HORMONE ANALYSES OF BLACK-FOOTED ALBATROSS (PHOEBASTRIA NIGRIPES) AT MIDWAY ATOLL NWR AND TERN ISLAND, HAWAIIAN ISLANDS NWR

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PREFACE

Black-footed albatross (*Phoebastria nigripes*) are among the many species of seabirds whose numbers are affected every year by anthropogenic impacts. Until now, no one has looked at the transfer of contaminants via soil to *P. nigripes*. However, studies have been done on how the Laysan albatross (*P. immutabilis*) is affected by lead in the soil (Work and Smith, 1996; Finkelstein et al., 2003). Studies on endocrine disruption and effects of PCB contamination in wildlife have shown that estradiol and testosterone levels may be altered and skewed as a result of anthropogenic sources (Fry and Toone, 1981; Grasman et al., 1996; Auman et al., 1997). Thus, the levels of gonadal steroids may function as indicators of exposure to polychlorinated biphenyls in the soil affecting these long-lived seabirds. The objective of this study was to determine if nesting in PCB-contaminated areas affects hormone balance due to endocrine disruption in both adult and chick black-footed albatross on Midway Atoll National Wildlife Refuge (NWR) and black-footed albatross chicks on Tern Island, Hawaiian Islands National Wildlife Refuge (HINWR).

This thesis is divided into four parts. Chapter 1 contains the literature review and information on research background. The results for each island are presented in following chapters with the methods section similar at both locations. Chapter 2 compares hormone variation and soil contamination in two nesting sites on Midway Atoll. Chapter 3 discusses hormone variation between two nesting sites on Tern Island. Chapter 4 summarizes the work done in this study and suggests research that might be done in the future.
CHAPTER 1
LITERATURE REVIEW

NATURAL HISTORY

Albatross are a unique group of long-lived pelagic seabirds. There are thirteen species of albatross worldwide (Tickell, 2000), with 3 species confined to the Northern Hemisphere: Laysan albatross (*Phoebastria immutabilis*), black-footed albatross (*P. nigripes*) and the endangered short-tailed albatross (*P. albatrus*). Some albatross species may have crossed the equator in the past, but at present each species is specific to its own hemisphere (Nunn et al., 1996). The three northern hemisphere species of albatross are members of the class Procellariiformes or “tube noses,” family Diomedeidae. The word “albatross” is derived from *alcatraz*, the word for *pelican* in Portuguese and Spanish (Harrison, 1990). The earliest identified petrel or albatross fossils came from the Oligocene epoch, about 32 million years ago (Tickell, 2000). Fossils from the west coast of North America and Japan indicate that albatross may have been found throughout the North Pacific in the mid-Miocene to late Pliocene periods (Tickell, 2000). The black-footed albatross is the only albatross with dark plumage at all stages of development (Whittow, 1993). Adults of this species have dusky-brown bodies, black webbed feet, and a white border around the base of the bill (Whittow, 1993). Black-footed albatross have an average wingspan of 190-220 cm (6-7 ft) and an average life span of 12-40 years (Whittow, 1993). While these are averages, exceptions are also found: during the field season of 2001, I observed a bird that was first banded 42 years ago.
Black-footed albatross breeding is primarily confined to the northwestern Hawaiian island chain of small islands and atolls, although small colonies exist off Japan and Mexico. In 2002, Laysan Island (25°42'41"N, 171°44'06"W), located in the Hawaiian Island National Wildlife Refuge, held the largest breeding population in the world (19,520 breeding pairs), followed by Midway Atoll National Wildlife Refuge (28°12'N, 177°20'W), with 19,331 breeding pairs (Flint, 2003). At one time, black-footed albatross breeding pairs may have also been found in the main Hawaiian Islands on Kaula Rock (21°40'N, 160°32'W) (Harrison, 1990), although no recent nests have been found (Flint, 2003). Past records indicate that they once nested throughout the Pacific at Wake Island, Marcus Island, Johnston Island, Volcano Island, Marshall Islands, and the Northern Marianas Islands (Rice and Kenyon, 1962). Feather hunters and World War II military occupation later destroyed these populations (Rice and Kenyon, 1962).

Black-footed albatross range throughout the North Pacific between 20° and 58° N. However, dependent on the time of year the majority of the species will concentrate in a few areas. During the non-breeding months (July-October), they are commonly observed between Alaska and Hawaii, and tend to stay 20-30 km offshore. During the chick-incubation and chick-rearing months (January-June), their pelagic feeding range is more restricted, and they tend to stay near the breeding islands (Whittow, 1993).

Albatross are philopatric monogamous breeders; the same pair returns to the same nest site year after year once a pair bond has been established. The pair bond remains unbroken until one of the pair is no longer present. Visually, birds are monomorphic. The estimated age of first breeding is from 3-5 years old. They lay a single sub-elliptical egg per year (Whittow, 1993). If the egg is not fertile or breaks, it is not replaced that
year. Copulation occurs on land. Following copulation, pairs forage at sea for 2-3 weeks, allowing a buildup of lipid and nutrient storage. Fertilization probably occurs at sea, with sperm stored in special glands (Whittow, 2002). Eggs are incubated immediately after laying. Incubation is approximately 65 days (Whittow, 1993). Members of pairs trade off incubating and chick-rearing duties. Chicks are hatched semi-precocial (confined to the nest, fed by parents). Chicks are fed by regurgitation, surviving on a diet of lipid-rich stomach oil and remnants of flying-fish eggs and squid. Black-footed albatross are diurnal surface-prey seizers (Harrison, 1990), feeding mainly on flying fish (family, Exocoetidae) eggs, and squid (family, Lolignidae), both of which are seized from the surface of the ocean. They rely on their well-developed olfactory and visual systems to locate food during the day. Black-foots have limited night vision, lacking high levels of rhodopsin in their retinas. They will also forage opportunistically on floating marine debris, ship offal and carcasses; they have been called “feathered pigs” by sailors (Harrison, 1990).

NEST DESCRIPTION

Albatross build their nest cups directly on the ground. Their nests are basically scooped-out depressions of soil, sand, and surrounding ground substrate that they scrape together using their beaks and feet and keep up throughout the breeding cycle. Adults and chicks are constantly grooming their nests cups. From early development inside the
eggs to growth in the nests, albatross young spend months sitting in their nests. It is common to observe chicks grooming themselves and the ground area around the nest cups and playing with the nest soil, rocks, plastic, metal, and vegetation in the vicinity of their nests.

**MIDWAY ATOLL NWR**

Midway Atoll National Wildlife Refuge (NWR) is a coral atoll, with three islands located in a semi-circular lagoon. Midway Atoll was discovered in 1859 when Captain N.C. Brooks of the *Gambia* ran aground and named the islands “Middlebrooks Islands”. Midway hosts the world’s largest colony of nesting Laysan albatross (*P. immutabilis*), and the second-largest colony of black-footed albatross (*P. nigripes*).

Midway has seen much of human and military disturbances. At the turn of the 20th century, Japanese feather and egg poachers decimated both albatross species. In 1903, President Theodore Roosevelt assigned jurisdiction and control of Midway, including the surrounding reefs and territorial waters, to the US Navy (Presidential Executive Order 199-A, January 20, 1903) (Rauzon, 2001). Additionally, in 1903, US Marines were sent to Midway to enforce the protection of the Cable Company and birds (Cousins and Cooper, 1998). In the 1930’s Sand Island, Midway, became a destination stopover for the Pan American Clipper seaplane service flights. During World War II, Midway had a population of approximately 10,000 military personnel. On 4 June 1942, Japan launched a major invasion of Midway, and World War II continued as the “Battle of Midway” was fought. Following World War II the facility was almost abandoned, and the airfield on Sand Island was operated by the Civil Aeronautics Administration. In
1957 the airfield facilities on Sand Island were greatly expanded to create a Pacific airborne early-warning base. Eastern Island was converted to a communication and intelligence-gathering base in the early 1960’s until it was abandoned in 1970. By 1978 military operations had drastically decreased, and Midway was downsized to a Naval Air Facility. In 1988 through an agreement between the US Navy and the US Fish and Wildlife Service, Midway became an overlay National Wildlife Refuge, a unit of the Hawaiian and Pacific Islands National Wildlife Refuge Complex. In 1997, all military operations ceased, and Midway was turned over to the Department of the Interior US Fish and Wildlife Service where “guns for goonies” were traded. From 1997-2002, US Fish and Wildlife Service and a subcontractor ran an eco-tourism public visitation outfit that included wildlife viewing and sport fishing and diving. Today, Midway Atoll continues to be part of the National Wildlife Refuge System managed by the US Fish and Wildlife Service.

CONTAMINATION AT MIDWAY ATOLL NWR

Midway Atoll may have PCB soil contamination of concern. Since 1903 Midway has been the site of a transpacific cable management facility and various military missions. In 1993, US Navy Midway Airfield began closure under the Base Realignment and Closure Act of 1990 (BRAC). During the BRAC operation, over 120 underground and aboveground storage fuel tanks were removed, buildings were demolished, and active landfills were investigated. During this remediation, soil contaminated with DDT\(\{1,1,1\text{-trichloro-2,2-bis(\rho\text{-chlorophenyl})ethane}\}\), petroleum, and polychlorinated
biphenyls (PCBs) was removed from all over the island. Despite the large remediation effort, one site called the old bulky waste landfill (OBWLF) could still be of concern since evidence of contamination of that site has surfaced since cleanup of the atoll. Investigations have found elevated levels of pesticides, PCBs, and metals in all environmental media at the OBWLF site, possibly due to the disposal of wastes that contained PCBs (Ogden Environmental and Energy Services Co., 1997). Old bulky waste landfill was formed by depositing bulky metal wastes, construction debris, scrap metal, old machinery, and salvaged vehicles into the peninsula and offshore (Ogden Environmental and Energy Services Co., 1997). During closure of the landfill and BRAC a 2.5-4 foot thick cap of clean soil was placed on OBWLF. However, in 1996, 2.4-80 mg/kg (ppm) of Arochlor-1260 (PCB) were found in subsurface soil samples (Ogden Environmental and Energy Services Co., 1997), and during preliminary analyses in 2000, PCB concentrations as high as 8.61 mg/kg were found in OBWLF soil samples. Previous research indicated that suspected contaminants in the soil at the dump are PCBs and pesticides such as DDT (Ogden Environmental and Energy Services Co., 1997). Possible exposure pathways exist to terrestrial receptors such as seabirds that nest directly on the soil, for example the black-footed albatross. Direct exposure to PCB-contaminated soil through dermal contact, incidental ingestion, inhalation of vapors, or ingestion of PCB-contaminated prey could be the primary exposure pathways of PCB contaminants in the soil currently at OBWLF. Preliminary soil analyses in October 2000 at OBWLF for this research concluded that there was a need for further investigation for contaminants such as PCBs in the soil at OBWLF on Midway Atoll NWR.
TERN ISLAND, HAWAIIAN ISLANDS NWR

Tern Island is the main island among approximately nine islets located in French Frigate Shoals (23°45'N, 166°15'W) located in the northwestern Hawaiian islands, part of the Hawaiian Islands National Wildlife Refuge (HINWR). French Frigate Shoals was discovered in 1786 by a French explorer La Perouse (Amerson, 1971). On 4 January 1859 the United States took formal possession of the atoll under the Guano Act of 1856. In 1891 Henry Palmer and George C. Munro made the first biological survey of French Frigate Shoals (Amerson, 1971). In 1909, President Theodore Roosevelt signed Executive Order 1019 (Rauzon, 2001) designating French Frigate Shoals, as well as the rest of the northwestern Hawaiian islands, excluding Midway Atoll, as bird reservations. Beginning in 1942, the land area of Tern Island was increased by dredging, during construction of a naval seaplane-refueling base. The US Navy controlled Tern until it was turned over to the US Coast Guard on 9 June 1946 (Woodward-Clyde, 1999). In 1952, the US Coast Guard LORAN station was transferred to Tern Island and operated until 1979. Since 1979, Tern Island and French Frigate Shoals have been under the jurisdiction of the US Fish and Wildlife Service with no public access (Miao et al., 2000). Today, Tern Island is home to the fourth-largest population of breeding black-footed albatross (Flint, 2003). Many of these birds nest directly on the soil of the island. There is a small human population of refuge staff and volunteers and researchers who live on Tern and monitor the wildlife.
CONTAMINATION AT TERN ISLAND, HAWAIIAN ISLANDS NWR

Like Midway Atoll, Tern Island has seen its share of anthropogenic alterations. There have been a number of investigations dealing with environmental issues as a result of past US military usage (Woodward-Clyde, 1999). In 1991 as part of the Defense Environmental Restoration Program, 21 underground fuel storage tanks were closed (Woodward-Clyde, 1999). Over the years, large quantities of unclassified debris have been disposed of in a landfill on Tern Island and pushed into the sea around the island (Miao et al., 2000). On Tern the major contaminants of concern in the soil where albatross are nesting are from an eroding US Coast Guard landfill known to contain scrap metal, capacitors, batteries, and transformers. A geophysical survey revealed an area thought to be composed of landfill debris approximately 2,690 feet in the east-west direction, and from 30-140 feet in the north-south direction (Woodward-Clyde, 1999). This old landfill has been the center of concern for years, and past evidence has shown that there is still contamination in the soil at Tern. During an environmental investigation conducted by the US Coast Guard, 346 soil samples were collected from Tern Island. Of these samples, 278 samples were collected from the surface (0-6") of the soil and 68 samples were collected from the subsurface (Woodward-Clyde, 1999). The majority of these samples were collected directly from two sites known to be “hotspots” for PCB contamination (Woodward-Clyde, 1999), both of these were found in the old landfill area mentioned above. In this investigation PCBs were detected in 200 surface soil samples with concentrations of 0.1 to 2300 mg/kg, and PCBs were detected in 44 of the 65
subsurface samples with detection limits ranging from 0.0099 to 24 mg/kg (Woodward-Clyde, 1999). This study at Tern investigates if the hormone levels of black-footed albatross (*Phoebastria nigripes*) could be affected by nesting in PCB-contaminated soil.

**ANTHROPOGENIC THREATS**

Polychlorinated biphenyls (PCBs) are halogenated aromatic hydrocarbons. PCBs are environmentally persistent, resisting bacterial breakdown, and readily absorbed from water into the fats of plankton and other biota, entering the aquatic food chain and biomagnifying up to the top predators, including fish-eating birds such as albatross (Guruge et al., 2001). In the oceanic environment PCBs accumulate in organisms based on the trophic food chain levels (Guruge et al., 2001). Albatross, feeding on fatty lipid-rich food sources of squid and fish eggs, and are at the top of the food chain containing significant amounts of contaminants (Auman et al., 1997; Ludwig et al., 1998; Guruge et al., 2001). Polychlorinated biphenyls have been known to elicit a variety of biologic and toxic effects including death, reproductive failure, and endocrine disruption in avian species (Murk et al., 1994; Fry, 1995; Grasman et al., 1996). A past study (Ludwig et al., 1998) looking at the possible impacts of global pollution of organochlorine chemicals in oceanic organisms found that both Laysan and black-footed albatross were contaminated with high concentrations of PCBs. While the source of most of the PCB contamination to albatross is via the aquatic foodweb, the high levels of PCBs found in the nesting soil, the behavioral characteristics of the birds on the nest, and the long duration (9 months) spent on the nest indicates a potential for direct exposure of black-footed albatross to PCBs in contaminated soil.
An investigation of possible adverse effects to Red-tailed tropicbirds (*Pheathon rubricauda*) nesting in an area with soil contaminated with a herbicide containing the dioxin 2,3,7,8-tetrachlorodibenzodioxin (TCDD) on Johnston Island found chicks were experiencing developmental and neurological disruption from their nesting area (Fry, 1998). Like albatross, tropicbirds nest directly on the ground, and will pick at objects nearby. Fry (1998) detected TCDD in the serum of tropicbird chicks which indicated a possible exposure route to the dioxins from their nesting soil. In addition to the detection of TCDD in the serum, an incidence of brain asymmetry was found in one of the chicks. Fry concluded that the measured exposure in the chicks was probably due to the consequence of dust inhalation or incidental soil ingestion from the nest soil.

**Endocrine Disrupters**

Recent work has shown that a number of anthropogenic chemicals have the capability to interfere with the endocrine system. These endocrine disrupting compounds (EDCs) may have the capability to mimic or antagonize estrogens in the female and androgens in the male as well as inhibit or alter the thyroid hormone activity (Fry and Toone, 1981; Murk et al., 1994; Porterfield, 1994; Facemire et al., 1995; Fry, 1995; Grasman et al., 1996; Niminen et al., 2000; Zhou et al., 2000). Thyroid hormones affect growth, reproduction, metabolism, molting and migratory behavior in birds (Norris, 1985). Most studies on avian endocrinology have concentrated on domestic birds, and few data are available on wild species (Norris, 1985). Therefore it is possible that levels of gonadal steroids and thyroid hormones may function as indicators for exposure to wildlife affected by EDCs such as PCBs (Murk et al., 1994). A few examples of bird
species that have already been shown to be affected by EDCs are the Western Gull, Caspian Tern, Herring Gull, and Double-Crested Cormorant (Fry and Toone, 1981; Grasman et al., 1996; Ludwig et al., 1996). These effects are not always obvious at the time of exposure and may show up later or even in the next generation. It is thought that delayed reproductive effects associated with endocrine disruption of the developmental processes may occur when the birds mature, and thus the long-term effects will only become obvious once mature birds reach reproductive age or when the population is in decline.

Developmental disruption in the endocrine system will also produce permanent modifications in the reproductive and neurological systems (Fry and Toone, 1981; Fry, 1998; Ludwig et al., 1996). Therefore, possible indicators of adverse effects of suspected EDCs such as PCBs may be found by assessing if the targeted hormones are correlated with exposure.

**Estrogens and Androgens**

In birds, estradiol 17-β, testosterone, and progesterone are the three major gonadal steroid hormones (Tanaka, 1980). Demasculinization and feminization of males resulting from exposure to EDCs have been documented in a variety of organisms such as alligators and turtles (Guillette et al., 1994), western gulls (Fry and Toone, 1981) and Florida panthers (Facemire et al., 1995). Past research on wild bird populations, especially focused on fish-eating birds of the Great Lakes region of the United States, has demonstrated that EDCs may impair the reproduction, sex ratios, and development of wild populations (Fry and Toone, 1981; Ludwig et al., 1996).
Thyroid Hormones

Thyroid hormones are useful biomarkers for EDC effects on wildlife (Fry and Toone, 1981; Grasman et al., 1996; Auman et al., 1997). Thyroid hormones are critical for physiological processes. Thyroid hormones in avian species are synthesized similarly to those in mammals. Once in the target tissues thyroxine (T4) is converted to triiodothyronine (T3), which is the more biologically active form (Sturkie, 1986). Thyroid hormones are necessary for normal growth and development, and for regulating oxidative metabolism (Sturkie, 1986). Thyroid hormones can be influenced by exposure to EDCs such as PCBs (Murk et al., 1994; Nieminen et al., 2000; Zhou et al., 2000). Altered plasma T4 and T3 concentrations have been observed associated with PCB contamination (Murk et al., 1994; Nieminen et al., 2000; Chiba et al., 2001). This endocrine disruption of thyroid hormones may be due to the similarity of PCBs to thyroxine. PCB metabolites are structurally similar to thyroxine thus PCBs are most likely competing for binding sites on the transport protein chain transthyretin (TTR) (Lans et al., 1993; Murk et al., 1994; Nieminen et al., 2000; Zhou et al., 2000). This competitive binding could cause toxic side effects such as thyroid disfunction resulting from chemical interference. Minks (Mustela vison) exposed to PCBs and mummichogs (Fundulus heteroclitus) sampled from a contaminated area had higher plasma total T4 levels than those from the reference site (Nieminen et al., 2000; Zhou et al., 2000).
SUMMARY

Midway Atoll and Tern Island are remote land areas in the northwestern Hawaiian islands; both are past sites of military and other human disturbance, and home to millions of seabirds. Albatross nest directly on the ground on these atolls and may be experiencing endocrine disruption from PCB contamination in the soil. To date, no study has been done on the correlation between PCB-contaminated soil and the endocrine system of pelagic seabirds that nest directly on this soil. Soil contamination is potentially an important exposure pathway that has not been adequately addressed. Currently, we lack the information necessary to know if there is a cause for concern regarding contamination in nesting soil affecting the albatross. This research was aimed to determine if seabird population health is affected by PCB-contaminated nest soil in the northwestern Hawaiian islands. The objective of this study was to determine if nesting in PCB-contaminated soil affects the hormone balance in both adult and chick black-footed albatross (Phoebastria nigripes).
CHAPTER 2
HORMONAL EFFECTS OF SOIL CONTAMINATION ON BLACK-FOOTED ALBATROSS AT MIDWAY ATOLL NATIONAL WILDLIFE REFUGE

ABSTRACT
Nesting activities of black-footed albatross (Phoebastria nigripes) were monitored on Midway Atoll National Wildlife Refuge during the 2001 breeding season. Blood samples were taken from adult and chick albatross, and analyzed for hormones indicative of endocrine disruption. Adults nesting at Bulky Dump (contaminated site) had significantly higher estradiol levels ($P < 0.05$) than those nesting at Black-foot 8 (reference site), whereas those nesting at Bulky Dump had significantly lower ($P < 0.001$) testosterone levels. Adults nesting at Bulky Dump had significantly higher ($P < 0.001$) total thyroxine (T4) levels than those nesting at Black-foot 8. In contrast, the adult albatross nesting at Bulky Dump had significantly lower ($P < 0.05$) total tri-iodothyronine (T3) levels than those nesting at Black-foot 8. There was no significant difference in progesterone, free T3 or T4 concentrations in adult albatross between the two sites. Chicks sampled from Bulky Dump had significantly higher ($P < 0.001$) levels of free T3 than chicks nesting at Black-foot 8. When PCB soil concentrations and plasma hormone levels were analyzed no relationship was found. These results indicate that although there are significant differences in the plasma hormone profiles in both adult and chick black-footed albatross nesting at two sites at Midway Atoll, no conclusive biological evidence of endocrine disruption from the nesting soil at the sites was obtained.
INTRODUCTION

Black-footed albatross (*Phoebastria nigripes*) are one of the three species of albatross found in the North Pacific. Their breeding is primarily confined to the northwestern Hawaiian island chain of small islands and atolls. The estimated age of the first breeding is from 3-5 years old. They lay a single sub-elliptical egg per year directly on the ground (Whittow, 1993). All of these attributes are indicative of a species that is slow to react to changes in its environment. Studies on endocrine disruption and effects of PCB contamination in wildlife have shown that estradiol and testosterone levels may be altered and skewed as a result of anthropogenic sources (Fry and Toone, 1981, Grasman et al., 1996; Auman et al., 1997). Thus, the levels of gonadal steroids may function as indicators for exposure of PCBs in the soil affecting these long-lived seabirds. The hypothesis that albatross hormone levels, especially of sex steroid origin, would vary on an area with varied PCB-contamination in the soil was examined.

Albatross are ground nesters, and the importance of having non-contaminated nest sites starts at early development inside the eggs followed by rapid growth in the nests. Albatross nests are basically scooped-out depressions of soil, sand, and surrounding ground substrate, in which they lay a single egg directly on the nest cup soil. Both adults and chicks are constantly grooming their nest cups by scraping the soil out of the nest cups with their beaks and feet throughout the breeding cycle. It is common to observe chicks and adults grooming themselves and the ground area around the nest cups and playing with the actual nest soil, rocks, plastic, metal, and vegetation in the vicinity of their nests. Due to the nest location, directly on the ground, there may be an exposure risk to albatross adults and chicks from the inhalation of PCB-contaminated dust or from...
pica (ingestion of PCB-contaminated soil particles). Pica has long been known to be an exposure pathway in human children and fossorial animals. It has not been extensively evaluated for ground-nesting birds.

The objective of this study was to determine if nesting in PCB-contaminated areas affects the hormone balance in adult or chick black-footed albatross nesting directly on the soil on Midway Atoll National Wildlife Refuge (NWR).

MATERIALS AND METHODS

Birds

Once an egg was laid, the nest was marked with a number painted on a rock. Both parent bird bands were read at least three times throughout the breeding season. I was not able to identify the sex of the adult birds or chicks. Once a bird was selected, the band number was examined to ensure that it belonged at that nest cup. If the band number did not match the field sheet, the bird was not sampled.

Blood Sampling

Blood samples were collected from adult albatross on Sand Island, Midway Atoll NWR located in the north-central Pacific Ocean (28° 13’N, 177° 22’W) during the 2001 nesting season. Adult data collection consisted of hand-capturing incubating or chick-brooding adults and drawing 5 ml of blood from the ulnar wing vein using 21-gauge needles and Vacutainer tubes (BD Vacutainer™ containing freeze-dried lithium heparin, Becton, Dickinson and Company, Franklin Lakes, NJ). For blood sampling, two assistants were required, one holding the bird’s beak and body across their lap and the
other holding the feet and the wing to be sampled flat against a padded mat. This position allowed the wing to be draped outward and the blood to rapidly flow to the vein to be sampled. At times, blood from both wings was sampled. In addition, birds were banded (if not previously banded) and weighed. Blood was kept in a cooler with ice packs and then centrifuged for 10 minutes at 3,000 x g within 4 hours of sampling. Approximately 2 ml of plasma were obtained from 5 ml blood collected. Plasma was divided into two microcentrifuge tubes and kept frozen at -40°C for later analysis of hormones and contaminants.

Chick blood was collected by hand-capturing temporarily color-banded chicks (monitored from day of egg-lay) in late May 2001. Five ml of blood were drawn from the ulnar wing vein using 21-gauge needles and vacutainers containing lithium heparin anticoagulant, as described for adult birds.

Sampling of parent birds took place February 12 to February 23, 2001. During this time blood was collected from 174 adults and we were able to obtain samples from approximately 25 pairs of albatross at both plots. When the same plot was examined the following day, the brooding parents were usually switched. During the breeding season, the parents were sharing brooding duties and exchanged places daily or every few days.

Sampling Sites

Preliminary Soil Analyses; Fall 2000

Eleven soil samples were collected from old bulky waste landfill on Sand Island, Midway Atoll NWR in October, 2000. For this study, old bulky waste landfill was divided into eleven sections for sampling and a reference plot was located on the
shoreline approximately 150 meters west of OBWLF. Twelve composite surface soil samples were collected using disposable sampling spoons. Sampling involved collecting a portion of soil at even intervals along a paced-off grid within a section. Preferentially soil was collected from a Bonin petrel (*Pterodroma hypoleuca*) burrow entrance if it was at or near a sample location. All samples were collected in the same manner.

Soil samples were sieved through mesh sieves to sort out the fine soil for analysis. Samples were extracted with methanol using the SDI Sample Extraction Kit (Strategic Diagnostics Inc., Newark, Delaware). Extracted soil samples were analyzed using a PCB RaPID Assay Kit; (Strategic Diagnostics Inc., Newark, Delaware).

### 2001 Study Sites

Two study sites were designated for the breeding year of 2001: Bulky Dump (contaminated site) and Black-foot 8 (reference site). The reference site was a 20 x 20 m plot that had been monitored by the US Fish and Wildlife Service since 1996. In preliminary analysis, this site showed no detectable PCB contamination in the soil. The contaminated site was an old landfill that the Navy used to dispose of unwanted debris. This plot at Bulky Dump was approximately 130 x 300 m. The plots were located near each other on the southern shore facing the east on Sand Island, Midway Atoll. The main difference was the age groups that occupied the plots. Bulky Dump was constructed recently, and seemed to have only a younger age-class nesting. Black-foot 8 is a plot that the US Fish and Wildlife Service had been monitoring since 1996, and Bulky Dump was a plot set up for the first time in 2001.
Soil Collection

In January 2001 nest-soil samples were collected from active nests at Black-foot 8 and Bulky Dump. Surface soil was collected using nitrile gloves and scooping 3-4 teaspoons of soil into individual plastic bags. The nest soil was collected from the middle of the nest cup while the adult albatross was incubating an egg or newly hatched chick. A total of 64 soil samples from Black-foot 8 and 100 soil samples from Bulky Dump were collected.

Soil Analyses

Soil analyses were conducted by Sarah Caccamise in the laboratory of Dr. Qing X. Li in the Department of Molecular Biosciences and Bioengineering, University of Hawai‘i. Air-dried soil samples were extracted with a pressurized fluid extraction (PFE) instrument. The instrument used was a Dionex (Sunnyvale, CA) ASE 200 extractor. Nest cup soil samples of ~10 g soil were loaded into the extraction cell, and were extracted with hexane: acetone (1:1, v/v) at a pressure of 2000 psi and a temperature of 100 °C, with two static cycles of 5 mins. The samples were analyzed on a Varian Saturn 2000 gas chromatograph (GC) with simultaneous mass spectrometric (ms) and electron capture detection (GC-MS and GC-ECD, respectively). The column flow was split between the ECD and the MS in a 1:10 ratio. The column used was a capillary column ZB 1 (60 m x 0.25 mm i.d. x 0.25 μm film; Phenomenex, Torrance, CA). Helium grade 5.5 was used as the carrier gas, and nitrogen was used as the make-up gas for the ECD. The initial oven temperature was 120 °C and it was linearly ramped at 2 °C/min to
275 °C. The injector and ECD detector were set to 280 °C and 330 °C, respectively. Quantitation of PCB concentrations were performed using external PCB standards (Accustandard Inc., New Haven, CT). Samples were then quantitated with ECD data and confirmed with mass spectrometry.

**Radioimmunoassays of Steroid Hormones**

Plasma concentrations of estradiol-17ß, testosterone, progesterone, total and free thyroxine (T4), and tri-iodothyronine (T3) were determined by radioimmunoassay using commercially available kits with modifications (see below). Since albatross feed primarily on flying-fish eggs and squid with high lipid content, it was necessary to separate interfering substances in the plasma by using Sep-Pak C-18 light cartridges (Waters, Milford, MA) to estimate steroid hormones. Albatross chick plasma was used in validation for both adult and chick radioimmunoassays.

Estradiol, testosterone, and progesterone were extracted from 250 μl of plasma with Sep-Pak C-18 cartridges. Briefly, the cartridge, preconditioned with 2 ml of 2-propanol and 2 ml of 0.1% aqueous trifluoroacetic acid (TFA), was loaded with the plasma sample. The cartridges were washed with 2 ml of 0.1% TFA, followed by 2 ml of 25% acetonitrile in 0.1% TFA. Estradiol and testosterone were eluted with 1.5 ml 40% acetonitrile in 0.1% TFA, and progesterone with 1.5 ml of 60% acetonitrile in 0.1% TFA. The eluates were evaporated to dryness using a SpeedVac (Savant, Atens, GA), and the dried residues were reconstituted with 250 μl of assay buffer consisting of 10 mM phosphate buffer (pH 7.3), containing 0.1% Triton X-100 (Sigma), 1% bovine serum albumin (Sigma), and 0.01% sodium azide (Sigma).
Estradiol 17-β

Plasma concentrations of estradiol-17β were determined by radioimmunoassay using a commercially available kit with modification (Immuchem Double Antibody Direct Estradiol-17β ¹²⁵I RIA Kit; ICN Biochemicals, Costa Mesa, CA). Estradiol-17β standards were prepared by dissolving estradiol-17β (Sigma) in 100% ethanol (1 mg/ml) and then diluting to the desired concentrations with an assay buffer consisting of 10 mM phosphate buffer (pH 7.3), containing 0.1% Triton X-100 (Sigma), 1% bovine serum albumin (Sigma), and 0.01% sodium azide (Sigma). Glass tubes (12 x 75 mm) were used. To each tube were added 50 μl of standard or plasma sample, 250 μl of ¹²⁵I estradiol-17β and 250 μl of anti-estradiol-17β (provided with the kit). After incubation at 37°C for 90 minutes, 250 μl of precipitant solution (provided with the kit) was added. After centrifugation at 2000 x g for 20 min at 4°C, the tubes were aspirated and ¹²⁵I was counted in a gamma counter (Cobra II, Packard, Meriden, CT).

The validity of the assays was assessed by demonstrating that parallel displacement curves are generated with serial dilutions of plasma samples and also by the absence of cross-reaction with stripped plasma. In the preliminary experiment, dilution of neat plasma of the chick was not parallel with estradiol standards (Figure 2.1). Plasma was then extracted with ether, following the procedure established in this laboratory for Potter's angelfish (Centropyge potteri) plasma. Briefly, 2 ml of ethyl ether (Anhydrous, Fisher Scientific, Pittsburgh, PA) was added to the plasma sample. The tubes were vortexed and frozen at -80°C for 10 min, and the aqueous organic layer was decanted into a new glass tube. The ether extract was evaporated to dryness in a water bath at 40°C for
Figure 2.1. Displacement curves for estradiol 17-β and serial dilution of albatross chick plasma. Closed circles represent estradiol 17-β standards. Other symbols represent diluted albatross plasma after various treatments. Each E₂ point represents the mean of duplicate determinations.

Figure 2.2. Elution profile of ³H-estradiol 17-β through Sep-Pak C18 cartridge. 10 μCi ³H-estradiol 17-β in 10 μl ethanol was diluted with 250 μl assay buffer and added to the cartridge.
1 hour, and then placed under nitrogen for 5 min to ensure complete evaporation.

Extracts were then reconstituted with assay buffer. For stripping, an aliquot of the pooled plasma was incubated at room temperature for 15 min with 2% activated carbon (Norit A, Aldrich, Milwaukee, WI). As shown in Figure 2.1, however, the ether-extracted and stripped plasma still cross-reacted with the estradiol antibody, indicating the presence of interfering substances after ether extraction. Thus, estradiol 17-β was extracted from 250 µl of albatross plasma with a Sep-Pak C-18 cartridge, and a fraction was eluted with 25-40% acetonitrile. In a preliminary experiment, 10 µCi of \(^{3}H\)-estradiol ([2, 4, 6, 7, 16, 17-\(^{3}H\)] oestradiol, Amersham, Piscataway, NJ) in 10 µl ethanol was diluted with 250 µl assay buffer, and added to the cartridge. As shown in Figure 2.2, estradiol was eluted in fractions between 25% and 35% acetonitrile. Serial dilution of the chick plasma after the Sep-Pak purification was parallel with the estradiol standard, and no cross-reaction was seen with the stripped plasma (Figure 2.1). The interassay and intra-assay coefficients of variation were 14.3% (n = 5) and 23% (n=10), respectively.

**Testosterone**

Plasma concentrations of testosterone were determined by radioimmunoassay using a commercially available kit with modification (Immuchem Double Antibody Testosterone \(^{125}I\) RIA Kit; ICN Biochemicals). Testosterone standards were prepared by dissolving testosterone (Sigma) in 100% ethanol (1 mg/ml) and then diluting to desired concentrations with assay buffer as above. To each tube were added 50 µl of standard or plasma sample, 250 µl of \(^{125}I\) testosterone, and 250 µl of anti-testosterone (provided
Figure 2.3. Displacement curves for testosterone and serial dilution of albatross chick plasma. Closed circles represent testosterone standards. Other symbols represent diluted albatross plasma after various treatments. Each point represents the mean of duplicate determinations.

Figure 2.4. Elution profile of testosterone through Sep-Pak C-18 cartridge. Testosterone (200 ng in 400 µl assay buffer) was applied to the cartridge.
with the kit). After incubation at 37°C for 120 min, 50 μl of the second antibody (provided with the kit) was added, and incubated again at 37°C for 60 min. After centrifugation at 2000 x g for 20 min at 4°C, the tubes were aspirated and ^{125}\text{I} was counted in a gamma counter.

At first, ether extract of the chick plasma was prepared as described above for estradiol radioimmunoassay. The dilution curve of the ether-extracted plasma seemed parallel with testosterone standard (Figure 2.3). However, there was still a significant cross-reaction after stripping. Therefore the plasma was purified by a Sep-Pak C-18 cartridge. In order to examine the elution profile of testosterone, 500 ng testosterone dissolved in 400 μl assay buffer was applied to a Sep-Pak C-18 cartridge, and eluted by a stepwise increase in acetonitrile. Testosterone concentrations in each fraction were determined by radioimmunoassay as described above. As shown in Figure 2.4, testosterone was eluted exclusively in fractions between 20% and 40% acetonitrile. Thus, the same plasma fraction as used for estradiol (25-40% acetonitrile) was used for the assay. The serial dilution of this fraction was parallel with the testosterone standard, and no cross reaction was seen after stripping (Figure 2.3). The interassay and intra-assay coefficients of variation were 47% (n=6) and 0.8% (n=10), respectively.

**Progesterone**

Plasma concentrations of progesterone were determined by radioimmunoassay using a commercially available kit with modification (Immuchem Double Antibody Progesterone ^{125}\text{I} RIA Kit; ICN Biochemicals). Progesterone standards were prepared by
Figure 2.5. Elution profile of progesterone through Sep-Pak C-18 cartridge. Progesterone (200 ng in 400 μl assay buffer) was applied to the cartridge.

Figure 2.6. Separation of sex steroids by reverse-phase HPLC. Packing: μBONDAPAK C18, Solvent: 55% acetonitrile, Flow rate: 2 ml/min, Detector: UV at 254 nm.
dissolving progesterone (Sigma) in 100% ethanol (1 mg/ml) and then diluting to the desired concentrations with the assay buffer as above. To each tube were added 50 μl of standard or plasma sample, 250 μl of anti-progesterone, and 100 μl of 125I progesterone (provided with the kit). After incubation at 37°C for 60 min, 250 μl of precipitant solution (provided with the kit) was added. After centrifugation at 2000 x g for 20 min at 4°C, the tubes were aspirated and counted for 125I in a gamma counter.

In order to examine the elution profile of progesterone, 1000 ng progesterone in 100 μl assay buffer was applied to Sep-Pak C-18 cartridge, and eluted by stepwise increase in acetonitrile. Progesterone concentrations in each fraction were determined by radioimmunoassay as described above. As shown in Figure 2.5, progesterone was eluted exclusively in fractions between 40% and 60% acetonitrile. This is in accord with the elution profile of progesterone in reverse-phase high performance liquid chromatograph (HPLC), the progesterone peak appearing toward the end of the elution using 55% acetonitrile, and estradiol and testosterone appearing between 25-40% acetonitrile (Figure 2.6). The inter assay and intra-assay coefficients of variation were 13% (n=5) and 11% (n=10) respectively.

In summary, estradiol 17-β, testosterone and progesterone were extracted from albatross plasma with the Sep-Pak C-18 cartridge as follows: the cartridge, preconditioned with 2-propanol and 0.1% TFA, was loaded with 250 μl of plasma sample. The cartridge was washed with 2 ml of 0.1% TFA, followed by 2 ml of 25% acetonitrile in 0.1% TFA. Estradiol and testosterone were eluted with 1.5 ml 40%
acetonitrile in 0.1% TFA, and then progesterone was eluted with 1.5 ml 60% acetonitrile in 0.1% TFA. The eluates were evaporated to dryness, and the dried residues were reconstituted with 250 μl of assay buffer.

**Total thyroxine (T4) and tri-iodothyronine (T3)**

Plasma concentrations of total T4 were determined by radioimmunoassay using a commercially available kit with modifications (T4 Monoclonal solid Phase Radioimmunoassay; ICN Biochemicals). To each tube were added 12.5 μl of T4 standard or plasma sample, and then 500 μl of 125I T4 (provided with the kit). After incubation at room temperature for 60 min, the tubes were aspirated and counted for 125I in a gamma counter.

Similarly, plasma concentrations of total T3 were determined by radioimmunoassay using a commercially available kit with modification (T3 Solid Phase Radioimmunoassay; ICN Biochemicals). To each tube were added 50 μl of T3 standard or plasma sample, and then 500 μl of 125I T3 (provided with the kit). After incubation at 37°C for 60 min, the tubes were aspirated and counted for 125I in a gamma counter. These assays were validated using parallel dilution curves of albatross plasma with T4 or T3 standards (Figure 2.7). Interassay and intra-assay coefficients of variation for total T4 were 13% (n=3) and 13% (n=10), respectively. The interassay and intra-assay coefficients of variation for total T3 were 8% (n=4) and 14% (n=7), respectively.
Figure 2.7. Displacement curves for total T4 (A) and T3 (B) and serial dilution of albatross chick plasma. Closed circles represent T4 and T3 standards. Open circles represent diluted albatross plasma. Each point represents the mean of duplicate determinations.
Figure 2.8. Displacement curves for free T4 (A) and T3 (B) and serial dilution of albatross chick plasma. Closed circles represent T4 and T3 standards. Open circles represent diluted albatross plasma. Each point represents the mean of duplicate determinations.
Free T4 and T3

Plasma concentrations of free T4 were determined by radioimmunoassay using a commercially available kit with modification (Free T4 Solid Phase Radioimmunoassay; ICN Biochemicals). To each tube were added 25 µl of T4 standard or plasma sample, and then 500 µl of \(^{125}\text{I}\) T4 (provided with the kit). After incubation at 37°C for 90 min, the tubes were aspirated and rinsed with 1 ml of distilled water. After aspiration, the tubes were counted for \(^{125}\text{I}\) in a gamma counter.

Similarly, plasma concentrations of free T3 were determined by radioimmunoassay using a commercially available kit with modification (Free T3 Solid Phase Radioimmunoassay; ICN Biochemicals). To each tube were added 50 µl of T3 standard or plasma sample, and then 500 µl of \(^{125}\text{I}\) T4 (provided with the kit). After incubation at 37°C for 2.5 hours, the tubes were aspirated and rinsed with 1 ml of distilled water. After aspiration, the tubes were counted for \(^{125}\text{I}\) in a gamma counter. These assays were validated by parallel dilution curve of albatross plasma with the T4 or T3 standards. As shown in Figure 2.8, serial dilution of chick plasma was parallel with the T3 standard, whereas no parallelism was seen with the T4 standard. This seems to be unavoidable, as plasma samples could not be diluted with either serum or buffer, because of the nature of the T4/protein equilibrium. Interassay coefficients of variation for free T4 were 5% (n=5) and free T3 10% (n=3).
STATISTICAL ANALYSIS

Statistical analyses were carried out using one-way analysis of variance (ANOVA) followed by Fisher’s least significant test. Student’s t-test was used to compare the mean concentrations of hormones between Black-foot 8 and Bulky Dump. Calculations were performed using a computer program, Statistica (Stat Soft, Tulsa, OK). A probability of < 0.05 was used to assess statistically significant differences.

RESULTS

Estradiol 17-β

Levels of estradiol 17-β for adult and chick albatross at the two sites on Midway Atoll NWR ranged from 15 to 40 pg/ml (Figure 2.9). The mean levels of estradiol 17-β in the adults at Bulky Dump (BD) (contaminated) were significantly higher than the levels in those nesting at Black-foot 8 (B8) (reference site) (P < 0.05). There was no significant difference (P = 0.274) in estradiol 17-β in chicks from the two sites.

Testosterone

The testosterone levels for adults and chicks at the two sites ranged from 10 to 50 pg/ml (Figure 2.10). In contrast to estradiol 17-β results, plasma concentrations of testosterone in adults at Bulky Dump were significantly lower than the levels found at Black-foot 8 (P < 0.001). There was no significant difference (P = 0.624) in testosterone levels in chicks from the two sites. However, testosterone levels in the chicks at both sites were significantly lower than those of adults in Black-foot 8 and Bulky Dump (P < 0.001).
Figure 2.9. Estradiol levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adults, n=75; BD adults, n=72; B8 chicks, n=48; BD chicks, n=43). Values not sharing common letters are significantly different (P < 0.05).

Figure 2.10. Testosterone levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adults, n=75; BD adults, n=72; B8 chicks, n=48; BD chicks, n=43). Values not sharing common letters are significantly different (P < 0.05).
Figure 2.11. Progesterone levels (ng/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD) Vertical bars represent means ± SEM (B8 adults, n=75; BD adults, n=72; B8 chicks, n=48; BD chicks, n=43). Values not sharing common letters are significantly different (P < 0.05).
**Progesterone**

Plasma levels of progesterone for adults and chicks at the two sites were 0.1 to 0.3 ng/ml. No significant difference ($P = 0.890$) was observed among levels in adults at the two sites. Similarly, there was no significant difference ($P = 0.776$) in progesterone levels in chicks between the two sites (Figure 2.11).

**Total T3 and T4**

Total T3 levels in adult and chicks ranged from 1.3 to 2.2 ng/ml. Total T3 in the adults nesting at Bulky Dump was significantly lower ($P < 0.05$) than in those at Black-foot 8. There was no significant difference ($P = 0.772$) in total T3 levels in chicks between the two sites, although these levels in the chicks were significantly higher ($P < 0.05$) than in adults (Figure 2.12).

Plasma concentrations of total T4 for adults and chicks were between 45 and 75 ng/ml. The total T4 levels were significantly higher ($P < 0.001$) in adults nesting at Bulky Dump compared with adults nesting at Black-foot 8. There was no significant difference ($P = 0.366$) in total T4 concentrations in chicks. However, the levels in the chicks were significantly higher ($P < 0.001$) than in the adults at Black-foot 8 (Figure 2.13).
Figure 2.12. Total T3 levels (ng/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adults, n=75; BD adults, n=72; B8 chicks, n=48; BD chicks, n=43). Values not sharing common letters are significantly different (P < 0.05).

Figure 2.13. Total T4 levels (ng/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adults, n=75; BD adults, n=72; B8 chicks, n=48; BD chicks, n=43). Values not sharing common letters are significantly different (P < 0.05).
Figure 2.14. Free T3 levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adults, n=75; BD adults, n=72; B8 chicks, n=48; BD chicks, n=43). Values not sharing common letters are significantly different (P < 0.05).

Figure 2.15. Free T4 levels (pg/ml) of adult and chick albatross at two sites on Midway Atoll, Black-foot 8 (B8) and Bulky Dump (BD). Vertical bars represent means ± SEM (B8 adults, n=75; BD adults, n=72; B8 chicks, n=48; BD chicks, n=43). Values not sharing common letters are significantly different (P < 0.05).
Free T3 and T4

Free T3 levels for adult and chick albatross at both sites were 6-10 pg/ml. There was no significance difference (P = 0.302) between adults at Black-foot 8 and those at Bulky Dump. Free T3 levels in chicks at Bulky Dump were significantly higher (P < 0.001) than those at Black-foot 8. The levels in chicks were significantly higher (P < 0.001) than those in adults at both sites (Figure 2.14).

Free T4 levels for adults and chicks ranged from 6-8 pg/ml. There was no significant difference (P = 0.795) among adults at Black-foot 8 and those at Bulky Dump. There was also no significant difference (P = 0.537) observed among chicks nesting at Black-foot 8 and Bulky Dump. Chicks nesting at both Black-foot 8 and Bulky Dump had significantly higher (P < 0.001) free T4 levels than adults nesting at both sites (Figure 2.15).

Correlations Between PCB Concentrations in the Nest Cup and Plasma Hormone Levels

In January 2001, soil samples were collected from each nest cup for the analysis of PCB concentrations. Soil PCB concentrations are available for only 12 of 100 nests from Bulky Dump and 10 of 64 nests from Black-foot 8. Available data show no significant difference in PCB concentrations between Bulky Dump (32.8 ± 6.9 μg/kg, Mean ± SEM, n = 12) and Black-foot 8 (73.5 ± 27.0 μg/kg, Mean ± SEM, n = 10). Correlations between PCB concentrations in the nest cup and plasma hormone levels in
Figure 2.16. Correlations between PCB levels in the nest cup and plasma levels of estradiol, testosterone, and progesterone in albatross chicks from Bulky Dump (BD, filled circles) and Black-foot 8 (B8, open circles).
Figure 2.17. Correlations between PCB levels in the nest cup and plasma levels of total T3 and total T4 in the albatross chicks from Bulky Dump (BD, filled circles) and Black-footed (B8, open circles).

Total T3 = 2.39 - 0.059 PCB
R = 0.380
P = 0.081

Total T4 = 72.1 - 0.014 PCB
R = 0.513
P = 0.082
Free T3 = 10.15 - 0.021 PCB  
R = -0.370  
P = 0.091

Free T4 = 7.91 + 0.0005 PCB  
R = 0.010  
P = 0.963

Figure 2.18. Correlations between PCB levels in the nest cup and plasma levels of free T3 and free T4 in the albatross chicks from Bulky Dump (BD, filled circles) and Black-foot 8 (B8, open circles).
the chicks were analyzed by combining the data obtained from Bulky Dump and Black-foot 8, since there was no difference in PCB levels between the two sites. As shown in Figures 2.16-2.18, no significant correlation was observed between nest cup PCB levels and plasma levels of estradiol (P = 0.826, R = 0.051), testosterone (P = 0.963, R = 0.010), progesterone (P = 0.630, R = -0.109), total T3 (P = 0.081, R = -0.380), total T4 (P = 0.082, R = -0.513), free T3 (P = 0.091, R = -0.370), and free T4 (P = 0.963, R = 0.010) in the chicks. When the relationship was analyzed for Bulky Dump and Black-foot 8 separately, there were no significant correlations either, except for total T4 levels at Bulky Dump (Total T4 = 88.8 – 0.463 PCB, R = -0.578, P < 0.05).

DISCUSSION

This study was intended to investigate the possible effect of PCB contamination in the soil on albatross nesting in the Midway Atoll NWR. The complete survey took place from October 2000 to June 2001. There were significant differences in plasma estradiol and testosterone level in the adult albatross between Bulky Dump and Black-foot 8. The significantly higher estradiol (P < 0.05) and lower testosterone (P < 0.001) in the adult birds may suggest that there may be endocrine disruption at Bulky Dump and/or Black-foot 8, both areas with a large population of albatross nesting, although no such difference was found in the chick plasma.

In birds, estradiol, testosterone, and progesterone are the three major gonadal steroid hormones (Tanaka, 1980). It has been well documented among seabirds, particularly in wandering albatross (Diomedea exulans) and black-browed albatross (Diomedea melanophris) that gonads undergo a regression once the egg has been laid.
(Hector et al., 1986; Williams, 1992; Vleck et al., 1999; Whittow, 2002). Associated with this gonadal regression is a drop in plasma levels of estradiol, testosterone, and progesterone. In the Adélie penguin, there was a drop in plasma testosterone levels in males to less than 2% of the levels prior to egg-laying as soon as the eggs were laid (Vleck et al., 1999). In female penguins, there was also an 84% decrease in estradiol immediately after eggs were laid (Vleck et al., 1999). In black-browed albatross, a similar pattern of decreased steroid hormones was observed immediately after egg laying (Hector et al., 1986). Hormone profiles in wandering albatross (D. exulans) and black-browed albatross (D. melanophris) showed that gonads regressed shortly after breeding, and a dramatic decrease was found in plasma progesterone and estradiol levels (Hector et al., 1985; Hector et al., 1986). Hector and coworkers (1986) found that at 70-90 days after egg lay, female black-browed albatross had estradiol levels of 400 ng/ml, testosterone levels of 500 pg/ml, and progesterone levels of 100 pg/ml. They also found that at 70-90 days after egg lay, male black-browed albatross had testosterone levels of 200 pg/ml and 500 pg/ml progesterone.

Plasma samples collected for this study at Midway Atoll were obtained during the late stages of incubation or at chick hatching, approximately 70-90 days after the egg was laid. Highest estradiol levels determined at Midway Atoll were in chicks at 40 pg/ml. The highest levels of testosterone determined were in adults at 54 pg/ml, and highest progesterone was in chicks at 3.1 ng/ml. The levels of sex steroids observed in black-footed albatross at Midway Atoll were about 1/10 lower than values observed in black-browed albatross nesting at South Georgia Island. This variation could be due to species differentiation or possibly extraction methods in each study.
Adults at Midway Atoll were not individually sexed so mean plasma levels observed are both sexes combined. It is also probable that more of one gender was grabbed for the sample collections at each plot. It is impossible to identify the genders of the albatross externally. Although the gender of the adult bird sampled from each plot was not identified, there were no apparent bimodal peaks in either estradiol or testosterone levels. Unpaired birds were included in the analyses and may have skewed the sex ratios.

In addition to support the results presented, factors that should be taken into consideration to explain the hormone levels observed relate to the natural history of the birds. Potential explanations for adults at Bulky Dump having lower testosterone levels may reflect nesting territories. Testosterone plays a pivotal role in nest defense (Hector et al., 1986; Vleck et al., 1999) and Bulky Dump was a relatively new site with many unclaimed nesting territories and nests widely spread out. Due to the recent formation of Bulky Dump, it is estimated that birds breeding at Bulky Dump are of the younger age class, validated by finding very few previously banded birds. Thus, there may be less aggressive nest defense behavior at larger, more spacious Bulky Dump (nests are more spread out) compared to Black-foot 8, thus leading to lower testosterone levels at Bulky Dump.

Little is known about thyroid hormones in wild avian populations, and there seems to be no information on levels of thyroid hormones of seabirds nesting on PCB-contaminated soil. Few studies have been conducted on polar seabird thyroid levels (Groscolas and Leloup, 1986). In male Emperor penguins (Aptenodytes forsteri) there was no significant difference in plasma T4 and T3 levels throughout courtship, egg
incubation and chick feeding (Groscolas and Leloup, 1986). In female Emperor penguins, levels of plasma T3 during feeding were not different than the levels observed in courtship (Groscolas and Leloup, 1986). During chick rearing Groscolas and Leloup (1986) found that plasma T3 levels in male Emperor penguins were 5 ng/ml and 8 pg/ml in females. In breeding penguins, plasma T3 (0.1 to 2.2 ng/ml) and T4 (14.8 ng/ml) levels are within the reported range of other avian species. In this research at Midway Atoll, plasma levels of T3 in breeding adults during incubation were 1.3 to 1.63 ng/ml and T4 ranged from 47 to 68 ng/ml.

Thyroid hormones are critical for the normal growth and development as well as for the physiological metabolic process including lipid metabolism (Norris, 1985). Chicks for this research were sampled when they were being infrequently fed by parents and were in a fasting mode prior to fledging. In the present study, adults nesting at Bulky Dump had significantly higher ($P < 0.001$) total T4 levels than those nesting at Black-foot 8. In contrast, the adult albatross nesting at Bulky Dump had significantly lower ($P < 0.05$) total T3 levels than those nesting at Black-foot 8. There was no difference in free T3 or T4 concentrations in adult albatross between the two sites. Chicks sampled from Bulky Dump had significantly higher ($P < 0.001$) levels of free T3 than chicks nesting at Black-foot 8. Thus, there was no consistent difference in plasma thyroid hormone levels in the adults and chicks between the two sites. It is possible that soil contamination and gonadal steroid levels at Midway could lead to a much larger concern at other locations where seabirds nest in the Pacific. In avian species, behavioral aspects such as reproduction, parental care, nest attentiveness, and migration are all controlled by neuroendocrine systems, and the disruption of these behaviors would have long-term
effects on populations. The possible hazards posed by ingestion of PCB-contaminated soil particles include decreased reproductive success and population decline. In avian species such as albatross the bioaccumulation of PCBs is related to the PCB concentration and composition of prey, sex, and age of the bird, and residence time in contaminated areas (Struger and Weseloh, 1985).

Soil samples collected from Bulky Dump in October 2000 were found to contain a significant amount of PCB ranging from 0.2 to 8.6 mg/kg (2.05 ± 0.77 mg/kg, Mean ± SEM, n = 11). It was then concluded that further investigation is needed for contaminants such as PCBs of the soil at old bulky waste landfill on Midway Atoll NWR. It is suspected that birds burrowing in the dump are bringing contaminated soil to the surface.

In the present study, soil samples were collected from each nest cup for the analysis of PCB concentrations. Soil PCB concentrations are available for only 12 of 100 nests from Bulky Dump and 10 of 64 nests from Black-foot 8. Available data show no differences in PCB concentrations between Bulky Dump and Black-foot 8. Correlations between PCB concentrations in the nest cup and plasma hormone levels in the chick were analyzed by combining the data obtained from Bulky Dump and Black-foot 8. As shown in Figures 2.16 through 2.18, no significant correlation was observed between nest cup PCB levels and plasma hormone levels in the chicks. When the correlations were determined for Bulky Dump and Black-foot 8 separately, there was no significant relationship either, except for total T4 levels at Bulky Dump. Thus, no conclusive biological evidence was obtained on endocrine disruption from the nesting soil, although it should be noted these results are based on an incomplete data set.
CHAPTER 3
HORMONAL EFFECTS OF SOIL CONTAMINATION ON BLACK-FOOTED ALBATROSS CHICKS AT TERN ISLAND, HAWAIIAN ISLANDS NATIONAL WILDLIFE REFUGE

ABSTRACT

Hormonal profiles of chicks of black-footed albatross (Phoebastria nigripes) were examined at two sites on Tern Island, Hawaiian Islands National Wildlife Refuge (HINWR) during the 2001 breeding season. Plasma samples were analyzed for estradiol, testosterone, progesterone, total and free thyroxine (T4) and tri-iodothyronine (T3) as indicators of endocrine disruption. Chicks nesting at the Old Landfill (contaminated site) had significantly higher (P < 0.05) estradiol levels than those nesting at the East End (reference site), whereas chicks nesting at Old Landfill had significantly lower (P < 0.05) testosterone levels than those nesting at East End. On the other hand, chicks nesting at Old Landfill had significantly higher (P < 0.001) total T4 and (P < 0.001) free T4 than those nesting at East End. There was no difference in the albatross chicks between the two sites in progesterone, total T3, and free T3 concentrations. When correlations between soil PCB and plasma hormone levels were determined, no relationship was found in any of the hormones. These data indicate that although there were significant differences in the plasma hormone profiles in the chicks of black-footed albatross nesting at two sites at Tern Island, no conclusive biological evidence of endocrine disruption from the nesting soil at the sites was obtained based on this limited data set.
INTRODUCTION

Black-footed albatross (*Phoebastria nigripes*) are one of the three species of albatross found in the northwestern Hawaiian islands. Tern Island is the main island among nine islets located inside the coral atoll of French Frigate Shoals (FFS) (23°45'N, 166°15'W) located in the northwestern Hawaiian islands, part of the Hawaiian Islands National Wildlife Refuge (HINWR). Historical and background information have been discussed in Chapter 1. Today, Tern Island continues to be home to the fourth-largest population of black-footed albatross as well as hundreds of thousands of nesting Pacific seabirds of other species. On Tern Island, albatross as well as other species of seabirds nest directly on the ground. Due to their nest location, directly on the ground, there may be an exposure risk to adults and their chicks from the inhalation of PCB-contaminated dust or from pica (ingestion of contaminated soil particles).

Large quantities of unclassified debris were disposed of in a landfill on Tern Island and pushed into the sea throughout the atoll (Miao et al., 2000). High levels of polychlorinated biphenyls (PCBs) have been found in soil samples from Tern Island (Woodward-Clyde, 1999). Investigations done by the US Coast Guard found PCB soil concentrations as high as 2300 mg/kg (Woodward-Clyde, 1999). Possible contaminators of the soil where albatross are nesting on Tern Island and French Frigate Shoals are scrap metal, capacitors, batteries, and air conditioners all inside a degenerating unstable landfill. Suspected contaminants in the soil at Tern Island are polychlorinated biphenyls (PCBs), dioxins, and pesticides (Ogden Environmental and Energy Services Co., 1997; Woodward-Clyde, 1999; Miao et al., 2000).
The objective of this study was to determine if nesting in PCB-contaminated soil atop a degenerating landfill affected the hormone balance in black-footed albatross chicks nesting directly on the soil on Tern Island, HINWR.

MATERIALS AND METHODS

Birds

Chicks of black-footed albatross (*Phoebastria nigripes*), 12-15 weeks of age, were selected based on the probable location of their nest cup. I was not able to identify the sex of chicks. From April 16 to May 12, 2001, blood samples were taken from a total of 90 chicks. They were selected from two sites on Tern Island, Old Landfill and East End. In addition to blood samples, 86 soil samples were collected from directly under each chick.

Blood Sampling

Blood samples were collected from chick albatross on Tern Island, HINWR during the 2001 nesting season. Chick data collection consisted of hand-capturing chicks located within the designated plots and drawing 5 ml of blood from the ulnar wing vein using 21-gauge needles and Vacutainer tubes (BD Vacutainer™ containing freeze-dried lithium heparin, Becton, Dickinson and Company, Franklin Lakes, NJ). For blood sampling, two assistants were required, one holding the bird’s beak and body across their lap and the other holding the feet and the wing to be sampled flat against a padded mat. This position allowed the wing to be draped outward and the blood to rapidly flow to the vein to be sampled. At times, blood from both wings was sampled. In addition, chicks
were banded and weighed. Blood was kept in a cooler with ice packs and then centrifuged for 10 minutes at 3,000 x g within 4 hours of sampling. Approximately 2 ml of plasma were obtained from 5 ml blood collected. Plasma was divided into two microcentrifuge tubes and kept frozen at -40° C for later analysis of hormones and contaminants. During this sampling period 45 chicks were sampled from both sites on Tern for a total of 90 blood samples collected.

Soil Collection

In May of 2001, soil samples were collected from active nest cups located near chicks within designated plots. Immediately after blood sampling, nest soil samples were collected using nitrile gloves and scooping 3-4 teaspoons of soil into individual plastic bags.

Study Sites

The Old Landfill (contaminated site) was selected based on findings described in Chapter 1 from investigations that pinpointed “hotspots” of PCB soil contamination (Ogden Environmental and Energy Services Co., 1997; Woodward-Clyde, 1999) on the landfill used for years by the US Coast Guard to dispose of unwanted debris. The plot at Old Landfill was approximately 123 x 45 m. East End (reference site) was a 25 x 33 m plot that was located the farthest from the buildings and the landfill site and shown not to be contaminated in an investigation in 2000 (Chase Environmental Group, 2002).
Radioimmunoassays of Steroid Hormones

Plasma concentrations of estradiol, testosterone, progesterone, total and free thyroxine (T4), and tri-iodothyronine (T3) were determined by radioimmunoassays using commercially available kits with some modifications as described in Chapter 2. Since albatross chicks feed on regurgitated flying-fish eggs and squid with high lipid content, it was necessary to separate interfering substances in the plasma by using Sep-Pak C-18 light cartridges to estimate steroid hormones.

STATISTICAL ANALYSIS

Student’s t-test was used to compare the mean concentrations of hormones between chicks at East End and the Old Landfill. Calculations were performed using a computer program, Statistica (Stat Soft, Tulsa, OK). A probability of $< 0.05$ was used to assess statistically significant differences.

RESULTS

Estradiol, Testosterone and Progesterone

The estradiol levels for chicks at the two sites on Tern Island ranged from 30 to 48 pg/ml. There was a significant difference ($P < 0.05$) in estradiol and testosterone levels in chick albatross from nests at two locations on Tern Island. As shown in Figure 3.1, the mean levels of estradiol in the chicks at Old Landfill were significantly higher than the levels of estradiol in those at East End ($P < 0.05$).
The testosterone levels for chicks at the two sites ranged from 6 to 22 pg/ml. In contrast to estradiol, plasma concentration of testosterone in chicks at the Old Landfill was significantly \( P < 0.05 \) lower than the levels found at East End (Figure 3.2).

On the other hand, plasma levels of progesterone for chicks at the two sites were .25 to .27 ng/ml. There was no significant difference \( P = 0.691 \) observed in progesterone levels in chicks between the two sites (Figure 3.3).

**Total T3 and T4**

Total T3 levels in chicks ranged from 1.9 to 2.2 ng/ml, and there was no significant difference \( P = 0.064 \) observed in total T3 levels in chicks between the two sites on Tern Island (Figure 3.4).

Plasma concentrations of total T4 in chicks were between 54 to 95 ng/ml. The total T4 levels were significantly higher \( P < 0.001 \) in chicks nesting at Old Landfill than in chicks nesting at East End (Figure 3.5).

**Free T3 and T4**

Free T3 levels for chick albatross at both sites were about 10 pg/ml. There was no significant difference \( P = 0.201 \) between chicks at East End and those at Old Landfill (Figure 3.6).

Free T4 levels for chicks at Tern ranged from 4 to 6 pg/ml. There was a significant difference between the nesting sites in free T4 levels in chick albatross in Tern Island. Chicks from Old Landfill had significantly \( P < 0.001 \) higher free T4 levels than chicks from East End (Figure 3.7).
Figure 3.1. Estradiol levels (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chicks, n=45; OLF chicks, n=45). Values not sharing common letters are significantly different (P < 0.05).

Figure 3.2. Testosterone levels (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chicks, n=45; OLF chicks, n=45). Values not sharing common letters are significantly different (P < 0.05).
Figure 3.3. Progesterone levels (ng/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chicks, n=45; OLF chicks, n=45).
Figure 3.4. Total T3 (ng/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chicks, n=45; OLF chicks, n=45).

Figure 3.5. Total T4 (ng/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chicks, n=45; OLF chicks, n=45). Values not sharing common letters are significantly different (P < 0.05).
Figure 3.6. Free T3 (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chicks, n=45; OLF chicks, n=45).

Figure 3.7. Free T4 (pg/ml) of albatross chicks at East End (EE) and Old Landfill (OLF) on Tern Island. Vertical bars represent means ± SEM (EE chicks, n=45; OLF chicks, n=45). Values not sharing common letters are significantly different (P < 0.05).
Figure 3.8. Correlations between PCB levels in the nest cup and plasma levels of estradiol, testosterone, and progesterone in the albatross chicks from Old Landfill.
Figure 3.9. Correlations between PCB levels in the nest cup and plasma levels of total T3 and total T4 in the albatross chicks from Old Landfill.
Free T3 = 9.11 + 1.25 PCB
R = 0.425
P = 0.078

Free T4 = 6.24 + 0.26 PCB
R = 0.180
P = 0.490

Figure 3.10. Correlations between PCB levels in the nest cup and plasma levels of free T3 and free T4 in the albatross chicks from Old Landfill.
Correlations Between PCB Concentrations in the Nest Cup and Plasma Hormone Levels

In May of 2001, soil samples were collected from active nest cups located near chicks within designated plots. Soil PCB concentrations are available for areas around 18 nests in Old Landfill from a previous investigation (Woodward-Clyde, 1999), ranging from 0.01 to 0.54 mg/kg (0.84 ± 0.29 mg/kg, Mean ± SEM, n = 18). Results of correlation analyses between PCB concentrations in the nest cup and plasma hormone levels in the chicks are shown in Figures 3.8 to 3.10. No significant correlation was observed between soil of a nest area PCB levels and plasma levels of estradiol (P = 0.831, R = 0.054), testosterone (P = 0.852, R = -0.047), progesterone (P = 0.831, R = -0.054), total T3 (P = 0.074, R = 0.430), free T3 (P = 0.078, R = 0.425), and free T4 (P = 0.490, R = 0.180) in the chicks.

DISCUSSION

Endocrinology of southern hemisphere albatross species has been investigated in the past ten years. These include Diomedea exulans (Hector et al., 1985), D. melanophрис and D. chrysostoma (Hector et al., 1986). These studies examined the circulating levels of some hormones and gonadal size variation in adult birds. Albatross research in the northern hemisphere has been concentrated mostly on natural history, and no previous documentation of circulating levels of hormones has been recorded in the three species in the northern hemisphere: P. albatrus, P. immutabilis, and P. nigripes.

In the present study, plasma levels of estradiol, testosterone, progesterone, and total and free T3 and T4 were examined in black-footed albatross chicks of
approximately 12-15 weeks of age at Tern Island, HINWR. It is important to note here that plasma samples were taken during the latter period of chick rearing, when most chicks were receiving infrequent parental feedings and were under greater heat and physiological stress. Once albatross young have fledged and reach breeding age, they will return year after year to the same location where they were reared, and if there is PCB contamination in the soil, the long-term effects could be widespread for that population. Studies on endocrine disruption and effects of PCB contamination in wildlife have shown that estradiol and testosterone levels may be altered and skewed as a result of anthropogenic sources (Fry and Toone, 1981; Grasman et al., 1996; Auman et al., 1997). Thus, the levels of gonadal steroids may function as indicators for exposure of PCBs in the soil affecting these long-lived seabirds. I examined the hypothesis that albatross hormone levels, especially of sex steroid origin, would vary on an area with PCB-contamination in the soil.

As shown in Figures 3.1 and 3.2, hormonal profiles of chicks of black-footed albatross were examined at two sites on Tern Island. Chicks nesting at Old Landfill (contaminated site) had significantly higher estradiol levels ($P < 0.05$), than those nesting at East End (reference site) whereas chicks nesting at Old Landfill had significantly lower ($P < 0.05$) testosterone levels than those nesting at East End. Similarly, significant differences were observed in the adult black-footed albatross on Midway Atoll between the plasma estradiol ($P < 0.05$), and testosterone ($P < 0.001$), in birds from Black-foot (reference site) and Bulky Dump (contaminated site), although no such difference was seen in the chick. There was no difference in either estradiol or testosterone levels between the chicks on Tern Island and Midway Atoll. The reason that there was no
difference in the chicks on Midway Atoll is unknown. Minimally, these results are consistent with other reports on endocrine disruption, showing demasculinization and feminization patterns in hormone levels occurring in wildlife populations (Fry and Toone, 1981; Guillette et al., 1994; and Facemire et al., 1995). However, without knowing the gender of the birds, it is difficult to conclude that the differences in estradiol and testosterone levels between the two sites at Tern Island and Midway Atoll are due to endocrine disruption from the nesting soil. Data on PCB concentrations of the soil were available for 18 nesting sites at Old Landfill. When the correlations between soil PCB and plasma levels of estradiol and testosterone were analyzed, no correlation was found for either of the hormones. These results indicate that although there were differences in the plasma hormone profiles in the chicks nesting at the two sites, it is difficult to conclude that the observed differences are due to endocrine disruptors from the nesting soil.

Thyroid hormone levels have also become indicators for endocrine disruption in wildlife (Fry and Toone, 1981; Grasman et al., 1996; Auman et al., 1997). Thyroid hormones are necessary for normal growth and development, and for regulating oxidative metabolism (Sturkie, 1986). Thyroid hormones can be influenced by exposure to endocrine disruptors such as PCBs (Lans et al., 1993; Murk et al., 1994; Nieminen et al., 2000; Zhou et al., 2000). Altered plasma T4 and T3 concentrations have been associated with PCB contamination (Murk et al., 1994; Nieminen et al., 2000; Chiba et al., 2001). This endocrine disruption of thyroid hormones may be due to the similarity of PCBs to thyroxine. Polychlorinated biphenyl metabolites are structurally similar to thyroxine; thus PCBs are most likely competing for binding sites on the transport protein chain.
transthyretin (TTR) (Lans et al., 1993; Murk et al., 1994; Nieminen et al., 2000; Zhou et al., 2000). Lans et al., (1993) found in vitro that hydroxylated metabolites of PCBs had binding affinities 10 times greater for TTR than for thyroxine. In this study, chicks nesting at Old Landfill had significantly higher (P < 0.001) total T4 and free T4 than those nesting at East End. There was no significant difference in the albatross chicks between the two sites in total T3, and free T3 concentrations. Chicks at Bulky Dump in Midway Atoll had significantly higher (P < 0.001) free T3 levels than those at Black-foot 8. The plasma levels of thyroid hormones were essentially the same between the chicks on the two islands. The reason that there were differences in the thyroid hormone profiles between the chicks on Tern Island and those on Midway Atoll is not clear.

Overall, plasma levels of total T3 (1.5-2 ng/ml) and total T4 (50-80 ng/ml) observed in this study at Tern Island and Midway Atoll were much higher than normal levels observed in other avian species (Astier, 1980). Most thyroid research in seabirds has focused on the adult levels of thyroid hormones (Hector et al., 1985; Groscolas and Leloup, 1986; Hector et al., 1986). In male Emperor penguins, Groscolas and Leloup (1986) found levels of 11 to 0.6 ng/ml during the 4-month fasting period of the breeding cycle, and in fasting females the levels were 10 ng/ml. During chick rearing, plasma total T3 levels in male Emperor penguins were 5 ng/ml and 8 pg/ml in females. The differences in plasma thyroid hormone profiles between the black-foot albatross and Emperor penguin may be due to the differences in their basal metabolism, age, species, or family differentiation.
Thyroid hormone levels patterns documented at Tern between the two sites, East End and Old Landfill, may suggest that elevated levels of total T4 observed at Old Landfill might be due to the PCB contamination in the environment and possible ingestion of PCBs. Nieminen et al. (2000) found higher total T4 levels in minks (*Mustela vison*) fed freshwater fish and PCBs compared with the control animal fed with food without PCBs. It may be possible that the transport mechanism of T4 in circulation in the black-foot albatross could be similar to that of the mink fed with PCBs. In common tern chicks (*Sterna hirundo*), Murk et al. (1994) found no difference in plasma total T4, total T3, or free T4 levels in areas where there was polyhalogenated aromatic hydrocarbons (PHAH) contamination. As stated above, data on PCB concentrations of the soil were available for 18 nesting sites at Old Landfill. When correlations between soil PCB and plasma thyroid hormone levels were analyzed, there was no significant correlation in any of the hormones. These data indicate that although total and free T4 levels in the chicks nesting at Old Landfill were significantly higher (P < 0.001) than in those at East End, it is difficult to conclude from the limited data that the observed differences are due to endocrine disruptors from the nesting soil, as in the case of plasma estradiol and testosterone levels.

On Tern Island, the plots East End and Old Landfill were set up based on the documented high levels of PCB-contamination in the Old Landfill soil from previous investigations (Woodward-Clyde, 1999). On Tern a one-time sampling of birds nesting on these areas was conducted due to the unexpected availability of the site. Chick nest cups were estimated and soil samples were taken from the assumed nest cups, where the chicks were found at time of blood collections. Chicks sampled at this time were in the
semi-mobile stage and often roamed around or went in search of shade. It is likely that some chicks were not in their exact nest cup, and this could have led to error in exact nest locations. An additional difficulty in this study was the limited sample size provided by the one-time collection of blood and soil. While the original study did include an early blood sample from the adults prior to brooding, only one sample was acquired during brooding. As stated above, it has been well documented among seabirds, particularly in wandering albatross (*Diomedea exulans*) and black-browed albatross (*D. melanophris*) that gonads undergo a regression once the egg has been laid (Hector et al., 1986; Williams, 1992; Vleck et al., 1999; Whittow, 2002). Associated with this gonadal regression is a drop in plasma levels of estradiol, testosterone, and progesterone. Therefore, it would be critically important to obtain information on plasma hormone profiles of adult birds out of the breeding season in order to understand further the effect of endocrine disruptors on the black-footed albatross. Knowing the gender and age of the birds would also provide additional information necessary for conclusive evidence in support of endocrine disruption. Finally, the analysis and comparison of actual PCB concentrations of the plasma of the study birds with plasma hormone levels may be a better indicator of the potential for endocrine disruption.
CHAPTER 4
SUMMARY AND CONCLUSION

Midway Atoll and Tern Island are remote land areas in the northwestern Hawaiian islands; both are past sites of military and other human disturbance, and home to millions of seabirds. Albatross nest directly on the ground on these atolls and may be experiencing endocrine disruption from PCB contaminants in the soil. Black-footed albatross are among the many species of seabirds whose numbers are affected every year by anthropogenic exposure. Currently, we lack the information necessary to know if there is a cause for concern regarding contaminants in nesting soil affecting the albatross. This research was aimed to assess if nest soil contamination could alter hormones which could affect the future reproductive success and health of seabird populations in the northwestern Hawaiian islands.

The objective of this study was to determine if nesting in PCB-contaminated areas affects hormone balance due to endocrine disruption in both adult and chick black-footed albatross on Midway Atoll National Wildlife Refuge (NWR) and chick black-footed albatross on Tern Island, Hawaiian Islands National Wildlife Refuge (HINWR). A total of 333 plasma samples were processed, validated and analyzed for plasma hormone concentrations over the course of two years.

To my knowledge, this is the first study to examine the possible transfer of PCB contaminants to any seabird, via the pathway of incidental ingestion or inhalation from PCB-contaminated soil. Although past research has shown that albatross in the Pacific are contaminated with PCBs from their food sources (Auman et al., 1997; Ludwig et al., 1998; Guruge et al., 2001), no study has been done on alternate sources of contamination.
such as nest soil. This is a newly identified pathway of contamination for species such as albatross that spend most of their lives on the sea and return to land to nest once a year. Most of the wildlife refuges where seabirds nest in the Pacific are decommissioned military bases and have signs of human presence, even though they have been cleaned up. As this and past investigations indicate, contamination can still be present, affecting wildlife (Ogden Environmental and Energy Services Co., 1997; Woodward-Clyde, 1999).

On Midway Atoll, adults nesting at Bulky Dump (contaminated site) had significantly higher ($P < 0.05$) estradiol levels than those nesting at Black-foot 8 (reference site), whereas those nesting at Bulky Dump had significantly lower ($P < 0.001$) testosterone levels. Adults nesting at Bulky Dump had significantly higher ($P < 0.001$) total thyroxine (T4) levels than those nesting at Black-foot 8. In contrast, the adult albatross nesting at Bulky Dump had significantly lower ($P < 0.05$) total tri-iodothyronine (T3) levels than those nesting at Black-foot 8. There was no significant difference in free T3 or T4 concentrations in adult albatross between the two sites. Chicks sampled from Bulky Dump had significantly higher ($P < 0.001$) levels of free T3 than chicks nesting at Black-foot 8. Polychlorinated biphenyl concentrations of the soil were available for 12 nests from Bulky Dump and 10 nests from Black-foot 8. When correlations between soil PCB and plasma hormone levels were determined, there were no significant relationships between any of the hormones. Daily field logs and observations gave no obvious signs of visual or physical contaminant effects in albatross at either Bulky Dump or Black-foot 8.
These results indicate that although there are significant differences in the plasma hormone profiles in both adult and chick black-footed albatross nesting at two sites at Midway Atoll, no conclusive biological evidence of endocrine disruption from the nesting soil at the sites was obtained.

On Tern Island, plasma hormone profiles were examined in chicks of approximately 12-15 weeks of age. Chicks nesting at Old Landfill (contaminated site) had significantly higher ($P < 0.05$) estradiol levels than those nesting at East End (reference site), whereas chicks nesting at Old Landfill had significantly lower ($P < 0.05$) testosterone levels than those nesting at East End. On the other hand, chicks nesting at Old Landfill had significantly higher total T4 ($P < 0.001$) and free T4 ($P < 0.001$), than those nesting at East End. There was no significant difference in albatross chicks between the two sites in progesterone, total T3, and free T3 concentrations. PCB concentrations of the soil were available for 18 nesting sites at Old Landfill. When correlations between soil PCB and plasma hormone levels were determined, no significant relationship was found with any of the hormones. As in the case of Midway Atoll, it is difficult to conclude that the observed differences are due to endocrine disruptors from the nesting soil, although there were significant differences in the plasma hormone profiles in the chicks nesting at two sites at Tern Island.

The hypothesis that hormone levels in seabirds that spend most of their life at sea could be altered due to anthropogenic inputs in their nesting environment was proposed. This research is the first of its kind to look at endocrine disruption in albatross chicks. This study has documented levels of seven endocrine hormones in hatch-year black-
footed albatross chicks from Midway Atoll and Tern Island. Although samples were
collected only for one year, these data may provide valuable insight to the viability of
levels of endocrine hormones in chicks of a similar age-class.

Endocrine disruption has been receiving considerable attention recently, and there
is an increasing body of evidence relating wildlife and human decline in health to
environmental endocrine disruptors. The results obtained in the present study indicate
that there were differences in certain hormone profiles in chick and adult albatross
between the contaminated sites and the reference sites on both Midway Atoll and Tern
Island. I hope that this research will serve as a guide to help monitor and increase the
survival of other species of seabirds, such as terns, petrels, shearwaters, and other
burrowing seabirds that are also directly exposed to soils, like petrels and shearwaters. I
also hope that the field collections and procedures for assay validations acquired during
this research can be further used in future research with albatross as well as other seabird
species.
REFERENCES


Cousins, K. and J. Cooper. 1998. The population biology of the Black-footed Albatross in relation to mortality caused by longline fishing, Western Pacific Regional Fishery Management Council, Honolulu, HI. pp. 120.


