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Potential Role of Environmental Contaminants in the Pathology of Beak Deformities among Black-capped Chickadees in South-central Alaska[1]

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ABSTRACT.—More than 1,400 individual Black-capped Chickadees (*Poecile atricapillus*) with beak deformities were recorded in south-central Alaska between 1991 and 2005. Over 200 individuals of 27 other species of birds were also recorded in Alaska with similar beak deformities. Affected birds included 17 resident species and 11 migrants. Beak deformities among chickadees generally involved overgrowth of the keratin sheath of the maxilla, the mandible, or both, with variable amounts of curvature and crossing. The keratin was often thickened with irregular growth ridges or flaking. In one case the underlying bone was notably deformed, being strongly curved laterally from the base of the beak. Some chickadees with beak deformities also had scaly legs or patches of dry or reddened skin or missing feathers, mostly in the head or neck region.

Five deformed adult chickadees were sent to the USGS National Wildlife Health Center for necropsy, histopathological examination, and viral testing in comparison to two normal adults. There was no evidence of disease, parasites, or chronic vitamin or mineral deficiency relating to the deformities. Two nestlings with leg deformities and one with a slightly overgrown beak were also sent in for examination. The two nestlings had folding leg fractures and all three showed epithelial disorganization in the overgrown beak with possible inclusions of excess keratin. No cause of the deformities was determined. Analysis of blood samples using flow cytometry suggested that adults with deformed beaks had higher rates of chromosomal damage than adults with normal beaks.

In 1999 a study was initiated to determine the prevalence of beak deformities among Black-capped Chickadees in south-central Alaska, determine at what age the deformities were developing, and investigate contaminants as a potential cause of the deformities.

From 1999–2005 chickadees were captured and banded at three to five sites in the Anchorage and Mat-Su Valley region every two months between September and April. From 2000–2004 about 500 nest boxes were erected in parks and residential areas within the region and were monitored to estimate prevalence of beak deformities among breeding adults, examine nestlings for evidence of deformities, and determine the effects of beak deformities on reproductive success.

Among 1,820 12-day-old nestling chickadees examined, only 0.05% had a crossed beak; an additional 0.6% had slight maxillary overgrowth of the keratin sheath. Among 2,186 hatching-year and after-hatching-year birds captured during all banding efforts, 8.1% had moderate to severe beak deformities; an additional 2.3% had possible incipient deformities. Prevalence varied seasonally, with lowest rates following the coldest winter months, and overall prevalence increased annually over the five years. Of the 178 deformed birds captured, 46% were male and 54% were female, which were not significantly different from the proportions of normal male and female chickadees captured. The youngest bird captured with a beak deformity was about six months old. Fifty-four cases were documented in which a chickadee that was first captured with an apparently normal beak was subsequently recaptured with a deformed beak. For some chickadees the deformities developed during their first year of life; others were known to be at least two years old. The probability of developing a deformity appeared greatest in late winter. Six cases were documented in which a chickadee with a beak deformity was subsequently recaptured with an apparently normal beak.

Reproductive success of Black-capped Chickadees in south-central Alaska was similar to that documented within other portions of their breeding range, but there were significant differences between normal and deformed birds. Clutch sizes averaged 7.8 $\pm$ 0.08 eggs across all pairs, but clutches tended by deformed males were significantly larger than those of pairs in which both adults were normal or the female was deformed. Genetic analysis of parentage showed that deformed males were cuckolded more often than normal males and also had more eggs dumped in their nests by unrelated females.

Almost all nests of normal pairs (96%) hatched at least one egg; among pairs in which the female was deformed, only 86% hatched any eggs. In nests that did hatch, the proportion of eggs hatching was also lower for deformed females than normal females. Several deformed females exhibited aberrant incubation behavior, with eggs strewn erratically across the nest, which may have contributed to lower hatching success. About 94% of all pairs with broods raised at least one young to fledging. Among these, broods tended by deformed males had proportionately fewer young fledge than broods tended by pairs in which both adults were normal or the female was deformed. Deformed males likely had more difficulty procuring enough food for young.

Thirty-six adult chickadees, 49 nestlings, and 39 eggs were screened for metals, trace elements, and organochlorine compounds to determine if there was an association between beak deformities or hatchability and any of the contaminants. Selenium, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) were of particular interest because they were known to have caused congenital beak malformations in other species of birds. γ-

Hexachlorocyclohexane (HCH–lindane) was also of interest because of its use to control spruce beetles during a recent epidemic in south-central Alaska. Contaminant concentrations were compared between normal and deformed adults; normal adults and nestlings; nestlings and eggs from nests of normal and deformed adults; and nestlings and eggs from nests in which all eggs hatched vs. those in which at least one egg failed to hatch. Separate comparisons involving eggs and chicks were made for nests in which the female parent was deformed and those in which either parent was deformed to test for trans-generational and environmental effects, respectively.

Concentrations of heavy metals, other potentially toxic elements, and methylmercury were all below levels of concern. There was no association between selenium, any other element, or methylmercury and the presence or absence of beak deformities in adult chickadees. Concentrations in nestlings were not related to hatchability of the clutch or to the presence or absence of a deformity in a parent.

Organochlorine pesticide residues most frequently detected in tissues of adults, nestlings, and eggs were DDT and its metabolites; hexachlorobenzene (HCB); dieldrin; heptachlor epoxide; and *trans*-nonachlor. γ-HCH (lindane) was rarely detected. PCBs, including the highly toxic, coplanar congeners, were detected in almost every bird and egg tested. Concentrations of the coplanar congener PCB 123 and of heptachlor epoxide were significantly higher in adults with beak deformities than in normal adults. Concentrations of total PCBs, HCB, and metabolites of DDT were all higher in eggs from clutches with eggs that failed to hatch than in those from clutches in which all hatched.

Compared to nestlings with normal parents, those with deformed parents had higher concentrations of PCB 126 and PCB 77, two of the most toxic congeners.

Laboratory detection limits for PCDDs and PCDFs were too high to test for meaningful differences between normal and deformed adults. Concentrations of these highly toxic compounds in eggs and nestlings were not associated with hatchability of clutches. Concentrations of 1,2,3,4,6,7,8-HpCDD were higher in eggs of normal parents than those of deformed parents for no explainable reason, but high rates of extra-pair paternity and egg-dumping made trans-generational comparisons suspect.

Eighteen samples of sunflower seeds, commonly eaten by chickadees at residential feeders, were tested for contaminants. None had detectable concentrations of 24 organophosphate or 6 carbamate pesticides. Concentrations of most organochlorines were very low, with HCB being the most commonly detected. All 19 metals and trace elements except mercury had detectable concentrations. A few samples had high concentrations of arsenic, selenium, and lead. Chickadees may be ingesting HCB and lead from sunflower seeds but are more likely accumulating PCBs from other sources.

There was no support for selenium as the cause for beak deformities among chickadees. We propose to test the hypothesis experimentally that low levels of PCBs contribute to beak deformities. PCBs were ubiquitous in chickadee eggs, nestlings, and adults, and were correlated with beak deformities in adults and with decreased hatchability. Deprivation of sunlight and reliance on a calcium-deficient seed diet during winter may trigger development of beak deformities among chickadees exposed *in ovo* to low concentrations of PCBs or to the PCDDs and PCDFs associated with them.

INTRODUCTION

Recently, large numbers of Black-capped Chickadees (*Poecile atricapillus*) and smaller numbers of many other species of birds have been observed with pronounced beak deformities in Alaska, primarily in the south-central region of the state. The etiology of these deformities is not yet understood. Beak deformities have been documented for many species of birds but the prevalence is generally rare, occurring in less than 0.5% of a normal, wild population (Pomeroy 1962, Hayes and Risebrough 1972, Craves 1994). Causes of beak deformities are difficult to determine but may be influenced by disease, parasites, blunt trauma, extreme heat, genetic abnormalities, or nutritional deficiencies (West 1959, Pomeroy 1962, Ritchie et al. 1994, Altman et al. 1997). Genetic mutations can be caused by environmental teratogens, and high frequencies of beak and feather defects have been associated with exposure to contaminants (Hays and Risebrough 1972, Gochfeld 1975, Sharp and Neil 1979).

Large clusters of birds with beak deformities rarely occur; those that have occurred have been associated with exposure to contaminants. Since the early 1970s, several species of colonial fish-eating birds breeding in the Great Lakes basin have exhibited low hatchability and high rates of congenital beak malformations, particularly crossed mandibles, as part of the Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS). Evidence suggests that GLEMEDS is caused by exposure of embryos to chick-edema active compounds, particularly polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs), which have been

discharged into rivers from industrial sources and then bioaccumulated in the food-chain of the Great Lakes (Gilbertson et al. 1991, Ludwig et al. 1996).

A large concentration of congenital beak malformations was also documented in central California during 1983–1985 among several species of aquatic birds that were exposed to high concentrations of selenium from subsurface agricultural drainage water (Ohlendorf et al. 1986a, b; Ohlendorf 1989; Hoffman et al. 1988). Embryonic mortality was extremely high and embryos often had multiple gross malformations of the beak, feet, brain, and eyes (Hoffman et al. 1988). Embryonic beak defects were usually characterized by incomplete development of the mandible and sometimes incomplete development of the maxilla as well. Some adults had abnormal feather development on the head and were sloughing off external layers of the rhamphotheca, the external keratin sheath covering the maxilla and mandible (Ohlendorf et al. 1988). In Alaska, high concentrations of selenium have been measured in several waterfowl species, including the threatened Spectacled Eider (*Somateria fisheri*) and Emperor Goose (*Chen canagica*) (Franson et al. 1999, Grand et al. 2002). Although populations of both species have been declining, deformities have not been documented for either species.

Beak deformities were also recently reported in 15 adult Willow Flycatchers (*Empidonax traillii*), with an estimated prevalence of 1.4%. This species is strongly associated with riparian habitats; selenium, mercury, or other agricultural chemicals accumulated through the aquatic food-chain are considered a possible cause of the deformities and tests are currently underway to determine the factors responsible (Sogge and Paxton 2000, Mora et al. 2003).

The Black-capped Chickadee, one of the most familiar and widespread songbirds across North America, is associated more with terrestrial than aquatic habitats, generally preferring deciduous and mixed deciduous/coniferous forests (Smith 1993). This species is a permanent year-round resident in Alaska; thus the factor causing the beak deformities, whether it be a disease organism, parasite, nutritional imbalance, or contaminant, should be found within this northern region.

The primary goal of this study was to determine whether exposure to contaminants is likely causing beak deformities in Black-capped Chickadees in Alaska. The specific objectives were to (1) estimate the prevalence and geographic distribution of beak deformities in the population; (2) document bioindicators of contaminant exposure (crossed beaks, malformed nestlings, poor hatching success, excessive rate of nest abandonment); (3) determine the life-stages at which beak deformities occur; (4) compare concentrations of metals, trace elements, and organochlorine compounds in adults with deformed and normal beaks; (5) compare concentrations of contaminants in eggs and nestlings relative to hatchability and to presence or absence of a deformity in a parent; (6) analyze potential food items for contaminant concentrations; and (7) determine distances individuals typically move during different life stages to gauge how local any exposure was likely to be. Because beak deformities have also been observed among Boreal Chickadees (*Poecile hudsonica*) and Red-breasted Nuthatches (*Sitta canadensis*), which co-occur with Black-capped Chickadees, we collected parallel information on these species. We concomitantly examined deformed chickadees for evidence of disease, parasites, and nutritional imbalances as alternative causes of the deformities.

BACKGROUND

The Black-capped Chickadee is a small, North American passerine that ranges across forested regions of Alaska, Canada, and the northern two-thirds of the contiguous United States (AOU 1998). Chickadees are resident throughout their range, although large numbers of individuals may move south in autumn during some irruption years (Elder and Zimmerman 1983, Smith 1989). Black-capped Chickadees are socially monogamous, with pair-bonds often persisting across several years; non-breeding flocks are highly structured and winter flock territories average 9.5–14.6 ha in size (Odum 1942, Glase 1973, Smith 1993). Distances between breeding sites and wintering territories were generally small (less than 2.4 km) for a small resident population in Wisconsin (Weise and Meyer 1979). Juvenile dispersal distances from natal sites to subsequent breeding locations were less than 12 km for this Wisconsin population (Weise and Meyer 1979) but a subordinate yearling female was observed 39 km from its natal site in Alberta, Canada (Desrochers et al. 1988).

Chickadees are primary cavity nesters, excavating holes predominantly in rotten wood of softwood trees (Smith 1991). Chickadees also roost at night or during cold periods in cavities or in dense vegetation of coniferous trees (Smith 1993). Natural foods taken by chickadees in winter include about half animal matter, mostly insects and spiders, and half plant matter, primarily seeds (Smith 1991). In residential areas, chickadees readily forage on whole black sunflower seeds, suet, and peanut butter at residential feeders. During the breeding season, the diet shifts markedly to 80–90% animal sources, primarily caterpillars and other larvae (Smith 1991), and chickadees

rarely frequent bird feeders. Chickadees forage regularly on trunks, branches, and leaves of deciduous and coniferous trees and rarely on the ground (Smith 1993).

Black-capped Chickadees have several adaptations for surviving the extreme cold and short photoperiod characteristic of winter at high latitudes. Chickadees often enter a state of regulated hypothermia at night (Chaplin 1974, 1976; Sharbaugh 2001), store and metabolize large amounts of fat daily (Chaplin 1974), and have a well developed spatial memory to relocate cached food (Hitchcock and Sherry 1990, Pravosudov and Lucas 2000, Pravosudov and Clayton 2002).

The average lifespan of Black-capped Chickadees has been estimated at 2.5 years and the current longevity record is 12 years 5 months (Smith 1991). No major outbreaks of any disease have been reported for the species although blood parasites (hematozoa) have been reported at low levels (Smith 1991). The availability of winter food may strongly influence overwinter survival, especially during periods of severe weather, and chickadees with access to supplemental feeding stations have higher survival rates (Brittingham and Temple 1988, Desrochers et al. 1988).

Prior to this study, there was one published record of a Black-capped Chickadee with a beak deformity (Kinch 1998). A single bird was observed at a feeder from November to December 1997 at Mountain Chutes Camp, Ontario, Canada. Its maxilla was about 2–3 times normal length and decurved; the lower mandible may have been slightly elongated but was not curved. The chickadee was noticeably aggressive towards other birds at the feeder and its body size appeared larger than normal. A few other published reports exist for individuals with similar beak deformities among closely related parids in

Europe, including the Blue Tit (*Parus caeruleus*; Pomeroy 1962, Carpenter 1986, Jebbett 1991), the Great Tit (*Parus major*; Pomeroy 1962), and the Willow Tit (*Parus montanus*, Magnusson 1978).

Only two birds had previously been documented from Alaska with beak deformities. One American Tree Sparrow (*Spizella arborea*) caged experimentally at high ambient temperatures developed an overgrown rhamphotheca (West 1959). A single White-winged Crossbill (*Loxia luecoptera*) was found dead with an extremely overgrown maxilla (West 1974). There was no apparent abnormality in the underlying bone and the deformity was thought to have been caused by unusually rapid growth of the rhamphotheca, the outer keratin sheath overlying the bone.

There have been no documented episodes of avian disease in Alaska within the past decade. One major environmental change of note within south-central Alaska has been a recent outbreak of spruce beetles (*Dendroctonus rufipennis*), which resulted in the loss of about 1.3 million ha of forest in the region between 1989 and 2000 (Ross et al. 2001, Wittwer 2001, Werner et al. *in press*). To protect white spruce (*Picea glauca*) from this insect in residential areas of the region, most commercial operators have used carbaryl, a carbamate insecticide highly effective in control of this beetle (Hastings et al. 2001), whereas many individual homeowners have sprayed trees with the moderately toxic and persistent organochlorine pesticide lindane (γ–hexachlorocyclohexane, γ–HCH; historically known as γ–BHC or benzene hexachloride).

Other potential sources of contaminants within south-central Alaska include two military installations in Anchorage (Fort Richardson and Elmendorf Air Force Base)

complete with Superfund sites; another private Superfund site on the banks of Ship Creek in Anchorage (Standard Steel); contaminated seed or suet from local suppliers; fungicides and insecticides used in agricultural areas of the region; fire-retardant chemicals that have been sprayed to control several very large fires in the affected region within the past decade; and a host of Alaska state toxic release sites.

Long-distance atmospheric transport could also be a significant source of contaminants within south-central Alaska. Persistent, semi-volatile organochlorine compounds, including agricultural pesticides and industrial pollutants such as PCBs, have been transported long distances through the atmosphere to remote regions of the earth (Blais et al. 1998). Atmospheric back-trajectory models showed significant transpacific transport corridors between east Asia and Yukon Territory, Canada, based on analysis of wind-field data (Bailey et al. 2000). Transport was strongest during winter, moderately strong during spring and fall, and weakest during summer; such transport was thought to be the major source of several organochlorine compounds modeled, including α-HCH, γ-HCH, and heptachlor epoxide (Bailey et al. 2000). Concentrations of such compounds are greater at high latitudes and in high mountains because of increased precipitation and 'cold condensation'—the increasing volatization in warmer locations and subsequent condensation in colder locations (Blais et al. 1998).

Black-capped Chickadees could be exposed to contaminants through direct ingestion of contaminated foods, natural or human-provided; by foraging on contaminated substrates, such as tree foliage or bark; through ingestion of chemicals during cavity excavation; or through inhalation of airborne contaminants. Chickadee embryos could be

exposed to contaminants deposited in eggs by females, with resulting teratogenic effects. Nestlings of the closely related Great Tit have been shown to accumulate high concentrations of organochlorine compounds that reflect both contamination from the mother (via the egg) and contamination from ingested foods (Winter and Streit 1992). The Great Tit has been used to monitor local levels of both organochlorine and heavy metal pollution in terrestrial environments because these parids accumulate contaminants, are ubiquitous, are fairly sedentary, and readily use nest boxes (Hogstad 2001, Dauwe et al. 2003, Veerle et al. 2004).

METHODS

FIELD METHODS

Cataloguing and mapping of observations.—To determine the numbers and locations of birds in Alaska with beak deformities or other abnormalities, we solicited observations from biologists and from the public through local media coverage and requests in newsletters of local Alaska Audubon Society chapters. To determine the number of beak deformities among Black-capped Chickadees outside of Alaska, we searched the published literature and sought observations through colleagues, national media coverage, collaboration with the Cornell FeederWatch Program, and monitoring of a variety of Internet list-servers used by banders, birders, and contaminants specialists.

We attempted to contact all observers reporting deformed birds personally via email or telephone. We requested the following information about each deformed bird: species, age or sex if known, detailed description and photographs of the deformity,

date(s) observed, location, behavior, and any foods eaten. We entered all observations into a relational database and geographic information system to produce maps of distribution. We examined records to determine which ones were likely resightings of the same individual birds, based on descriptions of the deformities and the locations and dates of the sightings. Total number of deformed birds observed excludes such probable resightings of the same individuals within a given year. Names of primary sites in Alaska where deformed birds were observed or sampled are shown in Figure 1.

Nest box study.—We conducted a study of chickadees and nuthatches breeding in nest boxes in south-central Alaska to examine nesting adults for deformities, monitor productivity, and monitor nestling development from 2000–2004. Nest boxes were constructed of untreated native white spruce (Figure 2); most were built by high school students in a cooperative education project at the King Career Center, a vocational school in the Anchorage School District. We attached all boxes except for those at a few at residences to paper birch (*Betula papyrifera*) trees, placed on the trunk in a randomly selected orientation and at a randomly selected height between 3 and 5 m. Nest boxes were filled with fresh spruce sawdust to a depth of about 8 cm when first erected and then refilled with fresh sawdust after all nest materials had been removed at the end of each field season.

We monitored a total of 496 nest boxes, including 9 commercially purchased cedar boxes that had been erected by homeowners prior to our study (Table 1). We placed 266 boxes on municipal and state park lands, 66 boxes on large tracts of privately owned rural property, and 164 boxes in yards of homeowners in residential areas. Among these, 289

boxes were in Anchorage and 207 boxes were in Eagle River and the Matanuska-Susitna (Mat-Su) Valley, areas in which large numbers of deformed birds had been reported. Boxes were spaced a minimum distance apart of 400 m on park lands, at a randomly selected distance of 10–30 m from the nearest trail. During 2000, most effort was concentrated in Anchorage; only eight boxes were monitored in Eagle River and none in the Mat-Su Valley due to staff limitations. All available boxes were monitored during 2001, but effort in Eagle River and the Mat-Su Valley was scaled back during subsequent years.

We checked nest boxes every 7–10 days from late March through late May to determine if boxes were occupied. Once nest-building had begun, we checked boxes every 2–4 days to determine date of initiation of egg-laying and final clutch size. We monitored nests generally every 4–6 days during incubation and every 1–2 days close to expected date of hatch to determine chronology and success. During the nestling period we measured, weighed, and examined nestlings for deformities on day 0 (date of hatch) or 1 and generally at day 4, 7, 10, 12, and 14. At day 12, we banded nestlings with an aluminum USGS band. After day 14, we checked nest boxes every 1–2 days for behavioral evidence of activity (parents provisioning nestlings or removing fecal sacs; nestlings begging) but did not open boxes to avoid premature fledging. If no activity was detected, we searched the surrounding area for banded parents and young and visually inspected boxes for evidence of failure (dead nestlings or remains) or success (flattened nest materials indicating fledging). All nest boxes were rechecked during late June or early July for late initial or second nesting attempts.

For each nesting pair, we attempted to capture the female during incubation and the male during the nestling period. Most birds were captured by placing a hoop of net attached to a long handle over the entrance hole while the adult was inside incubating (female) or provisioning young (male). Some wary birds were captured by sliding a trap door across the entrance hole with a string pulled remotely. A few adults were too skittish to trap. Each adult was given a unique combination of colored leg bands in addition to the USGS aluminum band. We weighed, measured, and examined adults for beak and other deformities.

From each adult and 12-day-old nestling we collected 5–50 μL of blood in 70-μL non-heparinized microhematocrit capillary tubes by ulnar venipuncture with a sterilized 27.5-ga needle after disinfecting the skin with 70% isopropyl alcohol. Pressure was applied to the puncture wound with a sterile cotton ball for 1 min or until bleeding stopped. We followed guidelines for handling and sampling blood from wild birds provided by the Ornithological Council (Gaunt and Oring 1999). Blood samples were immediately transferred to 400 μL of Longmire buffer solution in 1.5 mL microcentrifuge tubes and stored at room temperature for 1–8 months until we extracted DNA for sexing, microsatellite genotyping, and sequencing of mitochondrial DNA (Handel et al. *in press*). We also transferred 1–2 drops of each blood sample into 50 μL of citrate buffer solution in a 1 mL or 2 mL cryogenic tube, gently inverted the tube to mix the buffer with the blood, immediately stored it on dry ice, and then stored in a freezer at -80°C until flow cytometry analysis for evidence of genotoxicity (Custer et al. 1994, Easton et al. 1997). During 2003 we tested and perfected a method of collecting DNA samples from 4-day-old nestlings using buccal swabs (Handel et al. *in press*).

During 2000, we collected a sample of 10 unhatched eggs and 30 12-day-old nestlings for contaminants analysis. Nestlings were collected from north (n = 9), west (n = 8) and south Anchorage (n = 13). Among these, six were collected from nests with a deformed parent, one from a nest with extremely low hatchability, and the remainder were selected randomly from nest boxes within each geographic area. Three of the 10 eggs were from clutches of deformed parents; six were from clutches in which 60–100% of the eggs failed to hatch; one was from a clutch in which two of eight eggs failed to hatch. For eight of the 10 eggs analyzed, we submitted a second unhatched egg from the same clutch for analysis of lipid and moisture content. For the remaining two, we substituted values from whichever of the eight eggs most closely matched the developmental stage of the embryo. In five cases we collected eggs and a nestling from the same clutch. Whenever possible, eggs were selected randomly from within each clutch for analysis of contaminants and lipid content.

During 2001, we collected 29 pairs of eggs and 19 12-day-old nestlings for contaminants analysis and 24 newly laid eggs for analysis of retinoids, vitamin E, and carotenoids. For 22 clutches we were able to collect newly laid eggs for comparative analysis of both contaminants and vitamins; for 10 clutches we were also able to collect a nestling. We randomly selected nests for egg sampling allocated geographically as follows: two from north Anchorage, eight from west Anchorage, six from south Anchorage, and six from the Mat-Su Valley. These included four clutches in which one or both parents had a deformed beak. We augmented contaminant samples with unhatched eggs from four clutches with deformed parents and three with high rates of unhatchability; we analyzed an additional five nestlings from deformed parents and four

from normal parents. To obtain fresh eggs, we monitored nest boxes daily during egg laying. To avoid causing desertion, we waited until three eggs had been laid and then collected one for vitamin analysis; 2–3 days later we collected two more eggs for analysis of contaminants and lipid and moisture content.

To track post-fledging movements, we attached conventional radio transmitters (Holohil BD-2A, 0.62 g, Holohil Systems, Ontario, Canada) to single nestlings from nine randomly selected broods at 15–17 days of age. We initially tried to attach radios using figure-eight style leg harnesses (Rappole and Tipton 1991), but found that we could not tighten the loops enough on the still growing birds and the radios would not stay on. Instead we glued the radios with superglue to contour feathers along the center of the back between the wings. We monitored each nest daily until fledging and every 1–3 days afterward until radios were shed or we could no longer find the signal.

Winter banding study.—Beginning in winter 1999–2000, we captured Black-capped and Boreal chickadees and Red-breasted Nuthatches with mistnets at various residential feeders where deformed birds had been reported. This work was done primarily to examine birds with deformities and to collect specimens for testing.

Beginning in November 2001, we began a systematic winter study to determine the seasonal and interannual prevalence of deformities by sex and age class. We established two study areas in Anchorage, two in Eagle River, and one in the Mat-Su Valley. Each was on a tract of protected lands surrounded by residential areas. At each site we suspended 10 rectangular funnel traps on pulleys at a height of 3–5 m in paper birch trees. Pairs of traps were placed 15–20 m apart and pairs were spaced 100–250 m apart

along a trail loop. Traps were constructed of plastic-coated wire mesh and designed per Senar et al. (1997) except that the funnel opening was made smaller (2 cm diameter) to prevent red squirrels (*Tamiascurius hudsonicus*) from entering and killing the birds. Traps were baited with trays of unhulled black oil sunflower seeds and peanut butter 10–14 days before each trapping session.

In November 2001, we trapped at each site for two consecutive days, then immediately repeated one day of trapping at each site. During subsequent sessions we trapped at each site for two consecutive days, completing each round within 15–19 days. Trapping sessions were repeated each winter from 2001–2002 through 2004–2005 on approximately the same dates: mid-September (beginning in 2002), late November, late January–early February, and late March–early April. We also trapped twice at locations on the Kenai Peninsula, south of our main study area, in parts of the Kenai National Wildlife Refuge that were at least 15 km away from any residential areas with bird feeders. We did this to test association between beak deformities and feeder foods.

We set the traps at sunrise each day and kept them open for 6 hours. We checked each trap every 40–60 min and brought birds back in individual, laundered and bleached cloth bags to a central indoor banding station. Each bird was examined for deformities, weighed, measured, and banded with an aluminum USGS band. We alternated legs each season to facilitate identification of same-year recaptures. We drew a blood sample from each bird upon initial capture for genetic analysis. Any birds recaptured during the same two-day session were reweighed and released. A subsample of birds was remeasured to

assess reliability of measurements among banders. After banding, birds were released at the banding station.

Collection of specimens.—Whenever possible we salvaged specimens of birds with deformed beaks that had been found dead by the public or by wildlife rehabilitators from Alaska (most notably Bird Treatment and Learning Center). We also salvaged all unhatched eggs, nestlings found dead before fledging, and the few adults killed by predators in winter traps during our field studies.

From 1999–2002 we collected a series of specimens of normal and deformed adult Black-capped Chickadees for necropsy, histopathology, blood chemistry, and contaminants analysis. Adults were captured in mist nets or in single-cell Potter traps suspended from trees and baited with sunflower seeds and peanut butter. Birds were euthanized by cervical dislocation or asphyxiated in a small chamber with CO_2.

Measurements, aging, and sexing.—For each adult that we banded or collected, we recorded the following measurements: fresh mass (to 0.1 g), unflattened wing chord (to 1 mm), tail length (to 1 mm), brood patch score (0–5), cloacal protuberance score (0–3), lipid score (0–7), presence or absence of wing, rectrix, and body molt, and extent of skull pneumaticization (score 0–8) (DeSante et al. 2005). We measured with digital calipers the distance from the distal end of one nare (left or right, depending on measurer) to the tip of the maxilla (to 0.1 mm). We also measured with digital calipers the total tarsus and diagonal tarsus (to 0.1 mm) on each bird collected.

For birds with elongated or crossed mandibles, we recorded the following additional beak measurements. Exposed culmen was the straight chord distance from the base of the foremost feathers on the forehead to the tip of the maxilla (measured with digital calipers to 0.1 mm). The gonys was the straight chord distance between the distal end of the notch along the centerline of the lower mandible to the tip (to 0.1 mm). For individuals with extremely curved mandibles, we measured (to 1 mm) with a piece of thread the curved distance between the distal end of basal feathering to the tip of the maxilla (upper curved beak) and between the distal end of the gonyl notch to the tip of the mandible (lower curved beak). We also measured any overbite (straight chord distance of maxilla beyond mandible) or underbite (straight chord distance of mandible beyond maxilla). Direction and extent of lateral crossing of maxilla and mandible were noted and measured in extreme cases. Terminology and measurements of the beak follow Svensson (1992), Proctor and Lynch (1993), and Pyle (1997).

In the fall, passerines were aged by the extent of pneumaticization of the skull and by species-specific plumage characteristics (Pyle 1997). For Black-capped Chickadees, we recorded the shape and presence or absence of white on the tip of the outermost rectrices to assist in aging of hatching-year and second-year birds; for Red-breasted Nuthatches we recorded the color of the crown and back to assist in sexing and aging birds (Meigs et al. 1983, Pyle 1997). Live passerines were sexed externally based on the presence of a brood patch (female) or cloacal protuberance (male) during the breeding season. For specimens, we used digital calipers to measure the largest ovum (female) or length and width of left testis (male). We also determined gender of live adult and

nestling chickadees and nuthatches genetically from blood or buccal samples (Handel et al. *in press*).

LABORATORY ANALYSES

Contaminants in potential foods.—In 2000 we collected 18 samples of whole black oil sunflower seeds, the primary food eaten by Black-capped Chickadees at residential winter feeders. Sample sites included 12 major retail outlets in Anchorage and the Mat-Su Valley and supplies that had been stored at six residences in the area, including four where several deformed adult chickadees had been observed and two where none had yet been seen (Table 2). One bag of seeds was randomly selected from each retail source. The manufacturer, retail source, and year purchased were recorded for each sample. A 30-g sample of seeds was collected from each supply, stored in a chemically-clean (ICHEM™) jar, and submitted for analysis. Samples were screened for 18 organochlorine (OC) pesticides, total polychlorinated biphenyls (PCBs) and four commercial mixtures of PCB congeners, 24 organophosphate pesticides, six carbamates, and 19 metals and trace elements (Table 3).

Necropsy and histopathology of deformed birds.— We submitted five adults with deformed beaks, and one adult and one juvenile with normal beaks to the USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin, for necropsy and histopathology (Case #16169 and #18458). We also submitted two nestlings each with a twisted leg and one nestling with a slightly overgrown maxilla (Case #18224). Fresh carcasses were weighed and measured externally, then air-shipped to the laboratory in insulated coolers surrounded by frozen gel packs. We also took whole-body radiographs

using high-resolution mammography film of nine adult Black-capped Chickadees and one adult Red-breasted Nuthatch with beak deformities, 17 normal adult Black-capped Chickadees, three normal 12-day-old nestling Black-capped Chickadees, and one 12-day-old nestling with a deformed leg.

Clinical chemistry of blood samples.— We collected blood samples from 7 adult Black-capped Chickadees with severely deformed beaks and 10 adults with normal beaks for clinical chemistry analysis as an indication of immunological response. Blood was drawn from the jugular vein into a 1-cc syringe with a sterilized 27-ga needle after disinfecting the skin with 70% isopropyl alcohol. Each bird was then immediately euthanized by cervical dislocation and the carcass was saved for subsequent contaminants analysis or necropsy and histopathology. Blood was placed in red-topped centrifuge tubes and allowed to clot for 30 min. Tubes were then centrifuged for 5–10 min at 2500 rpm. Refrigerated tubes were air-shipped immediately to Marshfield Laboratories, Marshfield, Wisconsin, and submitted for protein electrophoresis and screening of the blood serum using the avian profile of tests.

Contaminant analyses of chickadee tissues.— For all adult and nestling birds to be analyzed for contaminants, we recorded fresh mass and all external measurements (see above) immediately after birds had died or been euthanized. Most specimens were then frozen individually at -80°C in chemically clean ICHEM jars until ready for processing. After thawing, we removed the legs below the tibiotarsal joint, clipped the wings at the distal end of the ulna, and clipped the beak at the base of the skull. To minimize external contamination when collecting tissue samples, we used a new chemically cleaned set of

instruments and powder-free latex gloves to excise tissues after the bird had been opened. Scissors, forceps, and scalpel handles were rinsed once in a 1% nitric acid solution, rinsed three times in distilled water, rinsed once in acetone, rinsed three times in distilled water, air dried and then wrapped in aluminum foil that had been wiped with acetone. Scalpel blades were not chemically cleaned but a new blade was used for removing internal organs. We excised the whole liver for analysis of metals and trace elements and used the remainder of the carcass for analysis of organochlorine pesticides, PCBs, PCDDs, and PCDFs. Each tissue sample was placed into a tared ICHEM jar, weighed, and stored at -80°C until air-shipped on dry ice for laboratory analysis.

Each whole egg collected for contaminants analysis was wrapped in acetone-wiped aluminum foil and refrigerated for 1–4 days until laboratory processing. We measured mass of whole eggs with an electronic balance (to 0.001 g), averaged three replicate measurements each of length and breadth taken at their greatest dimensions with digital calipers (0.05 mm), and measured displacement volume in a graduated cylinder filled with distilled, deionized water (to 0.001 cm^3). We then scored the eggshell with a chemically cleaned scalpel around the equator, cut the membrane, and poured the contents into a tared ICHEM jar. We determined the mass of the contents on an electronic balance (0.001 g) and noted any embryonic development. Samples were stored at -80° C until shipped on dry ice for laboratory analysis.

We submitted four catalogs through the Patuxent Analytical Control Facility (PACF) for analysis of various contaminant compounds in tissues of Black-capped Chickadees. Catalog #7020052 consisted of 14 normal adults and 6 adults with beak deformities

collected during late winter 1998–1999. Liver tissue of all birds was screened for metals and trace elements and the remaining carcasses of all but one deformed bird were screened for organochlorine pesticides and commercial mixtures of PCBs. Catalog #7020058 included 10 eggs and 30 12-day-old nestlings collected during 2000; catalog #7020083 included 29 eggs and 19 12-day-old nestlings collected during 2001. For both catalogs, all eggs and nestling carcasses (minus the liver) were analyzed for organochlorine pesticides and specific congeners of PCBs, PCDDs and PCDFs. Livers of nestlings were analyzed for metals and trace elements. Catalog #7020090 consisted of eight normal adults and eight adult chickadees collected from 2001–2002 that had developed beak deformities 1–5 months after having been originally banded as "normal" birds. Whole carcasses minus the liver were analyzed for organochlorine pesticides, specific congeners of PCBs, PCDDs, and PCDFs, the organophosphate chlorpyrifos, and semivolatile organic compounds 1,2,3,4-tetrachlorobenzene and 1,2,4,5-tetrachlorobenzene. For the same suite of analyses we also submitted two samples of liver tissue pooled over the normal and deformed birds.

All analyses of metals and trace elements were conducted according to PACF protocols by Research Triangle Institute, Research Triangle Park, North Carolina. Analyses of organochlorine pesticides, PCBs, and other related compounds were conducted according to PACF protocols by AXYS Analytical Services Ltd., Sidney, British Columbia (catalog #7020052, #7020058, #7020083) and by Geochemical Environmental Research Group, Texas A&M Research Foundation, College Station, Texas (catalog #7020090).

Analysis of retinoids, Vitamin E, and carotenoids.—Each whole egg collected for analysis of vitamins was refrigerated and processed within 24 h of collection. We measured mass of whole eggs with an electronic balance (to 0.01 g) and measured length and breadth at their greatest dimensions with digital calipers (to 0.1 mm). We then scored each eggshell with a scalpel, cut the membrane, and emptied the contents into a tared 1.5 mL cryotube. After weighing the egg contents, we stored samples at -80°C until air-shipped on dry ice for laboratory analysis. We submitted 24 samples to Jeffrey J. Whyte and Donald E. Tillitt, USGS Columbia Environmental Research Center, Columbia, Missouri, for extraction and chromatographic analysis of retinoids (3,4-dehydroretinol, all-*trans*-retinol, all-*trans*-retinal, and all-*trans*-retinol-palmitate), vitamin E (α-tocopherol), and carotenoids (astaxanthin, canthaxanthin, and β-carotene). Laboratory methods and results are presented in Whyte and Tillitt (2003), which is attached as Appendix A.

Blood flow cytometry.—Blood samples from 9 deformed and 35 normal Black-capped Chickadees were submitted to Michael Easton, International EcoGen, Inc., Vancouver, BC, for flow cytometry analysis to look for evidence of genotoxicity in the form of clastogenic damage. Most samples had been stored on dry ice immediately after collection, but samples from 12 normal birds had been stored on snow in a cooler for 4 h before being transferred to an ultracold (-80° C) freezer. Flow cytometry was used to determine the net amount of DNA in a populaton of about 10,000 blood cells from each individual bird. Any coefficient of variation (CV) higher relative to controls or reference values was used to indicate that a significant degree of DNA damage had occurred in the

cells of that tissue. Specific methods and results of these analyses are presented in Easton (1999), which is attached as Appendix B.

STATISTICAL ANALYSES

Reproductive success.—We used logistic regression (PROC LOGISTIC; SAS Institute Inc. 1990) to model success of nests as a function of geographic area, year, nest initiation date, and presence or absence of a beak deformity in the male or female. We first modeled effects of experimental treatments, including collection of three eggs during laying and early incubation for nutritional and contaminants analysis, collection of one 12-day-old nestling for contaminants analysis, and use of a video camera on the nest box during the nestling period. If modeling results suggested any experimental treatment negatively affected nest survival, nests subjected to that treatment were excluded from final modeling efforts. We also modeled the effect of presence or absence of parasitic blowflies (*Protocalliphora* spp.) or fleas (Siphonaptera) on nest success during the nestling period; if survival decreased, then the factor was also included in the final analysis and nests missing such data were excluded.

We evaluated candidate models using the Akaike information criterion (AIC, Burnham and Anderson 2002). The model with the smallest AIC value was judged to be the best-approximating model, given the data. The remaining models were ranked on the basis of the difference in AIC values compared with the best model (ΔAIC). We calculated an AIC weight (w_i) for each model in the balanced candidate set and calculated a relative importance measure for each variable by summing the Akaike weights across the models in which that variable appeared (Burnham and Anderson 2002).

Other reproductive parameters.—We used general linear models (PROC GLM; SAS Institute Inc. 1990) to model number of eggs hatched and number of nestlings alive at day 12 as a function of geographic area, year, nest initiation date, and presence or absence of a beak deformity in the male or female. We excluded from all analyses nests from which we had collected eggs during laying; we excluded nests with video cameras from analyses related to nestlings. We included number of eggs hatched as a covariate for analysis of brood size at day 12. We also modeled the effect of presence of parasitic blowflies (*Protocalliphora* spp.) or fleas (Siphonaptera) on brood size; if survival decreased, then the factor was also included in the final analysis and nests missing such data were excluded. We compared candidate models using AIC values.

Blood chemistry.—Because of small sample sizes and nonnormal distribution of the data, we used the Mann-Whitney U test to compare various clinical chemistry parameters of blood from normal and deformed adults.

Contaminants analysis.—We present medians and interquartile ranges in summaries because of small sample sizes and nonnormal distributions. For most of the contaminants analyzed, varying numbers of samples had concentrations that were below the minimum detection limit of the laboratory equipment, and detection limits also varied among samples based on quantity of tissue being analyzed and other variables. For organochlorine pesticides, PCBs, and other organic compounds, we used nonparametric Kendall's tau to test for correlation between concentrations of contaminants and percent lipid in the sample (Helsel 2005). If correlation was significant, we used PROC RELIABILITY (SAS Institute Inc. 1990) to compare groups of samples with a

maximum-likelihood censored regression model that included percent lipid as a covariate (cf. Hebert and Keenleyside 1995). We log-transformed concentrations when necessary to meet the assumption of normality of residuals. For compounds not correlated with lipid content, we used the nonparametric generalized Wilcoxon score test to compare concentrations of a contaminant among two or more groups for censored data with multiple detection limits (Helsel 2005). Data were first "flipped" by subtracting the known concentration or detection limit from a value greater than the maximum concentration. We then used PROC LIFETEST (SAS Institute Inc. 1990) to analyze the censored data.

For adults, we compared contaminant concentrations in the following groups: (1) those with vs. without deformed beaks and (2) those recently deformed (developed within past 2–5 months) vs. those with normal beaks. We also compared concentrations between normal adults and nestlings. For eggs and nestlings, we compared the following groups: (1) those in which both parents had normal beaks vs. those in which either parent had a beak deformity; (2) those with vs. without a female parent with a beak deformity; and (3) those from nests in which all eggs hatched vs. those in which at least one egg failed to hatch. In the last comparison, eggs from clutches abandoned by females due to investigator disturbance were classified as viable; eggs from clutches abandoned by females due to aberrant incubation behavior (e.g., abnormal scattering of eggs across nest or erratic incubation pattern) were classified as failed eggs. For all tests, a significance level of $P < 0.05$ was used.

Seasonal prevalence.—For each capture period (May–June for nesting; each winter banding session) we calculated the percent of individuals captured with a beak deformity by species, age, and sex. We also noted other individuals that had a slight overgrowth of maxilla or mandible (<0.5 mm) that may have been an incipient deformity. Finally, we documented several individuals that had had a normal beak when originally captured but had a deformed beak when later recaptured. For those in which the deformity was known to have developed in less than a year, we calculated the monthly probability of developing a deformity. To do so, we allocated the probability uniformly across the number of days between captures for each bird, summed the probabilities across all individuals for each month, standardized the probabilities to sum to 100% across the year, and then plotted the relative probabilities for each month.

Movements.—We recorded geographical coordinates for each site at which chickadees and nuthatches were banded, recaptured, or resighted. We obtained several resightings from the public, primarily at winter feeders, of birds that we had uniquely color-banded during the nesting season. We used a geographical information system to plot the sightings and compute the minimum distances traveled and examined these in relation to season and age and gender of birds.

RESULTS

OBSERVATIONS OF BIRDS WITH BEAK DEFORMITIES

Numbers and geographic distribution of sightings.—As of June 30, 2005, we had documented 2,153 reports of Black-capped Chickadees with deformed beaks in Alaska;

these birds had been observed between November 1991 and May 2005 (Table 4). We estimate that these represent a minimum of 1,441 different individuals, based on their locations and descriptions of the deformities. By comparison, few responses from outside of Alaska have been received from inquiries through Project FeederWatch (Cornell Laboratory of Ornithology), bulletin boards (BirdBander, CavNet, WildlifeHealth, and FWS-EC-Tech), and response to national media coverage. Only 21 reports of Black-capped Chickadees with deformed beaks, representing 17 unique individuals, have been obtained from outside of Alaska (Table 4). Deformed chickadees were observed in scattered years between 1986 and 2005 and include four birds from Ontario, Canada, four from Washington, two from Pennsylvania, two from Wisconsin, and single birds from Vermont, Connecticut, New Hampshire, New York, and Michigan (Figure 3). One of the observations in Ontario constitutes the single published record of a beak deformity in this species; neither the ultimate fate of the bird nor the cause of the abnormality, however, was determined (Kinch 1998).

We have also documented 281 observations of individuals of 27 other species of birds in Alaska with similar beak deformities. We estimated that these represent a minimum of 209 different individuals, based on the locations of the birds and descriptions of their deformities (Table 5). Affected birds include 17 resident species and 11 migrants. In addition to Black-capped Chickadees, the most commonly reported residents include several species of corvids, two species of woodpeckers, and the Red-breasted Nuthatch. Only five Boreal Chickadees and one Chestnut-backed Chickadee (*P. cincta*) have been reported with beak deformities. A total of 13 individuals of nine species of migratory passerines has been documented with beak deformities. Among these, all but a single

Varied Thrush (*Ixoreus naevius*), Orange-crowned Warbler (*Vermivora celata*), Yellow-rumped Warbler (*Dendroica coronata*), and Savannah Sparrow (*Passerculus sandwichensis*) were juvenile birds captured or observed during autumn; it is almost certain that these juveniles had been produced in Alaska. Deformed beaks have also been recorded from waterbirds and raptors, including two gosling Cackling Geese (*Branta hutchinsii*) banded on the Semidi Islands, at least three Pelagic Cormorants (*Phalacrocorax pelagicus*) seen near Sitka, one adult Bald Eagle (*Haliaeetus leucocephalus*) on the Kenai Peninsula, and one nestling Peregrine Falcon (*Falco peregrinus*) on the Colville River in northern Alaska.

Sightings by year.— We observed our first deformed Black-capped Chickadees in January 1998, when a cluster of three birds with distinctly deformed beaks was recorded at a residential feeder in Anchorage (S. L. Talbot, pers. observ.). We captured one of the birds in March, banded it, trimmed its beak, released it, and never saw it again. In April of 1998 we saw a photograph published in the Anchorage Daily News of a second deformed chickadee from near Big Lake, in the Mat-Su Valley of south-central Alaska (Figure 1). After that observation, we began soliciting observations from the scientists and the public, through a series of presentations, newspaper articles, radio programs, and televised news reports, of any other beak deformities in Alaska.

The earliest observations of beak deformities in Alaska that we have documented historically were of a Downy Woodpecker (*Picoides pubescens*) in Homer on the Kenai Peninsula and of a Northwestern Crow (*Corvus caurinus*) on Kodiak Island, both during winter 1979–1980 (Table 5). No other birds with deformed beaks were documented until

winter 1991–1992, when the first deformed Black-capped Chickadees were observed (Figure 4). That winter, single chickadees with deformed beaks were seen in King Salmon and Naknek in the Bristol Bay region and in Wasilla and near Nancy Lakes in the Mat-Su Valley (Figure 5).

Observations of deformed birds increased exponentially between 1991 and 2000 (Figure 4), coincident with a surge of media coverage. Since winter 2000–2001, when almost 500 individual birds with deformed beaks were reported, the number of observations received each year has declined and leveled off at about half that peak rate.

Maps of cumulative sightings of deformed chickadees indicate a clear increase in geographic distribution as the years progressed (Figures 5, 6). Observations were reported increasingly southward along the Kenai Peninsula, northward through the Mat-Su Valley, eventually reaching Fairbanks, and westward along the Alaska Peninsula and out to the Yukon-Kuskokwim Delta. Maps of other species with beak deformities show a similarly increasing geographic distribution (Figures 7, 8), with additional expansion throughout southeastern Alaska, where Black-capped Chickadees rarely occur. Three individuals were also reported from adjacent Yukon Territory, Canada, including a Black-billed Magpie (*Pica hudsonia*), Hammond's Flycatcher (*Empidonax hammondii*), and Snow Bunting (*Plectrophenax nivalis*).

Description of Deformities

Black-capped Chickadee.—In a normal chickadee, the maxilla and mandible are straight and meet at the tips (Figure 9a). Beaks of normal male chickadees are longer in

all measurements (all $P < 0.05$) than those of normal females (Table 6). Measurement of the maxilla from nares to tip does not normally exceed 8.4 mm; the exposed culmen is less than 11.5 mm; and the gonyl measurement of the mandible is less than 7.5 mm.

We classified a beak as deformed if (a) the maxilla and mandible were laterally offset from each other, (b) the maxilla or mandible extended more than 1 mm beyond the other, (c) both maxilla and mandible were straight but abnormally long (>8.5 mm from nares to tip), or (d) there was a gap >0.2 mm between the maxilla and mandible. Overgrowths of 0.5–1.0 mm or smaller gaps were classified as possible incipient deformities. In most deformed chickadees, the maxilla was overgrown, often with a pronounced decurvature (Figure 9b–d). In some cases, the mandible was also overgrown and often upcurved, resulting in a markedly crossed beak (Figure 9e–h). It was relatively rare to have only the lower mandible overgrown (Figure 9i), and we documented only a single case in which the maxilla was curved in a lateral arc, almost from its base (Figure 9j). In some cases both the maxilla and mandible were overgrown but not crossing; this generally resulted in a pronounced gap (Figure 9k).

In many cases the overgrowth, particularly of the maxilla, was thin and brittle (e.g., Figure 9d). In a few cases, however, the beak was severely thickened along its entire length (Figure 9l). Deformed beaks often had a series of semi-elliptical or longitudinal ridges along the maxilla distal of the nares, which suggested patterns of irregular growth of the rhamphotheca. In many cases the tomium of either the maxilla or mandible or both had irregular, often thin edges.

Among 178 deformed chickadees of known sex that we measured, the maxilla ranged from 5.9–40.3 mm from nares to tip, averaging about 4–5 mm longer than that on normal birds (Table 6). The gonyl measurement of the lower mandible ranged from 5.7–28.4 mm, averaging about 3 mm longer than normal. Measurement along the curvature of the maxilla ranged from 6.5–43.0 mm and averaged 17.7 $\pm$ 0.99 mm (n = 62). Among 14 birds with an upcurved mandible, the length along the curvature ranged from 7.4–21.5 mm, averaging 14.3 $\pm$ 1.1 mm. Beaks of deformed chickadees did not differ significantly in any measurements between males and females.

There were a few other abnormal physical conditions observed among some of the chickadees with deformed beaks. About 12% of the birds (23 of 194) had patches of feathers missing, particularly from the forehead or occipital region, or contour feathers that were sparse, abnormally long, and plumaceous. About 2% (n = 4) of the deformed chickadees had one to several white feathers instead of black at the base of the maxilla; another 3% (n = 5) had patches of brown feathers in the same area. We noted markedly dry, flaky, or reddened skin in 5% (n = 10) of the deformed chickadees. One of the more pronounced cases is shown in Figure 10a. Some birds (2%, n = 4) also had notably scaly legs, with the keratin sheath easily flaking off in layers (Figure 10b). One incubating female with a severe beak deformity had a large, subcutaneous lipoma on the abdomen along the brood patch (Figure 10c–d) and another deformed individual had a tumor on its tarsometatarsus.

Boreal Chickadee.—Although we did not capture any deformed Boreal Chickadees, one reported individual that was banded and released in Fairbanks had an overgrown

maxilla, with an exposed culmen length of about 20 mm; this would have been about twice the length of that on a normal individual (Table 6). We received a report of another Boreal Chickadee found dead whose maxilla and mandible were both straight, with an exposed culmen of about 15–20 mm.

Red-breasted Nuthatch.—We measured two female Red-breasted Nuthatches with deformed beaks (Table 6). In one case the maxilla and mandible were both slightly overgrown and crossed at the tips (Figure 11a); in the other both were straight but overgrown by about 7 mm, with the tips meeting. Most of the reports of nuthatches that we received from others were of individuals with both maxilla and mandible overgrown, generally straight but sometimes with slight curvature or crossing. The beak of one nuthatch was overgrown and slightly upcurved (e.g., Figure 11b); one individual was reported with a normal maxilla but a mandible about 5 mm shorter than normal.

Woodpeckers.—Based on photographs and descriptions from others, beak deformities among Downy and Hairy woodpeckers were similar to those of nuthatches, generally involving extreme overgrowth with little crossing or curvature (e.g., Figure 11d). One Downy Woodpecker we measured had an exposed culmen of 41.1 mm and gonys of 22.6 mm, with the maxilla extending 14.0 mm beyond the mandible. A few deformed beaks of both species were reported with a gap at the tip. The elongated beaks of some woodpeckers were reported as abnormally thickened; others were noticeably thin.

Small passerines.—Most individuals of other small passerines had beaks crossed at the tips with varying amounts of overgrowth; these included Ruby-crowned Kinglet, American Robin, Varied Thrush, Orange-crowned Warbler, Yellow-rumped Warbler,

Savannah Sparrow, Lincoln's Sparrow, Dark-eyed Junco, Pine Grosbeak, White-winged Crossbill, Common Redpoll, and Pine Siskin (Figure 11). Several songbirds were also observed or reported with a normal mandible but an overgrown and decurved maxilla: Yellow-rumped Warbler, American Tree Sparrow, Pine Grosbeak, Common Redpoll, and Pine Siskin. One American Robin was photographed with an elongated and upcurved maxilla but normal mandible. One Chestnut-backed Chickadee was reported with a deformed beak but we were unable to obtain a detailed description.

Corvids.—Based on many photographs, beak deformities of corvid species were quite varied in character and similar to those of Black-capped Chickadees. Most beak deformities among Northwestern Crows involved elongation and decurvature of the maxilla (Figure 11e) or elongation and crossing of both maxilla and mandible. Only two cases were documented with only an overgrown mandible (Figure 11f) and a single case was reported in which the maxilla was shorter than normal. In some crows the entire beak was thickened but in many individuals the elongated tips were notably thin. Most Black-billed Magpies had a long, decurved maxilla; one individual had only a long mandible; several had crossed beaks; and in one both the maxilla and mandible were reported to be long and decurved. Two of three Common Ravens had an overgrown, decurved maxilla; one had a crossed beak.

Beak deformities reported among Gray and Steller's jays were also quite variable. One Gray Jay had likely suffered from a physical injury to the maxilla, which was half the normal size. The beak of the second bird appeared malformed; the maxilla was very narrow and slightly shorter than the mandible, which itself was only about a third to half

the normal length. This individual was also missing several claws on both feet. Among the many deformed Steller's Jays reported, most had an elongated maxilla that was either straight or decurved. In a few cases both the maxilla and mandible were long and either crossed or straight; one individual was reported with only an elongated mandible. In four cases the maxilla was either completely missing or very short; these might have been the result of physical injury.

Other species.— The single adult Bald Eagle and nestling Peregrine Falcon reported both had crossed beaks; the falcon was also missing one eye. The Pelagic Cormorants also all had crossed beaks. The beak deformities of gosling Cackling Geese were noted when banded but not described.

PREVALENCE OF BEAK DEFORMITIES AMONG BLACK-CAPPED CHICKADEES

Prevalence by gender and age.—Among 2,186 individual post-fledging Black-capped Chickadees that we examined through all our trapping efforts between 1999 and 2005, we found evidence of a beak deformity in 178 (8.1%) individuals. We also documented an additional 50 (2.3%) individuals with possible incipient deformities. Among 178 definitely deformed individuals of known gender, 81 (46%) were males and 97 (54%) were females. We trapped fewer males (48.1%) than females (51.9% of 1,988 birds of known gender), and the proportion of individuals with deformities did not differ significantly between the sexes ($\chi^2 = 0.54$, df = 1, $P = 0.46$).

Among 1,820 12-day-old nestlings, we found only one individual (0.05%) in which the maxilla was slightly longer than the mandible and the tips of the mandibles were

slightly crossed. In an additional 10 nestlings (0.6%) the maxilla was slightly (<0.6 mm) longer than the mandible but there was no sign of crossing. These were all from a single brood whose male parent had an elongated maxilla and whose female parent was normal. One of these nestlings was collected at age 15 days and found by NWHC to have abnormal inclusions in the beak epithelium (see below). Another nestling was held in captivity for 34 days; the beak grew normally and the overgrowth disappeared, so the bird was released. There were also seven 12-day-old nestlings (0.38%) with either folding leg fractures or dislocated legs.

None of the 229 known hatching-year (HY) Black-capped Chickadees captured during September of any year was found to have a deformed beak. The youngest Black-capped Chickadee that we captured with a beak deformity was about 6 months old. The bird had originally been banded in September 2001 with a normal beak at a fall migration banding station in Anchorage and was aged as a HY bird with an incompletely pneumaticized skull (B. Seppi, Bureau of Land Management, pers. comm.). We recaptured it in November at one of our nearby research sites with a normal beak and then in December with both mandibles longer and the maxilla slightly overhanging (0.5 mm overbite). We captured it again in February 2002, by which time the tips of the mandibles had crossed, the lower mandible was 1.6 mm longer than the maxilla, and there was a pronounced gap between the mandibles. We captured four other individuals between mid-November and late December with deformed beaks that were likely HY birds, based on tail characteristics, but the skull on each was completely ossified so age could not be confirmed.

Prevalence by year and season.—Prevalence of deformities varied both among years and by season within years (Figure 12). Prevalence was also strongly influenced by the proportion of juveniles captured each session, because deformities did not generally develop until birds were older (see above). The proportion of adults breeding in nest boxes with beak deformities increased from 6–7% in 2000 and 2001 to about 14% in summer 2004 (Figure 12, bottom). Seasonal prevalence among adults during the non-breeding period varied markedly from 0–16% across our capture sessions, with a pronounced decline during January–February in three of four winters. When we trapped on the Kenai Peninsula in areas at least 15 km away from any residential feeders, one (8%) of 13 Black-capped Chickadees we caught in October 2002 had a severe beak deformity; in April 2004, none of the nine Black-capped Chickades captured was deformed.

Seasonal development and disappearance of deformities.—We documented 54 cases in which an individual that was captured originally with a normal beak was subsequently recaptured with a deformed beak. For 19 of these individuals, 12–35 months had elapsed between their last capture as normal and first recapture as deformed. The remaining 35 deformed individuals were recaptured within 10 months of last being recorded as normal; 17 of these were recaptured within 6 months. Deformities developed throughout the annual cycle but the average probability that the deformities of these individuals had developed in any given month appeared to be slightly higher during late winter than during the remainder of the year (Figure 13).

We also documented six cases in which individuals with slight to moderate beak deformities were subsequently recaptured with apparently normal beaks. The most severe case was of an adult male captured in February 2002 whose maxilla and mandible were both straight but overgrown (12.2 mm nares to tip; 11.3 mm gonys); the tips of the mandibles met and there was a pronounced gap between them. When we recaptured the bird in the fall seven months later, measurements were within the normal range (8.5 mm nares to tip) and there was no evidence of a gap. We recaptured this bird three more times through April 2005; once it appeared normal but twice it showed evidence of a gap or overgrowth, but never as severe as when first captured. In the remaining five cases the original overgrowth was less pronounced. We recaptured these individuals from 1–16 times; one of these vacillated between an obviously deformed state and an apparently normal state whereas the other four became indiscernible from normal individuals.

We also noted several cases of individuals with severely overgrown beaks whose brittle tips had obviously broken off. This may have been the mechanism through which evidence of deformities was largely erased.

Prevalence of Beak Deformities among Other Species Captured

Prevalence by gender, age, and season.—Among 178 post-fledging Boreal Chickadees captured one or more times during our banding efforts, none had a deformed beak and only 2 (1.1%) had possible incipient deformities (both adult breeding females). We recorded 1 (0.5%) post-fledging Red-breasted Nuthatch (an adult female in April) among 198 individuals captured with a beak deformity. No nestlings of either species showed any evidence of crossed or overgrown beaks. Among 109 Common Redpolls

captured once or more during our winter banding efforts, only 1 (0.9%) had a deformed beak; the maxilla and mandible were crossed, with the mandible slightly longer than the maxilla (0.4 mm) and with a pronounced gap (0.8 mm) between them.

Reproductive Success

Nest-box occupancy.—During 2001, the year of maximum effort for monitoring nest boxes, we found 28.4% of 268 nest boxes occupied in the Anchorage area and 16.9% of 207 boxes occupied in the Eagle River/Mat-Su Valley areas. We monitored a total of 420 nesting attempts during the five-year period (Table 7). Among these, Black-capped Chickadees comprised 72.6%; Boreal Chickadees, 13.3%; unidentified chickadees, 2.4%; and Red-breasted Nuthatches, 11.7%. Both Boreal Chickadees and Red-breasted Nuthatches rarely used nest boxes in the Eagle River/Mat-Su Valley areas.

Among the 305 nesting attempts by Black-capped Chickadees, 33 (10.8%) had one or both adults with a definite beak deformity and another 21 (6.9%) had one adult with a possible incipient deformity (Table 8). Most (69%) of these deformed chickadees were females. None of the Red-breasted Nuthatches nesting in boxes had a deformed beak; only two of the Boreal Chickadees, both females, had possible incipient deformities (Table 8).

Nesting chronology of Black-capped Chickadees.—Black-capped Chickadees initiated egg-laying between 27 April and 3 June, with a mean initiation date of 10 May ($\pm$ 0.4 days, n = 283; Figure 14). Egg-laying dates for nest boxes within Anchorage, which were monitored each year, differed significantly among years ($F_{4,247} = 43.94$, $P < 0.001$)

and among areas ($F_{2,247} = 6.30$, $P = 0.002$); these patterns were consistent across years and areas ($F_{8,247} = 0.91$, $P = 0.91$). Nesting was earliest in 2003 and latest in 2002 and 2004 (Figure 14). Nest initiation dates averaged 3 days earlier in north Anchorage compared with those in south or west Anchorage. Nests in Mat-Su Valley and Eagle River, which were monitored only from 2001–2003, were similar in timing to those in west and south Anchorage.

We recorded three cases for Black-capped Chickadees in which a second clutch was initiated in the box in the same year, all after the first clutch had failed during incubation. In one case, a new female laid a new clutch on top of the first clutch, which had been abandoned after the initial female was banded. In the other two cases, we don't know if the same or a different female laid the second clutch, since the initial females were unbanded. One appeared to be a continuation of laying after the initial clutch of seven eggs had been collected after being found broken; the two new eggs were subsequently abandoned. In the other attempt, a second clutch of six eggs was laid after the initial clutch of six eggs had been abandoned and collected; two nestlings hatched but failed to fledge from the second clutch. All three replacement clutches were initiated within the range of initial clutches.

The incubation period, from last egg laid to first egg hatched, ranged from 11–20 days and averaged 13.3 $\pm$ 0.1 days ($n = 203$). Across all years, hatching dates ranged from 19 May–22 June, with the mean on 30 May ($\pm$ 0.4 days, $n = 270$). Fledging occurred 14–21 days after hatching, with the nestling period averaging 18.0 $\pm$ 0.1 days ($n = 176$).

Fledging dates ranged from 6 June–10 July, with the mean on 17 June (± 0.4 days, *n* = 236).

Nesting chronology of Boreal Chickadees.—Boreal Chickadees initiated egg-laying of first clutches from 3–24 May, with a mean initiation date of 12 May (± 0.8 days, *n* = 52). Three additional clutches were renesting attempts, in which the same pair laid a second clutch in the box after their first brood had successfully fledged; these were initiated from 18–21 June. Initiation of first clutches varied significantly among years ($F_{4,51} = 8.39$, $P < 0.0001$), with timing in 2002 averaging about nine days later than during other years. The incubation period of Boreal Chickadees averaged 13.6 ± 0.1 days (*n* = 35) and the nestling period averaged 17.3 ± 0.2 days (*n* = 27). Eggs of initial clutches hatched from 21 May–11 June (*n* = 50) and nestlings fledged from 11–28 June (*n* = 46). Second clutches hatched from 2–10 July (*n* = 3) and fledged on 25 July (*n* = 2).

Nesting chronology of Red-breasted Nuthatches.—Red-breasted Nuthatches began laying first clutches before both species of chickadee, from 23 April–13 June, with a mean initiation date of 6 May (± 2.1 days, *n* = 39). Seven additional clutches were second attempts in the same box; average initiation date of these was 18 June (± 1.6 days; range 13–22 June; *n* = 6). Five of these were second clutches after the first brood had successfully fledged; at least one member of the original pair was identified with the second clutch in each case. In the sixth case, the second clutch was laid by a new female after the first had died after becoming wedged in the sap-laden entrance hole after laying one egg. In the last case, we collected a clutch of seven eggs that a banded female had abandoned after incubating for 20 days; the same female, with the same male, laid a

replacement clutch of seven eggs, which she then incubated for 12 days before abandoning it. Timing of initiation of first clutches did not vary significantly among years ($F_{4,38} = 0.98$, $P = 0.43$), although nest initiation was latest during 2002, as it was for Boreal and Black-capped chickadees.

The incubation period of Red-breasted Nuthatches averaged 14.0 $\pm$ 0.3 days ($n = 15$) and the nestling period averaged 19.1 $\pm$ 0.4 days ($n = 15$). Eggs of initial clutches hatched from 8 May–2 July ($n = 36$) and nestlings fledged from 28 May–9 July ($n = 30$). Second clutches hatched from 1–11 July ($n = 5$) and fledged from 22–29 July ($n = 4$).

Clutch size.—Black-capped Chickadees with normal beaks laid 2–11 eggs per clutch, averaging 7.83 $\pm$ 0.08 ($n = 216$; Table 9). The model receiving the greatest support for explaining variation in clutch size included clutch initiation date, male deformity, and year ($w_i = 0.71$, ΔAIC = 0.00, AIC = 36.49; Table 10). Across all candidate models, clutch initiation date was unconditionally supported ($\Sigma w_i = 1.00$); clutch size decreased by 0.06 $\pm$ 0.01 egg per day as the season progressed. There was strong evidence for an additional difference unrelated to laying date among years ($\Sigma w_i = 0.86$), with clutches being largest in 2000, smallest in 2002, and intermediate during the other years.

There was very strong evidence ($\Sigma w_i = 0.90$) that clutches in Black-capped Chickadee nest boxes with male parents exhibiting any type of beak deformity were larger than those of normal males but almost no support ($\Sigma w_i = 0.16$) for clutch sizes being different for deformed females (Table 10). Nest boxes attended by deformed males had clutch sizes that averaged 0.7 eggs larger than those attended by normal males (Table 9).

Initial clutches of Boreal Chickadees ranged from 1–10 eggs and averaged 7.6 ± 0.2 eggs (n = 51). Replacement clutches ranged from 2–7 eggs and averaged 4.7 ± 1.5 eggs (n = 3). Clutch size was strongly correlated with initiation date, decreasing by 0.13 ± 0.03 eggs per day through the season. There was little evidence for additional differences among years. Both nests of females with incipient beak deformities had 8 eggs; thus, there was no evidence that these deformities influenced clutch size.

Initial clutches of Red-breasted Nuthatches ranged from 5–8 eggs and averaged 6.8 ± 0.1 eggs (n = 39). Replacement clutches ranged from 5–7 eggs and averaged 6.4 ± 0.3 eggs (n = 7). There was no evidence that clutch size was correlated with initiation date or differed among years. No deformed nuthatches nested in any boxes.

Hatching success of Black-capped Chickadees.—For Black-capped Chickadee nests in which both parents were normal (n = 235), the percent of clutches hatching at least one young was extremely high (95.7%). The model receiving the greatest support for explaining variation in hatching success included the presence or absence of a beak deformity in the female parent (w_i = 0.20, ΔAIC = 0.00, AIC = 103.40), although several other models that included clutch initiation date, year, and/or area in addition to female deformity also were well supported (Table 11). Across all models, there was strong evidence (Σw_i = 0.67) that hatching success of nests with deformed female parents was lower than that of normal parents and some support (Σw_i = 0.46) for success to vary with date (Table 11). Only 85.7% of 17 nests with a deformed female parent hatched one or more young; in contrast, 100% of 10 nests with a deformed male parent successfully hatched.

Evidence was stronger that hatching success of individual eggs was lower within clutches of females with beak deformities than among clutches of normal parents. For clutches that hatched at least one young, brood size at hatch averaged 7.2 $\pm$ 0.1 young (n = 200) for normal chickadee parents but only 6.1 $\pm$ 0.5 young (n = 14) for deformed female parents (Table 9). Brood size in nests of deformed males was similar to that of normal parents (Table 9). The model with the greatest support for explaining variation in brood size at hatch, after controlling for initial clutch size, included the presence or absence of a female deformity (w_i = 0.41, ΔAIC = 0.00, AIC = 135.66). Across all models (Table 11), evidence for the effect of female deformity was almost unequivocal (Σw_i = 0.97); there was little support for effect of male deformity (Σw_i = 0.33), date (Σw_i = 0.32), or year (Σw_i = 0.08).

Fledging success of Black-capped Chickadees.—Among Black-capped Chickadee nests that had successfully hatched and in which both parents had normal beaks (n = 225), 93.8% of the broods fledged at least one young. Among 14 broods of females with a beak deformity, 86% produced at least one fledgling; success was similar for broods with deformed male parents (90% of 10 broods). The most parsimonious model included no variables other than brood size at hatch to explain variation in fledging success of nests (w_i = 0.29, ΔAIC = 0.00, AIC = 126.08); across all models (Table 11), there was little support for the effect of deformities among either females (Σw_i = 0.38) or males (Σw_i = 0.29), or for the influence of date (Σw_i = 0.32) or year (Σw_i = 0.06).

Brood size of nestlings 12 days old, after which there was generally little loss before fledging, averaged 6.7 $\pm$ 0.1 young (n = 207). Females with beak deformities raised the

fewest young (5.9 ± 0.6, n = 13) and deformed males raised an intermediate number (Table 9). After controlling for the number of young hatched, the model that best explained the number of young raised included variables for year and the presence or absence of a beak deformity in the male (w_i = 0.33, ΔAIC = 0.00, AIC = 7.05). Across all models (Table 12), the effect of year was consistently strong (Σw_i = 0.98) and there was also substantial evidence that males with deformed beaks raised proportionally fewer young (Σw_i = 0.64). There was little support for the influence of beak deformity of a female parent (Σw_i = 0.28) or clutch initiation date (Σw_i = 0.28) affecting the number of young being raised relative to the number hatched.

Reproductive success of Boreal Chickadees.—Among 53 initial clutches, 94% hatched one or more young; 100% of 3 second clutches hatched. Brood size at hatch ranged from 2–9 and averaged 7.2 ± 0.2 young for initial clutches (n = 50). Second broods were smaller (4.3 ± 1.8, range 1–7, n = 3). Almost all (98% of 50) first broods fledged one or more young; only 2 of 3 second broods fledged. Very few nestlings were lost between hatch and day 12; thus, sizes of initial broods surviving to day 12 were similar to those at hatch (7.2 ± 0.2, range 2–9, n = 49). No young were lost from the two second broods that survived. The two clutches from adult females with incipient beak deformities each had 8 eggs, all of which hatched and survived to day 12.

Reproductive success of Red-breasted Nuthatches.—Among 40 initial clutches, 90% hatched one or more young; 71% of 7 second clutches hatched. Brood size at hatch ranged from 3–8 and averaged 6.1 ± 0.2 young for initial attempts (n = 35) and was slightly smaller for second efforts (4.8 ± 0.8, range 2–6, n = 5). Almost all initial (92%)

and second (80%) broods fledged one or more young. Some broods did suffer partial loss after hatch, however, averaging 5.6 ± 0.3 young on day 12 for initial broods (range 2–8, *n* = 33) and 5.3 ± 0.8 young for second broods (range 3–6, *n* = 4).

MOVEMENTS OF MARKED BIRDS

Radio-marked nestlings.—Of the nine radio transmitters we attached to nestling chickadees, only two remained attached long enough to the birds for us to track post-fledging movements. Three radios were shed inside the nest box before the nestlings fledged and four were shed within 50 m of the nest box 1–2 days after fledging. It appeared that either the adults or nestlings themselves removed the radios with their beaks since radios that were shed had significant pieces of contour feathers still attached. One radioed nestling was tracked for 8 days and the other for 15 days after fledging. During that period adults remained with fledglings within a distance of 500 m of the nest box. After that, no signal could be detected within a distance of 1 km of the last known location of the birds.

Twenty-eight banded Black-capped Chickadees, one Boreal Chickadee, and one Red-breasted Nuthatch were recaptured or resighted with unique color-band combinations away from original banding locations. Twelve adult female Black-capped Chickadees moved an average of 2,300 ± 800 m (range 50–9,300 m) between breeding and wintering sites; seven adult males moved an average distance of 8,100 ± 1,600 m (range 2,400–15,000). One male Black-capped Chickadee was recaptured breeding in a nest box 4,900 m away from its natal site, and four juveniles moved an average distance of 8,200 ± 6,800 m (range 900–28,700) from their natal sites to their wintering territory.

Movements away from natal sites were 2,700 m and 1,500 m for a single female Boreal Chickadee and a single male Red-breasted Nuthatch, respectively.

TEST RESULTS

Necropsies and histopathology.—In Case #16169, three cross-beaked (one male, two females) adults and one normal-beaked (female) adult Black-capped Chickadee were examined by pathologist Roger E. Brannian, DVM, MS Diplomate, American College of Zoological Medicine, at NWHC. The pathology report indicated that cross-beak deformities were obvious grossly and were also demonstrated through Faxitron radiography (R. E. Brannian *in litt.*). There was no apparent difference in radiographic bone density or in the tibiotarsal snap test between the deformed birds and the control bird. Two of the deformed birds were devoid of reservoir fat. Histopathology showed no abnormalities in bone or other tissues. All affected birds were cultured for salmonella with negative results. There was no evidence of parasites or nasal mites (*Cnemidocoptes* sp.). The entire head of each bird was sectioned serially but no abnormalities were found. A cause for the beak deformities was not determined.

In Case #18548, one adult male and one adult female each with an overgrown maxilla and one normal juvenile female Black-capped Chickadee were examined by Rex Sohn, DVM, at NWHC. His pathology report indicated that the maxilla of the adult female was overgrown and possibly broken at the tip (R. Sohn *in litt.*). The bird was in fair body condition and fair post mortem condition. The maxilla of the adult male was significantly overgrown. Several unidentified feather mites were present at the base of the beak and there was some dryness and crusting of the skin at the thoracic inlet. For

both deformed birds, the thyroid appeared normal in size and shape. The normal-beaked juvenile female was in fair body condition and fair post mortem condition; the body had no external lesions. Radiographs showed no significant osteological abnormalities of the cranium for any of the three birds. No viruses were cultured from the beaks or from the pooled spleen/kidney or pooled spleen/liver for any of the birds. Diagnostic pathologist Lou Sileo, Ph. D., reported that there were no microscopic abnormalities noted in any of the three birds (L. Sileo *in litt.*). Deeper sections of the head for the two deformed birds did not reveal any pathology. There were multiple and various sections of feather mites on the superficial aspect of the cranial tissues of the two deformed birds, along with occasional feather mite sections within the tissues, but these had not provoked any type of inflammatory response. The tissue feather mite sections may have been dragged in as a sectioning artifact from the surface. A cause of the beak deformities was not determined for these chickadees.

In Case #18224, two 12-day-old nestling Black-capped Chickadees each with a twisted, dangling leg and one 15-day-old nestling with a slightly overgrown maxilla (0.5 mm longer than mandible) were examined by Carol U. Meteyer, DVM, Diplomate, American College of Veterinary Pathologist, at NWHC. The pathology report indicated that there was no obvious cause of death or fatal illness in these nestlings (C. U. Meteyer *in litt.*). All were in good body condition. The two nestlings with deformed legs both had folding fractures of the legs without evidence of trauma in the surrounding tissue. Such problems sometimes occur in domestic birds when the diet does not contain the correct balance of calcium, phosphorus, vitamin D and sometimes vitamin A. It was difficult to document or speculate about a cause for nutritional imbalance in wild birds

and the fractures may have been due to trauma at an early stage in bone growth and development before ossification was complete.

Cross sections of the beaks of all three nestlings showed occasional areas of epithelial disorganization (C. U. Meteyer *in litt.*). Similar cells were also found in the beak epithelium of cross-beaked adults from Case #16169. One of the nestlings with a deformed leg also had similar inclusions in the feather follicle. Although there was some suggestion of viral particles in these cells, the inclusions could also have been accumulation of a cell product such as keratin. Slides were sent to Kansas State University for electron microscopy and no viral inclusion bodies were found (R. Sohn *in litt.*)

Blood chemistry.—Adult Black-capped Chickadees with deformed beaks had significantly lower concentrations of uric acid in their blood than did chickadees with normal beaks ($U = 2.50$, $n = 13$, $P = 0.008$). There were no other differences between normal and deformed chickadees (all $P > 0.10$; Tables 13, 14). One apparently normal adult had unusually high values for glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase, and total protein.

Radiography.—High-resolution radiographs of nine adult Black-capped Chickadees and one adult Red-breasted Nuthatch with beak deformities showed no evidence of skeletal malformations when compared to radiographs of normal chickadees. In chickadees with greatly elongated and crossed beaks, the rhamphotheca was not only overgrown distally but thicker than normal along the distal end of the maxillary and

mandibular bones. Radiographs of the nestlings clearly showed the folding leg fracture that resulted in the twisted leg.

CONTAMINANTS ANALYSES

Food samples.—Among the 18 samples of sunflower seeds tested, none had a detectable concentration of any of the 24 organophosphate pesticides or six carbamates for which they were screened (all concentrations <0.500 µg/g wet weight). Only 6 of 18 organochlorine pesticides were found in detectable concentrations (Table 15). Most of these were detected in less than 20% of the samples. The Aroclor 1242 mixture of PCBs was detected in three samples and DDT metabolites were detected in two. γ-HCH (lindane) was detected in only three samples, with a maximum concentration of 0.00684 µg/g. Hexachlorobenzene (HCB) was the most prevalent organochlorine detected, occurring in seven (44%) of the samples. HCB was detected in four of five samples that had been purchased at Alaska Mill & Feed in Anchorage; in both samples from Valley Feed & Seed in Eagle River; and in the single sample from Eagle Hardware in Anchorage. These included at least two different suppliers from different states (Table 2). HCB was detected in residential samples from two of six residences reporting deformed chickadees and in both samples from homes where no deformities had been noted.

We found detectable concentrations in sunflower seeds of all 19 metals and trace elements for which we screened except for inorganic mercury (Table 16). Among the others, all elements except two were found in all 18 samples tested; aluminum occurred in 16 and lead in only four of the samples tested. Concentrations of a few elements were

unusually high among individual samples. One commercial sample from Anchorage had 10 times the median concentration of aluminum and twice the median concentration of arsenic. One commercial sample from the Mat-Su Valley (different retailer) had four times the median concentration of selenium and twice the median concentration of arsenic. Seeds from the same retailer in Anchorage had three times the median concentration of selenium, suggesting this may have been a characteristic of the supplier rather than retailer storage. Among the four samples with detectable concentrations of lead, one residential sample had twice the median concentration. Notably, two severely deformed chickadees from this same residence had concentrations of lead in their livers (0.417–0.488 μg/g dry weight) that were twice the median concentration detected in adults (see below).

Metals and trace elements in adult and nestling chickadees. —Adult Black-capped Chickadees had detectable concentrations in liver tissue of methylmercury and 12 of 19 elements for which we tested (Table 17). The only significant difference we found between normal adults and those with deformed beaks was in the concentration of iron, which was significantly lower among deformed adults (median 1180 μg/g dry weight, IQR 604–1573, $n = 6$) than among normal adults (median 1865 μg/g dry weight, IQR 1373–2274, $n = 14$; generalized Wilcoxon score (W) = 4.54, df = 1, $P = 0.03$; Figure 15).

We found detectable concentrations of 16 of 19 metals and trace elements in liver tissue of 12-day-old nestling Black-capped Chickadees (Table 18), including barium, boron, chromium, and strontium, which had not been detected in adults. Nestlings had significantly lower concentrations of cadmium, iron, molybdenum, lead, and selenium

than normal adults; however, concentrations of arsenic, magnesium, manganese, and zinc were significantly higher among nestlings (Wilcoxon score test; Figures 15,16; Tables 17, 18). We found no significant difference in concentration of any element among nestlings with both parents normal vs. those with either parent deformed; among nestlings with a deformed female parent vs. those with a normal female parent; or among nestlings from clutches in which all eggs hatched vs. those in which at least one egg was inviable.

Organochlorine pesticides in adult and nestling chickadees.—We found detectable concentrations of 12 of 25 organochlorine pesticides for which we tested among adults (Table 19) and 13 of 18 organochlorines tested for among 12-day-old nestling Black-capped Chickadees (Table 20). The most ubiquitous compounds were DDT and its metabolites, HCB, dieldrin, heptachlor epoxide, *trans*-nonachlor, and mirex. Only 6% of the adults and 14% of nestlings had detectable concentrations of γ-HCH (lindane).

Concentrations of several organochlorine compounds were significantly higher among normal adults than among nestlings (Figure 17, Tables 19, 20). These included four compounds that were correlated with percent lipid in body tissues: dieldrin ($Z = -4.62$, $P < 0.001$); HCB ($Z = -10.50$, $P < 0.001$); *p,p′*-DDE ($Z = –3.27$, $P = 0.001$); and *trans*-nonachlor ($Z = -7.34$, $P < 0.001$). Concentrations of two other compounds, not correlated with lipid loads, also were higher among normal adults than nestlings: heptachlor epoxide ($W = 23.73$, $P < 0.001$) and mirex ($W = 33.23$, $P < 0.001$).

Two compounds were directly associated with beak deformities in adult chickadees. Heptachlor epoxide, which was not correlated with percent lipids in adult body tissue

(Kendall's tau = 0.17, z = 1.48, *n* = 35, *P* = 0.14), occurred in significantly higher concentrations among deformed adults (median 0.000540 µg/g wet weight, IQR 0.000408–0.000913, *n* = 13) than among normal adults (median 0.000355 µg/g wet weight, IQR 0.000334–0.000522, *n* = 22; W = 4.89, df = 1, *P* = 0.03; Figure 17). The semivolatile organic compound 1,2,3,4-tetrachlorobenzene, a derivative of the pesticide lindane, was tested for only among a sample of banded adults that were known to have developed beak deformities within the previous 1–5 months in comparison with a matched sample of normal adults. Concentrations of this compound among the recently deformed adults (median 0.000623 µg/g wet weight, IQR 0.000350–0.000794, *n* = 8) were marginally higher (W = 3.52, *P* = 0.061) than those among the eight normal birds, among which only a single individual had a concentration (0.000723 µg/g wet weight) above detection limits (0.000490–0.000545 µg/g wet weight). Concentrations of 1,2,3,4-tetrachlorobenzene were not correlated with lipid concentrations in body tissue (Kendall's tau = 0.07, *P* = 0.74).

Concentrations in nestlings did not vary significantly for any of the organochlorine compounds relative to the presence of a beak deformity in the nestling's male or female parent, or relative to hatchability of other eggs in the nestling's clutch.

Organochlorines in chickadee eggs.—There were detectable concentrations of 12 of 19 organochlorine pesticides in eggs of Black-capped Chickadees (Table 21). The most pervasive were HCB and *p,p′*-DDE, which were measurable in all 39 eggs tested. *Trans*-nonachlor and γ-chlordane were detected in more than half the eggs; dieldrin,

heptachlor epoxide, and *p,p′*-DDT occurred in more than 25% of the eggs; and γ-HCH (lindane) was detected in only 10% of the eggs.

None of these compounds differed in concentration between eggs from clutches of normal parents and those from clutches of either deformed females or deformed males. Two organochlorine pesticides were associated with hatching failure. HCB occurred in significantly higher concentrations in eggs from clutches in which at least one egg failed to hatch (median 0.00467 µg/g wet weight, IQR 0.00327–0.00550, $n = 23$) than in eggs from clutches in which all eggs hatched (median 0.003525 µg/g wet weight, IQR 0.00327–0.00467, $n = 16$; W = 6.59, df = 1, $P = 0.01$; Figure 18). The association for *p,p′*-DDD with hatching failure was equivocal. Concentrations were detectable in 4 of 23 clutches with unhatched eggs and only in 1 of 16 clutches with all viable eggs (W = 4.06, df = 1, $P = 0.04$; Figure 18); however, all concentrations except one were close to detection limits. The exception was a single egg with a concentration of 0.00265 µg/g wet weight, which was more than three times the detection limit. This egg was from a clutch of six eggs that was abandoned during egg-laying and in which one of the eggs had dry, shriveled contents and a very thin shell. This egg also had the highest concentration of *o,p′*-DDE (0.00119 µg/g wet weight) and the third and fourth highest concentrations of *p,p′*-DDE (0.06010 µg/g wet weight) and *p,p′*-DDT (0.00153 µg/g wet weight), respectively, among all 39 eggs tested.

PCB mixtures in adult and nestling chickadees.—There were detectable concentrations of PCBs in all adult Black-capped Chickadees ($n = 35$) and in 96% of 12-day-old nestlings ($n = 49$; Tables 20, 22). The most prevalent commercial mixture in tissue

samples was Aroclor 1260, which was found in 95–96% of both adults and nestlings and occurred in greater concentrations than any of the other mixtures. Aroclor 1254 was detected in all adults but only about half of the nestlings; Aroclor 1242 was detected in only 16% of the adults; and Aroclor 1248 was not detected in any chickadees. PCB concentrations were not correlated with lipid loads in either adults or nestlings.

Normal adults had significantly higher concentrations than nestlings of total PCBs (W = 11.29, df = 1, $P = 0.001$), Aroclor 1254 (W = 4.23, df = 1, $P = 0.04$), and Aroclor 1242 (W = 6.05, df = 1, $P = 0.01$). However, concentrations of Aroclor 1260 did not differ by age (W = 0.58, df = 1, $P = 0.45$; Figure 19). Concentrations of neither total PCBs nor any of the mixtures differed significantly between normal adults and those with beak deformities (Figure 19). Concentrations in nestlings did not vary significantly for either total PCBs or any of the Aroclor mixtures relative to the presence of a beak deformity in the nestling's male or female parent, or relative to hatchability of other eggs in the nestling's clutch.

PCB mixtures in chickadee eggs.—PCBs were detected in all 39 eggs tested (Table 21). The median concentration of total PCBs in all eggs (0.03930 µg/g wet weight) was about five times the median concentration measured in 12-day-old nestlings (0.00707 µg/g wet weight) and almost twice the median concentration measured in adults (0.02100 µg/g wet weight). Concentrations of total PCBs were significantly higher (W = 4.70, df = 1, $P = 0.03$) among eggs from clutches with one or more inviable eggs (median 0.02535 µg/g wet weight, IQR 0.01173–0.07050, $n = 16$) than among eggs from clutches

in which all eggs hatched (median 0.04240 µg/g wet weight, IQR 0.02740–0.10900, *n* = 23; Figure 18).

We tested for specific Aroclor mixtures in only 10 eggs, all of which had failed to hatch. Aroclor 1260 was detected in 100% of these eggs and occurred in a median concentration about five times higher than that of Aroclor 1254, which occurred in 50% of the egg samples. Aroclor 1242 and 1248 were not detected at all. There was no significant difference in concentration of total PCBs or of any of the Aroclor mixtures between eggs from clutches attended by normal vs. deformed parents, either male or female.

Specific PCB congeners in adults and nestlings.—Eight normal and eight newly deformed adult Black-capped Chickadees were analyzed for 12 PCB congeners that have been found to have dioxin-like effects (Table 23). Five of eight mono-*ortho* PCBs were detected in all 16 adults and a sixth was detected in 15 adults. Among the four non-*ortho* PCBs, only one (PCB 77) was detected, and only in a single bird. We found similar patterns of occurrence among the 49 12-day-old nestlings we tested (Table 24). All eight of the mono-*ortho* PCBs were detected in nestlings, all of which occurred in the majority of the birds tested. All of the non-*ortho* PCBs were also found, although these were detected in only 6–18% of the individuals tested.

Concentrations were significantly higher among normal adults than nestlings for PCB 167 (W = 7.35, df = 1, *P* = 0.007; Figure 20); no age differences were found for PCB 77, 126, 105, 118, or 189. Direct comparisons could not be made between adults and

nestlings for the other congeners because of analytical differences in coelution or in detection limits.

Only one congener, PCB 123 (which was coeluted with PCB 149), occurred in significantly higher concentrations among adults with beak deformities (median 0.2565 ng/g wet weight, IQR 0.1335–0.5440, $n = 8$) than among those with normal beaks (median 0.1205 ng/g wet weight, IQR 0.0380–0.1875, $n = 8$; W = 4.18, df = 1, $P = 0.04$; Figure 20). All three nestlings with detectable concentrations of PCB 126 had parents with deformed beaks; these same individuals were three of the four nestlings with detectable concentrations of PCB 77.

Specific PCB congeners in chickadee eggs.—We detected all eight of the mono-*ortho* PCB congeners in almost every egg we tested (Table 25). Similar to the patterns shown by adults and nestlings, we detected each of the four non-*ortho* PCBs in <25% of the 39 eggs tested. Median concentrations in eggs were 2–3 times greater than those found in adults and 3–7 times greater than those found in 12-day-old nestlings (Tables 23–25). There were no significant differences in concentrations of any specific congeners between eggs from clutches attended by normal vs. deformed parents (male or female). Concentrations in eggs from clutches with one or more unhatched eggs did not differ from concentrations in eggs from clutches in which all eggs hatched.

PCDDs and PCDFs in adults and nestlings.—Very few of the 16 adult Black-capped Chickadees tested had detectable concentrations of any of the most highly toxic congeners of the PCDDs or PCDFs, although laboratory detection limits were relatively high (Table 26). We were unable to compare concentrations between normal and newly

deformed adults or between adults and nestlings. Detection limits for nestlings were 10–20 times lower than those for adults, and all of the most highly toxic PCDDs and PCDFs were detected in one or more 12-day-old nestlings, dioxins more frequently than dibenzofurans (Table 27). The most pervasive congener was 1,2,3,4,6,7,8-HpCDD, which was detected in 90% of the 29 nestlings tested. There were no differences in concentrations of any of these individual compounds between nestlings with normal vs. deformed parents (either male or female) or between nestlings from clutches with at least one unhatched egg vs. those in which all eggs hatched.

PCDDs and PCDFs in chickadee eggs.—Although laboratory detection limits were higher for eggs than for nestlings, PCDDs and PCDFs were detected in about the same proportions of each cohort. PCDDs were detected in 8–90% of the 39 eggs tested; 1,2,3,4,6,7,8-HpCDD was again the most pervasive of these compounds (Table 28). Among the PCDFs, only 1,2,3,4,6,7,8-HpCDF was detected in more than one egg; it was found in 33% of the eggs tested. Median concentrations of PCDDs were about 5–10 times higher in eggs than in nestlings; median concentrations of 1,2,3,4,7,8,9-HpCDF were similar in the two cohorts.

Eggs from clutches tended by deformed parents had significantly lower total concentrations of PCDDs with seven chlorine atoms (HpCDDs) than did eggs from clutches tended by normal parents. This held true whether it was the female ($W = 4.17$, $df = 1$, $P = 0.04$) or either parent ($W = 7.69$, $df = 1$, $P = 0.006$) that had a beak deformity. Median concentration of these compounds was 0.03600 ng/g wet weight (IQR 0.01850–0.06200, $n = 29$) in eggs of normal pairs and 0.00715 ng/g wet weight (IQR 0.00540–

0.02100, $n = 10$) in eggs of deformed adults. Much of this effect was due to the specific congener 1,2,3,4,6,7,8-HpCDD (Figure 21), which occurred in significantly higher concentrations in eggs of normal parents vs. deformed parents ($W = 5.20$, df = 1, $P = 0.02$). Concentrations of PCDDs and PCDFs did not differ between eggs from clutches in which all eggs hatched and those that had one or more fail to hatch.

Organophosphate pesticides.—We tested for only one organophosphate pesticide, chlorpyrifos, in samples of eight normal and eight newly deformed adult Black-capped Chickadees. This compound was detected in only two individuals, both normal adults, at concentrations just above the detection limits (Table 22).

Analysis of Vitamins in Eggs

All-*trans*-retinol concentrations were the highest of the retinoids in the chickadee eggs, ranging from 491 to 9250 ng/g wet weight (Whyte and Tillitt 2003; Appendix A). The mean chickadee egg concentration of the antioxidant α-tocopherol was 85 $\pm$ 16 µg/g wet weight. For carotenoids, both astaxanthin and canthaxanthin concentrations in eggs were below the detection limit, while the mean β-carotene concentration was 638 $\pm$ 115 ng/g wet weight.

Blood Flow Cytometry

Flow cytometry analysis of the Black-capped Chickadee blood indicated that the adults with beak deformities had a highly significant amount of DNA damage compared to the normal adults ($\chi^2 = 152.4$, df = 1, $P < 0.001$; Easton 1999; Appendix B). In addition, the samples from normal birds in Palmer that were kept in snow for 4 hours

may have suffered degradation, since their CV DIF values varied significantly from the other samples from normal birds that had been frozen immediately on dry ice ($\chi^2 = 34.8$, df = 1, $P < 0.001$; Easton 1999; Appendix B).

DISCUSSION

PREVALENCE

The prevalence (8.1%) and concentration (over 1,400 individuals) of beak deformities among Black-capped Chickadees in Alaska are the highest ever recorded among adults within a wild bird population anywhere. Abnormal beaks are relatively rare among adult birds, with most published reports being of single aberrant individuals (see Craves 1994 for review). The prevalence among adult chickadees was four times higher than the maximum rate documented for any passerine (1.84% of 271 Blue Tits; Pomeroy 1962) and 20 times higher than the frequency of abnormal beaks (0.38%) found in a sample of about 10,000 European Starlings (*Sturnus vulgaris*) (Hicks 1934).

It is possible that our estimate of prevalence was positively biased if the probability of capture was higher for chickadees with beak deformities than that for normal birds. During the summer, deformed birds may be more likely to nest in boxes than in natural cavities, simply because of the difficulty in excavating a cavity. Both members of the pair typically take part in excavation, with the female taking the lead in nest site selection (Odum 1941, Smith 1991). During the winter, deformed birds may be more attracted to baited traps because of difficulty in procuring natural foods. Normal chickadees,

however, are adept at finding supplemental food sources and will regularly forage on them when available (Brittingham and Temple 1988, Desrochers et al. 1988).

Since the 1990s, prevalence of beak deformities has clearly increased in the Anchorage area among Black-capped Chickadees. During 1992–1999 we found no beak deformities among 491 Black-capped Chickadees, 83 Boreal Chickadees, and 8 Red-breasted Nuthatches captured in mist nets at three sites in the Anchorage area during the breeding season (C. Handel, unpubl. data). The prevalence of beak deformities among Black-capped Chickadees in nest boxes has shown a fairly linear increase since then, from about 7% in 2000 to 14% in 2004. In contrast, prevalence of beak deformities at our capture sites during the current decade has remained at low, background levels for both Boreal Chickadees (about 1% with possible incipient deformities) and Red-breasted Nuthatches (0.5%). This difference among species was surprising, since all three cavity-nesting species occur within the same affected geographic areas, with only slight differences in use of habitats and foraging substrates. Red-breasted Nuthatches are relatively uncommon in our study areas, however, and the large number of observations reported by the public of nuthatches with overgrown beaks suggests that prevalence might be higher in other parts of the region.

Although we have no objective measure of prevalence of beak deformities among the corvids, the large number of sightings of Northwestern Crows, Black-billed Magpies, and Steller's Jays suggests that prevalence is higher than the normal background level for these species. Although they overlap geographically with Black-capped Chickadees within the same broad region, there are significant differences in habitats used,

particularly with crows foraging most frequently in marine intertidal areas (Verbeek and Butler 1999). Reporting rates for deformed Downy Woodpeckers have also been relatively high, but this cavity-nesting species uses much of the same habitats and foraging substrates as the chickadees.

DISTRIBUTION

Geographically, there appeared to be two epicenters from which the deformities may have spread: one in the Mat-Su Valley and one in Bristol Bay. It is difficult to determine how much of the apparent temporal changes in distribution were due to changes in observer effort, which was strongly affected by media coverage. Regardless of this potential bias, the initial deformities recorded did occur in areas separated by more than 400 km. Our research suggests that Black-capped Chickadees in Alaska are unlikely to move such long distances. We found that they are highly faithful to breeding and wintering territories, few adults move long distances once established, and males move farther than females, confirming patterns found elsewhere within their range (Weise and Meyer 1979, Desrochers et al. 1988, Smith 1991). The greatest distance we recorded was for a juvenile that dispersed 28 km from its natal site; this is comparable to the maximum juvenile dispersal distance of 39 km recorded in Alberta (Desrochers et al. 1988). Thus, the two broadly separated records at the apparent onset of this phenomenon suggest that the causative agent was widely spread across the region rather than that the chickadees themselves transported it.

SEASONAL PATTERNS

Seasonal prevalence of beak deformities was a function primarily of the rate at which deformed birds died and less so of the rate at which new deformities developed or existing deformities apparently reversed. There appeared to be a higher probability of deformities developing during late winter than during other parts of the year, although this pattern was not strong and sample sizes were small. The rate at which deformities apparently reversed was quite low, although we were surprised to encounter this situation at all.

Prevalence was extremely variable during the nonbreeding months, and was strongly influenced by how cold the weather was during the depths of winter (January–February). Studies of chickadees at lower latitudes also found both interannual and seasonal variation in survival, much of which could be attributed to differences in temperature and the availability of supplemental food resources (Brittingham and Temple 1988, Desrochers et al. 1988). During the shortest winter days in Alaska, chickadees increase their foraging intensity, which is more strongly influenced by temperature than daylength (Kessel 1976). During winter, Black-capped Chickadees accumulate fat reserves each day that constitute about 10% of their body mass, which they then metabolize at night to keep warm; rates of mass increase are most rapid during the shortest days (C. Handel, unpubl. data). Daily patterns of mass gain are an important survival mechanism among other species of parids as well (e.g., Pravosudov and Grubb 1998, Pravosudov and Lucas 2000).

Severely deformed birds had difficulty foraging and often spent prolonged periods at feeders manipulating foods. They often foraged on the snow beneath feeders, picking up scraps dropped by other birds. Because of their increased time away from protected foliage, they were likely more susceptible to predation. Deformed chickadees also had difficulty preening and many had dirty, matted plumage by late winter. Mortality rates of deformed chickadees were probably proportionately higher than those of normal birds during the shortest, coldest days of winter. A few deformed chickadees with dirty, almost jet black breast feathers were found dead at residences in winter, most likely due to starvation or hypothermia.

Developmental Onset of Deformities

We found little evidence that the beak deformities in chickadees were congenital in nature. In contrast, Ohlendorf et al. (1986a) found 61% of nests of aquatic birds with at least one dead embryo or at least one embryo or chick with an obvious malformation due to selenium toxicity. The prevalence of beak malformations among Double-crested Cormorant (*Phalacrocorax auritus*) chicks from colonies in the Great Lakes affected by PCBs, PCDDs, and PCDFs was 0.52% (Fox et al. 1991), ten times higher than that recorded among the chickadee nestlings (0.05%). It might have been problematic to detect developmental malformations because of the short fledging period, however, especially since the beak is not fully grown by the time young leave the nest (C. Handel, unpubl. data). But if prevalence of congenital malformations were indeed high, reproductive success would have been much lower or we would have detected a higher prevalence of beak deformities among juvenile birds in the fall.

We were surprised to recapture so many birds that changed from apparently normal to significantly deformed. This finding, coupled with the low prevalence of deformities among hatching-year birds, suggests that either some factor alters a chickadee's truly normal status at older stages or that there is a delayed onset of an underlying abnormal condition. A similar case was recorded for a Great Tit in Europe, which had been observed for four years with a normal beak and then developed within four months a maxilla that was twice normal length (Pomeroy 1962). Blood flow cytometry indicated that chickadees with beak deformities had a significantly greater amount of chromosomal damage than normal chickadees (Easton 1999). Such damage to the DNA can result from exposure to contaminants (Custer et al. 1994) or some other specific mutagen, such as a disease organism. We did collect blood flow cytometry samples from several individuals both before and after they had changed from "normal" to deformed. Analysis of these samples, matched with pairs from birds that remained normal, would reveal whether any chromosomal damage was associated with the apparent change in state.

Most beak deformities for which we witnessed development were fairly subtle in form at the onset, with a slight overgrowth or asymmetry of the mandibles. Thus, the incipient condition could have been congenital and latent, only becoming obvious as the bird aged. In only one case did we document an adult in which there was a definite congenital malformation, a pronounced lateral curvature of the maxilla that involved the bone.

Clinical Signs

In all but the above case, beak deformities in Black-capped Chickadees appeared to be restricted to the rhamphotheca and neither the cranium nor the underlying bones of the beak itself were malformed. Cross-sections of the beaks revealed occasional areas of epithelial disorganization and abnormal inclusions of what may have been keratin; irregular growth ridges, scaly plates, brittleness, and abnormal thickening of the keratin layers all suggested abnormal growth patterns of the rhamphotheca, including extremely rapid growth in some individuals. In almost all cases the rhamphothecal layer of either the maxilla or mandible or both was overgrown. This characteristic suggests that the deformities were not due to selenium toxicity, which generally results in necrosis of the beak (Ohlendorf 2002).

The rhamphotheca constantly grows throughout a bird's life and is continually worn down through the process of pecking and feeding (Stettenheim 1972). The maxillary keratin layer grows in sheets in a continuous cranioventral manner and has various growth centers in the vascular layer that overlies the bone; the number and locations of the growth centers vary among species (Clipsham 1994). The keratin layer of the mandibular beak grows at a faster rate than the maxillary layer, with a broader-based, more uniform contact surface that extends caudally, following the margin of the jaw (Lucas and Stettenheim 1972; Clipsham 1989, 1994). Several studies of passerines have documented seasonal changes in beak length, which have been related primarily to changes in diet and the concomitant rates of wear and abrasion (Clancey 1948; Davis

1954, 1961; Gosler 1987; Morton and Morton 1987). Variation in beak size may also be linked to hormonally mediated growth rates (Matthysen 1989).

If the jaws are not aligned properly, the rhamphotheca may overgrow (Stettenheim 1972). Apposition of the tips of the mandibles inhibits overgrowth. Any factors that affect bone growth or cause malocclusion of the maxilla and mandible can result in overgrowth of the rhamphothecal covering of the beak. Beak elongation can be genetic or developmental in origin (Thompson and Terkanian 1991). BMP4, a protein that is normally associated with the development of the skull and other bones, is one of the molecules that helps control the shape of the beak during development; experimental manipulation of the gene for this protein can alter the shape of the beak (Wu et al. 2004). Thus, it is possible that a mutagen affecting this or related genes could induce malformation of the beak.

Overgrowth of the beak can be a symptom of liver disease (Harrison 1986). Necropsy and histopathology of deformed chickadees did not reveal any abnormal pathology of internal organs, including liver, spleen, and thyroid. Similarly, there was no other evidence of the gross pathology characteristic of GLEMEDS, including subcutaneous, pericardial, and peritoneal edema, club feet, or missing eyes (Gilbertson et al.1991).

There was no evidence of infection with the parasitic scaly mite *Cnemidocoptes pilae*, which can cause lesions on the beak (Tully et al. 2000). Tests were negative for both avian polyomavirus and the circovirus that causes psittacine beak and feather disease, which is not currently known to infect passerine birds (Tully et al. 2000). Tests included

isolation attempts through cell cultures and electron microscopic inspection of skin scrapings for inclusion bodies. Lack of melanin in some of the feathers near the base of the beak could have been caused by a deficiency of copper (Tully et al. 2000), but this was not tested for.

Blood serum biochemistry did not indicate that deformed chickadees were responding to any type of infection. The only difference between normal and deformed chickadees was in uric acid, which was highly elevated among some of the normal birds. High levels of uric acid could be indicative of renal disease but were more likely the result of dehydration while being held in captivity for testing (Ritchie et al. 1994). The single normal chickadee with elevated levels of enzyme activity, glucose, and total protein could have been responding to an undiagnosed disease condition. Elevated levels of AST can be indicative of liver disease or pesticide intoxication (Ritchie et al. 1994).

Overgrowth of the beak can also be caused by nutritional deficiencies of vitamin A, vitamin D_3, or calcium, or by an imbalanced ratio of calcium and phosphorus (Altman 1986, Harrison and Harrison 1986). The dry, reddened skin and flaky keratin layers of the beak and legs could be symptomatic of a deficiency of vitamin A or calcium (Tully et al. 2000). However, there were no abscesses in the oral cavity or respiratory tract, or discharges from the nares, which are symptomatic of vitamin A deficiency (Tully et al. 2000).

Vitamin D_3 (cholecalciferol), which is the form of vitamin D used by birds, is synthesized in the skin when 7-dehydrocholesterol is exposed to sunlight and is then absorbed through the skin or ingested during preening (Ritchie et al. 1994). Vitamin D_3

helps stimulate the absorption of calcium in the gastrointestinal tract and regulates the balance of calcium and phosphorus (Ritchie et al. 1994). Juvenile birds that were housed outdoors in shady flights showed overt signs of rickets, a developmental disease caused by calcium deficiency, when fed diets low in calcium and vitamin D_3 (Ritchie et al. 1994). Wild birds living at high latitudes experience long periods of low sunlight but are thought to consume diets with high levels of vitamin D_3 (Klasing 1998).

Seed-based diets are usually deficient in vitamin A and calcium; in addition, the high fat content of seeds can interfere with calcium uptake (Harper and Skinner 1998). Thus, if chickadees are overly reliant on sunflower seeds at feeders, nutritional deficiencies could develop. Chickadees exhibited several conditions that have been found to be symptoms of vitamin D_3 or calcium deficiency in poultry: thin-shelled eggs, folding fractures of legs in nestlings, and bowing or rotation of the tibiotarsus in nestlings (Austic and Scott 1984). Such deficiencies can also cause a lackluster, loose appearance of feathers due to breakdown of interlocking barbules (Harrison and Harrison 1986); abnormal, loose feathers were also found among some of the adult chickadees with beak deformities. There was no evidence, however, of rickets in nestling chickadees or osteomalacia in adults, conditions due to improper calcification of the skeleton and symptomatic of chronic imbalances of calcium, phosphorus, or vitamin D_3 (Tully et al. 2000). A high frequency of lipomas, such as that found in one severely deformed female, are often found among domestic birds maintained on a high fat diet, such as sunflower seeds (Harrison and Harrison 1986).

Effects of Deformities on Reproductive Success

Reproductive success, in terms of clutch size, hatching success, and fledging success, was extremely high among Black-capped Chickadees, but there were different, significant effects linked to the presence of a beak deformity in the male and female parents. The average clutch size was 7.8 eggs for Alaskan chickadees, which was similar to that reported in other parts of their range, in which 6–8 were the most common clutch sizes (Smith 1993). Surprisingly, clutches tended by males with deformed beaks averaged 0.7 egg larger than those of normal pairs or pairs in which the female was deformed. Genetic studies of these offspring revealed that there were high rates of extra-pair young in the broods; nests commonly included not only within-pair young, but young from the same mother but different father; young from the same father but different mother; and young dumped by a different female that did not belong to either parent (L. Pajot, unpubl. data). Deformed males were not only cuckolded more frequently but also raised a higher proportion of dumped eggs unrelated to either parent. Deformed females supported a higher proportion of extra-pair young but a similar number of dumped young compared to nests of normal females (L. Pajot, unpubl. data).

Hatching success of Alaskan chickadees was higher than that recorded for populations at lower latitudes (4.0–5.0 nestlings/clutch; Smith 1993); there was no evidence of high embryonic mortality typical of the chick-edema syndrome of the Great Lakes or high selenium exposure. Among deformed females, however, hatching success was significantly depressed, both in terms of proportion of nests hatching any young and proportion of eggs hatching in successful nests. In addition, we documented several

additional cases in which the deformed female parent abandoned the nest after she was banded; most normal females tolerated such disturbance quite well and returned to incubate eggs immediately upon release.

Several deformed females exhibited abnormal incubation behavior as well, with eggs scattered across the nesting material instead of being arranged neatly in a nest cup. It is unclear whether this abnormal behavior was due to physical limitations imposed by the deformity itself or to hormonal disruption of incubation behavior. Both male and female Ringed Turtle-Doves (*Streptopelia risoria*) showed reduced courtship activity after being exposed experimentally to DDE (Haegele and Hudson 1977). Tree Swallows (*Tachycineta bicolor*) breeding in areas highly contaminated with PCBs exhibited abnormal nest-building behavior (McCarty and Secord 1999). Herring Gulls (*Larus argentatus*) breeding in the Great Lakes were significantly less attentive during incubation than those from less PCB-contaminated colonies (Fox et al. 1978). The reduced hatching success shown by deformed female chickadees could have been a result of either reduced quality of the eggs or of impaired incubation.

Although fledging success of normal chickadees in Alaska (6.7 young per successful brood) was high relative to that in other parts of their range (4.0–6.6; Smith 1993), deformed males raised a significantly smaller proportion of the young in their nests than did either normal pairs or pairs with deformed females. It is likely that the physical deformity impaired the male's ability to gather enough food for the nestlings. Brood sizes of deformed females were smaller than those of either normal pairs or deformed

males, so it's possible that the deformity of the female had less of an impact on her ability to gather sufficient food for the brood.

Potential Effects of Contaminants

Analysis of contaminants concentrations offered no support for the hypothesis that selenium or any other element was responsible for beak deformities among chickadees, and some support for organochlorine compounds as a potential cause. Concentrations of selenium in liver tissues of adults and nestlings ranged from <0.26–6.66 µg/g dry weight, all less than 10 µg/g, which is considered the background level for this element (Ohlendorf 2002). Levels of arsenic, boron, copper, mercury, and zinc were all well below levels thought to have any effects in birds (U. S. Department of Interior 1998). Concentrations of all elements were low in every chickadee tested and they did not differ between normal adults and those with deformed beaks.

The compound 1,2,3,4-tetrachlorobenzene, which is used in dielectric fluids and is also a metabolite of the pesticide γ-HCH (lindane), occurred in marginally higher concentrations among newly deformed adults compared with normal adults, but sample sizes were small. Little is known about the potentially xenoestrogenic effects of this compound. Additional samples of newly deformed and normal adults would help determine whether this compound is likely to be involved.

Concentrations of heptachlor epoxide were significantly higher among deformed than normal chickadees, but there is no evidence that this compound causes beak deformities and concentration levels were extremely low compared to known toxicity levels (Blus

2002). Heptachlor epoxide is a highly persistent metabolite of heptachlor, a cyclodiene insecticide that was once widely used but gradually phased out because of its extreme toxicity to wildlife (Blus 2002). With the advent of more sensitive tests, recent research has shown that heptachlor epoxide, a xenoestrogen, can induce an increase in oxidants and DNA damage in human tissues; concentrations in adipose breast tissue were positively correlated with the prevalence of breast cancer in the biopsies (Richard et al. 2005). Rapid proliferation of keratin cells in overgrown beaks may represent a type of cancerous growth that is induced by a xenoestrogenic compound. Clastogenic activity, DNA damage as evidenced by flow cytometry, has been shown to be closely associated with cancer incidence (Easton 1999). In addition, immunocompetence may be reduced because of damage to the lymphocytes (Easton 1999).

PCB 123 was the only congener to occur in significantly greater concentrations among newly deformed adults compared with normal adults among all of the non-*ortho* and mono-*ortho* PCB congeners tested. Chickadee tissue concentrations, however, were extremely low. Tree Swallows in Hudson Bay that showed severe reproductive impairment linked to PCBs had concentrations of all congeners that were more than 100 times greater than those found in chickadees (Secord et al. 1999). This pattern held whether comparing tissues of adults, nestlings, or eggs. PCB concentrations in eggs from various aquatic bird species in the Great Lakes Basin that showed embryonic teratogenicity were also generally more than 100 times greater than concentrations recorded in the chickadees (e.g., Gilbertson et al. 1976, Gilman et al. 1977, Ellenton and McPherson 1983). Given such low concentrations of PCBs in chickadee eggs and

nestlings, it is not surprising that few congenital beak malformations were found and that reproductive success was very high.

There is some evidence, however, that exposure to low levels of PCBs *in ovo* could result in a delayed onset of rhamphothecal overgrowth. Cormorants from Great Lakes colonies that were exposed to low PCB levels developed bill malformations only after being held in captivity for two weeks without natural daylight (Kuiken et al. 1999). A deficiency of the hormone derived from vitamin D_3, which is used for calcium regulation, may be involved in PCB-related beak malformations (Rice et al. 2002). Chickadees in Alaska may be deficient in vitamin D_3 during the shortest days of winter, and relying on calcium-deficient sunflower seeds may further exacerbate this condition. Thus, there could be a synergistic condition involving low levels of PCBs and deficiency of calcium and vitamin D_3. The delayed onset of deformities and increased frequency of their development during late winter would be consistent with such a mechanism.

We were limited in our ability to test the potential of the most toxic organochlorine compounds, the PCDDs and PCDFs, as causes of beak deformities among adult chickadees. Laboratory detection limits for these compounds were very high and most concentrations fell below them; so we were unable to make meaningful comparisons between normal adults and newly deformed adults. Unfortunately, the detection limits coincided with the levels at which concentrations for Double-crested Cormorant chicks were correlated with PCDD-related brain asymmetry (Henshel et al. 1997). Given the high toxicity of PCDDs and PCDFs and the potential for both local and long-distance sources, they deserve further investigation as possible causes of deformities.

Although hatching success was relatively high among chickadees, those clutches that had one or more eggs fail to hatch were associated with higher concentrations of total PCBs, HCB, and DDT metabolites. Concentrations of total PCBs were very low, however, relative to those found in Tree Swallows in Hudson Bay (Secord et al. 1999) and in aquatic birds in the Great Lakes Basin (Gilbertson et al. 1976, Gilman et al. 1977, Ellenton and McPherson 1983), where rates of reproductive failure were high.

HCB is a highly persistent organochlorine that had been widely used as a fungicide to protect seeds until it was banned in 1965 in the U. S. It is also formed as a by-product in the production of other chemicals and in burning of municipal waste. HCB exposure can induce porphyria, a severe skin disorder, and cancer of the liver, kidneys, and thyroid (ATSDR 1996). The effects of the organochlorine pesticide DDT, and its metabolites DDD and DDE, on eggshell thinning, embryotoxicity, and related reproductive failures have been well documented for many species of birds (Blus 2002). Although the use of DDT was banned in the U. S. in 1972, several other countries continue to use it around the world, primarily for mosquito control, and the metabolites are highly persistent in the environment (Blus 2002). The *p,p′*-DDE isomer is the most potent of these, but concentrations of the various isomers are generally correlated. The chickadee egg with the highest concentration of *p,p′*-DDD was from a clutch in which one of the eggs had a very thin shell and whose contents were desiccated, quite likely from increased porosity of the eggshell. *p,p′* DDE has been shown to alter the metabolism of calcium and prostaglandin in the eggshell gland (Lundholm 1997).

It was puzzling to find significantly higher concentrations of the some of the highly toxic PCDDs in eggs of normal parents than in eggs of deformed parents. One possible partial explanation would be that normal females could be more efficient at excreting these compounds into their eggs, thereby lowering their own body burdens. This would not, however, explain why concentrations would also be lower in eggs from clutches of deformed males mated to normal females. The occurrence of high rates of extra-pair young within broods has important implications for a study of this nature, particularly when attempting to draw inferences about trans-generational effects or relationships between members of a clutch or brood.

One cannot assume that concentrations within a particular egg or nestling are related to concentrations within the putative female parent. Nor can one assume that concentrations between putative siblings should be correlated. Parentage in birds can now be determined genetically using samples of saliva, skin, feathers, eggshell membranes, and other tissues (e.g., Ellegren 1992, Pearce et al. 1997, Groombridge et al. 2000, Strausberger and Ashley 2001, Handel et al. *in press*). Such testing should be undertaken simultaneously with contaminants sampling to verify that familial relationships have been correctly inferred.

An important question that remains to be resolved is what is the source of the contaminants that chickadees are incorporating into their body tissues? Comparison of concentrations in sunflower seeds with those in adults, eggs, and nestlings suggests that chickadees are more likely to be accumulating these compounds from natural foods than from seeds provided at residential bird feeders. The most notable exception might be

HCB, which occurred in detectable concentrations in about half of the seed samples, and was detected in every adult, egg, and nestling, with associated negative effects on hatching success. Our data also suggested that adults were picking up lead from sunflower seeds at residential feeders. Suet, another common feeder food, should also be tested as a possible source of contaminants or growth hormones.

Additional testing should be undertaken to determine if chickadees are ingesting PCBs, PCDDs, or PCDFs from natural foods and if there is a geographical pattern of contamination that might match the distribution of beak deformities. Long-distance transport across the Pacific Ocean from Asia could be an important source of contaminants for south-central Alaska (Bailey et al. 2000). Such transport corridors could help explain why there were apparently disjunct epicenters for the chickadee deformities and why beak deformities have so rarely been recorded north of the Alaska Range. Changes since the early 1990s in the composition or abundance of semi-volatile organochlorine compounds discharged from Asia might signal an important link to the increase in beak deformities in Alaska during that period.

Finally, more research should be done to determine at which developmental stage individuals are truly being affected and whether or not there is a delayed onset to the deformed condition. Obviously affected birds should be carefully examined to determine if there are more subtle clinical signs associated with the beak deformities, particularly since the state can be apparently labile. These may include measurements of brain and cranial asymmetry. Analysis of flow cytometry samples of individuals pre- and post-deformity can be analyzed to help determine the true timing of affliction.

Genetic paternity analysis should be completed on tissues for which we have contaminants samples to determine correct trans-generational and sibling relationships. Additional samples should be analyzed for specific PCB congeners, PCDDs, and PCDFs with detection limits sufficiently low to test if concentrations differ between normal and deformed adults. The role that calcium and vitamins A and D may play in this syndrome merits further research. Parallel tests on other affected species, particularly the Northwestern Crow, would be especially informative. Given its different ecology but similar beak deformities, such tests could help confirm the likely etiology of this condition. Other classes of contaminants, including polycyclic aromatic hydrocarbons, polybrominated flame-retardants, and perfluorinated compounds should also be tested.

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Table 1. Number of nest boxes erected each year in Anchorage, Eagle River, and the Mat-Su Valley for study of Black-capped Chickadees, Boreal Chickadees, and Red-breasted Nuthatches in south-central Alaska.

Year	Anchorage	Eagle River/Mat-Su Valley	Total
<2000	9	0	9
2000	248	45	293
2001	11	162	173
2002	3	0	3
2003	16	0	16
2004	2	0	2
Total	289	207	496

Table 2. Source of sunflower seeds submitted for contaminants screening. The most commonly used retail outlets supplying bird seed were selected from various areas in Anchorage and the Mat-Su Valley. All six residences sampled had large numbers of chickadees coming to feeders regularly during winter. The first four had each reported several chickadees with deformed beaks; the latter two had not observed any deformities before testing began.

Source	Location	Supplier	Year purchased
Retail			
Alaska Mill & Feed	Anchorage	Bulk supply, unknown	2000
Walmart	Anchorage	Penn Pak, Inc., GA	2000
Walmart	Wasilla	Penn Pak, Inc., GA	2000
Fred Meyer	Anchorage	F. M., Inc., OR	2000
Fred Meyer	Wasilla	F. M., Inc., OR	2000
Valley Feed & Seed	Eagle River	Animal Supply Co., WA	2000
Valley Feed & Seed	Wasilla	Animal Supply Co., WA	2000
Animal Food Warehouse	Wasilla	Animal Supply Co., WA	2000
Eagle Hardware	Anchorage	Kaytee Products, Inc., WI	2000
H&C Farm & Garden	Palmer	Bemis Co., WA	2000
Budget Feed & Farm	Palmer	Seawest	2000
Budget Feed & Farm	Palmer	Bulk supply, unknown	2000
Residential			
Animal Food Warehouse	Trapper Creek	Unknown	1999
Alaska Mill & Feed	Anchorage	Unknown	1997–98
Alaska Mill & Feed	Wasilla	Unknown	1999
Alaska Mill & Feed	Anchorage	Unknown	1999
Valley Feed & Seed	Eagle River	Unknown	1997
Alaska Mill & Feed	Anchorage	Unknown	1997

Table 3. List of organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), organophosphate (OP) pesticides, carbamates, and elements for which 18 samples of sunflower seeds were screened.

OC pesticides	PCBs	OP pesticides	Carbamates	Elements
o,p'-DDD	Aroclor 1242	acephate	aldicarb	aluminum
o,p'-DDE	Aroclor 1248	azinphos-methyl	carbaryl	arsenic
o,p'-DDT	Aroclor 1254	chlorpyrifos	carbofuran	barium
p,p'-DDD	Aroclor 1260	coumaphos	methiocarb	beryllium
p,p'-DDE	Total PCBs	demeton	methonyl	boron
p,p'-DDT		diazinon	oxamyl	cadmium
HCB		dichlorvos		chromium
α-HCH		dichrotophos		copper
γ-HCH (Lindane)		dimethoate		iron
dieldrin		disulfoton		lead
endrin		EPN		magnesium
heptachlor epoxide		ethoprop		manganese
α-chlordane		famphur		mercury
γ-chlordane		fensulfothion		molybdenum
oxychlordane		fenthion		nickel
trans-nonachlor		malathion		selenium
toxaphene		methamidophos		strontium
mirex		methyl parathion		vanadium
		mevinphos		zinc
		monocrotophos		
		parathion		
		phorate		
		terbufos		
		trichlorfon		

Table 4. Number of reports received of Black-capped Chickadees with beak deformities observed at different locations in North America between November 1991 and May 2005. The minimum number of individuals excludes known or probable replicate sightings of individual birds during multiple years at the same locations.

Location	Number of reports	Minimum number of individuals
Alaska	2,153	1,441
Other states	17	13
Canada	4	4
Total	2,174	1,458

Table 5. Estimated minimum number of individual birds by species reported with beak deformities from Alaska between 1979 and 2005. These exclude probable resightings of the same individual birds, based on locations of the birds and descriptions of the deformities.

Species	Scientific name	Years reported	Number of individuals
Cackling Goose	*Branta hutchinsii*	1991	2
Pelagic Cormorant	*Phalacrocorax pelagicus*	2001–2003	3
Bald Eagle	*Haliaeetus leucocephalus*	2000	1
Peregrine Falcon	*Falco peregrinus*	2000	1
Downy Woodpecker	*Picoides pubescens*	1979–2005	24
Hairy Woodpecker	*Picoides villosus*	1999–2003	9
Gray Jay	*Perisoreus canadensis*	2000–2001	2
Steller's Jay	*Cyanocitta stelleri*	1997–2004	24
Black-billed Magpie	*Pica hudsonia*	1997–2005	35
Northwestern Crow	*Corvus caurinus*	1979–2005	44
Common Raven	*Corvus corax*	1992–2004	3
Black-capped Chickadee	*Poecile atricapillus*	1991–2005	1,441
Chestnut-backed Chickadee	*Poecile rufescens*	Unknown	1
Boreal Chickadee	*Poecile hudsonica*	1994–2002	5
Red-breasted Nuthatch	*Sitta canadensis*	1998–2005	29
Ruby-crowned Kinglet	*Regulus calendula*	1999	2
American Robin	*Turdus migratorius*	1999–2000	2
Varied Thrush	*Ixoreus naevius*	2001	1
Orange-crowned Warbler	*Vermivora celata*	2002–2004	2
Yellow-rumped Warbler	*Dendroica coronata*	1998–2004	2
American Tree Sparrow	*Spizella arborea*	2002	1
Savannah Sparrow	*Passerculus sandwichensis*	1999	1
Lincoln's Sparrow	*Melospiza lincolnii*	2002	1
Dark-eyed Junco	*Junco hyemalis*	2002	1
Pine Grosbeak	*Pinicola enucleator*	1999–2002	4
White-winged Crossbill	*Loxia leucoptera*	2002	1
Common Redpoll	*Carduelis flammea*	1999–2002	5
Pine Siskin	*Carduelis pinus*	1999–2002	3

Table 6. Beak measurements of normal and deformed Black-capped and Boreal chickadees and Red-breasted Nuthatches captured in the Anchorage and Mat-Su Valley region, Alaska, 1998–2005. For individuals recaptured multiple times during study, only those from first capture were included in calculations. Birds captured as both normal and deformed were included once each time.

			Normal					Deformed				
Species	Measurement[a]	Sex	N	Mean	SE	Min	Max	N	Mean	SE	Min	Max
Black-capped Chickadee	Nares to tip	M	827	7.29	0.01	6.0	8.4	81	11.56	0.55	5.9	31.6
		F	897	7.15	0.01	6.1	8.4	97	12.46	0.61	7.3	40.3
	Culmen	M	420	9.75	0.03	7.2	11.4	61	14.69	0.68	7.6	34.0
		F	419	9.57	0.02	8.0	11.1	66	14.90	0.66	9.8	34.4
	Gonys	M	10	6.62	0.15	6.1	7.3	60	9.60	0.53	6.1	28.3
		F	14	6.52	0.13	5.8	7.4	76	9.64	0.50	5.7	28.4
Boreal Chickadee	Nares to tip	M	84	7.48	0.03	6.9	8.2					
		F	60	7.40	0.04	6.8	8.2					
	Culmen	M	44	9.93	0.08	8.5	10.9					
		F	27	10.09	0.07	9.5	10.7					
Red-breasted Nuthatch	Nares to tip	M	90	10.74	0.06	9.5	12.4					
		F	79	10.51	0.06	9.2	12.0	2	15.21	3.10	12.1	18.3
	Culmen	M	46	14.46	0.12	13.2	16.2					
		F	34	13.96	0.15	11.7	15.4					
	Gonys	M										
		F						2	15.24	2.96	12.3	18.2

[a] Nares to tip = chord measurement from distal end of nare to tip of maxilla; exposed culmen = chord measurement from base of foremost feathers on forehead to tip of maxilla; gonys = chord measurement between distal end of notch along centerline of lower mandible to tip of mandible.

Table 7. Number of occupied nest boxes monitored each year by species and location[a] in south-central Alaska from 2000–2004.

	Black-capped Chickadee			Boreal Chickadee			Unidentified chickadee			Red-breasted Nuthatch		
Year	NW	S	ER/MS	NW	S	ER/MS	NW	S	ER/MS	NW	S	ER/MS
2000	30	19	0	4	12	0	0	0	0	7	8	1
2001	28	24	33	4	10	1	0	0	1	5	5	0
2002	35	29	7	1	9	0	1	0	0	5	6	0
2003	26	33	6	2	5	0	0	4	3	6	3	0
2004	10	26	0	2	6	0	0	1	0	2	1	0
Total	128	131	46	13	42	1	1	5	4	25	23	1

[a]NW=North and West Anchorage; S=South Anchorage; ER/MS=Eagle River and Mat-Su Valley.

Table 8. Number of nest boxes in south-central Alaska that were occupied by chickadees with deformed beaks, 2000–2004. No deformed Red-breasted Nuthatches nested in any of the boxes.

	Black-capped Chickadee		Boreal Chickadee
Extent of deformity	Male	Female	Female
Severe	4	9[a]	0
Moderate	8[a]	13	0
Possible incipient	5	16	2
Total	17	38	2

[a]In one pair the male had a moderate beak deformity and the female was severely deformed.

Table 9. Comparison of clutch and brood sizes of Black-capped Chickadees relative to presence or absence of beak deformity in parents in south-central Alaska, 2000–2004.

Parameter/Parental status	*n*	Mean ± SE	Minimum	Maximum
Clutch size				
Normal parents	216	7.83 ± 0.08	2	11
Deformed female	30	8.13 ± 0.18	6	10
Deformed male	15	8.47 ± 0.27	7	11
Brood size at hatch				
Normal parents	220	7.15 ± 0.12	1	10
Deformed female	14	6.14 ± 0.51	2	10
Deformed male	10	7.00 ± 0.42	5	9
Brood size at 12 days old				
Normal parents	207	6.74 ± 0.13	2	10
Deformed female	13	5.85 ± 0.58	2	10
Deformed male	8	6.13 ± 0.35	5	8

Table 10. Model selection for clutch size (n = 260) of Black-capped Chickadee nests from 2000–2004 in south-central Alaska. Models with the lowest ΔAIC and the greatest Akaike weights (w_i) have the most support. K is the number of parameters in each model, including the intercept and explanatory variables. Areas include NW and S Anchorage and Eagle River/Mat-Su Valley. Deformity indicates adult male (M) or female (F) with beak deformity. Date is clutch initiation date.

Model	K	ΔAIC	w_i
Deformity_M+date+year	5	0.00	0.709
Deformity_M+deformity_F+date+year	8	4.37	0.080
Deformity_M+date	3	5.19	0.053
Deformity_M+deformity_F+date	4	5.56	0.044
Date+year	6	7.24	0.019
Deformity_M+date+year+area	9	7.58	0.016
Date	2	7.76	0.015
Deformity_F+ date+ year	7	7.98	0.013
Deformity_M+deformity_F+date+year+area	10	8.02	0.013
Deformity_M+date+area	5	8.43	0.010
Deformity_F+date	3	8.51	0.010
Deformity_M+deformity_F+date+area	6	8.94	0.008
Date+year+area	8	10.71	0.003
Date+area	4	11.06	0.003
Deformity_F+date+year+area	9	11.53	0.002
Deformity_F+date+area	5	11.91	0.002

Table 11. Model selection for hatching and fledging success of Black-capped Chickadee nests from 2000–2004 in south-central Alaska. Models with the lowest ΔAIC and the greatest Akaike weights (w_i) have the most support. *K* is the number of parameters in each model, including the intercept and explanatory variables; *n* = total number of nests monitored. Areas include NW and S Anchorage and Eagle River/Mat-Su Valley. Deformity indicates adult female (F) or male (M) with beak deformity. Date is nest initiation date.

Hatching success (n = 262)[a]				Fledging success (n = 249)[b]			
Model	*K*	ΔAIC	w_i	Model	*K*	ΔAIC	w_i
Deformity_F	2	0.00	0.199	Constant	1	0.00	0.286
Deformity_F+area	4	0.51	0.154	Deformity_F	2	0.97	0.176
Deformity_F+date	3	1.02	0.119	Date	2	1.52	0.134
Deformity_F+date+year	7	1.53	0.092	Deformity_M	2	1.85	0.113
Deformity_F+date+area	5	1.94	0.075	Deformity_F+date	3	2.62	0.077
Constant	1	2.03	0.072	Deformity_F+deformity_M	3	2.77	0.072
Date	2	2.46	0.058	Deformity_M+date	3	3.38	0.053
Area	3	2.87	0.047	Deformity_F+deformity_M+date	4	4.43	0.031
Deformity_F+ date+area+year	9	3.23	0.040	Year	5	5.87	0.015
Deformity_F+year	6	3.58	0.033	Date+year	6	6.64	0.010
Date+year	6	3.65	0.032	Deformity_F+year	6	6.79	0.010
Date+area	4	3.70	0.031	Deformity_F+date+year	7	7.68	0.006
Deformity_F+area+year	8	4.83	0.018	Deformity_M+year	6	7.77	0.006
Date+area+year	8	5.17	0.015	Deformity_M+date+year	7	8.51	0.004
Year	5	6.16	0.009	Deformity_F+deformity_M+year	7	8.64	0.004
Area+year	7	7.39	0.005	Deformity_F+deformity_M+date+year	8	9.50	0.002

[a]Unable to model effects of male deformity because no failures occurred during incubation for deformed males.
[b]Unable to model effects of area because no failures occurred in the Mat-Su/Eagle River area during nestling period.

Table 12. Model selection for brood size at hatch and brood size at 12 days old for Black-capped Chickadee nests from 2000–2004 in south-central Alaska. All models for brood size at hatch also include clutch size as a controlling variable; models for brood size at 12 days old control for brood size at hatch. Models with the lowest ΔAIC and the greatest Akaike weights (w_i) have the most support. *K* is the number of parameters in each model, including the intercept and explanatory variables; *n* = total number of nests monitored. Area could not be modeled because no broods were lost in Mat-Su Valley/Eagle River. Deformity indicates adult male (M) or female (F) with beak deformity. Date is clutch initiation date.

Brood size at hatch (*n* = 244)				Brood size at 12 days old (*n* = 227)			
Model	*K*	ΔAIC	w_i	Model	*K*	ΔAIC	w_i
Deformity_F	3	0.00	0.408	Deformity_M+year	7	0.00	0.327
Deformity_F+deformity_M	4	1.43	0.200	Year	6	1.14	0.185
Deformity_F+date	4	1.53	0.190	Deformity_M+deformity_F+year	8	1.87	0.129
Deformity_F+deformity_M+date	5	2.92	0.095	Deformity_M+date+year	8	2.00	0.120
Deformity_F+year	7	4.86	0.036	Deformity_F+year	7	2.95	0.075
Deformity_F+deformity_M+year	8	6.15	0.019	Date+year	7	3.13	0.068
Constant	2	6.59	0.015	Deformity_M+deformity_F+date+year	9	3.86	0.048
Deformity_F+ date+ year	8	6.82	0.013	Deformity_F+date+year	8	4.95	0.028
Deformity_F+deformity_M+date+year	9	8.11	0.007	Deformity_M+date	4	7.71	0.007
Deformity_M	3	8.24	0.007	Deformity_M	3	8.96	0.004
Date	3	8.45	0.006	Date	3	9.20	0.003
Deformity_M+date	4	10.09	0.003	Deformity_M+deformity_F+date	5	9.65	0.003
Year	6	12.25	0.001	Constant	2	10.70	0.002
Deformity_M+year	7	13.80	0.000	Deformity_M+deformity_F	4	10.81	0.001
Date+year	7	14.01	0.000	Deformity_F+date	4	11.09	0.001
Deformity_M+date+year	8	15.56	0.000	Deformity_F	3	12.47	0.001

Table 13. Levels of enzyme activity and concentrations of nutrients, metabolites, and electrolytes in blood serum from adult Black-capped Chickadees with deformed and normal beaks during winter 1999.

		Deformed beaks					Normal beaks				
Parameter[a]	Units	Median	IQR[b]	Minimum	Maximum	*n*	Median	IQR[b]	Minimum	Maximum	*n*
ALT	U/L	95.00	98.75	40.00	315.00	6	59.50	302.75	4.00	555.00	6
AP	U/L	55.00	34.00	3.00	100.00	7	75.00	110.75	3.00	245.00	6
AST	U/L	563.00	510.00	285.00	1235.00	7	624.00	460.00	435.00	2295.00	7
ALT	U/L	95.00	98.75	40.00	315.00	6	59.50	302.75	4.00	555.00	6
CK	U/L	3030.00	2665.00	840.00	5385.00	7	2635.00	2675.00	1290.00	20490.00	7
GGT	U/L	4.00	13.25	3.00	20.00	6	3.00	1.00	3.00	5.00	5
LDH	U/L	1957.50	576.25	940.00	2690.00	6	1752.50	890.00	800.00	2320.00	6
Calcium	mg/dL	5.90	3.00	2.70	7.60	7	6.90	2.00	4.70	7.70	5
Cholesterol	mg/dL	201.00	150.00	1.50	295.00	7	214.00	70.00	75.00	2085.00	7
Glucose	mg/dL	490.00	370.00	45.00	630.00	7	425.00	95.00	295.00	775.00	7
Phosphorus	mg/dL	4.25	—	3.60	4.90	2	4.85	8.28	4.10	15.10	4
Total protein	g/dL	3.20	1.60	2.00	3.90	7	2.60	1.48	1.00	5.00	10
Uric Acid	mg/dL	8.00	6.60	4.50	13.80	7	17.75	24.78	11.90	42.60	6
Chloride	mmol/L	118.50	—	116.00	121.00	2	122.00	—	122.00	122.00	2
Potassium	mmol/L	11.05	—	10.50	11.60	2	9.55	—	8.80	10.30	2
Sodium	mmol/L	155.50	—	153.00	158.00	2	158.50	—	157.00	160.00	2
Bicarbonate	mmol/L	43.00	—	42.00	44.00	2	37.00	—	37.00	37.00	1
Anion Gap	mmol/L	5.50	—	5.00	6.00	2	10.00	—	10.00	10.00	1

[a]ALT=alanine aminotransferase; AP=alkaline phosphatase; AST=aspartate aminotransferase; CK=creatinine kinase; GGT=gamma glutamyl transferase; LDH=lactate dehydrogenase.
[b]Interquartile range.

Table 14. Percentages of different protein fractions, determined from electrophoresis, in blood serum of adult Black-capped Chickadees with deformed and normal beaks during winter 1999.

	Deformed beaks				Normal beaks			
Protein fraction	Median	Minimum	Maximum	*n*	Median	Minimum	Maximum	*n*
Prealbumin	28.98	27.23	34.13	4	33.94	19.81	34.37	3
Albumin	36.20	30.34	38.54	4	36.06	23.06	41.87	3
α1-globulin	5.65	4.84	7.75	4	5.14	4.64	5.54	3
α2-globulin	4.73	3.07	6.21	4	4.02	3.96	4.10	3
β-globulin	10.91	5.15	13.95	4	6.32	4.10	19.35	3
γ-globulin	13.70	10.26	18.23	4	13.62	11.05	28.53	3

Table 15. Maximum concentrations (µg/g wet weight) of organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) found in samples of sunflower seeds (*n*=18) collected from different sources in the Anchorage and Mat-Su Valley region, Alaska, 1997–2000. The minimum detection limit (DL) for a given analyte varied among samples, so the highest DL is listed, along with the number of samples with any detectable concentrations.

Compound	# above sample DL	Highest sample DL	Maximum concentration
OC pesticides			
o,p'-DDD	0	0.000103	
o,p'-DDE	0	0.000312	
o,p'-DDT	1	0.000200	0.000268
p,p'-DDD	0	0.000108	
p,p'-DDE	1	0.000291	0.000106
p,p'-DDT	2	0.000238	0.000296
HCB	7	0.000202	0.000129
α-HCH	1	0.000852	0.002360
γ-HCH (Lindane)	3	0.000923	0.006840
Dieldrin	0	0.000161	
Endrin	0	0.000242	
Heptachlor epoxide	0	0.000115	
α-Chlordane	0	0.000150	
γ-Chlordane	0	0.000134	
Oxychlordane	0	0.002020	
Trans-Nonachlor	0	0.000129	
Toxaphene	0	0.024700	
Mirex	0	0.000126	
PCBs			
Aroclor 1242	3	0.002790	0.004330
Aroclor 1248	0	0.002790	
Aroclor 1254	0	0.003590	
Aroclor 1260	0	0.002030	
Total PCBs	3	0.000129	0.004330

Table 16. Concentrations (µg/g dry weight) of metals and trace elements in samples of sunflower seeds (*n* = 18) collected from different sources in the Anchorage and Mat-Su Valley region, Alaska, 1997–2000. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples.

Analyte	# above DL	Median concentration	IQR[a]	Minimum concentration	Maximum concentration
Aluminum	16	7.975	4.220	<4.970[b]	67.400
Arsenic	18	0.059	0.005	0.053	0.098
Barium	18	3.290	2.780	1.880	10.300
Beryllium	18	0.101	0.003	0.095	0.106
Boron	18	14.950	3.000	12.100	18.800
Cadmium	18	0.433	0.127	0.308	0.878
Chromium	18	7.280	3.300	4.160	16.800
Copper	18	16.050	1.500	12.000	20.600
Iron	18	76.850	23.000	62.200	118.000
Lead	4	<0.021[b]	—	<0.021[b]	0.084
Magnesium	18	3274.000	474.000	2869.000	3626.000
Manganese	18	20.400	5.000	15.200	27.300
Mercury	0	<0.021[b]	—	<0.021[b]	<0.021[b]
Molybdenum	18	0.741	0.184	0.578	1.090
Nickel	18	5.740	1.980	3.830	8.880
Selenium	18	1.135	0.360	0.708	3.910
Strontium	18	7.370	9.270	3.610	29.100
Vanadium	18	1.130	0.130	0.946	1.270
Zinc	18	48.550	8.300	35.800	58.200

[a]Interquartile range.
[b]Maximum DL among samples in which analyte was not detected.

Table 17. Concentrations (µg/g dry weight) of metals, trace elements, and methyl mercury in livers of adult Black-capped Chickadees from the Anchorage and Mat-Su Valley region, Alaska, in 1999. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

			Concentration		
Analyte	*n*	# above DL	Median	Minimum[a]	Maximum
Aluminum	20	0	–	<4.9800–19.8413	
Arsenic	20	13	0.0218	<0.0180–0.0397	0.0496
Barium	20	0	–	<0.4980–1.9841	
Beryllium	20	0	–	<0.0996–0.3968	
Boron	20	0	–	<0.4980–1.9841	
Cadmium	20	20	0.8290	0.1160	1.6300
Chromium	20	0	–	<1.3944–5.5556	
Copper	20	20	21.9500	18.9000	34.3000
Iron	20	20	1696.5000	488.0000	3007.0000
Lead	20	18	0.2445	<0.0116–0.4255	0.6110
Magnesium	20	20	815.5000	661.0000	909.0000
Manganese	20	20	5.7500	4.0500	9.3700
Mercury	20	17	0.0411	<0.0100–0.0314	0.1500
Molybdenum	20	15	3.2748	<2.5180–5.5556	5.5556
Nickel	20	1	–	<1.3944–5.5556	
Selenium	20	20	4.2350	2.3600	6.6600
Strontium	20	0	–	<0.5858–2.2222	
Vanadium	20	0	–	<0.4980–1.9841	
Zinc	20	20	80.0000	67.1000	108.0000
Methyl mercury	9	3	–	<0.0200–0.0444	0.0652

[a]Minimum concentration for analytes that were detected in all samples; range of DLs for analytes that were not detected in some or any of the samples.

Table 18. Concentrations (µg/g dry weight) of metals and trace elements in livers of nestling Black-capped Chickadees from the Anchorage and Mat-Su Valley region, Alaska, from 1999–2001. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

			Concentration		
Analyte	*n*	# above DL	Median	Minimum[a]	Maximum
Aluminum	30	0	–	<20.8000–46.6000	
Arsenic	49	32	0.1320	<0.1030–0.2220	1.2300
Barium	49	15	0.3678[b]	<0.1030–4.6600	1.8400
Beryllium	49	0	–	<0.0261–0.9310	
Boron	30	3	–	<8.3100–14.7000	219.0000
Cadmium	49	45	0.0986	<0.0469–0.1090	0.3180
Chromium	49	15	0.4029[b]	<0.2060–4.6600	0.6930
Copper	30	30	21.7000	12.0000	59.8000
Iron	30	30	1456.0000	673.0000	2574.0000
Lead	49	16	0.0199[b]	<0.0415–0.1110	1.1300
Magnesium	30	30	899.5000	700.0000	1136.0000
Manganese	49	49	6.2800	3.7600	11.2000
Mercury	49	7	0.0338[b]	<0.0435–0.1860	0.4830
Molybdenum	49	20	1.4422[b]	<2.0800–4.6600	3.1600
Nickel	49	7	0.1551[b]	<0.2060–4.6600	3.2400
Selenium	49	48	1.1100	<0.2670	6.4900
Strontium	30	4	–	<0.8310–1.8600	1.6700
Vanadium	49	0	–	<0.0435–4.6600	
Zinc	30	30	95.4500	69.7000	119.0000

[a]Minimum concentration for analytes that were detected in all samples; range of DLs for analytes that were not detected in some or any of the samples.
[b]Median estimated through robust regression on order statistics (Helsel 2005:68*ff*).

Table 19. Concentrations (µg/g wet weight) of organochlorine (OC) pesticides in samples of adult Black-capped Chickadees from the Anchorage and Mat-Su Valley region, Alaska, from 1999–2002. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

Compound	*n*	# above DL	Concentration		
			Median	Minimum[a]	Maximum
OC pesticides					
o,p'-DDD	35	0	–	<0.00007–0.00057	
o,p'-DDE	35	0	–	<0.00017–0.00057	
o,p'-DDT	35	0	–	<0.00028–0.00057	
p,p'-DDD	35	8	0.00020[b]	<0.00014–0.00057	0.00048
p,p'-DDE	35	35	0.00542	0.00121	0.04700
p,p'-DDT	35	10	0.00023[b]	<0.00018–0.00057	0.00070
HCB	35	35	0.00198	0.00063	0.00720
1,2,3,4-TeCB	16	6	0.00320[b]	<0.00049–0.00057	0.00125
1,2,4,5-TeCB	16	16	0.00538	0.00121	0.02241
α-HCH	35	0	–	<0.00046–0.00230	
β-HCH	16	1	–	<0.00046–0.00057	0.00091
γ-HCH (Lindane)	35	2	–	<0.00046–0.00210	0.00600
δ-HCH	16	0	–	<0.00046–0.00057	
Aldrin	16	0	–	<0.00046–0.00057	
Dieldrin	35	20	0.00028[b]	<0.00046–0.00057	0.01800
Endrin	35	1	–	<0.00007–0.00057	0.00065
Endosulfan-II	16	0	–	<0.00046–0.00057	
Heptachlor epoxide	35	24	0.00040[b]	<0.00051–0.00056	0.01100
Heptachlor	16	0	–	<0.00046–0.00057	
Pentachlor	16	1	–	<0.00046–0.00056	0.00328
α-Chlordane	35	0	–	<0.00026–0.00057	
γ-Chlordane	35	0	–	<0.00016–0.00057	
Oxychlordane	35	1	–	<0.00046–0.00360	0.00430
Cis-nonachlor	16	0	–	<0.00046–0.00057	
Trans-nonachlor	35	24	0.00061[b]	<0.00051–0.00057	0.00350
Toxaphene	35	0	–	<0.00455–0.03400	
Mirex	35	16	0.00026[b]	<0.00015–0.00057	0.00052

[a]Minimum concentration for compounds that were detected in all samples; range of DLs for compounds that were not detected in some of the samples.
[b]Median estimated through robust regression on order statistics (Helsel 2005:68*ff*).

Table 20. Concentrations (µg/g wet weight) of organochlorine (OC) pesticides and polychlorinated biphenyl (PCB) mixtures in samples of Black-capped Chickadee nestlings from the Anchorage and Mat-Su Valley region, Alaska, from 2000–2001. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

Compound	*n*	# above DL	Concentration		
			Median	Minimum[a]	Maximum
OC pesticides					
o,p'-DDD	49	0	–	<0.00008–0.00053	
o,p'-DDE	49	0	–	<0.00005–0.00040	
o,p'-DDT	49	1	–	<0.00011–0.00073	0.00096
p,p'-DDD	49	15	0.00010[b]	<0.00010–0.00053	0.00092
p,p'-DDE	49	49	0.00308	0.00039	0.07640
p,p'-DDT	49	21	0.00019[b]	<0.00013–0.00088	0.00188
HCB	49	49	0.00071	0.00052	0.00117
α-HCH	49	0	–	<0.00028–0.00310	
γ-HCH (Lindane)	49	7	0.00032[b]	<0.00034–0.00294	0.00307
Dieldrin	49	20	0.00012[b]	<0.00002–0.00041	0.00264
Endrin	49	0	–	<0.00008–0.00065	
Heptachlor epoxide	49	22	0.00010[b]	<0.00003–0.00067	0.00086
α-Chlordane	49	1	–	<0.00008–0.00060	0.00014
γ-Chlordane	49	7	0.00014[b]	<0.00016–0.00051	0.00023
Oxychlordane	49	1	–	<0.00113–0.06070	0.00613
Trans-nonachlor	49	29	0.00013[b]	<0.00008–0.00035	0.00193
Toxaphene	49	1	–	<0.00816–0.04730	0.04400
Mirex	49	0	–	<0.00014–0.00085	
PCB mixtures					
Aroclor 1242	30	0	–	<0.00175–0.00472	
Aroclor 1248	30	0	–	<0.00175–0.00472	
Aroclor 1254	30	17	0.00321[b]	<0.00208–0.00787	0.03990
Aroclor 1260	30	29	0.01033	<0.00340	0.08470
Total PCBs	49	47	0.00707	<0.00405–0.00714	0.12300

[a]Minimum concentration for compounds that were detected in all samples; range of DLs for compounds that were not detected in some of the samples.
[b]Median estimated through robust regression on order statistics (Helsel 2005:68*ff*).

Table 21. Concentrations (µg/g wet weight) of organochlorine (OC) pesticides and polychlorinated biphenyl (PCB) mixtures in samples of Black-capped Chickadee eggs from the Anchorage and Mat-Su Valley region, Alaska, from 2000–2001. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

Compound	*n*	# above DL	Concentration		
			Median	Minimum[a]	Maximum
OC pesticides					
o,p'-DDD	39	0	–	<0.00027–0.00148	
o,p'-DDE	39	2	–	<0.00019–0.00370	0.00119
o,p'-DDT	39	0	–	<0.00040–0.00593	
p,p'-DDD	39	5	0.00016[b]	<0.00048–0.00296	0.00265
p,p'-DDE	39	39	0.01980	0.00389	0.07690
p,p'-DDT	39	11	0.00022[b]	<0.00015–0.00333	0.00556
HCB	39	39	0.00405	0.00163	0.01320
α-HCH	39	0	–	<0.00044–0.02890	
γ-HCH (Lindane)	39	4	0.00076[b]	<0.00075–0.02740	0.00215
Dieldrin	39	15	0.00024[b]	<0.00013–0.00142	0.14000
Endrin	39	0	–	<0.00043–0.00133	
Heptachlor epoxide	39	10	0.00025[b]	<0.00015–0.00234	0.08600
α-Chlordane	39	0	–	<0.00037–0.00556	
γ-Chlordane	39	21	0.00047[b]	<0.00038–0.00333	0.00117
Oxychlordane	39	0	–	<0.00087–0.04670	
Trans-nonachlor	39	30	0.00067[b]	<0.00040–0.00148	0.00891
Toxaphene	39	1	–	<0.02750–0.47100	0.11100
Mirex	39	7	0.00034[b]	<0.00026–0.00259	0.00073
PCB mixtures					
Aroclor 1242	10	0	–	<0.01050–0.03300	
Aroclor 1248	10	0	–	<0.01050–0.03300	
Aroclor 1254	10	5	0.01846[b]	<0.03070	0.03940
Aroclor 1260	10	10	0.08460	0.01830	0.19100
Total PCBs	39	39	0.03930	0.00477	0.23400

[a]Minimum concentration for compounds that were detected in all samples; range of DLs for compounds that were not detected in some of the samples.
[b]Median estimated through robust regression on order statistics (Helsel 2005:68*ff*).

Table 22. Concentrations (µg/g wet weight) of polychlorinated biphenyl (PCB) mixtures and one organophosphate (OP) pesticide in samples of adult Black-capped Chickadees from the Anchorage and Mat-Su Valley region, Alaska, from 1999–2002. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

Compound	*n*	# above DL	Concentration		
			Median	Minimum[a]	Maximum
PCB mixture					
Aroclor 1242	19	3	–	<0.00150–0.00320	0.00780
Aroclor 1248	19	0	–	<0.00150–0.00320	
Aroclor 1254	19	19	0.00770	0.00470	0.11000
Aroclor 1260	19	18	0.01200	<0.00430	0.02400
Total PCBs	35	35	0.02100	0.00740	0.15000
OP pesticide					
Chlorpyrifos	16	2	–	<0.00046–0.00057	0.00064

[a]Minimum concentration for compounds that were detected in all samples; range of DLs for compounds that were not detected in some of the samples.

Table 23. Concentrations (ng/g wet weight) of dioxin-like polychlorinated biphenyl (PCB) congeners in tissues of adult Black-capped Chickadees from the Anchorage and Mat-Su Valley region, Alaska, from 2001–2002. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

			Concentration		
Analyte	*n*	# above DL	Median	Minimum[a]	Maximum
Non-*ortho* PCBs					
Congener 77	16	1	–	<0.0182–0.0229	0.0340
Congener 81	16	0	–	<0.0182–0.0229	–
Congener 126	16	0	–	<0.0182–0.0229	–
Congener 169	16	0	–	<0.0182–0.0229	–
Mono-*ortho* PCBs					
Congener 105	16	16	0.2415	0.1190	0.4780
Congener 114	16	0	–	<0.0182–0.0229	–
Congener 118	16	16	0.8585	0.3450	2.9170
Congener 123[b]	16	16	0.1470	0.0290	0.8640
Congener 156	16	15	0.1200	<0.0220	0.3870
Congener 157[c]	16	16	0.1140	0.0770	0.1800
Congener 167	16	16	0.1235	0.0550	0.3090
Congener 189	16	1	–	<0.0182–0.0229	0.0220

[a]Range of DLs for compounds that were not detected in some or any of the samples.
[b]Coeluted with congener 149.
[c]Coeluted with congeners 173 and 201.

Table 24. Concentrations (ng/g wet weight) of dioxin-like polychlorinated biphenyl (PCB) congeners in tissues of nestling Black-capped Chickadees from the Anchorage and Mat-Su Valley region, Alaska, from 2000–2001. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

			Concentration		
Analyte	*n*	# above DL	Median	Minimum[a]	Maximum
Non-*ortho* PCBs					
Congener 77	49	4	–	<0.0030–0.0190	0.0330
Congener 81	49	9	–	<0.0012–0.0057	0.0056
Congener 126	49	3	–	<0.0020–0.0200	0.0510
Congener 169	49	9	–	<0.0011–0.0067	0.0077
Mono-*ortho* PCBs					
Congener 105	49	49	0.0970	0.0175	3.6000
Congener 114	49	45	0.0120	<0.0019–0.0035	0.2100
Congener 118	49	49	0.4600	0.0644	13.0000
Congener 123	49	36	0.0154	<0.0044–0.0108	0.1900
Congener 156/157[b]	49	48	0.0676	<0.0032	2.3000
Congener 167	49	49	0.0404	0.0064	0.9700
Congener 189	49	44	0.0122	<0.0018–0.0041	0.1200

[a]Range of DLs for compounds that were not detected in some of the samples.
[b]Congeners coeluted.

Table 25. Concentrations (ng/g wet weight) of dioxin-like polychlorinated biphenyl (PCB) congeners in Black-capped Chickadee eggs from the Anchorage and Mat-Su Valley region, Alaska, from 2000–2001. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

			Concentration		
Analyte	*n*	# above DL	Median	Minimum[a]	Maximum
Non-*ortho* PCBs					
Congener 77	39	2	–	<0.0092–0.1800	0.0240
Congener 81	39	1	–	<0.0074–0.0250	0.0251
Congener 126	39	4	–	<0.0062–0.1900	0.0316
Congener 169	39	8	–	<0.0052–0.0275	0.0480
Mono-*ortho* PCBs					
Congener 105	39	39	0.4630	0.0721	4.3900
Congener 114	39	39	0.0525	0.0080	0.7150
Congener 118	39	39	2.7000	0.3040	34.8000
Congener 123	39	33	0.0526	0.0140	0.4840
Congener 156/157[b]	39	39	0.4870	0.0434	4.0000
Congener 167	39	39	0.2920	0.0306	1.7000
Congener 189	39	39	0.0718	0.0088	0.3300

[a]Range of DLs for compounds that were not detected in some of the samples.
[b]Congeners coeluted.

Table 26. Concentrations (ng/g wet weight) of polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners in tissues of adult Black-capped Chickadees from Anchorage and Mat-Su Valley, Alaska, in 2001–2002. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound.

			Concentration	
Analyte	*n*	# above DL	Minimum[a]	Maximum
2,3,7,8-TCDD	16	0	<0.00182–0.00229	
1,2,3,7,8-PeCDD	16	0	<0.00911–0.01144	
1,2,3,4,7,8-HxCDD	16	0	<0.00911–0.01144	
1,2,3,6,7,8-HxCDD	16	2	<0.00911–0.01111	0.01590
1,2,3,7,8,9-HxCDD	16	1	<0.00911–0.01111	0.01360
1,2,3,4,6,7,8-HpCDD	16	3	<0.00911–0.01111	0.15620
OCDD	16	6	<0.01820–0.02160	0.69310
2,3,7,8-TCDF	16	0	<0.00182–0.00229	
1,2,3,7,8-PeCDF	16	0	<0.00911–0.01144	
2,3,4,7,8-PeCDF	16	0	<0.00911–0.01144	
1,2,3,4,7,8-HxCDF	16	1	<0.00911–0.01144	0.01080
1,2,3,6,7,8-HxCDF	16	0	<0.00911–0.01144	
1,2,3,7,8,9-HxCDF	16	0	<0.00911–0.01144	
2,3,4,6,7,8-HxCDF	16	0	<0.00911–0.01144	
1,2,3,4,6,7,8-HpCDF	16	1	<0.00911–0.01111	0.02710
1,2,3,4,7,8,9-HpCDF	16	0	<0.00911–0.01144	
OCDF	16	0	<0.01820–0.02288	

[a]Range of DLs for compounds that were not detected in some or any of the samples.

Table 27. Concentrations (ng/g wet weight) of polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners in tissues of nestling Black-capped Chickadees from Anchorage and Mat-Su Valley, Alaska, from 2000–2001. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound. Cl-4 through Cl-7 are concentrations of all PCDDs or PCDFs with 4–7 chlorine atoms, respectively.

			Concentration		
Analyte	*n*	# above DL	Median	Minimum[a]	Maximum
2,3,7,8-TCDD	29	7	0.00005[b]	<0.0002–0.0010	0.0072
1,2,3,7,8-PeCDD	29	10	0.00033[b]	<0.0006–0.0015	0.0510
1,2,3,4,7,8-HxCDD	29	5	–	<0.0015–0.0043	0.0390
1,2,3,6,7,8-HxCDD	29	10	0.00138[b]	<0.0018–0.0043	0.0740
1,2,3,7,8,9-HxCDD	29	3	–	<0.0015–0.0043	0.0370
1,2,3,4,6,7,8-HpCDD	29	26	0.00530	<0.0012–0.0022	0.1900
Cl-4 PCDD	29	7	0.00005[b]	<0.0002–0.0010	0.0072
Cl-5 PCDD	29	11	0.00030[b]	<0.0007–0.0015	0.0510
Cl-6 PCDD	29	13	0.00088[b]	<0.0018–0.0043	0.1500
Cl-7 PCDD	29	24	0.00500	<0.0011–0.0022	0.2000
OCDD	29	13	0.00715[b]	<0.0072–0.0170	0.2000
2,3,7,8-TCDF	29	3	–	<0.0004–0.0013	0.0040
1,2,3,7,8-PeCDF	29	3	–	<0.0004–0.0016	0.0100
2,3,4,7,8-PeCDF	29	6	0.00015[b]	<0.0005–0.0016	0.0070
1,2,3,4,7,8-HxCDF	29	5	–	<0.0006–0.0090	0.0120
1,2,3,6,7,8-HxCDF	29	4	–	<0.0005–0.0090	0.0120
1,2,3,7,8,9-HxCDF	29	2	–	<0.0005–0.0090	0.0054
2,3,4,6,7,8-HxCDF	29	4	–	<0.0005–0.0090	0.0047
1,2,3,4,6,7,8-HpCDF	29	15	0.00120	<0.0009–0.0018	0.0280
1,2,3,4,7,8,9-HpCDF	29	2	–	<0.0005–0.0018	0.0040
Cl-4 PCDF	29	2	–	<0.0003–0.0013	0.0043
Cl-5 PCDF	29	7	0.00009[b]	<0.0005–0.0016	0.0160
Cl-6 PCDF	29	10	0.00068[b]	<0.0007–0.0090	0.0300
Cl-7 PCDF	29	5	–	<0.0005–0.0018	0.0340
OCDF	29	1	–	<0.0040–0.0130	0.0210

[a]Range of DLs for compounds that were not detected in some or any of the samples.
[b]Median estimated through robust regression on order statistics (Helsel 2005:68*ff*).

Table 28. Concentrations (ng/g wet weight) of polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners in eggs of Black-capped Chickadees from Anchorage and Mat-Su Valley, Alaska, from 2000–2001. Frequency of detection is indicated by the number of samples above the detection limit (DL), which varied among samples for a given compound. Cl-4 through Cl-7 are concentrations of all PCDDs or PCDFs with 4–7 chlorine atoms, respectively.

			Concentration		
Analyte	*n*	# above DL	Median	Minimum[a]	Maximum
2,3,7,8-TCDD	39	12	0.00114[b]	<0.0012–0.0037	0.0043
1,2,3,7,8-PeCDD	39	21	0.00377	<0.0030–0.0089	0.0180
1,2,3,4,7,8-HxCDD	39	3	–	<0.0080–0.0250	0.0240
1,2,3,6,7,8-HxCDD	39	16	0.00679[b]	<0.0080–0.0250	0.0840
1,2,3,7,8,9-HxCDD	39	3	–	<0.0080–0.0250	0.0270
1,2,3,4,6,7,8-HpCDD	39	35	0.02800	<0.0044–0.0077	0.1900
Cl-4 PCDD	39	10	0.00086[b]	<0.0012–0.0033	0.0092
Cl-5 PCDD	39	21	0.00359	<0.0030–0.0069	0.0180
Cl-6 PCDD	39	18	0.00767	<0.0080–0.0200	0.1400
Cl-7 PCDD	39	33	0.02800	<0.0044–0.0081	0.6500
OCDD	39	26	0.06200	<0.0340–0.1000	0.6500
2,3,7,8-TCDF	39	0	–	<0.0023–0.0074	
1,2,3,7,8-PeCDF	39	1	–	<0.0029–0.0093	0.0099
2,3,4,7,8-PeCDF	39	0	–	<0.0029–0.0093	
1,2,3,4,7,8-HxCDF	39	1	–	<0.0034–0.0110	0.0230
1,2,3,6,7,8-HxCDF	39	1	–	<0.0034–0.0110	0.0130
1,2,3,7,8,9-HxCDF	39	0	–	<0.0034–0.0110	
2,3,4,6,7,8-HxCDF	39	0	–	<0.0034–0.0110	
1,2,3,4,6,7,8-HpCDF	39	13	0.00139[b]	<0.0033–0.0100	0.1000
1,2,3,4,7,8,9-HpCDF	39	0	–	<0.0033–0.0100	
Cl-4 PCDF	39	0	–	<0.0023–0.0074	
Cl-5 PCDF	39	1	–	<0.0029–0.0093	0.0099
Cl-6 PCDF	39	6	–	<0.0034–0.0110	0.0460
Cl-7 PCDF	39	11	0.00096[b]	<0.0033–0.0100	0.1100
OCDF	39	0	–	<0.0240–0.0760	

[a]Range of DLs for compounds that were not detected in some or any of the samples.
[b]Median estimated through robust regression on order statistics (Helsel 2005:68*ff*).

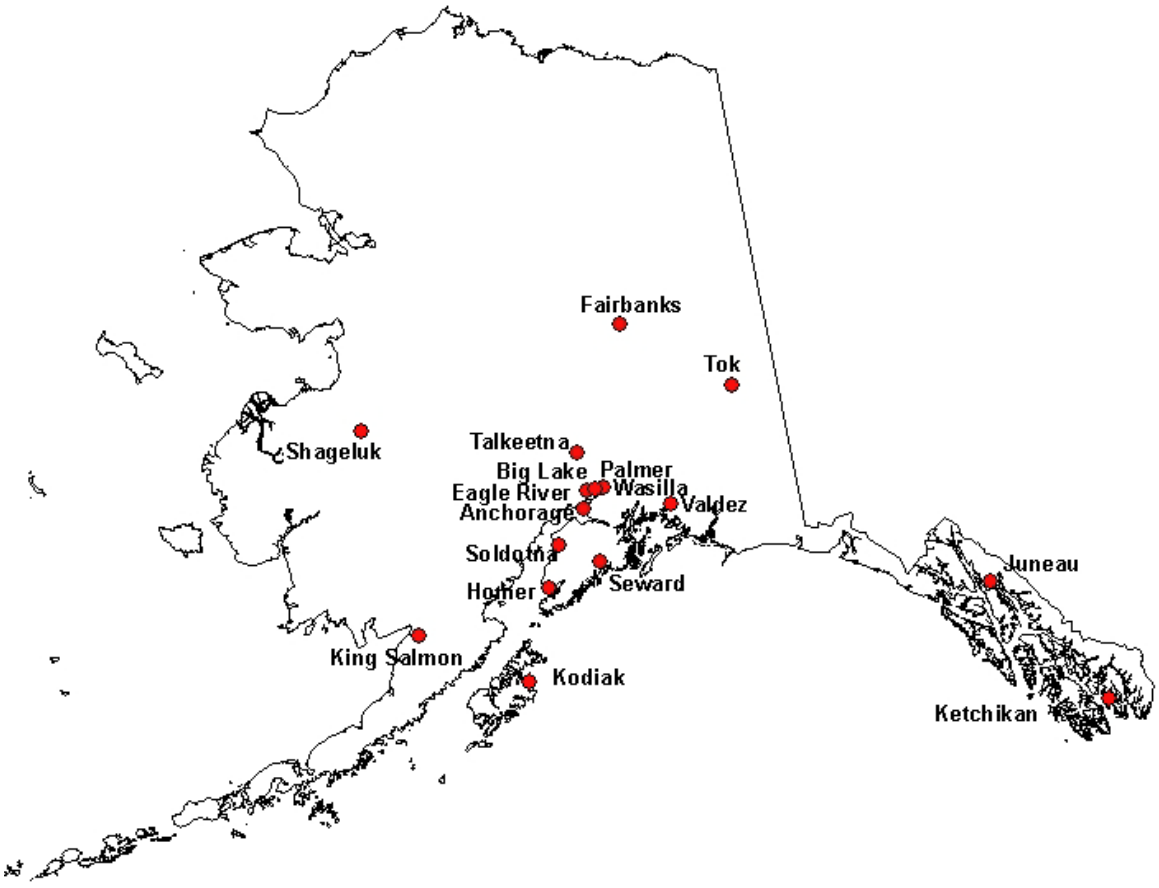

Figure 1. Map of Alaska showing primary sites where deformed birds have been observed or sampled.

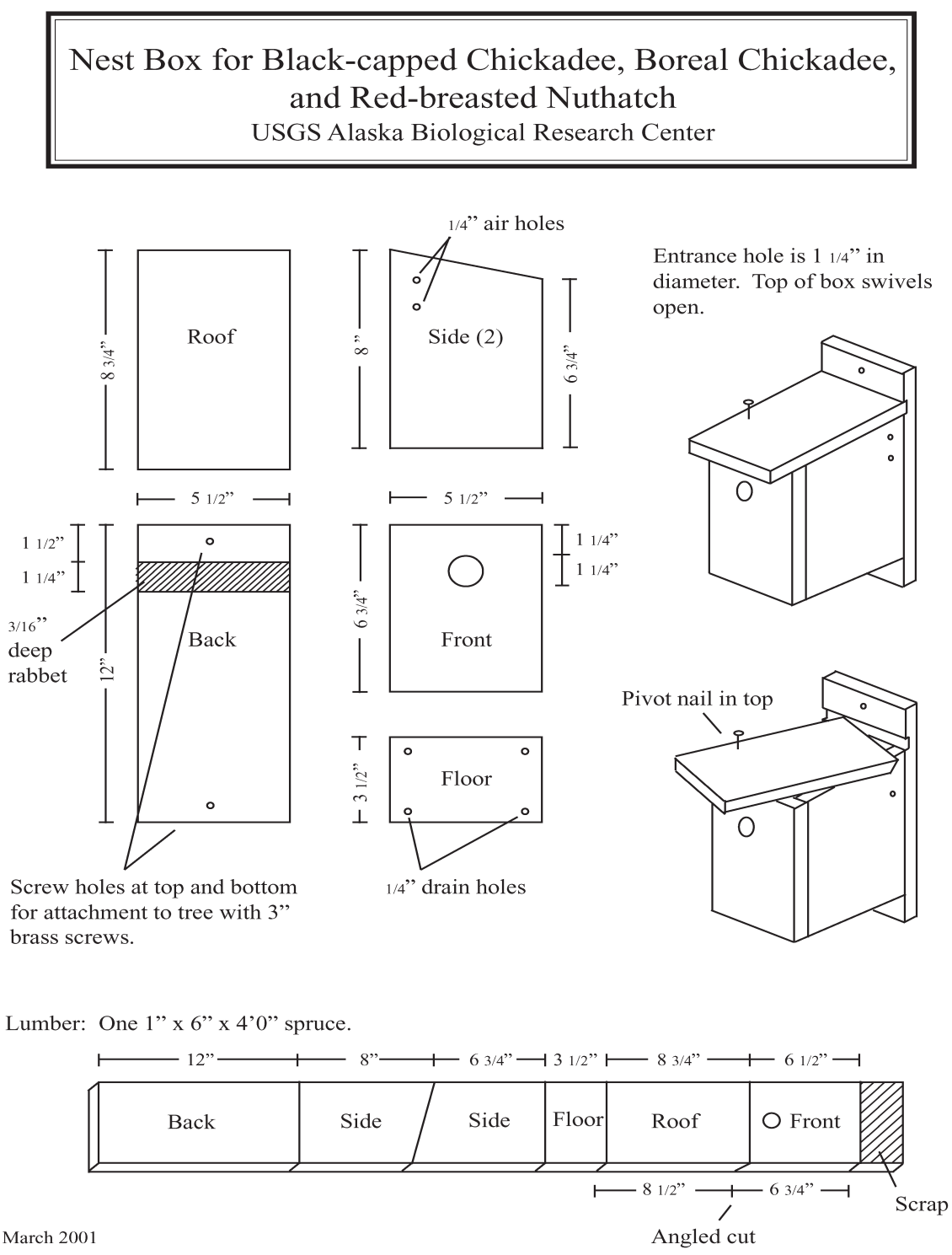

Figure 2. Design of nest boxes used in study of Black-capped Chickadees, south-central Alaska, 2000-2004.

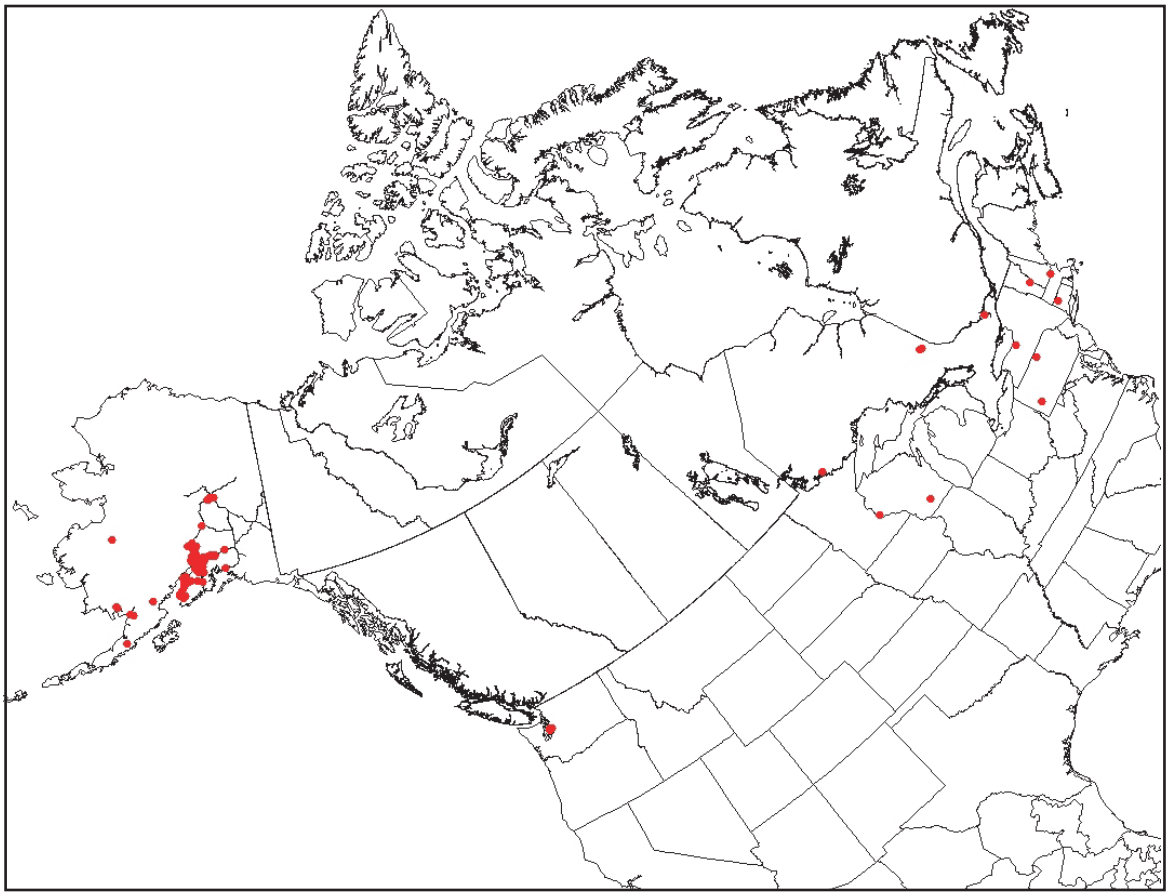

Figure 3. Cumulative distribution of Black-capped Chickadees reported from North America with beak deformities between winter 1991-1992 and winter 2004-2005.

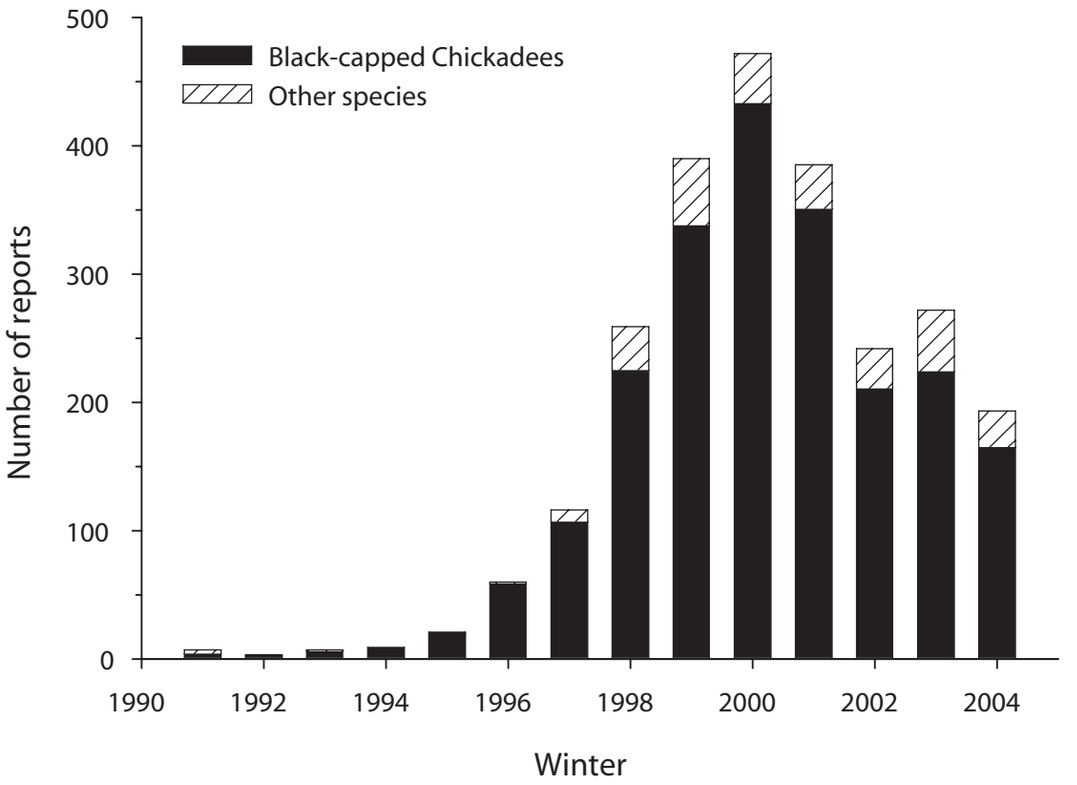

Figure 4. Number of reports received of Black-capped Chickadees and 27 other species of birds with beak deformities in Alaska each year from 1991-2004. Each year brackets a winter, spanning from 1 July to 30 June.

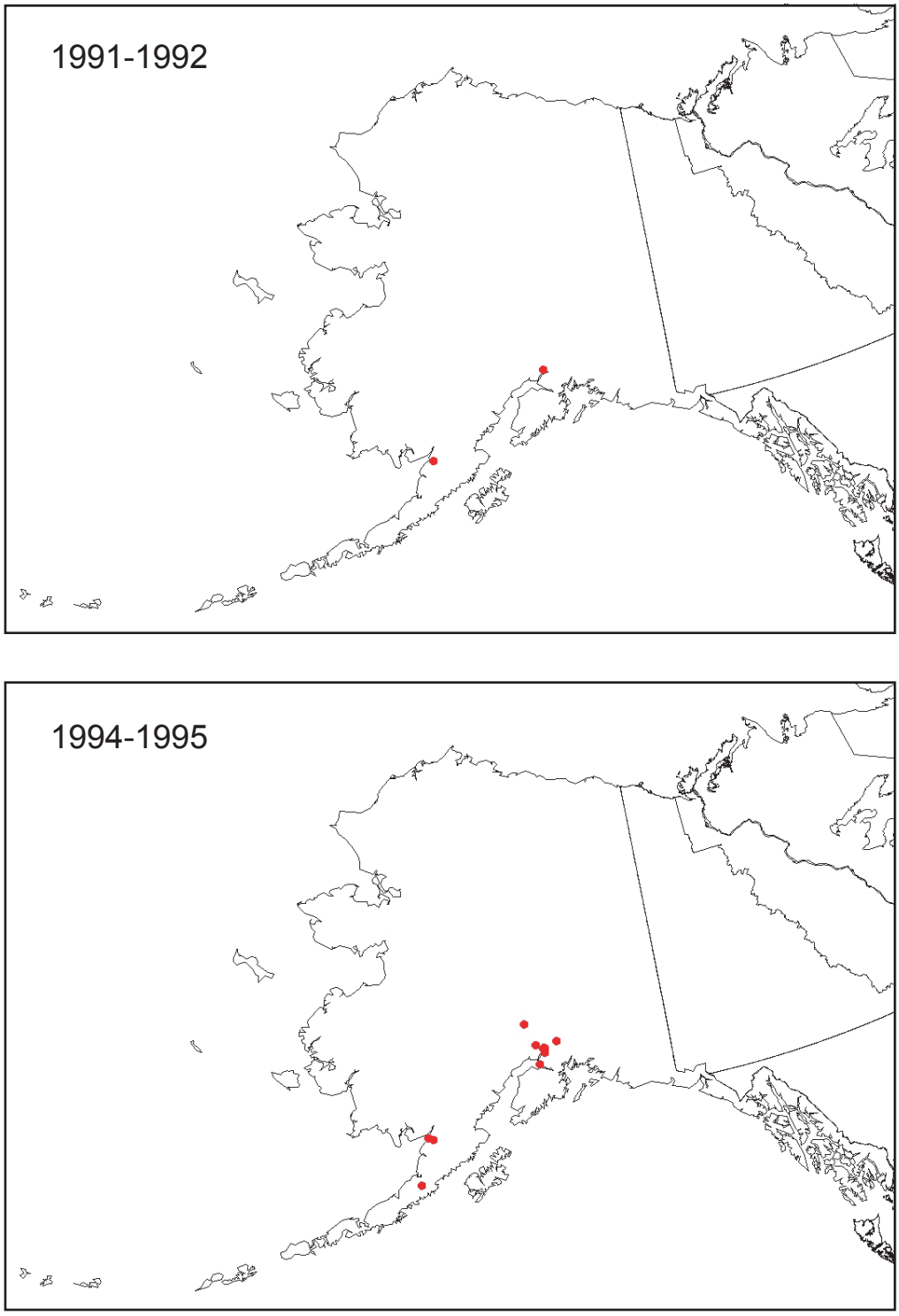

Figure 5. Distribution of Black-capped Chickadees reported from Alaska with beak deformities during winter 1991-1992 (top) and cumulatively through winter 1994-1995 (bottom).

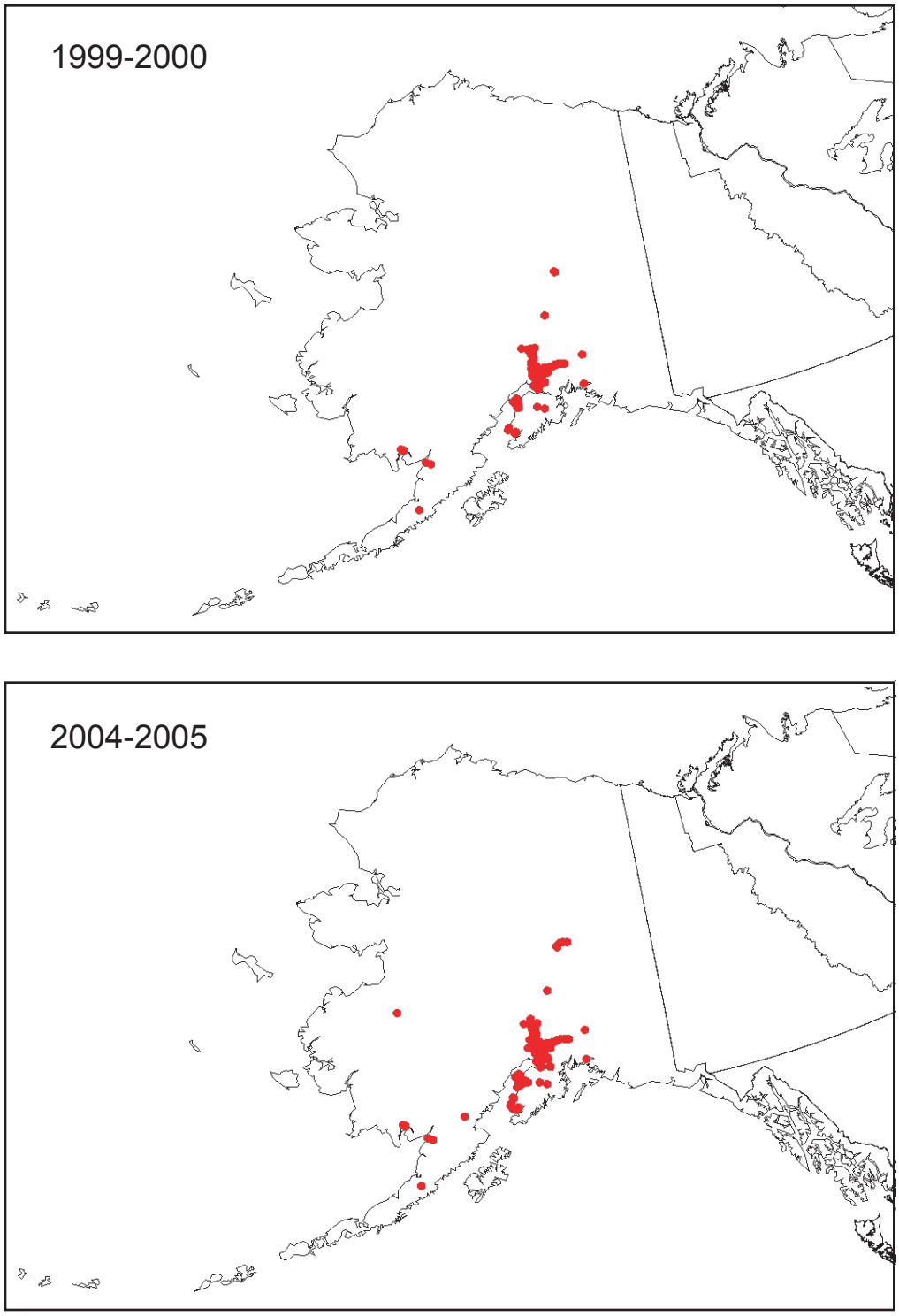

Figure 6. Distribution of Black-capped Chickadees reported from Alaska with beak deformities cumulatively through winter 1999-2000 (top) and winter 2004-2005 (bottom).

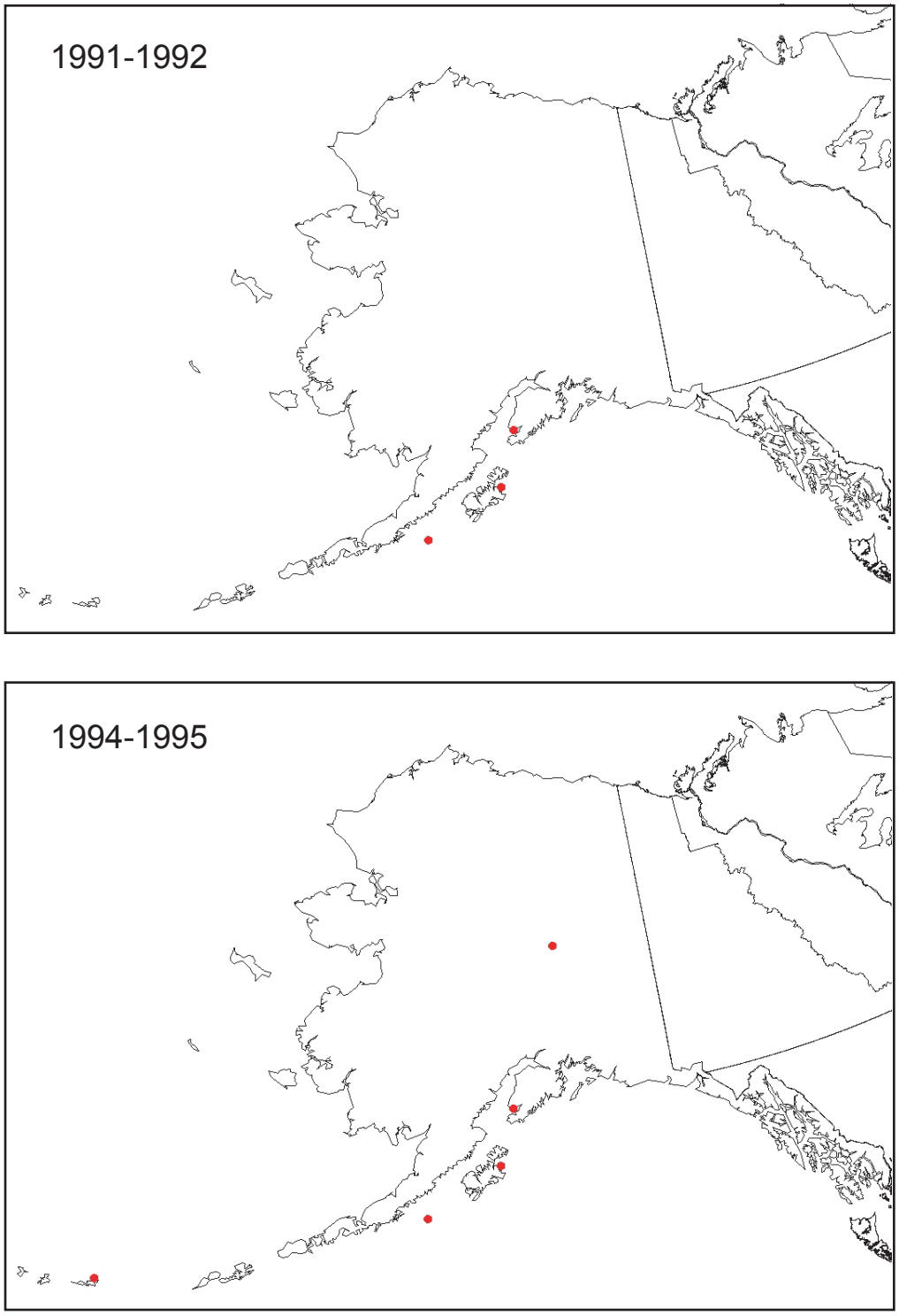

Figure 7. Distribution of birds other than Black-capped Chickadees reported from Alaska with beak deformities cumulatively through winter 1991-1992 (top) and winter 1994-1995 (bottom).

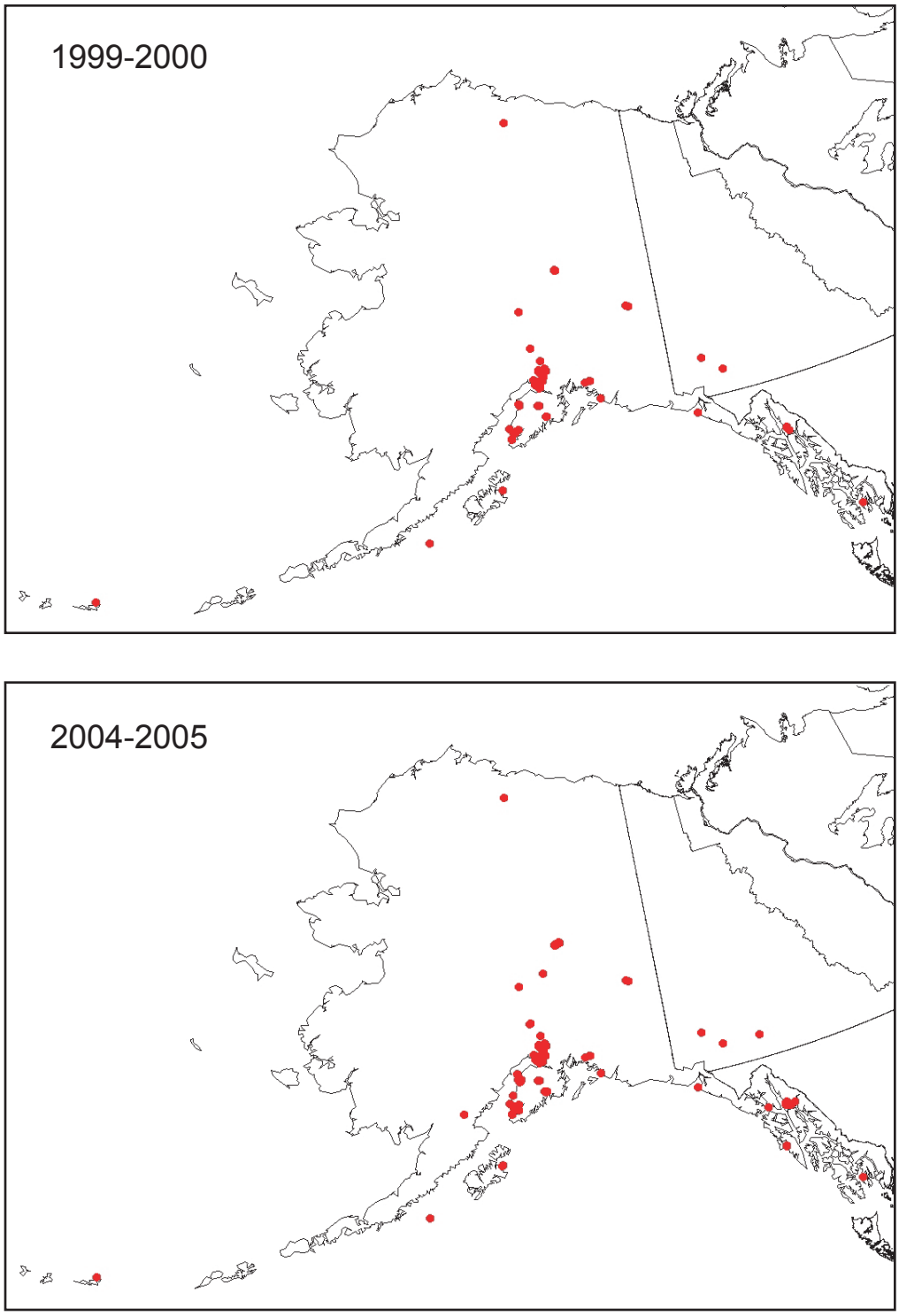

Figure 8. Distribution of birds other than Black-capped Chickadees reported from Alaska and Yukon Territory with beak deformities cumulatively through winter 1999-2000 (top) and winter 2004-2005 (bottom).

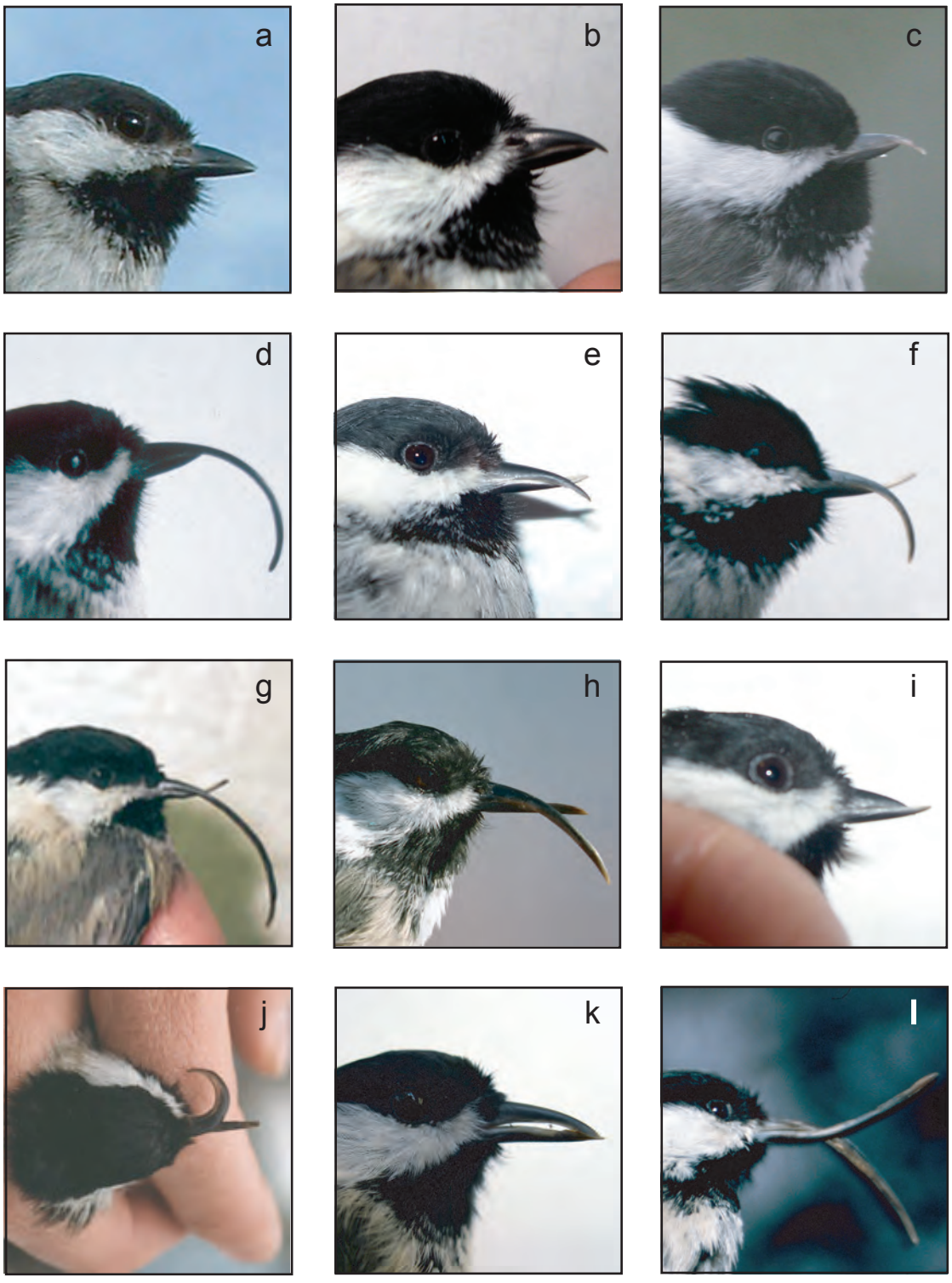

Figure 9. Black-capped Chickadees with normal beak (a), overgrown maxilla (b-d), overgrown and crossed maxilla and mandible (e-h), overgrown mandible (i), laterally curved maxilla (j), overgrown maxilla and mandible with pronounced gap (k), and overgrown, crossed, and greatly thickened maxilla and mandible (l).

Figure 10. Other abnormal conditions associated with beak deformities among Black-capped Chickadees in Alaska. Many chickadees had dry, reddened skin and patches of missing feathers, usually in the head region (a). On others, the keratin sheath on the tarsometarsus was dry and flaky (b). One female chickadee with a grossly overgrown beak (c) also had a large lipoma on the abdomen on the brood patch (d). Several nestlings had a folding fracture of the tibiofibula (e).

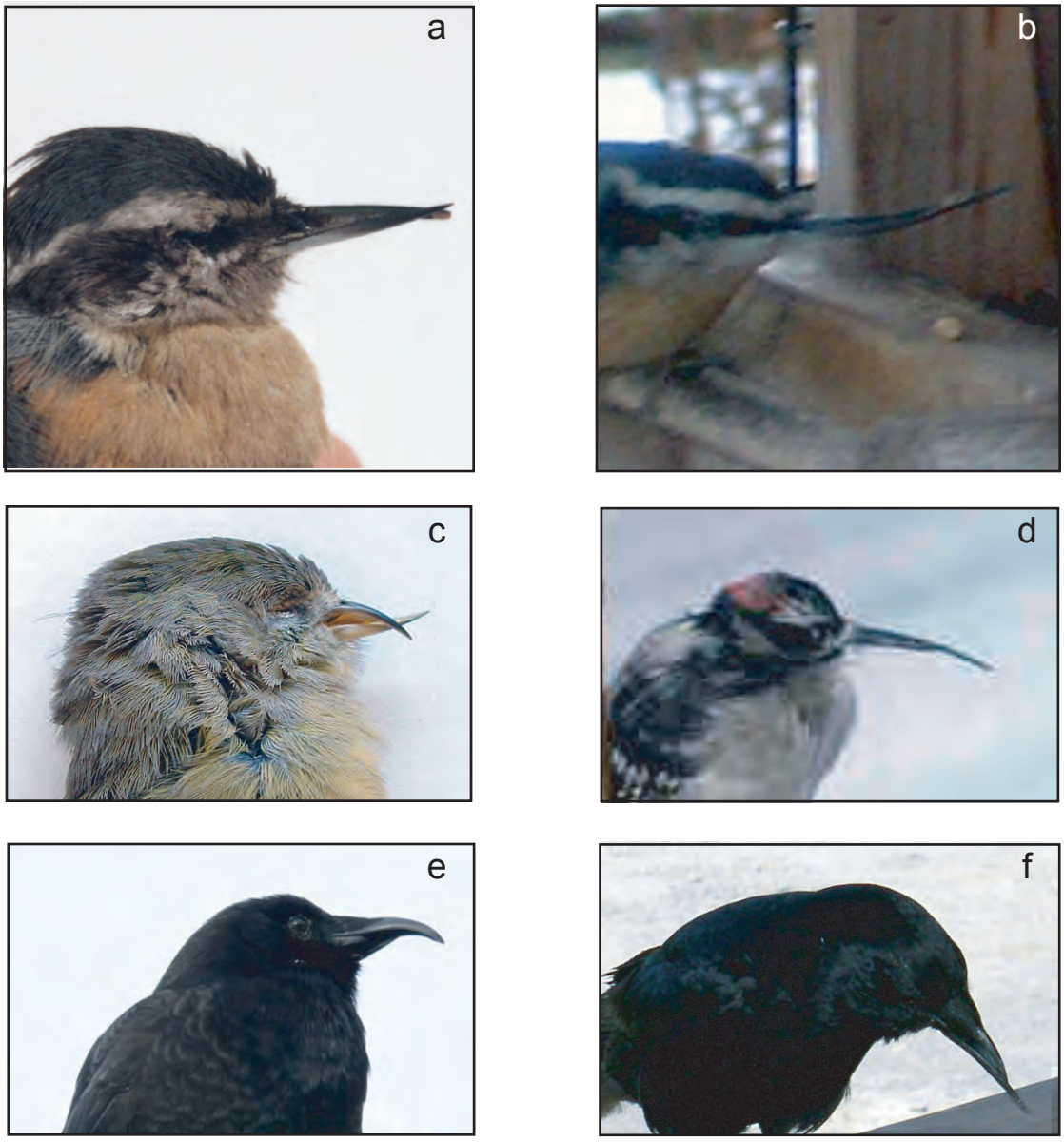

Figure 11. Examples of other species with beak deformities in Alaska. Red-breasted Nuthatch with elongated and crossed maxilla and mandible (a), Red-breasted Nuthatch with elongated, upcurved, and crossed maxilla and mandible (b), Orange-crowned Warbler with decurved and crossed maxilla (c), Downy Woodpecker with elongated maxilla and mandible (d), Northwestern Crow with slightly elongated and decurved maxilla (e), and Northwestern Crow with elongated mandible and slight crossing of maxilla and mandible (f). Photos courtesy of Stephanie Cristal (b), Owen Hughes (d), Paul Suchanek (e), and Donna Dewhurst (f).

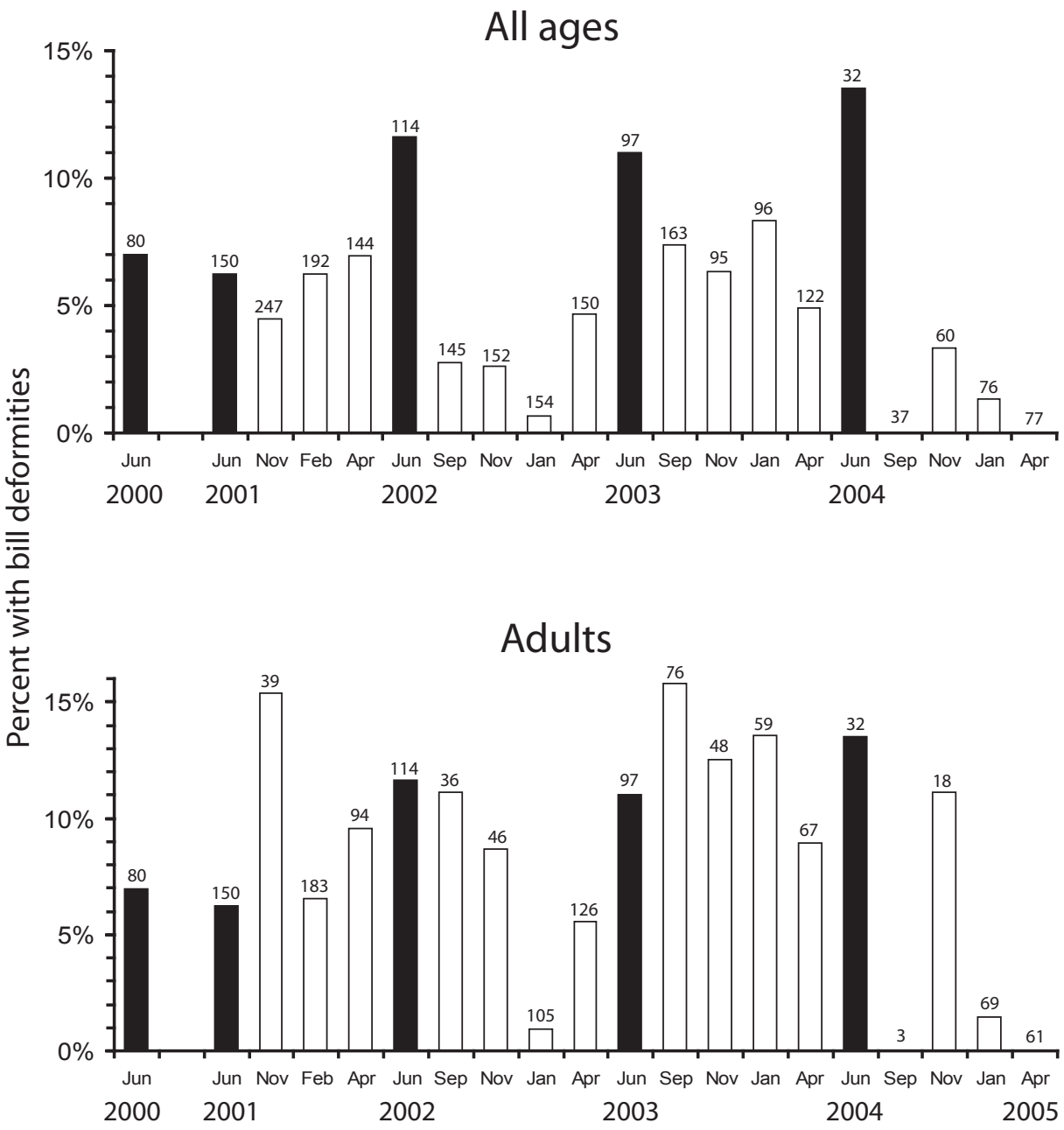

Figure 12. Percent of individual Black-capped Chickadees captured at nest boxes (solid bars) or at winter feeder traps (open bars) each month from 2000-2005 with beak deformities in south-central Alaska. Top graph includes all adults and juveniles captured and lower graph includes only known adults (excludes all hatching-year and second-year birds and birds of uncertain age in November). Number of birds captured is given above each bar.

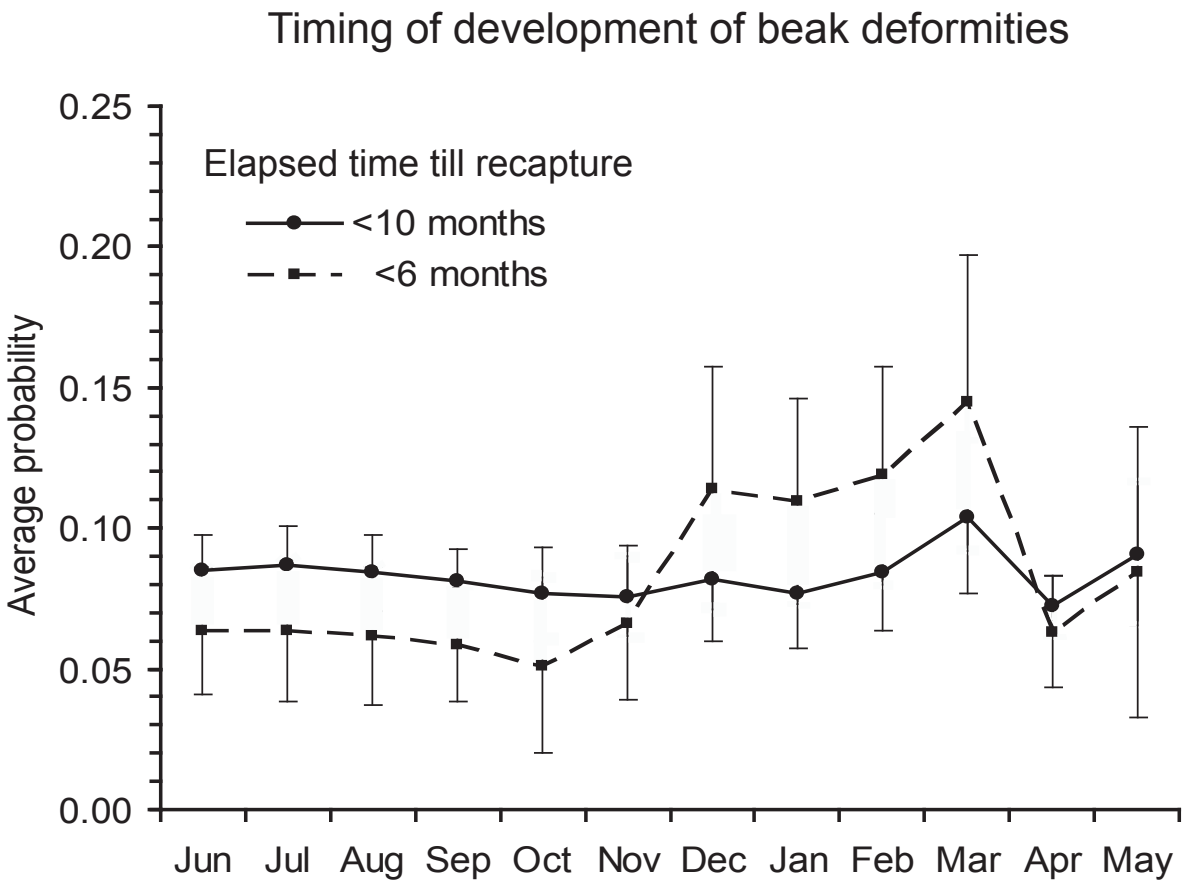

Figure 13. We recaptured 54 individual Black-capped Chickadees that had normal beaks when originally captured but had deformed beaks upon a subsequent recapture. This illustrates the estimated average probability (± SE) that these deformities developed during a given month. Estimates are shown for 35 individuals that were known to have changed sometime within a 10-month period (solid line) and for the subset of 17 individuals that were recaptured within 6 months and for which timing of development of the deformity was more precisely known (dashed line). For each individual, the probability of becoming deformed was allocated evenly to the days between capture and recapture. Monthly probabilities were then averaged across all individuals.

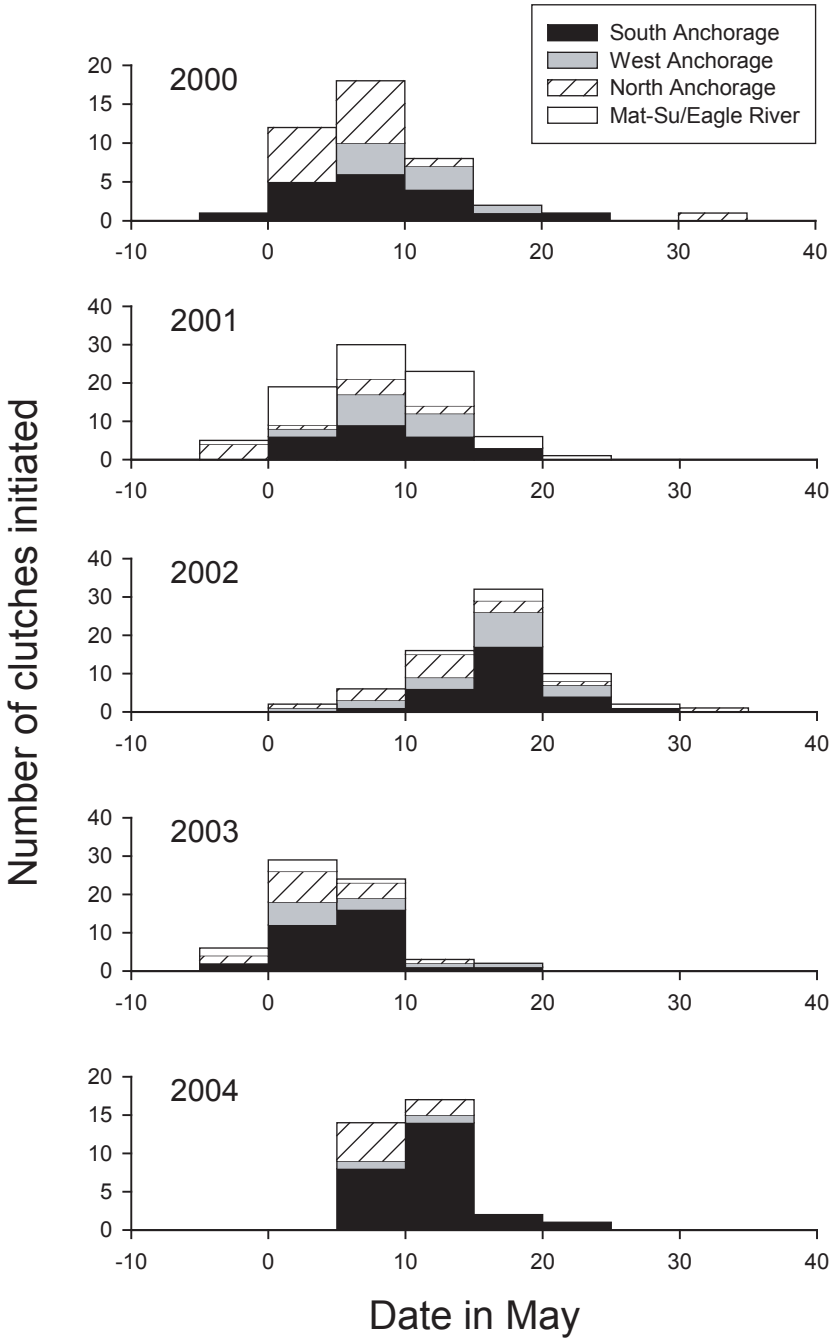

Figure 14. Timing of clutch initiation among Black-capped Chickadees nesting in nest boxes in south-central Alaska from 2000-2004 by geographic area. No nest boxes were monitored in the Mat-Su Valley or Eagle River in 2000 or 2004.

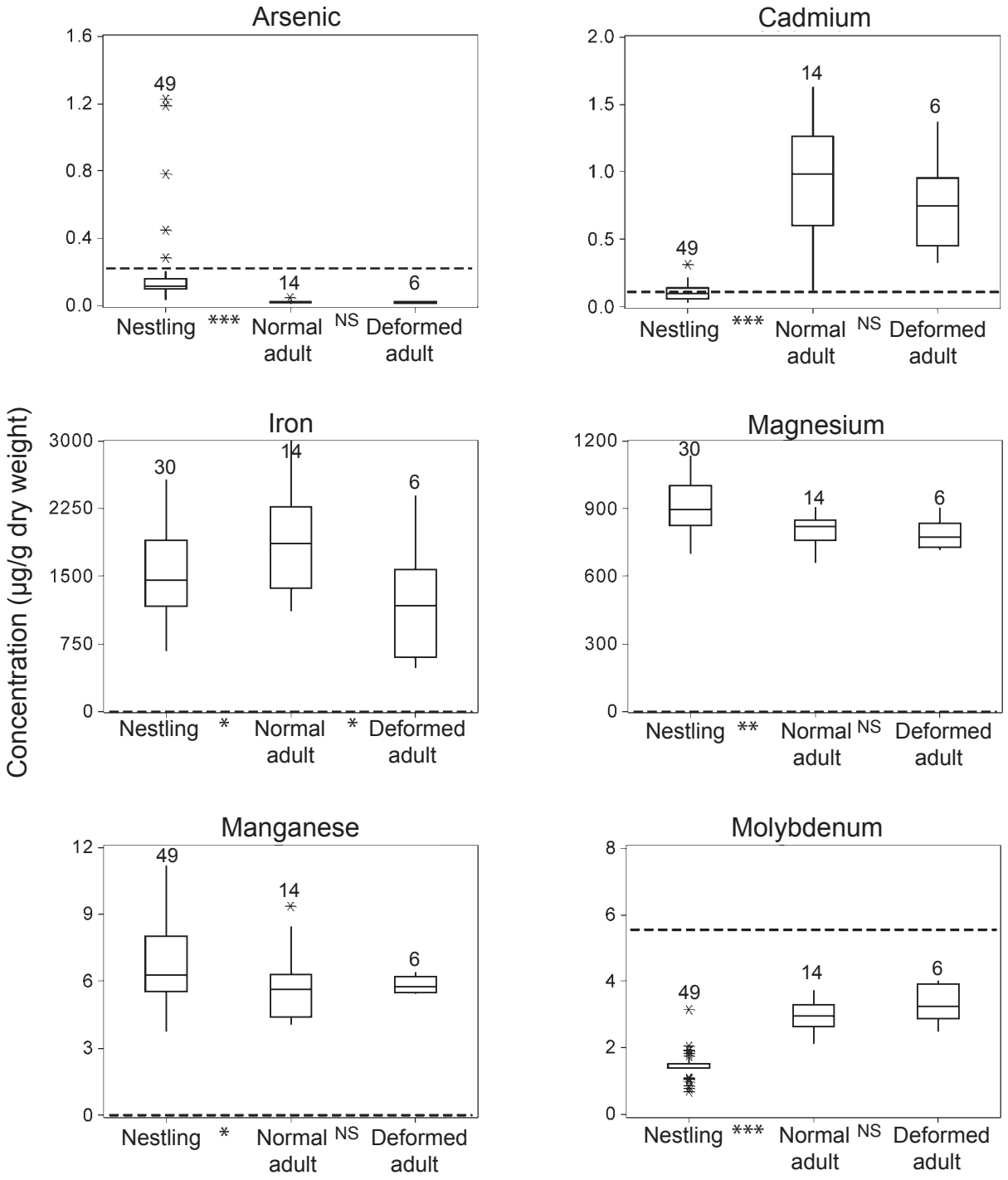

Figure 15. Censored box plots of concentrations (µg/g dry weight) of six elements in livers of nestling Black-capped Chickadees, normal adults, and adults with deformed beaks. Horizontal bar shows median, box shows first and third quartiles, whiskers include 1.5 times IQR beyond first and third quartiles, asterisks indicate outliers, and sample sizes are above plots. Dashed line shows maximum detection limit. Differences between nestlings and normal adults and between normal and deformed adults are shown: ***$P <$ 0.001, **$P < 0.01$, * $P < 0.05$, NS = not significant.

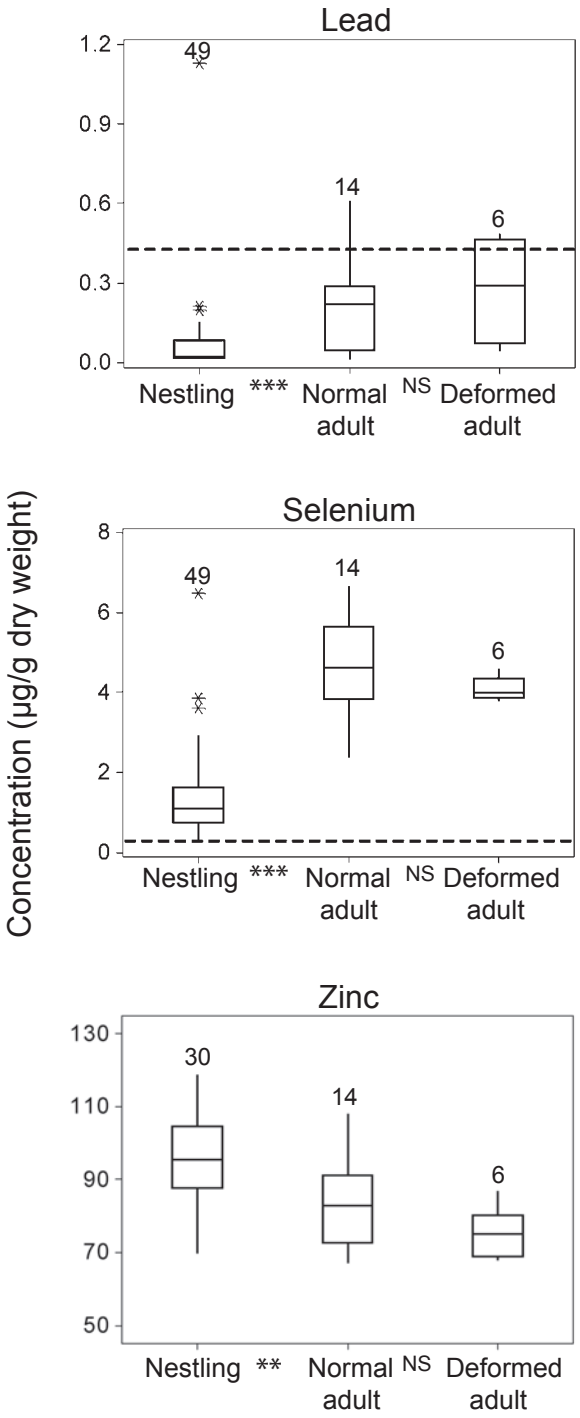

Figure 16. Censored box plots of concentrations (µg/g dry weight) of three elements in livers of nestling Black-capped Chickadees, normal adults, and adults with deformed beaks. Horizontal bar shows median, box shows first and third quartiles, whiskers include 1.5 times IQR beyond first and third quartiles, asterisks indicate outliers, and sample sizes are above plots. Dashed line shows maximum detection limit. Differences between nestlings and normal adults and between normal and deformed adults are shown: ***$P < 0.001$, **$P < 0.01$, NS = not significant.

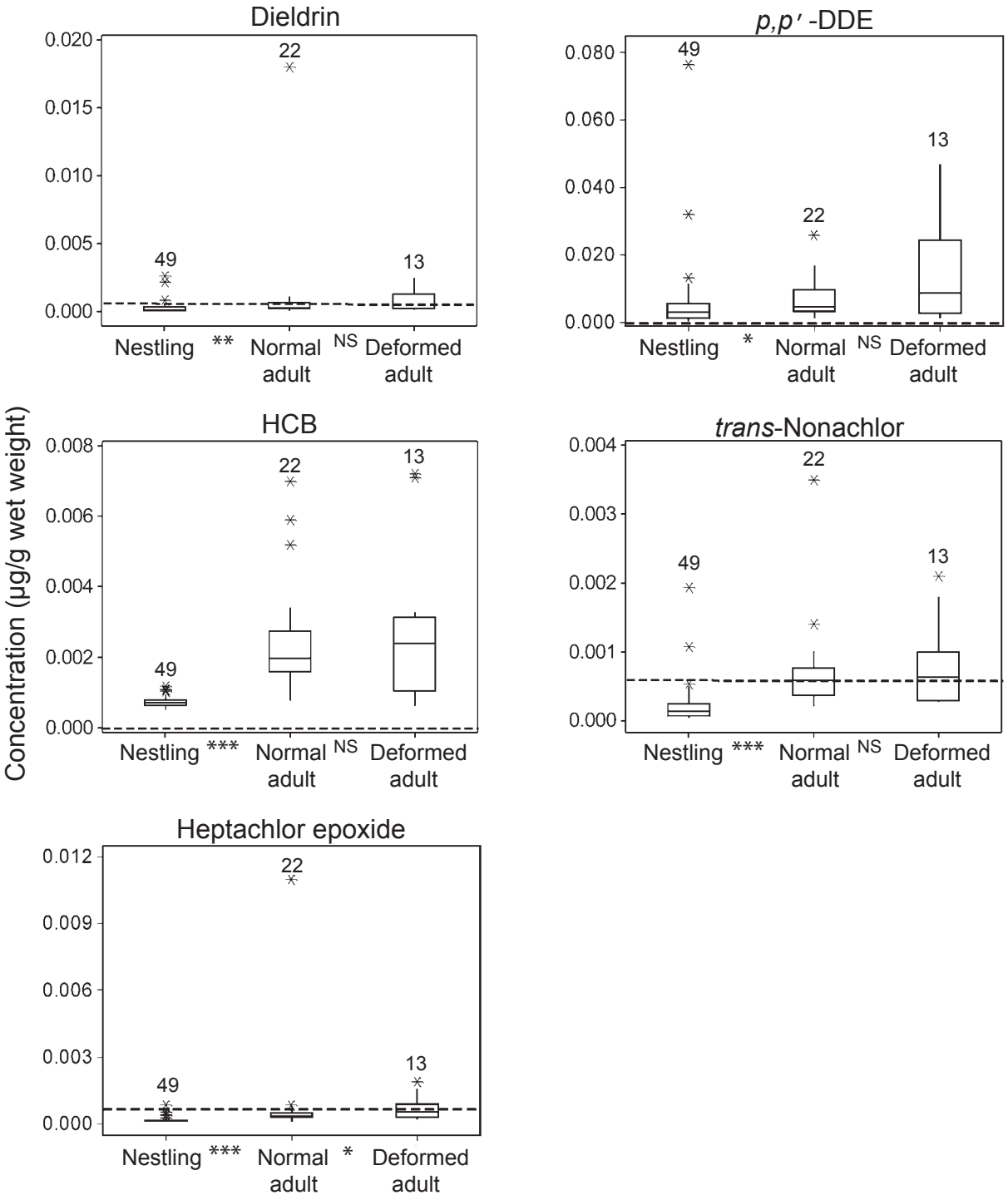

Figure 17. Censored box plots of concentrations (µg/g wet weight) of organochlorine compounds in tissues of nestling Black-capped Chickadees, normal adults, and adults with deformed beaks. Horizontal bar shows median, box shows first and third quartiles, whiskers include 1.5 times IQR beyond first and third quartiles, asterisks indicate outliers, and sample sizes are above plots. Dashed line shows maximum detection limit. Differences between nestlings and normal adults and between normal and deformed adults are shown: ***$P < 0.001$, **$P < 0.01$, * $P < 0.05$, NS = not significant.

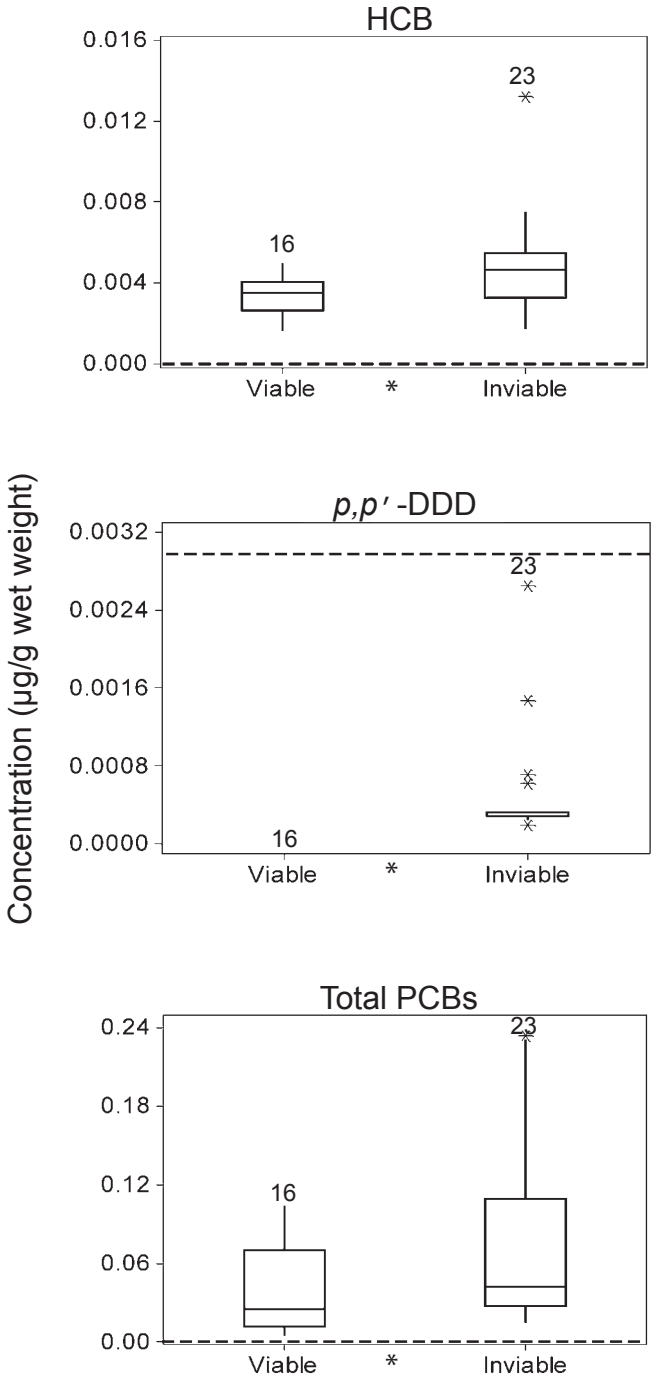

Figure 18. Censored box plots of concentrations (µg/g wet weight) of three contaminants in Black-capped Chickadee eggs from clutches in which all eggs were viable compared with those in which at least one egg was inviable. Horizontal bar shows median, box shows first and third quartiles, whiskers include 1.5 times IQR beyond first and third quartiles, asterisks indicate outliers, and sample sizes are above plots. Dashed line shows maximum detection limit. Differences between groups are shown: * $P < 0.05$.

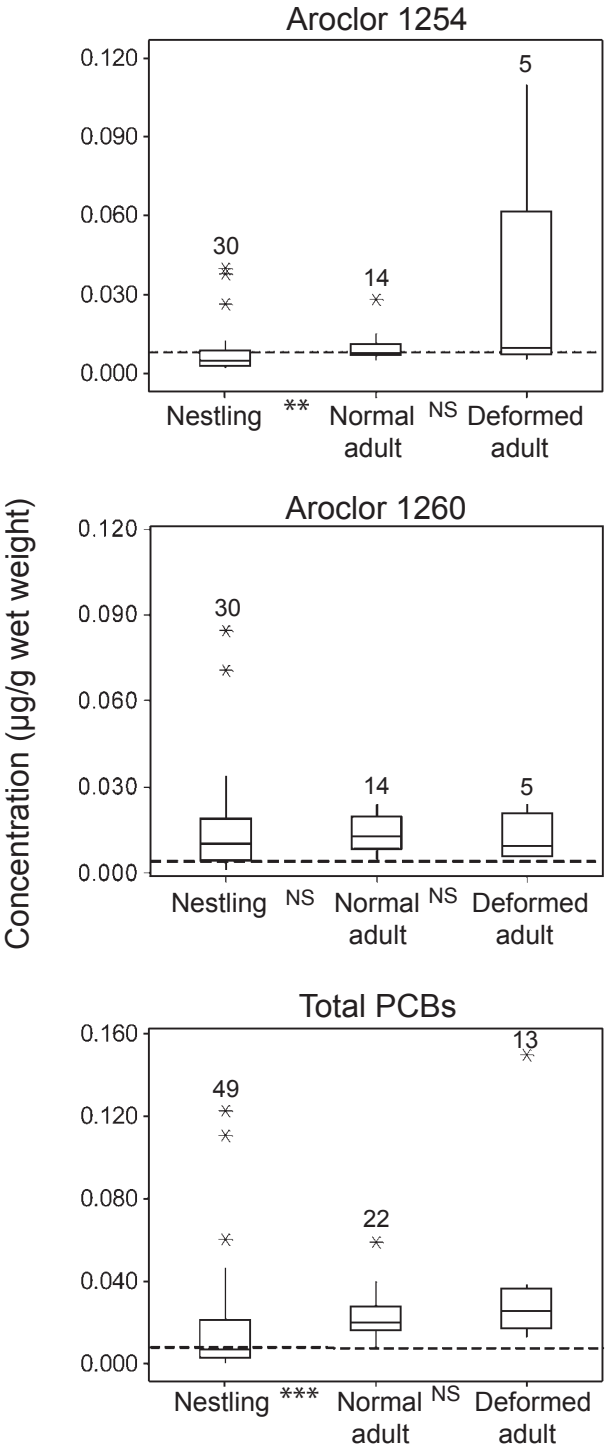

Figure 19. Censored box plots of concentrations (µg/g wet weight) of two Arochlor mixtures and total PCBs in tissues of nestling Black-capped Chickadees, normal adults, and adults with deformed beaks. Horizontal bar shows median, box shows first and third quartiles, whiskers include 1.5 times IQR beyond first and third quartiles, asterisks indicate outliers, and sample sizes are above plots. Dashed line shows maximum detection limit. Differences between nestlings and normal adults and between normal and deformed adults are shown: ***$P < 0.001$, **$P < 0.01$, * $P < 0.05$, NS = not significant.

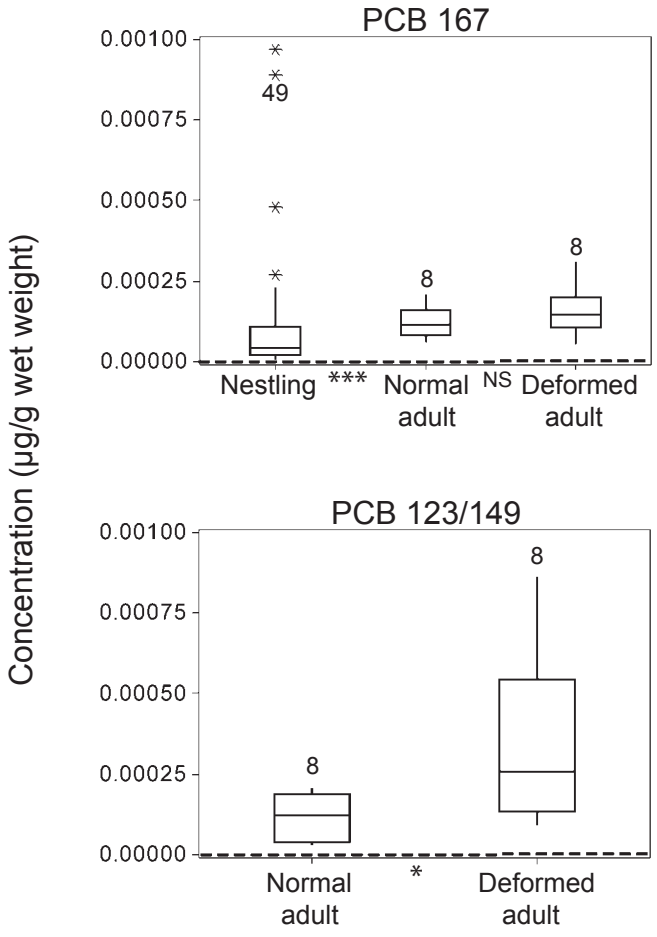

Figure 20. Censored box plots of concentrations (µg/g wet weight) of PCB congeners in tissues of nestling Black-capped Chickadees, normal adults, and adults with deformed beaks. Horizontal bar shows median, box shows first and third quartiles, whiskers include 1.5 times IQR beyond first and third quartiles, asterisks indicate outliers, and sample sizes are above plots. Dashed line shows maximum detection limit. Differences between groups are shown: $*P < 0.05$, $***P < 0.001$, NS = not significant.

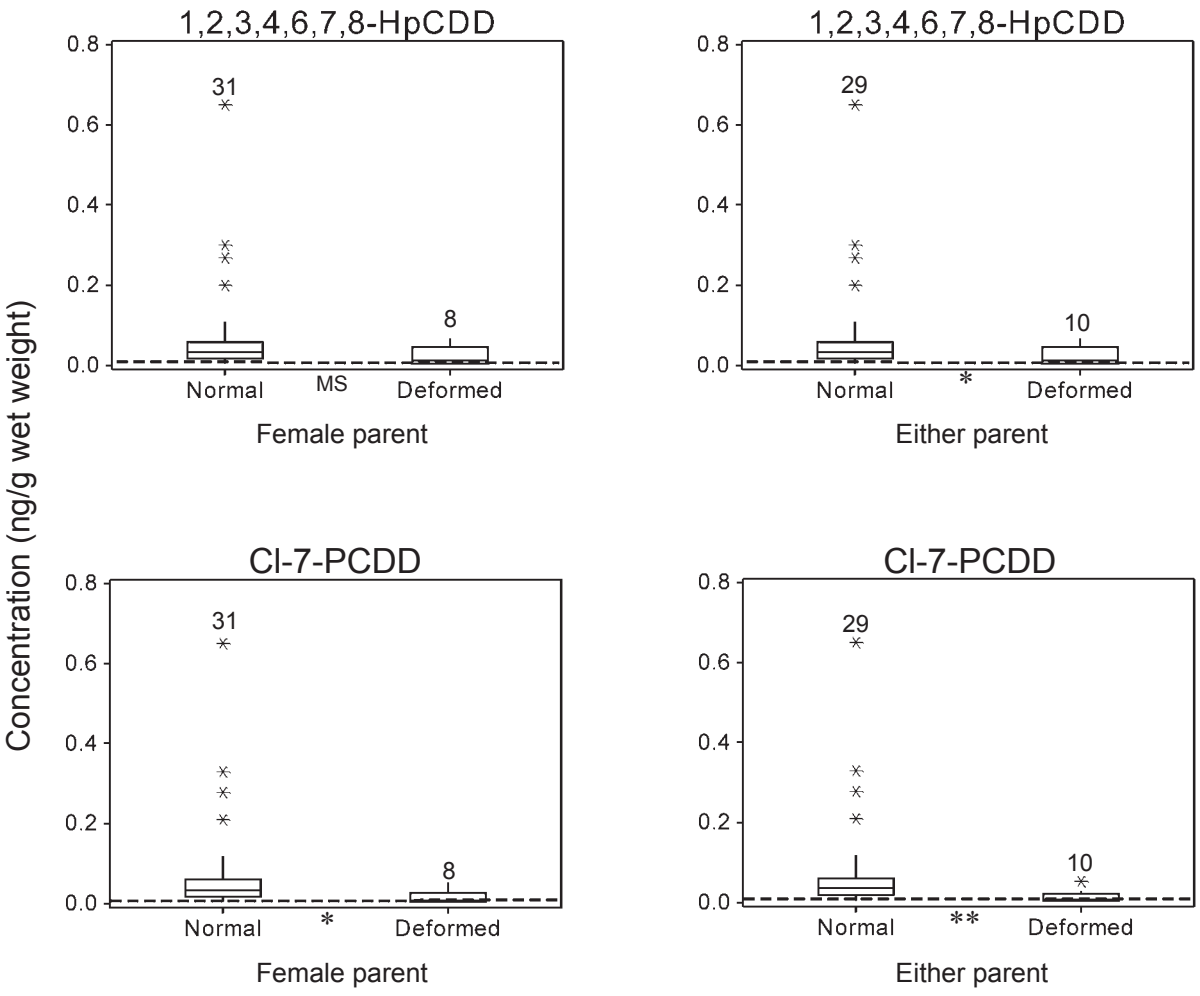

Figure 21. Censored box plots of concentrations (ng/g wet weight) of polychlorinated dibenzo-*p*-dioxins in Black-capped Chickadee eggs from parents (female or either parent) with normal vs. deformed beaks. Horizontal bar shows median, box shows first and third quartiles, whiskers include 1.5 times IQR beyond first and third quartiles, asterisks indicate outliers, and sample sizes are above plots. Dashed line shows maximum detection limit. Differences between groups are shown: $^{**}P < 0.01$, $^{*}P < 0.05$; MS = marginally significant ($0.05 < P < 0.10$).

Appendix A

Determination of Retinoids, Vitamin E and Carotenoids in Eggs from Black-capped Chickadees from the Regions of Anchorage, AK and Mat-Su Valley, AK Collected in 2001.

Biochemistry & Physiology Branch Final Laboratory Report FY 2003 - 30 - 12

30 May 2003

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SUBJECT:

Results from the determination of retinoids (3,4-dehydroretinol, all-trans-retinol, all-trans-retinal, and all-trans-retinol-palmitate), vitamin E (alpha-tocopherol), and carotenoids (astaxanthin, canthaxanthin, and beta-carotene) in black-capped chickadees (*Poecile atricapillus*) from the regions of Anchorage and Mat-Su Valley, Alaska collected in 2001.

INTRODUCTION:

Retinoids (vitamin A) is a general term applied to a group of isoprenoid compounds originated either from animal products, the retinoids, or from plant isoprenoid pigments, the carotenoids. These compounds are involved in biochemical pathways which involve diverse biological functions (Collins & Mao 1999). Sufficient levels of retinoids are essential for (i) vision, (ii) effective reproduction, (iii) the growth and development of young, (iv) nerve function, (v) epithelial function, and (vi) an effective immune response in mammals and birds (Thompson, 1976). Imbalances in retinoid homeostasis have been hypothesized as part of the mechanism of action of dioxin-like chemicals, due to their capacity to reduce vitamin A stores following exposure in different groups of vertebrates, including birds (Martinovic et al. 2003).

This report summarizes the findings of the measurement of retinoids, a form of vitamin E (alpha-tocopherol), and carotenoids in black-capped chickadees (*Poecile atricapillus*) from the regions of Anchorage and Mat-Su Valley, Alaska collected in 2001.

MATERIALS AND METHODS:

Eggs shipped from the Alaska Biological Science Center, Anchorage, AK in 1.5 mL cryotubes were stored at –80ºC until extraction and chromatographic analysis.

Chickadee eggs were analyzed for selected retinoids, carotenoids, and vitamin E based on the method recently described by Carvalho (2002). Individual eggs were used in the analysis of retinoids and carotenoids. Analytical procedures were performed under yellow fluorescent lights, and on ice. Each individual chickadee egg was weighed, and then 15% of this egg mass was placed in a separate tube. This egg fraction was homogenized in 2 mL of deionized water using a Omni TH homogenizer. The homogenate was split into 2 mL cryotubes for retinoid and carotenoid analysis. An internal standard mixture of retinol acetate and alpha-tocopherol-acetate was added to the retinoid samples for recovery estimates. A stock solution of Beta-Apo-8'-carotenal was used as the internal standard for recovery estimates in carotenoid samples. All samples were vortexed, and extracted with a 3:2 (v/v) ethyl acetate:hexane (both HPLC grade) solvent mixture 3 times. Samples were then centrifuged at 7500 rpm in a microcentrifuge (Fisher Scientific, Pittsburgh, model 16KM) and the upper organic phase was carefully transferred to a 1.5 mL amber cryo-tube. All samples were evaporated to dryness under a stream of dry N_2, and stored at –80ºC until chromatographic analysis. Each homogenate

from a single egg was analyzed in duplicate for each analyte. In addition, one sample was analyzed in triplicate (i.e. three portions from a sample).

Separate chromatographic procedures were used for retinoids and vitamin E, and another for carotenoids. Chromatographic peaks were identified by comparing retention times of the different analytes. The standards used during retinoid and vitamin E analysis were 3,4-dehydroretinol (gift from F. Hoffman-La Roche Ltd.), all-trans-retinol 95% pure (Sigma), all-trans-retinal 98% pure (Sigma), retinol-acetate (internal standard), the vitamin E alpha-tocopherol 95% pure (Sigma), the internal standard alpha-tocopherol-acetate 96% pure (Sigma), and all-trans-retinol-palmitate 1,800,000 USP units/g (Sigma). The standards for carotenoid analyses were: astaxanthin 99% pure (Sigma), canthaxanthin (gift from F. Hoffman-La Roche Ltd.), the internal standard beta-apo-8'-carotenal 99% pure (Sigma) and beta-carotene 95% pure (Sigma).

The HPLC system (Agilent Technologies, Wilmington, mode 1100) consisted of a binary pump, a variable wavelength UV absorbance detector and an autosampler. A reverse-phase Adsorbosphere C18 column (4.6 mm internal diameter, 150 mm length) equipped with a column guard (Altech Associates, Deerfield, IL) was employed for all analyses. The detector was set at 325 nm for the retinoids, 292 nm for alpha-tocopherols, and 474 nm for carotenoids. Flow rate was 1.0mL.min-1 during all analytical procedures. Both the retinoid and carotenoid analysis employed the same basic solvent mixtures, composed of methanol:water:ethyl-acetate 90:10:0 (mixture A) and methanol:water:ethyl-acetate 29:1:70 (mixture B). Retinoids were eluted with an isocratic flow during the first 10 min with 100%A:0%B. At 10 min the flow was changed to a linear elution gradient starting at 100%A:0%B and changing to 0%A: 100%B over 21.2 min. Each retinoid run lasted 30 min, with 4 min reequilibration time between samples. Carotenoids were eluted with a linear elution gradient starting with 80%A: 20%B, and changing to 0%A: 100%B after 19 min. Each carotenoid analysis lasted 20 min, with 4 min reequilibration time between samples. Recovery rates were 73 ± 12% (mean ± standard deviation) for carotenoids, 58 ± 21% (mean ± standard deviation) for smaller retinoids, and 74 ± 11% (mean ± standard deviation) for longer chain vitamin E and retinol-palmitate. Results were corrected based on the recovery of these internal standards. Concentrations are reported per gram egg.

The limits of detection (LOD) and quantitation (LOQ) for the method were calculated as described by Keith et al. (1983). These parameters were calculated using the daily assay method blanks. The lowest definable area of a peak for each analyte (based on a signal-to-noise ratio of two) was determined for each method blank. The LOD was defined to be equal to the mean concentration (n = 5 per method) for the lowest definable peak area plus 3 times the standard deviation of the mean associated with that peak area. The LOD was defined to be equal to the mean concentration for the lowest definable peak area plus 3 times the standard deviation of the mean associated with that peak area. These measures were used to evaluate the sample data results and to determine whether they were detectable or measurable above that of the background. Detection limits were very consistent among blanks and thus, the mean detection limit was used for analyses. Mean detection limits for each analyte method are as follows: 3,4-dehydroretinol, retinol, and retinal (0.519 ± .000627 ng); alpha-tocopherol (49.9 ± 0.487 ng); retinol-palmitate (0.168

± 0.022 ng); astaxanthin (2.41 ± 0.098 ng); canthaxanthin (0.908 ± 0.00340 ng); and beta-carotene (0.010 ± 0.00130 ng). Note that detection limits are for the raw chromatogram values measured, and not the egg-mass corrected concentrations reported in Tables 2 and 3.

QUALITY ASSURANCE AND QUALITY CONTROL:

The objective of the quality assurance plan of this study was to assure that the extraction and biochemical analyses were accurate and precise measurements of the samples collected in the field portion of this study. The general scheme included replication of assayed samples during each extraction, comparison of calibration against known standards, proper maintenance and calibration of equipment, proper documentation at all steps of sample processing and other considerations of Good Laboratory Practice (GLP). The specific aspects of the QA plan related to the retinoid and carotenoid extraction and evaluation procedures are given below.

All experimental information was recorded in bound laboratory notebooks and electronic copies maintained in a separate, secured area (network drive backup). Instrumental printouts and computer generated data tables were uniquely labeled and cross-referenced to the project notebook. Hard copies of computerized data files were maintained in a project notebook. Computer files were copied to a network storage area and by archived tape back up. All equipment used in this study was routinely inspected and preventative maintenance performed. A logbook was kept for each instrument to document its use, performance and maintenance.

Replication and subsequent performance checks were performed at different stages of the retinoid/carotenoid determination procedure. During the extraction, one sample was randomly selected to be extracted in triplicate (i.e. three portions from the sample). Five point standard curves for each analyte and internal standard were prepared each week. In addition, two retinoid and carotenoid standards were analyzed in conjunction with each HPLC analysis of extracted chickadee egg samples to verify analyte retention times. Sample calibration standard curves for each analyte are displayed in Figures 1 through 8. The sources and lot numbers of the retinol, retinyl acetate and retinyl palmitate used as standards were recorded in the laboratory notebook. Data summaries for retinoid, vitamin E, and carotenoid standard curves have been included with this report to demonstrate the consistency of the detector response with quantity of analyte over the time course of the study. In addition to allowing quantitative determination of analyte present, the weekly standard curves also provided a performance check of the operating system.

DATA ANALYSIS:

In order to verify the consistency and consequently the reliability of the sample data as a whole, the weekly standard curve data for each analyte were recorded and monitored over time. In each case a mean and standard deviation were calculated for each standard curve (Table 1). Standard curve slopes and r-squared were very consistent among weeks. Y-intercept values were more variable (data not shown), but did not influence estimates of analyte concentrations in samples. Values are reported to three significant digits.

Table 1. Mean, standard deviation, and coefficient of variation of parameters for weekly standard curves of retinoid and carotenoid analytes (*n* = 3 for each analyte).

Standard Curve	Slope mean (S.D.) CV%	y-intercept mean (S.D.) CV%	r-squared mean (S.D.) CV%
3,4-dehydroretinol	307000 (9350) 3%	-89300 (28400) 32%	0.999 (0.000192) 0%
all-trans-retinol	485000 (21700) 4%	-133000 (75700) 57%	0.999 (0.000688) 0%
all-trans-retinal	119000 (10100) 8%	-49800 (20200) 41%	0.999 (0.00106) 0%
alpha-tocopherol	3200 (57.7) 2%	-132000 (40800) 31%	0.998 (0.00143) 0%
alpha-tocopherol acetate (int)	241000 (13300) 6%	-79400 (30700) 39%	0.999 (0.00223) 0%
all-trans-retinol-palmitate	221000 (26800) 12%	-40900 (62400) 153%	0.996 (0.00721) 1%
astaxanthin	130000 (10000) 8%	-48000 (24800) 52%	0.999 (0.000162) 0%
canthaxanthin	147000 (8730) 6%	-57900 (11500) 20%	0.999 (0.000135) 0%
beta-apo-8'-carotenal (int)	247000 (11900) 5%	-110000 (47500) 43%	0.999 (0.000119) 0%
beta-carotene	107000 (5590) 5%	29400 (29100) 99%	0.998 (0.00229) 0%

Estimates of the efficiency of recovery based on internal standards are reported in the data tables. Due to solvent changes during extraction, only 14% of the extracted sample was analyzed by HPLC. It was necessary to adjust the measured retinoid, vitamin E, and carotenoid values accordingly. This was done as indicated in equation 1.

$$\text{Analyte content in egg} = \frac{\text{(mass of analyte measured)}}{(0.14 * \text{mass of egg extracted})} \quad [1]$$

RESULTS AND DISCUSSION:

The error associated with the slopes measured for the routine standard curves were relatively small, ranging from 1% to 12%. This suggests that the sensitivity of the detector, instrumental response and standard solutions were inherently unchanged through out the time course of the measurements. The error associated with the y-intercepts for the composite standard curves were significant, ranging from 20% to 153%. This is why we run standards on a regular basis. This variability may reflect the weekly variability in environmental and instrumental conditions as opposed to anything inherent in the detection method or the system. Sample chromatograms for retinoid and carotenoid analyses are displayed in Figures 9 through 12. Duplicate procedural extraction blanks measured with each HPLC run revealed that no analytes were present in the extraction reagents or introduced during the extraction procedure.

Data for retinoids, vitamin E, and carotenoids determined in the chickadee eggs are reported in Tables 2 and 3 along with their associated estimated recoveries of retinol acetate, alpha-tocopherol acetate, and beta-apo-8'-carotenal. For the analytes measured, greater than 85% of the duplicate samples had an associated coefficient of variance of less than 20%. Egg samples examined in triplicate had coefficients of variation less than 15% for the majority of analytes (all-trans-retinol-palmitate had a higher coefficient of variation at 37%).

For all samples, 3,4-dehydroretinol was below the limit-of-detection in the eggs analyzed. This is expected, because 3,4-dehydroretinol is found mainly in fish, although terrestrial vertebrates can obtain it through fish-based diets (Kakela et al. 2003). Retinoids stored in the avian egg are essential for normal development. All-trans retinol concentrations were the highest of the retinoids in the chickadee eggs, ranging from 491 to 9250 ng/g egg (Table 2). This is supported in developmental studies of other avian species. For example, the vitamin A content in freshly-laid eggs of normal domestic hens was analyzed to be approximately 80% retinol (Parrish et al. 1951). The mean chickadee egg concentration of the antioxidant, alpha-tocopherol, was 85.2 µg/g egg. This is only slightly higher than normally reported values for hen eggs (Chen et al. 1998). For carotenoids, both astaxanthin and canthaxanthin concentrations in eggs were below the method limit-of-detection, while the mean beta-carotene concentration was 638 ng/g egg. Other studies have reported beta-carotene as the major carotenoid present in bird eggs (Surai et al. 2001), but the reason for the absence of astaxanthin and canthaxanthin in the chickadee samples is unknown (no literature reference values found for comparison).

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Appendix 1 – Sample calibration curves and chromatograms for analytes measured.

Figure 1 - 3,4-Dehydroretinol

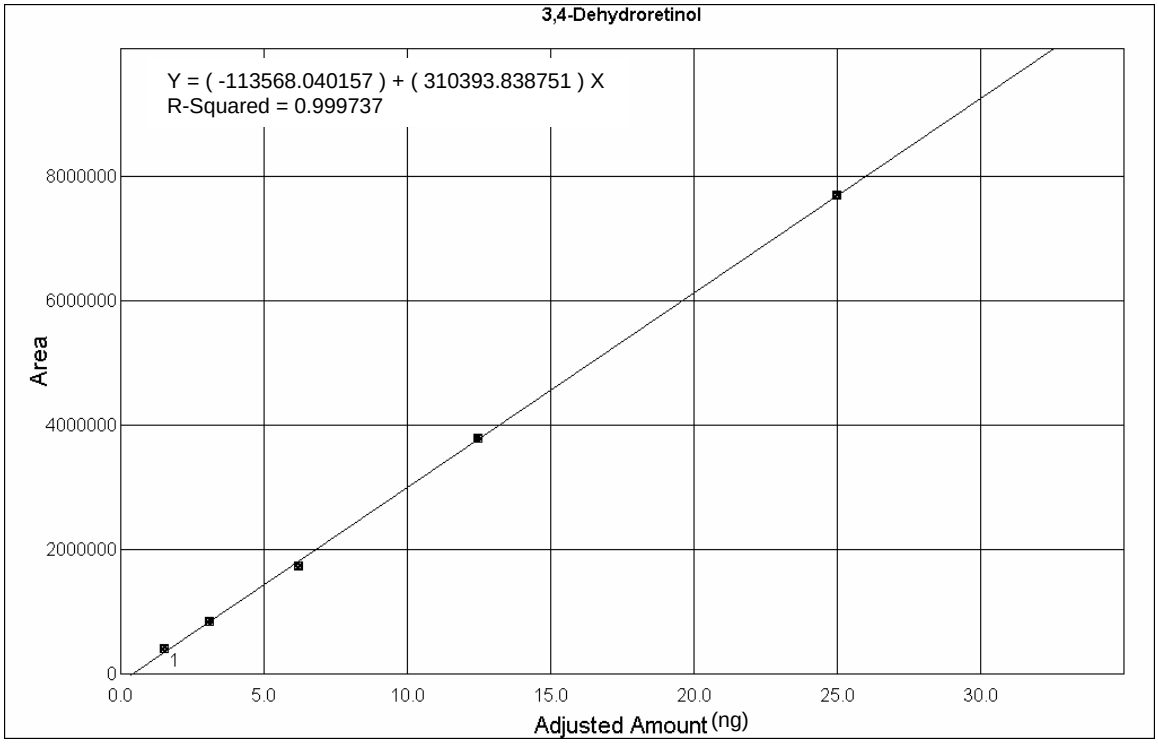

Figure 2 - All-trans-retinol

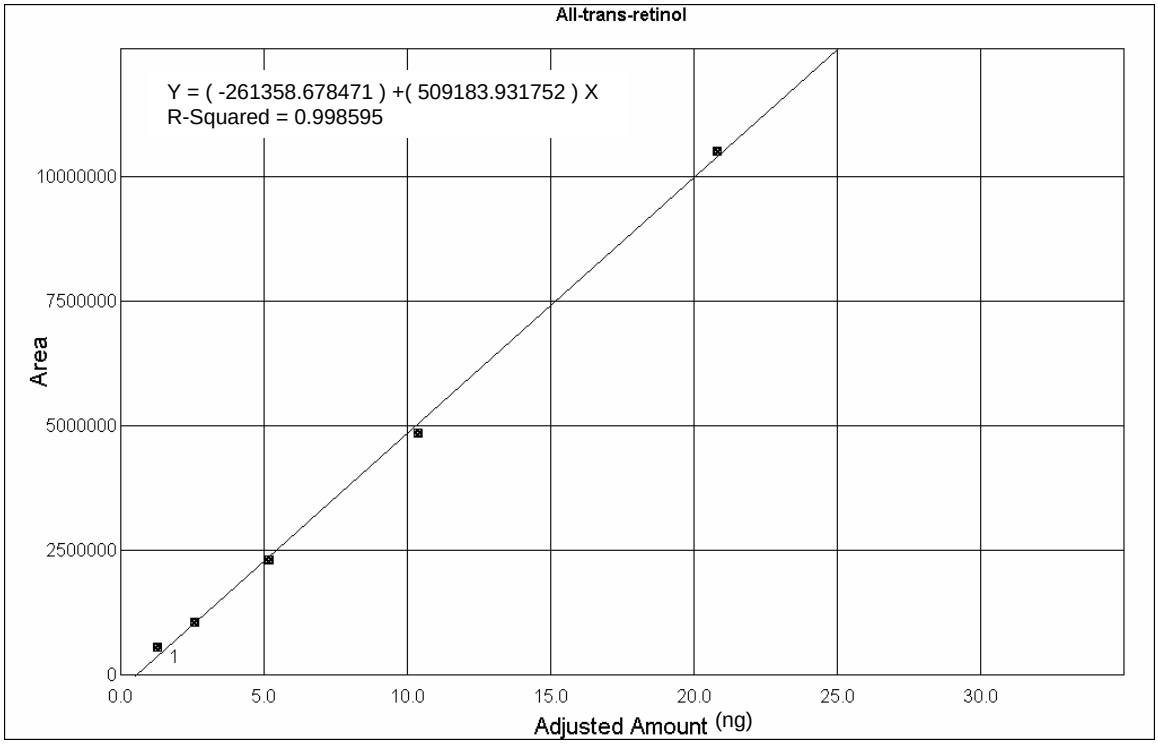

Figure 3 - All-trans-retinal

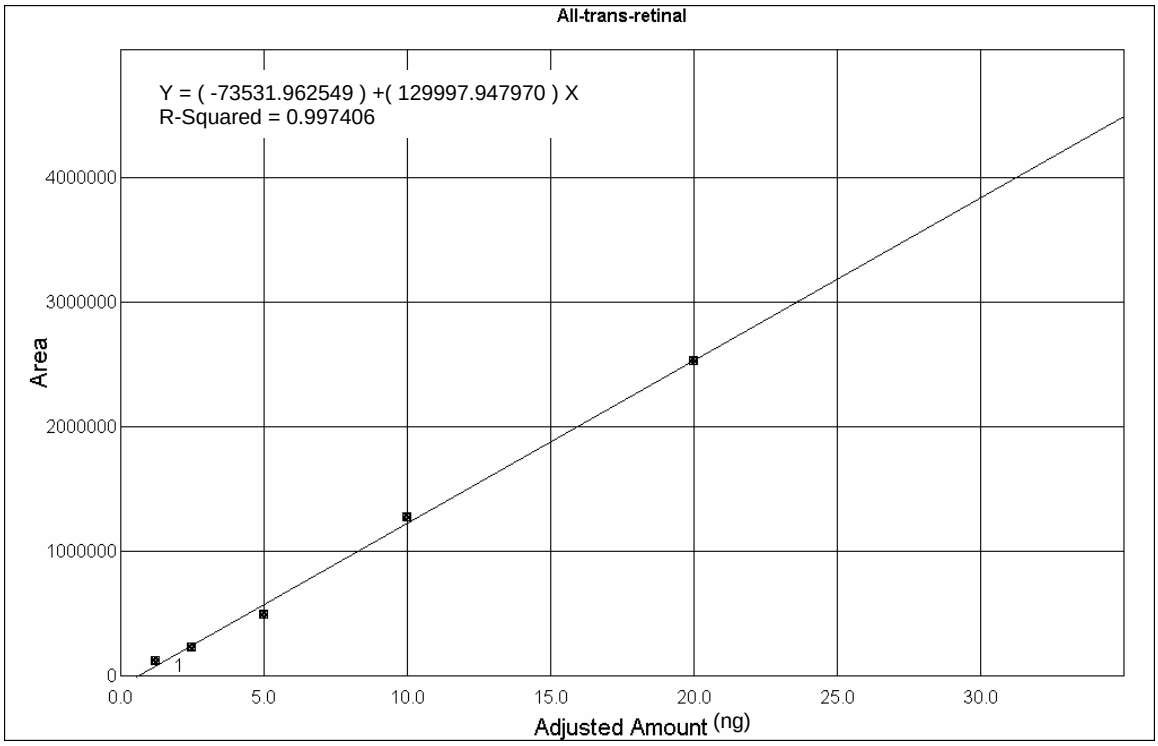

Figure 4 - Alpha-tocopherol

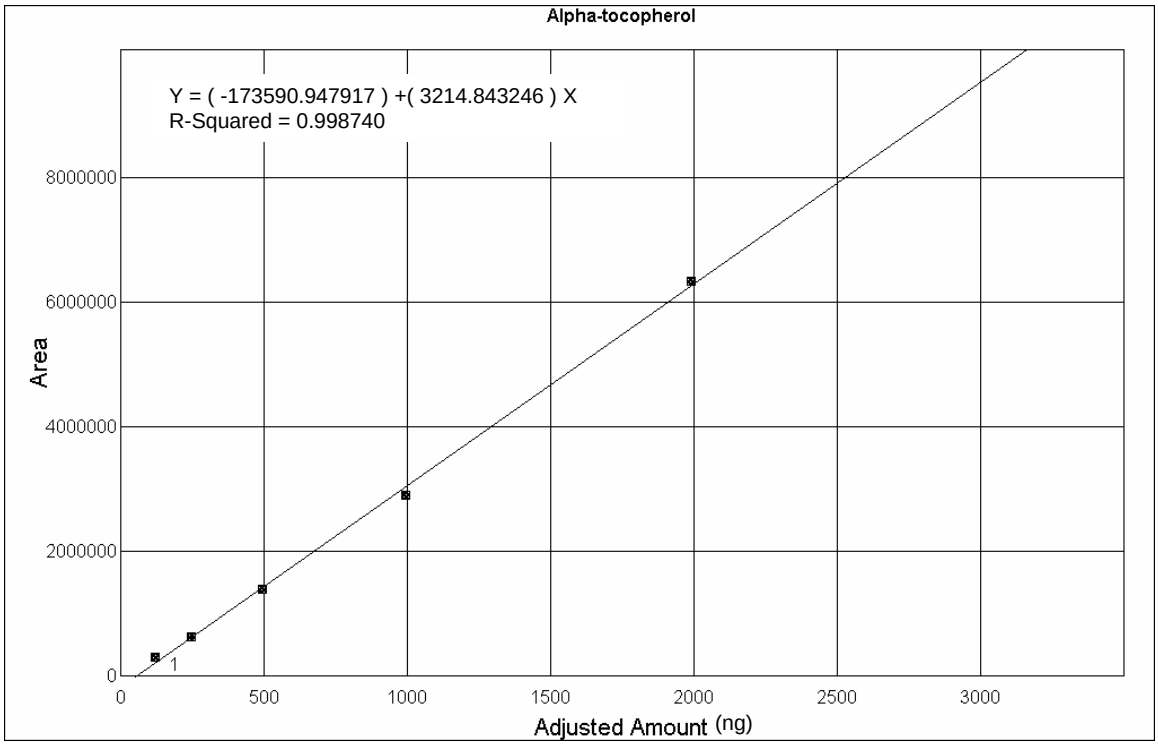

Figure 5 - Retinol-palmitate

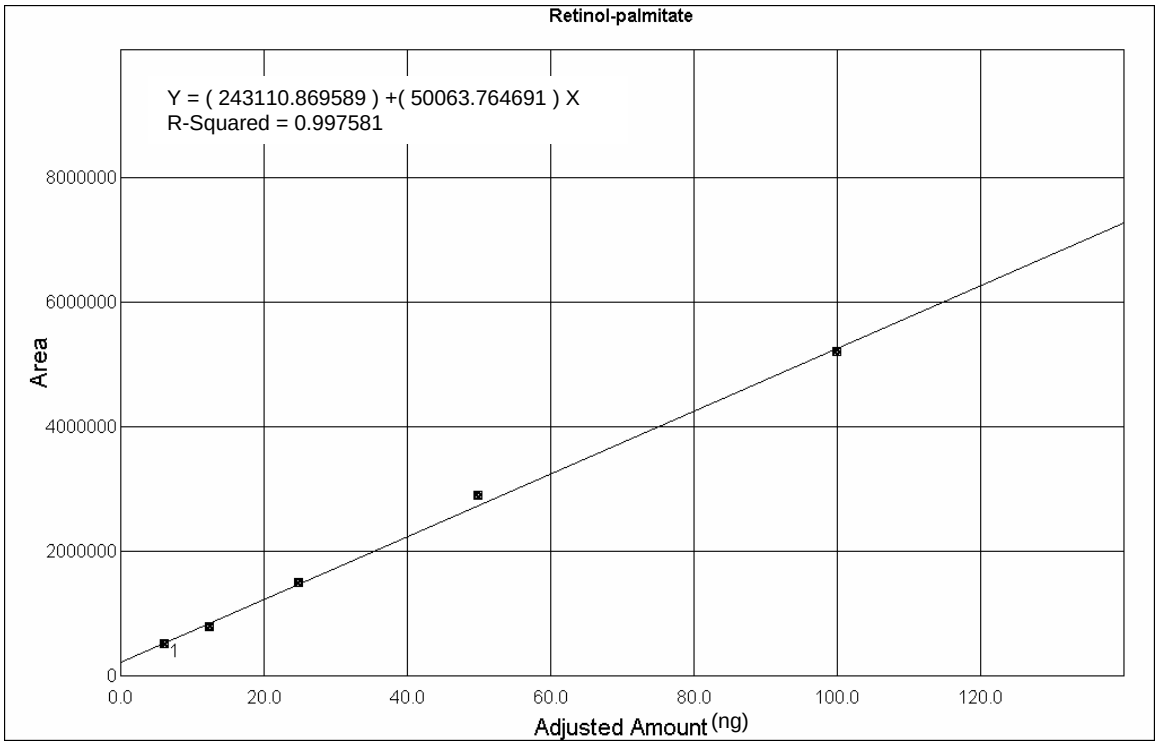

Figure 6 - Astaxanthin

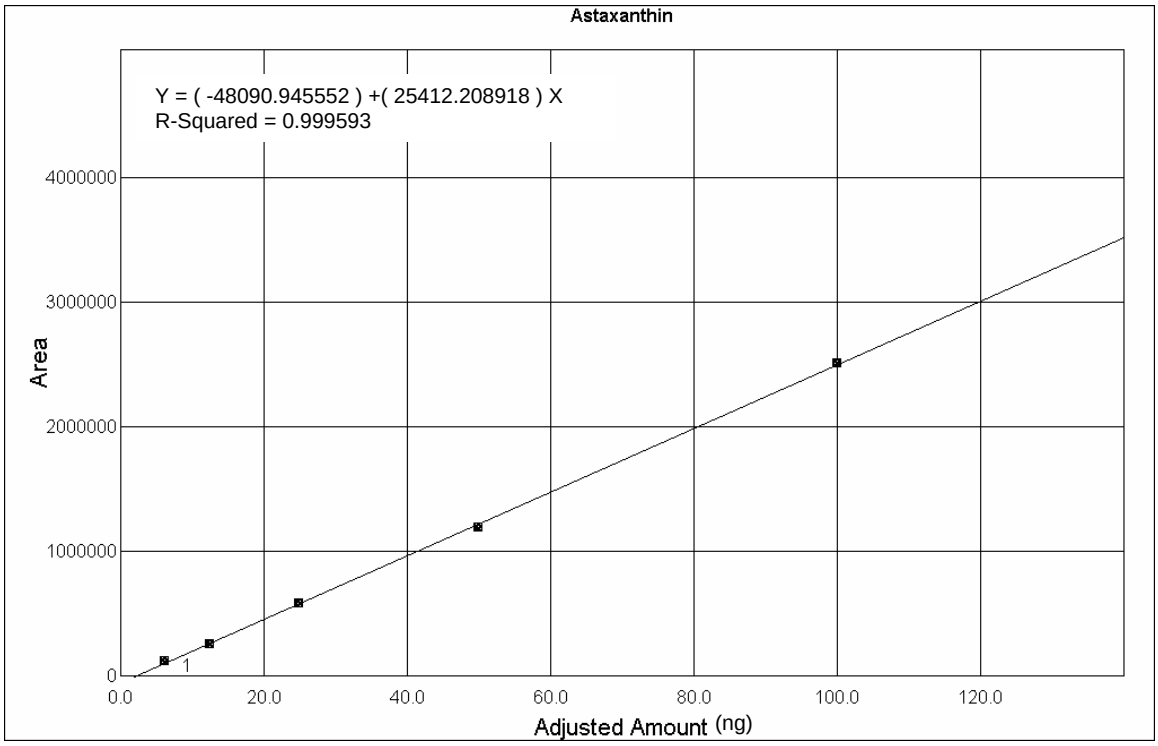

Figure 7 - Canthaxanthin

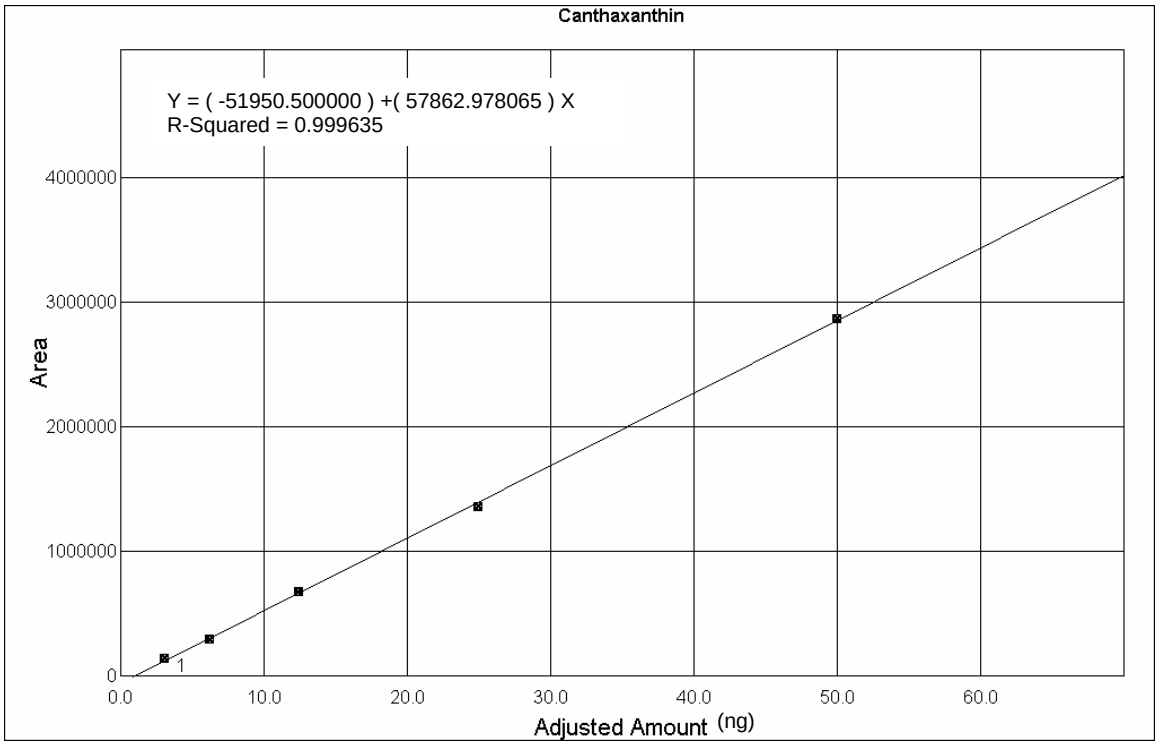

Figure 8 - Beta-carotene

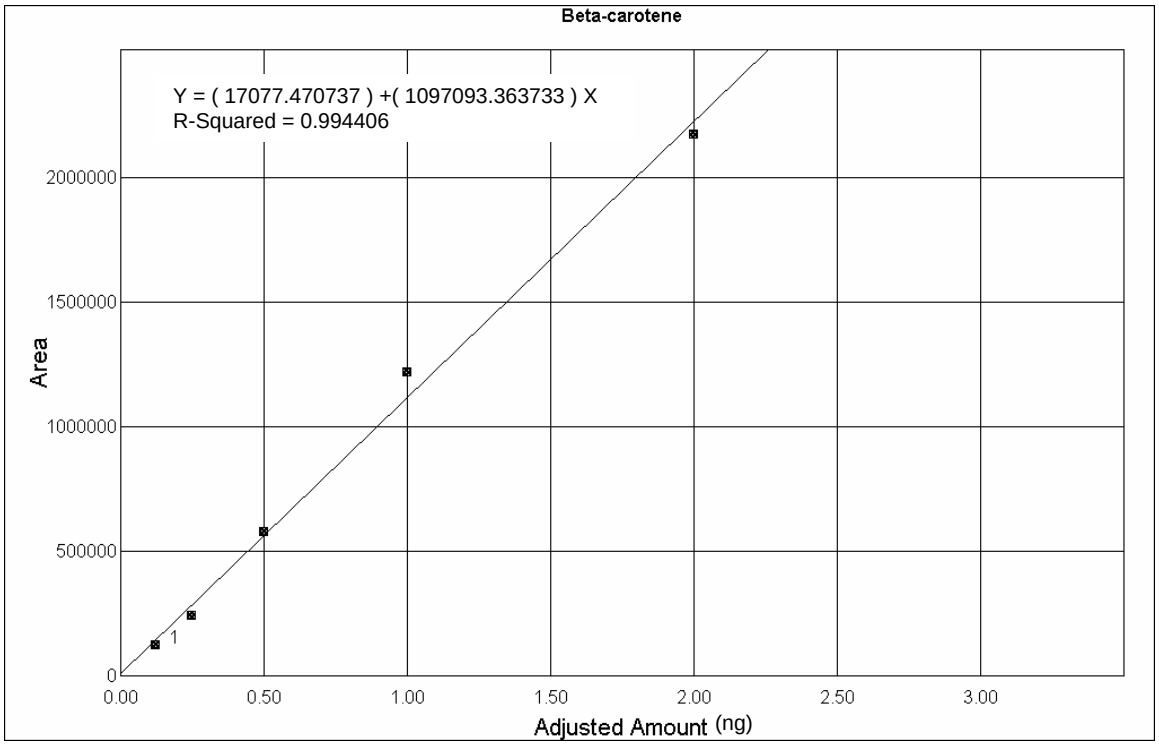

Figure 9 - Sample Chromatogram
Retinoid Calibration Standards

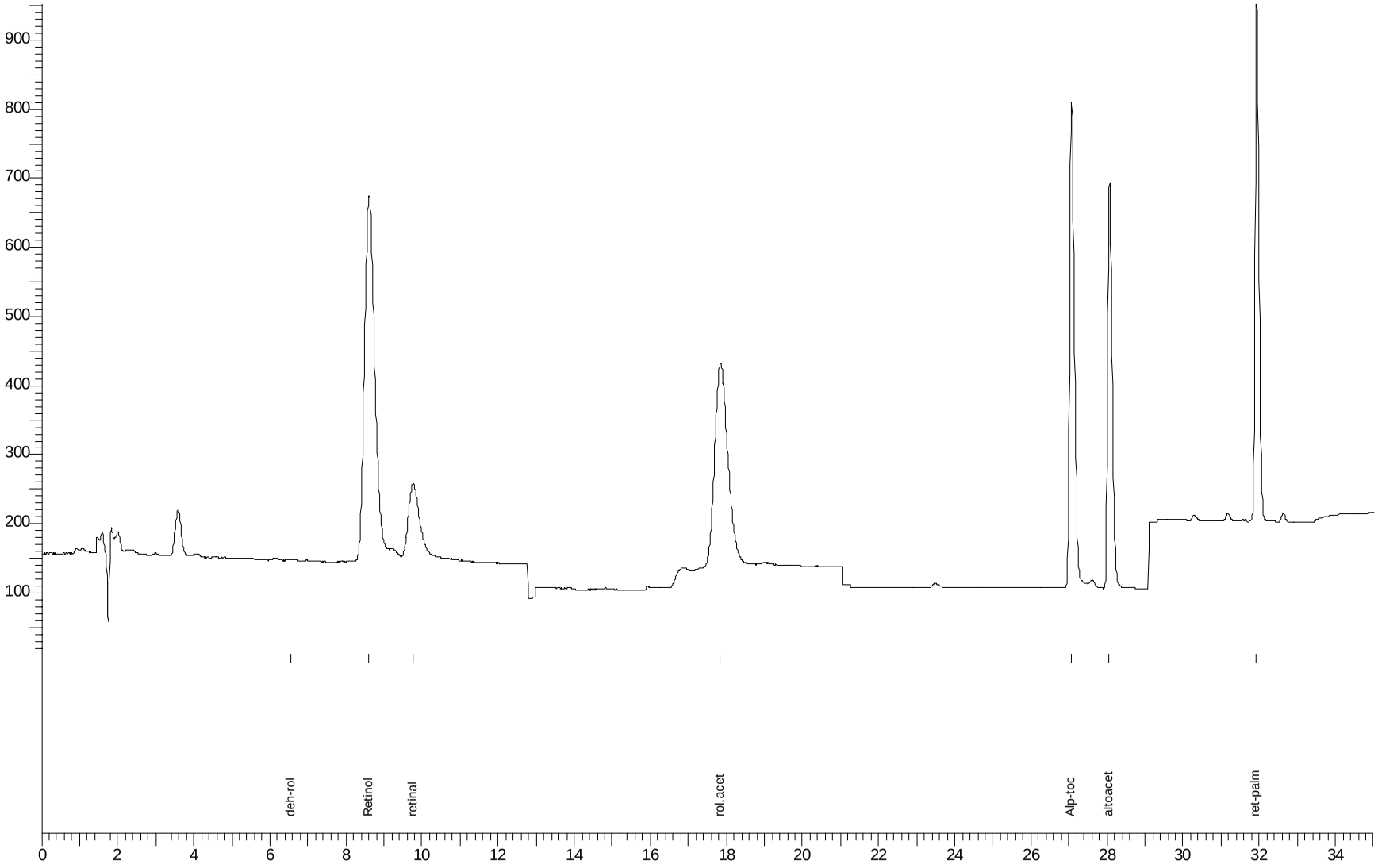

Figure 10 - Sample Chromatogram
Retinoid Analysis – Black-capped Chickadee Sample 4 (Replicate 1)

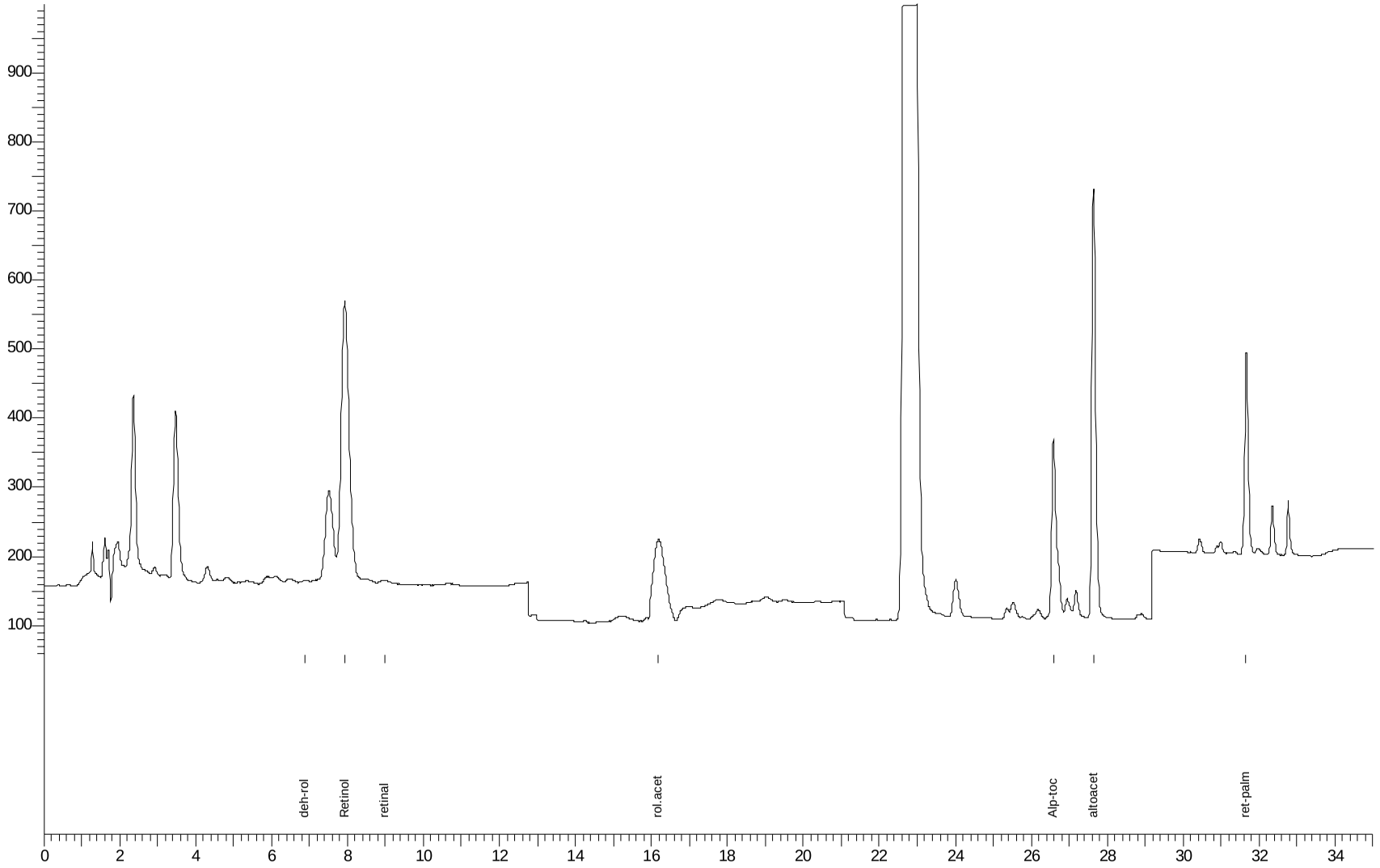

Figure 11 - Sample Chromatogram
Carotenoid Calibration Standards

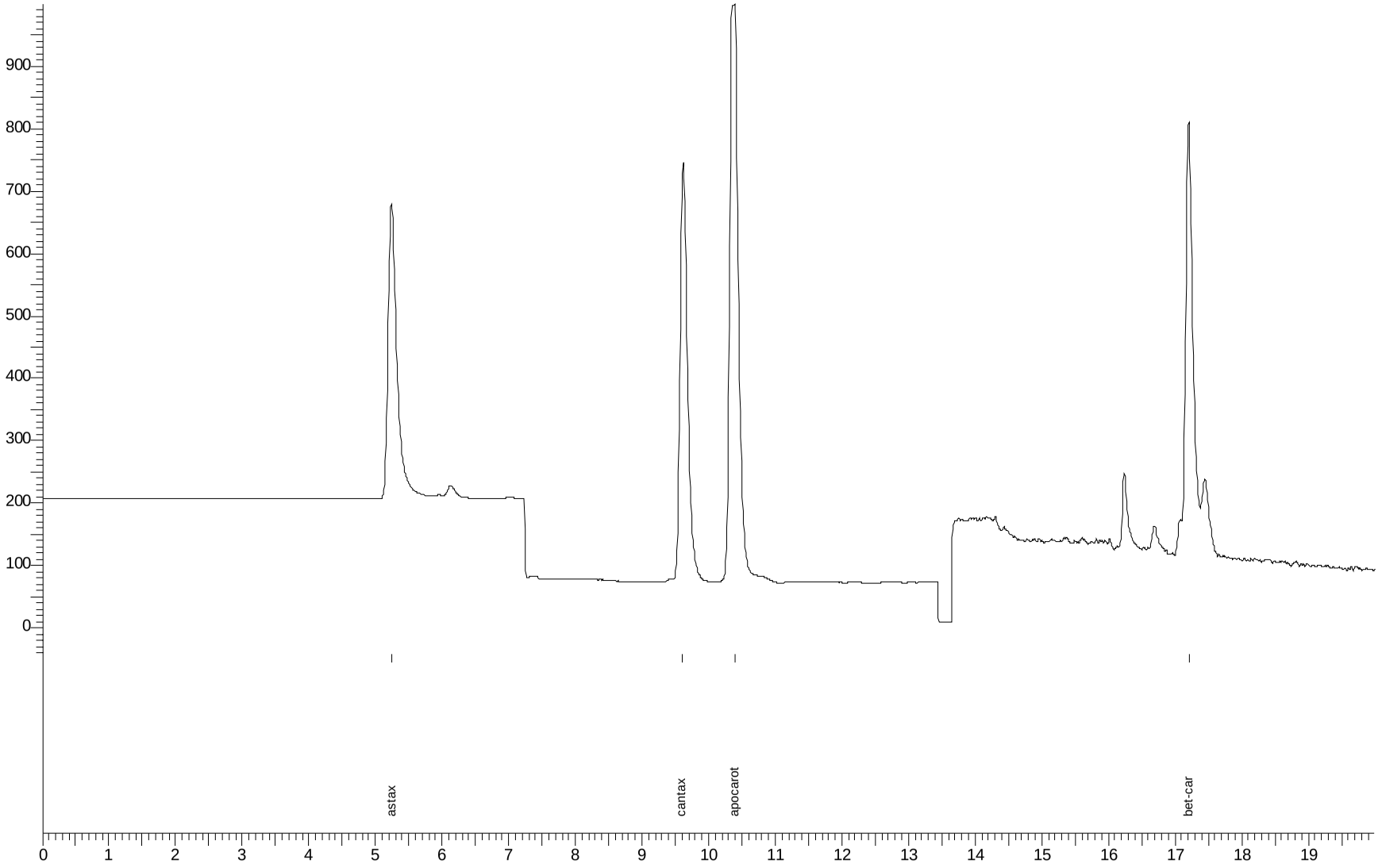

Figure 12 - Sample Chromatogram
Carotenoid Analysis - Black-capped Chickadee Sample 1 (Replicate 1)

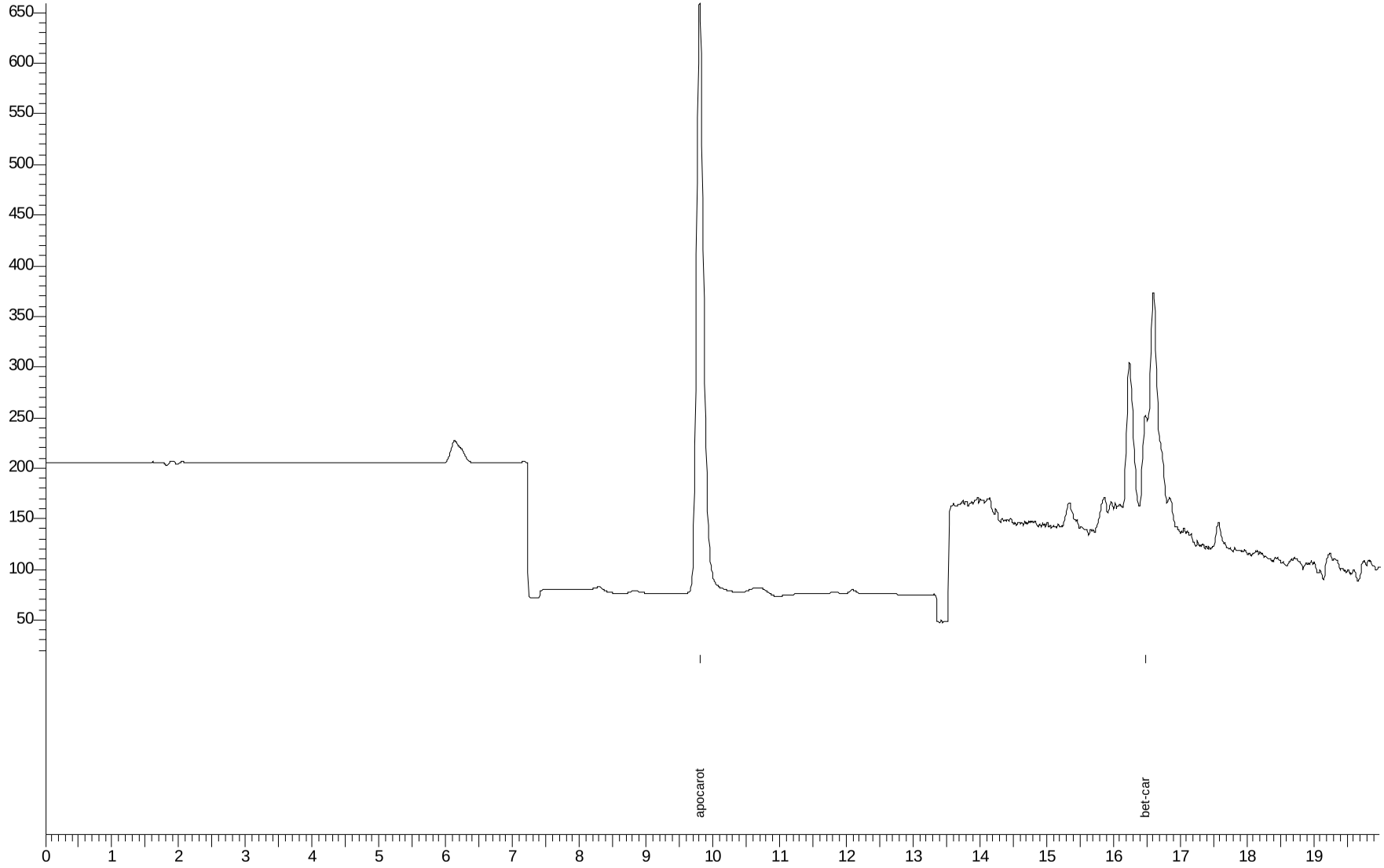

Table 2. Retinoid vitamins and vitamin E concentrations in black-capped chickadee eggs collected from the Anchorage, AK and Mat-Su Valley, AK. Values shown are corrected for percent recovery of internal standard. All egg homogenate samples were measured in duplicate. Procedural triplicate samples are highlighted in bold.

Sample Number	Sample Code	retinol acetate % recovery	3,4-dehydroretinol (ng/g egg)	S.D.	all-trans-retinol (ng/g egg)	S.D.	all-trans-retinal (ng/g egg)	S.D.	acetate % recovery	alpha-tocopherol (ug/g egg)	S.D.	retinol-palmitate (ug/g egg)	S.D.
1	1TEEL02	93%	13.5	(2.03)[1]	491	(38.7)	52.5	(7.66)	73%	64.7	(0.942)	4.29	(0.267)
2	1STER01	54%	25	(1.19)[1]	759	(10.3)	240	(38.4)	87%	55.3	(0.901)	1.79	(0.0209)
3	1SIMA01	68%	19.9	(2.14)[1]	1190	(27.8)	19.9	(2.14)[1]	68%	77.1	(1.5)	1.97	(0.0607)
4	1RACR05	64%	22.4	(4.97)[1]	1110	(140)	108	(5.14)	80%	58.2	(0.461)	2.33	(0.143)
5	1PTWO10	36%	49.1	(19.1)[1]	1640	(236)	391	(49.8)	67%	51.3	(0.209)	2.92	(0.166)
6	1PTWO07	39%	56.7	(9.57)[1]	3340	(1370)	403	(157)	67%	34.7	(4.89)	4.81	(0.247)
7	1PTWO04	44%	28.3	(5.14)[1]	940	(171)	177	(32.3)	78%	61.1	(0.322)	3.22	(0.0576)
8	1POPO02	42%	35	(3.82)[1]	1060	(29.4)	255	(29.4)	82%	77.5	(7.72)	3.66	(0.148)
9	1POMA05	47%	34.8	(1.67)[1]	1970	(181)	183	(25.6)	61%	136	(3.18)	4.73	(0.0238)
10	1PAJO11	72%	30.3	(2.31)[1]	2130	(62.9)	130	(14)	78%	96.9	(1.35)	4.7	(0.129)
11	1MUPA05	97%	13.1	(0.346)[1]	516	(37)	58.5	(1.09)	82%	42.5	(0.106)	4.81	(0.0778)
12	1LAMT11	60%	24.7	(1.63)[1]	1270	(30.4)	97.6	(10.8)	78%	65.2	(0.694)	3.7	(0.0137)
13	1KULE01	41%	30.6	(2.49)[1]	1240	(1.4)	127	(14.8)	79%	59.4	(0.547)	3.49	(0.0722)
14	1KIPA16	39%	35.3	(0.0997)[1]	1340	(12.1)	35.3	(0.0997)	76%	70.4	(2.31)	1.1	(0.104)
15	1KIPA05	32%	46.9	(2.75)[1]	3250	(80.9)	894	(55.5)	38%	140	(4.4)	1.48	(0.121)
16	1JOTR01	59%	23.8	(4.59)[1]	925	(62.4)	113	(8.3)	78%	42.6	(3.39)	4.06	(0.0372)
17	1HIPA01	90%	25.2	(16.5)[1]	620	(63.6)	61.5	(0.813)	76%	36.1	(2.61)	3.01	(0.0828)
18	1HANS03	55%	24.3	(1.59)[1]	1850	(67.1)	90.4	(5.45)	82%	94.7	(2.19)	3.55	(0.18)
19	1DEWH01	70%	19.1	(1.03)[1]	911	(0.261)	77.3	(9.18)	83%	65.5	(0.828)	4.39	(0.157)
20	1CACR06	53%	37.7	(2.01)[1]	2530	(121)	231	(34)	78%	122	(1.58)	8.97	(0.367)
21	1CACR04	91%	16.3	(2.65)[1]	1070	(69.8)	90.4	(31.7)	70%	64.2	(1.34)	3.97	(0.436)
22	1BRUD01	51%	27.1	(2.26)[1]	1760	(137)	131	(3.97)	74%	67.2	(0.504)	3.43	(0.106)
23	1BLUM04	51%	72.3	(3.37)[1]	9250	(26.1)	459	(35.6)	69%	417	(7.3)	13.3	(0.312)
24A*	1BISS03 (rep 1)	51%	25.5	(2.61)[1]	1710	(105)	180	(2.86)	82%	39.2	(0.875)	3.69	(0.125)
24B*	1BISS03 (rep 2)	51%	25.7	(5.11)[1]	1800	(352)	195	(35.1)	62%	49.9	(2.07)	1.75	(0.0683)
24C*	1BISS03 (rep 3)	51%	25.7	(2.25)[1]	1820	(94.1)	156	(7.37)	76%	45.6	(0.311)	3.73	(0.222)
		Mean	30.7	(14)	1790	(1760)	192	(190)		85.2	(76.3)	4.03	(2.51)
			min	max	min	max	min	max		min	max	min	max
		Range	13.1	72.3	491	9250	19.9	894		34.7	417	1.1	13.3

[1] Below method limit-of-detection (LOD). One-half LOD used for calculation purposes.

* Overall mean was calculated using the mean of the procedural triplicate samples, not the individual triplicate values.

Table 3. Carotenoid vitamin concentrations in black-capped chickadee eggs collected from the Anchorage, AK and Mat-Su Valley, AK. Values shown are corrected for percent recovery of internal standard. All egg homogenate samples were measured in duplicate. Procedural triplicate samples are highlighted in bold.

Sample Number	Sample Code	beta-apo-8'-carotenal % recovery	astaxanthin (ng/g egg)	S.D.	canthaxanthin (ng/g egg)	S.D.	beta-carotene (ng/g egg)	S.D.
1	1TEEL02	79%	74.9	(18.6) [1]	28.2	(6.99) [1]	26	(17.3)
2	1STER01	89%	69.8	(1.29) [1]	26.3	(0.486) [1]	29.1	(2.78)
3	1SIMA01	66%	94	(7.47) [1]	35.4	(2.82) [1]	69.7	(9.61)
4	1RACR05	63%	103	(4.94) [1]	38.9	(1.86) [1]	51.1	(0.58)
5	1PTWO10	89%	85.9	(4.74) [1]	32.4	(1.79) [1]	709	(59.1)
6	1PTWO07	71%	92.8	(2.46) [1]	35	(0.927) [1]	414	(16.9)
7	1PTWO04	87%	65.6	(4.26) [1]	24.7	(1.61) [1]	308	(42.3)
8	1POPO02	66%	102	(7.18) [1]	38.6	(2.7) [1]	320	(49.1)
9	1POMA05	65%	116	(8.89) [1]	43.7	(3.35) [1]	1680	(272)
10	1PAJO11	46%	221	(29.6) [1]	83.1	(11.1) [1]	887	(11.5)
11	1MUPA05	90%	65.9	(6.84) [1]	24.8	(2.58) [1]	192	(47.5)
12	1LAMT11	68%	101	(2.35) [1]	38.1	(0.884) [1]	738	(752)
13	1KULE01	63%	93	(5.56) [1]	35	(2.1) [1]	752	(130)
14	1KIPA16	79%	83.1	(20.5) [1]	31.3	(7.73) [1]	320	(55.3)
15	1KIPA05	68%	101	(5.49) [1]	38.1	(2.07) [1]	1230	(26.5)
16	1JOTR01	64%	99.8	(1.48) [1]	37.6	(0.557) [1]	367	(59.2)
17	1HIPA01	68%	89.1	(3.92) [1]	33.6	(1.48) [1]	422	(82.7)
18	1HANS03	68%	91.4	(7.19) [1]	34.4	(2.71) [1]	223	(18.7)
19	1DEWH01	64%	97.3	(2.12) [1]	36.7	(0.801) [1]	995	(65.9)
20	1CACR06	78%	118	(4.77) [1]	44.3	(1.8) [1]	1210	(93.4)
21	1CACR04	100%	68.3	(5.45) [1]	25.7	(2.05) [1]	509	(75.4)
22	1BRUD01	76%	82.8	(4.24) [1]	31.2	(1.6) [1]	438	(30.4)
23	1BLUM04	70%	244	(18.2) [1]	92.1	(6.86) [1]	2280	(88.5)
24A*	1BISS03 (rep 1)	67%	89.9	(7.55) [1]	33.9	(2.85) [1]	988	(175)
24B*	1BISS03 (rep 2)	75%	80.5	(2.66) [1]	30.3	(1) [1]	1150	(13.4)
24C*	1BISS03 (rep 3)	70%	86.4	(6.08) [1]	32.6	(2.29) [1]	1260	(48.6)
		Mean	102	(42.8)	38.4	(16.1)	638	(561)
			min	max	min	max	min	max
		Range	65.6	244	24.7	92.1	26	2280

[1] Below method limit-of-detection (LOD). One-half LOD used for calculation purposes.

* Overall mean was calculated using the mean of the procedural triplicate samples, not the individual triplicate values.

Appendix B

A Report to the

United States Fish and Wildlife Service

On

An Investigation of DNA Damage

In Black-capped Chickadees.

By

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July 22nd, 1999.

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SUMMARY

The flow cytometry analysis of the black-capped chickadee blood indicated that the beak-deformed birds had a highly significant amount of DNA damage compared to the normal bird samples (X^2 = 152.4, 1 df, P=0). In addition, the normal Palmer samples that were kept in snow for 4 hours may have suffered degradation, since their CV DIF values varied significantly from the normal bird samples that were frozen immediately on dry ice (X^2 = 34.8, 1 df, P=0). It is suggested that additional statistical analyses be carried out to further understanding about the causation of the observed DNA damage.

Introduction

This study examines the possibility that the chickadee population with beak deformities in the Anchorage region also show evidence of genotoxicity in the form of clastogenic damage. The latter is defined as the breakage and subsequent rearrangement of chromosomal material. This rearrangement involves either reattaching to another chromosome (translocation) or remaining as a separate entity. At cell division, these separate entities are lost from the nucleus and may becomed enveloped in a membrane to form a micronucleus. The end result is that the subsequent daughter cells receive an unequal amount of parental DNA. This change in DNA content is then perpetuated and increased in the general cell (i.e. red blood cell) population by further divisions of these corrupted cell lines until the terminal division which ends in the formation of functional somatic cells such as blood cells. Thus the cell incidence of the initial clastogenic damage is multiplied many times before the damaged cells are released to the general cell population of the tissue (i.e. peripheral blood circulation). The function of these cells may be impaired as a consequence of the loss (or gain) of genetic material. This loss of function likely impacts the animal's fitness. In addition genotoxicity of the blood may be an indicator of genotoxic activity in cells from other tissues.

Flow cytometry can be used to determine the net amount of DNA in each cell to a very high degree of accuracy. In examining a population of about 10,000 cells from an individual animal, a very clear measurement of the degree of variation of DNA content within this population of cells can be determined. This value is called the coefficient of variation (CV) and is computed by dividing the standard deviation by the mean. A CV value significantly higher than the control or reference value indicates a significant degree of DNA damage has occurred in the cells of that tissue. This increase in variation was shown to be dose dependent (Otto et al, 1981; Easton et al, 1997). The mechanisms for producing such a state may or may not involve direct acting mutagens. Some genotoxins such as the heavy metals mercury, lead, arsenic and cadmium act by blocking the DNA repair capability of the cell, thus enabling normal breakage events to go unrepaired.

Flow cytometry has been used to detect genotoxic damage in field populations of fish (Easton, 1997; Lingenfelser *et al*, 1997), birds (Custer *et al*, 1994; George *et al.*, 1991), turtles (Lamb *et al.*, 1991; Bickham *et al.*, 1988) frogs (Lowcock *et al*, 1997) and mice (McBee and Bickham, 1988) where contaminated sites were compared with reference sites. Results have been verified by other cytogenetic methods (Bickham *et al.*, 1992; McBee and Bickham, 1988).

Methods

Populations Sampled

Samples of chickadees were collected from 4 locations (see Figure 1 and Table 1), Anchorage (4 sites), Trapper Creek (1 site), Talkeetna (1 site) and Palmer (1 site). In all 44 samples were collected. There were two questions of interest:

1. Did the abnormal cross-billed group also have DNA damage?
2. Did the samples stored in snow for 4 hours differ qualitatively from the normal population?

To answer the above questions the samples were divided into 3 groups regardless of location. These groups consisted of:

- Birds with nornal beaks (normal) - 22 samples;
- Birds with deformed cross-beaks (abnormal) - 8 samples;
- Normal birds whose blood sample was stored in snow for 4 hours prior to freezing (snow) - 11 samples.

Flow Cytometry

All samples were stored at -80^{o}C until used. The 44 samples were processed as a single batch. This ensured that all staining times and length of exposed cells to staining reagents were kept constant. The samples were all run on an Epic Elite Flow Cytometer (Coulter Corp.) using an argon laser (488 nanometers).

The instrument was aligned prior to the running of a specimen batch first using DNA Check Beads (Coulter Corp) followed by human lymphocytes and chicken erythrocyte nuclei (CENs, BioSure Controls) as external biological controls for the staining protocol and to fine tune the alignment and ensure stability of the instrument. The mean channels for DNA content for the chickadee blood and the human lymphocytes was established in the instrument's most sensitive range and maintained throughout for all 44 specimens.

The samples were run on the cytometer at a rate of about 150 cells per second, the data being collected as both Histogram and Listmode files in a double gated environment to ensure only nuclei were being measured. The subsequent data was analyzed with Elite Software (Coulter Corp.) to produce the mean channel and full peak CV values used in the subsequent data analysis.

All cells were stained using a modified whole cell method (Clevenger *et al*, 1985). The frozen chickadee red blood cells (CRBCs) were thawed rapidly at 37^{o}C and then placed on ice. They were washed twice in phosphate buffered saline (PBS) and the cells were counted in order to adjust the numbers to the optimum level

for the staining protocol ($2x10^6$ cells per ml). A known volume of standard human lymphocytes was then added to the CRBCs as an internal control. Separate tubes of CENs and human lymphocytes were stained at the same time as external controls.

The cells were fixed in 1.0 ml 0.5% paraformaldehyde for 10 minutes at 4°C. the cells were then centrifuged and the supernatant removed. Membrane perforation was achieved by the addition of 1.0 ml 0.1% Triton X-100 (Phoenix Flow Systems) for 3 minutes at 4°C followed by centrifugation and removal of the supernatant. The RNA, which, if present, would take up the fluorescent dye and result in a false DNA reading, was removed by the addition of 0.1 ml of 1.0 mg/ml Rnase and incubation for 20 minutes at 37°C. The cells were then centrifuged and the supernatant removed. The cells were then resuspended in 1.0 ml of 50 mg/ml propidium iodide (PI), a fluorescent stain that is proportionately bound to the nuclear DNA. The samples were allowed to stand for 1 hour in total darkness at 4°C. The PI is excited to fluoresce at 488 nanometers. Just prior to running on the flow cytometer, the cells from each specimen were passed through a fine insulin syringe and then filtered through a 47 micron screen (Phoenix Flow Systems) to remove any cell debris and any unbroken cell clumps.

Statistical Analysis

Recently, Misra and Easton (1999) have developed the only statistically valid protocol for analyzing CV values from flow cytometry data. Since the CV value is itself a summary statistic derived from the division of the standard deviation by the mean, an approach was required that recognized this unique feature of the collection of CV values that comprised the data set. A computer program was written by R.K. Misra to utilize the statistically well recognized weighted least squares procedure (i.e. see Johnson and Wichern, 1988) for analyzing the CV values. This procedure increases the sensitivity of the flow cytometry analysis by at least an order of magnitude in comparison to the inappropriate ANOVA and non-parametric methods formerly used.

The CV values from the chickadees were standardized by subtraction from the human lymphocyte CV internal control values. This value is referred to as DIF. The DIF value is the parameter that was analyzed using the least squares weighted procedure and is the value represented in the following tables and figures. The flow cytometry data summary for all samples analyzed is presented in APPENDIX 1.

Results

A density plot of the DIF values are shown in Figure 1, the larger the DIF value the greater is the amount of clastogenic damage. Subsequent analysis for outliers (Hoaglin *et al*, 1983) using probability plots (Figure 2) identified one anomalous data point from the abnormal treatment group which could abnormally skew the analysis in favour of a greater difference. This data point was subsequently removed before applying the least squares weighted analysis.

FIGURE 1. A jitter plot showing the distribution of the flow cytometry results for each group. The data points were jittered in order to show their actual density relationship without overlap of identical values. The data point far to the right in the abnormal group was treated as an outlier and not utilized in the weighted least squares analysis.

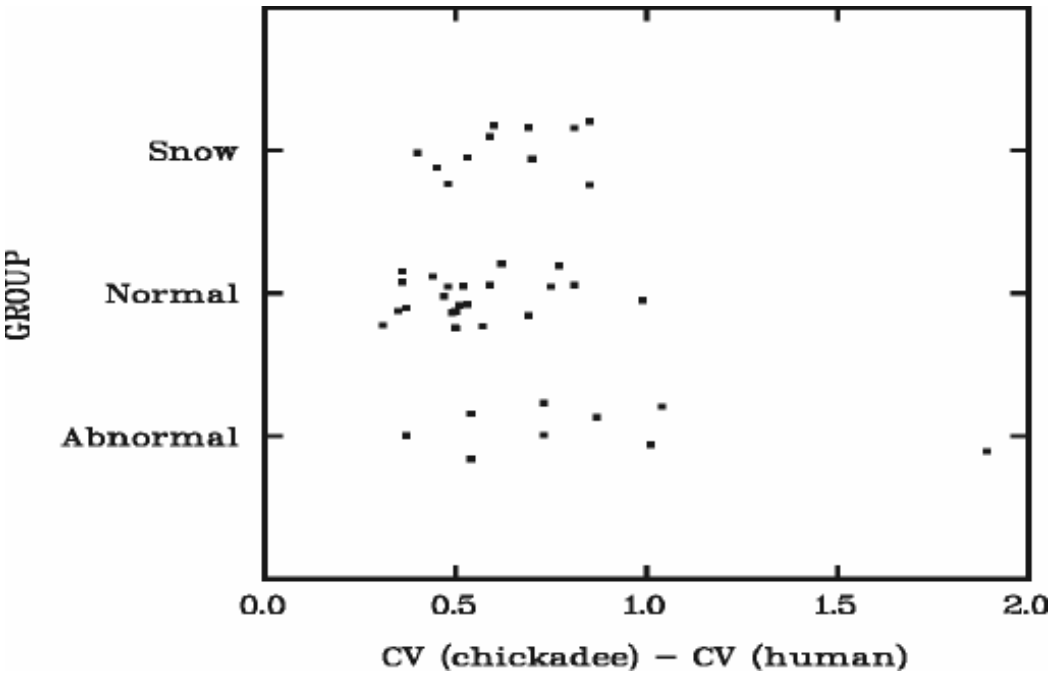
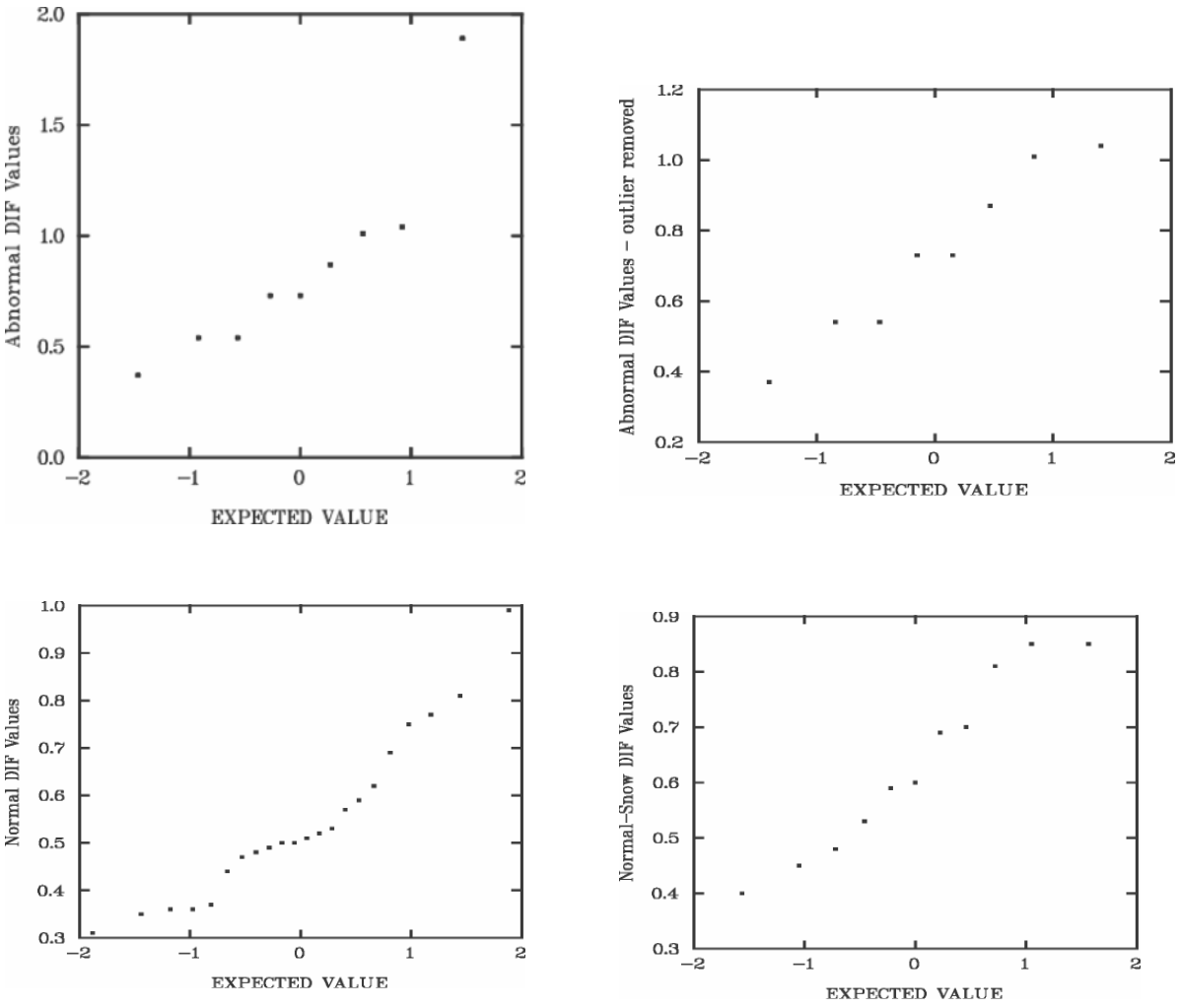


FIGURE 2. The Probability plots of the DIF data by group to isolate outlying data points for exclusion from the subsequent analysis.



The results of the weighted analysis (Table 1) indicates that there are highly significant differences between the treatment groups (X^2 =155.4 with 2 degrees of freedom). A comparison of the individual treatments demonstrates that the abnormal birds show a very high degree of red blood cell DNA damage (X^2 = 152.4, P=0) relative to the normal reference group. In addition there is a highly significant difference between the normal samples that were frozen immediately upon collection and those that were kept in snow for 4 hours prior to freezing (X^2 = 34.8, P=0). The snow samples were still significantly different than those of the abnormal birds, but the Chi-square value has diminished (X^2 = 37.6 vs X^2 =

152.4). When all the normal birds (snow + normals) are combined, the resulting Chi-square value is still highly significant ($X^2 = 101$, P=0).

TABLE 1. Results of the weighted analysis from the Alaska chickadee flow cytometry data.

Treatment	**N**	**Mean CV DIF**
Abnormal (A)	8	0.72875
Normal (N)	22	0.54455
Snow-normal (SN)	11	0.63182

Source	**Chi-square**	**DF**	**Probability**
Between treatments	155.4	2	0.00000000
A vs N	152.4	1	0.00000000
A vs SN	34.8	1	0.00000007
N vs SN	37.6	1	0.00000002
A vs N+SN	101.0	1	0.00000000

Discussion

The genetic damage found in the abnormal chickadee populations are the first reported for this type of phenomenon. Given the range of observed DNA damage in the beak-deformed chickadees, there may be variation in:

- genetic resistance to the clastogenic action of the unknown chemicals;
- dose received of the genetically active material.

The main source of variation could be determined by a regression analysis of the multifactoral quantitative chemical analysis of individual birds with the individual CV DIF values. A strong relationship with one or more chemicals would indicate a dose effect, while a non-significant relationship may indicate:

- differential genetic resistance
- or the principal clastogenic material was not effectively measured because
  - the material has been excreted from the body
  - or is still present, but not yet identified.

The difference in CV of the snow samples from the normal samples may either be:

- a function of the sample collection protocol (i.e. 4 hours in snow before freezing)

- or a function of a real difference in genotoxic effect between the Tattlow site at Palmer, Alaska and the normal samples that came from several sites in Anchorage.

The confounding of these parameters may be cleared up by the regression analysis of chemical contaminant information (or possibly another indicator of exposure such as MFO induction) collected separately for each bird and the CV DIF values for the non-snow samples.

If a relationship exists:

- then the relationship would be expected to differ between non-snow and snow samples when the collection procedure was responsible for the difference in observed CV values;
- the relationship would not be expected to differ between non-snow and snow samples when the observed genotoxic effect at the Tattlow site was real.

We can do these analyses if the appropriate chemical analysis data is available.

Implications of Genetic Damage to Blood Cells

DNA damage is its own significant endpoint.

1. The exact direct negative physiological event that may be triggered by this damage is unknown, but can be determined through further research. Perhaps the red blood cells do not have the normal variety of haemoglobins or the oxygen binding properties have been damaged which would affect flying stamina and possibly the ability to withstand cold temperature. In addition immunocompetence may be reduced because of damage to the lymphocytes.
2. One of the main consequences of mutagenicity on somatic cells is the greatly increased risk of cancer in the bird. Clastogenic activity has been shown to be closely associated with cancer incidence (IPCEMC, 1988).

3. All the above activities and others ultimately have a direct effect on the fitness of the individual and set the stage for natural selection to bring about a genetic adaptation to the genotoxic stress. The very act of responding genetically to the contaminants, is in itself a form of genetic damage.

   Selection response by its very nature is a form of controlled change in the genetic structure of the population whereby formerly rare genes become common and common genes become rarer and rare genes may be completely lost from the population. This latter event especially can reduce the population's genetic flexibility to respond to new population stressors in the future. These phenomenon have been studied in vertebrate populations using fish because of cost, convenience and generation time. Laboratory studies with fathead minnows (Diamond et al, 1995a) and mosquitofish (Boyd and Ferguson, 1964; Angus, 1983) have clearly shown that animals have the ability

to genetically respond by selection to chemical pollutants. This has been corroborated by field studies with Killifish (Weiss and Weiss, 1984) and mosquitofish (Angus, 1983). Recent work on the genetic structure of selected and unselected fish populations have shown the significant changes in gene frequency occur within these populations (Diamond et al, 1995b; Theodorakis and Shugart, 1995).

Recommendations

1. Additional statistical analysis using a multiple regression approach to relate contaminant and effect if data from individual birds is available. This analysis will require a special method to eliminate the colinearity problem with correlated contaminant values and to take advantage of the unique features of the DNA damage estimator, the coefficient of variation DIF value. Dr. R.K. Misra knows this problem well and its solution.

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APPENDIX 1 The data used in the statistical analysis.

		Black Capped Chickadee Blood				Human Blood (internal control)				
Sample	**Group**	# of Cells	Mean	S.D.	C.V.	# of Cells	Mean	S.D.	C.V.	**Difference (DIF)**
1	Abnormal	12390	200.9	4.2	2.10	4596	559.1	9.7	1.73	0.37
2	Normal	9843	195.5	4.4	2.23	5611	545.8	9.4	1.72	0.51
3	Abnormal	11637	195.1	4.4	2.24	2724	551.8	9.4	1.70	0.54
6	Normal	8947	190.1	5.0	2.64	4383	541.1	11.7	2.16	0.48
8	Abnormal	13836	174.6	5.5	3.12	1185	504.0	10.6	2.11	1.01
9	Normal	10011	194.6	5.3	2.72	3620	542.3	12.0	2.22	0.50
11	Normal	11191	189.7	5.8	3.04	2827	533.0	13.4	2.51	0.53
12	Abnormal	25943	204.1	4.8	2.33	857	562.2	8.2	1.46	0.87
14	Abnormal	12486	199.6	4.4	2.23	1503	556.3	8.3	1.50	0.73
15	Abnormal	14903	195.0	4.1	2.08	1042	550.7	7.4	1.35	0.73
16	Abnormal	24083	179.9	5.0	2.76	1744	522.2	9.0	1.72	1.04
17	Normal	10513	187.3	5.5	2.92	4292	535.6	11.3	2.11	0.81
18	Normal	15508	183.1	3.6	1.99	673	527.4	7.2	1.37	0.62
19	Abnormal	9909	194.6	7.6	3.91	2531	543.9	11.0	2.02	1.89
20	Normal	7449	171.5	5.5	3.19	2695	501.2	11.0	2.20	0.99
21	Normal	9871	206.5	5.8	2.81	2423	551.3	13.5	2.45	0.36
23	Snow	13247	195.5	4.2	2.12	1363	547.4	8.3	1.52	0.60
24	Snow	9688	187.4	4.6	2.47	1223	533.7	9.5	1.78	0.69
25	Snow	12021	195.9	5.4	2.77	2605	544.7	10.7	1.96	0.81
26	Snow	9064	188.9	5.9	3.13	3046	533.6	12.2	2.28	0.85
27	Snow	11469	192.1	6.1	3.17	1862	543.6	13.4	2.47	0.70
28	Snow	9937	211.7	5.7	2.71	2212	574.0	10.7	1.86	0.85
29	Snow	5803	197.9	5.3	2.66	2884	546.0	11.9	2.18	0.48
30	Snow	11762	203.8	5.5	2.70	2468	562.0	12.2	2.17	0.53
31	Snow	15763	210.6	4.4	2.09	1088	571.8	9.7	1.69	0.40
32	Snow	13305	202.9	5.0	2.45	1646	552.3	10.3	1.86	0.59
33	Snow	8024	194.3	5.0	2.59	2409	545.5	11.7	2.14	0.45
34	Normal	11079	186.5	4.9	2.60	1416	537.8	11.5	2.13	0.47
36	Normal	5325	196.9	7.1	3.63	4348	548.5	16.1	2.94	0.69
37	Normal	13215	198.9	4.7	2.34	826	555.2	10.1	1.82	0.52
38	Normal	2480	187.4	6.7	3.59	4562	521.3	16.2	3.10	0.49
39	Normal	2251	203.7	6.8	3.34	5178	552.0	16.5	2.99	0.35
40	Normal	4883	180.8	6.5	3.59	3360	514.4	14.6	2.84	0.75
41	Normal	6387	190.8	5.8	3.05	3566	534.6	14.6	2.74	0.31
42	Normal	24175	191.7	5.4	2.84	1296	536.9	12.1	2.25	0.59
43	Normal	10964	197.8	4.9	2.48	1686	554.5	11.0	1.98	0.50
44	Normal	9739	187.3	5.9	3.13	2980	521.5	14.4	2.76	0.37
45	Normal	8214	192.1	5.8	3.04	2685	537.9	14.4	2.68	0.36
46	Abnormal	14902	196.2	4.5	2.31	1204	546.7	9.7	1.77	0.54
47	Normal	12434	191.9	4.1	2.12	1156	539.5	8.4	1.55	0.57
48	Normal	26484	202.9	4.2	2.07	1712	555.3	9.1	1.63	0.44
49	Normal	18425	191.1	4.3	2.23	1366	539.4	7.9	1.46	0.77