HEALTH ASSESSMENTS AND STRESS RESPONSE OF XANTUS'S MURRELETS TO CAPTURE, HANDLING AND RADIO-MARKING

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SUMMARY

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Physiologic health of Xantus's Murrelets *Synthliboramphus hypoleucus,* a seabird of conservation concern in the United States and Mexico, has received little attention. During 1994–1997, we collected blood samples from murrelets attending nocturnal at-sea congregations adjacent to Santa Barbara Island, California, to establish baseline health indices. Hematologic and serum biochemistry indices were similar to those in other small alcids, except for lower creatine kinase activity, higher lactate dehydrogenase activity and higher uric acid concentration. To determine whether minimally invasive procedures for capture, handling and radio-marking pose a health risk to murrelets, we also assessed the acute stress response. Serum corticosterone, white blood cell counts (heterophils, lymphocytes, monocytes, eosinophils and basophils), and heterophil:lymphocyte ratio (H:L ratio) were used as biochemical and cellular indicators of stress. Corticosterone levels measured 30 minutes after capture (49 ± 22 ng/dL) were significantly higher than those measured within 3 minutes of capture (22 ± 21 ng/dL), indicating a stress response. However, at 30 minutes after capture, neither handling (before blood collection) nor sedation with isoflurane gas and radio-marking (subcutaneous anchor method) resulted in significantly higher mean corticosterone levels, indicating that the stress response was not greater in magnitude because of these additional procedures. White blood cell counts did not differ between any of the 30-minute study groups, but heterophil counts were higher, lymphocyte counts lower, and the H:L ratio higher for birds that were sedated and radio-marked. The magnitude of the stress response was not excessive relative to other handling protocols and probably did not cause changes to bird behavior after release.

Key words: Xantus's Murrelet, blood, health, stress, hematology, biochemistry, corticosterone, heterophil, telemetry, radio-marking, *Synthliboramphus hypoleucus*

INTRODUCTION

The Xantus's Murrelet (*Synthliboramphus hypoleucus*) is a small diving seabird (family Alcidae) that spends most of the year at sea and visits land only for a few months to breed (Murray *et al.* 1983, Drost & Lewis 1995). It has a relatively small global population and a geographically restricted breeding range in southern California, USA, and northwestern Baja California, Mexico. Populations appear to have declined considerably at several colonies since the late 19th century mainly because of predation and habitat degradation from introduced mammals, but marine threats (e.g. oil spills and light pollution) probably have seriously affected some colonies (McChesney & Tershy 1998, Carter *et al.* 2000, Burkett *et al.* 2003). World population estimates range from 10 000 to 20 000 breeding individuals (Drost & Lewis 1995, Carter *et al.* 2000, Burkett et al. 2003, Karnovsky *et al.* 2005, Keitt 2005), but potential threats from changes in local oceanographic conditions and prey availability, global climate change, and diseases have not been well described.

To better understand at-sea foraging and colony attendance, at-sea captures and two major radio-telemetry studies were conducted at Santa Barbara and Anacapa Islands, California (Whitworth *et al.* 1997, 2000a, 2003; Newman *et al.* 1999; Hamilton *et al.* 2005). During 1994–1997, we captured Xantus's Murrelets from at-sea congregations on the water beside Santa Barbara Island at night. Blood samples were collected

- to establish baseline health reference ranges (hematology and biochemistry) for Xantus's Murrelets, a species for which no such data exist and a species at risk of exposure to oil spills and possibly requiring biomedical care in the future.
- to determine the sex of individuals for telemetry studies.
- to obtain genetic material for assessment of genetic diversity.

We also gathered additional blood samples so that we could use blood corticosterone concentration, white blood cell estimates, and heterophil:lymphocyte ratios to evaluate acute stress response to various handling protocols. These techniques have been useful in

evaluating stress in other birds and mammals (Beuving & Vonder 1978; Harvey *et al.* 1980, 1984; Gross & Siegel 1983; Jain 1993; Maxwell 1993; Rijnberk & Mol 1997; Wingfield *et al.* 1997). In this paper, we report results from the health assessments and the acute stress response of Xantus's Murrelets to various handling protocols.

METHODS

Blood samples

We captured Xantus's Murrelets between 22h00 and 05h30 (PDT) off Santa Barbara Island, the largest breeding colony in the Southern California Bight (Drost & Lewis 1995), using the night-lighting technique (Whitworth *et al.* 1997). Birds (one or occasionally two at a time) were captured with a dip net and individually placed in cardboard holding boxes. Time of capture was recorded for every bird to the nearest minute. Before blood collection, all birds were evaluated for evidence of external trauma, physical impairments, emaciation or disease (e.g. oral or nasal discharge, respiratory compromise, infection, fecal discoloration) and for normal mucosal perfusion and handling response.

Blood samples (0.5–1 mL) were collected aseptically by a trained veterinarian (SHN) from the metatarsal vein using a 25-gauge needle and 3 cc syringe. After blood collection, a gauze sponge was used to apply pressure to the venipuncture site until hemostasis was complete. Immediately after blood collection, two blood smears were made using standard protocols (Jain 1986) and two Pre-Cal Microhematocrit tubes (Becton Dickinson, Rutherford, NJ, USA) were filled and clay capped for hematocrit and total solid determinations (Jain 1986). The remainder of the blood was placed in a Microtainer serum separator tube (Becton Dickinson) and centrifuged for 15 minutes at 3500 rpm using a Triac Centrifuge (Clay Adams, Sparks, MD, USA) to separate the serum from the cellular fraction. Disposable polyethylene pipettes were used to pipette serum into 1.5 mL cryovials (Out Patient Services, Petaluma, CA, USA). Samples were placed in liquid nitrogen until frozen and then stored in a –80°C freezer until analyzed.

Health assessments

Blood for hematology and serum biochemistry testing was collected during 6–11 May 1994, 26–29 April 1995, and 20–21 May 1995. Analyses were performed at Consolidated Veterinary Diagnostics Incorporated (CVD Inc., West Sacramento, CA, USA). White blood cell (WBC) estimates and differential WBC counts (heterophils, lymphocytes, monocytes, eosinophils and basophils) were performed from Wright–Giemsa-stained blood smears. White blood cells were counted in an area on the smear where red blood cells (RBCs) were adjacent to one another (membranes touching but not overlapping). The average number of WBCs in ten high-power microscope fields (40×) was multiplied by 2000 to obtain the WBC estimate. Differential WBC counts were determined by counting 200 WBCs (at 40×) and multiplying the percentage of a particular cell type by the overall WBC estimate. Blood smears were also examined for RBC morphology, thrombocytes, reticulocytes and RBC parasites. Packed cell volume (PCV) and buffy coat were determined by microhematocrit centrifugation (Jain 1986). Total solids (TS) of plasma from centrifuged microhematocrit tubes were measured using a handheld temperature-regulated Schuco clinical refractometer (American Calduceus Industries, Carle Place, New York, NY, USA).

Serum was analyzed using routine biochemical methods to determine activities of alkaline phosphatase (Alk Phos), aspartate amino transferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH). Concentrations of albumin, globulin, total protein (TP), cholesterol, blood urea nitrogen (BUN), glucose, Ca, inorganic P, total CO_2, Cl, K, Na and uric acid (UA) were also determined. Albumin:globulin ratio (A:G ratio) was calculated. Protein electrophoresis was conducted to determine concentrations of the following globulins (alpha 1 and 2, beta 1 and 2, gamma 1 and 2), albumin and pre-albumin.

Stress study groups

Blood was collected from birds for part of the stress study during 26–29 April 1995, 20–21 May 1995, 15–19 April 1996 and 13–17 May 1996. Birds were transported to the larger support vessel within 10 minutes of capture and were randomly assigned into one of three experimental groups. In Group 1 birds (n = 32), blood samples were collected (see "Methods") within 10 minutes of capture. Group 2 birds (n = 54) remained in the holding box until 30 minutes after capture before blood sampling. Group 3 birds (n = 47) were removed from the holding box after capture and "handled" [i.e. morphometric measurements (culmen, tarsus, mass), brood patch inspection, photographs or facial plumage inspection, and banding] for up to 10 minutes before blood sampling at 30 minutes after capture.

TABLE 1
Xantus's Murrelet study groups, sample sizes and blood tests performed

Group	Blood collection time	n	WBC & differential cell count[a]	Serum biochemistry	Corticosterone assay
1	Sample at less than 10 minutes	32	Yes	Yes	Yes
2	Sample at 30 minutes	54	Yes	Yes	Yes
3	Handle & sample at 30 minutes	47	Yes	No	Yes
4	Radio-mark & sample at 30 minutes	56	Yes	No	Yes
5	Sample at less than 3 minutes	28	No	No	Yes
6[b]	Sample at 60 minutes	28	No	No	Yes

[a] As part of the stress evaluation, heterophil:lymphocyte ratios were calculated in samples when WBC and differential cell counts were performed.

[b] Samples collected at 60 minutes are from the same birds sampled at less than 3 min. Because the sampling of individuals is repeated, statistical comparisons were not performed, and results are reported only for purposes of comparison.

WBC = white blood cell.

To obtain birds for two other study groups, other capture efforts took place during 10–12 April 1997 and 5–7 May 1997. Group 4 birds (n = 56) were radio-marked using subcutaneous anchor attachment under isoflurane sedation (Newman *et al.* 1999) before blood sampling 30 minutes after capture. Group 5 birds (n = 28) were transported to the support vessel within two minutes of capture for immediate blood sampling within three minutes of capture, and were returned to a cardboard pet carrier. At 60 minutes after capture, birds were removed from the box and an additional blood sample was collected (Group 6). Because group 6 birds represented a repeated sampling of individuals, statistical comparisons of blood results were not performed, but results are reported for comparative purposes. To prevent possible predation, all murrelets were released approximately 500–1000 m away from the island at the completion of all procedures.

TABLE 2

Baseline health (hematology, biochemistry and electrophoresis) reference intervals for Xantus's Murrelets at Santa Barbara Island, California, 1994/95

Analyte	n	Mean±SD	Range
PCV (%)	49	51±3	48–55
TS	49	3.8±0.7	1.9–5.2
WBCs (10^3/μL)	60	6274±1918	4300–9500
Heterophils (10^3/μL)	60	3272±1835	1169–5180
Lymphocytes (10^3/μL)	60	2543±1329	1300–3990
Monocytes (10^3/μL)	60	228±216	12–480
Eosinophils (10^3/μL)	60	0±0	0–0
Basophils (10^3/μL)	60	160±236	13–424
Na (mEq/L)	12	154±3	148–160
K (mEq/L)	13	6.3±2.0	3.2–10.2
Cl (mEq/L)	13	116±6	102–122
Calcium (mg/dL)	17	8.0±0.7	6.8–9.6
P (mg/dL)	17	3.6±1.9	0.6–7.0
Total CO_2 (mEq/L)	17	23±4	14–30
Alk Phos (IU/L)	26	48±48	5–165
AST (IU/L)	32	287±174	41–855
CK (IU/L)	31	46±45	15–348
LDH (IU/L)	31	396±232	112–839
Uric acid (mg/dL)	13	20.4±5.9	12.2–27.6
BUN (mg/dL)	26	3.6±0.9	1.2–5.8
Albumin (g/dL)	31	1.0±0.2	0.4–1.8
Total protein (g/dL)	32	3.2±0.4	2.2–4.7
Globulin (g/dL)	32	2.2±0.4	1.4–3.3
A:G ratio	32	0.5±0.2	0.3–0.9
Glucose (mg/dL)	32	374±85	158–564
Cholesterol (mg/dL)	31	288±50	160–351
Alpha 1 globulin (g/dL)	10	0.6±0.3	0.2–1.0
Alpha 2 globulin (g/dL)	10	0.1±0.2	0.0–0.5
Beta 1 globulin (g/dL)	8	0.4±0.15	0.2–0.6
Beta 2 globulin (g/dL)	9	0.0±0.1	0.0–0.2
Gamma 1 globulin (g/dL)	10	0.6±0.25	0.2–1.0
Gamma 2 globulin (g/dL)	10	0.0±0.0	0.0–0.0
Albumin (g/dL)	10	2.1±0.35	1.6–2.6
Pre-albumin (g/dL)	10	0.1±0.05	0.0–0.1
Mass (g)	35	168±11	150–186

PCV = packed cell volume; TS = total plasma solids; WBCs = white blood cells; Alk Phos = alkaline phosphatase; AST = aspartate amino transferase; CK = creatine kinase; LDH = lactate dehydrogenase; BUN = blood urea nitrogen; A:G = albumin:globulin.

Stress parameters

Corticosterone concentrations were determined using the ImmuChem Corticosterone ^{125}I radioimmunoassay (Cat. 07–120102: ICN Biomedicals, Costa Mesa, CA, USA), which has been validated for accuracy and precision in avian samples (Spano *et al.* 1987, Vleck *et al.* 2000). All corticosterone assays were performed in duplicate; if paired results differed by more than 10%, the assay was repeated. Differential cell counts [heterophil (H), lymphocyte (L), monocyte, eosinophil and basophil] were performed, and the H:L ratio was calculated. Because 1995 and 1996 WBC estimates did not differ among study groups, this test was not performed on samples collected in 1997 (Table 1).

Statistical analyses

Descriptive statistics were performed using the BMDP Statistical Software (Los Angeles, CA, USA). Outliers were identified and removed using the range test (PetitClerc & Kelly 1981, Solberg 1994). Individual serum biochemical values were removed from the data set if the difference between the two highest (or lowest) values in the distribution exceeded one third of the range of all values. Hematologic intervals were established using the same method; however, individual differential WBC counts were not removed from the data set unless the overall WBC estimate for the individual bird was determined to be an outlier. Kruskal–Wallis analysis of variance and a modified Mann–Whitney rank sum test (Hollander & Wolfe 1973) were performed to determine whether statistical differences ($P < 0.05$) existed between groups 1–5.

TABLE 3

Mean corticosterone concentration (ng/dL) in Xantus's Murrelet study groups

Venipuncture time	Study group	n	Mean±SD	Range
Sample at less than 3 minutes	5	28	22±21[a]	4–83
Sample at less than 10 minutes	1	32	33±12[a,b]	13–57
Sample at 60 minutes[d]	6	28	46±29	1–114
Sample at 30 minutes	2	54	49±22[b,c]	14–112
Handle & sample at 30 minutes	3	47	55±17[c]	23–09
Radio-mark & sample at 30 minutes	4	56	79±61[c]	20–269

[a,b,c] Means marked with a common superscript are not statistically different (Kruskal–Wallis ANOVA and modified Mann–Whitney *U*-test, $P < 0.05$).

[d] Samples collected at 60 minutes are from the same birds sampled at less than 3 minutes. Because sampling of individuals is repeated, statistical comparisons were not performed, and results are reported here only for purposes of comparison.

RESULTS

We calculated mean baseline health indices (hematology, biochemistry, electrophoresis and mass) from Xantus's Murrelets sampled in 1995 and 1996 (Table 2). Blood health indices indicate immune competence (WBC and cell counts, A:G ratio, globulin and gamma globulin concentrations), inflammation (WBC and cell counts, alpha and beta globulins), electrolyte and acid–base balance (Na, K, Ca, Cl, P, total CO_2), liver structure and function (AST, LDH, albumin and TP), kidney structure and function (AST, BUN, UA), muscle exertion or damage (Alk Phos, AST, LDH, CK), and nutritional status (cholesterol, glucose, TP).

Statistical differences ($P < 0.05$) the groups were identified for corticosterone concentration (Table 3). Lowest mean corticosterone concentration was measured from birds sampled within three minutes of capture (group 5), differing from all other study groups except for birds sampled 10 minutes after capture (group 1). Highest mean corticosterone concentration was found in birds that were sedated, radio-marked and sampled at 30 minutes (group 4), but significant differences were not found when those birds were compared to others sampled at 30 minutes regardless of handling procedures (groups 2 and 3).

Hematologic results that varied between the groups included relative frequencies of heterophils, lymphocytes, basophils and TS. Birds that were sedated, radio-marked, and sampled at 30 minutes (group 4) had significantly higher heterophil counts ($P < 0.05$; Table 4) and lower lymphocyte counts (and therefore a higher calculated H:L ratio, 3.79) than did

- group 1 birds sampled less than 10 minutes after capture (H:L ratio: 1.11).
- group 2 birds sampled at 30 minutes (H:L ratio:1.34).
- group 3 birds handled and sampled 30 minutes after capture (H:L ratio: 1.91).

Group 4 birds also had a significantly higher basophil count than did other study groups. No differences were measured for monocyte counts, eosinophil counts or PCV.

DISCUSSION

Health assessments

One of the greatest threats to the health of marine birds is oil pollution, and increased efforts to rehabilitate oiled wildlife effectively have been a major reason for recent interest in establishing baseline blood health intervals. Because Xantus's Murrelets are highly vulnerable to oil pollution in the Southern California Bight and off central California (Carter *et al.* 2000, Carter 2003), they may require biomedical care in the future because of petroleum exposure or other environmental contaminants. They also may fall ill because of diseases or algal blooms. Baseline blood reference intervals will serve as the health standard for determining how ill birds are while in care and when they will be healthy enough for release (Newman & Zinkl 1998, Mazet *et al.* 2002, Newman *et al.* 2003).

TABLE 4
Hematology (mean ± standard deviation) of Xantus's Murrelets at Santa Barbara Island, California, 1994/95

Analyte	Group 1 Sample at less than 10 min	Group 2 Sample at 30 min	Group 3 Handle & sample at 30 min	Group 4 Radio-mark & sample at 30 min
WBCs ($10^3/\mu L$)	6824±1996[a]	6134±1835[a]	7028±2396[a]	6377±2490[a]
	(n=34)	(n=53)	(n=45)	(n=57)
Heterophils (cells/μL)	3383±1897[a]	3312±1522[a]	4237±1787[a,b]	4604±2122[b]
	(n=34)	(n=53)	(n=45)	(n=56)
Lymphocytes (cells/μL)	3047±1350[a]	2467±1193[a,b]	2217±1342[b]	1214±1077[c]
	(n=34)	(n=53)	(n=45)	(n=56)
Monocytes (cells/μL)	330±236[a]	212±184[a]	275±254[a]	314±318[a]
	(n=34)	(n=53)	(n=45)	(n=56)
Eosinophils (cells/μL)	2±10[a]	0[a]	0[a]	3±20[a]
	(n=34)	(n=53)	(n=45)	(n=56)
Basophils (cells/μL)	62±158[a]	136±218[a]	295±342[a]	328±113[a]
	(n=34)	(n=53)	(n=45)	(n=56)
PCV (%)	51±4[a]	50±4[a]	49±4[a]	50±6[a]
	(n=28)	(n=32)	(n=24)	(n=52)
Field TS (g/dL)	3.8±0.7[a]	4.0±0.7[a,b]	4.1±0.9[a,b]	4.4±1.1[b]
	(n=28)	(n=32)	(n=24)	(n=52)

[a,b,c] Means marked with a common superscript in the same row are not statistically different (Kruskal–Wallis ANOVA and modified Mann–Whitney *U*-test, $P \leq 0.05$)
WBCs = white blood cells; PCV = packed cell volume; TS = total plasma solids.

Diseases such as avian botulism, West Nile virus, and exotic Newcastle disease have been documented in shorebirds and marine birds in southern California (Reece 1989, Docherty & Friend 1999, Hansen 1999, Rocke & Friend 1999) and also pose a real threat to the health of Xantus's Murrelets. The recent emergence of the highly pathogenic H5N1 avian influenza has further increased concern about the effects that diseases may have on free-ranging bird populations such as Xantus's Murrelets. From this perspective, baseline health intervals also will serve as a valuable tool to determine whether rehabilitation is an option or whether euthanasia is more humane. As better knowledge of the small population size of Xantus's Murrelets at specific colonies is gained, it may be extremely important to treat individuals if they represent a unique genetic component of the species. In this case, baseline health intervals will be invaluable.

Baseline health indices have been established for many avian species, but information on diving marine birds, particularly alcids, is lacking because of the difficulty of capturing and sampling these species. Fortunately, some comparative data are available for other small alcids, including Marbled Murrelets *Brachyramphus marmoratus* and Cassin's Auklets *Ptychoramphus aleuticus* (Newman *et al.* 1997, Newman & Zinkl 1998). Xantus's Murrelets have generally similar reference-range blood results to those of Marbled Murrelets and Cassin's Auklets with the following exceptions:

- Xantus's Murrelets had lower CK activity than both Marbled Murrelets and Cassin's Auklets.
- Xantus's Murrelets had higher LDH activity level than Marbled Murrelets.

CK and LDH both become elevated with muscle contraction and exertion or with physical struggling, and higher LDH activity in Xantus's Murrelets may indicate that this enzyme is a more sensitive indicator of physical exertion for this species. Based on LDH alone, our results suggest that Xantus's Murrelets undergo a greater level of struggling during capture by night-lighting than either Marbled Murrelets captured by night-lighting or Cassin's Auklets captured by hand or mist net.

Xantus's Murrelet hematology and serum biochemistry reference intervals were established from birds sampled in 1994 and 1995, the latter being a poor year for food availability, which led to colony abandonment (Whitworth *et al.* 2000a, Roth *et al.* 2005, Schwemm *et al.* 2005). Although food resources were not adequate near Santa Barbara Island for successful reproduction by many birds in 1995, Xantus's Murrelets preserved their own health by maintaining physiologic homeostasis, apparently by foraging very far from the colony and greatly reducing colony attendance by abandoning incubation duties. If birds were undergoing emaciation and muscle catabolism associated with starvation and ecologic conditions, one would expect both CK and LDH to be elevated, and other blood chemistry changes to be apparent, but this was not the case. Although sample sizes were small in both 1994 and 1995, no noticeable differences in blood results were observed between years, and the birds were considered healthy in both years. In 1996 and 1997, when additional samples were collected for the stress study, breeding success and prey availability were much improved (Whitworth *et al.* 2000a, Roth *et al.* 2005, Schwemm *et al.* 2005).

Stress response

Stress is defined as the physiologic response to stimuli perceived as a threat (real or anticipated). The stress response is manifested through physical, behavioral and physiologic changes, including increased heart and respiratory rates and nearly simultaneous release of corticosterone and epinephrine (Harvey *et al.* 1984). Acute stress results in corticosterone release mediated through activation of the hypothalamic-pituitary–adrenal axis (Harvey *et al.* 1984). Avian blood samples collected less than three minutes after capture (group 5) represent background corticosterone levels for unstressed conditions (Beuving & Vonder 1978, Schoech *et al.* 1998, Wingfield & Romero 2000, Romero & Romero 2002). Corticosterone concentration in blood is believed to rise continuously until it reaches a plateau, at which time the animal either escapes from the stressful situation, adapts to the stressor, suffers adrenal exhaustion or undergoes pathologic changes that can result in death (Harvey *et al.* 1984, Smith *et al.* 1994, Rijnberk & Mol 1997). The rise in corticosterone associated with handling is routinely used to assess the magnitude of the stress response, with more stressful situations causing a greater rise in corticosterone (Silverin 1998). Concurrent with adrenal response in birds is a cellular response in which heterophil counts increase and lymphocyte numbers decrease (Maxwell 1993).

Mean corticosterone concentration from Xantus's Murrelets in this study revealed that the stress response was initiated within 10 minutes of capture, but mean corticosterone concentration was not significantly higher than background level (under three minutes) until 30 minutes after capture (Table 3). At 60 minutes, mean corticosterone concentration was slightly lower than at 30 minutes. This observation suggests that the capture-associated stress response peaked between 10 and 30 minutes, and reached a plateau between 30 and 60 minutes when birds behaviorally and physiologically adapted to being captured. Without ongoing or additional stressful stimuli, Xantus's Murrelets showed no additional increase in corticosterone levels, suggesting that murrelets could continue to be held under appropriate conditions [i.e. in the dark in pet carriers, with little exposure to auditory or visual stimuli, and at moderate temperatures (10°C–15°C) without a subsequent increase in stress response]. However, to ensure that murrelets were not affected, we did not examine the stress response beyond 30 minutes for birds that underwent handling and procedures.

Corticosterone levels measured from all study groups bled at 30 minutes after capture (Table 3) suggested that neither handling birds before blood collection, nor sedating them with isoflurane gas and radio-marking them with subcutaneous anchors resulted in significantly higher mean corticosterone concentrations than those seen in birds kept in holding boxes until sampling at 30 minutes. However, a cellular response was detected as heterophils increased and lymphocytes decreased in association with sedation and subcutaneous anchor radio-marking. This observation demonstrated that the H:L ratio was a sensitive marker of cellular change associated with handling procedures in Xantus's Murrelets.

The subcutaneous anchor attachment method (Newman *et al.* 1999) is commonly used to evaluate habitat use and movements of small alcids (Whitworth *et al.* 1999, 2000a, 2000b; McFarlane Tranquilla *et al.* 2003; Ackerman *et al.* 2004; Adams *et al.* 2004; Peery *et al.* 2004) and to conduct post-release survival studies on oiled and rehabilitated seabirds (Anderson *et al.* 2000, Golightly *et al.* 2002). In most cases, this technique has not been considered to have had significant short- or long-term behavioral or physiologic effects on birds, but this conclusion has been difficult to prove. In our study, although radio-marked Xantus's Murrelets (group 4) had the highest mean corticosterone concentration, the level was not statistically

different from that of all other birds sampled at 30 minutes after capture. This finding suggests that the stress associated with sedation and subcutaneous anchor attachment was not greater than the stress associated with being captured, handled and blood-sampled at 30 minutes. In other radio-marked bird species, the stress response also has been found to be limited. For instance, fecal glucocorticoid levels of Dickcissels (*Spiza americana*) were elevated for only 24 hours after harness radio-marking (Suedkamp Wells *et al.* 2003).

Sedation using isoflurane inhalant anesthetic probably reduced the pain and stress associated with radio attachment using subcutaneous anchors and may even reduce the stress associated with capture and handling. Heatley *et al.* (2000) found that corticosterone concentrations in manually restrained Amazon Parrots *Amazona ventralis* were significantly higher than for birds anesthetized using isoflurane. Once sedated, the "perceived threat" that regulates corticosterone levels is impeded and, if stress occurs, sedation can be important in preventing resulting effects.

Some debate continues about the degree of behavioral effects associated with the subcutaneous anchor radio attachment technique (McFarlane Tranquilla *et al.* 2003, Ackerman *et al.* 2004, Hamel *et al.* 2004, Peery *et al.* 2004). For Xantus's Murrelets, the magnitude of the immediate stress response associated with this procedure was not found to be extreme. In fact, transitory corticosterone levels observed in Xantus's Murrelets undergoing radio-marking were comparable to those in Black-legged Kittiwakes *Rissa tridactyla* during chick rearing (Kitaysky *et al.* 1999). Although differences in stress response between species can be expected, it is highly unlikely that the stress response associated with a once-per-life event (e.g. subcutaneous anchor radio-marking) could result in a life-threatening outcome. These levels of stress can be experienced by birds annually for months during periods of low prey availability, although such conditions typically develop gradually.

Because handled Xantus's Murrelets had only a slightly higher mean corticosterone level than non-handled birds and because the stress response did not continue to rise between 30 and 60 minutes after capture, birds apparently did not experience an overwhelmingly detrimental stress response associated with these techniques. Based on the stress-mediated corticosterone response, our handling protocols did not pose any immediate danger to the health of Xantus's Murrelets and can be safely used in future studies. However, possible behavioral effects may occur from various other sources (e.g. physical effects of radio attachment on flying or diving, temporary changes in waterproofing, and brief disruption of social activity).

CONCLUDING REMARK

Although baseline health indices have been established for Xantus's Murrelets at Santa Barbara Island, California, we recommend that additional baseline health assessments be conducted at other major colonies in southern California, USA, and northwestern Baja California, Mexico. Health and stress issues can vary between colonies, and establishing baseline information for each colony will allow detection of subtle differences and changes in health before catastrophic mortality conditions occur—especially oil spills. With the potential emergence of avian influenza in North America, disease screening should also become a standard part of future health assessments. Such research will promote a greater understanding of how diseases and other stressors affect the health of Xantus's Murrelets throughout their breeding range.

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