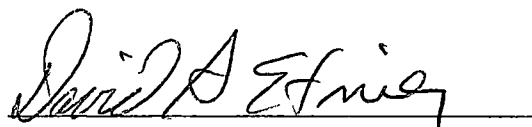


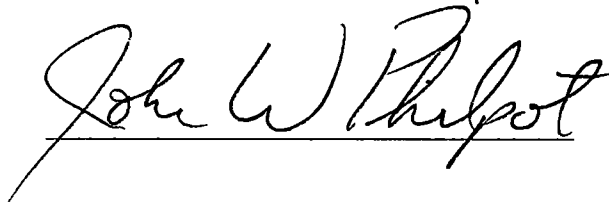
To the Graduate Council:

We are submitting herewith a thesis written by Robert L. Brewer, Jr. entitled "PCB and Mercury Contamination in Great Blue Herons Associated with the Oak Ridge Reservation, Tennessee" We have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree Master of Science, with a major in Ecology and Evolutionary Biology.


David A. Buehler, Major Professor

We have read this thesis
and recommend its acceptance:





Accepted for the Council:


Associate Vice Chancellor
and Dean of the Graduate School

**PCB AND MERCURY CONTAMINATION IN GREAT BLUE
HERONS ASSOCIATED WITH THE OAK RIDGE
RESERVATION, TENNESSEE**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Robert L. Brewer, Jr.
May, 1999

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ABSTRACT

I collected eggs and chicks from four great blue heron (*Ardea herodias*) colonies on and around the Oak Ridge Reservation, Tennessee, in 1993 and 1994. I analyzed eggs for both PCB and mercury levels, while also measuring a suite of biomarkers aimed at exposing biological effects of these contaminants. Chicks were necropsied, and muscle, liver, feather, and fat samples were collected for contaminant analysis. A group of biomarkers was also examined to ascertain any effects of these contaminants on growing chicks. Reproductive output was also monitored in an effort to correlate it with contaminant loads at specific sites. Fish collected at the colonies were also examined for PCB and mercury content.

The K-25 colony was located closest to the sources of contaminants; the Melton Hill colony was on the Oak Ridge Reservation, but upstream from contaminants. The Long Island colony was located >15 km from Oak Ridge and the Looney Island colony was located on Fort Loudon Lake, >15 km from Oak Ridge. Eggs from Melton Hill had lower mercury concentrations than eggs from K-25 ($P \leq 0.05$). No other colonies differed. PCB concentrations were higher in eggs

from K-25, when compared to eggs from Long Island and Looney Island ($P \leq 0.05$). The only biomarker that differed between colonies was circumference ($P \leq 0.05$), which was greatest in the K-25 colony.

In chicks, liver, muscle, and feathers showed differences between colonies for mercury concentration ($P = 0.0002, 0.0996, \text{ and } 0.0002$, respectively). Overall, K-25 samples showed the highest concentrations, when compared to the other colonies. Total PCB concentrations in chicks also differed between colonies in both muscle and fat samples ($P = 0.0373 \text{ and } 0.0396$, respectively), with the highest concentrations coming from the K-25 colony. The same was true for PCB congener 1260 in fat, muscle, and liver samples ($P = 0.0099, 0.0069, \text{ and } 0.0275$, respectively).

The only biomarker to differ marginally between colonies was the liver enzyme, MPRO ($P = 0.0630$). The activity of this enzyme was lowest in the K-25 colony. The other biomarkers, including another liver enzyme, did not differ between colonies.

The highest mean clutch size ($\bar{x} = 3.71, n=17$) was found in the Melton Hill colony. The lowest clutch size ($\bar{x} = 3.19, n = 32$) was found in the Looney Island colony.

v

Overall, it appears that clutches were larger in on-site colonies than they were in off-site colonies. Mean mortality (chicks lost/nest) was highest in the Looney Island colony ($\bar{x} = 0.67$), and lowest in the Long Island and Melton Hill colonies ($\bar{x} = 0.44$ for both).

PCB concentrations in fish did not differ between sites, although, mercury concentrations differed in fish ($P = 0.03$). Fish from on-site colonies had mean mercury concentrations of 0.06 ± 0.02 ppm, while fish from off-site colonies had a mean level of 0.02 ± 0.00 ppm.

Results indicated that clutch sizes from these colonies were comparable to those reported by other researchers across North America. Contaminant loads found in this study are below those reported by other investigators in areas where bird populations show measurable effects from these contaminants. It appears that the contaminant levels found in this study are not adversely affecting reproduction, growth, or survival.

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Chapter 1

Introduction

The Clinch River-Environmental Restoration Program (CR-ERP)

The Clinch River Resource Conservation Recovery Act Facility Investigation (CRRFI) was initiated in 1989, in compliance with section 3004(v) of the Hazardous and Solid Waste Amendments of 1984 to the Resource Conservation and Recovery Act of 1976. The CRRFI focused on areas of the Clinch and Tennessee Rivers which may have been adversely affected by contaminants released from the Oak Ridge Reservation (ORR) from 1943 to present. The contaminants of concern originated from research, industrial, and waste disposal activities conducted at the three plant sites (Figure 1)*:

- 1) Oak Ridge National Laboratory (ORNL), an energy research and development facility formerly known as X-10,
- 2) The Oak Ridge Gaseous Diffusion Plant (ORGDP), a uranium enrichment facility until closure in 1986, formerly known as K-25, and
- 3) The Y-12 plant, a facility for producing nuclear weapons components.

* All tables and figures may be found in the appendix.

The contaminants released from these sites included radionuclides, heavy metals, and organic compounds. While some liquid wastes were discharged into streams on the ORR, the majority of contamination arose from seepage of old waste storage pits and trenches into shallow ground water. This ground water ultimately flowed into the Clinch River via streams on the ORR. The Clinch River enters the Tennessee River 34 km downstream of the ORR in the Watts Bar Reservoir (Figures 2 and 3).

The objectives of the CRRFI were:

- 1) Define the nature and extent of contamination of the off-site surface-water and sediment environment downstream of the ORR;
- 2) Quantify any risk to human health and to the environment resulting from the existing off-site contamination; and
- 3) Preliminarily identify and evaluate potential corrective measures/remedial action alternatives (ERD 1990).

The CRRFI was carried out in several phases. Phase I included a synthesis of extant data, and a screening level risk analysis to determine what additional data collection was necessary for site characterization, risk assessment, and evaluation of remedial action alternatives. Phase I identified the great blue heron (*Ardea herodias*) as being at

risk from polychlorinated biphenyls (PCBs) and mercury. Phase II involved preliminary sampling and analysis in selected areas that were representative of different levels of contamination. It was during this phase that the great blue heron was selected as an indicator species for the aquatic systems around the ORR, and the Great Blue Heron Project was launched.

The overall goal of the project was to determine the extent to which great blue herons nesting in colonies associated with the ORR were contaminated by past and present releases from DOE-OR operations. The specific objectives were 1) determine contaminant concentrations in great blue heron eggs and chicks, 2) determine great blue heron reproductive success, and 3) measure a suite of biomarkers, including DNA damage and microsomal enzyme analysis, to assess population health. This portion of the Clinch River-Environmental Restoration Program ended in June 1995 due to a lack of funding.

The colonies situated near ORR were of concern because the birds using these colonies were in direct contact with the contaminated areas on ORR. These birds may have consumed fish with elevated levels of PCBs and mercury. Evaluation of contaminant loads, and their effects on this species, will allow the assessment of risk associated with the consumption of fish from the ORR.

Chapter 2

Literature Review

The Great Blue Heron

Great blue heron populations in Tennessee underwent drastic declines during the 1960s, apparently as a result of habitat destruction (Pullin 1990), or direct disturbance (Gersbacher 1964). However, many remnant populations persisted allowing population growth across the state (Pullin 1990). The great blue heron population of Tennessee increased in ten years from 180 individuals in four colonies in 1977 to 2124 individuals in 24 colonies in 1987 (Pullin 1990).

Nesting Ecology of the Great Blue Heron

Most North American herons do not attempt to breed until the breeding season of their second year (Pratt 1970). Pratt (1970) reported two nesting attempts by juvenile great blue herons, although both failed.

Great blue heron nests are exposed platforms built mainly of dry twigs (Pratt 1970). Nests that remain intact from year-to-year are repaired and re-used. Nests sometimes occur in close proximity, but territorial defense keeps

nests at least as far apart as opposing adults can extend their necks and bills (>2 m) (Pratt 1970).

Pratt (1970) reported that breeding herons first appeared in a colony in central California on or about the first of February. Based on data collected prior to this study, this is comparable to the onset of breeding in east Tennessee. The onset of nesting occurs much later at more northern latitudes (Henny 1972).

Great blue herons have one brood per year. Clutch size varies from 3 to 7, with typically 4 eggs (Henny 1972). If the eggs or young are lost, a new clutch may be attempted without an apparent break in occupancy (Miller 1944, Pratt 1970). Pratt and Winkler (1985) found an average clutch size of 3.16 ± 0.04 , (range = 1-5 eggs) in central California. Pratt and Winkler (1985) also demonstrated a weak correlation between clutch size and time of laying, with early clutches being larger. Great blue heron clutch size also varies geographically, usually increasing with latitude (Palmer 1962, Henny and Bethers 1971, McAloney 1973) (Table 1).

Nestling mortality of 40% (Miller 1944), 46.8% (Pratt and Winkler 1985), and 17-19% (Collazo 1981) have been reported in the literature. Miller (1944) further stated that he had never found a nest with more than 3 young more than 1/3 grown. The number of young fledged was shown to

increase with clutch size (Pratt and Winkler 1985). The second through fourth weeks of a chick's life were the most hazardous; starvation was the primary cause of mortality (Collazo 1981, Pratt and Winkler 1985).

Effects of Mercury on Wildlife

Although mercury poisoning causes obvious neurological dysfunction, lower levels of exposure may produce subtle, yet significant differences in behavior and reproduction (Spyker et al. 1972). Tejning (1967a) showed that as mercury concentrations in food increased, egg production and food intake decreased in chickens. Also, as mercury intake increased, so did the tendency to deposit eggs outside the nest. Mercury has also been shown to affect chromosome distribution during cell cleavage, as well as the hatchability of eggs (Tejning 1967a).

Some of the highest levels of mercury in wildlife have been found in piscivorous birds (Hoffman and Curnow 1979). Fimreite et al. (1971) demonstrated higher concentrations of mercury in piscivorous birds than in their prey. Many investigators have shown this pattern of trophic bioaccumulation, where mercury concentrations increased in successively higher trophic levels (Johnels and Westermarck 1969, Hanks et al. 1970, Fimreite et al. 1971, Huckabee et al. 1973, Hoffman and Curnow 1979).

Dustman et al. (1972) found mercury concentrations as high as 175 ppm in livers of great blue herons from Lake St. Clair, Canada. Mercury concentrations as high as 59.4 ppm were reported in juvenile great white herons (*Ardea herodias occidentalis*) in southern Florida (Spalding et al. 1994). Low levels of mercury (0.12-2.0 ppm) were found in great blue herons in the Tennessee Valley during 1980-1981 (Fleming et al. 1984).

As the amount of mercury in the body increases, different symptoms of mercury poisoning are exhibited. In pheasants (*Phasianus colchicus*) mercury levels of 1.3-4.4 ppm in the diet significantly reduced egg hatchability (Borg et al. 1969), whereas concentrations of 9.0 ppm reduced egg production (Tejning 1967a).

Tejning (1967a) also showed that mercury concentrations in eggs increased proportionately with intake of mercury coated seed for the first 30 days of the study. After 30 days, the mercury content in eggs decreased, possibly because of toxic effects on the hen. Concentration of mercury in tissues has also been shown to differ significantly between tissue types. Tejning (1967a) found that breast muscle contained greater concentrations than thigh muscle. Median mercury levels were shown to increase from brain tissue to breast muscle to liver and wing feathers in herons, egrets, and mallard ducks (*Anas*

platyrhynchus) (Hough and Zabick 1972, Hoffman and Curnow 1979). Berglund and Berlin (1969) felt that a blood-brain barrier hindered mercury accumulation in the brain.

Hair or feather analysis has been suggested as a reliable index to tissue stores of metals (Huckabee et al. 1973). The concentration of heavy metals (i.e. mercury) in hair or feathers has been correlated with environmental concentrations of these metals (Huckabee et al. 1973). Tejning (1967b) also found that mercury deposited strongly in feathers. Feathers accurately reflect the body burdens of mercury at the time the feather is grown (Johnels et al. 1968). With these facts, the mercury in the environment around a heron colony can be assessed through the analysis of feathers from chicks in that colony.

Mercury Release History for the ORR

Beginning in the early 1950's, the Oak Ridge Y-12 Plant used chemical mercury in the production of nuclear weapon components (Solomon 1985). The mercury from this process was eventually released into East Fork Poplar Creek (EFPC), adjacent to the plant. Total mercury released has been estimated around 150 metric tons (ERD 1990). The largest releases of mercury, 33 and 29 metric tons, respectively, occurred in 1957 and 1958 (ERD 1990) (Figure 4).

The East Fork of Poplar Creek carries its contaminant load from the eastern end of the Y-12 plant westward to the

ORGDP (formerly K-25), and eventually to the Clinch River. Monitoring by Rogers et al. (1987) showed a relatively low concentration ($\bar{x} \leq 0.002$ mg/l) of mercury in water samples from the East Fork and the Clinch River. However, Loar et al. (1981) reported that mercury concentrations in lower Poplar Creek were "very high" (ERD 1990).

Effects of PCBs on Wildlife

Polychlorinated biphenyls are persistent in the environment, lipid soluble, and undergo bioaccumulation (Davidson and Nettles 1988), similar to the properties of DDT. Although PCBs can be taken up through inhalation or absorption, ingestion appears to be the most important route of uptake in animals (Shaw and Connell 1986).

Polychlorinated biphenyls have been shown to affect reproduction in birds. Blus et al. (1980) suggested a negative correlation between PCB levels and eggshell thickness. However, many other investigators have shown that PCBs do not significantly decrease eggshell thickness (Koeman et al. 1973, Vermeer and Reynolds 1970, Ohlendorf et al. 1978, Fleming et al. 1984, Steidl et al. 1991).

Kubiak et al. (1989) noted several population effects as the result of high body burden of PCBs in Forster's terns (*Sterna forsteri*). Among these were: 1) a higher rate of nest abandonment, 2) decreased total weight of chicks, and 3) a lower hatching success. Hoffman et al. (1986) also

noted lower embryonic weights of chicks from PCB contaminated mothers.

It appears that there is variability in species' susceptibility to PCB toxicity (Hoffman et al. 1986). In general, PCBs have a low toxicity to birds (Peakall 1986), although they may produce measurable changes in the structure and function of the avian body. Polychlorinated biphenyls have been shown to produce increased weights as well as histological changes in the livers of contaminated individuals (Linder et al. 1974). These compounds have also been shown to effect the comb size and testes weight in cockerels (Platanow and Funnell 1971). Decreased semen volume, sperm concentration, and testes weight were also noted in white leghorn chickens (Ahmed et al. 1977). Bird et al. (1983) also noted a significant decrease in the sperm concentrations of American kestrels (*Falco sparva*) when exposed to PCBs.

Several investigators have noted teratogenic effects related to PCB exposure. Embryos exposed to PCBs at conception developed mutations including eye, leg, toe, and neck deformities, edema, hydrocephalus, incomplete abdominal closure, unabsorbed yolk, small body, and deformed beaks (Carlson and Duby 1973, Tumasonis et al. 1973, Cecil et al. 1974). Although PCBs have been shown to cause immunosuppression, tumor promotion, porphyria, estrogenic

activity, and hepatic induction (Bitman et al. 1972, Vos 1972), Peakall (1986) stated that PCBs are not sufficiently toxic in either acute or chronic feedings to pose a hazard to birds.

The toxicity of PCBs in mammals seems to be much greater. Increased mobility of PCBs within the organism occurs during pregnancy, lactation, stress, and starvation (Fuller and Hobson 1986). Prenatal exposure to PCBs, although not considered to be teratogenic, has been shown to cause birth defects, including cleft palate and lip, brachydactyly, and syndactyly (Fuller and Hobson 1986). Gellart and Wilson (1979) noted a significant increase in testis weight in rats exposed to PCBs prenatally.

Fertility in males has not been shown to be affected, unless the daily uptake is extremely high (500 ppm in the diet) (Fuller and Hobson 1986). In females, chronic, low levels of PCBs strongly affect reproduction. PCBs have been shown to affect the reproductive tract, neuroendocrine function, and general reproductive performance (Fuller and Hobson 1986).

The total amount of PCBs released into the environment of the ORR is unknown. There are so many possible sources for PCBs that it is impossible to determine when, where, or how much was released into the environment. Unlike mercury, PCBs were not a byproduct of production. Therefore, records

of release were not kept. Preliminary studies by Suter (1991), however, did report the presence of PCBs in Poplar Creek. Mean sediment levels were 0.94 mg/kg, while mean levels in fish flesh were 1.03 mg/kg. The maximum level noted in fish flesh during Suter's study was 3.4 mg/kg.

Chapter 3

Study Area

The Oak Ridge Reservation

The Oak Ridge Reservation is located in Roane and Anderson counties, Tennessee, 40 km west of Knoxville (Figure 5). The area is approximately 14,260 ha in size, and is bounded on the north and east by the city of Oak Ridge, and on the south and west by the Clinch River. The area serves as an environmental research park, along with other government operations.

The ORR lies within the Ridge and Valley physiographic province, just east of the Cumberland Plateau. Mean annual temperature is 15 °C, with a January mean of 3.5 °C and a July mean of 25 °C. Precipitation is seasonally distributed, with peaks occurring in late winter-early spring and in mid-to-late summer (McMaster 1967). Mean annual precipitation is approximately 136 cm. Precipitation gauges distributed throughout the ORR indicate a decrease in rainfall from northwest to southeast. Average precipitation ranges from 150 cm in the west to about 115 cm in the east.

The ORR and its three major facilities were acquired for the Manhattan Project in 1942. X-10 (ORNL) and Y-12 were initiated in February 1943, and K-25 (ORGDP) was

initiated in September, 1943. Production at the Y-12 plant began in 1944. At the height of the Manhattan Project the population of Oak Ridge was approximately 75,000.

The U.S. Atomic Energy Commission (AEC) assumed control of the Oak Ridge facilities in 1947. The AEC then became the Energy Research and Development Administration (ERDA) and the Nuclear Regulatory Commission (NRC) in 1975. In 1977, all ERDA programs became part of the U.S. Department of Energy (DOE). The emphasis at the Oak Ridge facilities is now to investigate both nuclear and non-nuclear energy technologies and the environmental consequences of such technologies.

Study Colonies

The four colonies used in this study were chosen based on their distance from the ORR. Two 'On-Site' colonies were chosen ≤ 3 km from the ORR, whereas two 'Off-Site' colonies were chosen ≥ 15 km from the ORR. The two on-site colonies were K-25 and Melton Hill, whereas the two off-site colonies were Long Island and Looney Island.

The K-25 colony was located at the K-25 plant, in Roane County. This colony was on a small peninsula in Poplar Creek, approximately 4.4 km upstream from the confluence with the Clinch River (Figure 6). This site is characterized by a stand of sycamores (*Platanus occidentalis*) adjacent to a large mud flat. The soil in

this colony is of the Pope very fine sandy loam type, an alluvium derived primarily from sandstone and shale parent material. This colony relocated downstream to an upland pine stand (*Pinus* spp.) in 1995, approximately 2.5 km from the confluence with the Clinch River.

The Melton Hill colony is located in Knox County, adjacent to the ORR, on an island in Melton Hill Reservoir (Figure 7). The colony island is located in a large embayment, between Clinch River Miles (CRM) 32-34. This colony is approximately 8 km north of the intersection of Interstate 40 and 75, west of Knoxville, Tennessee. This colony site consists of hackberries (*Celtis occidentalis*), with black cherry (*Prunus serotina*), black willow (*Salix nigra*), and yellow-poplar (*Liriodendron tulipifera*) intermixed. The soil of this island is of the Wolftever silty clay loam, eroding undulating phase type, which is derived from mixed general alluvium.

The Long Island Colony is located within the Long Island Wildlife Management Area on Watts Bar Reservoir in Roane County, Tennessee (Figure 8). This colony is located on Long Island, approximately 6.7 km south of the intersection of Interstate 40 and State Highway 58 in Kingston, Tennessee. Nests in this colony occur in many different species of trees including sycamore, boxelder (*Acer negundo*), and yellow-poplar. This colony is underlain

by Sequatchie very fine sandy loam, and alluvium derived from shale parent material.

The Looney Island colony is located in Knox County, on Fort Loudon Reservoir, approximately 4 km southwest of the University of Tennessee, Knoxville (Figure 9). This colony is composed of many different tree species, including boxelder, hackberry, and sycamore, with no one species used predominantly over another. The soil of this island is alluvium dredged from the river channel for navigation purposes. Unlike the other study colonies, this colony is located very close (<1 km) to metropolitan Knoxville, Tennessee, and is subject to human disturbance year-around.

Chapter 4

Materials and Methods

Reproduction

We monitored reproduction at the four colonies by visiting each site an average of four times per breeding season in 1993 and 1994. The first visit of the season occurred during the first week of April each year, coinciding with the onset of incubation. We then visited each colony every 2-3 weeks, until nestlings fledged around the first of June.

Reproduction at the various colonies was determined by counting eggs and chicks in nests during visits to collect for contaminant analysis. During visits to collect eggs, each accessible and/or visible nest was marked with a stake beneath it on the ground. The climber/observer would tell workers on the ground the contents of each nest, and these were recorded by nest number and date. If an egg or chick was collected from the nest, it was also noted on the stake with "-1". Subsequent visits to colonies for the collection of chicks were handled in the same manner.

In 1993 and 1994, siblings of collected chicks were leg-banded with USFWS leg bands and color coded ORNL leg bands. The K-25 colony was monitored during the 1993 and

1994 nesting seasons with a time-lapse video recorder. These video tapes allowed me to determine nesting chronology as well as the influence of extrinsic factors, such as disturbance. In all, mean clutch size, number of young hatched, nesting chronology, and number of young fledged was determined for one year during this study.

Egg Collection and Sample Preparation

Eggs were collected by climbing into the nest tree. Once the climber obtained an egg, it was wrapped carefully in a towel, inserted into a mesh bag, and lowered to the ground. Workers on the ground marked both ends of the egg with a unique alpha-numeric code. The alpha-numeric code denoted site, sample type, and sample number. For example, LE501 was the code for off-site (L) egg (E) number 501. An on-site (K) egg (E) numbered 001 would be KE001. All off-site samples were numbered ≥ 500 , while all on-site samples were numbered 001-499.

After labeling, the eggs were carefully packed in towels in a Styrofoam cooler to keep them warm. Upon return to the laboratory, the eggs were incubated at 37° C for 24 hours prior to measuring and tissue collection. After incubation, the eggs were cleaned and weighed (± 0.01 g). Circumference, diameter, and length measurements were taken (± 1 mm). Any damage to the shell was noted as was any evidence of pipping. If a chick was pipping, it was

euthanized, via asphyxiation with ether, before the egg was opened. The eggs were then opened along the equator using a triangular file and scalpel. The contents were emptied into an acid washed glass dish for inspection.

If an embryo was developing, it was carefully examined for any abnormalities (i.e. crossed bill, deformed limbs, or enlarged head), and the age was approximated. After examination, the contents were placed into a clean blender for homogenization. The liquid homogenate was then separated into two ≥ 20 g aliquots. The first aliquot was sent for metals analysis, the second for PCB analysis. Excess homogenate was archived in a third aliquot. All egg homogenates were placed into plastic sample bottles labeled with the alpha-numeric code for that egg, and stored in a locked super-cool (-20° C) freezer until shipment to an independent laboratory for analysis.

The eggshells were placed on blotting paper, and allowed to dry for at least 72 hours at room temperature. The shells were then weighed, and thickness was measured at three places along the equator of each half, to determine thickness. The measurements were taken with a digital micrometer (± 0.001 mm) at ORNL Engineering. After measurement, eggshells were archived at room temperature.

All dissection equipment was washed thoroughly with Alconox and distilled water after the dissection of each

egg. One final rinse water sample was collected in a plastic sample bottle for PCB and metals analysis, as a quality control.

Chick Collection and Sample Preparation

Chicks were collected from nests in the same fashion eggs were. The chicks were lowered in a backpack, and a metal tag marked with a unique alpha-numeric code was attached to their leg. The alpha-numeric code was similar to the one used on eggs, but the sample type was "C" for chick. For example, on-site chick number 001 was KC001. After labeling, chicks were placed in cardboard boxes and returned to the laboratory alive. When necessary, the chicks were cooled with water.

Upon arrival at the laboratory, blood samples were taken from the brachial vein in the wing. Samples taken included 3 hematocrit tubes for blood volume (stored on ice), 1 blood smear (air dried), and 1 sample for flow cytometry (~0.5 ml) in a heparin-washed tube. After blood was drawn, the chicks were euthanized via CO₂ asphyxiation.

After euthanization, the chicks were weighed (± 1 g) and measured (± 1 mm). The measurements included bill length, wing length, total length, tarsometatarsus length, and tibiotarsus length. The chicks were then necropsied. The chicks were placed on their back, and the abdomen was

opened by cutting up the ventral body wall, beginning above the cloaca.

The bird was sexed by locating the gonads on the dorsal body wall of the abdomen. A small section of kidney was then removed and preserved in Formalin for histological analysis. The liver was then removed, sectioned, and frozen as quickly as possible to prevent decay. The liver was sectioned to include one ≥ 20 g sample for mercury analysis, one ≥ 20 g sample for PCB analysis, one 1-3 g sample for histological analysis, one 1-3 g sample for DNA strand break analysis, and one 1-3 g sample for microsomal enzyme analysis (EROD/MPRO). The gall bladder was archived along with a small piece of liver tissue.

The spleen was then removed, and one-half was preserved for histological analysis while the other half was archived. A small section of lung (≥ 1 g) was removed and preserved for histological analysis.

The heart was removed, weighed (± 0.1 g), rated for heart fat, and archived. The digestive system (esophagus, proventriculus, gizzard, intestines, and pancreas) was removed, opened, and examined for content. Any identifiable material was noted, and fish were preserved for analysis. The incision was then continued up the neck. The thymus glands were removed and archived in a supercool freezer.

Muscle was removed from the breast and/or legs of the bird, and separated into two >20 g samples for PCB and mercury analysis. One >2 g sample of abdominal fat was also taken for PCB analysis. Feathers were collected from the bird's back and nape for mercury analysis (2-3 g dry wt.). Feather samples were, if necessary, dried for 24 hours at 37° C and stored at room temperature.

With the exception of feathers, liver for DNA strand break analysis, liver for microsomal enzyme analysis, and histological samples, all samples were stored in a locked super-cool freezer. Liver samples for DNA strand break analysis and microsomal enzyme analysis were immediately frozen in liquid nitrogen, and stored in an ultra-cool (-120° C) freezer.

After necropsy, all tools were washed thoroughly with Alconox and distilled water. One final rinse water sample was collected in a plastic sample bottle for PCB and mercury analysis, as a quality control. Hematocrit tubes were then spun for 15 minutes in a microhematocrit centrifuge, and the percentage of formed elements was recorded. The mean percentage of formed elements was recorded for each chick. The blood samples in heparin were placed in a refrigerator (4° C) for storage.

Fish Collection and Sample Preparation

Fish were collected in a variety of ways. While in the colonies, fish were collected from the ground beneath nests, in the nests, or from captured chicks. All of these fish were marked to tie them as closely as possible to a specific colony, nest, or chick. These fish were frozen whole and stored in a super-cool freezer until analysis.

Fish collected for a related Michigan State University study on mink (*Mustela vison*) were also analyzed for this study. Some of the fish were analyzed as whole fish samples for PCBs and mercury, while others were ground into homogenous mixtures. The mixtures were also tested for PCBs and mercury. The fish used in the mink study were collected by gill-netting and electro-fishing in Poplar Creek and Melton Hill Reservoir.

Sample Analysis

All PCB and metals samples were sent to an independent contractor (Southwest Laboratories, San Antonio, Texas) for analysis. DNA strand break analysis was done by Dr. Richard Halbrook and myself. Microsomal enzyme analysis was done by Dr. Richard Halbrook, myself, and Rhonda Epler of ORNL. Histological samples were prepared by the Biology Division at Y-12, and, along with blood smears, were sent to an independent pathologist for interpretation.

DNA Strand Break Analysis

DNA Strand Break Analysis was done to determine if genetic trauma was happening as a result of PCB or mercury contamination (Shugart 1988). Twelve liver samples were removed from the ultra-cool freezer per run. These samples were kept on ice throughout the entire process. Each liver sample was weighed (± 0.01 g) and placed in a Dounce homogenizer. One milliliter of 1N NH_4OH /0.2% Triton X-100 was added per 200-400 mg of tissue. The tissue was then homogenized, with care being taken to homogenize only enough to disrupt the tissue. Samples were then poured into labeled centrifuge tubes.

Distilled water was added at a rate of 1 ml per 200 mg of tissue to the homogenizer. This solution was plunged 2-3 times to suspend any tissue clinging to the homogenizer tube. The distilled water from the homogenizer tube was then added to the centrifuge tubes.

CIP (25 parts liquified phenol, 24 parts chloroform, and 1 part isoamyl alcohol) was added to the centrifuge tubes at a ratio of 3 ml/3 ml of sample or 6 ml/>3 ml of sample. The centrifuge tubes were then capped and slowly inverted 10 times to fully denature all proteins. Care was taken to invert all samples the same number of times.

Capped samples were stored under the fume hood, on ice, for 30 minutes. After storage, the samples were centrifuged

at 8000 RPM for 30 minutes, at a temperature of 10-15 °C. Upon removal, care was taken not to mix the three resultant layers. The top aqueous layer, containing the DNA, was carefully removed and placed into clean, labeled, culture tubes. The culture tubes were then covered with parafilm and stored at 4° C until further processing.

Sephadex G-50 columns were prepared for DNA isolation. Approximately 50 g of Sephadex was added to 1500 ml of distilled water in a 3 L flask. This mixture was autoclaved for 20 minutes to swell the Sephadex, and allowed to cool to room temperature. The Sephadex was degassed for 3-5 minutes prior to column preparation. When not in use, Sephadex was stored at 4° C.

Columns 13 mm in diameter and 30 mm long were used. To each column was added one wet plastic frit and a slurry of G-50 in distilled water. This was allowed to settle to a packed bed volume of 3.50-3.75 ml. The outlets of the columns were plugged and another plastic frit was placed on top and pushed down to the 3.5 ml level. The outlet was then unplugged and rinsed with two 5 ml washes of distilled water and 2-3 washes of G-50 buffer.

After column preparation, labeled 1.5 ml eppendorf tubes were placed below the columns. A sample quantity of 1.25 ml was then allowed to pass into the column. To this, 1.25 mls of G-50 buffer was added and allowed to pass

completely through the unplugged column. The collected DNA was stored in the capped 1.5 ml eppendorf tubes in the refrigerator until analysis.

Total DNA was determined by adding 25 μ l of DNA solution to disposable culture tubes containing 100 μ l of 0.1 M NaOH. Each sample was run in duplicate. Duplicate calf thymus samples and blanks were also run as controls. Culture tubes were covered with parafilm (wax side down), paper towels, and weights, and placed in hot plate holders. They were then incubated at 80-85° C for 60 minutes.

After incubation, the culture tubes were removed and spun for 10-15 seconds. Three milliliters of PO_4 buffer with dye (3 μ l PO_4 buffer and 1 μ l dye) was then added to each culture tube, and the tubes were placed in the dark for 15 minutes. After 15 minutes, I removed each culture tube and poured the contents into a 1 cm X 1 cm cuvette. I read the fluorescence in a Fluorescence Spectrophotometer set with; Excitation slit =10, Emission'slit =10, Excitation wavelength =360, and Emission wavelength =450. The cuvette was washed with distilled water between each sample.

The dilutions required for single strand DNA determination, double strand DNA determination, and the alkaline unwinding assay were then determined based on total DNA (Table 2).

The amount of double stranded DNA was determined by adding 100 μ l of DNA sample, diluted as recommended, to culture tubes containing 50 μ l 0.05N NaOH, 50 μ l 0.05N HCl, 5 μ l 1/10 dilution SDS/EDTA, and 3 ml 0.2M PO_4 plus dye. Again, these culture tubes were vortexed and placed in the dark for 15 minutes. After 15 minutes, the fluorescence was read as described for total DNA determination.

Single strand DNA was determined by adding 100 μ l of sample, with the appropriate dilution, and 50 μ l 0.05N NaOH to labeled culture tubes, and vortexing. After vortexing, these tubes were incubated for 90 minutes at 80-85° C. After 90 minutes, I spun the culture tubes for 10-15 seconds and then added 3 ml of PO_4 buffer and dye to each sample. These were placed in the dark for 15 minutes, then the fluorescence was read.

The alkaline unwinding assay was done by adding 100 μ l of sample, with the appropriate dilution, and 50 μ l 0.05N NaOH to labeled culture tubes. These tubes were then incubated for 30 minutes at 38° C. After incubation, 50 μ l 0.05N HCl and 5 μ l 1/10 dilution of SDS/EDTA was added to each culture tube. The content of each tube was then passed through a syringe 5 times. I then added 3 ml of 0.2M PO_4 buffer with dye to each tube and vortexed. Culture tubes were then placed into the dark for 15 minutes, after which time, the fluorescence was read.

Microsomal Enzyme Analysis Sample Preparation

The level of liver enzymes can often indicate adverse effects of toxins in the body. Microsomal Enzyme analysis is a measure of these enzymes. I weighed frozen liver samples and placed them into labeled homogenization tubes. To these tubes I added the Working Homogenization Buffer (WHB) at a ratio of 1:6 (weight:volume). With the homogenization tube placed in ice, I homogenized each sample with six plunges of the pestel. Homogenates were then transferred to labeled centrifuge tubes, and pairs were balanced with WHB. I then centrifuged these samples at 5000 RPM for 10 minutes in a cold centrifuge. At completion of the 10 minute run, the centrifuge was allowed to stop, and the samples were then spun for 20 minutes at 12,000 RPM.

After the second centrifugation, the supernatants were transferred into clean, labeled, centrifuge tubes, and the pellets were discarded. The supernatants were then centrifuged for 1 hour at 40,000 RPM in the cold centrifuge. After the run, I carefully removed the supernatants (Cytosol fraction) and placed them into color coded (orange), labeled, Nunc cryotubes. These were placed immediately into liquid nitrogen for storage.

A 1:1 (weight:volume) ratio of working resuspension buffer was then added to each pellet. The pellets were then dislodged and resuspended using a clean glass rod. The

tubes were then placed in a beaker of ice, and sonicated for a maximum of 15 seconds to resuspend any residual solids. This solution (Microsome fraction) was then split equally between two color-coded (blue), labeled Nunc cryotubes, and stored with the Cytosol fractions. All samples were then sent to Rhonda Epler of ORNL for analysis.

Chapter 5

Results

Reproductive Data Analysis

Analysis by Site

Clutch sizes were determined in 1993 and 1994 for both sites. On-site colonies did not differ from off-site colonies ($P \geq 0.10$). Mean on-site clutch size was 3.65 ($n = 40$, $s.d. = 0.6222$) while off-site clutch size was 3.31 ($n = 42$, $s.d. = 0.8692$).

Analysis by Colony

No differences in clutch size occurred among colonies ($P \geq 0.10$). The Melton Hill colony had the highest mean with 3.71 ($n = 17$, $s.d. = 0.4697$), while K-25 had the second highest with 3.61 ($n = 23$, $s.d. = 0.7223$). Of the off-site colonies, Looney Island had the highest mean clutch size with a mean of 3.60 ($n = 10$, $s.d. = 0.6992$), while Long Island had the lowest with a mean of 3.19 ($n = 32$, $s.d. = 0.8958$). Total reproductive output of the entire colony was greater in off-site colonies simply because these colonies were much larger than the on-site colonies.

Chick mortality was not monitored in 1993. In 1994, however, Looney Island had the highest mean mortality ($\bar{x} = 0.67$ chicks/nest) when compared to K-25 ($\bar{x} = 0.56$), Long

Island, ($\bar{x} = 0.44$), and Melton Hill ($\bar{x} = 0.44$). These values indicated higher reproduction in this study than in other studies (Table 1).

Egg Biomarker Analysis

Based on a two-way analysis of variance (ANOVA), circumference was the only biomarker that appeared to differ between colonies ($P = 0.0648$, $F = 2.56$, $df = 56$). A colony-by-colony comparison revealed that the mean circumference of eggs from the K-25 colony ($\bar{x} = 146.80$ mm) was larger than the mean circumference of eggs from the Long Island colony ($\bar{x} = 143.30$ mm) ($P = 0.05$). Total weight, length, width, shell weight, and shell thickness did not differ between colonies ($P > 0.05$) (Table 3).

Egg Contaminant Analysis

Based on analysis of variance, mercury differed between colonies ($P = 0.0874$, $F = 2.29$, $df = 65$). Mercury concentrations in eggs from the Melton Hill colony ($\bar{x} = 0.07$ ppm) were lower than mercury concentrations in eggs from the K-25 colony ($\bar{x} = 0.17$ ppm) ($P \leq 0.05$). No other colony-by-colony comparison differed ($P \geq 0.05$) (Table 3).

PCB concentrations differed between colonies ($P = 0.027$, $F = 3.27$, $df = 65$). The K-25 colony had higher PCB concentrations than the Long Island and Looney Island colonies ($\bar{x} = 4.64$, 0.89 , and 0.44 ppm, respectively) ($P = 0.05$). An apparent difference between the K-25 ($\bar{x} = 4.64$

ppm, $n = 24$) and Melton Hill ($\bar{x} = 0.93$ ppm, $n = 7$) colonies was not significant ($P > 0.10$), possibly because of the small sample size for Melton Hill.

Chick Biomarker Analysis

Of the biomarkers analyzed for chicks, only one differed between colonies. The liver enzyme, MPRO, levels differed between colonies ($P = 0.0630$; $F = 2.79$; $df = 26$). Colony-by-colony comparisons revealed that the K-25 colony ($\bar{x} = 18.54$) differed from the Melton Hill colony ($\bar{x} = 25.06$), as well as the Long Island colony ($\bar{x} = 24.44$) ($P = 0.05$). The other biomarkers, EROD, hematocrit, and DNA strand break showed no differences between colonies (Table 4).

Chick Contaminant Analysis

Nearly all sample types showed differences for PCB concentration among colonies. Total PCB levels differed between colonies for both muscle ($P = 0.0373$; $F = 3.01$; $df = 61$) and fat samples ($P = 0.0396$; $F = 2.94$; $df = 69$). Colony-by-colony comparisons revealed that the K-25 colony ($\bar{x} = 1.10$ ppm) had higher levels of total PCB in muscle than the Melton Hill ($\bar{x} = 0.18$ ppm), Long Island ($\bar{x} = 0.41$ ppm), and Looney Island ($\bar{x} = 0.23$) colonies ($P \leq 0.05$). Colony-by-colony comparisons of fat samples revealed higher concentrations of total PCBs in samples from K-25 ($\bar{x} = 53.28$

ppm) when compared to Looney Island ($\bar{x} = 15.37$ ppm) and Long Island ($\bar{x} = 14.90$ ppm) ($P \leq 0.05$).

All sample types showed differences between colonies for PCB congener 1260: Fat ($P = 0.0099$; $F = 4.28$; $df = 46$), muscle ($P = 0.0069$; $F = 4.58$; $df = 49$), and liver ($P = 0.0275$; $F = 3.32$; $df = 51$). K-25 fat samples ($\bar{x} = 48.63$ ppm) were higher in PCB 1260 than fat samples from Melton Hill ($\bar{x} = 10.78$ ppm), Long Island ($\bar{x} = 16.10$ ppm), and Looney Island ($\bar{x} = 19.04$ ppm). K-25 muscle samples ($\bar{x} = 1.05$ ppm) again had higher concentrations of PCB 1260 than Melton Hill ($\bar{x} = 0.16$ ppm), Looney Island ($\bar{x} = 0.23$ ppm), and Long Island ($\bar{x} = 0.41$ ppm) muscle samples. K-25 colony liver samples ($\bar{x} = 1.09$ ppm) had higher PCB 1260 concentrations than Melton Hill ($\bar{x} = 0.25$ ppm), Looney Island ($\bar{x} = 0.20$ ppm), or Long Island ($\bar{x} = 0.24$ ppm) liver samples (Table 4).

All sample types showed differences between colonies for mercury concentrations; liver ($P = 0.0002$; $F = 7.38$; $df = 72$), muscle ($P = 0.0996$; $F = 2.17$; $df = 70$), and feather ($P = 0.0002$; $F = 7.47$; $df = 72$). Looney Island liver samples ($\bar{x} = 0.25$ ppm) had higher concentrations of mercury than samples from Long Island ($\bar{x} = 0.12$ ppm). The K-25 colony had higher mercury concentrations in liver samples ($\bar{x} = 0.29$ ppm) when compared to the Melton Hill ($\bar{x} = 0.15$ ppm) and

Long Island colonies ($\bar{x} = 0.12$ ppm). Looney Island and K-25 liver mercury samples did not differ.

Looney Island and K-25 muscle samples did not differ in mercury content ($\bar{x} = 0.13$ and 0.09 , respectively). The Looney Island samples, however, were higher than both Long Island ($\bar{x} = 0.07$ ppm) and Melton Hill ($\bar{x} = 0.05$ ppm). Muscle was the only sample type that did not show the highest concentration in the K-25 colony. K-25 feather samples had higher mercury concentrations ($\bar{x} = 2.02$ ppm), than feathers from Melton Hill ($\bar{x} = 1.02$ ppm), Long Island ($\bar{x} = 0.87$ ppm), and Looney Island ($\bar{x} = 0.97$ ppm) (Table 4).

Fish Contaminant Analysis

Fish collected from beneath nests in colonies, or from stomachs of chicks collected in this study were analyzed for PCBs (Arochlor 1260) and mercury. A by-colony analysis was not done because of small sample sizes. PCBs did not differ between sites ($P = 0.269$). Mean concentrations from on-site fish were 0.20 ± 0.04 ppm ($n = 13$), while mean concentrations from off-site fish were 0.15 ± 0.03 ppm ($n = 15$). Limited sample sizes may have reduced our ability to detect differences in PCBs in fish.

Mercury levels, however, differed between sites in spite of small sample sizes ($P = 0.03$). On-site fish had mean levels of 0.06 ± 0.02 ppm ($n = 13$), while off-site fish had mean levels of 0.02 ± 0.00 ppm ($n = 15$).

Chapter 6

Discussion

Reproduction Results

The clutch sizes observed during this study are comparable to those reported by other investigators across North America (Table 1). This comparison suggests that the contaminant burdens these birds are carrying are not affecting reproduction, at least not as far as clutch size is concerned. However, the number of chicks fledged per nesting pair was not determined. Kubiak et al. (1989) suggested that PCBs in general can cause aberrant nesting behavior in contaminated adults, resulting in lowered reproductive output from normal sized clutches.

Throughout the study, only one egg was found that definitely would not have hatched. This egg had an extreme concentration of mercury (0.601 ppm), and came from the Melton Hill colony. One other egg had an extremely large concentration of mercury (0.596 ppm), but it showed early signs of development. This egg came from the Long Island colony.

Unhatched pheasant and partridge (*Perdix perdix*) eggs collected from abandoned nests had levels of 3 ppm mercury, while unhatched white-tailed eagle (*Haliaeetus albicilla*)

eggs have been found with mercury levels of 3.5 to 11.0 ppm mercury (Borg et al. 1969).

Biomarker Results

Eggs

There were no biologically significant differences in egg biomarkers between colonies. It appears that the only biomarker that differed was circumference. This could lead one to believe that the embryos in eggs from K-25 may be larger. If so, it remains unclear whether this was a result of the contaminant burdens.

Chicks

The only biomarker to show a difference between colonies was the liver enzyme MPRO. Liver samples from the K-25 colony showed the lowest liver enzyme activity ($x = 18.54$). This was unexpected, given the PCB levels found in chicks from this colony. PCBs generally induce liver enzyme activity. A surprise was the fact that the EROD levels did not differ between colonies ($P = 0.8203$). One would expect both liver enzymes to be effected, but they were not.

The lack of difference in DNA strand breakage between colonies indicated that there were no apparent genetic effects associated with the levels of contaminants found in this study. No data were available for comparison to other populations of this species, so the relative importance of these values is unknown. This is one area where further

investigation is warranted, to determine the genetic viability of this population.

Contaminant Results

Eggs

The mean PCB concentrations from K-25 eggs ($\bar{x} = 4.64$ ppm) were over five-times higher than those of eggs from Long Island ($\bar{x} = 0.89$ ppm) and Looney Island ($\bar{x} = 0.44$ ppm). Hoffman et al. (1986) reported a negative correlation between embryonic weight and log-transformed PCB residues in eggs of black-crowned night herons in California, with a geometric mean of 4.1 ppm in eggs collected for their study. The geometric mean PCB value for K-25 eggs in this study, however, was only 1.52 ppm, significantly lower than the values found by Hoffman et al. (1986). Geometric mean PCB values from the other colonies were an order of magnitude lower than Hoffman et al. (1986). Ohlendorf et al. (1978) reported geometric mean PCB levels of 13.65, 2.95, 2.42, and 1.70 ppm for great blue herons in the Great Lakes, Inland, Southern Atlantic, and Gulf Coast regions, respectively.

PCBs are both carcinogenic and teratogenic (Patnaik 1992). Specifically, animals exposed to PCBs develop tumors in livers, gastro-intestinal tracts, and lymphatic tissues (Patnaik 1992). Aroclor 1260 specifically is listed as a moderately toxic mutagen, with an LD₅₀ of 1315 mg/kg in rats (Patnaik 1992). PCBs have also been shown to cause aberrant

parental behavior, resulting in lowered reproductive success (Kubiak et al. 1989). Eggs with ≥ 10 -15 ppm PCBs in the yolk produced leg, toe, and neck deformities in chicks from those eggs (Tumasonis et al. 1973). Increasing levels of PCBs in eggs effected the embryos of chickens and resulted in decreased hatchability of those eggs (Carlson and DUBY 1973).

While many investigators have shown PCBs to contribute to mutation and lowered reproduction, all of them were dealing with much higher contaminant loads than those seen in this study. Mora (1996) found mean PCB levels of 0.396 ppm (range = 0.058-1.99 ppm) in great blue herons in Texas, but stated that these levels did not have detrimental effects on the birds. It appears, that while PCB levels are significantly higher at K-25 than at the other colonies monitored in this study, the PCB loads are not having apparent detrimental effects upon the developing embryos. No deformed embryos were found during this study.

The mercury concentrations found during this study were well below those expected, given the amount of mercury released into the environment of the ORR over the years. The levels found during this study fall into the lower end of the range of levels previously reported for the Tennessee Valley by Fleming et al. (1984) (0.12-2.0 ppm). The K-25 mean of 0.17 ppm was higher than the mean from Melton Hill

eggs ($x = 0.007$ ppm), but not to the extent expected. This suggests that either herons are not feeding in the mercury contaminated environments prior to egg formation, or some mechanism is preventing the uptake of mercury during the egg synthesis and laying stages or the mercury is not being mobilized into the food chain.

The means found during this study are comparable to those reported by Mora (1996) from great blue herons in Texas ($x = 0.09$ ppm range = $0.02-0.15$ ppm). However, they are below the levels reported to affect reproduction. Heinz (1979) found that levels of 0.8 ppm reduced the reproductive success of mallards (*Anas platyrhynchos*), and Borg et al. (1969) indicated that levels of $1.3-2.0$ ppm in eggs inhibited hatching.

Chicks

With mean PCB concentrations significantly higher in all tissues from K-25 chicks, there is little doubt that K-25 chicks are more exposed to the contaminant than chicks from other colonies. The K-25 colony is closest to the source of contamination, and it appears that herons from other colonies are not using the contaminated on-site environment, then traveling off-site.

The highest total PCB concentrations were found in abdominal fat samples in all colonies, with the maximum concentrations coming from the K-25 colony in 1993 (400 ppm)

and the Melton Hill colony in 1992 (170 ppm). Total PCBs in muscle samples were also the highest in the K-25 colony ($\bar{x}$ = 1.10 ppm). The highest single value was 4.00 ppm, from the K-25 colony in 1994. The means for Long Island, Looney Island, and Melton Hill ($\bar{x}$ = 0.41, 0.23, and 0.18 ppm, respectively) were all significantly smaller than for the K-25 colony ($P < 0.05$). Abdominal fat samples from Looney Island in 1994 had PCB concentrations that ranged from 16.0 to 48.0 ppm, with a mean of 30.5 ppm. The same samples from 1992 and 1993 had means of 6.28 and 7.58 respectively. This could be due to the disturbance and/or redistribution of PCBs known to be present in Fort Loudon Reservoir. I found no other studies dealing with chicks that could be used for comparison.

Feather samples proved to contain the highest concentrations of mercury. Overall, as expected, mercury was in significantly higher concentrations in on-site feather samples. The highest mean mercury concentration was found in 1994 feather samples from the K-25 colony. The observed concentrations during that year were higher than those previously reported by Fleming et al. (1984). However, the mean of 2.40 ppm (range = 0.79 to 4.66 ppm) fell well below those associated with illness and reproductive inhibition. While these levels are of concern,

there is no evidence that the population is being dramatically influenced around the ORR.

The mean mercury content of feathers from female Goshawks (*Accipiter gentilis*) collected in Sweden between 1966 and 1972 was 3.36 ppm (Odsjo 1975). Tejning (1967a) reported feather mercury concentrations of 3.0, 2.9-56.0, and 12.8-41.0 ppm in partridges, peregrine falcons (*Falco peregrinus*), and eagle owls (*Bubo bubo*) respectively.

Fish

I expected to find significantly higher concentrations of both PCBs and mercury in on-site fish, given that on-site chicks had significantly higher concentrations of both contaminants. Also, on-site eggs had significantly higher PCB concentrations. However, analysis revealed that the only significant difference between sites was mercury concentration. As expected, on-site fish had significantly higher concentrations of mercury. The small sample sizes, although taken from chicks and from beneath nests, may not reflect the true contamination burden these birds are exposed to. While no identifiable organisms other than fish were found, it is well known that great blue herons feed on many other things, including frogs and toads, tadpoles, newts, reptiles, small mammals, birds, insects, crabs, crawfish, mollusks, fish, and carrion (Bent 1926, Roberts 1936, Martin et al. 1951, Krebs 1974, Kushlan 1978). Perhaps

the contamination arose from the consumption of softer bodied benthic prey, which was digested quickly, leaving no trace in the digestive tract.

Chapter 7

Conclusions and Recommendations

While obvious differences exist in contamination between colonies, they do not appear to be affecting reproduction in great blue herons. However, it is unknown if these contaminants are affecting survival of chicks. Future studies should concentrate on what happens once the chicks leave the nest. Observations during this study showed that chicks tend to feed very close to the natal colony immediately after fledging. Detrimental contaminant loads do not appear to be obtained before the young fledge and begin foraging for themselves, but they may develop later.

While this study has shown significant contamination of on-site eggs and chicks, it appears that this contamination is not having population-level effects. In general, heron populations are increasing in the area. Therefore, whatever effects these contaminants may be having, they are not enough to negate the intrinsic population growth. The contaminant loads found in this study are all below those reported from areas where bird populations are suffering from these contaminants.

reported from areas where bird populations are suffering from these contaminants.

While we examined PCBs and mercury, we did not examine many other contaminants known to be present on the ORR, such as arsenic, chromium, and radionuclides. While not probable, it is possible that these contaminants are causing population damage we did not uncover. There were many questions left unanswered when this project was terminated.

Hopefully, future researchers can answer these questions so we can better manage and restore aquatic systems like those of the ORR.

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Appendix

**TABLE 1: Clutch size and reproductive success reported
for Great Blue Heron colonies**

LOCATION	LAT °N	YEARS	CLUTCH SIZE	SUC. EGGS	FLEDGED/ NEST	FLEDGED/ SUC. NEST	SOURCE
SOUTHERN ALBERTA	49-55	1967- 1968	5.00	---	----	2.2-2.5	VERMEER (1969)
BRITISH COLUMBIA	49	1977- 1979	-----	---	----	2.3-2.9	KELSALL AND SIMPSON (1980)
IDAHO	47	1977- 1978	-----	---	----	2.20	COLLAZO (1981)
IDAHO	47	1977- 1978	-----	---	0.5-2.5	2.6-3.4	WARREN (1979)
COLUMBIA RIVER	46	1978	4.45- 4.49	41-50%	1.88-2.29	2.29-2.79	BLUS ET AL. (1980)
WESTERN OREGON	42-46	1974	-----	---	-----	2.18-2.70	WERSCHK UL ET AL. (1977)
NOVA SCOTIA	45	1971	4.17	---	-----	3.09	McALONE Y (1973)
CENTRAL OREGON	45	1975	-----	---	2.17-2.71	-----	ENGLISH (1978)
WESTERN OREGON	44	1970	4.19	---	-----	2.61	HENNY AND BETHERS (1971)
LAKE ERIE	42	1974- 1975	3.39	---	2.27	2.46	EDFORD (1976)
CENTRAL CALIF- ORNIA	38	1971	3.36	59%	2.14	2.50	GILL (1973)
CENTRAL CALIF- ORNIA	38	1967- 1973	3.0-3.7	---	-----	1.8-2.3	PRATT (1974)
CENTRAL CALIF- ORNIA	38	1967- 1979	3.16	47%	1.45	2.20	PRATT AND WINKLER (1985)
TEXAS	28	1979	3.58	45%	-----	-----	MITCHELL (1981)
NORTHERN IDAHO	47	1977	----	---	-----	2.14	COLLAZO (1981)
FLORIDA BAY	28	1981	2.88-3.5	---	1.55-2.04	-----	POWELL (1983)

TABLE 2: Dilution regime for DNA Strand Break Samples.

Total DNA	Dilution	Receipt
<75	No Dilution	
75-100	1/2	50 ul sample + 50 ul G-50 Buffer
101-150	1/4	25 ul sample + 75 ul G-50 Buffer
>150	1/10	10 ul sample + 90 ul G-50 Buffer
Blank		100 ul G-50 Buffer

Table 3: Great Blue Heron Egg Characteristics and Contaminant Loads, 1993-1994, Knox and Roane Counties, Tennessee.*

	Colony					P			
Variable	K-25		Melton Hill	Long Island	Looney Island				
	n	μ	n	μ	n	μ			
Total Weight (g)	24	69.14	7	68.06	25	66.71	10	65.42	NS
Length (mm)	24	63.92	7	63.57	25	62.44	10	64.20	NS
Width (mm)	24	45.21	7	45.43	25	44.40	10	45.60	NS
Circumference (mm)	24	146.80 ¹	7	144.14	25	143.30 ¹	10	145.30	0.0648 ¹
Shell Weight (g)	24	6.07	7	5.98	25	5.76	10	6.05	NS
Shell Thick. (μm)	24	0.42	7	0.43	25	0.42	10	0.43	NS
Mercury (ppm)	24	0.17 ¹	7	0.07 ¹	25	0.13	10	0.12	0.0874 ¹
Total PCB (ppm)	24	4.64 ^{1,2}	7	0.93	25	0.89 ¹	10	0.44 ²	0.0270

*Entries with like superscripts differed at the $p = 0.05$ level.

Table 4: Great Blue Heron Chick Biomarker and Contaminant Load Results, 1993-1994, Knox and Roane Counties, Tennessee.*

	Colony								
Variable	K-25		Melton Hill		Long Island		Loon Island		P
	n	μ	n	μ	n	μ	N	μ	
EROD	12	20.01	26	17.14	23	20.68	12	20.38	NS
MPRO	10	18.54 ^{1,2}	4	25.06 ¹	9	24.44 ²	4	21.84	0.0630
Hematocrit (%RBC)	12	31.28	26	32.34	23	30.31	11	32.38	NS
Liver PCB (Total)	12	0.431	26	0.836	16	0.274	10	0.204	NS
Liver PCB1260	18	1.09 ^{1,2,3}	26	0.25 ¹	16	0.24 ²	8	0.20 ³	0.0275
Muscle PCB (Total)	12	0.18 ^{1,2,3}	26	1.1 ¹	16	0.41 ²	8	0.23 ³	0.0373
Muscle PCB1260	18	1.05 ^{1,2,3}	8	0.16 ¹	16	0.41 ²	8	0.23 ³	0.0069
Fat PCB (Total)	12	30.52 ^{1,2}	24	57.72	22	14.90 ¹	12	15.37 ²	0.0396
Fat PCB1260	16	48.63 ^{1,2,3}	8	10.78 ¹	15	16.10 ²	8	19.04 ³	0.0099
Liver Mercury	12	0.15 ^{1,2}	26	0.29 ¹	23	0.12 ^{2,3}	12	0.25 ³	0.0002
Muscle Mercury	24	0.09	12	0.05 ¹	23	0.07 ²	12	0.13 ^{1,2}	0.0996
Feather Mercury	26	2.02 ^{1,2,3}	12	1.02 ¹	23	0.87 ²	12	0.97 ³	0.0002
DNA Strand Break (f-value)	23	66.43	9	66.96	23	74.19	11	83.24	NS

* Entries with like superscripts differed at the P = 0.05 level.

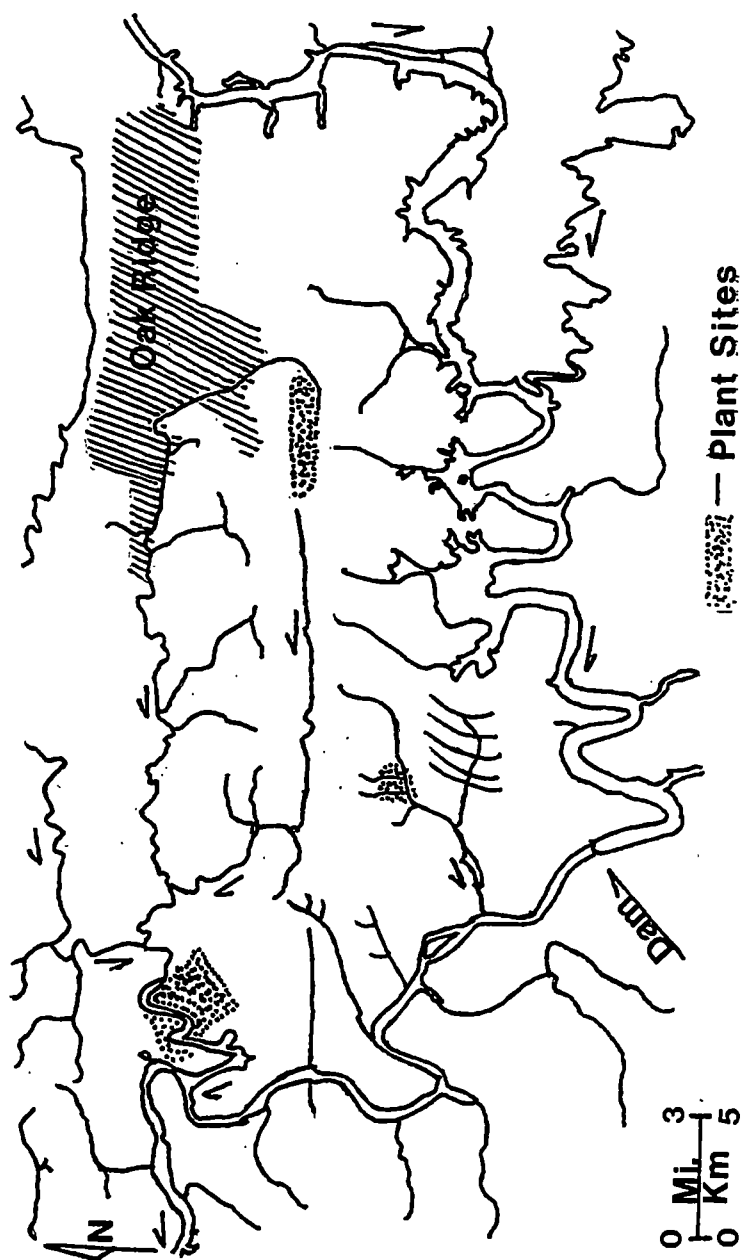


Figure 1: Location of the three plant sites within the ORR.

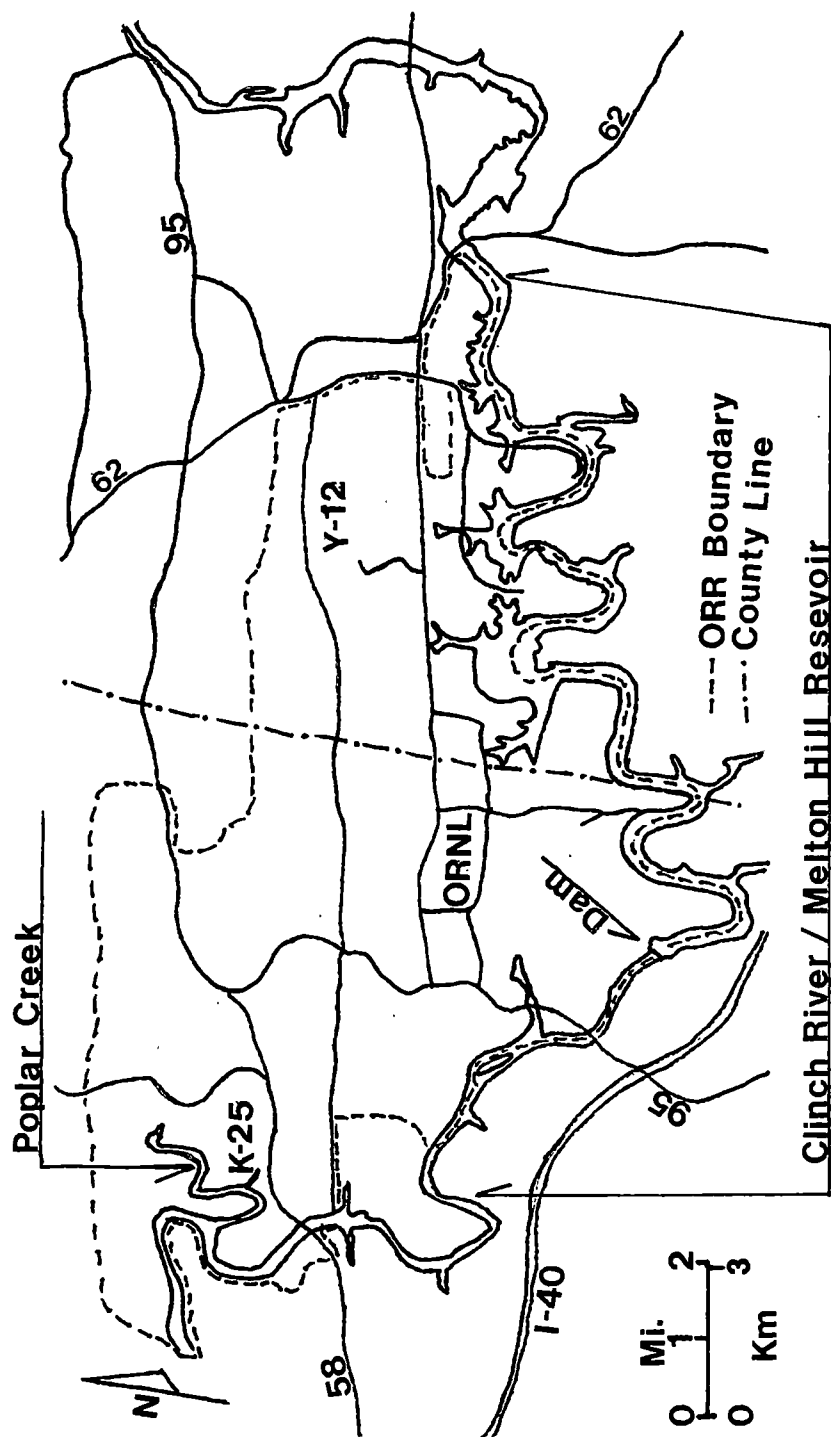


Figure 2: Waterways of the ORR, and their drainage to the Clinch River. (Adapted from ORNL Drawing 87.705.1)

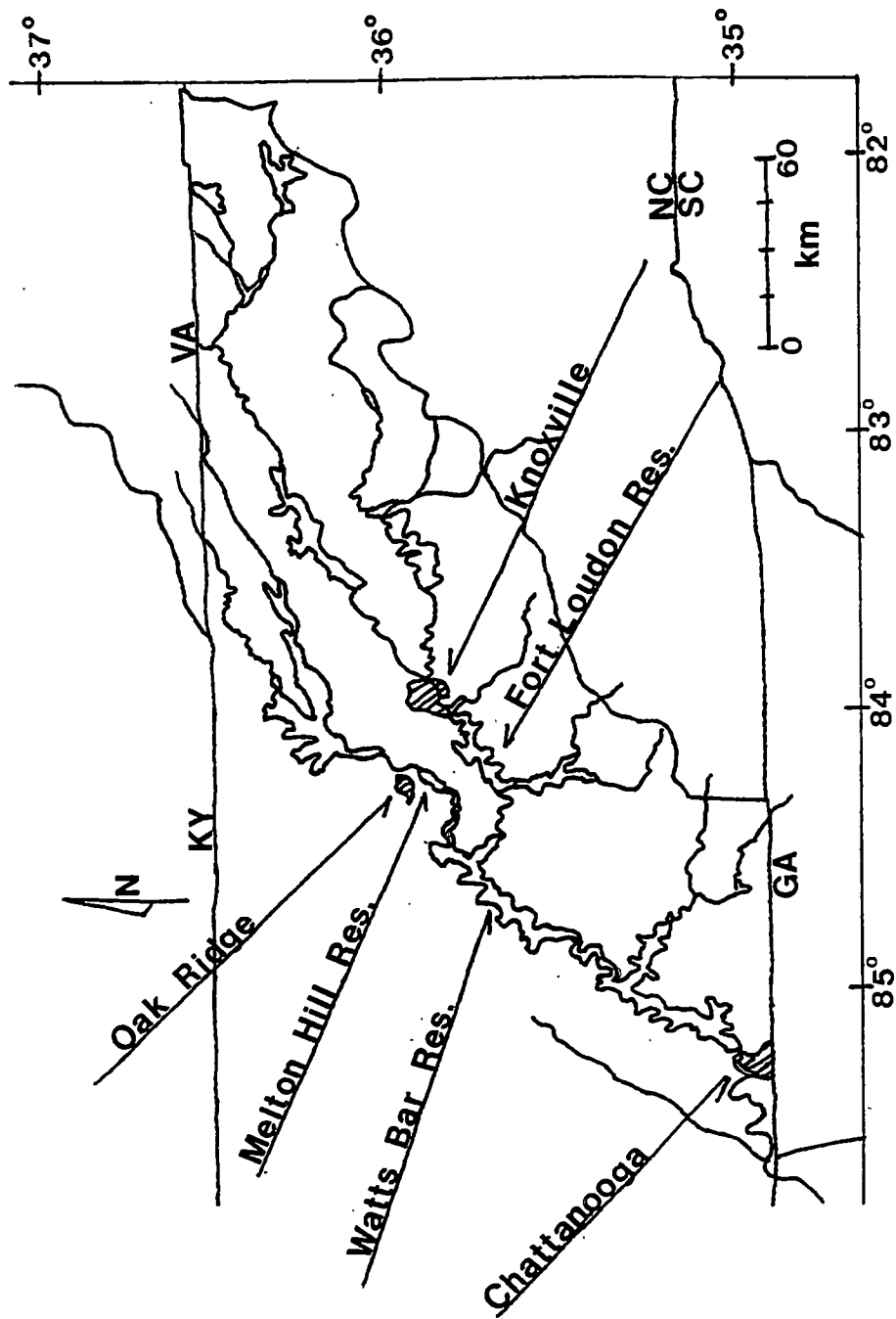


Figure 3: Waterways of East Tennessee, in Relation to Knoxville and the ORR.

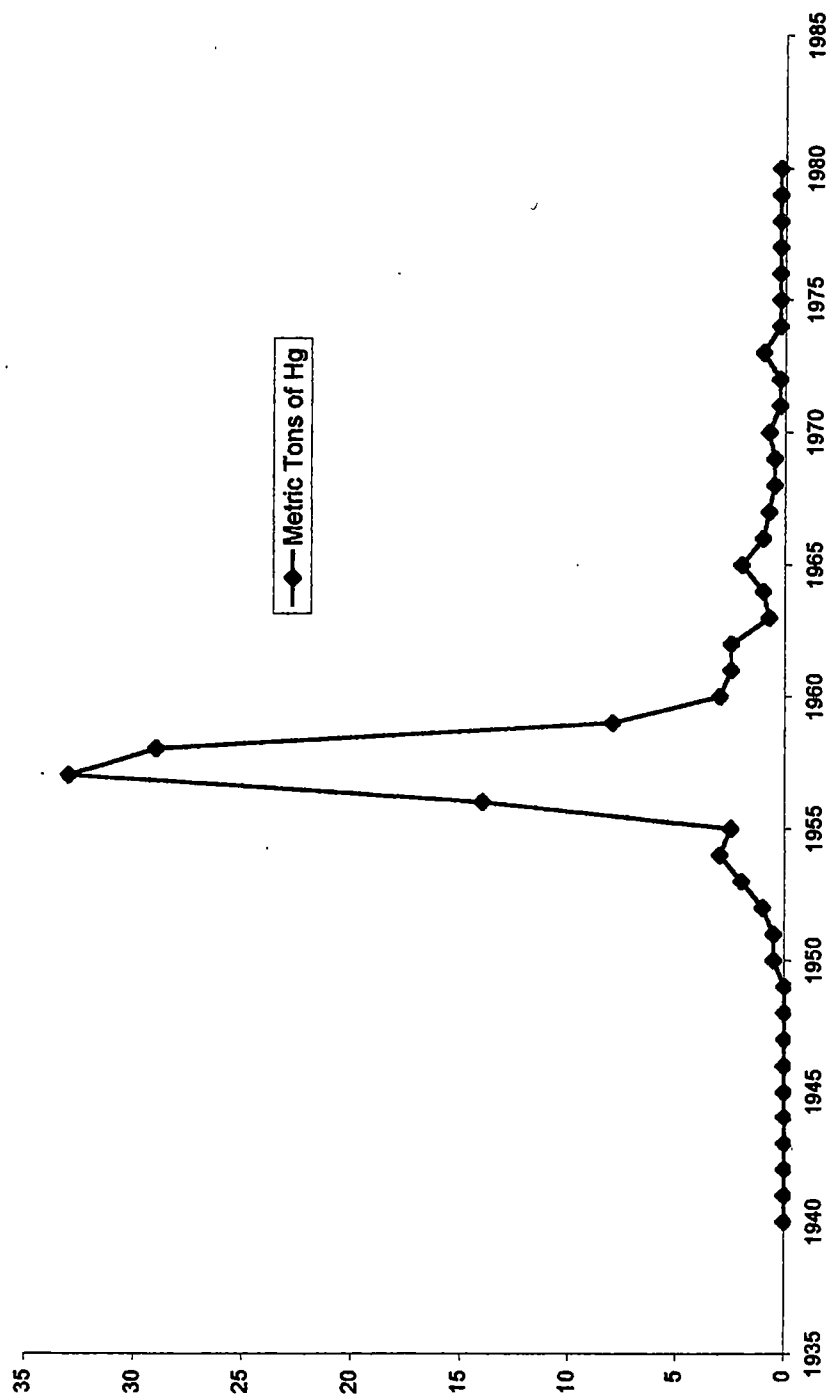


Figure 4: Mercury release history for the Y-12 plant.
(Adapted from ORNL-DWG 84-10074)

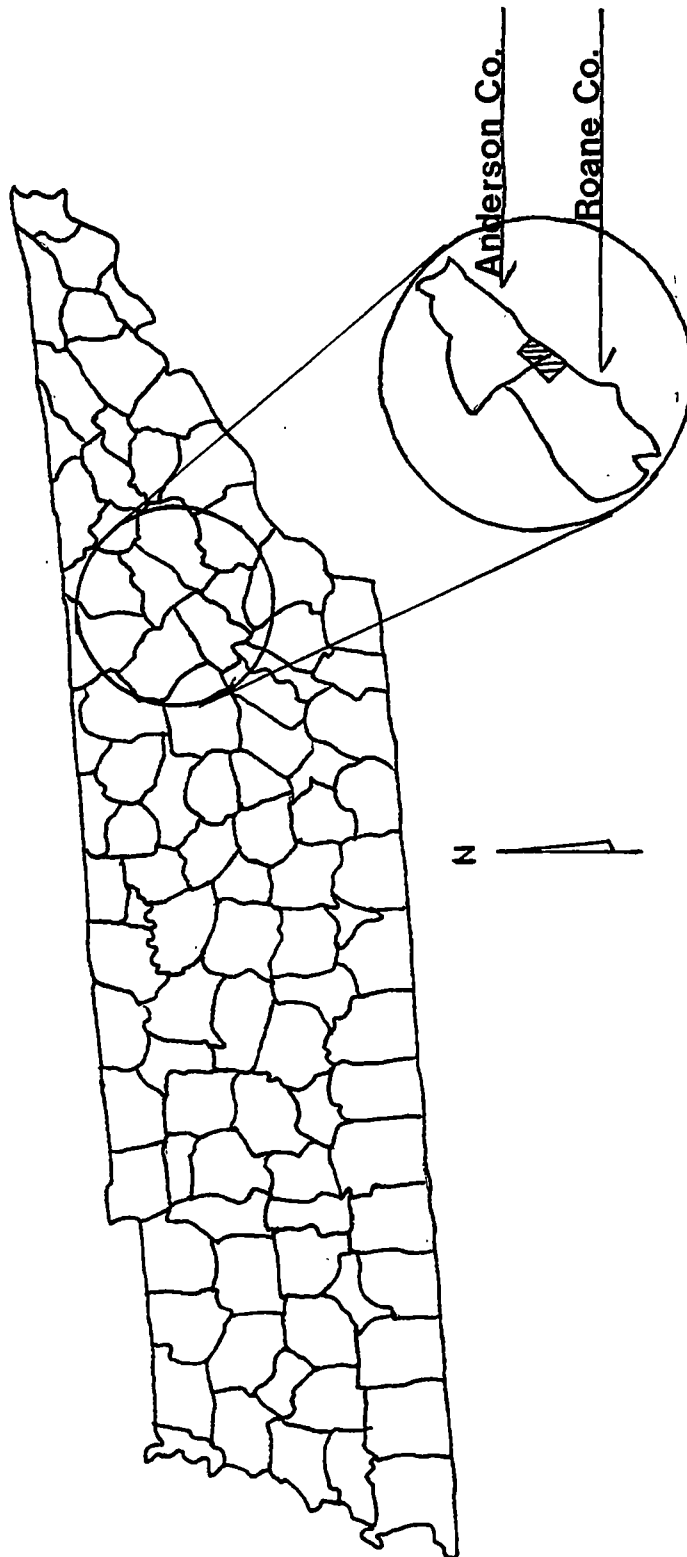


Figure 5: Location of the Oak Ridge Reservation in Anderson and Roane Counties, Tennessee

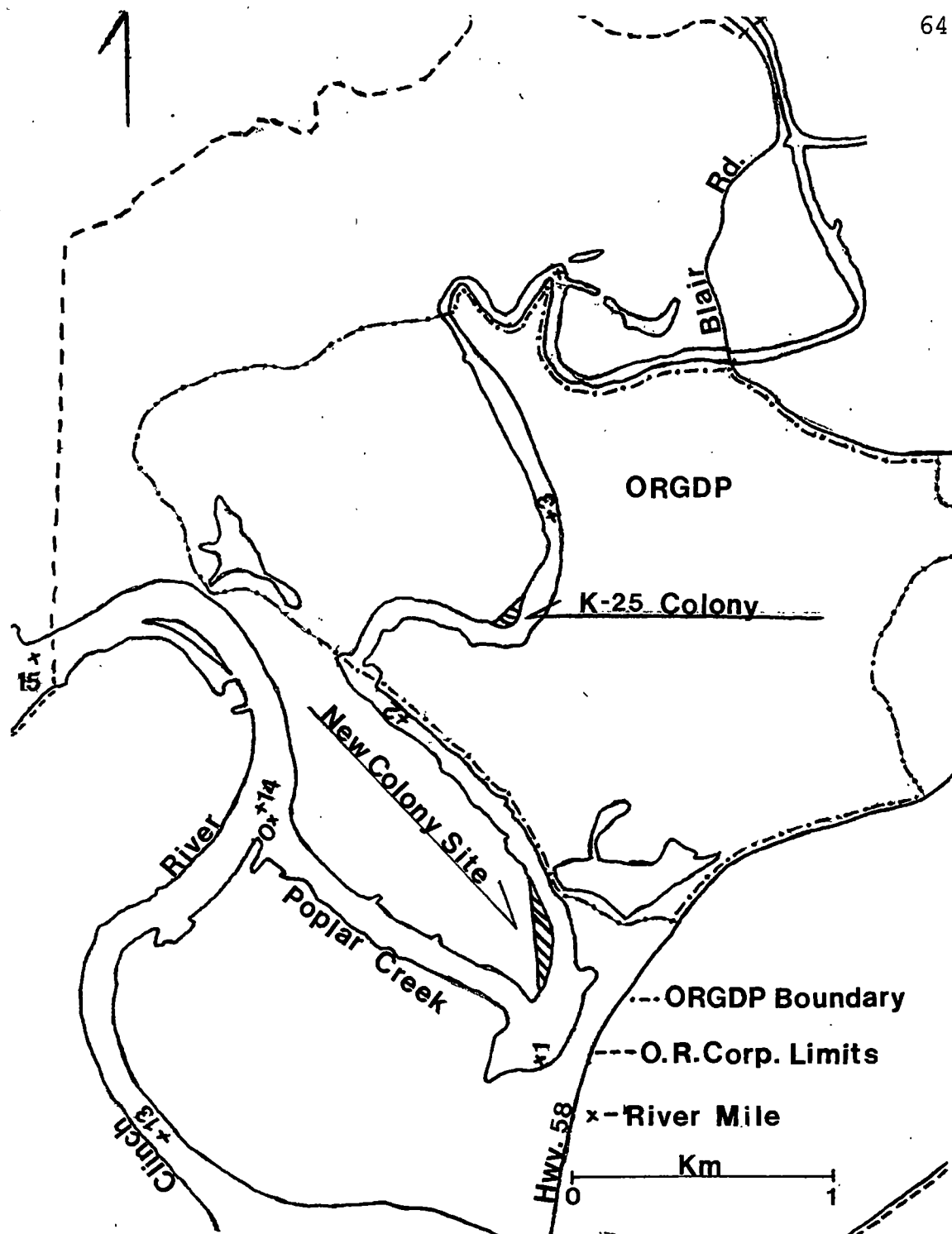


Figure 6: Location of the Original and New K-25 Colonies adjacent to the Oak Ridge Gaseous Diffusion Plant.

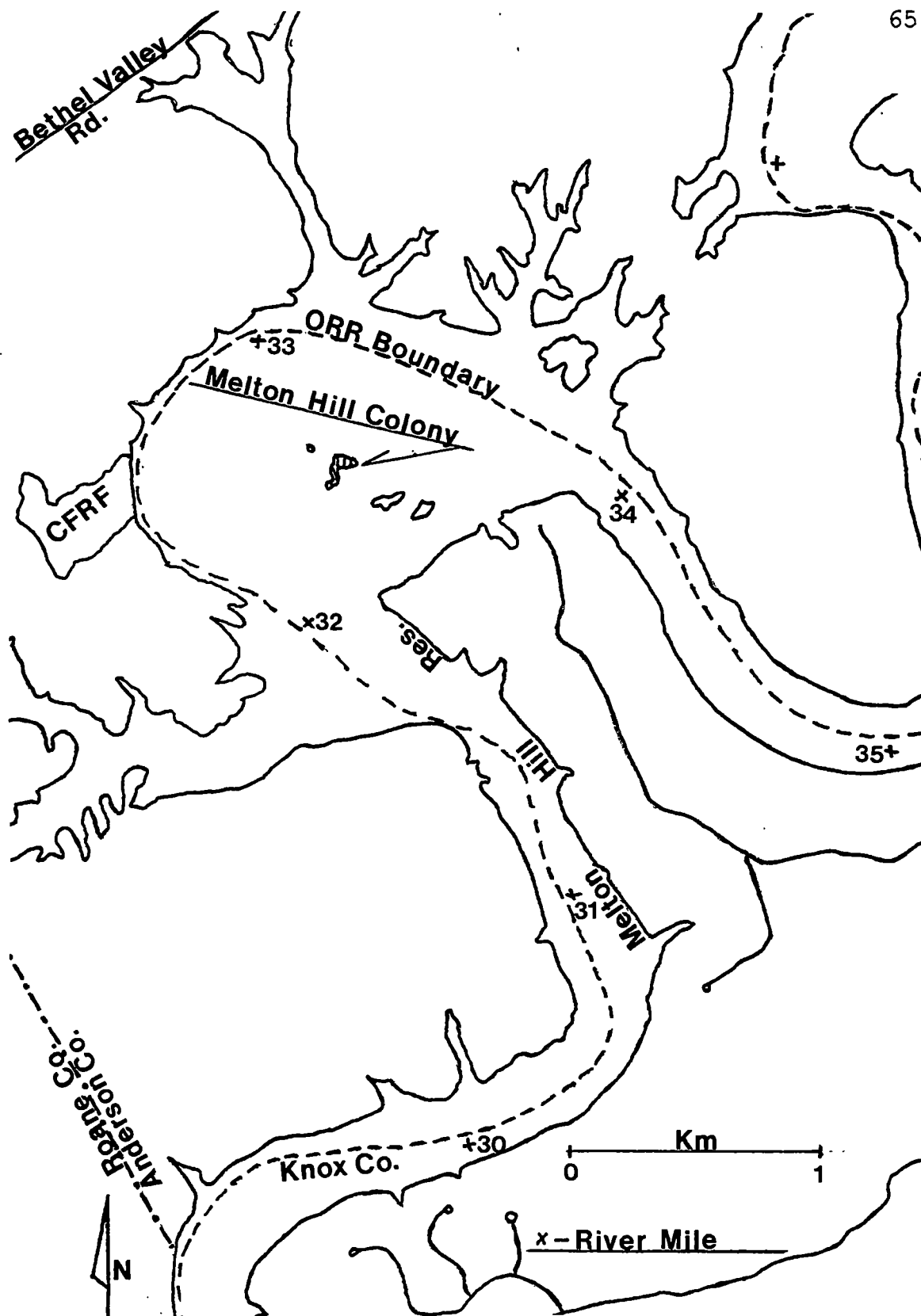


Figure 7: Location of the Melton Hill Colony on Melton Hill Reservoir, Adjacent to the ORR.

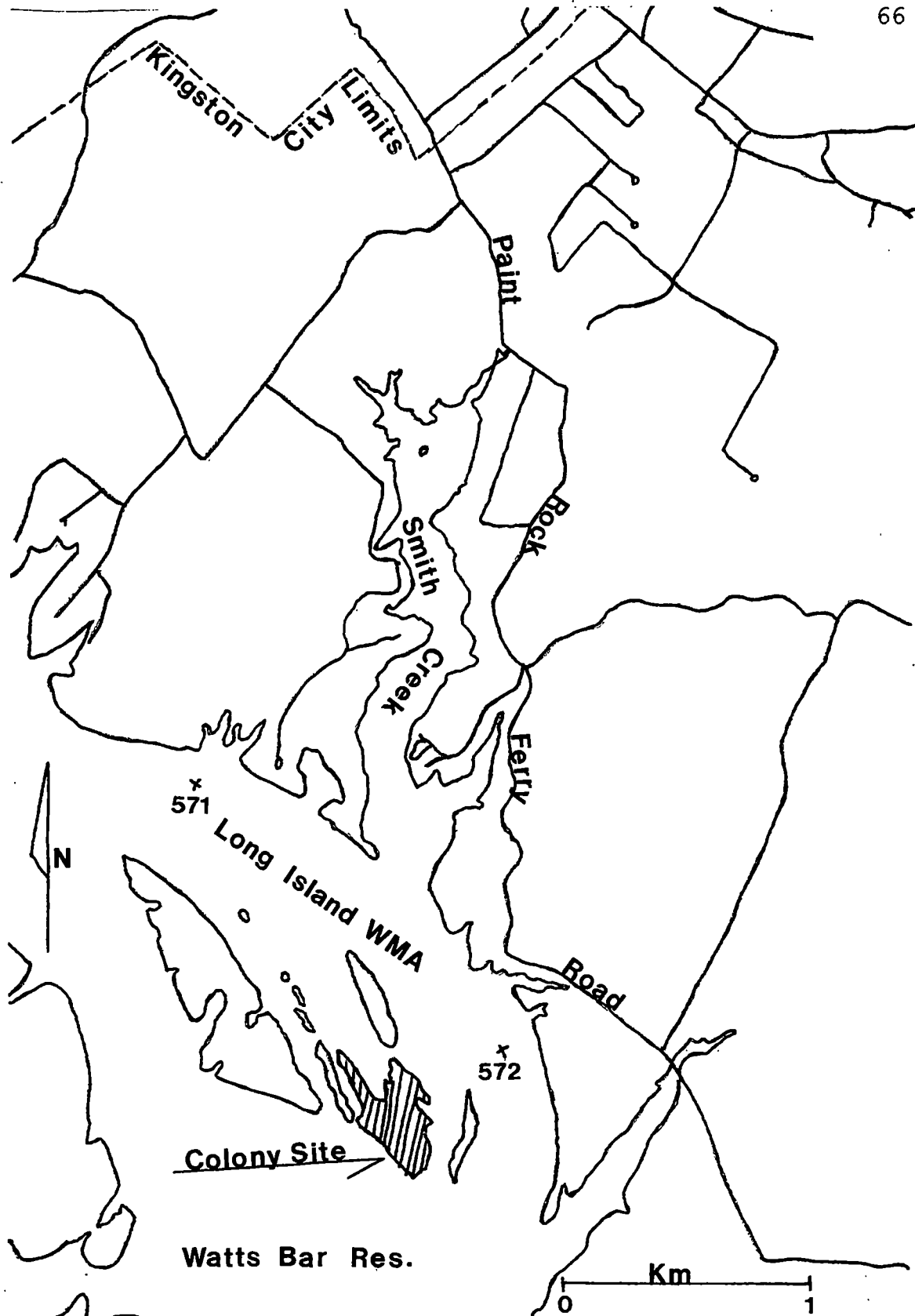


Figure 8: Location of the Long Island Colony on Watts Bar Reservoir in Roane County, Tennessee.

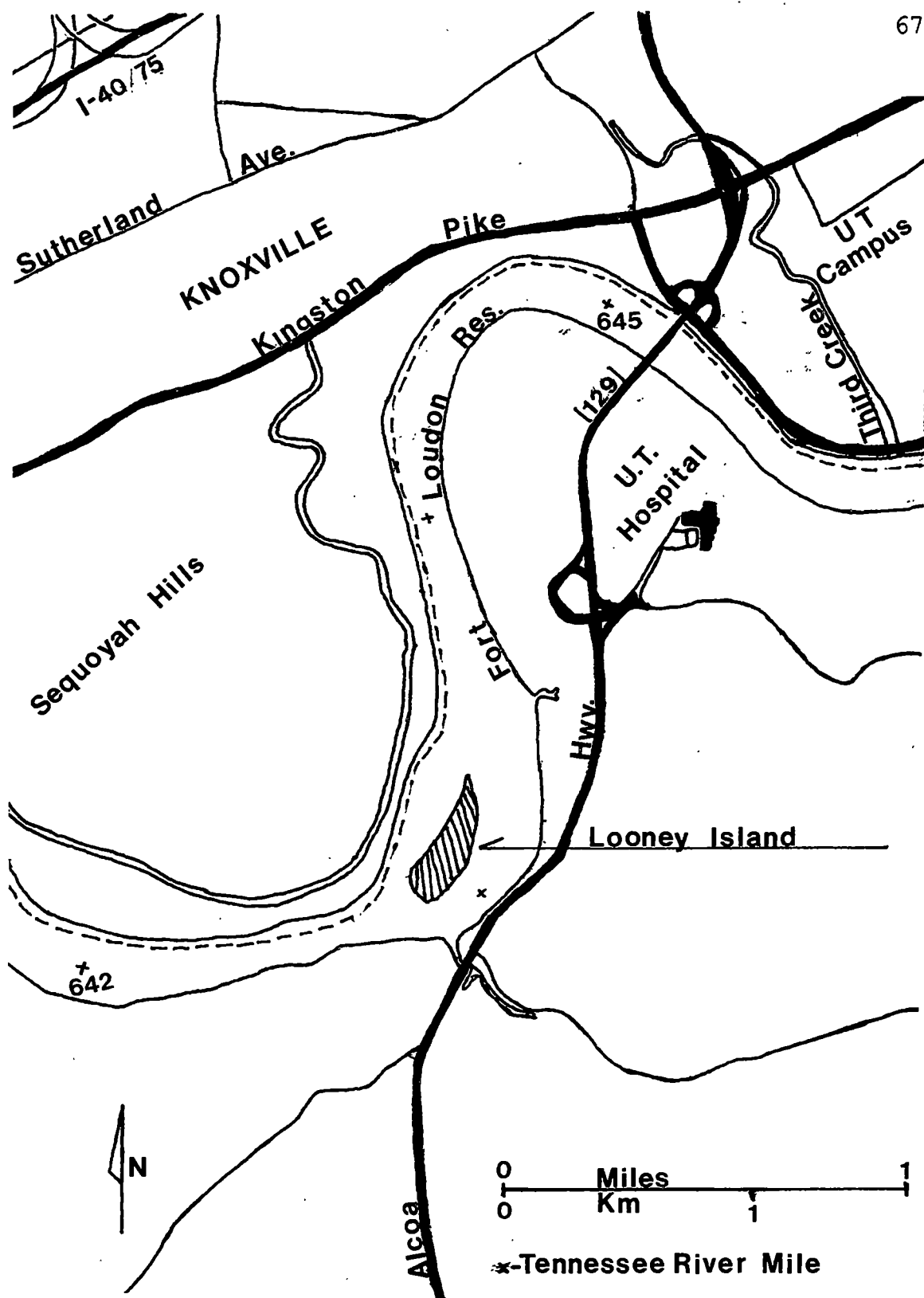


Figure 9: Location of the Looney Island Colony on Fort Loudon Reservoir, Adjacent to Knoxville, Tennessee.

VITA

Robert Brewer was born in Knoxville, Tennessee in 1967, and was raised in Fountain City. As a youth he developed a love of the outdoors, fostered by his father, grandfather (Curt Underwood), and the Boy Scouts of America. Robert graduated from Knoxville Central High School in 1985. He received a Bachelor of Science degree in Wildlife and Fisheries Science from The University of Tennessee, Knoxville, in May 1990.

Robert's experiences in both the public and private sectors ingrained in him a concern for the future of all wildlife species and their environments. He received a Master of Science Degree from The University of Tennessee, Knoxville, in Ecology and Evolutionary Biology in December 1998. Robert has committed himself to our natural resources, hoping to ensure their well being for future generations.