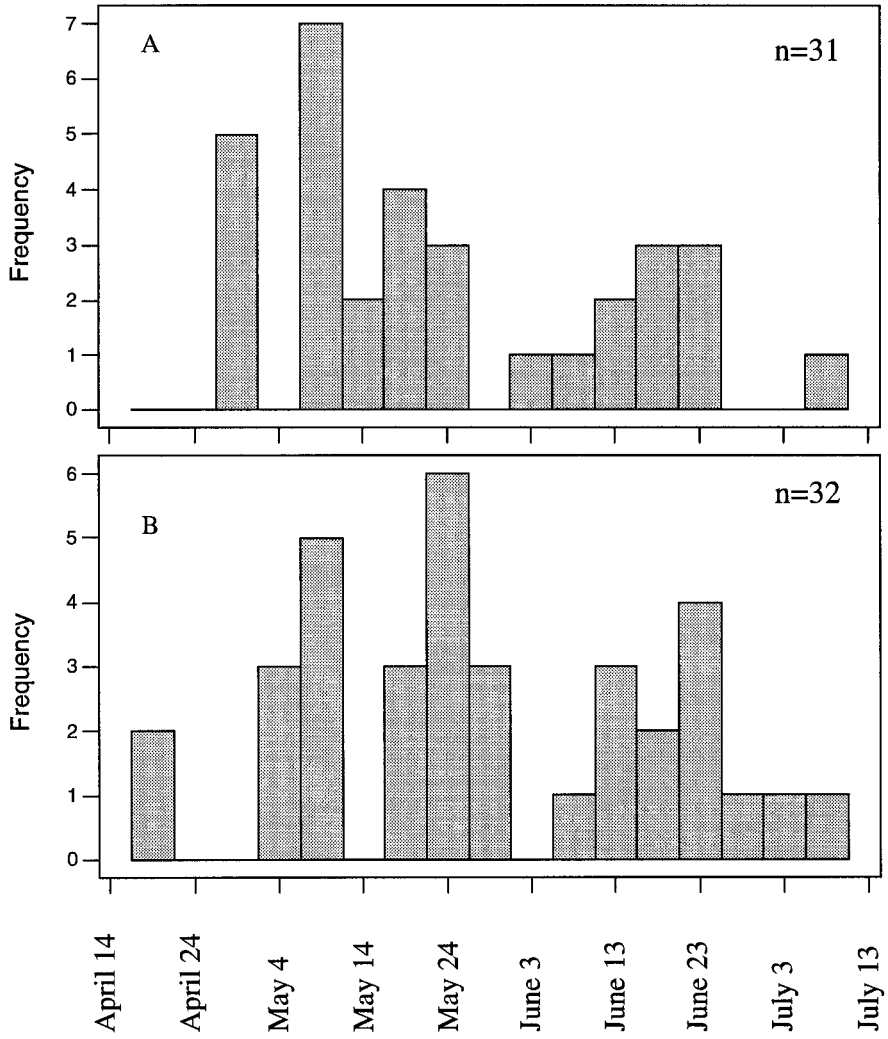


Table 1.2: Phenology Data with predictions for breeding stage timing in Marbled Murrelets. Data are ± S.D. (days) unless otherwise stated. Included are data from Vanderkist (1999) as comparison of previous study using VTG-Zn, and Lougheed (2000) as a comparison for estimating phenology using multiple methods (at-sea surveys, forest observations, radiotelemetry (Lougheed 2000), VTG-analysis (Vanderkist 1999)).

Study	Year	Start of captures	First egg-producer caught	Last egg-producer caught	Mean egg-producing date ± S.E.	Mean egg producing period	Mean initiation of incubation period	Mean chick-rearing period	Mean predicted fledging period *	First juvenile predicted **	First juvenile seen (dip-netting)
This study	1999	20 Apr	27 April	6 July	24 May ± 3.6	24 May ± 19	June 1± 19	July 1 ± 19	28 July ± 20	1 July	30 June
	2000	19 Apr	20 April	6 July	29 May ± 3.5	29 May ± 21	June 5 ± 21	July 5 ± 21	2 Aug ± 20	24 June	25 June
	Pooled	-	-	-	27 May ± 2.5	27 May ± 20	June 3 ± 20	July 3 ± 20	31 July ± 20	-	-
Vanderkist 1999	1997	14 May	14 May	3 July	-	21May-10 July -			-	11 July	27 Jun
Lougheed 2000	1996-1998	-	-	-					July 20 or July 30♦	-	-
Hamer & Nelson 1995	Breeding records	-	-	-			2 May-4 July	1 June-30 August			

* mean predicted fledging period = (mean egg-laying period ± SD) + 7 days to egg lay + 30 days (incubation) + 28 days (chick-rearing).
**first juvenile predicted = date first egg-producer caught + 7 days to egg lay + 30 days (incubation) + 28 days (chick-rearing).
♦ indicates median date only

Table 1.3: Percentages of egg-producing female Marbled Murrelets caught using the dipnet method during 1999 and 2000. Dates are arranged in 20-day standard deviations around the mean egg-laying date (**27 May**), which was determined using the seasonal occurrence of only the egg-producing birds (see Figure 1.2). Sample sizes are in brackets. Percentages in each period did not differ by year (i.e. across columns), except in row 1. Rows with the letter 'A' are not significantly different from each other. Rows with the letter 'B' are significantly different from those with the letter 'A' (p=0.00 in 1999, p=0.01 in 2000)

Dates, in 20-day periods (each period is 1SD from mean egg-laying date, in bold)	Percentage of females that were producing eggs		
	1999	2000	
17 April to 7 May	41.7% (12)	83.3% (6)	A
8 May to **27 May**	67.7% (21)	87.5% (16)	A
28 May to 16 June	41.7% (12)	47.1% (17)	A
17 June to 6 July	54.6% (11)	50% (16)	A
7 July to 26 July	0% (6)	0% (10)	B
27 July to 15 August	0% (19)	0% (2)	B

1.4 Discussion

This is the first study to use physiology to describe inter-annual variation in egg-production in Marbled Murrelets. From this assessment, inter- and intra-annual asynchrony in egg-production was examined, incubation and chick-rearing phases were predicted, and proportions of egg-producers were estimated. This study also assessed some methodological questions regarding capture methods, and VTG analysis itself. It is necessary to catch the bird at the correct time in its egg-producing phase in order to detect VTG in the blood. Females captured well before or after they were producing eggs would be missed, which almost certainly has caused under-estimation of the number of egg-producers in the population. However, the duration and range of egg-production should be accurate, if we assume an equal probability of catching any female throughout the season.

Comparison of Capture Methods

Most capture methods used by biologists usually assume random sampling of the population. However, this assumption has been challenged in the Desolation Sound study area, due to a significant male bias found in birds caught in mistnets (Vanderkist 1999, Vanderkist *et al.* 1999; Bradley *et al.* 2002). It has been suggested that dipnetting and mistnetting catch marbled murrelets at different reproductive stages (Vanderkist 1999, Bradley *et al.* 2002). Results from this study support this hypothesis, detecting no egg-producing murrelets in the mistnet sample in two years of study. VTG in the dipnetted females was significantly higher than in all other groups, reinforcing the idea

that mistnet females are either from a different subset of the population, or are using the area only after egg-production. My data support Vanderkist's (1999, also Vanderkist *et al.* 1999) finding that one cannot assume that different capture methods sample populations randomly.

The Egg-Producing Period in the Marbled Murrelet

When reproductive status is difficult to assess, analysis of plasma VTG is a valuable tool to identify the reproductive status of individuals (Mitchell & Carlisle 1991), and provides information on breeding status (Vanderkist *et al.* 2000, this study) and chronology at a population level. We were able to take blood samples from Marbled Murrelets spanning the duration of the breeding season, capturing murrelets earlier and later in the Desolation Sound study area than ever before. Egg-laying in Marbled Murrelets has been previously estimated to last from early May to mid July in B.C. (Nelson 1997). The present study extends Nelson's (1997) early egg-laying estimate by about 2 weeks. The earliest estimate for the egg-laying in the Desolation Sound area is 14 April (Lougheed 2000), inferred from fledglings seen at sea. It is possible that we missed some early egg-producing activity (note skew in Figure 1.2, Figure 1.3). While the highest frequency of egg-producers came earlier than the mean egg-producing date in both years of our study, it is normal for egg-laying to be skewed to the earlier part of the egg-laying period in other seabirds (Ainley and Boekelheide 1990, Birkhead and Nettleship 1987), and is associated with within-season synchrony of laying date (i.e. nesting synchrony between individuals in a colony) (Ainley and Boekelheide 1990). In our study, predictions of juvenile appearance based on early egg-production were closely followed by actual

juvenile appearance at the study site, suggesting that the start of egg-production was not missed.

While some colonial alcids have been found to lay eggs synchronously (within 10 days in some colonies of Razorbills), others are more asynchronous within years (50 days in Common Murres; 35-40 days in Thick-billed Murres; ~ 21 days in Dovekies; ~ 42 days in Pigeon Guillemots) (Gaston and Jones 1998). In populations of other murrelets, egg-laying can take 14-21 days (Kittlitz' Murrelet), 45 days (Ancient Murrelet), or can show an extreme range of up to 4 months egg-laying (Xantus' Murrelet) (Gaston and Jones 1998). Ainley and Boekelheide (1990) report a prolonged initiation of laying (of the first eggs) over three months in Cassin's Auklets, but found that the timing of termination of egg-laying was much more regular, observing that colonies of auklets finished their clutches within 7 days, for most of 14 years. Similarly, the present study found a prolonged egg-production period (assumed to correspond closely to laying period) for Marbled Murrelets, of 70-77 days, while the last egg-producers were caught on 6 July in both years. This compares closely with Lougheed (2000), who found that mean laying period in Desolation Sound was 79 days, based on a three-year average using predictions from multiple techniques (radiotelemetry, truncated VTG analysis, at-sea surveys, and forest observations).

Inter-individual variation such as age and experience (Western Gulls, Sydeman *et al.* 1991; Wandering Albatross, Wiemerskirch 1992; Thick-billed Murre, Gaston *et al.* 1994, DeForest & Gaston 1996), body condition (Common Murre, Cassin's Auklet, Ainley and Boekelheide 1990), and body size (Snowy Petrel, Barbraud *et al.* 2000) could play a role in variation of onset of breeding. For this study, no environmental data were

collected, nor was it possible to determine age or breeding experience due to low recapture rates in the study area. In addition, it has not been possible to link variation in body mass in Marbled Murrelets with inter-individual variation in timing of breeding activities (Hull *et al.* in press; R. Bradley, unpubl. data; see also chapter 4). The selective forces that shape breeding synchrony and asynchrony in so many other seabirds are not yet clear for Marbled Murrelets, and require further study.

In spite of the within-year asynchrony seen in Marbled Murrelets, the present study found that mean egg-producing date and duration did not vary between the two study years. Thus, it seems that, while egg-laying in Marbled Murrelets is significantly more asynchronous (intra-annually) than most other Alcidae (Lougheed 2000), between-year variation in egg-laying was not pronounced, at least at our study site. Given that one force suggested to reinforce synchronous breeding is conspecific information exchange at the colony nest site (Buckley 1997, Danchin & Wagner 1997), non-colonial Marbled Murrelets should be expected to exhibit low intra-annual breeding synchrony (Lougheed 2000). However, one might expect a higher rate of between-year asynchrony if there are no conspecific cues. This suggests that the state of the individuals, or the environmental conditions in the study area, are constant enough to cue individuals to breed at approximately the same time each year. It may be that high prey concentrations in coastal fjords during the summer relates to non-coloniality in this species (Kaiser 1994), and that this prey is relatively constant between years in these inshore waters.

Unlike the mistnet method, the dipnet method captured murrelets in all phases of reproduction. It was assumed that the murrelets caught in the study area using the dipnet method were representative of the population and thus, the proportion of egg-producing

females caught should also have been representative. However, the actual structure (i.e. age and breeding status) of the Marbled Murrelet population in most areas is poorly known (Gaston and Jones 1998, Cooke 1999, Cam *et al.* in review). We have found few data on the relative distributions of breeding, nonbreeding, or prebreeding Marbled Murrelets, as are available for other seabirds (eg., see Ainley and Boekelheide 1990, Gaston *et al.* 1994). Thus, there is no information on social associations between breeders and nonbreeders. They may be spatially or temporally segregated within the study area or more broadly, along the BC coast; or they may not be segregated at all, aggregating and mixing freely in Desolation Sound. Distribution of breeders and nonbreeders in the study area is an important factor influencing the probability of encountering a breeding bird when captures are taking place, and could thus influence results reported here. This requires further study.

Breeding Chronology in Desolation Sound

This study was able to predict breeding chronology for two years based on one simple physiological analysis, and to make comparisons with other studies (such as Hamer & Nelson 1995, Lougheed 2000) which used a compiling approach to make predictions based on breeding records and at-sea observations. The appearance of juveniles in the study area allowed an independent assessment of the VTG-derived estimate of breeding chronology, and corresponded within one day to the predicted juvenile appearance in both years.

The duration of the breeding season calculated in this study, from the first presence of egg-producing females to the last predicted juvenile fledged, was 142 days in

total. This corresponds closely to that calculated by Lougheed (2000), which averaged 137 days (calculated using a combination of methods, including at-sea surveys, fish-holding behaviour, radio telemetry, forest observations, and VTG data from Vanderkist (1999)).

Using breeding chronology, it can be possible to make direct comparisons of inter-annual variation in the onset, and duration of breeding stages. In the present study, mean egg-production dates and presence of juveniles did not differ significantly between 1999 and 2000. In contrast, Lougheed (2000) found a trend towards earlier breeding in the same study area from 1996 to 1998, but it is difficult to directly compare our results with hers, since hers come from a compiled dataset with multiple breeding season endpoints. She found that the trend coincided with significant inter-annual increases of sea-surface temperature during the breeding season in Desolation Sound. This is consistent with Ainley and Boekelheide (1990), who found that temperature, along with food and nest site availability, are often causes for inter-annual variation in timing of breeding in many seabirds. Although we found no annual variation (eg., Speckman *et al.* 2000), we are confident that using VTG to track egg-production and predict breeding phenology would detect any inter-annual variation caused by environmental change, if blood sampling began early enough and continued throughout the breeding season. This method could also be used to address changes in breeding phenology across latitudes. Causes of inter-annual variability (or local variation across latitudes) along with their impacts on life-history characteristics, could then be assessed. Because there was no variation in breeding chronology across years, we feel that the environment the murrelets encountered in Desolation Sound did not vary significantly during the two years of study.

Proportions of Egg-producing Marbled Murrelets

Predictions of population sizes and demographic trends must be calculated using some measure of breeding individuals in the population (Ebert 1999). However, assessing the proportion of reproductive individuals in the population can be difficult, as pre-breeders often disappear from the colony (Ainley and Boekelheide 1990), and non-breeders may or may not attend a colony ('floater populations', Ainley and Boekelheide 1990). In the present study, non-egg-producers (as defined by VTG analyses) could have laid eggs prior to or after sampling. Thus, the proportions of egg-producers in this study do not represent true 'fecundity' measurements, but are certainly low estimates.

Other studies have counted breeders using the percentage of occupied sites at a colony, or the percentage of occupied sites at which eggs were eventually laid (Ainley *et al.* 1990). For example, Brandt's Cormorant on the Farallones laid eggs at only 69-71% of occupied sites (Ainley *et al.* 1990). The proportion of breeders has been shown to vary with the frequency of return rate of mature adults to the colony, which can vary from 25-100% (Williams & Rodway 1992). Returns can be related to food availability and the previous years' breeding success (Williams & Rodway 1992, Weimerskirch 1992). A better understanding of Marbled Murrelet attendance in our study area is needed, as well as increased information on food resources and nest site availability. From our results, we suggest that the population of Marbled Murrelets in Desolation Sound should be assessed as if it included prebreeders (physiologically immature), as well as the reproductively mature birds, including non-breeders (who may be in poor body condition, recovering from a previous breeding season or failed breeding attempt, or experiencing

nest-site competition). In a non-colonial system, and away from nest sites, there is no reason to believe that breeders in the at-sea study area would be caught any more frequently than non-breeders or pre-breeders. However, as discussed earlier, we do not have any information on relative distributions of breeders, nonbreeders, or pre-breeders. There is little information on recruitment of murrelets (Divoky and Horton 1995) to our study area (see Cam *et al.* in review). Although egg-producing females seem to have made up a small and fluctuating proportion (38-87%) of birds we caught, they may have been swimming in a local population that includes resident and immigrant nonbreeders and prebreeders. More work to determine age structure and breeding propensity of Marbled Murrelet populations is needed to gain a clear understanding of their reproductive potential.

Chapter 2

Are Brood Patches Reliable? Examining the Use of Brood Patches to Describe Reproductive Status in Marbled Murrelets

2.1 Introduction

A brood patch (BP) is an area of highly vascularized, bare skin found on the abdomen of incubating birds (Bailey 1952, Phillips *et al.* 1985). Parent birds use the BP to transfer heat directly to the egg during incubation, or to keep newly-hatched chicks warm. Using BP to assess parental status is common practice (eg; see Ainley *et al.* 1990, Deviche 1997). Passerines and many other taxa of birds lose abdominal down within a few days of egg-laying (Bailey 1952, Gill 1995, Manuwal 1974), and the close timing of vascularization of the BP with the onset of incubation can provide criteria for determining when the eggs are laid (Ainley *et al.* 1990). However, in a few species of seabirds, pre-breeders lose abdominal down (Lange 1928 *in* Bailey 1952, Ainley *et al.* 1990, Gaston & Jones 1998, I. Jones pers. comm), which challenges the reliability of using early BP stages to indicate reproductive status. In most colonial seabirds nesting results are obvious; breeding can be directly assessed or, if BP is used to infer breeding, incubation status can be confirmed at the colony. However, breeding status is not easily confirmed, especially when the study species is not colonial. This study assessed the accuracy of BP to infer reproductive status in Marbled Murrelets (*Brachyramphus marmoratus*) for which breeding status is difficult to confirm.

In the case of the Marbled Murrelet, which is classified as threatened in Canada and the United States, inclusion of individual reproductive potential would allow more reliable estimates of demographic parameters. Thus, scoring BP to provide reliable information on reproductive status would be very valuable. However, Marbled Murrelets nest at largely inaccessible sites in old-growth coniferous trees (Nelson 1997), leaving few opportunities to observe morphological or physiological characteristics of adults coinciding with specific breeding activities such as egg-laying, incubation, or chick-feeding. Marbled Murrelets are usually captured off the nest during the breeding season (see Kaiser *et al.* 1995, Vanderkist *et al.* 2000, Lougheed 2000), and thus, are examined in the absence of any information on breeding status.

Variation in timing of incubation, chick-rearing, and chick-fledging, is often studied (Murphy 1995, Birkhead & Nettleship 1982, Hipfner 1997, Daunt *et al.* 2001), but few studies have ever tried to assess timing of breeding remotely by assessing adults away from the nest site, colony or chick, simply because they have not needed to. With Marbled Murrelets, remote assessment is the only option (Speckman *et al.* 2000), and because both pre-breeders and breeders exhibit alternate plumage during the summer months, BP scores are the only physical sign that can be used to infer breeding status at the time of capture.

During the breeding season, Marbled Murrelets caught in Desolation Sound could be breeders, nonbreeders, or pre-breeders; and breeders could be caught in any reproductive stage (egg-production, incubation, or chick-rearing). It would be advantageous to be able to use BP scores to distinguish between each of these groups in

the field. Thus, this study attempted to correlate the BP of Marbled Murrelets, to the timing of breeding found using physiological analyses and radio telemetry.

Multiple research approaches (none infallible, and with varying degrees of accuracy) are used in this study to assess breeding status and provide independent estimates with which to compare BP data. These include: (1) analysis of vitellogenin (VTG), an egg-yolk protein present in the plasma of egg-producing females (Deeley *et al.* 1975, Redshaw & Follett 1976), which allows identification of fecund females (Vanderkist *et al.* 2000); (2) Radio telemetry, used to study survival, movement, and habitat use (Bunck & Pollack 1993), is particularly useful in Marbled Murrelets, who are highly mobile, commuting daily over distances of up to 100 km between nest sites and foraging areas (Hamer & Nelson 1995, Hull *et al.* 2001, Centre for Wildlife Ecology, unpubl. data). In this study, radio telemetry was used to track the daily activities of Marbled Murrelets throughout the breeding season, thereby allowing us to predict the onset of incubation (laydate). Since we do not know how long it takes for Marbled Murrelets to make an egg, prediction of laydate for individuals would be impossible without radio telemetry.

The objectives of this study were to test how well BP correlated with nesting events (egg-production and incubation), and whether BP could be used to correctly infer reproductive status of individuals. We did this by (1) reporting the timing of specific BP scores in the capture sample; (2) using plasma VTG to describe the link between egg development and BP development; (3) using radio telemetry to track individuals, predict the egg-laying date, and relate this to BP score and VTG at the time of capture; and (4)

comparing dates and durations of egg-production, egg-laying, and fully-developed brood patches using different methods.

2.2 Methods

Blood samples for physiological analyses and BP scores were taken at the time of capture, while laydates were determined using radio telemetry after capture. Thus, all BP and physiological measurements were made without any prior knowledge of reproductive status or laydate information.

Captures and Blood-Sampling

Captures occurred in Desolation Sound, British Columbia (centre 50° 05'N, 124° 40'W), from 20 April to 4 September in 1999, and 19 April to 26 August in 2000. "Dipnetting" (Whitworth *et al.* 1997, Lougheed *et al.* 1998) was used to catch birds at night between 22:00 and 05:00. Birds were captured from the surface of the water, using a spotlight and a salmon landing net. Mistnetting was also employed to catch birds (8 June to 30 July in 1999, 14 June to 29 July in 2000) at the mouth of Theodosia Inlet (50°04'N, 124° 42W) (Kaiser *et al.* 1995), adjacent to Desolation Sound. Dipnet and mistnet techniques were employed concurrently after June 1; mistnets do not catch murrelets any earlier than this (Kaiser *et al.* 1995).

Radio transmitters (weight 3 g, Advanced Telemetry Systems, Model No. 386) were attached with subcutaneous anchors, following the methods of Newman *et al.* (1999) but without sutures or anaesthetic; instead of sutures, each radio was glued with a

small amount of Bird Epoxy (Titan Corporation, USA) to the dorsal feathers. Radio transmitters were attached to murrelets early in the season, with the criteria to have all radios attached before the end of June (1999) or the end of May (2000). Four out of every five murrelets caught had a transmitter attached (the remaining one had only a blood sample taken , for another part of the study). Radios are secure for 3-4 months at least, and are known to fall off between breeding seasons (CWE unpubl. data), probably during pre-basic moult. Blood samples were taken approximately 5 minutes after radio attachment and birds were released immediately after glue on the radio had dried.

Marbled Murrelets captured using both methods were bled. Birds were bled with no knowledge of their sex or breeding status at the time of sampling. Blood samples (1-2 mls) were taken from the brachial vein using a heparinized 1/2" 26 guage hypodermic needle with a 3 cc syringe. Blood was centrifuged at 6,000 rpm for 10 minutes, plasma removed, and red blood cells and plasma were frozen at -20°C until they could be transported to laboratory facilities for further analysis.

Scoring Brood Patches

Marbled Murrelets have one large central brood patch (Sealy 1972, LMT pers. obs), worth noting because in Nelson 1997 and Gaston and Jones 1998, it is incorrectly reported as two lateral patches. Brood patches (BP) were scored from 0-6, according to Sealy's (1972) classification:

BP 0=no evidence of defeathering (similar in appearance to BP 6, but occurs early in breeding season).

BP 1=beginning loss of down and contour feathers.

BP 2=almost complete loss of down and most contour feathers, vascularization beginning.

BP 3=complete loss of feathers, heavy vascularization.

BP 4=regression beginning with down appearing, especially around the edges, sheaths of new contour feathers appearing.

BP 5=most of the area down-covered, contour feathers beginning to break out of sheaths

BP 6=complete regression, appearance as in BP 0, but at the end of the breeding season.

For some analyses, I pooled BP scores according to developmental stage (Bailey 1952 for description of changes to the abdominal skin in BP development in passerines): absent (BP 0), developing (BP 1-2), fully-developed (BP3-4) and regressing/regressed (BP 5-6). BP 4 was included in the 'fully developed' category because in the field, it was sometimes difficult to make a definite distinction between BP 3 and 4. For clarification, this study uses the following terms, based on presumptions of what BP scores indicate, to describe the breeding status of birds: 'pre-breeder' (expected to have BP 0) is used to describe young birds that are still reproductively immature; 'early breeders' (expected to have BP 0) are reproductively mature, but have not yet initiated breeding; 'breeders' (could have BP 1-5) are reproductively mature and have begun a breeding attempt; and 'post-breeders' (BP 6) have presumably completed their breeding attempt for a given year.

Vitellogenin Analysis

Vitellogenin (VTG) concentration in plasma is determined indirectly, with an assay for vitellogenic zinc (following Mitchell & Carlisle 1991), using a diagnostic kit from Wako Chemicals (Zn, Cat. No. 435-14909). Vitellogenic zinc (VTG-Zn) is used as an index for VTG, as described and validated for Marbled Murrelets by Vanderkist (1999) and Vanderkist *et al.* (1999). VTG-Zn is selectively precipitated from the plasma with dextran sulphate (Griffin & Mitchell 1984); the difference between the original (total) plasma zinc and the remaining zinc (albumen-bound) following precipitation provides an index of VTG-Zn (Mitchell & Carlisle 1991, Williams 1999).

DNA Sexing

Marbled Murrelets were sexed following the methods described in Vanderkist (1999) (from Griffiths 1996), with a few modifications. Blood samples were either centrifuged red blood cells, or a drop of blood that had been dried on a small piece of filter paper. Unlike in Griffiths (1996), genomic DNA was isolated from erythrocytes without using a phenol/chloroform extraction. Instagene Matrix (Bio-Rad Laboratories, Hercules, Ca, Cat. No. 732-6030) was used to finish the extraction, following the procedures provided by the manufacturer. Extracted DNA was amplified using PCR, following the description in Vanderkist (1999a,b). Male birds were identified by the presence of a single 400-base pair band, and females by the presence of both 470 and 400 base pair bands.

Determining Laydate

Laydate (used synonymously with 'egg laying' and 'onset of incubation') was determined using radio telemetry. Telemetry from a helicopter tracked the presence of radio-tagged birds on the water (foraging/staging area) and in the forests (nest site). The presence or absence of individual radio-tagged birds on the water were noted daily. Because parent Marbled Murrelets incubated the egg in equal, regular incubation shifts of 24 hours (Nelson 1997, Bradley *et al.* 2002), radio telemetry was able to detect the patterns of presence or absence of radio-tagged birds in the foraging area (Desolation Sound). Once a radio-tagged bird began to leave the foraging area regularly, every second day, it was assumed to be incubating (R. Bradley, unpubl. data). Additional data to reinforce this assumption came from radio-tagged birds in which both members of the pair had a radio; these pair members disappeared regularly and alternately (one bird on the water one day, the other bird on the next, and so on).

Comparing dates of egg-production, egg-laying, and incubation

The following terms are used to describe dates of nesting activity: 'mean egg-production' was determined by the presence of females with elevated VTG (see chapter 1), and is the mean seasonal date when eggs are still being produced, not laid yet. 'Mean laydate' is the mean seasonal date of the start of incubation (as determined by radio telemetry, above). 'Mean BP 3' refers to the mean seasonal date when birds have fully-developed brood patches, and are thought to be incubating. If the latter is correct, 'mean BP 3' may also refer to mean incubation period in the population. Mean laydates were compared with mean egg-producing dates, and with mean date of fully-developed BP.

Statistical Analyses

All statistical analyses were done using Minitab 13 for Windows. There were no discernible differences in BP development in males and females (Sealy 1972, this study), so BP scores were pooled by sex (except in VTG analyses). All analyses are done on birds captured only once in the breeding season, except when recaptures are specifically mentioned (Table 2.2). Normality was tested using Anderson-Darling Normality tests, and visual assessment of frequency distributions. Two-sample t-tests were used to test differences between the ranges of dates that each BP score occurred, because their distributions did not have the same shape or standard deviations. ANOVA with Tukey's pairwise comparisons were used to test VTG in groups with different BP scores. Nonparametric tests (Mann-Whitney) were used to test differences between the distributions (by seasonal date) of egg-producers, initiation of incubation, and presence of fully-developed BP.

In all boxplots, the box represents the middle 50% of the data. The line through the box represents the median, and dotted lines or dots in the box indicate means. The lines (whiskers) extending from the box represent the upper and lower 25% of the data (excluding outliers). Outliers are represented by asterisks (*).

2.3 Results

Evidence of Brood Patches but No Incubation.

During this study, we were able to track the daily activity patterns of 139 radio-tagged Marbled Murrelets. Of all radio-tagged individuals, only 56% exhibited incubation

behaviour (Table 2.1), with approximately equal numbers of incubators and putative nonbreeders in each BP category. There was no obvious correlation between BP score and propensity for incubation, with about half of the birds in each BP category (including fully-developed BP 3) proceeding to the incubation stage (see Table 2.1). This finding prompted the following comparisons of BP scores with physiological analyses and radio telemetry.

Variation in Brood Patch Score with Date and Capture Method

Marbled Murrelets caught using the dipnet method throughout the capture period had BP in all stages of development, from 0-6 (Figure 2.1). Birds with BP 0 occurred throughout the season. Dates for BP scores 3, 4, 5, and 6 were significantly different from each other (two-sample t-tests, $p<0.001$ in all cases), but dates for BP 1 and 2 were not. This is probably due, in part, to the difficulty in grading the difference between BP 1 and BP 2 in the field. Birds with BP 3 (fully developed), were found from April 27 to August 6 (Figure 2.1, two years pooled data).

We wanted to determine how long it took for individuals to progress from one BP score to the next. Thus, the number of days between mean dates for each of the BP scores (Figure 2.1) was calculated (using only BP dates that were significantly different from each other, from 2-6, as above). Days elapsed between mean BP dates were as follows: BP 2–3=27 days; BP 3-4=30 days; BP 4-5=20 days; BP 5-6=16 days. Adding these estimates, an individual could have a BP in some stage of development (i.e. 2-5) for 93 days. How closely these average dates at the population level correspond to individuals is unknown. However, data from a few recaptured individuals are available

(Table 2.2), to describe the number of days between successive BP stages. For example, one individual was captured twice in the breeding season, the first time with a fully-developed BP (3), and 63 days later, with a regressing BP (4) (Table 2.1), suggesting that this individual had a BP for much longer than the duration of the incubation period alone (which is 30-days; Nelson 1997). Both datasets (from the entire capture sample, and from recaptured individuals) suggest that BP can be detected for a long time (months) in individual Marbled Murrelets, but that this timing is extremely variable from one individual to the next.

The number of breeding stages captured by each method (dipnet and mistnet) was assessed. The full range of BP scores (0-6) was seen in Marbled Murrelets that were captured with dipnets (Figure 2.2A), the largest proportion falling in the BP 3 (165/449) category. However, birds captured in the mistnets had BP that were either fully developed (63/70) or regressing (7/70) (i.e. ranged from BP 3-6 only, Figure 2.2B), with no birds having BP in stages of early development.

Variation in Brood Patch Score with VTG

We expected that a comparison of mean dates of egg-production (from VTG) and mean dates for birds presumed to be incubating (BP3) would provide insight into how closely these two events are timed. Because VTG is elevated before the egg is completed (i.e. during rapid yolk development; Challenger *et al.* 2001), the expectation was for BP to be fully developed after (or concurrent with) detection of egg-production (elevated VTG).

To test this, plasma VTG from 150 females was analysed (1999 and 2000). Egg-producers (i.e. in which VTG was higher than 0.96 ug/ml; see chapter 1) had BP 0-4, but

never BP 5-6. Plasma VTG levels corresponding to BP scores were as follows (data are in ug/ml of VTG ± SE): BP0=3.02 ± 1 (n=12); BP1=3.36 ± 1.2 (n=10); BP2=3.36 ± 0.8 (n=15); BP3=1.95 ± 0.2 (n=69); BP4 =1.7 ± 0.6 (n=16); BP5=0.27 ± 0.1 (n=16); BP6=0.40 ± 0.1 (n=12) (one-way ANOVA, F=4.45, p=0.000 with Tukey's pairwise comparisons). To make VTG analyses more applicable to BP developmental stage (i.e. absent, developing, fully-devloped, regressing), BP scores were pooled accordingly (see description in methods). VTG levels in each group were tested for significant differences: VTG in females with no BP (BP0, early in the season) was not significantly different from that in birds with developing BP (1-2) (Figure 2.3) (Mann-Whitney, W=141.5, p=0.83). VTG in birds with developing BP (1-2) was significantly higher than that in birds with fully-developed BP (3-4) (Mann-Whitney, W=1677.5, p=0.04), and regressing BP (5-6) (Mann-Whitney, W=5305.5, p=0.002).

Variation in Brood Patch Score with Estimated Laydate from Radio Telemetry

We were able to use estimated laydates from radio telemetry to test how closely they were timed to the development of BP, seen in radio-tagged individuals at the time of capture. Radio transmitters were used to calculate laydate for 24 birds in 1998, 36 birds in 1999, and 29 birds in 2000. Most radio-tagged birds were caught before their subsequent laydate, but in some, it was evident (from radio telemetry) that incubation had begun before they were captured. Radio-tagged birds caught before laying had BP ranging from 0-3 (Figure 2.4); in this sample, an unexpectedly large number of developing (BP1-2) or developed (BP3) BP occurred >20 days (up to 65 days) in advance of observed laydate (see Figure 2.4). While this finding suggests a long period of BP

development, other birds had BP 0-3 only 5 days before laydate, suggesting the ability to reach full BP development quickly. Birds caught after laydate had BP ranging from 1-3 (Figure 2.4). Overall, BP score did not vary significantly with nearness to laydate (ANOVA F=2.16, p=0.08), however, as expected there was a trend toward more-developed BP closer to laydate (only BP 0 was significantly earlier than BP 3; two-sample t-test, t=-3.0, p=0.006). Estimated time (days) between BP scores and subsequent laydate in incubating individuals are as follows (with SE): BP 0=25 $\pm$ 3.1; BP 1=23 $\pm$ 5.7; BP 2=17 $\pm$ 2.7; BP 3=13 $\pm$ 2.5 days before laydate.

Variation Between Dates of Egg-laying, Egg-Production and Brood Patch Development

For all individuals captured during this study, we were able to plot seasonal dates for the reproductive events observed (i.e. egg-production, laydates, or BP 3 to infer incubation). Frequency distributions of laydates (determined using radiotelemetry; data 1998, 1999, and 2000; n=89), egg-producers (determined using VTG analyses, data from 1999 and 2000; n=68; see chapter 1), and BP 3 (data from 1999 and 2000; n=191) were compared (Figure 2.5). Mean egg-producing date from all females was found to be May 27, ranging from April 19 to July 6 (n=68) (Figure 2.5A). Mean laydate from radio-tagged birds was calculated to be May 28, and ranged from April 29 to July 3 (n=89) (Figure 2.5B). These distributions were not significantly different (Mann-Whitney, W=7308, p=0.33). Mean date for BP 3 (i.e. putative incubators) was June 11, but ranged from April 27 to August 12 (Figure 2.5C), did not vary between years (1999 abd 2000), and was significantly different from egg-production (Mann-Whitney, W=25523, p=0.0001) and laying dates (Mann-Whitney, W=25418, p=0.0001). Assuming no capture effects,

mean date for BP 3 is our best estimate for 'mid-incubation period' in this population of murrelets.

Table 2.1: Brood patch (BP) score at the time of capture (for radio-tagged murrelets), with subsequent incubation information (1999 and 2000 data pooled) (see chapter 4 for a discussion of the remaining percentage that did *not* incubate). BP 5 and BP 6 were not present early in the season when radio transmitters were being attached.

BP	Number of radio-tagged murrelets	Number that went on to incubate
0	30	14 (47%)
1	19	13 (68%)
2	23	15 (65%)
3	65	35 (53%)
4	2	1 (50%)
5	n/a	n/a
6	n/a	n/a
Total	139	78 (56%)

Figure 2.1: Brood patch (BP) scores versus seasonal date in all birds captured using dipnets (1999 and 2000). Individuals were captured only once. Numbers inside boxes indicate sample size. Mean dates (indicated by dotted lines) for BP scores 3 through 6 were significantly different (p=0.00). Letters indicate boxes with means (tested with Tukey's pairwise comparisons) and medians (tested with Mann-Whitney tests) that are significantly different.

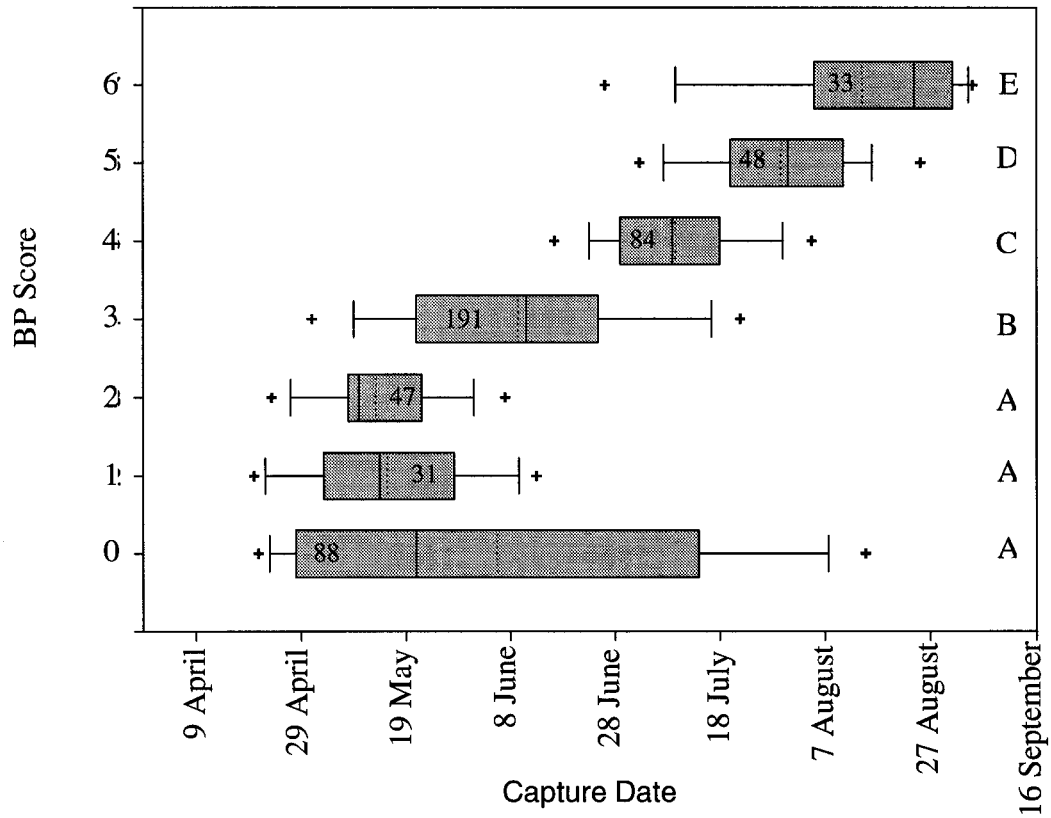


Table 2.2: Dates of brood patch scores in individuals captured twice. This data provides rough information on the timing of progression through successive BP scores in individuals. Days elapsed indicate number of days between BP scores indicated in the table.

Individual	BP	0	1	2	3	4	5	6	Days elapsed
1		28 Mar	-	-	31 Jul	-	-	-	125
2		27 Apr	-	-	-	6 Jul	-	-	70
3		-	-	17 May	-	-	14 Jul	-	58
4		-	-	6 June	-	-	-	4 Aug	59
5		-	-	-	5 May	7 Jul	-	-	63
6		-	-	-	12 Jul	24 Jul	-	-	12
7		-	-	-	10 June	15 Jul	-	-	35
8		-	-	-	5 May	-	-	31 Aug	118

Figure 2.2. Frequency of brood patches (BP) in birds captured using two capture methods: (A) dipnetting and (B) mistnetting. BP scores 0-6 progress from completely undeveloped to completely regressed; full development is scored as 'BP 3' when birds should be incubating. Data in both A and B are from two years, pooled (1999 & 2000).

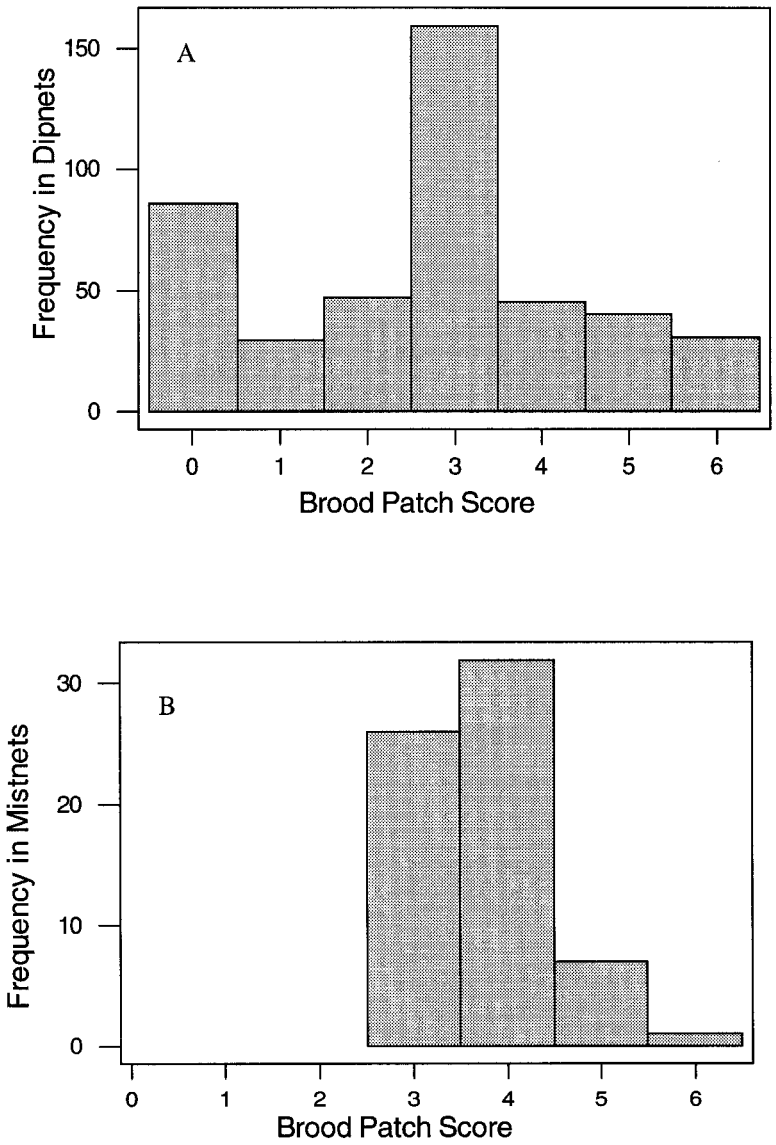


Figure 2.3. Vitellogenin (VTG) present in plasma of females, with corresponding BP score (1999 and 2000 pooled). All females were caught with dipnets. BP scores are pooled according to developmental stages (described in methods). VTG varies significantly with BP developmental stage (p=0.000). Letters above boxes indicate groups that are significantly different from each other (Mann-Whitney tests).

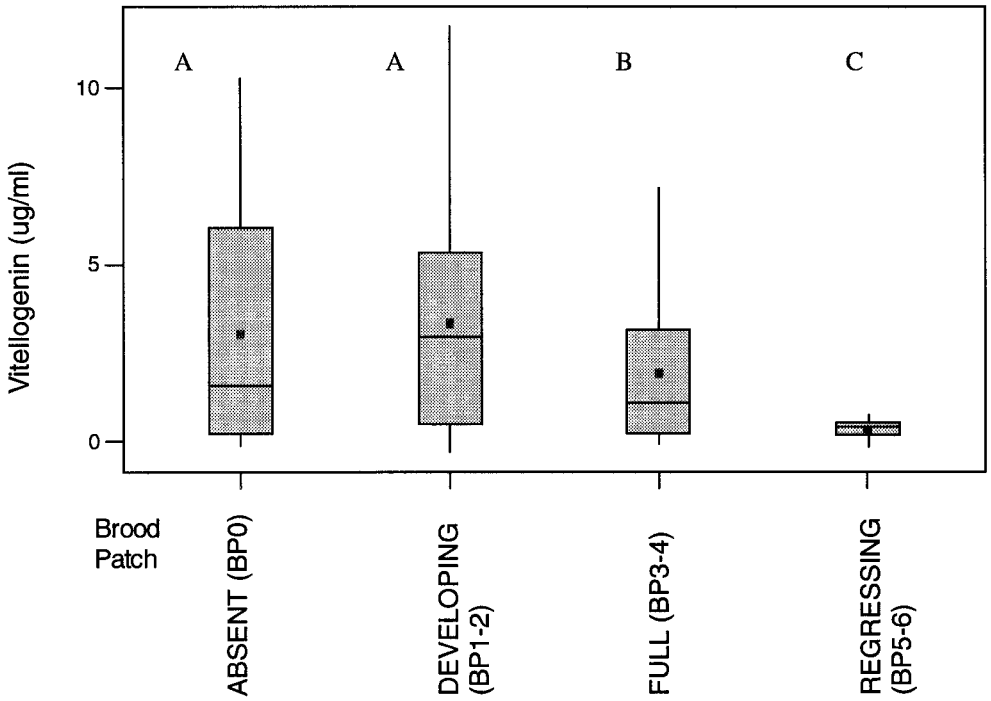


Figure 2.4. Brood patch (BP) with corresponding laydate (as determined by radiotelemetry) in radio-tagged individuals. Laydate=0 on the x-axis. Most birds were captured before laydates (and thus, have a negative number on the x-axis), but some were caught after they had started incubating (and thus, have a positive number on the x-axis). In general, brood patch does not vary significantly with laydate (p=0.081), however, mean dates of occurrence of BP 0 and BP 3 are significantly different (p=0.006). Numbers near boxes indicate sample sizes.

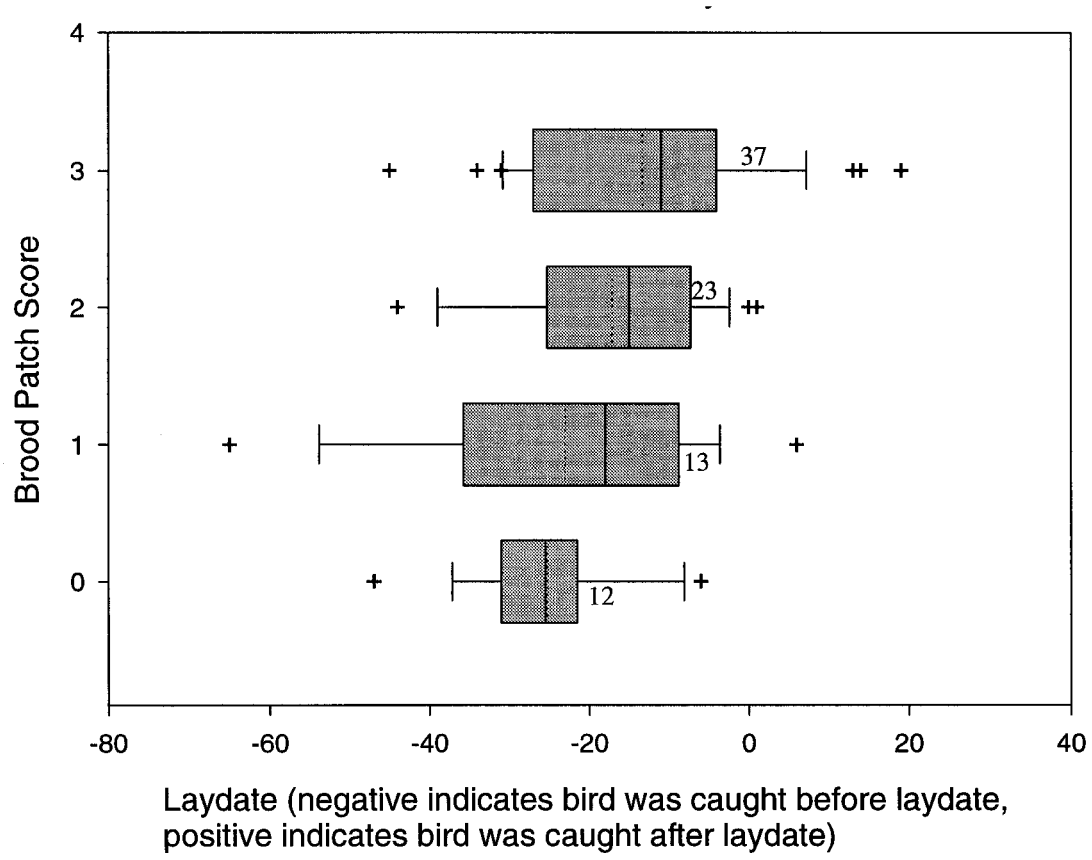
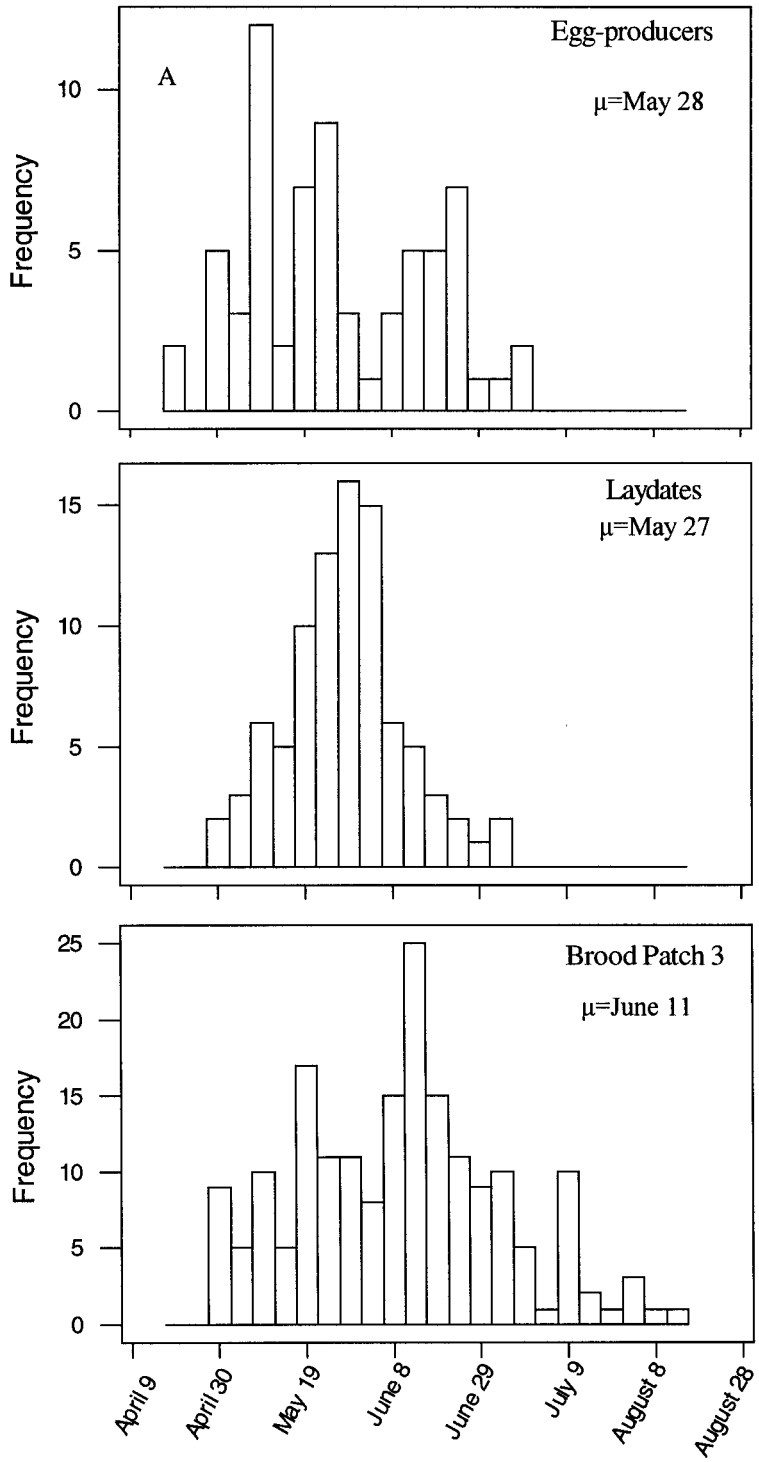


Figure 2.5. Frequency distribution, by date, of (A) Egg-producers, derived from VTG analyses; (B) Laydates, derived from radiotelemetry; and (C) BP 3, derived from physical examination at the time of capture. Data are grouped in 20-day intervals. Data for each graph were derived independently.



2.4 Discussion

During this study, it occurred to us that use of BP scores to infer breeding was not entirely reliable because in our sample of radio-tagged birds, only 54% of birds with fully-developed BP carried on into incubation. In fact, this pattern was consistent for birds in all BP categories, with only 56% of the all radio-tagged birds proceeding to the incubation stage. This in itself suggests that capture is influencing the probability of continuing with the nesting process. However, birds that were closer to anticipated laydate (as judged by BP score) were not more likely to be deterred from breeding; there was no obvious correlation between BP score and propensity to incubate, prompting a more in-depth assessment of BP with egg-production (physiological analyses) and the initiation of incubation (radio telemetry).

The accuracy of using BP to determine reproductive status was difficult to test when status could not be confirmed, but using physiological analyses and radio telemetry helped in assigning breeding status to individuals, with which to compare BP scores. For some Marbled Murrelets, breeding status was confirmed using radio telemetry, but these results may have been biased by the effects of capture or radio transmitter attachment on an individual's breeding attempt. In turn, it is difficult to quantify the effects of capture on individuals, because under field conditions the very act of capture and handling is stressful, making control groups impossible to obtain (Wingfield *et al.* 1992). A discussion of the effects of investigator disturbance is reserved for chapter 4 of this thesis.

Marbled Murrelets without BP were present in our study area throughout the breeding season, in both years (1999 and 2000); while some of these could have been pre-breeders, many females without BP at capture were actually producing an egg (i.e. had elevated VTG). In general, egg-yolk precursors (VTG) were highest before full BP development; while this suggests full BP development occurs as expected (i.e. after or concurrent with egg-production and just before egg-laying), data collected from radio-tagged individuals showed BP development starting, in some cases, up to 65 days before egg-laying. This may have been due to re-laying or egg replacement. Individuals that did have BP may have taken months to complete the process of BP development and regression. It is not clear if BP development takes a long time, or a short time, in Marbled Murrelets; and neither is it definite that a fully-developed BP indicates that a bird will start, or continue, to incubate.

Variation in Brood Patch with Date.

Birds without brood patches (BP 0) were captured throughout the season (from April to September). Sealy (1972) also found Marbled Murrelets at Langara Island (British Columbia) with BP 0 during much of the breeding season, from March 15-June 30. In the field, BP 0 can sometimes be confused with BP 6 since they look alike (Sealy 1972; LMT pers. obs.), but the distinction is made using relative timing, with BP 6 occurring at the end of the season. There was no way to determine if birds without BP were breeders caught pre- or post-incubation, or true pre-breeders. We suspect it is a combination of these possibilities. In our study, the 3.5-month occurrence of fully developed brood patches (BP 3), from April 27 to August 12, supports previous studies showing an

extended breeding season in Marbled Murrelets compared to other alcids (Lougheed 2000; this study, chapter 1). While Sealy (1972) saw BP 3 only from mid-May to mid-July (even though he was making observations from March to mid-August), we found birds with BP 3 almost a month before and after these dates. Sealy's small sample sizes may be the most likely explanation for this difference.

Individuals appeared to take a long time to progress from one BP score to the next. From a large sample of individuals captured once, days elapsed between each BP score ranged from 16-30 days; if each progression takes this long, individuals could have a BP in some stage of development (i.e. 2-6) for 80-120 days. How closely these population-level averages correspond to individuals is unknown. However, the available data from some recaptured individuals (Table 2.2) also described long periods of time between successive BP stages (Table 2.2). When using recaptured individuals to calculate the time between BP scores, we run into the potential problem of capture effects, which may delay a reproductive attempt (i.e. possibly extending the presence of BP in individuals?). Calculating the time between BP stages using singly-captured birds (as in Figure 2.1) may avoid this problem. Nevertheless, both datasets (from the entire capture sample, and from recaptured individuals) suggest that BP can be detected for a long time (months) in individual Marbled Murrelets.

Variation in BP Refeathering

Sealy (1972) reported BP refeathering in Marbled Murrelets in early July, at the time which first juveniles were appearing on the water, but he did not record complete BP refeathering. In contrast, we report refeathering BP (4 and 5) from late June until mid-August, and found refeathered brood patches (BP 6) from July to September, however, it

is possible that some pre-breeding birds (BP 0) were mis-classified as post-breeders (BP 6) (i.e. refeathered and unfeathered BP look the same). The dynamics of refeathering of BP is not widely reported for other species. However, of the few accounts, it seems that there is no consistent pattern of refeathering in relation to breeding activity: in Ancient Murrelets, little or no refeathering of the BP occurs before the adults and chicks depart the nest site for sea a few days after chick hatch (Sealy 1972). Auklets (Cassin's, Crested, Least, Whiskered and Parakeet) have fully-developed BP at the onset of incubation, which rapidly shrink and refeather after hatching (I. Jones, pers. comm.). The initiation of re-feathering in Leach's Storm Petrels occurs between two and ten days of hatch, and within three to four weeks the BP becomes completely refeathered with down (Ainley *et al.* 1990), while Cassin's Auklets BP begin refeathering mid-incubation (Manuwal 1974). In passerines, although BP skin returns to normal (i.e. vascularization and edema regresses) after chicks fledge, BP remained unfeathered until fall moult (Bailey 1952).

Because most other seabird studies do not have to rely on BP information to infer breeding status, there has been no need for careful study of the BP and thus, there are few reported data to compare with timing of BP development with laying in Marbled Murrelets. However, many studies have concentrated on the hormone which controls BP development: prolactin. In general, BP development tracks prolactin secretions, which can decline rapidly, or more slowly, corresponding to the length of post-hatching parental care (see descriptions for precocial species, Goldsmith & Williams 1980; semi-precocial species, Hector & Goldsmith 1985, Jouventin & Mauget 1996, Lormee *et al.* 1999, Vleck *et al.* 2000; or altricial species, Goldsmith 1982, Silverin & Goldsmith 1984). Because of

this, studies of prolactin tend to be reflective of breeding strategies and life-history patterns. We were unsuccessful in pursuing a study of prolactin, but feel that it would have helped describe the relationships between BP development and maintenance of parental behaviour in Marbled Murrelets, with their life history.

Variation in Brood Patch with Capture Method.

Previous studies have found that birds caught in mistnets in the Desolation Sound study area are a sex-biased sample, with a 2:1 male:female sex ratio (Vanderkist *et al.* 1999b) made up of birds primarily in the chick-feeding stage (Bradley *et al.* 2002). Bradley *et al.* (2002) suggested the male bias resulted from increased chick provisioning by the male, rather than not from the presence of prospecting nonbreeders in the area. The BP data from the mistnets supports this explanation, because birds in the mistnet sample show only fully-developed or regressing brood patches, as opposed to the full range of brood patches seen in the dipnet sample. Nonbreeding prospectors that might be increasing the male:female ratio in the mistnet sample would not be expected to have developed BP. However, if birds caught in the mistnets are primarily chick-feeding, one might ask why so many of them (BP3=37%; BP4=52%) had fully developed brood patches, and so few had regressing brood patches. If the previous assumptions about mistnetted bird are correct (i.e. that they are chick-rearing), this finding suggests that brood patches do not refeather in Marbled Murrelets until sometime during late chick-rearing. This is corroborated by the long duration of BP scores, as described above. Also, it is common for BP to extend into chick-rearing in other birds, as in the prolactin discussion (above). Manuwal (1974b) found that BP in Cassin's Auklets refeathered

during mid-incubation, and refeathering was partially inhibited in individuals laying replacement clutches. While Marbled Murrelets may have a similar ability to delay BP refeathering if they lay a replacement egg, it is still not clear why they would need BP well into chick-rearing, when they do not continue to brood the chick past ca. 5 days (Nelson 1995).

Variation in Brood Patch with Egg-Production (VTG)

It was expected that egg-production and BP development should coincide in Marbled Murrelets, with egg-production (indicated by elevated VTG) occurring a few days before full BP development. However, close timing of high VTG and BP 3 was not explicitly clear. Females without BP were producing eggs, as were females with BP 1-3 (developing or fully-developed), or BP 4 (just beginning to regress). It is possible that in the latter, egg-production or laying had been disrupted or delayed by some natural disturbance, and the egg being produced was a replacement; while BP development could not be completely suspended (but was partially inhibited, as in Manuwal 1974), a second egg could be produced.

While it is difficult to predict the exact timing of BP and egg development in the Marbled Murrelet, some comparisons can be made with other birds: in other alcids, total egg development takes about 14 days (eg; 14 days in Common Murres, Murphy 1995; 13-17 days in Brunnich's Guillemots, Hipfner *et al.* 1999; 14 days in Cassin's Auklets, Astheimer 1986). This pattern is also seen in domestic chickens, with elevated serum VTG preceeding oviposition by 10-15 days (Redshaw & Follett 1976). From these studies, we should expect Marbled Murrelets to complete egg-development in similar time, about 14 days. VTG data from the current study (Figure 2.3) suggests that even if a

Marbled Murrelet is developing an egg, she may not have started BP development. Therefore, we need not expect that BP development occur any earlier than 14 days before laying. Also, some Marbled Murrelets captured within 5 days of laying had BP 1-2 (not fully developed), suggesting that BP development could be completed rapidly, by the time egg-laying occurred.

Sealy (1972) dissected a few Marbled Murrelets, and found 2 females with BP 0 had half-developed primary oocytes. Others (n=5) with one fully-developed ovum still in the follicle had BP 1-2, one female with an unshelled egg in the oviduct had BP 2, and 10 females with one recently ovulated follicle had BP 2-3. During the current study, three eggs were laid at the time of capture; two were completely shelled with color deposition and came from females with BP 2 and 3. The other was not fully developed, (soft shell and no coloration, LMT pers. obs.), and came from a female captured with a male (assumed to be a mated pair), each with BP 2 (Centre for Wildlife Ecology, unpubl. data). Thus, from Sealy's (1972) and our study, it would seem that nearly- or fully-developed BP are well correlated with egg-production. However, the length of time it takes Marbled Murrelets to produce an egg and a BP remains unconfirmed, nor do we know if they accelerate BP development by pulling out feathers (but plucking of down probably not likely in adults that do not use feathers in the nest; Bailey 1952). In Auklets, BP defeathers once the egg is fully formed and the shell is being deposited; it is believed that defeathering of BP takes only a day or two just before the day of laying, and that the adult may pull out feathers just before or after laying (I. Jones, pers. comm.).

Variation in Brood Patch Score with Estimated Laydate

Confirmation of breeding status was possible in the radio-tagged Marbled Murrelets, to try to correlate BP scores with nearness to subsequent laydate in individuals. In Marbled Murrelets, defeathering of BP occurred in advance of laydates, but in some cases (in three years of data), an unexpected loss of down (BP 1-2) occurred up to 65 days before laying. With the onset of incubation, the skin of the brood patch becomes heavily vascularized; the close timing of these changes can provide criteria for determining when eggs are laid (eg; in Storm Petrels, Ainley *et al.* 1990; and Cassin's Auklets have fully-developed BP 1-2 days before laying, Gaston & Jones 1998). "This pattern, however, is not evident in storm-petrels or in [Adelie] penguins, in which breeding birds lose the down from their abdomens up to a month or more before egg laying" (Ainley *et al.* 1990). Pre-breeders in populations sometimes lose abdominal down; this has been recorded in physiologically immature, nonbreeding petrels and penguins (Ainley *et al.* 1990). Non-breeding auklets sometimes show feather loss in the abdominal region, but never show vascularization or thickening of the BP skin (I. Jones, pers. comm). Older pre-breeding Atlantic Puffins have also been found to develop BP (Gaston and Jones 1998). In our study, birds with early BP so long before egg-laying (up to 65 days; see Figure 2.4) could not have been pre-breeders, because they were confirmed, radio-tagged incubators (and hence, had laydates assigned). It may be possible that these birds were young, inexperienced breeders, less able to time BP development appropriately; or laydates could have been delayed by capture. In contrast, the presence of BP 0 (absent) or 1-2 (developing) within 5 days of egg-laying, suggesting that Marbled Murrelets can defeather BP quickly. Given this information, it is paradoxical that some individuals had fully-developed BP

>40 days before laydate (see Figure 2.4). These birds may have been re-nesters. Manuwal (1974b) reports that it is possible for eggs to be incubated with poorly developed BP, if environmental conditions permit (as in Cassin's Auklets on the Farallones). This may help explain why some Marbled Murrelets have poorly-developed BP within 5 days of laying (as above).

In seabirds, the energetic cost of developing a BP is presumably large due to heat loss while sitting on the ocean, but this is not well studied. Marbled Murrelets have a thick layer of outer feathers that interweave and cover the BP, and which has to be physically moved aside for BP examination (LMT pers. obs.). It is possible that maintenance of a BP does not result in significant heat loss in Marbled Murrelets, and consequently, an extended time during which BP are present has no energetic consequences. A comparative study of heat loss among temperate- and arctic-breeding alcids is required to test this hypothesis.

These results for Marbled Murrelets could be explained four ways: (1) individual variation in the timing of BP development and egg-laying; (2) individual variation in the timing between egg-development and laying; (3) laying replacement clutches occurs commonly, and could explain the combination of late BP scores with elevated VTG; (4) disturbance resulting from capture and/or radio attachment delayed breeding in birds that were preparing for egg-laying. Investigator disturbance at seabird colonies has been well-studied, common findings including reduced breeding success and delayed hatch dates (Safina & Burger 1983, Ellison & Cleary 1978, Cairns 1980, Anderson & Keith 1980, Fetterolf 1983, Piatt *et al.* 1990, Rodway *et al.* 1996). However, investigator disturbance has not been well studied when birds are captured or disturbed away from the

colony or nest site, as is the case for Marbled Murrelets. It is not known if the brief disturbance at capture (which occurs at sea in our case) versus disturbance at a colony have equivalent reproductive consequences. The possibility that individuals were induced to delay or abandon a breeding attempt after capture by researchers is probably the most likely explanation for the observed discrepancy between BP development and subsequent laydates in Marbled Murrelets (Figure 2.4). This possibility is troubling, and will be addressed in a future paper (see chapter 4).

Comparing Dates for Egg-Production, Laydate, and BP3

In addition to the radio-tagged sample of birds, we were able to make comparisons of data collected using a larger capture sample. Seasonal dates for three separate groups from this data were compared: all egg-producing birds (1999-2000), all birds with BP 3 (presumed to indicate incubation behaviour) (1999-2000), and all estimated laydates from radio-tagged birds (1998-2000). We found that the mean laydate calculated using radio telemetry (May 28) (Figure 2.5A) was not significantly different from the mean egg-producing date calculated using VTG analyses (May 26) (Figure 3.5B). This was expected, as elevated VTG occurs immediately prior to laying (Redshaw & Follett 1976). However, when considering explanation 4 (above), in which investigator-induced disturbance may have delayed breeding, one might expect mean laydate from radioed birds to be later than that calculated using VTG alone. This is not the case, and may indicate that investigator disturbance does not significantly influence timing of breeding.

For radioed individuals alone, BP 3 was not closely related to subsequent laydate determined by radio telemetry. Mean date of fully-developed BP (June 11), was significantly later than mean laydate, and extended for 40 days past the last laydate found

using radio telemetry. This extension is explainable because Marbled Murrelets incubate for approx. 30 days (Nelson 1997), while our results suggest that BP 3 can extend into chick-rearing (mistnet data, Figure 2.2).

While laydates potentially could be influenced by investigator disturbance, plasma VTG or BP measurements taken at the immediate time of capture would not be affected. As a result, if there was significant investigator-induced disturbance acting to delay laydate in Marbled Murrelets, we would expect mean VTG to be significantly earlier than subsequent laydate; likewise, mean dates for BP 3 would overlap earlier onto mean laydates. Contrary to this, our general findings were that egg-production, laydate, and BP 3 correlated well (Figure 2.5), and conformed to what would be expected if there was no investigator disturbance.

The results of this study are difficult to interpret because there are conflicting messages in the data: (1a) egg-production (via VTG) and BP show no consistent correlation with laydate in a small sample of radio-tagged individuals (Table 2.1, Figure2.4); (1b) on a broader scale, and with larger, independent samples, mean egg-producing date and mean laydate correspond closely, while mean date of fully-developed BP extends past laydate, corresponding to the expected time of incubation (Figure 2.5); (2a) BP can take a long time to develop (up to 60 days) in Marbled Murrelets (Table 2.2, Figure 2.4); (2b) BP can take a short time to develop (up to 3 days) (Figure 2.4); and (3a) capture may prevent or delay breeding in some individuals (Table 2.1, Figure 2.4); (3b) capture does not affect breeding in some individuals (Figures 2.4 and 2.5).

Our study suggests that BP usually indicates incubation behaviour. However, the prevalence of developing BP (1-2) so close to laydates may represent young,

inexperienced birds who may be inexperienced in timing BP development with incubation. Alternatively, this may not be a problem if it takes only a few days for BP to develop. The absence of BP does not mean the bird will not become a breeder, especially when BP 0 occurs early in the season (i.e. April-May). Likewise, the presence of BP does not absolutely mean the bird will become a breeder, a finding that researchers should be wary of when inferring breeding status using BP.

Because of the asynchrony in timing of breeding (chapter 1, this study; Lougheed 2000), it is not possible to be sure a bird caught mid-season with BP 0 has not or will not be a breeder. Can BP score be used to estimate laydate? The data presented here should caution anyone from using BP to predict the *timing* of a breeding attempt. Only fully-developed, vascularized BP should be used to infer incubation, in case pre-breeders show the beginnings of BP development. Disregarding capture effects, we suggest that fully-developed brood patches may occur in Marbled Murrelets within about 10 days of laydate (mean in Figure 2.4) but probably persist for 30-40 days post-lay, well into chick-rearing. Using BP to infer reproductive status in Marbled Murrelets can neglect reproductive individuals pre-or post-breeding, misinterpret re-laying attempts, and cannot pinpoint the exact laydate for individuals. We are confident only that fully-developed BP (3) can infer that the bird is incubating, or that it is about to incubate, but not that incubation will proceed uninterrupted.

Chapter 3

Stress Response in Marbled Murrelets

3.1 Introduction

Birds respond to stressful events by secreting the hormone corticosterone (Kitaysky *et al.* 1999b). A stressful event is any situation that elicits a defensive response (Siegel 1980); defense mechanisms may be required to cope with any combination of factors making up a bird's environment, including external (eg. temperature, daylength, food) and internal (eg. parasites, body condition) factors. Success in coping with the environmental stress depends on the severity of the stress stimuli (stressors) and the bird's physiological ability to respond appropriately (Siegel 1980). Variations in the response to stressors are known to correlate with a number of factors, including sex (Astheimer *et al.* 1995, Wingfield *et al.* 1995), body condition (Heath & Dufty 1998, Schoech *et al.* 1997), and reproductive stage (Kitaysky *et al.* 1999a). The stress response can be assessed by measuring corticosterone (CORT) released into the blood (Wingfield *et al.* 1995).

The sensitivity of the hypothalamo-pituitary-adrenal (HPA) axis to stress can be tested by measuring CORT concentrations in blood samples, taken in the first hour post-capture (Wingfield *et al.* 1995). Capture and handling is used as a standardized acute stressor, with the assumption that the responses to capture are comparable between species and individuals (Wingfield *et al.* 1995, Holberton & Able 2000). Analysis of HPA sensitivity can then be compared by measuring CORT release among individuals, between sexes, seasons, years, and study sites (Wingfield *et al.* 1995, Silverin & Wingfield 1998, Holberton & Able 2000). It has been suggested that variation in HPA

sensitivity can have an ecological basis, and may vary depending on the life-history of a particular species (Wingfield *et al.* 1998).

Increases in CORT redirect ongoing behavioural and physiological activities towards immediate life-saving activities such as an increase in food searching or food intake, and promote gluconeogenesis, using protein reserves as an energy source if needed (Holberton & Able 2000). Chronic high levels of CORT are believed to be immunosuppressive and incompatible with successful reproduction, growth, and development in a variety of vertebrate taxa (Siegel 1980, Harvey *et al.* 1984, Astheimer *et al.* 1995). If an individual is able to meet an energetic challenge successfully or if the perturbation passes, CORT concentrations in the plasma return to pre-disturbance levels (Holberton & Able 2000) and normal activities are re-established. Meeting the energetic challenge of a stressor is done in a variety of ways by different species, which modulate the stress response individually or seasonally (Wingfield *et al.* 1995).

Due to its status as an endangered species, there is considerable concern about the effects of capture and handling on the Marbled Murrelet (*Brachyramphus marmoratus*), which purportedly, could disrupt breeding or affect adult survivorship. However, survival parameters of species in the wild must be estimated by following individually marked animals through time (Lebreton *et al.* 1992), while the ability to attach instruments (like radio transmitters) allows knowledge of ecological and physiological variables that are otherwise unmeasurable (Culik & Wilson 1991). Good conservation and management decisions depend on the quality of the data for any species. Instead of ignoring or discounting capture effects, it is more useful to understand the stress response to capture.

This study is the first to investigate plasma CORT in Marbled Murrelets. Objectives were to assess (1) the release of CORT over time in captured individuals; (2) whether CORT release increases in Marbled Murrelets after the attachment of radio transmitters, compared to those handled only; and (3) the relationship of CORT with date, mass, year, breeding stage, and sex.

3.2 Methods

Captures and Blood Sampling

Captures took place in Desolation Sound (centre 50° 05'N, 124° 40'W) from 20 April to 4 September 1999, and 19 April to 26 August 2000. "Dipnetting" (Whitworth *et al.* 1997, Lougheed *et al.* 1998) was used at night between 22:00 and 05:00. Birds were captured from the surface of the water, using a long-handled salmon landing net, and put into dark cloth bags. Mistnetting was also employed to catch birds (8 June to 30 July 1999, 14 June to 29 July 2000) at the mouth of Theodosia Inlet (50°04'N, 124° 42W) (Kaiser *et al.* 1995).

Marbled Murrelets from both capture methods were blood-sampled, and some birds were tagged with radio transmitters (see 'assigning birds to sample groups', below). Thus, not all birds were bled following the same protocol. The groups were as follows:

1. radio-tagged birds with a single blood sample (brachial vein) taken at ca. 30 minutes post capture, after radio-attachment.
2. non-radio-tagged birds with a single blood sample (brachial vein), taken at a range of times, from immediately post-capture, up to 45 minutes post-capture.

3. non-radio-tagged birds that were caught within 5 minutes (from first seeing them on the water), on which a stress series was done. This required repeated (hence the term 'stress series') 40 ul blood samples (tarsal vein) to be taken at <5, 15, 30, and if possible, 45 minutes post-capture (eg. see 'capture stress series', Wingfield *et al.* 1994b).

Because it was not feasible to carry out the stress series protocol (i.e. repeated measures) on all birds, many individuals had samples taken at t_{max} (where t_{max} = time at maximum CORT release). The peak CORT level attained during the capture period is an indication of adrenocortical capacity (Astheimer *et al.* 1995) and has been found to vary seasonally (Wingfield *et al.* 1994b, Wingfield *et al.* 1992), with fat score or body mass (Wingfield *et al.* 1994b), and increase during storms (Astheimer *et al.* 1995, Smith *et al.* 1994). There may also be a phylogenetic component to species differences in max CORT levels attained during capture stress (Wingfield *et al.* 1995). Therefore, we feel that the measurement and comparisons of maximum CORT (such as in Holberton and Able 2000) in absence of other stress series samples is valid.

Assigning birds to sample groups:

Blood samples were taken from 25% of the radioed birds in 1999. Thus, every 4^{th} bird caught was assigned to category 1 (bled and radio-tagged), and every 5^{th} bird to category 2 (bled only). Birds were assigned to category 3 (stress series) solely on the basis of capture time: any bird caught within 5 min., regardless of its position in the 5-bird rotation, was assigned to category 3 (i.e. the 5-bird rotation was temporarily suspended when we caught a bird quickly). In 2000, 50% of radioed birds were bled, and birds were

similarly assigned to the three categories: every 2nd bird was assigned to category 1, every 3rd bird to category 2, and any quickly-caught bird to category 3.

For groups 1 and 2, 1-2 ml blood was collected (as described in chapter 1). For group 3, blood was collected in heparinized capillary tubes, from a pinprick in the tarsal vein, after skin was disinfected with an alcohol swab. Following sampling and radio attachment, birds were released as soon as possible. Blood collected in capillary tubes and Eppendorf tubes was kept cold in a small cooler with icepacks until returning to field camp. Blood was centrifuged, the plasma separated, and both blood and plasma were frozen at -20°C until they could be transported to laboratory facilities for analyses.

Attachment of Radio Transmitters

Transmitters (weighing 3 grams, Advanced Telemetry Systems, Model No. 386) were attached following the methods of Newman *et al.* (1999) but without sutures or anaesthetic. Transmitters were attached with a subcutaneous anchor, and the loose end secured with a small amount of Bird Epoxy (Titan Corporation, USA) to the dorsal feathers. Birds were bled approximately 5 minutes after radio attachment.

Radioimmunoassay

Plasma CORT levels were analysed using a specific radioimmunoassay (as described in Salvante 2000; see also detailed description in Wingfield *et al.* 1992). Each sample (5-20 ul of plasma) was equilibrated overnight at 4°C with 2000 cpm tritiated ([^{3}H]) CORT, to measure the percentage of recovery following the extraction procedure. Re-distilled dichloromethane was added to each sample to extract endogenous and [^{3}H]CORT, and

these extracts were dried and reconstituted with phosphate buffer. 100 ul of each sample was placed into a scintillation vial (along with 4.5 ml of scintillant), the cpm of tritium in which provided an estimate of percent recovery after extraction. Duplicate assay tubes received 200 ul and were incubated overnight with 10,000 cpm [^{3}H]CORT (for competitive binding) and 100 ul CORT antiserum. Dextran-coated charcoal was added to remove any unbound CORT (endogenous and [^{3}H]). After centrifugation, the supernatants (containing bound cpm) were decanted into scintillation vials, allowed to equilibrate, and counted for bound radioactivity. All samples were adjusted for percentage recovery of the internal standard. Plasma CORT concentrations were calculated as nanograms per milliliter (ng/ml).

Breeding stage categories.

In this part of the study, it was assumed that brood patches (BP) had true biological significance, that is, that birds would not develop a brood patch if they were not reproductively active. BP scores were modified from Sealy's (1972) 6-tiered classification, and used to categorize Murrelets into the following breeding stages (note that the definitions associated with each BP score are slightly different from those in chapter 2):

BP 0= completely undeveloped, classifies birds as 'pre-breeders', or 'early breeders' (Sealy's BP 0) (see chapter 2).

BP 1= developing with beginning loss of down and pinfeathers, classifies birds as 'pre-incubators' (Sealy's BP 1 and 2).

BP 2= fully developed (defeathered and vascularized), classifies birds as incubators (Sealy's BP 3 and 4).

BP 3= regressing or regressed brood patches, classifies birds as chick-rearers (Sealy's BP 5 and 6).

Because breeding is highly asynchronous in Murrelets (Lougheed 2000, this study chapter 1), all birds will not be in pre-breeding, pre-incubation, incubation, or chick-rearing periods at the same time in the season. There is a large overlap in the timing of these periods, making it impossible to categorize individual breeding Marbled Murrelets into any period based on seasonal time, so BP score was used instead.

Statistical analyses

Two groups of blood samples were assessed; firstly, the 'stress series' samples, which included repeated blood samples (up to 4) for each individual; these samples were compared using ANOVA repeated measures analysis. Secondly, the single blood samples, which did not use repeat blood samples from the same individual. For the single blood samples, an assessment of all independent variables (affecting CORT) was made using AIC (Aikike's Information Criterion) (Appendix 3.1). The resulting linear model was explored for covariance and interactions using SAS (version 8) and Minitab (version 13) statistical software, and provided a framework for exploring simpler models. Statistical significance was assumed at $p=0.05$. The original linear model included time as a quadratic term.

For all comparisons between individuals (no repeat sampling), CORT was corrected for the effects of time and mass by taking the residuals from a regression where

cort=time mass. Analyses using maximum CORT (samples taken at 30 ± 10 minutes post-capture) were most appropriate for individual samples. Time at maximum CORT release was denoted as t_{max} (30 ± 10 mins). Maximum CORT (at t_{max}) was used in comparisons with year, presence of radio transmitters, BP and sex, and was corrected (using residuals) for mass. Comparisons of CORT residuals with sex, year, and BP were made using ANOVA.

There was no detectable relationship between morphometric variation (tarsus, wing chord, or wing chord^3) and mass variation ($p>0.2$ in all cases), and thus, correcting mass for size would not change any of the analyses.

3.3 Results

Stress Series

Over two years (1999 and 2000), plasma CORT levels were analyzed from 22 stress series (some incomplete). Capture and handling resulted in a significant increase in plasma CORT from 0 to 30 minutes, and a significant decrease after 30 minutes. This pattern was seen in 5 individuals in which a complete stress series (4 repeat samples) was done (Figure 3.1, Table 3.1; p=0.0465 Wilks' Lambda). This pattern was also seen in the larger sample of individuals (n=22) with partially-complete stress series (2-3 repeated measures) (Figure 3.2; p=0.000 Wilks' Lambda). Mean CORT in each of the 4 time categories (1=<10 min, 2=~20 min, 3=~30 min, 4=~45 min) differed significantly, except mean CORT at time 2 and time 4, which were not significantly different (Figures 3.1, 3.2). Mean maximum CORT reached during stress series was 31.39 ± 2.33 (SE) ng/ml.

Because CORT in Marbled Murrelets peaks near 30 min, the measurement and comparisons of maximum CORT (at t_{max}) (such as in Holberton and Able 2000) in absence of other stress series samples is valid for use in the individual comparisons that follow.

Capture methods and radio attachment.

Over two years (1999 and 2000), plasma CORT levels from 58 radio-tagged and 132 non-radio-tagged Marbled Murrelets were analyzed. Maximum CORT did not vary (Krukall-Wallis, p=0.97) among birds that were bled only and those that were bled after radio attachment (Figure 3.3). Rate of CORT increase between the two groups was not addressed in this study. Time that samples were taken (post-capture) also did not differ between (Krukall-Wallis, p=0.71): 35.1 $\pm$ 1.5 (SE) min. for birds without radio transmitters and 35.2 $\pm$ 1.2 (SE) min. for birds with radio transmitters. Maximum CORT also did not vary (Krukall-Wallis, p=0.85) between birds caught in mistnets (n=11) or dipnets (n=80).

Specific Independent Variables, with Individual Samples

Variation with Mass. A significant increase in CORT with body mass was found in females (R^2=18%, p=0.002) but not in males (R^2=2%, p=0.506) (Figure 3.4, see also equations in Table 3.2). While the data do not fit closely to the regression line (i.e. very low R^2), the most interesting feature in this dataset are opposite trends seen for each sex. A significant interaction found between the sexes (sex*mass, p=0.001, general linear model; see Appendix 3.1) emphasizes that the CORT response with mass is not the same

in both sexes; the opposing directions of the regressions for each sex (Figure 3.4) illustrate this point. The relationship is made more complicated by an interaction with date (Appendix 3.1), with females (but not males) decreasing in mass as date progresses. All subsequent analyses control for mass (except BP analysis, see specifics below).

Variation with Date. Mean CORT release decreased over breeding season, but this effect disappeared (linear regression, R^2=0.6%, p=0.37) after correcting for the seasonal change in mass. This change was driven by the decrease in mass in females, as they progressed from egg-production to incubation and chick-rearing. CORT was not found to vary significantly with time of day (20:00 to 05:00 h) (linear regression, R^2=0.8% p=0.30).

Variation with Year. Maximum CORT was higher in 2000 than 1999, in males (Krukall-Wallis, p=0.004) but not in females (Krukall-Wallis, p=0.126), although the trend in females was toward higher values in 2000 (Figure 3.5).

Breeding Stage (as estimated by BP score). Variation in maximum CORT with breeding stage was not significant in females (p=0.245) or in males (p=0.194), neither before nor after controlling for mass. However, a trend for higher CORT was seen during the pre-incubation and incubation stages in both sexes. In females, CORT increased from pre-breeding (BP 0: 25.4 $\pm$ 8.8 ng/ml) to pre-incubating (BP 1: 44.6 $\pm$ 4.8 ng/ml) and incubating (BP 2: 43.1 $\pm$ 3.4 ng/ml), and then decreased at chick-rearing (BP 3: 30.9 $\pm$ 10.6 ng/ml) (Figure 3.6). Similarly, in males, CORT increased from pre-breeding (BP 0:

20.1± 4.4 ng/ml) to pre-incubating (BP 1: 33.4± 5.1 ng/ml) and incubating (BP 2: 36.9 ± 3.8), then decreased in chick-rearing (BP 3: 31.6 ± 9.2 ng/ml). This trend may be driven by its interaction with mass, which in females, increased significantly during pre-incubation and incubation (Figure 3.7; p=0.001), and females showed a positive increase in CORT with mass (Figure 3.4). However the trend with CORT and BP was also seen in males, which did not change mass between breeding stages (p=0.44) (Figure 3.7; also see interactions in Appendix 3.1).

Variation with Sex. Maximum CORT was not found to vary between sexes, except in covariance with mass and brood patch score (seen in Figures 3.4 and 3.7).

Figure 3.1. Corticosterone with time after capture, in repeated-measures analysis (n=5). Each point for individual birds is significantly different from the previous one. F=20.67, p=0.0465 (Wilks' Lambda).

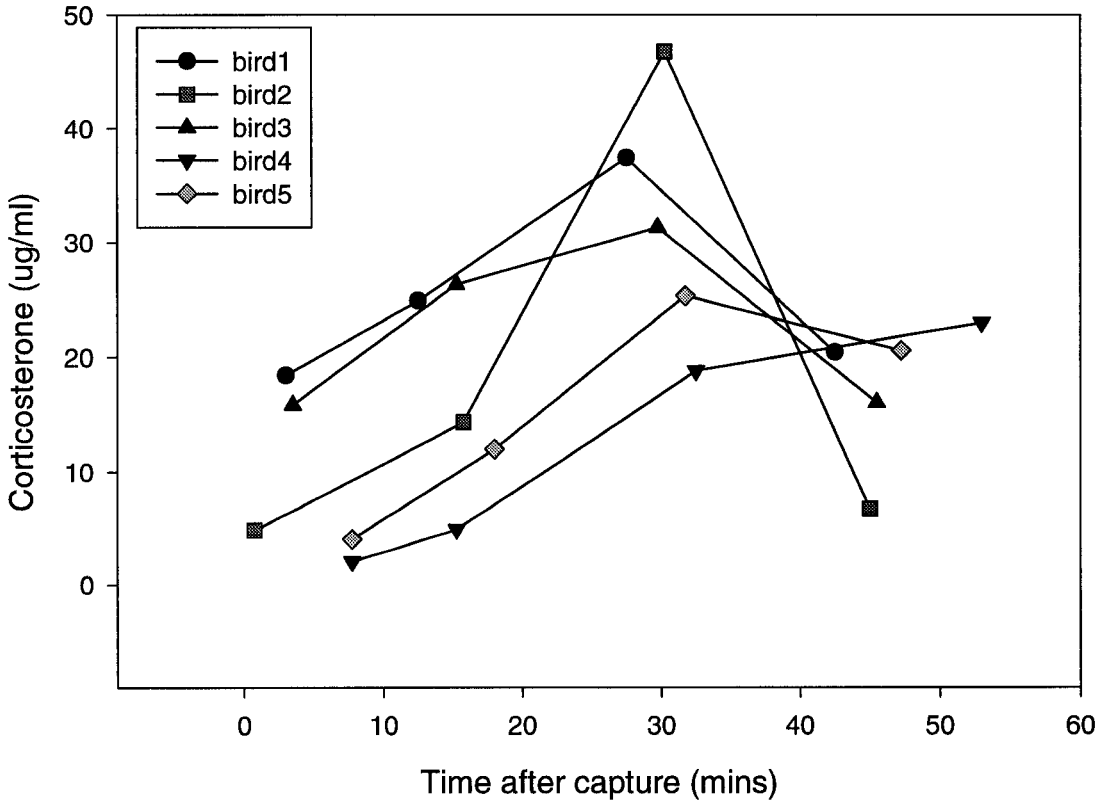


Table 3.1. Mean corticosterone at time intervals (1=<10 min, 2=~20 min, 3=~30 min, 4=~45 min), grouped from Figure 3.1. Equal repeated samples were taken for each individual. Letters indicate intervals that are significantly different from each other.

Level	N	Mean	SE	
1	5	9.044	3.36	A
2	5	16.504	4.04	B
3	5	31.950	4.82	C
4	5	17.366	2.89	B

Figure 3.2. CORT by time group (1=<10 min, 2=~20 min, 3=~30 min, 4=~45 min) for all available data (sample sizes in brackets). Includes repeated samples from individuals, but not all individuals had 4 repeated measures taken F=14.04, p=0.000 (Wilks' Lambda). Letters indicate groups that are significantly different from each other, asterisks indicate outliers.

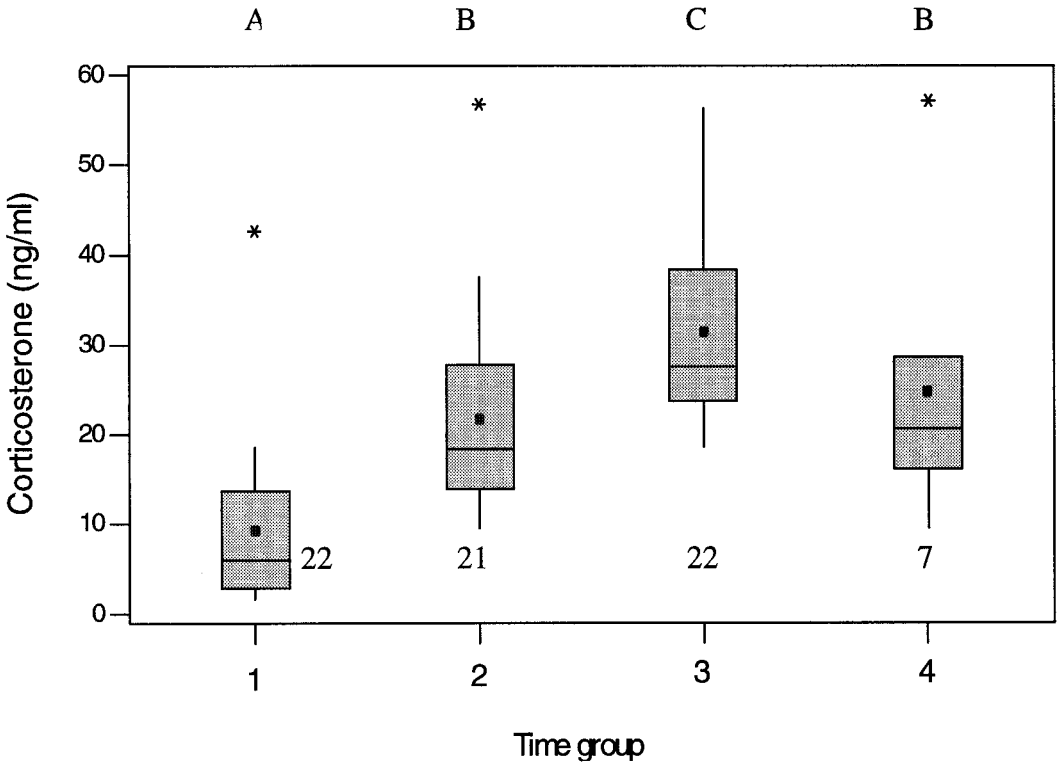


Figure 3.3. Maximum CORT (at t_{max}) (time-mass residuals) in radioed and non-radioed birds. No significant difference was found (Kruskall-Wallis, H=0.11, df=1, p=0.736).

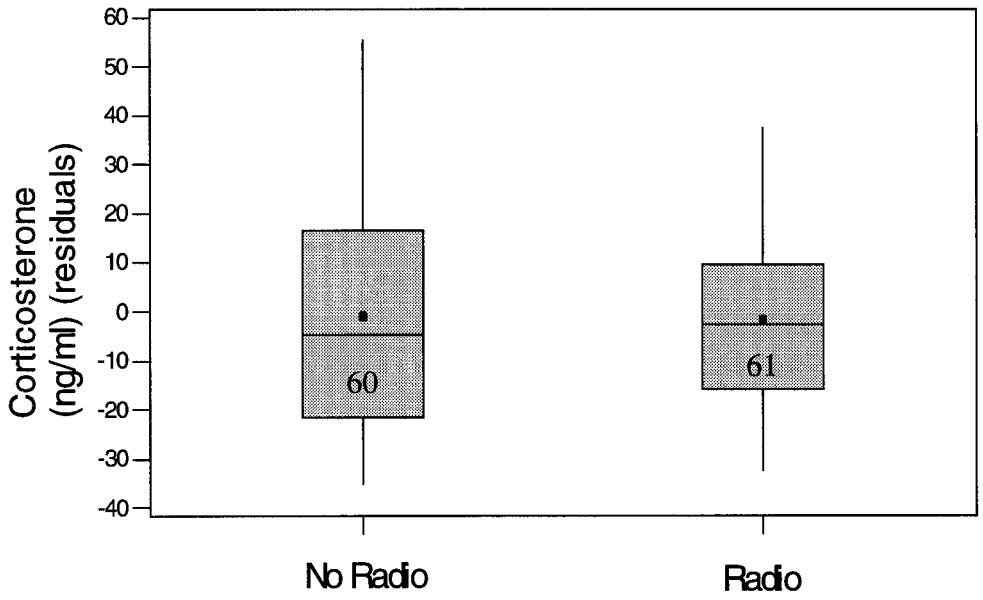


Figure 3.4. Corticosterone (time residuals, to 'control' for differences in sampling time) with mass, separated by sex (● females n=68, ▽ males n=65). Only female regression is significant.

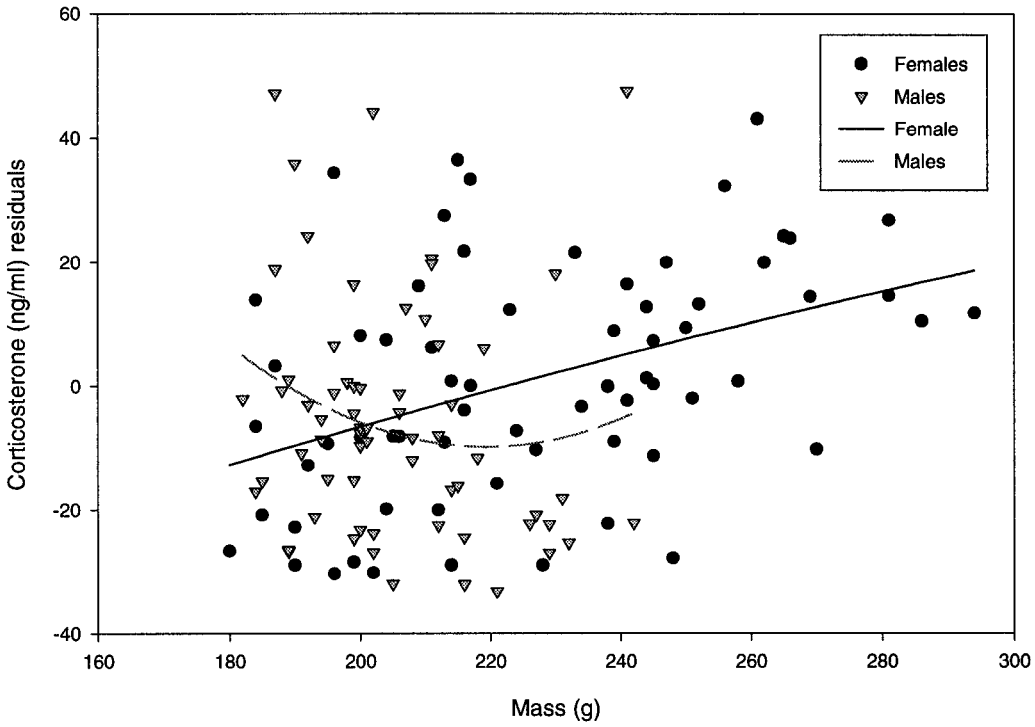


Table 3.2. Statistics for Regressions in Figure 3.4.

Group	R^2	Model	p	Regression equation*
Females	12.7%	Linear	0.003	Cort = -108.5 + 0.71 mass – 0.001 $(mass)^2$
		Quadratic	0.707	
Males	3.9%	Linear	0.299	Cort = 559.4 - 5.23 mass + 0.012 $(mass)^2$
		Quadratic	0.249	

*where Cort=cort-time residuals

Figure 3.5. Maximum corticosterone (at t_{max}) (time-mass residuals) by year, in each sex. Females (A) H = 1.83, df = 1, p = 0.176; Males (B) H = 8.17, df = 1, p = 0.004 Kruskall-Wallis test. Numbers in boxplots indicate sample sizes.

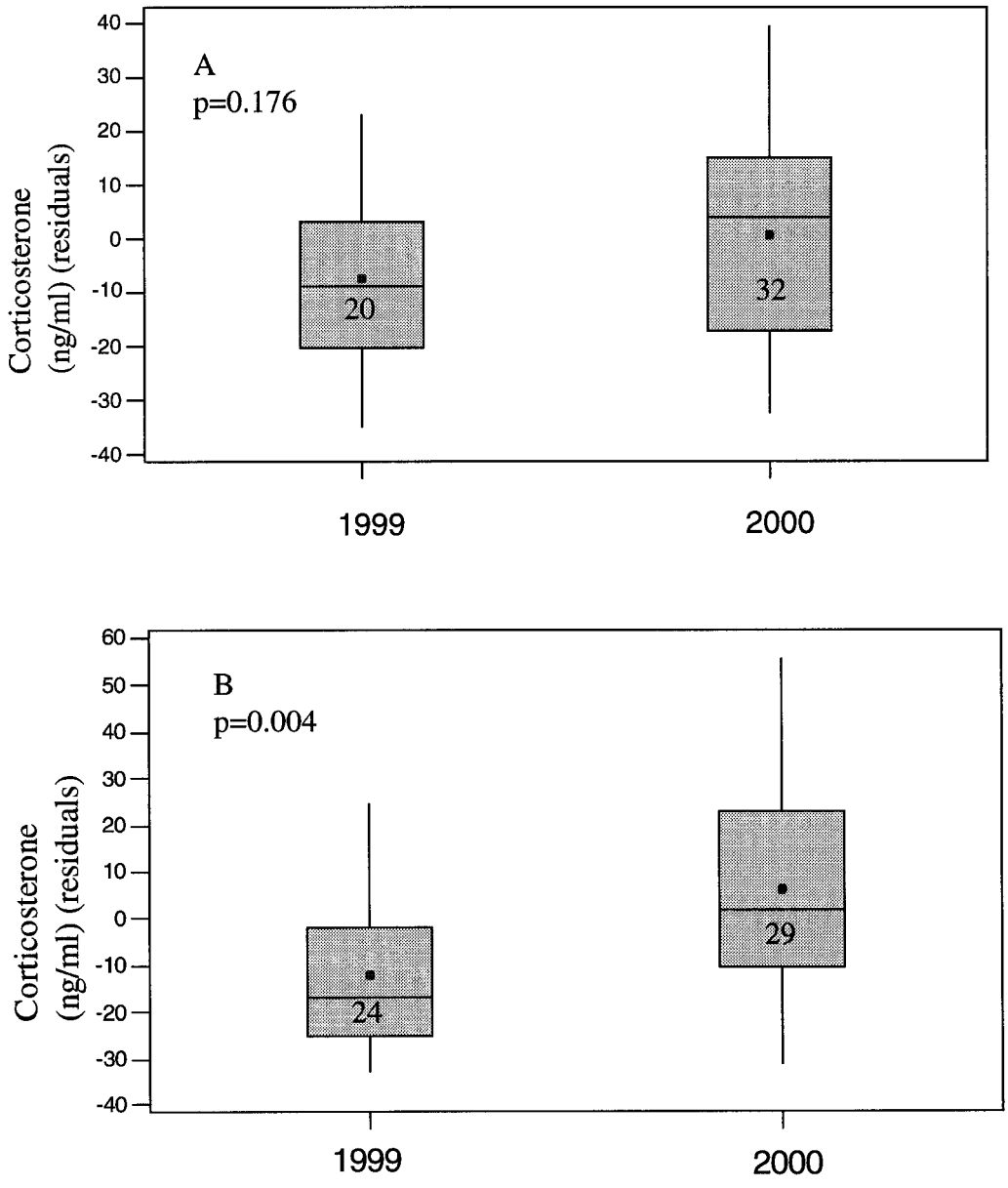


Figure 3.6. Maximum CORT (at time t_{max}) in each breeding period in (A) females (Kruskal-Wallis, H=4.16, df=3, p=0.245), and (B) Males (Kruskal-Wallis, H=4.71, df=3, p=0.194). Here, the effects of inter-individual variation in mass on CORT is not controlled. Numbers in boxplots indicate sample sizes.

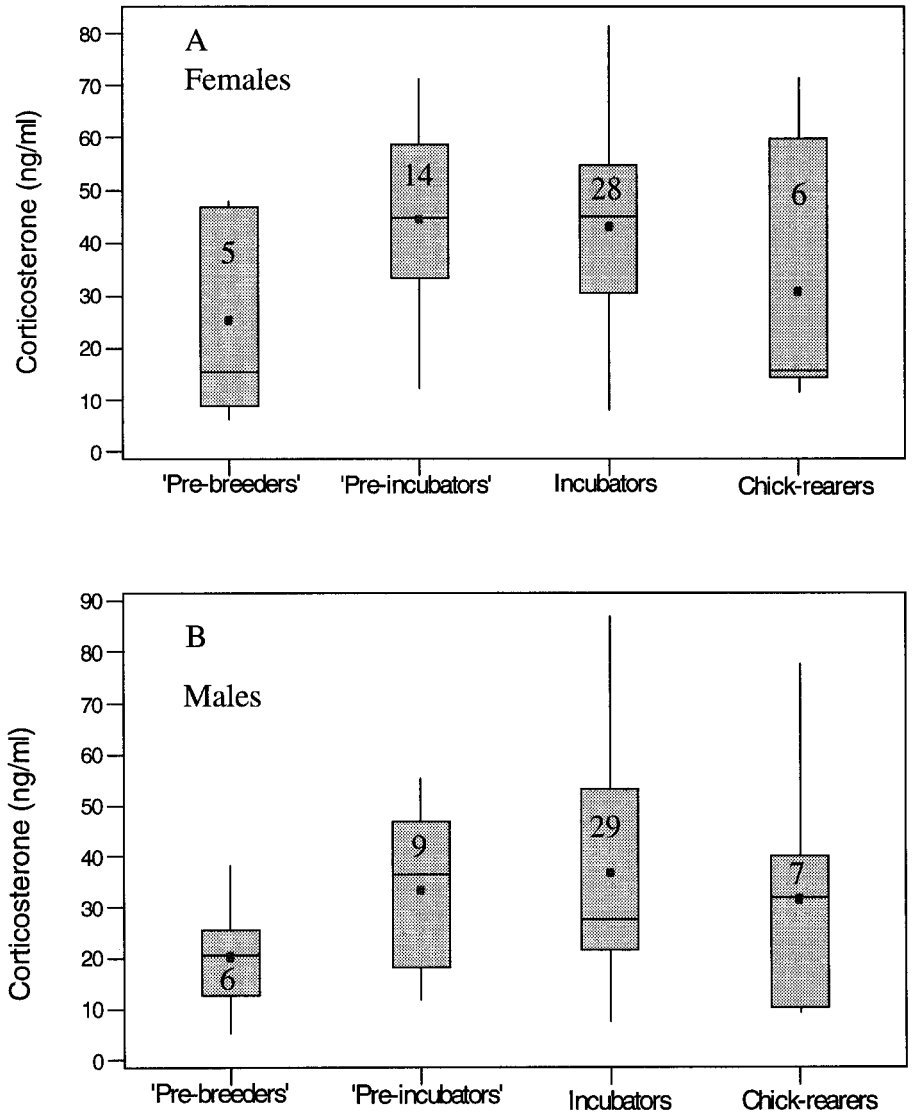
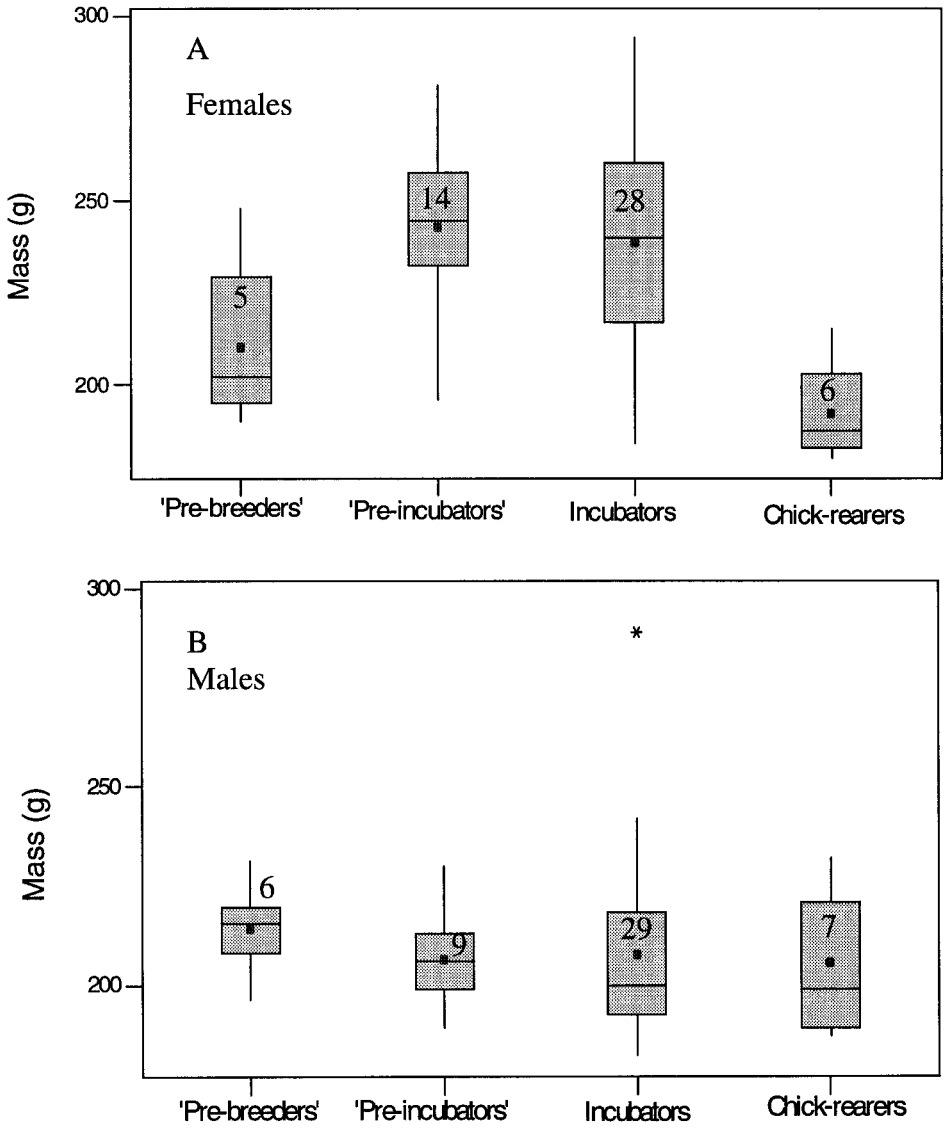


Figure 3.7. Mass in each breeding period (estimated using BP) in (A) females (Kruskall-Wallis, H=16.8, df=3, p=0.001) and (B) males (Kruskal-Wallis, H=2.68, df=3, p=0.44). Numbers in boxplots indicate sample sizes.



3.4 Discussion

This study is the first to report the response of Marbled Murrelets to a standardized stressor (capture, handling and restraint). Factors found to be biologically relevant in modulating the stress response in other studies (eg. date, year, mass, sex, breeding stage) were assessed. I also compared the stress responses to capture with and without radio transmitter attachment, to address concerns about the response of Marbled Murrelets to transmitter attachment (F. Cooke, S. Newman, pers. comm). Radio attachment did not cause any immediate additional increase in the stress response, compared to birds that were handled only.

The major cause of variation in CORT release by Marbled Murrelets appeared to be body mass. Mass differences drove the variation in CORT during breeding stages. The mass differences were probably due to inclusion of egg-producing females early in the sampling (see this thesis, chapter 1; Vanderkist 1999a), the overall decline in mass with date explainable by the gradual loss of gravid females, as they progressed from egg-production to chick rearing. However, the way in which mass variation affected CORT release was unclear, with trends differing between sexes. Furthermore, CORT was not correlated with physiological markers of egg-production and with nearness to laying date (LMT unpublished data; see chapter 4), suggesting that it is not egg-production alone that drives variation in CORT release. Other measurements of stress (eg., heart rate or body temperature, B. Culik pers. comm.) in Marbled Murrelets were not addressed here, but could be used in future research on their response to stressful events.

Stress series

This study demonstrates a robust stress response to capture and handling in Marbled Murrelets, in which CORT at t_{max} was significantly higher than CORT in earlier samples. The source of individual variation in the stress response (seen in Figure 3.1) was not statistically testable. The increase in CORT up to 30 minutes post-capture is similar to those recorded for other avian species, as was the range of plasma CORT (see Astheimer *et al.* 1995, Dufty & Belthoff 1997, Heath & Dufty 1998, Smith *et al.* 1994, Silverin & Wingfield 1998, Wingfield *et al.* 1992). Wingfield *et al.* (1995) predicted that capture stress elicits lower CORT per mass (g) in birds that exhibit more parental care than those showing less parental care. Consistent with their hypothesis, I found that mean maximum CORT level (at t_{max}) for Marbled Murrelets was lower than reported for other *Charadriiformes* (ranged from 70-120 ng/ml, with body weights from 20-70g) as predicted by Wingfield *et al.* (1995), based on arctic-breeding shorebirds. In comparison with Marbled Murrelets, these shorebirds would fall into the 'less parental care' category described in Wingfield *et al.* (1995), due to the differences in parental care during chick-rearing between the two groups: shorebirds fledge soon after hatch and usually feed themselves (as in Wilson 1994), while Marbled Murrelet chicks are fed by both parents for 30 days (Nelson 1997).

Capture and handling vs. capture, handling, and radio attachment:

Prior to this study, there have been no detected physiological effects (heat loss, infection) or physical effects (increased depredation, ability to fly) of radio attachment on Marbled Murrelets (Newman *et al.* 1999). The current study documents a physiological response

to capture, but not an increase in response when transmitters are attached; maximum CORT (at t_{max}) was equal in birds that were handled compared to birds that were handled and radio-tagged. A preliminary study with Xantus' Murrelets (Newman *et al.* 1997) described a similar increase in plasma CORT with time, and although the birds were certainly stressed in response to capture, the level of stress was not affected by an additional 10 minutes of handling, as opposed to an additional 10 minutes of being held in a pet carrier (Newman *et al.* 1997). My results support this finding, suggesting that the CORT release in Marbled Murrelets proceeds in a pre-determined pattern (probably associated with the capture event itself), with no adjustment to additional handling or invasive procedures.

Change in corticosterone with year

Mean maximum CORT in the second year of this study (2000) was higher than in the first (1999). Although this was statistically significant in males only, females showed a similar trend. Inter-annual differences in stress responses are not surprising, especially if years differ in weather conditions, availability of food or water, and thereby body condition, all of which has been found to influence the stress response (Wingfield 1984, Siegel 1980). During this study, I did not document environmental factors, and so was not able to use them to assess inter-annual variation in CORT. However, I suggest that for future studies, CORT analysis could be a useful tool for investigating the effects of environmental stochasticity on Marbled Murrelets.

Corticosterone release: Interactions across breeding season with sex and mass.

In some species, the CORT response is suppressed during the parental phase of breeding, to facilitate successful breeding by preventing inappropriate abandonment in response to short-term, non-lethal stressors (Astheimer *et al.* 1995, Holberton & Able 2000). However, this pattern is seen mainly in short-lived birds, particularly arctic breeders, where it is common to see a suppressed stress response during the breeding season, reducing the chances of an abandoned reproductive attempt stimulated by poor weather conditions or food shortage (Silverin & Wingfield 1998). Because short-lived birds have fewer breeding seasons during their lifetime than long-lived birds, they are expected to be more resistant to acute stress, even though this may affect survival (Wingfield *et al.* 1995). In comparison, it would not be adaptive for a long-lived bird to risk survival when many more breeding attempts are possible and thus, they are expected to maintain the stress response during reproduction (Wingfield *et al.* 1995, Kitaysky *et al.* 1999a). In fact, CORT release in some long-lived species has been shown to increase as the breeding season progresses, in accordance with intensive chick feeding at the end of the breeding season after a decline in parental body condition (Kitaysky *et al.* 1999a).

Adult Marbled Murrelets caught in pre-breeding, pre-incubation, incubation, and chick-rearing stages showed no statistically significant changes in the response to capture and handling. However, a trend toward higher CORT after the pre-breeding stage was seen in both sexes after they started defeathering BP (classified here as 'pre-incubators'). In both sexes, CORT increased after pre-breeding stage (BP 0), reached a peak during pre-incubation and incubation (BP 1 and 2), and decreased during chick-feeding (BP 3) (see Figure 3.6). Is this trend driven by its interaction with mass? For females, this

explanation cannot be refuted because they show an increase in CORT with mass (Figure 4), which probably corresponds to increased mass during pre-incubation and incubation (Figure 3.7). Thus, the change in CORT with breeding stage is probably due to the change in mass. However, the strength of the mass-CORT interaction is less convincing for males: mass affects neither CORT release (Figure 3.4), nor is there a change in mass between breeding stages in males (Figure 3.7), and yet, a trend toward increasing CORT after the pre-breeding stage still occurs.

A more detailed description of the change in CORT with breeding stage is needed for Marbled Murrelets. It is possible that our ability to correctly infer breeding stages (by BP score) is compromised by the presence of physiologically immature birds in the study area, who may undergo partial defeatheration of BP (seen occasionally in petrels and penguins, Ainley *et al.* 1990; auklets, I. Jones, pers. comm.; and Atlantic Puffins, Gaston and Jones 1998) (see chapter 2 for a more detailed discussion on brood patches). For future studies, sampling the same individuals at successive breeding stages would be best to assess the modification of CORT across the breeding season. Regardless, these data support the idea that long-lived birds should maintain their stress response throughout breeding (Kitaysky *et al.* 1999a).

Mass variation between sexes and breeding stages

Contrary to other studies, this study found that increased body mass in female Marbled Murrelets is accompanied by an increase in CORT. The result for females is surprising, given that in most other studies, CORT usually exhibits a negative relationship with body mass or fat reserves; birds with better energy reserves (larger mass or better body

condition) show reduced responsiveness to stress (Kitaysky *et al.* 1999a, Kitaysky *et al.* 1999b, Wingfield *et al.* 1994a, Wingfield *et al.* 1994b, Schoech *et al.* 1997, Wingfield *et al.* 1995, Astheimer *et al.* 1992, Wingfield *et al.* 1998). Better physical condition has also been associated with a quicker response to and recovery from stressors (Heath & Dufty 1998), while Schoech *et al.* (1997) found that body mass (in scrub jays) explained the variation in CORT secretion in response to capture and handling. Like Schoech *et al.* (1997), I found that body mass explains much of the individual variation in CORT. Because the relationship between CORT and mass in females was *reversed*, I can conclude that either (a) in this study, mass is not a good measure of body condition and/or (b) release of CORT in females does not have anything to do with body condition.

Female Marbled Murrelets were significantly heavier than males only during the second two stages of breeding: pre-incubation and incubation. While the 21-g difference in mass between males and females is not equal to the mass of an egg (36-41 g, Nelson 1997), it is just about right if we assume that pre-incubating females were halfway through egg-production. Female body mass declined during the breeding season, supporting the idea that increased mass early in the season is associated with egg-production. In this study, body mass increase in females probably represents egg-production, or preparation for egg production, and not necessarily any increase in endogenous fat reserves. Thus, the positive relationship between CORT and mass probably represents a time that is more stressful for females: egg-production. However, because baseline and maximum CORT are not necessarily correlated, the CORT release elicited by capture, may only be representative of the level of plasma CORT released during a *stressful* event, and not of normal, circulating levels ('baseline'). For most

cases, we were not able to measure baseline CORT, and so have to rely on inferences drawn from CORT at t_{max}.

In Lesser Black-backed Gulls, Houston *et al.* (1984) found that it was the females' protein reserves, not fat reserves, that declined significantly during egg production. In addition, they found that egg quality was significantly influenced by the female's protein reserves, as was the ability to relay. Because CORT is known to mobilize protein when energy is needed (Siegel 1980), an increase in CORT in female Marbled Murrelets during the pre-incubation phase may assist in mobilizing protein for use in the developing egg. CORT has also been shown to increase food-searching behaviour (Astheimer *et al.* 1992, Wingfield & Silverin 1986). Although this usually occurs in preparation for migration or in response to food shortages, elevated CORT early in the breeding season may promote feeding and thus, play a role in preparation for egg production in female Marbled Murrelets (if it is normal baseline CORT that is elevated, not just higher CORT elicited in response to a stressor). These possible functions of CORT in the regulation of reproductive effort in long-lived birds (as requested by Kitaysky *et al.* 1999a) require more investigation. I suggest that female Marbled Murrelets may be more susceptible to acute stressors, or unable to suppress their stress responses, early in the breeding season.

I detected no change in plasma CORT with body mass or breeding stage in males. Although mass in males varied by 60 g (from minimum to maximum mass, see Figure 3.4) with no detected change in plasma CORT levels, increases in body mass do not necessarily mean increase in fat stores. Because there was no correlation between skeletal size (tarsus, wing) and mass, I was unable to determine the extent to which

changes in body mass were explainable by skeletal size. In Desolation Sound, it is not known if or when Marbled Murrelets readily accumulate fat reserves, but it would be interesting to compare across study sites to address intra-species variation in fat accumulation (eg., the Marbled Murrelets in Clayoquot Sound, BC. seem to have greater subcutaneous fat deposits than those in Desolation Sound, BC.; L. Lougheed and N. Parker, pers. comm.). This would provide a basis for an assessment of stress response, correlated with site, food availability at each site, and fat reserves. Unlike females, males did not lose mass during the breeding season, even during chick-rearing. There was also no support that Marbled Murrelets in our study area lost mass due to reproductive stress or to minimise the cost of flight (Hull *et al.* in press). An abrupt decrease in mass at chick hatch, coinciding with the requirement for increased flights to feed the chick, when lowered wing-loading is advantageous, has been reported for auklets (Jones *et al.* 1994). Similar requirement to decrease wing loading in Marbled Murrelets could be expected to drive the mass change in adults during the chick-feeding period, as the chick can be fed 1-8 times per day (Nelson 1997). However, I found no mass decrease in males, which feed the chick as often, if not more (Bradley *et al.* 2002), than females. On the other hand, it may not be necessary for males to increase mass prior to the breeding season. It may be difficult for Marbled Murrelets to accumulate resources before the breeding season, since reserves would have to be laid down in late winter or early spring, a time when food is most scarce for resident species (Perrins & Birkhead 1983). The chick-rearing behaviour of Marbled Murrelets, involving inland flights of up to 100km from foraging areas (Hull *et al.* in press, Hamer & Nelson 1995) suggests a high energy output and thus, decreased wing-loading could be advantageous when chick-rearing. If males do

not increase wing loading in the first place, mass loss during chick-rearing would not be necessary. It is not clear whether inland flight incur significant energetic costs for Marbled Murrelets (Hull *et al.* 2001)

Future studies

Due to concerns about interrupting the breeding of individuals, I did not recapture radioed birds. In future, this would be an effective way to monitor the long-term response to carrying a radio and to track the stress response in individuals during different breeding stages. An analysis of stress responses during pre-basic moult may help to understand the energetic consequences of moult, after an intensive breeding season when energy resources may have been depleted. Finally, studying stress responses during the winter may reveal trends (in comparison with responses during the breeding season) that were not detected by the analysis during just the breeding months.

Chapter 4

Does Catching Marbled Murrelets Disrupt Breeding?

4.1 Introduction

Marbled Murrelets are unique seabirds in the alcid family (Gason and Jones 1998) because they nest solitarily on the mossy limbs of old-growth trees (Nelson 1997). As a result of this cryptic behaviour, much of their breeding biology, ecology, and demography remain poorly known (Nelson 1997, Ralph and Long 1995, Cam *et al.* in review). In spite of poor knowledge of its demographic trends (Cooke 1999), the Marbled Murrelet is listed as threatened in Canada (Committee on the Status of Endangered Wildlife in Canada), Washington, and Oregon (U.S. Fish and Wildlife Service 1992), and as endangered in California. Thus, there is an ongoing effort to improve demographic estimates, including a more accurate assessment of reproductive success, productivity and breeding population size.

Survival parameters of species in the wild must be estimated by following individually marked animals through time (Lebreton *et al.* 1992). However, free-living birds are often difficult to study, due to their mobility, wide range and freedom of movement. The ability to attach radio transmitters has greatly improved the knowledge of ecological and physiological variables that may otherwise have been unmeasurable (Culik & Wilson 1991). However, the use of such instruments may alter the behaviour and performance of the study animal, and these alterations may be reflected in the scientific conclusions drawn from the data collected (Culik *et al.* 1994, Kinkel 1989).

The effects of these instruments on the parameters researchers measure are rarely discussed (Culik *et al.* 1994); for Marbled Murrelets, the effects of capture, handling, and radio attachment, on breeding behaviour are unknown.

Assessing the severity of a stressful event is difficult. Without the help of dependable physiological or behavioural observations, it is impossible to accurately interpret how an individual responds to any stressful stimuli. With the help of radio-telemetry, individuals can be followed throughout the breeding season; hence, their breeding activities following investigator disturbance can be monitored. Likewise, analyses of blood taken at the time of capture helps to describe pre-capture reproductive state (Vanderkist *et al.* 2000), and the physiological response to a stressful event (see Wingfield *et al.* 1998). We also used brood patches in our assessment of disturbance, with the assumption that birds with developed brood patches, radio-tagged at capture, should be observed in incubation soon after capture.

Physiological analyses in this study involved vitellogenin (VTG) and corticosterone (CORT). Reproductive state can be assessed using VTG, a phospholipoprotein found only in the plasma of egg-producing females (Bergink *et al.* 1974, Deeley *et al.*1975). Analyzing VTG from Marbled Murrelets in our study area (Desolation Sound, B.C.) was used successfully by Vanderkist *et al.* (2000) to identify fecund females whose breeding status was otherwise unknown.

Birds respond to stressful events (such as reduced food availability, severe storms, or predator encounters) by releasing the hormone corticosterone (CORT) into the bloodstream (Wingfield *et al.* 1995), prompting birds to respond appropriately to a stressful event (Siegel 1980). Because capture mimics a predator encounter, measuring

CORT concentrations in blood samples, taken in the first hour post-capture, indicates sensitivity to acute stresses in general (Wingfield *et al.* 1995). The sensitivity of birds to a stressor can then be compared among individuals, between sexes, seasons, years, and study sites (Silverin & Wingfield 1998, Holberton & Able 2000).

Evidence that seabirds are detrimentally affected by human observers has been accumulating for years, but is traditionally measured at the colony (Anderson 1988, Fetterolf 1983, Piatt *et al.* 1990, Cairns 1980, Fraser *et al.* 1999, Rodway *et al.* 1996). Likewise, nest site disturbance of Marbled Murrelet has been a conservation concern in Washington, Oregon, and California (Long and Ralph 1998). However, information on disturbance of birds away from nest sites, or away from colonies is rarely presented (but see Brubeck *et al.* 1981, Whitworth *et al.* 2000). To this end, we assessed the effects of disturbing Marbled Murrelets away from their nest sites during the breeding season. Our objectives were to (1) assess the impact of capture and transmitter attachment on Marbled Murrelets; (2) compare breeding status (of nonbreeders, failed breeders, and incubating birds) found using three independent methods (radio telemetry, physiology, and brood patches); (3) assess egg and brood patch development in relation to laying date in radio-tagged female Marbled Murrelets; and (4) use plasma CORT to help characterize individual variation in the sensitivity to stress, especially between groups of nonbreeders, failed breeders, and incubating birds.

4.2 Methods

Captures, Blood-sampling, and Radio Transmitter Attachment

Captures occurred in Desolation Sound, British Columbia, (centre 50° 05'N, 124° 40'W) from 20 April to 4 September in 1999, and 19 April to 26 August in 2000. "Dipnetting" (Whitworth *et al.* 1997, Lougheed *et al.* 1998, Vanderkist *et al.* 1999b) was used to catch birds at night between 22:00 and 05:00. Birds were captured from the surface of the water, using a long-handled salmon net, and put into dark cloth bags. Blood sampling and radio attachment took place at the nearest shore, within 1 km of the capture site (always reached within 10 min).

Transmitters (weight 3 g, Model No. 386, Advanced Telemetry Systems, Isanti, MN) were attached following the methods of Newman *et al.* (1999) but without sutures or anaesthetic (Bradley and Cooke 2001). Transmitters were attached with a subcutaneous anchor, and the loose end secured with a small amount of Bird Epoxy (Titan Corporation, USA) to the dorsal feathers. Birds were bled approximately 5 minutes after radio attachment, and released immediately after glue on the radio had dried (ca. 3 min). Due to concerns regarding handling time and additive effects of invasive procedures, a cautionary approach was taken, in which not all birds with radio transmitters were bled; in two years of study, 25% (1999) and 50% (2000) of radio-tagged birds were bled. Radio-tagged birds from 1998 were also included in some of the analyses (Centre for Wildlife Ecology, SFU, unpubl. data), but these birds were not bled for VTG or CORT analyses.

Blood samples (1-2 ml) were taken from the brachial vein, and kept cold in a small cooler with icepacks until returning to the field camp. Blood was centrifuged at 6,000 rpm for 10 minutes, the plasma separated, and blood and plasma were frozen at -20°C until they could be transported to laboratory facilities for further analysis.

Physiological Analyses: Vitellogenin and Corticosterone Assays

Vitellogenin (VTG) concentration in the plasma was determined indirectly, with an assay for vitellogenic zinc (following Mitchell & Carlisle 1991), using a diagnostic kit from Wako Chemicals (Zn, Cat. No. 435-14909). Vitellogenic zinc (VTG-Zn) is used as an index for VTG, as described and validated for Marbled Murrelets by Vanderkist (1999a) and Vanderkist *et al.* (2000). VTG-Zn is selectively precipitated from the plasma with dextran sulphate (Griffin and Mitchell 1984); the difference between the original (total) plasma zinc and the remaining zinc (albumen-bound) following precipitation provides an index of VTG-Zn (Mitchell and Carlisle 1991, Williams 1999). VTG-Zn $\geq$ 0.96 ug/ml denotes egg-producing females (LMT unpublished data, see chapter 1).

Plasma CORT levels were analysed using a specific radioimmunoassay (as described in Salvante 2000; see also detailed description in Wingfield *et al.* 1992). Each sample (5-20 ul) was extracted with redistilled dicholoromethane, in duplicate, and adjusted for percentage recovery of the internal standard. Plasma CORT concentrations were calculated as nanograms per milliliter (ng/ml) (see chapter 2 for specific details).

Blood samples for physiological analyses were taken at the time of capture, while laydates were determined using radio telemetry after capture (Bradley, in prep). Thus, all brood patch and physiological measurements were made without any prior knowledge of

breeding status or laydate. Because it is not possible to determine breeding status in individual Marbled Murrelets without the help of radio transmitters, there could be no control groups for breeding status.

Brood Patch Scores

Brood patches (BP) were scored according to Sealy (1972):

BP 0=no evidence of defeathering.

BP 1=beginning loss of down and contour feathers.

BP 2=almost complete loss of down and most contour feathers, vascularization beginning.

BP 3=complete loss of feathers, heavy vascularization.

BP 4=regression beginning with down appearing, especially around the edges, sheaths of new contour feathers appearing.

BP 5=most of the area down-covered, contour feathers beginning to break out of sheaths.

BP 6=complete regression, appearance as in BP0, but at the end of the breeding season.

For one analysis, BP were pooled according to developmental stage: 'Absent' (BP0) indicates birds that could be pre-breeders (i.e. physiologically immature), or breeders pre-incubation; 'Slight' (BP 1), with some loss of abdominal down, occurs in true breeders but could also be seen in pre-breeders (Gaston & Jones 1998, I. Jones pers. comm, Ainley and Boekelheide 1990); and 'Present' (BP2-5) indicates birds that had obvious BP and were probably physiologically mature (but see chapter 2 for a discussion of BP).

DNA Sexing

Marbled Murrelets were sexed following the methods described in Vanderkist (1999a) (from Griffiths *et al.* 1996), with a few modifications. Blood samples were either wet red blood cells, or a dried drop of blood on filter paper. Unlike in Griffiths *et al.* (1996), genomic DNA was isolated from erythrocytes without using a phenol/chloroform extraction. Instagene Matrix (Bio-Rad Laboratories, Hercules, Ca, Cat. No. 732-6030) was used to finish the extraction, following the procedures provided by the manufacturer. Extracted DNA was amplified with a polymerase chain reaction (PCR) following the description in Vanderkist (1999a). Male birds were identified by the presence of a single 400-base pair band, and females by the presence of both 470 and 400 base pair bands.

Determining Laydate and Breeding Status

Laydate (used synonymously with 'onset of incubation' and 'egg laying') was determined using radio telemetry to track each bird's daily activity patterns. Aerial telemetry from a helicopter tracked the presence of radio-tagged birds on the water (foraging/staging area) and in the forests (nest site) (Hull *et al.* 2001). The presence or absence of individual radio-tagged birds found on the water were noted daily. Because parent Marbled Murrelets incubate the egg in regular incubation shifts of 24 hours (Nelson 1997, Bradley and Cooke 2001), radio telemetry was able to detect the patterns of presence or absence of radioed pair members in the foraging area (Desolation Sound). Once a radioed bird began to disappear from the foraging area regularly, every second day, it was assumed to be incubating (Bradley and Cooke 2001). Additional data to reinforce this assumption came from radioed birds in which both members of the pair

were radio-tagged; these pair members disappeared regularly and alternately (one bird on the water one day, the other bird on the next, and so on) (Centre for Wildlife Ecology, Simon Fraser University, unpubl. data).

Breeding status was determined by attendance patterns at the nest and foraging area with daily radio telemetry monitoring (as described above). If birds disappeared from the study area, or if their attendance in the foraging area was markedly sporadic, their status was classified 'unknown', and they were left out of the current analysis. If birds showed about 8-30 days (30 days = incubation period, Nelson 1997) of the presence-absence pattern in the study area, they were classified as 'breeders' (Bradley, in prep). Thus, for the purposes of this study, 'breeders' were birds that started to incubate; no further information on chick hatch or chick fledge was needed to determine if the bird was reproductively mature. Murrelets that reached incubation will henceforth be termed 'incubating birds' (IB). If birds were found at the foraging/staging area for most or all of the breeding season, they were classified as 'nonbreeders' (Bradley, in prep), henceforth 'NB'. These NB could have been true pre-breeders (i.e. physiologically immature) or nonbreeding adults. If females were found producing eggs (elevated VTG) at the time of capture, but never showed evidence of incubation behaviour (i.e. appeared to be NB from radio tracking), they were classified as 'failed breeders' (FB). Importantly, this classification of 'failure' could include birds with resorbed eggs (yolk resorption can occur after yolk is completed but before ovulation, Astheimer 1986) or egg loss (i.e. not deposited in a nest, but 'dumped' elsewhere), but does not make a statement about failure during incubation or chick rearing: FB in this classification did not start incubation.

There was no way to classify males as FB using physiological methods, as with females. Due to the classification criteria (i.e. using VTG) FB were all egg-producers. FB may have gone undetected if females were not captured during the egg-producing period, when VTG was not present in the plasma.

Laydates are used in two types of comparisons in this study: (1) The calendar date on which the bird started incubating was determined, and compared between birds. (2) The time between the day of capture and the day that subsequent incubation was initiated was determined for each bird in respect to its own breeding cycle.

Statistical Analyses

All statistical analyses were done using Minitab 13 for Windows and SAS (SAS Institute). Independent variables explaining variation in CORT were selected based on biological relationships suggested in the literature (ex. seasonal date, year, reproductive status, mass), and then assessed using Aikike's Information Criteria (AIC). Then, using the variables resulting from the AIC model selection process, CORT levels in each breeding status group (IB, NB, FB) were predicted with a general linear model (SAS). However, because of the numerous variables (10) and interactions (16) retained in the model, patterns in the data were obscured and trends were difficult to detect, making variation in CORT between FB, IB, and NB inconclusive.

We used t-tests and paired t-tests when data was normally distributed (Anderson-Darling Normality tests); when data was non-normal, nonparametric tests (Mann-Whitney, Kruskall-Wallis) were used. The latter were used to make all comparisons between breeding status groups (FB, NB, IB) and between 'delayed' and 'normal'

incubators. Due to incomplete information from radioed birds in 1998, they are not included in all data analyses. Because 'failed' status is determined by VTG analyses, only females that had been bled were included in the analyses using 'status' (i.e. could not include NB females if no blood samples were taken to determine if she was actually FB). This reduced the sample size significantly, from 74 to 35 females.

Significance levels were assumed at 5% (α=0.05). In all boxplots, the box represents the middle 50% of the data. The line through the box represents the median. The lines (whiskers) extending from the box represent the 95th percentile (excluding outliers). The point or dotted line on each plot inside the boxes represents the mean of the sample. All data are $\pm$ SE unless otherwise indicated.

4.3 Results

In 1998, 40 birds received radio transmitters but were only bled to determine sex (i.e. insufficient volume to analyze VTG or CORT). In 1999 and 2000, 174 birds received radio transmitters. The proportion of radio-tagged birds that were females (58%) was significantly higher than those that were males (42%) (test for two proportions Z= 2.86, p=0.004). Blood samples were available for only 40% (70) of the radioed birds.

Radio telemetry methods detected 84 IB and 62 NB murrelets (28 had unknown breeding status) (1999 and 2000), making 58% (84/146) radio-tagged birds putative 'parents'. Because those with unknown sex and status were removed, fewer IB and NB were available for some of the following analyses.

We originally assumed an equal probability of encountering and capturing males and females in our sample. The larger proportion of females in the dipnet sample (above) may be explainable because females (3.5 $\pm$0.3 SE min, n=88) were captured in slightly less time than males (4.7 $\pm$ 0.6 SE min, n=62) (ANOVA F=3.96, p=0.05). No birds were chased for longer than 12 minutes, to avoid exhausting the birds at sea. This protocol may have biased our sample, by only capturing birds that were less successful in evading our capture effort.

The use of independent methods to discover 'failed' breeders:

We expected that fecund females and birds with BP would eventually show incubation behaviour. However, some radio-tagged birds that appeared to be reproductively active at the time of capture (as determined by presence of BP and elevated VTG) never proceeded to incubation.

There were only 35 radio-tagged females with both radio telemetry and VTG data available (1999 and 2000). 68% (24) of these females had elevated VTG at capture, but of these, only 50% (12) became IB (Table 4.1), prompting the classification of 'failed breeders': those who were evidently reproductively active at capture but did not show any incubation behaviour when radio-tracked. A few (n=8) of the radio-tagged females did not have elevated VTG at capture but later became IB, indicating that they were not captured while producing an egg; only 3 birds showed neither evidence for egg-production nor incubation (Table 4.1).

To test the idea that egg-producers are more susceptible to disturbance from capture, (i.e. more likely subsequently to fail), we tested VTG levels between FB and IB.

There was no detectable difference between average plasma VTG in FB (3.34 ± 2.5 ug/ml, n=12) and IB (2.66 ± 2.5 ug/ml, n=20) (ANOVA F=1.98, p=0.16).

Radio-tagged males and females were assessed at capture for presence of BP. Similar to the finding of fecund but failed females, we found that 49% (25/51) male and female NB actually had BP at capture (Table 4.2).

Any explanations for 'failed status?

To assess reasons for breeding failure, we made some biological comparisons between FB, IB, and NB. Mass did not differ significantly between breeding status groups (1999 and 2000), for either females (ANOVA F=1.66, p=0.2) or males (ANOVA F=0.17, p=0.68). Date of capture for each group differed significantly for females, with FB (29 May ± 3.1 days, n=12) captured significantly later than IB (11 May ± 2.3 days, n=46) (ANOVA F=7.45, p=0.001) (Figure 4.1). Males showed no such trend with date (NB captured 17 May ± 3.1days, n=23; IB 16 May ± 2.7 days, n=32), however, there was no FB category for males.

CORT analyses were used to detect possible differences in the stress response to capture in birds that subsequently became FB, NB, or IB. Because CORT in Marbled Murrelets peaks near 30 min (see chapter 3), the measurement and comparisons of maximum CORT (such as in Holberton and Able 2000) in absence of other stress series samples is valid for use in the individual comparisons that follow. While the AIC model retained breeding status as an explanatory variable for the variation in CORT, there were no detectable differences in maximum CORT between FB, NB, or IB, in either sex (Figure 4.2) (Mann-Whitney test, p>0.2 in all cases). In addition, no significant

differences were found between the increase of CORT with time or predicted 'baseline' CORT (see y=0 in equations, Figure 4.3) between FB, NB, or IB. No significant trend in CORT was detected as birds approached their individual laydates (regression, F=2.9, p=0.100, R^2=10%; cort=33.6 –0.378laydate; n=28).

Incubating birds: evidence for delayed breeding

There was significant variation in the dates that radio-tagged birds were caught. There were 88 (three breeding seasons, 1998-2000) incubating, radio-tagged Marbled Murrelets, for which laydates were determined. The majority (95%) of these birds were caught before their subsequent incubation (i.e. before laydates), especially early in the season. Capture dates for IB ranged from 19 April to 8 June, a range of 50 days. More time elapsed between capture and laydate in birds caught in 2000 than previous years (ANOVA F=5.41. p=0.006).

In all IB, mean time between capture and laying was not significantly different between sexes (17 $\pm$ 2.4 days for females, n=45, 16 $\pm$ 2.0 days for males, n=37) (ANOVA F=0.38, p=0.541). Thus, duration between capture and laydate is not biased toward one sex. Also, the date on which incubation was initiated did not vary between years (24 May 1998, n=23; 30 May 1999, n=34; 31 May 2000, n=28) (Kruskall-Wallis H=4.03, p=0.133).

The correlation between egg-production (VTG) and laydate was assessed in 15 radio-tagged females for which data were available. First, laydate relative to capture date was calculated for each individual, and time elapsed between capture and laydate was plotted against VTG and BP for each female (Figure 4.5). Three females were caught

after they had laid the egg and had low VTG; two of these had fully developed BP. This pattern was expected for birds captured after their respective laydate. Six (40%) females were producing eggs within 9 days before laydate (thought to be the 'normal' pattern), while six (40%) other females were producing eggs 21-33 days before subsequent egg-laying (thought to be 'delayed') (Figure 4.5). These 12 females had developing or fully-developed BP well before respective laydates. Second, we determined julian date of egg-laying for all birds in these two groups (n=6, 9), and found that 'delayed' incubators laid their eggs significantly later than 'normal' incubators (Figure 4.6).

Table 4.1. Numbers of female Marbled Murrelets with vitellogenin (VTG) in each breeding status group. Breeding status of incubating birds and nonbreeders is determined remotely using radio telemetry, while failed breeders are categorized by the presence of elevated VTG with no subsequent incubation. 'High VTG' ($\geq$ 0.96 ug/ml, chapter 1) indicates an egg-producing female.

Breeding Status	High VTG	No VTG	Total
Incubating Birds	12	8	20
Nonbreeders	0	3	3
Failed Breeders*	12	0	12

*Failed breeders are differentiated from nonbreeders only by the presence of high VTG.

Table 4.2: Number of birds with present (BP 2-5), absent (BP0), and slightly developed (BP1) brood patches (Sealy 1972), by breeding status and sex (n=83). Data includes 1999 and 2000 breeding seasons, all radio-tagged males, and all radio-tagged females with VTG data available.

Sex	Status	BP:	Absent (BP 0)	Slight (BP1)	Present (BP 2-5)
Male	Incubating		6	4	19
	Nonbreeder		7	1	13
Female	Failed Breeder		2	1	9
	Incubating		1	1	16
	Nonbreeder		0	0	3

Figure 4.1. Date by breeding status in Males and Females. ANOVA shows significance only in females (F=7.45, p=0.001), between Failed * and Incubating females (Tukey's pairwise comparisons, Family error rate = 0.05, Individual error rate =0.02, critical value =3.38). Solid boxes=females, hatched boxes=males. Sample size in brackets.

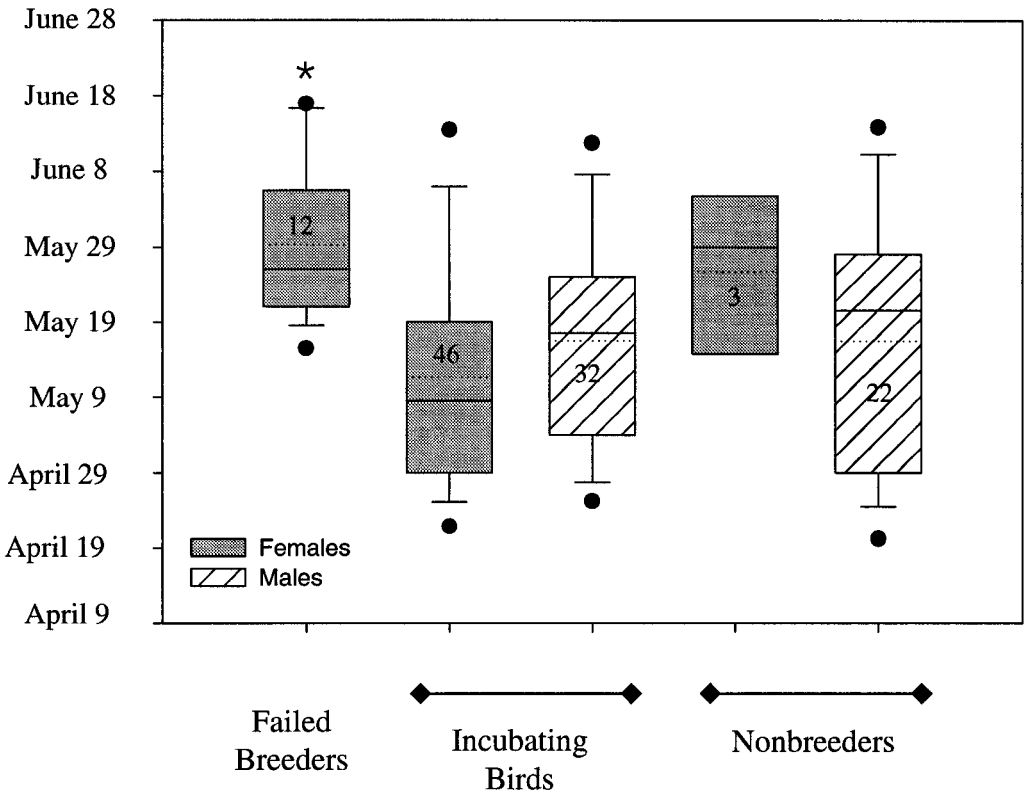


Figure 4.2: Maximum Corticosterone (at time=30 mins) by breeding status in females and males. No significant differences between any groups were found. Sample size in brackets.

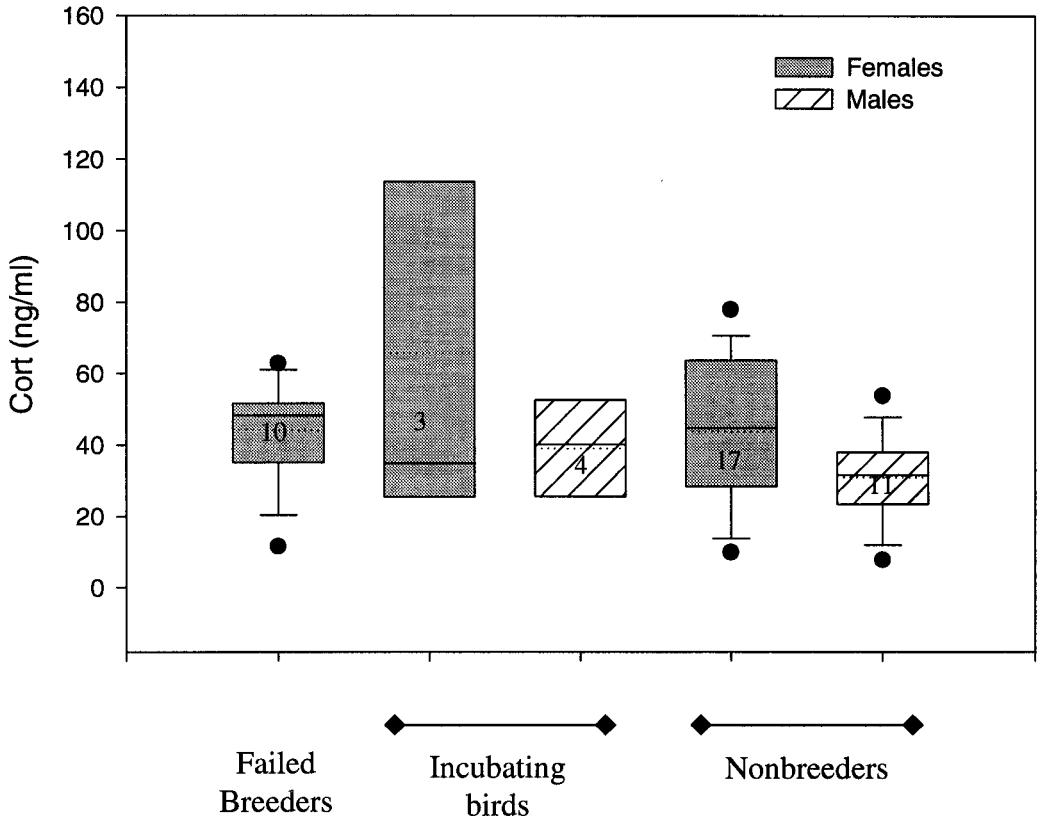


Figure 4.3: Predicted corticosterone, based on variables retained in an AIC model, for each breeding status group. Failed breeders y0=-42 $\pm$ 40.6, R^2=41%; Nonbreeders y0=48$\pm$ 347, R^2=35%; Incubating birds y0=-39$\pm$ 35, R^2=20%. Relationships between CORT and time did not vary significantly between status groups.

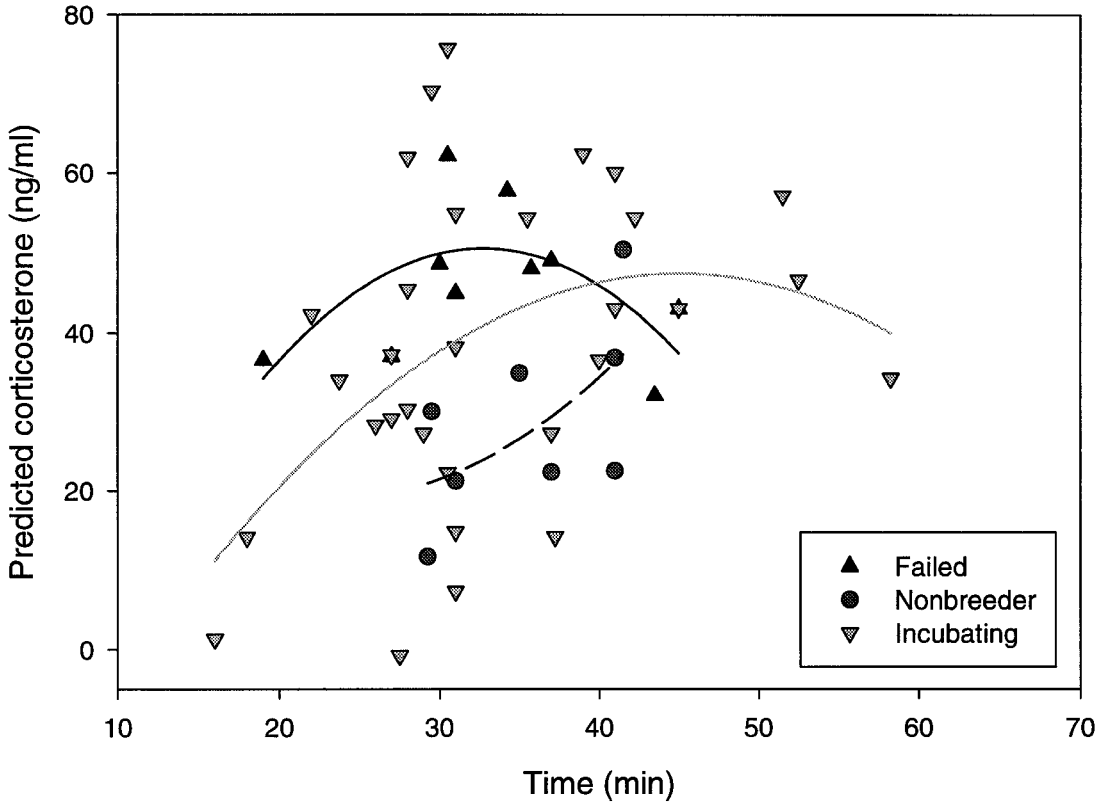


Table 4.3. Mean time elapsed from capture to laydate (in days) for individuals in relation to their own breeding initiative, separated by year. Time elapsed between capture and laydate 2000 was significantly longer than previous years (ANOVA F=5.41. p=0.006; Tukey's pairwise comparisons, Family error rate = 0.0500, Individual error rate = 0.0194, Critical value = 3.37). Letters indicate groups that are significantly different from each other.

Year	N	Days from capture to laydate (Mean ± SD)	Earliest before laydate	Latest after laydate	
1998	23	12.2 ± 10.0	-39	1	A
1999	34	15.1 ± 16.2	-45	19	A
2000	28	24.0 ± 16.0	-65	6	B

Figure 4.4. Female Marbled Murrelets with vitellogenin level (VTG) and brood patch (BP) scores in comparison with estimated laying date. Light grey dotted lines join observations corresponding to the same individual. VTG (▲) above horizontal line indicate egg-producers. Laydate (x=0, dark vertical dashed line) was determined using radio telemetry, while VTG levels were determined from blood samples taken at the time of capture. Brood patch 3 (• BP) is fully developed, vascularized brood patch.

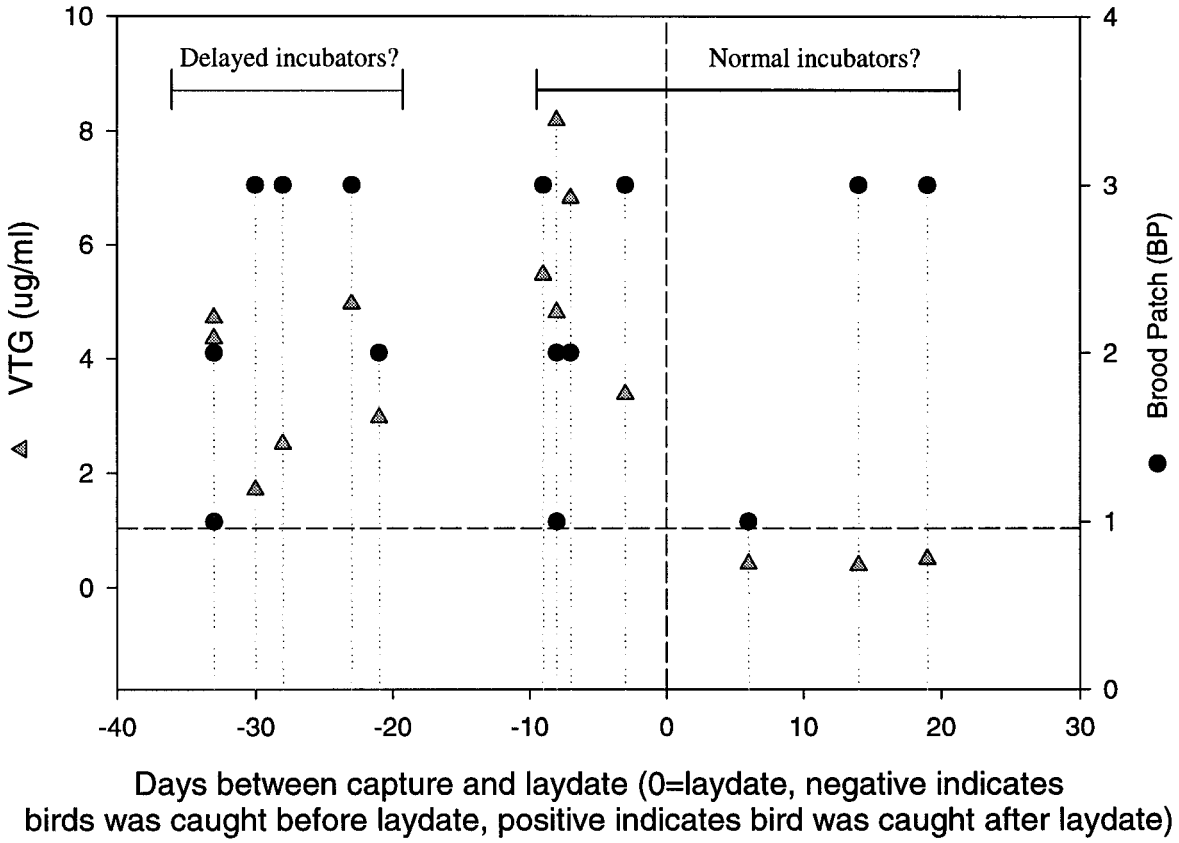
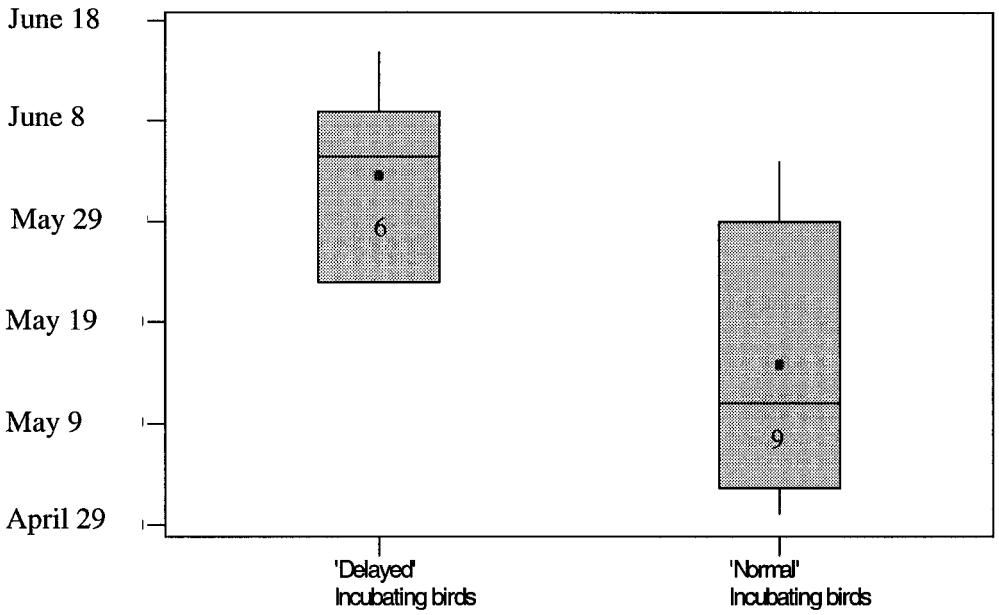


Figure 4.5: Seasonal dates for initiation of incubation in 'delayed' incubators vs. 'normal' incubators (as grouped in Fig. 4.4). 'Delayed' incubators had laydates that were significantly later than the 'normal' incubators, by 24 days (Mann-Whitney W=68, p=0.02). Numbers in the boxes indicate sample sizes.



4.4 Discussion

It is our opinion that capture, handling, and/or radio transmitter attachment sometimes affected breeding in Marbled Murrelets. However, investigator disturbance did not affect all birds in the same way, implying a number of factors can influence 'vulnerability' in Marbled Murrelets. Only 50% of egg-producing females proceeded to incubation, and 40% of a sample of incubators seemed to be delayed. However, it is not known if the same success rate in egg-production and incubation would have occurred had the birds never been captured; it is possible that the radio transmitters simply documented the natural rates of incubation and failure to nest. It may be that a certain percentage of birds always fail, however, this can not be measured unless birds are marked and tracked.

The quality of any investigation is dependent, in part, on choosing methods that do not influence the results (Kinkel 1989). However, influenced results do not necessarily translate directly to investigator disturbance, in which adult reproductive success or survival are affected (Nisbet 2000). In our study area, the average proportion of radio-tagged individuals that did not breed (42%, see results) was unexpectedly high (Cam *et al.* in review), however, estimates of breeding success from our radio telemetry data were among the highest reported for this species, and are consistent with the range specified for adult auks (Cam *et al.* in review). Still, we felt it important to examine some of the possible influences of our research methods on the Marbled Murrelet.

A contradiction in methods: discovering 'failed' breeders:

Radio telemetry alone was insufficient to understand the difference between NB, FB, and IB, because it would not have detected all potential egg-producers (as seen using VTG

and presence of BP). Similarly, physiology alone would have incorrectly classified all egg-producers as egg-layers and subsequent IB, had there been no radio telemetry monitoring to determine incubation patterns (or lack thereof).

While radio telemetry was essential to determining that a number of males and females in our study sample did not breed, it was unable to detect differences in the at-sea behaviour of NB and putative FB (the latter of which made up 34% of radio-tagged females). Notably, this study does not address FB which fail *during* incubation or chick-rearing; the behaviour patterns of these birds can be detected using radio telemetry (R. Bradley, in prep). Assessment of fecundity at capture identified the possibility of reproductive failure. Reproductive failure was also suggested by the presence of BP in 58% of the apparent NB (including FB). While the reasons for failure were unclear, investigator-induced failure remains a distinct possibility. This is not uncommon in colonial-nesting seabirds, with decreased breeding success attributed to investigator disturbance in many species of alcids (Rodway *et al.* 1996, Piatt *et al.* 1990, Cairns 1980) and other *Charadriiformes*, as well as *Sphenisciformes*, *Procellariiformes, Pelecaniformes,* and *Ciconiiformes* (review in Carney and Sydeman 1999, but see Nisbet 2000). Colony disturbance also been also reported having negative effects on incubation, hatching, and fledging success (Anderson 1988, Piatt *et al.* 1990; but see Manuwal 1979), chick growth (Cairns 1980, Fraser *et al.* 1999), and premature fledging (Safina and Burger 1983). However, these studies compared degrees of researcher disturbance at breeding colonies (for example, daily vs. weekly visits), with the overall conclusion that observer activity should be reduced to a minimum to avoid disturbance (Ainley and Boekelheide 1990, Carney and Sydeman 1999). Though Whitworth *et al.* (2000) were

not able to find evidence for long-term effects on radio-tagged Xantus' Murrelets (tagged away from the colony), and Brubeck *et al.* (1981) reported that patagial tags could be applied to terns away from nest sites without adverse effects, we were able to find no other studies that address the effects of investigator disturbance on breeding success when adults are caught *away* from nesting colonies. Also, we did not assess variable amounts of investigator disturbance, because disturbance was restricted to a single capture for radio attachment, and occurred in the foraging area, away from the nest site. Thus, comparisons of our study to those of colony disturbance may not be appropriate.

There are a number of studies documenting the influence of external devices on birds' behaviour. Instrumented birds spent more time absent from nests than controls (Culik and Wilson 1992), incurred higher costs of transport (Culik and Wilson 1991, Culik *et al.* 1993), decreased food intake (Gales *et al.* 1990, Pietz *et al.* 1991), and lost mass compared to controls (Pietz *et al.* 1993). Pain from injections or blood sampling may prevent birds from foraging optimally (Culik and Wilson 1992). While these studies rarely make direct associations with breeding success (but see Kinkel 1989), foraging success has a clear link to self-maintenance and chick provisioning. If energy consumption increases during a breeding attempt because of instrument attachment, there may be a reduction in breeding success, especially in years with low food availability (Culik *et al.* 1993). Gales *et al.* (1990) caution that the effects of carrying devices are probably not fixed, but may vary between seasons and locations. It may be that carrying a radio transmitter may incur different costs for Marbled Murrelets in winter versus summer, or at different times during their reproductive season. This requires further study.

The present study was not specifically designed to address the reaction to disturbance by adults at the breeding site, nor the effects of carrying a radio transmitter throughout the breeding season. Although we were able to determine breeding status of radioed individuals, we had no control groups (i.e. we had to catch and radio all birds to study them) and no direct physiological measurements on the costs of carrying transmitters (eg., double-labelled water, Culik and Wilson 1992). However, our observations may help to begin to understand what effects we could be having on Marbled Murrelets, and the source of individual variation in the response to a stressful event.

Why were the breeding attempts of some Marbled Murrelets failed or delayed?

Although our investigation of breeding failure was restricted to egg-producing females, it was not egg-production itself that made all females more vulnerable to failure: average VTG between FB and IB females, and between 'delayed' and 'normal' incubators, did not differ. While we caught egg-producing females which did not continue into incubation, we caught other egg-producing females that proceeded successfully to incubation. Logically, it would seem that disturbance alone is not the only reason for breeding failure. That is, females may have reacted to investigator disturbance by aborting or delaying their breeding attempts because of some predisposition to failure, or increased vulnerability, compared to the IB.

We found that the most significant factor influencing variation in breeding outcomes was date, in relation to capture. FB were captured significantly later (18 days) than IB (Figure 4.1). On average, FB were caught two days after the population's mean

egg-producing date of 27 May (two years pooled data) (see chapter 1). In contrast, most IB were captured before mean egg-producing date (Figure 4.4), on average by 16 days. This finding suggests that researchers should capture Marbled Murrelets early in the breeding season; if possible, it would be best to determine the mean egg-producing date for the study population, and try to capture most birds before this date. The influence of date has been reported in other seabirds: breeding success was lowest in late-laying Common and Thick-billed Murres (Birkhead and Nettleship 1987), and Manuwal (1979) reported high breeding success in Cassin's Auklets, for clutches laid before the population's mean laying date, after which success declined rapidly.

A similar pattern with date was found in the group of IB whose plasma VTG could be compared with each bird's individual laydate (Figure 4.5). In this group, six birds were found producing eggs 9 days before laydate. This fell within the expected duration of egg-production, thought to be about 15 days in many alcids (eg. Cassin's Auklet, Astheimer 1986; Common Murres, Murphy 1995; and Thick-billed Murres, Gaston and Nettleship 1981), but unknown for Marbled Murrelets specifically. Confusingly, we found females with elevated VTG and developed BP much earlier before laydate (21-33 days) than expected (Figure 4.5); the most likely explanation for this seems to be that these females had delayed incubation. Although VTG secretion patterns are not known for Marbled Murrelets, it seems unusual that VTG could be elevated a full month before actual laying of the egg. Generally, the pre-laying period and egg are considered energetically costly because females must redirect a certain proportion of dietary or stored nutrients to the egg (Monaghan and Nager 1997, Astheimer 1986); thus, elevated VTG before intended egg-production and subsequent

laydate seem unlikely, as these events are expected be associated with an energetic cost. When we compared the dates, we found that 'delayed' breeders laid eggs significantly later (24 days) than the 'normal' breeders which laid eggs within the expected window of time. Most birds, including Marbled Murrelets (Sealy 1972), develop accessory follicles in addition to the primary follicle, which become atretic following completion of the primary yolk or laying of the egg (Hipfner 1999, Astheimer 1986). A bird that loses her egg soon after laying may have a head start in the form of a larger second follicle with which to produce a replacement egg (Hipfner *et al.* 1999). It may be that delayed females in the current study were disturbed early enough in the breeding season to have time to relay. An advantage for laying eggs early in the breeding season may be the increased potential for renesting if the first egg is lost (Hannon *et al.* 1988, Gaston and Nettleship 1981). In many species, females that lay early (and thus have a better opportunity for renesting) are older and more experienced (Brunnich's Guillemot, Hipfner *et al.* 1999; Thick-billed Murres, Hipfner 1997, DeForest and Gaston 1996; Western Gull, Sydeman *et al.* 1991; Common Terns, Wendeln *et al.* 2000; but see Weimerskirch 1992). It follows that the later-breeders are the younger, less experienced, or poorer quality individuals (Moreno *et al.*, 1997, Sydeman *et al.* 1991, DeForest and Gaston 1996). With this corroborative evidence, we suggest that the failed and delayed murrelets in our study could have been younger or less experienced breeders than the successful breeders.

Another influence on delayed laydate seemed to be year. We found that more time elapsed between capture and laydate in 2000 than in the two previous years (Table 4.3). Environmental influences (eg. food availability, snow cover in forest, temperature) may compound the impact that capture has on individuals, or in fact, may be more

influential to inter-annual variability than the investigator disturbance. This finding suggests investigator disturbance should not be perceived as the only culprit in delaying breeding in Marbled Murrelets.

Are there sex differences in laying delay?

Males did not show the trend of reduced breeding frequency with increasing capture date as observed in females. Reasons for this may have been (i) males were not be affected by capture, or (ii) we were not be able to detect the effects, since we could not identify 'failed' males directly. However, there is some evidence to suggest that males failed too, beginning with the presence of fully-developed BP in 38% (Table 4.2) of the putative male NB. In comparing sexes, 42% males were NB; had we not been able to use physiology to re-classify some females as 'failed', 43% females would have been classified NB. If males in this study were not affected by human disturbance, we would expect the number of male NB to be the same as the female NB (which was 9%=NB- FB, Table 4.1); this is not the case. This suggests that we have incorrectly classified some males as NB, when in fact, some may have been FB. (i.e. failed before incubation began).

If males were less 'vulnerable' than females, we would also expect to see a discrepancy in the perceived delay in laydate between incubating males and females. In this scenario, we assume that a disturbed male is unable to halt egg development in his mate, and thus can do nothing to prevent her from laying and incubating the egg without delay. From 85 IB, we found no evidence that one sex is delayed more than the other. As with the presence of NB males with BP, these data suggest that males and females do not react differently to capture. But, it is possible that these data are biased because they

include only birds that successfully reached incubation; they may have been better quality individuals (Curio 1983), perhaps more experienced, and less prone to negative effects from human disturbance.

Can a change in corticosterone explain failures or delays?

CORT was assessed in this study to investigate possible differences in the stress response to capture among FB, NB, and IB. We hypothesized that if FB were more intrinsically 'vulnerable' than IB, we might have seen differences in the stress response to capture (for assessing sensitivity to stress, see Wingfield *et al.* 1998).

We found no pattern in variation of CORT with breeding status. CORT secretion is often reflective of intra-specific differences in body condition, often caused by differences in food availability (Kitaysky *et al.* 1999a and 1999b, Heath and Dufty 1998, Wingfield *et al.* 1994a). Mass, used in this study as an approximation of body condition, did not vary between breeding status groups. Females were slightly heavier than males probably due to the presence of eggs (Vanderkist *et al.* 2000, Hull *et al.* 2001) or recent post-ovulatory follicles (Sealy 1972), however, mass in female FB and IB did not differ significantly. Because there can be age-specific differences in foraging efficiency among birds (Sydeman *et al.* 1991, Daunt *et al.* 2001, Martin 1995), a lack of food may constrain younger birds more severely than older birds; younger birds may thus be in poorer body condition and more susceptible to reproductive failure (but see Williams 1996). In turn, the differences in body condition may show up in patterns of CORT release. However, the lack of evidence for mass variation between FB, NB, and IB prevented further explanation for failed Marbled Murrelets as younger or poorer foragers.

Seasonal changes in CORT secretion have been detected in a number of species (Wingfield *et al.* 1994a, Wingfield *et al.* 1992, Wingfield *et al.* 1998, Astheimer *et al.* 1995). In many birds, the pattern of CORT secretion changes as breeding progresses or with the degree of parental care (Kitaysky *et al.* 1999a, Wingfield *et al.* 1995), and is sometimes suppressed during reproductive seasons (Wingfield *et al.* 1998, Wingfield *et al.* 1992). Also, when disturbed during the breeding season, more experienced breeders may reach lower maximum levels of CORT than young breeders (in Storm Petrels, K. O'Reilly, pers. comm.). In our study, Marbled Murrelets showed no significant difference in CORT with seasonal date, or as they approached individual laydates. However, these data come from individual observations, not from repeated measures on individuals. Repeated-measures sampling, with recapture of individuals later in the breeding season, would be needed to detect such patterns if they were present. This would have been possible with the methods we used, by radio-tracking birds to their location at night, and recapturing them on the water. However, we were concerned with the vulnerability of the Marbled Murrelets, especially as they were approaching laydate, and did not want to risk further disturbance. The assessment of individual variation in the stress response as the season progresses, using the 'capture stress protocol' (see chapter 3; alsoWingfield *et al.* 1995, 1998), would be useful for further studies of how the capture response might be modified seasonally Marbled Murrelets.

While we detected delayed breeding only in the 2000 breeding season, mean laydate for all radio-tagged birds did not differ between years. It is possible that environmental conditions (ex. food availability, spring conditions, snow cover) in 2000 were such that birds had time to renest after a failed breeding attempt or re-make an

aborted egg. However, we collected no information on food availability or environmental conditions with which to compare this observation.

Age-specific differences in foraging efficiency (Sydeman *et al.* 1991, Daunt *et al.* 2001, Martin 1995), could be amplified with attachment of external devices, known to increase energy consumption during foraging (Culik *et al.* 1993). Similarly, if lack of food constrains younger birds more severely than older birds, especially in years with low food availability (Culik *et al.* 1993), the attachment of radio transmitters to younger breeders may have more significant effects on breeding success than for older birds. If the later breeders were also younger, it is possible that they were affected more by the external radio transmitters, which would help explain the variation in response to disturbance.

Our understanding of breeding failure in Marbled Murrelets is incomplete, with little or no knowledge about how or if young or inexperienced females experience partial or incomplete yolk development (with elevated VTG), partial BP development, nor if reproductively mature breeders naturally experience early reproductive failure due to nest site or food limitation. It is possible that a certain percentage of early breeding failure is common in this study species and/or study area, but this can not be measured unless birds are marked and tracked, at which point the study methods become suspect. It is likewise possible that temporary disturbance of breeding has no effect on lifetime reproductive success or survival of individuals (Nisbet 2000). While our study has suggested date as the most significant factor determining a successful start to incubation, we still do not know enough about recruitment age or delayed maturity in Marbled Murrelets to fully understand the age structure of the population, and the subsequent dynamics of

disturbance according to age and date. We recommend further study on Marbled Murrelets specifically designed to address timing of egg production, age-specific breeding success, and physiological costs of carrying radio transmitters at different reproductive stages.

General Synthesis

The primary goal of the research described in this thesis was to describe the breeding biology of a localized population of Marbled Murrelets for which reproductive potential was not well known. The major findings of this study were:

(1) The large proportion of Marbled Murrelets in our sample that did not breed. More research is needed to determine how prevalent natural nonbreeding is in Marbled Murrelets, and in other seabirds as well.

(2) The importance of assessing the impact of research techniques on the species studied.

(3) The implication that investigator disturbance was not the sole influence in causing breeding failure, which betters our understanding of the natural factors that may influence Marbled Murrelet reproductive success.

(4) The value of collaboration and integration of physiological techniques and radio telemetry in seabird research.

I studied Marbled Murrelets in Desolation Sound, B.C., during the breeding seasons of 1999 and 2000. Although the same physiological approach was used by Vanderkist (1999a) to identify fecund females in the Desolation Sound population, he was unable to track egg-production throughout the breeding season. My research (in chapter 1) extended his work, to describe egg-production for two complete breeding seasons, and as a result, determined that an indirect assessment of egg-production using vitellogenin analyses provides accurate dates for use in predicting the timing of other reproductive stages, namely, incubation, chick-feeding, and fledging. These results provide confirmation that physiological analysis of VTG produces large enough sample

sizes throughout the breeding season to allow the full description of breeding chronology (as suggested by Vanderkist 2000 and Lougheed 2000). In general, breeding chronology did not vary from 1999 to 2000. However, any variation in the timing of egg-production caused by environmental parameters (see Speckman *et al.* 2000) would be detected using this method, and thus, inter-annual comparisons of breeding chronology could be made. During the 'egg production period' (April to July), 55% of females captured were producing eggs.

In chapter 2, I conclude that researchers must be wary of assuming that presence of a BP means a bird is incubating: 53% Marbled Murrelets caught with fully-developed BP never incubated. A similar finding came in chapter 4, in which only half of the individuals that appeared to be reproductively mature (by presence of elevated VTG), and half of the radio-tagged individuals, initiated an incubation attempt. In addition, predicting incubation dates using BP measurements could be difficult, as outlined by the finding of full BP development occurring, in some cases, up to 30 days before predicted individual laydate; or from partial BP development which occurred up to 65 days before predicted individual laydate. In spite of this apparent 'delay' between BP development and subsequent laying, BP development seemed to be closely related to egg-production, with egg-production preceeding full BP development. However, I suggest for other Marbled Murrelet researchers that only fully-developed, vascularized BP should be used to infer incubation, in case pre-breeders show the beginnings of BP development. Disregarding capture effects, we suggest that fully-developed brood patches may occur in Marbled Murrelets within about 10 days of laydate, but probably persist for 30-40 days post-lay, well into chick-rearing (a pattern which is seen in other seabirds; Jouventin &

Mauget 1996, Lormee *et al.* 1999, Vleck *et al.* 2000). We are confident only that fully-developed BP (3) can infer that the bird is incubating, or that it is about to incubate, but not that breeding will proceed uninterrupted.

In chapter 3, I report that like other birds (Astheimer *et al.* 1995, Dufty & Belthoff 1997, Heath & Dufty 1998, Smith *et al.* 1994, Silverin & Wingfield 1998, Wingfield *et al.* 1992), Marbled Murrelets reach maximum corticosterone release at 30 min. However, unlike other studies, CORT increased with mass in females, but not in males, suggesting that females are more sensitive to stressful events when they are heaviest: during egg-production. In future, recapturing radioed birds a number of times during the season, would be an effective way to monitor the long-term stress response to carrying a radio and to track the stress response in individuals during different breeding stages.

Chapter 4 identified the potential for disturbance caused by capture and/or radio-tagging Marbled Murrelets. Results presented show that only 58% radio-tagged birds proceeded to incubation; 50% of the females with elevated VTG (i.e. egg-producers) did not incubate (aborted or dumped egg/resorbed follicle?); and 40% of a sample of fecund females started incubation shifts about 15 days later than expected (delayed incubators). There is no doubt that elevated VTG indicates egg-production (Deeley *et al.* 1975, Wang & Williams 1982, Challenger *et al.* 2001); thus, failure or delay in egg-producing birds is must be the explanation for the discrepancy seen between elevated VTG and subsequent laydate (or lack thereof). Similarly, if BP 3 truly means a bird has, or is about to, incubate, the discrepancy between fully-developed BP and subsequent laydate (or lack thereof) must be due to failure or delay.

Although investigator-induced failure and delay seemed prevalent in this study, it is not clear how often failure, delay, or renesting occurrs naturally in this population. The research reported here gives betters our understanding of the natural factors that may influence Marbled Murrelet reproductive success. For instance, failed incubators were captured 18 days later than successful incubators, and 'delayed' incubators initiated incubation 24 days later than 'normal' incubators, suggesting a seasonal decline in breeding success in Marbled Murrelets. Thus, while capturing murrelets sometimes affected individual breeding status, later breeders were affected more than earlier breeders. Because the murrelet's breeding season is so long (see chapter 1), early breeders have plenty of time to renest or lay a replacement egg if nesting is disturbed. This finding suggests that researchers should capture Marbled Murrelets early in the breeding season, to minimize possible capture effects on nesting murrelets.

It is possible that a certain percentage of early breeding failure is common in this study species and/or study area, but this can not be measured unless birds are marked and tracked, at which point the study methods become suspect. It is likewise possible that temporary disturbance of breeding has no effect on lifetime reproductive success or survival of individuals (Nisbet 2000). While our study has suggested an early date as the most significant factor determining a successful start to incubation, we still do not know enough about reproductive potential or the annual contribution by indivuduals to the productivity of Marbled Murrelet populations. Because studies of disturbance may overstate the significance of human disturbance, managers may unnecessarily restrict the activities of researchers (Nisbet 2000). Thus, I stress that researchers need a more thorough understanding of (1) processes that influence inter-annual variation in

productivity; (2) recruitment age and delayed maturity in Marbled Murrelets to fully understand the age structure of the population; and (3) the subsequent dynamics of investigator-induced disturbance according to age and date.

The knowledge gained by the research in this thesis challenges some general assumptions often made by researchers about influence of scientific methods on results (Kinkel 1989). Specifically, this applies to the presumed randomness of capture techniques, the use of physiological and morphological markers of reproductive status without confirmation of that status, and the neglect of assessing the impact of research methods (capture and attachment of instruments; Culik *et al.* 1994) on the conclusions drawn from the study.

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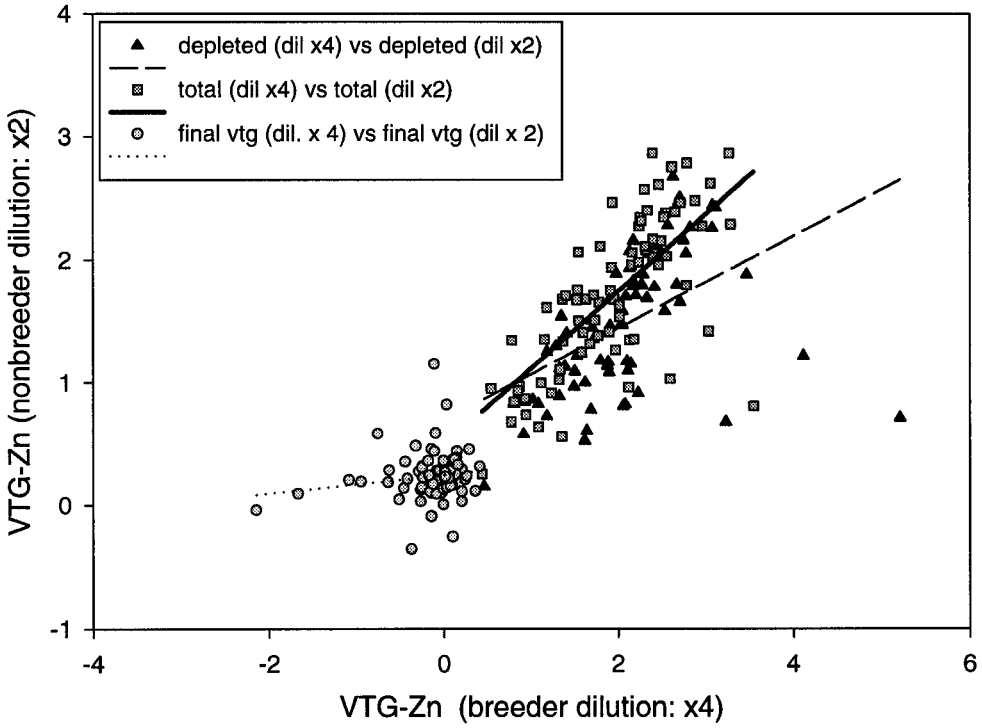
Appendix 1.1. Methodological assessment of VTG analyses: Results of plasma Zn assays at breeder dilution (x4) and nonbreeder dilution (x2). The sample plasma is used for each dilution. Data are in (ug/ml) $\pm$ SD.

	Average Total Plasma Zinc	Depleted (albumen-bound) Zinc	Final Vitellogenic Zinc
x4 Dilution (n=76)	1.94 $\pm$ 0.70	2.08 $\pm$0.758	-0.14 $\pm$ 0.40
x2 Dilution (n=76)	1.70$\pm$ 0.63	1.47 $\pm$ 0.57	0.23$\pm$ 0.20
Pearson Correlation	<0.0001	<0.0001	0.204

Appendix 1.2. Methodological assessment of VTG analyses: VTG-Zn analyzed using nonbreeder dilution (x2) vs. VTG-Zn analyzed using breeder dilution (x4).



Regression line for 'Total' values p=<0.0001, r^2=0.490; for 'depleted' values p=0.0001, r^2=0.252. Regression equations: ----- total plasma Zn= 0.4837 + 0.6297(x), - - - - depleted plasma Zn = 0.6909 + 0.3761(x).

Appendix 1.3. Methodological assessment of VTG analyses: Plasma VTG-Zn (ug/ml) in non-egg-producing females (after July 24) (n=41) and in males (n=103). Data are $\mu \pm$ SE.

Non-Laying Females		Males	
$\mu \pm$ SE	+3 SD	$\mu \pm$ SE	+3 SD
0.296 ± 0.03	0.90	0.26 ± 0.03	0.96

Appendix 1.4. Methodological assessment of VTG analyses: Decrease in detectable vitellogenin in plasma with time and freezing in egg-producing birds. Data are ± S.E. 5 month and 18 month total and final VTG-Zn are significantly different, but depleted Zn does not differ.

Approximate time in freezer	Avg. total Zn	Avg. depleted Zn	Avg. final VTG-Zn
5 months (n=14)	7.03 ± 0.40	1.77 ± 0.15	5.26 ± 0.35
18 months (n=14)	4.63 ± 0.39	1.79 ± 0.13	2.85 ± 0.47
Pearson Correlation	0.460	0.336	0.338
Mann-Whitney test	p=0.001	p=0.84	p=0.001

Appendix 3.1. Aikike's Information Criteria (AIC) was used to determine which independent variables (plus interactions) best described the variation in corticosterone. This table presents the variables selected using a stepwise AIC procedure. After the final selection, the variables were given p values using a general linear model. Because the resulting model (seen below) was too complex to represent graphically, data (in 'results' section) were explored for trends using simpler comparisons. However, the interpretation of data was made easier by an understanding of which variables interacted (denoted below by *) to explain variation in corticosterone.

Source	DF	Type III SS	F value	Pr>F
time	1	9649.46	24.43	<.0001
date	1	11438.48	28.96	<.0001
year	1	3377.21	8.55	0.0042
bp	1	1602.91	4.06	0.0463
mass	1	14978.18	37.92	<.0001
sex	1	11278.33	28.55	<.0001
$time^2$	1	5420.36	13.72	0.0003
$mass^2$	1	16807.42	42.55	<.0001
date*bp	1	787.07	1.99	0.1608
mass*sex	1	12659.92	32.05	<.0001
date*mass	1	13167.27	33.33	<.0001
date*sex	1	9000.61	22.79	<.0001
$mass^2$*sex	1	14224.12	36.01	<.0001
date*$mass^2$*sex	2	16942.75	21.45	<.0001
date*mass*sex	1	10292.64	26.06	<.0001