Ecology and contaminant exposure of Lake Calumet Black-crowned Night Herons: Population Levels and Nesting Ecology

Final Report

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30 June 2004

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I. FIELD ACTIVITIES

1. 2002

a) Population censuses were conducted at IRM on a weekly basis from 24 March to 26 May; a count was not conducted during the week of May 19. Censuses were conducted under conditions of no precipitation and winds < 15 mph by two observers beginning 1 hour before sunset and continuing until the rate of departure was < 2 herons/5 minute period. As days lengthened the herons became active earlier and beginning on May 2 censuses were initiated two hours before sunset. During this time BCNH were aged by plumage characteristics to the extent possible and the direction of flight as herons exited IRM was noted. Although no BCNH were noted at Heron Pond (HP) on March 24 and March 30, a number of herons were observed at this site on April 6. After mid-April it became apparent that some herons were nesting at HP and censuses were subsequently conducted on 4/26 and 5/12 at that site. The presence of other species of waders and any other noteworthy observations were also recorded during these counts.

b) The activities of BCNH at IRM, HP, Inland Steel, and Lake Renwick were observed for 2-4 hour periods on eight occasions during April 5 to May 10 to document the timing of breeding activities. Nest building, pairing, breeding, and nesting behaviors were recorded. During these observational periods the reaction of the herons to the observer(s) and other potential sources of disturbance was also noted. BCNH response behaviors were categorized as flutter and settle, circling flight, or departed area. The degree of response was categorized as none, momentary, or sustained. Other information recorded included BCNH activity level and inter- and intraspecific interactions.

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c) Nest monitoring was initiated at IRM on 15 May, when 30 nests were marked and the number of eggs and chicks was recorded for active nests; an additional 19 nests were subsequently included in monitoring efforts at that site. Nests were checked every 4 to 7 days depending on weather and staff availability. The number and disposition of eggs (cracking, pipping, hatched, fell out, depredated, missing) and nestlings (present, missing, depredated) was recorded during each visit. Eggs were measured (length and width) and numbered with non-toxic marker, and the culmen length of chicks was recorded on one or more occasions. Habitat variables, including water depth, height of nest above water, distance to open water/vegetation edge, canopy cover, and horizontal cover (visual obstruction), were also measured at ≥20 nest sites.

A sample of 15 nests located at HP was on May 30, with another 8 nests added on June 13; these nests were monitored every 10 to 14 days. Twelve tree nests were also monitored at a BCNH colony located at Inland Steel, East Chicago, IN. It was anticipated that a sample of nests at Lake Renwick Heron Rookery Preserve, Forest Preserve District of Will County, and McGinnis Slough in Will County would be monitored for this study. However, accessibility issues (LR) and absence of nesting BCNH (MS) precluded these activities.

d) We conducted bi-monthly post-breeding surveys at IRM during June-August 2002 to monitor the relative abundance of BCNH present by age class from about the time the first young became

flighted through dispersal. These surveys consisted of walking the access road west of the tracks from their intersection with 116th Street to the southern end the south end of the access road at a slow pace, counting and aging the herons as the observer passed by them. To avoid double-counting, herons that took flight at the observer's approach and landed within an unsurveyed portion of the colony were not counted at that time.

2. 2003

a) Weekly population surveys were conducted at IRM from 23 March to 1 June in 2003; methods followed those described above for 2002.

b) The activities of BCNH at IRM were observed for 1 to 2 ½ hour periods on 11 occasions during 9 April to 13 May. Nest building, pairing, breeding, and nesting behaviors were recorded.

c) Post-breeding surveys (i.e., counting number of adult and juvenile BCNH observed while walking a transect next to IRM) were initiated 12 June and concluded on 21 August.

d) Nest monitoring at IRM was initiated on 16 May; a total of 55 nests were marked (n= 20 on 5/16, 23 on 5/21, and 12 on 5/29). A total of 53 nests (n= 6 on 5/14, 4 on 5/21, 12 on 5/28, 14 on 6/11, and 17 on 6/18) located at ISPAT Inland Steel in Lake County Indiana were also monitored. Nest monitoring at Indian Ridge Marsh and Inland Steel, respectively, was concluded 10 and 23 July.

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II. POPULATION LEVELS

1. Annual Arrival of BCNH at LCW

Dates of first known arrival of BCNH at LCW are based on periodic checks each spring of known previous nesting locations. The earliest date during which a BCNH has been observed at LCW was on March 10 of 1994 (Table 1). At least 130 BCNH were present by March 29 of that year.

No BCNH were seen on one or more visits prior to the dates of first appearance in 1993, 1994, 1995, 2000, 2001, and 2003; the interval between the first known arrival date and the previous visit when no herons were observed averaged four days in these years. No previous visits were recorded for 1996, 1997, 1998, and 2002. No BCNH were observed on March 29 in 1992 or March 23 in 1999, thus the herons apparently began arriving after those dates. Although we can not be sure of the exact date of arrival based on these data, they do indicate that BCNH typically began arriving in substantial numbers during the latter half of March (Table 1).

BCNH may continue to arrive at LCW well after others have arrived and begun nest building and pairing. For example, in 2002 the number of BCNH at IRM greatly increased between April 26 and May 12, well after others had arrived and begun nesting. Also, no BCNH were observed at Heron Pond (HP) until April 6 of that year (see Current and Historic Population Levels below).

Similarly, BCNH were present at IRM as early as March 10 in 1994, but were not observed at Big Marsh (BM) as late as May 1, though were known to be successfully nesting there by June 9.

2. Current and Historic Population Levels

BCNH were first noted at IRM on March 17 of 2002; numbers there gradually increased from the start of evening population surveys on March 24 through April 17, leveling off between April 17 and 26 before increasing dramatically between April 26 and May 12, when they peaked at 608 herons (Fig. 1). The number of BCNH observed during evening surveys at IRM declined dramatically between May 12 and May 26; evening surveys of BCNH were discontinued after that time. This decline in BCNH observed leaving the colony during evening hours was undoubtedly due to the increasing numbers of herons remaining at nests to incubate eggs or brood young. Also, counting became more difficult as foraging trips became shorter in duration as BCNH returned to the colony to feed young or allow their mates to recess.

No BCNH were observed at Heron Pond on March 24 or 30 of 2002; however, 33 herons were counted there on April 6. Population surveys conducted at HP on the evenings of April 26 and May 12 (Fig. 1) yielded a maximum count of 170 BCNH. The peak count of BCNH at LCW in 2002 occurred during the week of May 12, when a total of 750 BCNH was observed.

In 2003, population censuses were initiated on 23 March when 105 BCNH were present. The population peaked on 10 May when 641 BCNH (750 in 2002) were observed leaving IRM (Fig. 1). The peak count was down 15% from 2002, but the estimated number of nests (641 total BCNH / 2 BCNH/nest = 320 nests) was within "normal" limits for the period 1997-2002 (306 to 404 nests). The results of population surveys showed a similar pattern of increase in both 2002 and 2003, except that numbers were slower to build during early April in 2003.

Peak numbers of BCNH during 1992-2003 were based on evening censuses of BCNH leaving nesting colonies to forage. Data for 1992-2001 represented an average of 2 to 3 counts conducted during late-April/early-May of each year; the 2002 and 2003 data represented the maximum of 11 counts conducted during March-May of each year; these were combined with counts of occupied tree nests (multiplied by 2 BCNH) conducted during 1987-89 and 1991-95.

The peak BCNH population at Lake Calumet wetlands varied considerably during 1992-1997, from a high of nearly 1,600 in 1992 to less than 600 in 2000 (Fig. 2). Although there was a declining population trend during much of the 1990s, numbers have fluctuated at between 600 and 800 herons in recent years.

We compiled the results of annual nest counts coordinated by Sue Elston of USEPA, conducted at BM during mid- to late-May of 1984-1991 (no data for 1990). These counts consisted of a line of 5-7 observers walking transects through the phragmites stands and counting nests seen to one side of each person. A portion of the colony was not surveyed in 1986 and 1987; it was estimated that < 15% of the nesting colony was missed, thus we added 15% to the nest counts in those

years. Direct counts of tree nests located in cottonwoods at IRM during 1987-91 were added to these counts.

The number of nests present during 1992-2003 were estimated by dividing peak counts of BCNH by two to represent a pair for each nest. We feel that this provides a reasonably accurate approximation of the number of breeding pairs (nests), as 1) our data suggests that prior to incubation the vast majority of BCNH are counted during evening surveys, 2) 99.25% of known-aged herons observed during our evening population surveys were > 1 year old, and 3) there are numerous accounts of nesting by one-year-old BCNH. In 1994 the herons began nesting at BM (in addition to phragmites and cottonwoods at IRM) late in the season, thus, a complete count was not conducted at that location. Consequently, the 1994 estimate of 410 nests represents a minimum and was not included in figure 3.

The number of nests varied considerably during 1985-97, from a low of 266 in 1984 to a high of 871 in 1992 (Fig. 3). The number of BCNH nests at LCW increased dramatically during the latter half of the 1980s and remained relatively high into the mid-1990s. Numbers of BCNH nests at LCW declined throughout much of the 1990s before essentially leveling off at 300-400 nests during the late 1990s and early 2000s (Fig. 3).

III. PRODUCTIVITY AND NESTING ECOLOGY 2002-2003

While comparisons of productivity between studies are useful, readers are cautioned that the ratio of breeders to non-breeders, food availability, local weather patterns, climate/growing season (latitude), predation, and a variety of density-dependent factors can influence productivity. Most powerful are comparisons with contemporary local/regional populations. The only sizable colony in the south Chicago area available to us for comparison during 2002 and 2003 was at ISPAT Inland Steel, Lake County, IN, located on the Lake Michigan shoreline only 15 km from the Lake Calumet colony. This colony is located at the same latitude as the LCW colonies and may also have a similar pattern of contaminant exposure. However, the IS colony nested in cottonwood trees during this study. Clutch size and nestling survival in Cattle Egrets nesting in shrubs was lower than those nesting in Phragmites in the same colony (Parson 1995). However, these parameters did not differ between shrub- and Phragmites-nesting Little Blue Herons in that study.

1. Clutch Size

Mean clutch size (all clutches) of BCNH nesting at Indian Ridge Marsh (IRM) in 2002 was similar to that of clutches in tree nests at nearby ISPAT Inland Steel (IS), Lake County, IN, as well as recently-studied colonies in MD and the Pacific Northwest (Table 2). The average clutch size at IRM was at the upper and lower ends, respectively, of the range of average clutch sizes observed in colonies in NV/OR/WA and ID that were thought to be impacted by DDE, and below the range of clutch sizes observed for "clean" colonies in NV/OR/WA, the upper east coast, and Quebec (Table 2). Mean clutch sizes (all clutches) at IRM in 2002 and 2003 were

very similar; clutch size declined considerably at IIS in 2003. the reasons for this decline, whether real or sampling artifact, are unknown.

A total of 6 monitored nests at IRM were recycled (one nest with second and third clutches) in 2002. Mean clutch size for initial clutches was slightly higher than for all clutches (Table 2). Interestingly, mean clutch size for first and second clutches differed by same amount as those in colonies in OR/WA. We did not document recycled nests at IRM in 2003.

Custer et al. (1983) noted that BCNH clutches initiated later in the season had a larger proportion of smaller clutches (i.e., < four eggs) than earlier clutches. When sorted by median date of initiation (5/13, see Nesting Phenology below), i.e., early nests ≤ median date, late nests > median date, the average clutch size in 2002 was 0.48 eggs larger in nests initiated on or prior to the median date. Thus, early clutches were nearly 16% larger than late clutches; however, we did not detect this difference statistically (t_{28}= 1.8, P= 0.08).

Mean clutch size at HP was considerable lower than for all clutches at IRM, though similar to first clutches initiated at IRM after the median date of initiation (Table 2). The nest-check interval was longer at HP ($\bar{x}$= 10.8 days) than IRM ($\bar{x}$= 5.7 days); the loss of one egg from an occasional nest at IRM within two weeks of clutch completion suggests that the average clutch size may have been slightly higher at HP had we utilized a shorter nest-check interval. Also, 64 % of monitored clutches at HP were estimated to have been initiated later than the median date at IRM and therefore mean clutch size at HP may have been biased low.

There were a total of four nests in 2002 at IRM that had additional eggs laid in them after the original clutches were complete (based on the time interval between last egg of the clutch laid and presence of new eggs). In two cases one additional egg was laid after clutches of three were complete; in the other two nests, one or more of the original eggs were found in the water and new eggs added to the nest. In one of these, a known total of five eggs were laid. In the other, the nest was recycled following the disappearance of the original clutch of three eggs. At least six eggs were subsequently found in the nest, with all eventually disappearing; four eggs (some from both clutches) were found in the water below this nest. We did not note any such "dump nests" at IRM in 2003.

2. Productivity

Daily nest survival rate was similar across the colonies we examined, and these were similar to contemporary colonies on the east and west coasts (Table 3). The survival rates of nests to hatch at HP and at IRM and IS during 2003 were relatively high; clutches at IRM and IS in 2002 had a lower likelihood of surviving the incubation period.

Daily survival rate of nests during the nestling period was similar across our colonies as well as those examined by others (Table 3). Survival of broods to 15 days of age was lower at IS than the other colonies examined in this or previous studies. Brood survival at IRM was similar between years, and was relatively high in comparison with past studies. Brood survival during

this period was higher at HP than at IS, though relatively low compared to colonies on the east and west coasts and at IRM.

In 2002, nest success (a function of survival rate to hatching and survival rate to fledging) at IRM and HP were similar, and both were greater than at IS, which was poor (Table 3). Nest success at both IRM and IS improved between 2002 and 2003, although nest success remained poor at the latter.

With the exception of IS in 2002, the probability of an egg hatching was relatively high at all sites in both years. Egg success was low at IS during 2002, though improved between 2002 and 2003 at this site and at IRM. Nestling survival was low at both IS and HP in 2002; survival of young to 15 days improved at both IRM and IS between years (Table 3). Lower productivity at HP was due in part to the destruction of 8 marked nests in a portion of that wetland (see below). We often noted eggs and carcasses of young on the ground below nesting trees at IS; this colony was directly exposed to winds off of Lake Michigan, and we assume that some of this loss may have been due to high winds and other mishaps causing nestlings to fall out of trees. In addition, nestling BCNH at IS were seen to regurgitate RBG chicks (a large RBG colony was located at the foot of the BCNH colony) on several occasions, and thus were feeding higher up on the food chain and may have had greater exposure to environmental contaminants.

The mean number of young surviving/nest was very low at IS, whereas IRM had the highest "recruitment" of the colonies we examined and was relatively high compared to colonies elsewhere (Table 3). Recruitment increased at both IRM and IS between 2002 and 2003. Henny (1972) determined that 2.0-2.1 young produced/breeding pair is needed to maintain BCNH populations, and Wolford and Boag (1971) calculated that a colony producing 1.1 fledged young/pair would disappear in 20 years. Thus, fledging rates (actually survival to 15 days) we observed during 2002 may not be high enough to maintain stable populations, particularly at HP and IS. Estimated recruitment improved at IRM and IS in 2003; recruitment at IRM in 2003 was among the highest reported in other studies (Table 3). It is not unusual for productivity of BCNH colonies to vary between years. For example, Greenwood (1981) reported annual production as 0.23, 0.57, and 2.20 fledglings/pair for a colony occupying a marsh in North Dakota; strong storms with hail and high winds were responsible for large losses of nests and young in two of those years. However, in the absence of such catastrophic events (including heavy losses to predation) productivity probably does not normally vary this dramatically between years (e.g., see Custer et al. 1983).

3. Nest, Egg, and Nestling Fate

2002

The largest cause of failed clutches at IRM in 2002 was through poorly constructed nests which allowed eggs to roll out into the water (Table 4). Predation was the next most-important cause of nest failure. The complete disappearance of two clutches/broods may also have represented predation events. Including these, seven of 51 (13.7%; includes second clutches) monitored

clutches/broods were lost to predation at IRM. This amounted to the loss of 13 eggs and seven chicks; coupled with two eggs with bills marks found in completed clutches, a minimum of 22 eggs or nestlings were lost to nest predators. It was difficult to determine the cause of death of most nestlings due to condition of carcasses upon discovery. Eight nestling carcasses amounted to articulated (intact) skeletons or carcasses in later stages of scavenging by invertebrates. The cause of death of these individuals could not be determined but may have represented abandoned, injured, or sick nestlings; e.g., BCNH nestlings may die from choking on fish (Custer et al. 1983). BCNH eggs hatch asynchronously and later nestlings to hatch may be disadvantaged, with competition by older siblings leading to starvation, drowning, or trampling of small nestlings. This was undoubtedly the cause of the disappearance of many individual nestlings. We frequently visited nests with 2 or 3 thriving nestlings, and a much smaller, less thrifty individual that was often missing at our next visit.

Several eggs had apparently been depredated by Ring-Billed Gulls (RBG), judging by bill marks, although in at least one case the Gull(s) may have scavenged a previously abandoned clutch. Large numbers of RBG frequented the area and were often seen fishing in open-water portions of IRM. We did not observe Gulls at BCNH nests, although we did note a BCNH briefly chasing a RBG that flew low directly over the colony. It is likely that RBG were responsible for the disappearance of some of the eggs and very young nestlings that we couldn't account for. However, predation by this species did not seem to be an important factor at IRM, particularly in light of the large numbers of RBG frequenting this wetland. In contrast, RBG were responsible for the poor productivity of some BCNH colonies in Alberta (Wolford and Boag 1971).

Three siblings < 5 days old that were found in a marked nest were killed by single, severe pecks that caved in the back of their skull; this may have represented intraspecific aggression, i.e., adults from neighboring nests. We also necropsied two older juveniles (520 and 750 g. body mass; ~3 and 4 weeks old) that were not associated with marked nests; one was found dead in a nest at HP with a broken bill and fractured spine; another received a broken neck after presumably colliding with overhead wires bordering IRM.

Eight marked and a number of unmarked nests in a shallow area of HP were destroyed sometime between 2 and 12 July 2002; judging by the degree of damage to nests and associated vegetation we presume this to be the work of either raccoons or human vandals. However, sufficient time had passed to obscure any definitive evidence as to the identity of the culprit(s). We did not observe damage to nests at IRM that might be indicative of raccoon depredation or vandalism; this may have been related to the deeper water at IRM, which also has a ditch separating the colony from the western shoreline (RR tracks). Much of the HP colony nested in Phragmites in shallow (in many places less than knee deep) water surrounding small potholes. Snapping turtles undoubtedly caused some losses of young, especially after the juvenile BCNH began to move about in the marsh. However, predation did not seem to be having a dramatic impact on the productivity of BCNH at IRM in 2002 or 2003, as has been documented for some colonies (e.g., Wolford and Boag 1971; Blus et al. 1997).

2003

Most nests successfully fledged at least 1 young (Table 5); there were no known or suspected losses of entire clutches or broods to predation. Although no eggs were observed in water, all eggs missing from two flat-topped nests were assumed to have rolled out into the water. One nest was presumed abandoned as the marked eggs were present well after they should have hatched. A total of 25 eggs were missing or otherwise failed to hatch. Twenty-three nestlings did not survive to fledging (i.e. 15 days post-hatch); of these, 21 were simply missing, one was found dead in nest (cause unknown), another had fallen out of the nest and was found with its neck caught between phragmites stems.

4. Nesting Phenology

2002

Observations of BCNH breeding behavior were recorded at IRM, HP, and Lake Renwick (Will County) during the early part of the breeding season. BCNH were first observed at LCW on 17 March of 2002, and were first seen (n= 4) carrying sticks on 6 April. Early in the breeding cycle this behavior signifies beginning of nest building by males just prior to courtship and accepting a female at the nest (Meyerriecks, 1960; Palmer, 1962). Later, after pair formation the male will present twigs to females prior to copulation and this behavior persists until after the eggs are laid. Gross (1923) reported that the first eggs were laid an average of 7 days after start of construction; based on this information the first eggs would have appeared at LCW around 13 April.

The first pairs were observed at nests at IRM on 4/16; the number of pairs seen from an established observation point increased from two on 16 April, to 10 on 23 April, and 22 on 2 May. Circling flights (synchronization of sexual behavior in early pair formation, Meyerriecks, 1960) were observed on 16 April; pre-copulatory displays (including billing, feather nibbling, twig presentation, erection of feathers, and neck stretch), and copulation were recorded as early as 18 April and 19 April. According to Allen and Mangels (1940) the first eggs are laid an average of 3.3 days after copulation or 4-5 days after pair formation. Based on this information the first eggs would have appeared on 20 or 21 April.

The phenology of BCNH nesting and juvenile fledging/dispersal at IRM was reconstructed based on direct observation (i.e., nest monitoring) and the following assumptions:

1) approximately two days to lay each egg (Gross 1923; Palmer 1962; Tremblay and Ellison 1980);

2) an average of incubation period of 24 (23.5) days for A-eggs (i.e., first egg laid; Custer et al. 1992);

3) hatching one day after pipping, two days after cracking (Custer and Peterson 1991);

4) B-egg hatches about one day after A-, and C- hatches two days after B- (Custer et al. 1992); we assumed D-egg hatched one day after the C-egg;

5) flight is attained at about 6 weeks (42 days) of age (Palmer 1962; Wolford and Boag 1971);

6) on average, young of the year disperse at 58 days of age (Erwin et al., 1995).

We initially marked 30 nests on 15 May and added new nests as encountered on that portion of the colony (n= 9 on 20 May, n= 6 on 26 May, n= 2 on 30 May, and n= 1 on 4 June). Based on our monitored sample of nests, we estimated that clutches were initiated as early as 20 April, with half being initiated by mid-May, and the last on 7 June (Fig. 4). The last clutches initiated were in recycled nests; excluding second and third clutches (i.e., recycled nests) the latest a clutch was initiated, based on our marked sample, was 23 May. This estimate of 20 April for the initiation of laying coincides with estimates of clutch initiation based on our observations of pre-copulatory displays and copulation and information provided in Allen and Mangels (1940). Given five days for completion of a 4-egg clutch, our estimate of 20 April to 12 June for the egg-laying period at IRM agrees very well with that of April 24 to 18 June for northern Illinois colonies by Graber et al. (1978).

The first eggs would have hatched by mid-May, with A-eggs from half of the monitored nests hatching by the second week in June; A-eggs from the last nests initiated would have hatched on the first of July (Fig. 4). Thus, eggs would have been present at IRM over a period of about two and one-half months (20 April to 4/5 July).

Based on a flightless period of 6 weeks (Palmer 1962), the first and last young, respectively, attained flight on 25 June and 10 August (Fig. 4). Erwin et al. (1996) reported that age of dispersal on BCNH averaged 55 and 60 days in the two years of their study. Thus, based on an average dispersal age of 58 days, dispersal of juvenile IRM BCNH began about mid-July and continued through late August, with most having reached dispersal age by the middle of August. This coincides well with the results of our post-breeding surveys which revealed a precipitous decline in juveniles observed at IRM between August 10 and 24 (see below).

2003

BCNH were first observed carrying sticks on 9 April (6 April in 2002) of 2003. According to Gross (1923), the first eggs were laid an average of 7 days after start of construction. Based on this the first eggs would have appeared at LCW around 16 April (13 April in 2002). Pairs were first observed at nests on 16 April. Pre-copulatory displays (including billing, feather nibbling, twig presentation, erection of feathers, and neck stretch) were recorded as early as 18 April. According to Allen and Mangels (1940) the first eggs are laid an average of 3.3 days after copulation or 4-5 days after pair formation. Based on this information the first eggs would have appeared on 21 April.

Nesting phenology was reconstructed as in 2002; nest monitoring at IRM was initiated on 16 May; a total of 55 nests were marked (n= 20 on 5/16, 23 on 5/21, and 12 on 5/29). Based on our sample of nests, the first clutches were initiated on 16 April (20 April in 2002), with half the nests initiated by 8 May (13 may in 2002), and the last on 23 May (same as in 2002 for initial clutches).

The first eggs in 2003 would have hatched by mid-May; A-eggs from half of the monitored nests would have hatched by early June; A-eggs from the last nests initiated would have hatched on 16 June (Fig. 5). Thus, eggs would have been present at IRM over a period of about two months (16 April to 16 June); this is a shorter egg period than in 2002 by about 2 weeks.

Based on a flightless period of 6 weeks (Palmer 1962), the first and last young, respectively, attained flight on 5 July and 3 August 2003 (Fig. 5). Erwin et al. (1996) reported that age of dispersal on BCNH averaged 55 and 60 days in the two years of their study. Thus, based on an average dispersal age of 58 days, dispersal of juvenile BCNH from IRM began the first week in July and continued through mid-August, with most having reached dispersal age by the end of July; this is about 2 weeks earlier than in 2002. This coincides well with the results of our post-breeding surveys, which showed an earlier decline in numbers of juveniles observed at IRM in 2003 than in 2002 (see below).

Post-breeding Surveys

The number of juveniles observed in 2002 increased dramatically between mid-July and mid-August. The first appearance of juveniles during the survey between June 29 and July 13 coincides rather well with our first observation of flighted juveniles on July 3 while performing nest checks and estimated dates that older chicks attained flight. The number of juveniles observed peaked on August 10, before declining precipitously between August 10 and 24 (Fig. 6). This reduction in juveniles is consistent with our expectation, based on nesting phenology (see below), that 97% of juveniles would have reached dispersal age (~58 days post-hatch) by August 24. The number of juveniles observed peaked earlier in 2003; most young were gone from IRM by mid-August of that year (Fig. 6).

III. NESTING HABITAT

1. General

BCNH have been known to nest at 5 locations at LCW during 1984-2003 (Table 6). The BCNH nested in the phragmites at Big Marsh for 15 consecutive years until prolonged high water levels in 1999 killed the phragmites used for nesting structure. Although the emergent vegetation has recovered, as of 2003 the BCNH have not returned to nest at Big Marsh. Assuming the phragmites cover is currently suitable for BCNH nesting, it is plausible that the tradition of nesting there has not re-developed after a hiatus of several years due to poor habitat conditions.

The cottonwoods at Indian Ridge Marsh were second in importance as a nesting site for this colony over the past 19 years. The herons nested in the cottonwoods along the Calumet River at IRM South (S) during 1987-89; their abandonment of that rookery coincided with nesting by Red-tailed Hawks at that location in 1990. The BCNH began nesting at the cottonwood grove at IRM North (N) near 122nd St. in 1991, but have not nested at that site since 1996 when a pair of Red-tailed Hawks nested there (Table 6). A lack of tree regeneration coupled with the death/poor

condition of larger trees due to beaver damage or high water levels has been suggested as a cause for the failure of BCNH to return to nest in the cottonwoods. A loss of tradition following abandonment of that site due to the presence of nesting Red-tailed Hawks may have also played a role. The phragmites at IRM N and Heron Pond (HP) have become important nesting sites in recent years.

During 2002 and 2003 the northern portion of Indian Ridge Marsh can be characterized as a hemimarsh condition, i.e, irregular-shaped and sized stands of phragmites, from narrow "fingers" and smaller isolated patches to larger blocks of cover. The denser stands were usually not more than 8-10 m wide; some areas of cover were dense enough so as to preclude one viewing through to the other side, other areas consisted of sparse cover of one or a few clumps. Thus, the area where the breeding colony was located was heterogenous with regard to available cover.

The BCNH colony can be characterized as being comprised of three "sub-colonies", each isolated from each other by an expanse of open water (Fig. 7). The northern subcolony was isolated from the nearest shoreline, i.e., to the west, where the closest nests were within ~15 m of shoreline, by the open, deeper water of the ditch bordering the railroad tracks and a screen of Phragmites growth that contained few nests. This subcolony extended onto the peninsula of Phragmites extending to the southeast (Fig. 7). This area had a greater number of nests than the southwestern and especially the eastern colony (personal observation of nesting adults).

The eastern subcolony contained fewest nests, separated from the northern subcolony by approximately 60 m of open water at nearest point (Fig 7). Considerably fewer nests were located here than at the other subcolonies.

The southern subcolony, like the northern, was "isolated" from western shore by the railroad ditch, which was wider at this point than to the north. This portion of the nesting colony was isolated by deeper (i.e., deeper than within the phragmites stands where nesting occurred) open water on all sides, with "narrows" separating the nesting herons from unoccupied emergent cover to the southeast and south (Fig. 7). This area also had a large number of nests (based on observation of adult BCNH), but had a smaller areal extent than the northern and so fewer nests.

Isolation by deeper water and emergent cover may have reduced the incidence of mammalian predation and vandalism/disturbance. Least isolated from shoreline by distance or deep, open water, nests in the eastern subcolony did experience greater predation loss in 2002 than in the monitored portion of northern subcolony.

Nest distribution was generally clumped. There were concentrations of nests in close proximity (some less than a meter apart), generally in areas of sparser cover (i.e., near edges or where Phragmites stems were less dense). Occasional individual nests were located in single clumps of reeds or scattered in denser cover. (There would appear to be a preference for relatively open nest sites, those which may allow nesting BCNH to have a less obstructed view of surroundings and/or provide easy access when approaching or departing the nest. A more open nest would seem to result in greater nest predation risk. However, 1) nest tend to be clumped in these more

open areas and there may be higher survival of individual nests within groups, and 2) there may be greater inclusive fitness in that parents may be better able to avoid predators at some risk of clutch/brood predation.

Although we did not intend to provide a detailed study of nesting habitat of BCNH, we collected a number of nesting habitat parameters to generally characterize the types of cover utilized at this site. Nests were selected systematically across the range of monitored nests. We monitored the change in water levels on a weekly basis at a station near the colony using a meter stick; measurements were taken at different stations in 2002 and 2003, precluding a direct comparison of depths between years.

Height of nest bowl above the water's surface and depth of water at nest (cm) were determined for a sample of nests in May and June of 2002; these parameters were later adjusted for changes in water levels, i.e, to determine maximum and minimum height/depth. We also measured the distance of the nest from the nearest edge, i.e., open water/cover in 2002.

Canopy and horizontal cover were determined at a sample of nests during May and June. Canopy cover was determined over the center of the nest bowl using a spherical densiometer, which uses a gridded mirror to estimate the amount of canopy closure. Horizontal cover (a measure of visual obstruction provided by vertical cover) was determined using a 2.5 m cover pole demarcated into 0.1 m sections.

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2. Water levels at IRM

The pattern of water level changes was similar in both years (Fig. 8). Abrupt declines in water levels at IRM resulted from clearing of debris that accumulated in the water outlet due to rising water levels during high runoff events. Overall the trend in both years was reflective of the typical pattern of precipitation and evaporation in Illinois, i.e., precipitation increasing into May before decreasing, coupled with evaporation increasing through July (unpubl State Climatological data).

In 2002 many of the lower nests were in danger of flooding during high peak water levels (Table 7). The average difference between minimum and maximum heights was nearly 17 cm. Water depth at nests also varied considerably among nests in 2002 (Table 7).

The goal for water level management at IRM for BCNH and other species with similar nesting habits (e.g., Great Egret) should be to emulate the natural cycle of higher water levels in early spring with slow decline to mid- to late-summer lows, and to lessen the possibility of dramatic increases in water levels during high run-off periods. Higher water levels in spring facilitate construction of nests high enough in vertical cover to minimize risks of flooding during high precipitation periods, lower water levels in mid- to late-summer to allow independent juveniles to effectively forage in shallows and along waters edge. Thus, the BCNH are particularly vulnerable to water-level fluctuations, especially during nesting and when juveniles must forage in the natal marsh (i.e., prior to dispersal).

3. Nesting Cover

There was considerable variation in canopy cover at nests during late May (Table 7, 8), a time when most BCNH have initiated clutches. By mid- to late- June, when most nests have one or more nestlings, new Phragmites growth has leafed out and canopy over nests became denser, with less variation (Table 7,8); there was a similar pattern in both 2002 and 2003.

Horizontal cover also increased between May and June in both years (Table 7, 8). In 2002, the range and variation where identical. The minimum percent cover in 2003, although higher than in 2002, was also identical in May and June; the much higher minimum in 2003 may have been the result of sampling error, and sample size for May of that year was rather small.

Egg survival (the number of eggs laid / number hatched) was not correlated with the amount (%) of canopy cover in June 2002 ($r= -0.53$, $P= 0.21$, $n= 7$) but was in June of 2003 ($r= 0.67$, $P= 0.004$, $n= 16$). Chick survival (number eggs hatched / chicks surviving 15 days) was moderately correlated with the amount (%) of horizontal cover in May 02 ($r= 0.62$, $P= 0.04$, $n= 11$); there was a nearly identical relationship in 2003, however sample size was small and the relationship was not significant ($r= 0.63$, $P= 0.37$, $n= 4$). Nest survival (number eggs laid / number young surviving 15 days) in 2002 was correlated with the amount of horizontal cover in May ($r= 0.70$, $P= 0.025$, $n= 10$). In 2003 nest survival was not significantly correlated with horizontal cover ($r= 0.44$, $P= 0.56$, $n= 4$), but was correlated with canopy cover in May of that year ($r= 0.56$, $P= 0.023$, $n= 16$)

There was considerable variation in the distance of nests from the edge of cover (Table 7). Some nests were exposed and isolated from stands of cover, with cover provided only by the Phragmites stems supporting the nest. Some nests were located approximately equidistant from open water in the denser cover of the larger stands. However, it appeared that preferred locations were those that allowed pairs to see their surroundings, and perhaps provided easy access when flying to and from nest. There was a high density of nests on edges or where cover was light to intermediate in density (stems); perhaps there is a trade-off between predator detection and concealment.

It would appear, based on previous studies, that the impact of nest location on nest success in colonial species varies across species, habitat characteristics, and annual differences in predator populations, weather, etc.. For example, hatching success was not greater in cattle egrets (Bubulcus ibis) nests centrally located within a colony as compared to those located on the periphery, although chick survival was greater in peripheral nests in one of four years Ranglack and Angus (1991). Cattle Egrets, which nested higher in trees, experienced greater nest success than Little Egrets which nested lower in the same trees (Hilaluddin et al. 2003).

Survival of Great Egret chicks was positively correlated with nest density (Ranglack and Angus 1991), whereas in another study survival of BCNH chicks was not affected by nest placement (height in tree and nearest neighbor), and Little Egrets were marginally affected (Kazantzidis et al. 1997). Our data did not permit us to rigorously test whether nests centrally located in clusters

of nests, or those isolated from such clusters, were more successful. To our knowledge this has not been examined in dense colonies of wading birds nesting in emergent cover.

Although we were not able to conclusively determine what habitat characteristics were being selected for (preferred), it appeared that sparse to moderately open cover (not thick cover, i.e., high stem density) was preferred. Cover becomes thicker later in the season as young begin to hatch, providing additional protection from aerial predators and the summer sun. The vegetation management goals for the conservation of BCNH, Great Egrets, and others at IRM should be to promote stands of stout emergent vegetation in a hemimarsh condition, allowing selection of nest sites along a continuum of habitat characteristics.

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V. ACKNOWLEDGMENTS

We gratefully acknowledge the following for their assistance: Tom Barnett, ISPAT International; Robert Page, EarthTech; Marv Piwoni, Julie Hafermann, WMRC; Suzanne Malec and Nicole Kamins, City of Chicago Dept. of Environment (DOE); John Dassow and Jyotsana Agarwal, INHS; Tom Custer, USGS; foraging survey volunteers. This project was funded by the Illinois Waste Management and Research Center, City of Chicago Department of Environment, and the Illinois Wildlife Preservation Fund (Illinois Department of Natural Resources); Three Rivers Environmental Assessments and the Illinois Department of Transportation - Aerial Surveys contributed services in-kind.

Table 1. Number at first sighting by week of occurrence of Black-Crowned Night Herons at Lake Calumet Wetlands, 1993-2003.

Year	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
1-7 March							No Data				
8-14 March		1									
15-21 March										16	
22-28 March			14		40	3		2			18
29 March-4 April	25			17					75		

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Table 2. Clutch size for Black-crowned Night-Herons nesting at Indian Ridge Marsh (IRM) Heron Pond (2002 only), and Inland Steel, 2002 and 2003, and as reported in previous studies.

Location/Status	n	Mean	S.E.	Min-Max	Mode	Comments	Citation
IRM 2002 - all clutches 2002	40	3.40	0.11	2-5	3	includes second clutches; nearly bimodal (3- and 4-egg clutches)	this study
IRM 2002 - first clutches only	36	3.42	0.12	2-5	3/4	excluding second clutches; bimodal	this study
IRM 2002 - first clutches ≤ 13 May	18	3.56	0.17	2-5	4	initiated prior to or on median date; nearly bimodal (3- and 4-egg clutches)	this study
IRM 2003 - all clutches		3.36					this study
IRM 2002- first clutches > 13 May	17	3.08	0.19	2-4	3	initiated after median date	this study
Heron Pond 2002	21	2.95	0.15	2-4	3	longer nest check interval and later nest initiation than IRM	this study
ISPAT Inland Steel, IN 2002 - first clutches	13	3.38	0.21	3-5	3	tree nests; exposure to environmental contaminants likely	this study
ISPAT Inland Steel, IN 2003		3.06				tree nests; exposure to environmental contaminants likely	this study
Maryland	69	3.46	NA	2-5	4	nearly bimodal (3- & 4-egg clutches); shrub/tree nests; no apparent impact environmental contaminants	Rattner et al., 2001
Oregon and Washington - all clutches	260	3.45[1,2]	0.08-0.11	2-5	NA	means 3.10 to 3.92; shrub/tree nests; no apparent impact environmental contaminants	Blus et al., 1997

Oregon and Washington - first clutches	208	3.47[1,2]	0.08-0.12	2-5	NA	means 3.09 to 3.93; shrub/tree nests; no apparent impact environmental contaminants	Blus et al., 1997
Idaho	281	3.6[1,2]	0.08-0.13	1-6	3/4[2]	means 3.4 to 3.7; shrub/trees; DDE impacts on reproduction	Findholt and Trost, 1985
NV, OR, WA - ≤ 8 ppm DDE	133	3.75[1,2,3]	0.15[1]	NA	NA	means 3.67 to 3.80; shrub/tree nests; no apparent impacts environmental contamination	Henny et al., 1984
NV, OR, WA - > 8 ppm DDE	50	3.17[1,2,3]	0.19[1]	NA	NA	means 3.00 to 3.41; shrub/tree nests; DDE impacts on reproduction	Henny et al., 1984
Massachusetts and Rhode Island	346	3.79[1,2,3]	NA	2-5	4	means 3.66 to 3.96; possible DDE impacts on reproduction	Custer et al., 1983
North Carolina	121	3.32[1,2,3]	NA	2-5	3	means 3.08 to 3.50; no apparent impacts environmental contaminants	Custer et al., 1983
Quebec	98	4.1[1,2,3]	0.1	1-6	NA	means 3.9 to 4.2; shrub/tree nests; no apparent impacts environmental contaminants	Tremblay and Ellison, 1979
Alberta - first clutches	116	3.60[1,2,3]	NA	1-6	3/4[2,3]	means 3.2 and 4.0; emergent vegetation nests	Wolford and Boag, 1971
CA - pre-1947	35	3.86	NA	NA	NA	unknown status	(see Henny et al., 1984)
NV, OR, WA - pre-1947	41	3.80[1,2,3]	NA	2-5	NA	unknown status	Henny et al., 1984
Utah - pre-1947	41	4.1	0.13	2-6	4	unknown status	Findholt and Trost, 1985

1 Grand mean; 2 Multiple colonies; 3 Multiple years

Table 3. Reproductive parameters in Black-crowned Night-Heron colonies in the Calumet region of Illinois and northwestern Indiana, 2002 and 2003.

Location	Indian Ridge Marsh -This Study 2002	Inland Steel -This Study 2002	Heron Pond -This Study 2002	Indian Ridge Marsh -This Study 2003	Inland Steel -This Study 2003	Maryland -Rattner et al. 2001	Pacific Northwest -Blus et al. 1997	San Francisco Bay - Hothem et al. 1995	East Coast -Custer et al. 1983
Egg Laying and Incubation (n)	46	15	28	55	17	69		485	531
Daily Survival Rate	0.9895	0.9855	0.9958	0.9985	0.9931	0.9936	0.9240-0.9934[4]		
Survival Rate to Hatch	0.7762[1]	0.7043[1]	0.9039[1]	0.9635	0.8474	0.8517[2]		0.475-0.701	0.547-0.902[2]
Nestling Period (n)	37	13	18	52	15	59		395	(<531?)
Daily Survival Rate	0.9922	0.9864	0.9830	0.9899	0.9809	0.9911	0.9782-1.000[5]		
Survival Rate to Fledging	0.8892[3]	0.6245[3]	0.7730[3]	0.8593	0.7485	0.8745[3]		0.691-0.896	0.758-1.000[3]
Nest Success	0.6920	0.4398	0.6987	0.8280	0.6343	0.7448			0.530-0.867
Probability of Egg Hatching	0.8860	0.6852	0.9643	0.9195	0.8333	0.8933			0.852-0.917
Probability Young Survived 15 days	0.8365	0.5143	0.5636	0.8688	0.7714	0.8917			0.771-1.000
Egg Success	0.5115	0.1550	0.3432	0.6614	0.4078	0.5932			0.385-0.716
Mean Clutch Size	3.40	3.38	2.95	3.36	3.12	3.46			3.08-3.96
Mean Number of Young Surviving 15 Days	1.74/nest	0.52/nest	1.02/nest	2.22/nest	1.27/nest	2.05/nest	0.41-1.83/nest	1.45-2.04/nest	1.19-2.65

1= 24 day laying and incubation period; 2= 25 day laying and incubation period; 3= 15 days post-hatch; 4= 27 day laying and incubation period; 5= 14 days post hatch

Table 4. Fate of Black-crowned Night-Heron Clutches/Broods at Indian Ridge Marsh, Cook County, Illinois, 2002.

Fate of Clutch	Number of Clutches	Percent of Clutches
Successful[1]	34	66.7
Eggs Rolled Out, Poorly Constructed Nest	7	13.7
Depredated[2]	5	9.8
Abandoned[3]	3	5.9
Eggs/Nestlings Gone, No Signs	2	3.9

1- at least one nestling survived to 15 days post hatch
2- one clutch assumed abandoned after one egg depredated
3- one damaged egg abandoned

Notes-
a) another 4 broods known to completely fail after 15 days
b) determined minimum 6 predation events (clutch/brood or single eggs/chicks)
c) 11 chicks dead in nest= 3 depredated (same nest), 8 unknown fate

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Table 5. Fate of Black-crowned Night Heron Clutches/Broods at Indian Ridge Marsh, Cook County, Illinois, 2003.		
Fate of Clutch	Number of Clutches	Percent of Clutches
Successful[1]	52	94.5
Eggs Rolled Out, Poorly Constructed Nest	2	3.6
Depredated	0	0
Abandoned	1	1.8
Eggs/Nestlings Gone, No Signs	0	0

1- at least one nestling survived to 15 days post-hatch
2- no confirmed or suspected predation of marked nests

Notes-
a) 23 chicks failed to survive 15 days= 21 missing, 2 chicks dead in nest (1 accidental death, 1 unknown cause of mortality)

Table 6. Known Black-Crowned Night-Heron Nesting Locations at Lake Calumet Wetlands, 1984-2003																				
Year	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
IRM phragmites										x	x					x	x		x	x
IRM S cottonwoods				x	x	x														
IRM N cottonwoods								x	x	x	x	x								
Big Marsh	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x					
Heron Pond												x	x					x	x	

Table 7. Black-crowned Night Heron Nesting Habitat Parameters at Indian Ridge Marsh, 2002

Parameter	N	Mean	Min-Max	SD
Min Hgt Nest Bowl Above Water (cm)	18	19.8	10.5-36.6	6.9
Max Hgt Nest Bowl Above Water (cm)	18	36.6	27.3-53.4	(6.9)
Min Water Depth at Nest (cm)	22	39.6	10.8-59.9	13.9
Max Water Hgt at Nest (cm)	22	55.7	27.6-76.7	14.5
% Canopy Cover at Nest - May	21	75.0	25.1-100	26.0
% Canopy Cover - June	7	98.8	91.7-100.0	3.1
Distance Nest to Edge (m)	18	1.77	0-4.20	1.17
% Horizontal Cover* - May	14	62.6	0-100	31.0
% Horizontal Cover* - June	13	80.6	0-100	36.6

* Percentage of 25 0.1 m pole sections more than 25% obscured

Table 8. Black-crowned Night Heron Nesting Habitat Parameters at Indian Ridge Marsh, 2003				
Parameter	N	Mean	Min-Max	SD
% Canopy Cover at Nest - May	12	71.2	25.0-91.7	24.0
% Canopy Cover - June	19	87.0	50.0-100.0	16.3
% Horizontal Cover* - May	4	68.0	52.0-88.0	17.0
% Horizontal Cover* - June	16	76.0	52.0-100.0	17.8

* Percentage of 25 0.1 m pole sections more than 25% obscured

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Figure 1. Black-crowned Night Herons numbers observed during evening surveys, 2002 and 2003.

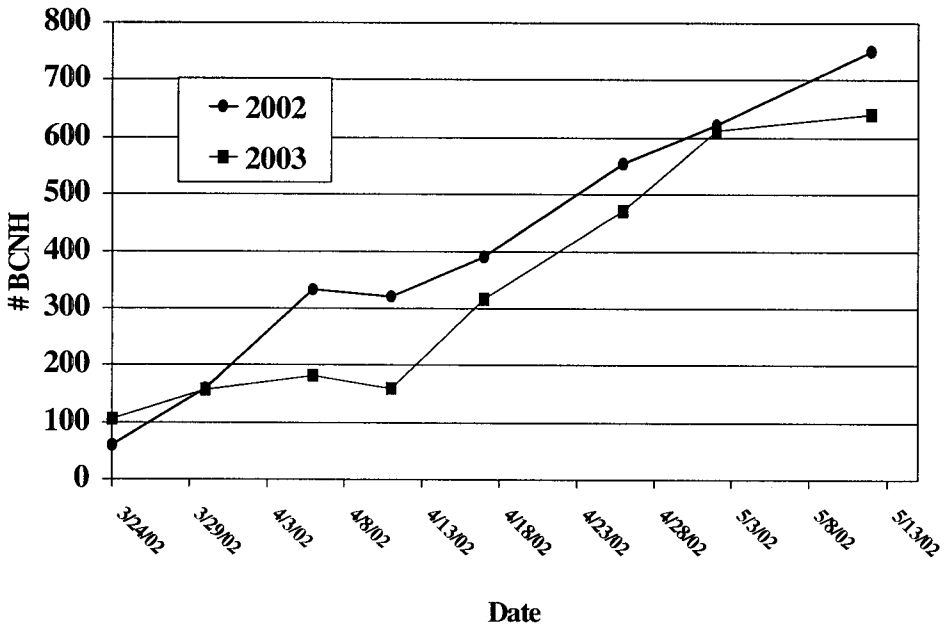

Figure 2. Peak numbers of Black-crowned Night Herons at Lake Calumet Wetlands, 1992-2003.

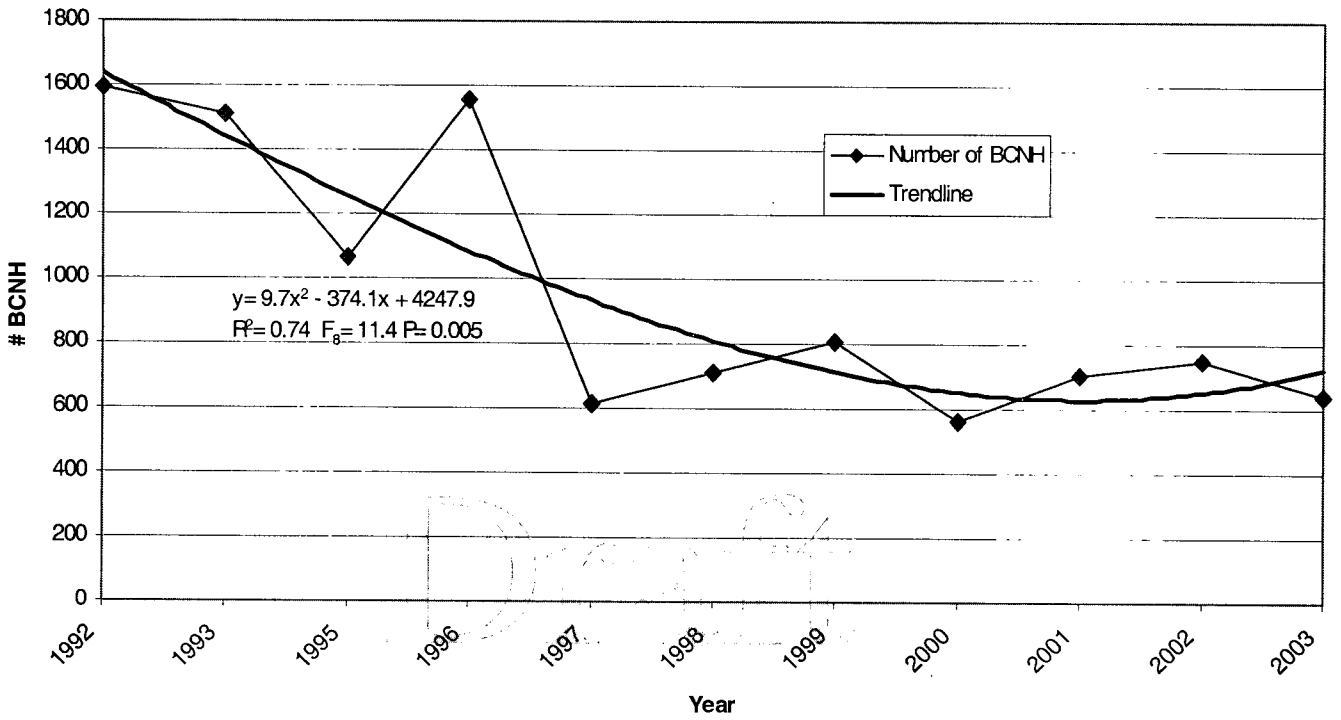

Figure 3. Estimated Number of Black-Crowned Night Heron Nests at Lake Calumet-area Wetlands, 1985-2003

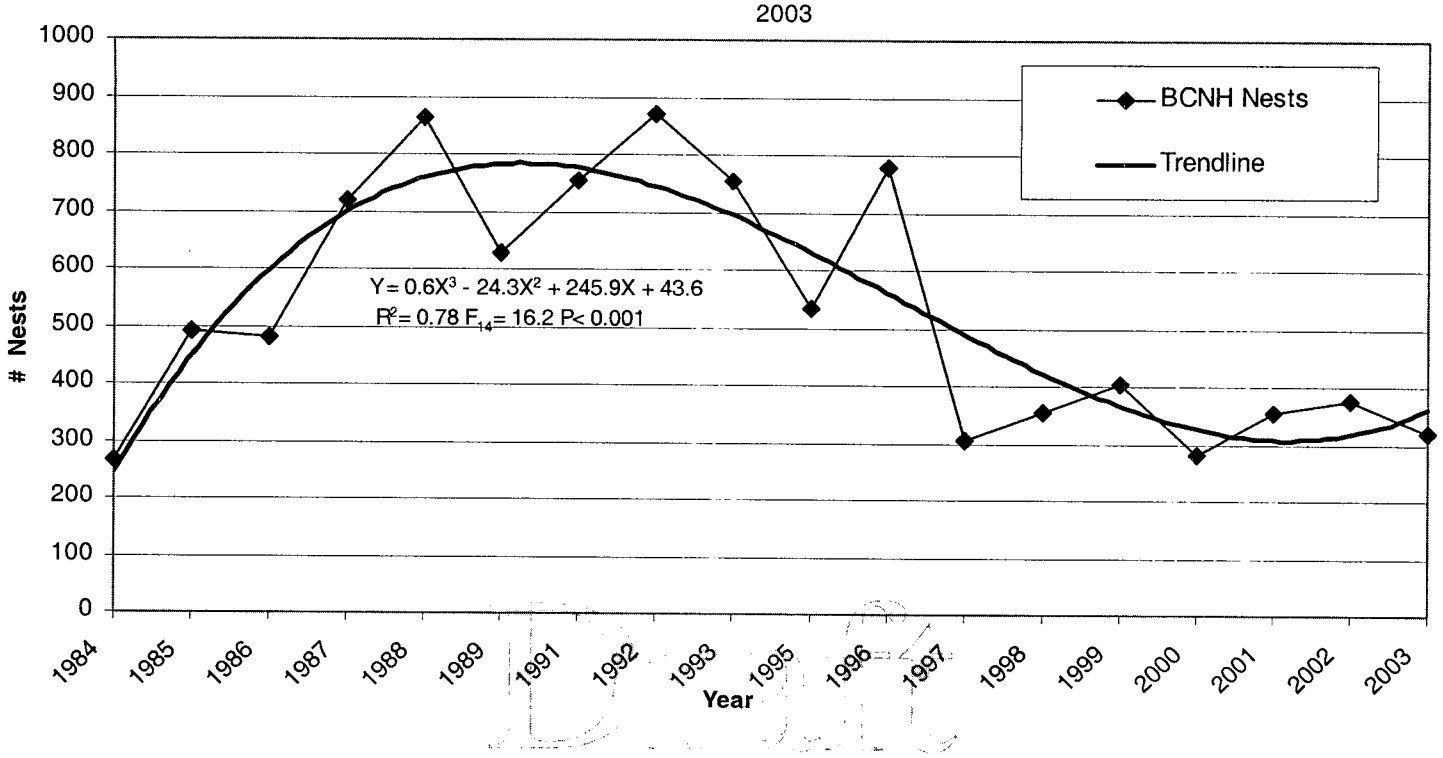

Figure 4 Nesting Phenology of Lake Calumet Black-crowned Night Herons, 2002

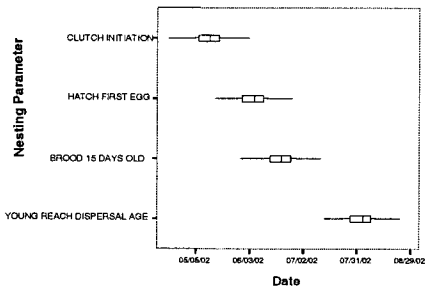

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Figure 5. Nesting and fledging phenology of Lake Calumet Black-crowned Night Herons, 2003

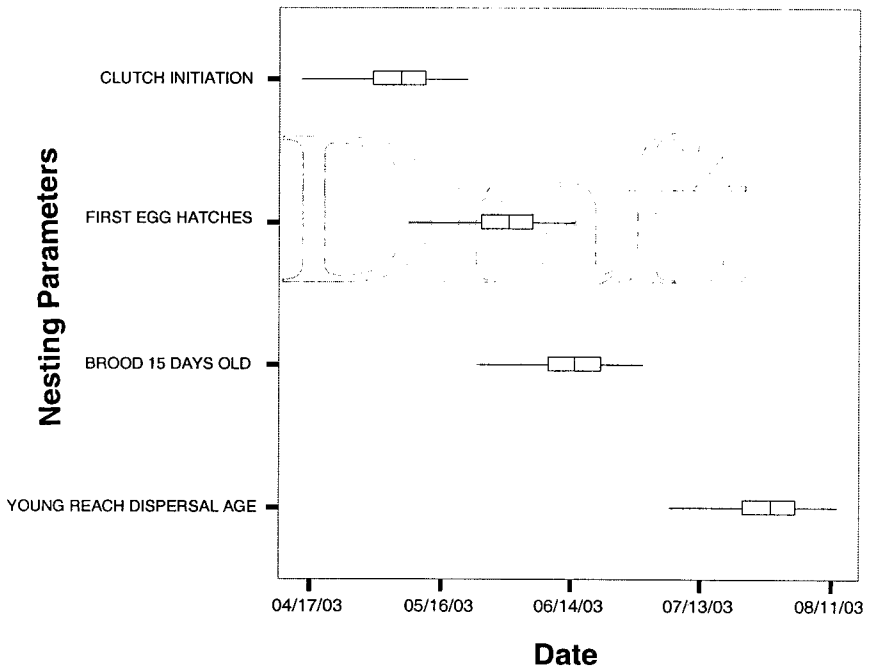
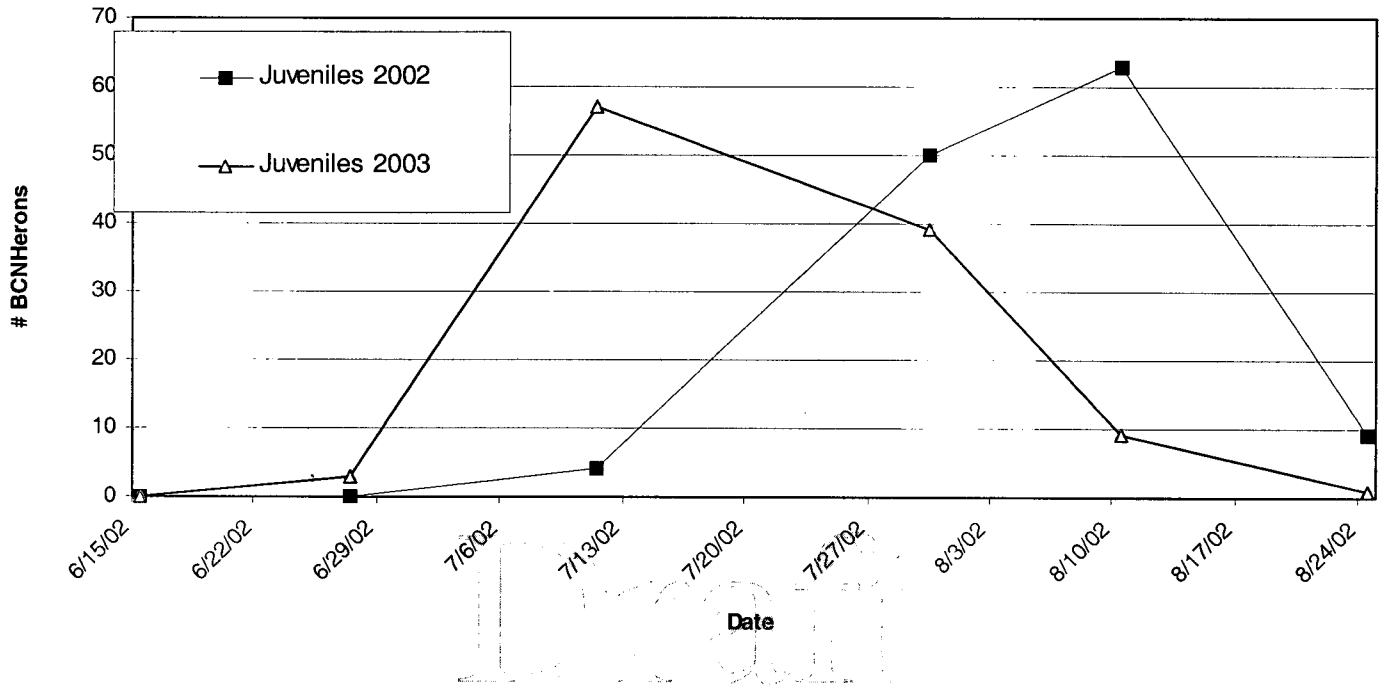


Figure 6. Numbers of juvenile Black-Crowned Night Herons observed at Indian Ridge Marsh during 2002 and 2003 post-breeding surveys



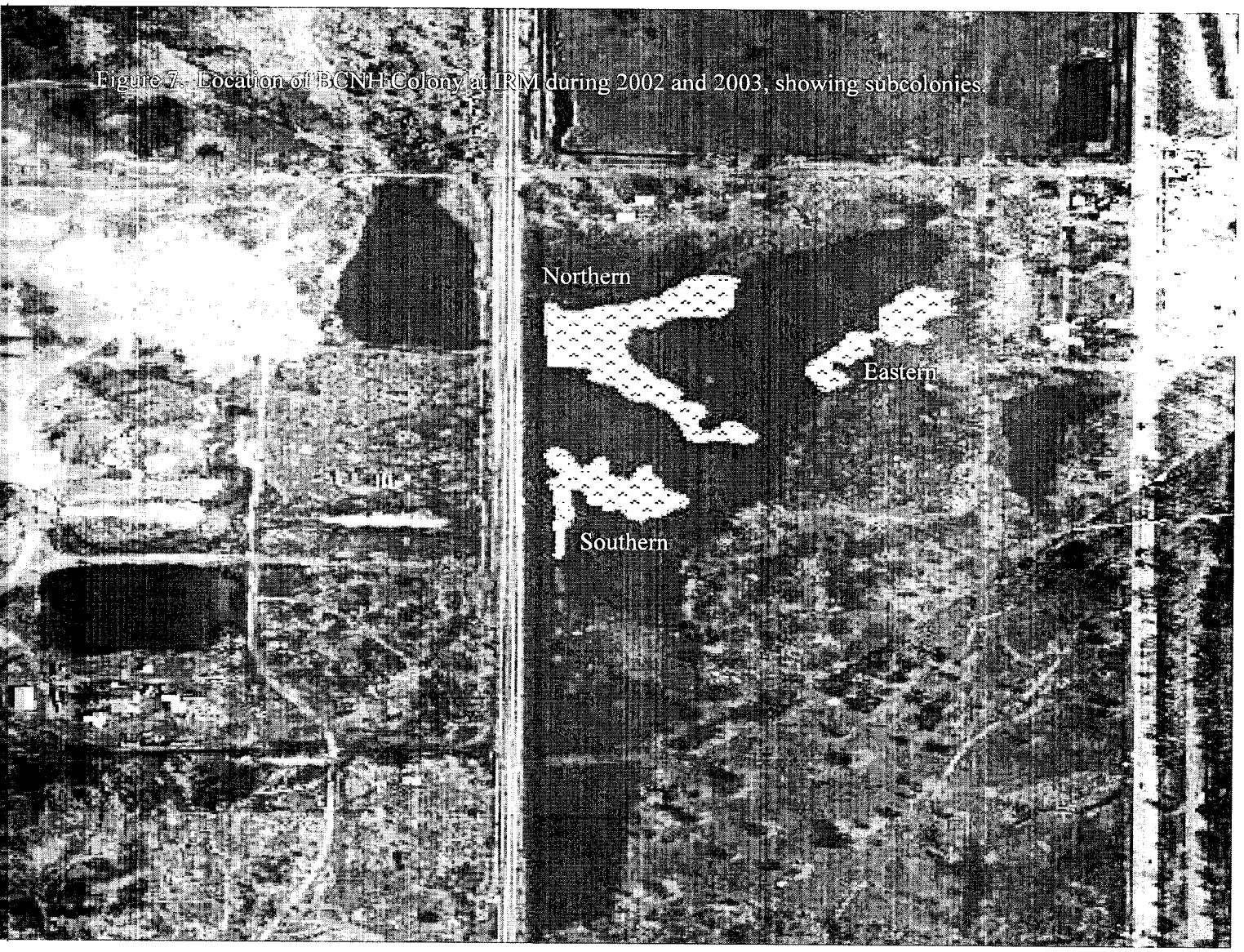
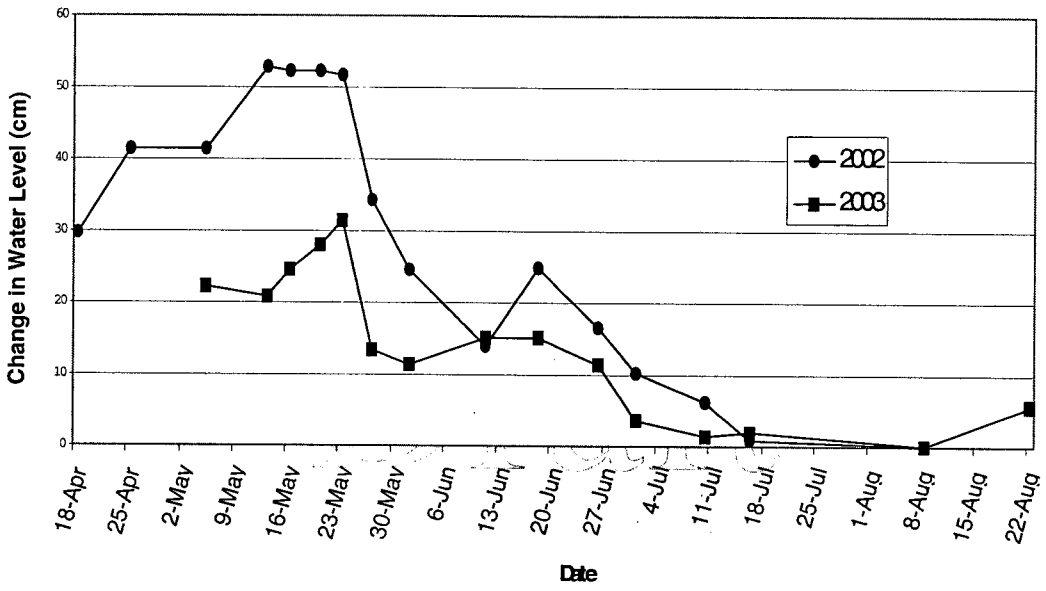


Figure 7. Location of BCNH Colony at IRM during 2002 and 2003, showing subcolonies.

Figure 8. Changes in Water Levels at IRM during 2002 and 2003



Selected Contaminants and Biomarkers of Exposure in Black-Crowned Night-Heron (*Nycticorax nycticorax*) embryos from Lake Calumet, Chicago, Illinois

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Abstract

We examined a suite of organic and inorganic analytes in a sample of Black-crowned Night-Heron (BCNH) embryos collected from a colony located adjacent to Lake Calumet in southeastern Chicago, Illinois, in 2002. Our objectives were to determine whether embryos from the Lake Calumet colony had greater exposure to environmental contaminants, when compared to samples from other contemporary populations, and if such exposure was great enough to produce changes in selected endpoints.

BCNH embryos from the Lake Calumet colony had greater exposure to PCBs, DDE and other organochlorine pesticides, cobalt, and selenium than those from colonies in northwestern MN and coastal VA. Contaminant levels in IL embryos were consistent with those reported for some colonies located in industrialized urban areas, though were not among the highest reported.

Activities of Cytochrome P450 monooxygenase proteins EROD and BROD were greatly increased in IL embryos, compared to reference colonies. ∑PCB concentrations were associated with EROD/BROD induction. ∑PCBs, DDE, transnonachlor, Co, and Se in combination were associated with variation in cellular DNA content; DDE contributed most to this relationship. Measures of oxidative stress did not differ among colonies, however, GSSG activity was elevated and associated with concentrations of the suite of elements examined in this study.

Concentrations of BZ#77, the most toxic of the PCB congeners we examined, in some IL specimens exceeded lower observed effects levels established for chickens. The concentrations of Se in eggs of BCNH from IL approached published effects thresholds. BCNH are relatively resistant to the effects of contaminant exposure; although there was increased exposure in this colony we did not observed any dramatic effects on the health of the embryos. Contaminants at these levels in eggs may be of concern in piscivorous waterbird species that are more sensitive to contaminant exposure than BCNH.

Introduction

The Calumet region of southwestern Lake Michigan was once a vast complex of glacial lakes, wetlands, and sand prairies. This region is now one of most heavily-industrialized in the US, and has been greatly impacted through industrial activities, waste disposal and discharge, urbanization, and changes to surface and groundwater hydrology. In spite of extensive habitat loss and degradation the area remains among the most biologically diverse in the state.

Sediments and other environmental media in this area are contaminated with heavy metals and organic compounds. Sinars (1999) determined that sediments at the Lake Calumet "cluster site" had elevated concentrations of several metals and PAHs. Cahill et al. (1999) found elevated concentrations of some metals, non-metallic elements, and PAHs in sediments along the West Branch of the Grand Calumet River. Based on concentrations of contaminants in sediments and chemical benchmarks, Ingersoll and MacDonald (1999) determined that total PCBs, chlordane, total DDT, heptachlor, and lindane in sediments along the West Branch posed risks to piscivorus wildlife.

A breeding colony of state-endangered Black-Crowned Night-Herons (BCNHerons) inhabits the marshes adjacent to Lake Calumet. This is one of the largest remaining nesting colonies of this species in the state, comprised of an estimated 750 BCNHerons in 2002. This high-profile population is of considerable interest to resource professionals, environmental groups, and the public.

Individuals from this population are thought to forage throughout the south Chicago region, including wetlands around Lake Calumet and along the Little and Grand Calumet Rivers, areas characterized by elevated concentrations of environmental contaminants in sediments. Some of these contaminants may be bioavailable to BCNH through bioaccumulation and transfer in the aquatic food chain.

Herons and egrets have been used extensively as bioindicators/biomonitors of environmental contamination (e.g. Blus et al. 1985; Custer et al. 1997; Fleming et al. 1984; Halbrook et al. 1999; Sundlof et al 1994). Their trophic position and aquatic foraging habits may put them in contact with prey that accumulate/bioconcentrate high concentrations of environmental contaminants such as organochlorine pesticides, PCBs, and metals found in sediments in the Calumet area. A variety of effects of exposure to such contaminants have been documented in BCNHerons, including eggshell thinning and reduced productivity (Price 1977; Ohlendorf et al. 1978; Henny et al. 1984; Findholt and Trost, 1985), hatching success (Custer et al. 1983), reduced embryo weights (Hoffmann et al. 1986), responses in biochemical markers of exposure (Rattner et al. 1997; 2000), possible teratogenic effects (Hothem et al. 1995), and cytogenetic damage (Custer et al. 1994).

The objectives of this study were to: 1) determine whether BCNH embryos from the Lake Calumet colony were exposed to elevated concentrations of environmental contaminants, 2) compare concentrations of selected environmental contaminants in embryos with other contemporary populations, and 3) whether such exposure, if it occurred, was great enough to produce changes in selected physiological endpoints.

Methods

In 2002, BCNH eggs (one per nest) were collected from Agassiz National Wildlife Refuge, MN, Lake Calumet-area marshes, Chicago, IL, and Chincoteague National Wildlife Refuge, VA. Eggs were placed in an incubator and removed at piping. Embryos were removed from the egg, weighed (±0.1 g), and euthanized by decapitation (Anonymous, 1993).

Blood for flow cytometry was collected in heparinized capillary tubes, aspirated into freezing media (Hams F10 media with18% fetal bovine serum and 10% glycerin), inverted several times, frozen in liquid nitrogen, and later transferred to an ultracold freezer and stored at -80°C until analysis. Immediately after death, the liver was removed, weighed (±0.1 g) and about 0.3 g placed into a cryotube for measurement of monooxygenase activity and another 0.3 g from the same nestling was placed into a cryotube for measurement of oxidative stress. After a few drops of glycerin were added to the cryotube for monooxygenase activity, both cryotubes were placed into liquid nitrogen. The cryotubes were later transferred from the liquid nitrogen to an ultracold freezer (-80°C) for storage until processing.

Hepatic microsomes were prepared from homogenates of thawed liver samples by differential centrifugation. The 11,000-*g* supernatant was centrifuged at 40,000 rpm for 60 min to obtain the microsomal pellet. Each 100,000-*g* pellet was resuspended in 2.0 ml/g of tissue weight of 0.05 M Na/K PO_4, 0.001 M disodium ethylenediamine tetraacetate, pH 7.6. Ethoxyresorufin-*O*-dealkylase (EROD) activities were assayed by the methods of Burke and Mayer (1983) as adapted to a fluorescence microwell plate scanner (Melancon, 1996). The 260-µl total assay volume contained microsomes equivalent to 0.65 mg liver, 2.5 µM substrate and 0.125 mM NADPH in 0.066 M Tris buffer, pH 7.4.

Microsomal protein was determined using the bicinchoninic acid method adapted to a microwell plate (Sorenson and Brodbeck, 1986). Ethoxyresorufin-*O*-dealkylase activities were calculated as pmol product/min/mg microsomal protein.

BCNH livers were analyzed for five measures of oxidative stress. Basic methods and assay conditions are described by Hoffman and Heinz (1998). Indicator assays included reduced glutathione (GSH), total sulfhydryl (TSH), protein bound thiol (PBSH), thiobarbituric acid reactive substances (TBARS), oxidized glutathione (GSSG), and GSSG/GSH. For flow cytometry, nuclear preparation and staining followed the method of Vindelov et al. (1983). Nuclear suspensions were prepared following lysis with detergent and trypsin digestion. Nuclear suspensions were treated with RNAse, and stained with propidium iodide. Nuclear deoxyribonucleic acid (DNA) content was analyzed in 10,000 cells per individual on a Coulter Elite flow cytometer by quantification of nuclear fluorescence. The half-peak coefficient of variation (HPCV) of the G1 peak was recorded for each individual. This value expresses the amount of variation in the DNA content among the 10,000 cells measured. Somatic chromosome damage is suggested when the HPCV in a population is significantly greater than the HPCV in a reference population (Bickham, 1990, 1994).

Samples for chemical analysis were ground under liquid nitrogen and mixed to achieve homogeneity; homogenates were stored frozen. Representative portions of each homogenate were freeze dried to determine percent solids. For metals analysis, a nitric acid microwave digestion procedure, equivalent to US EPA Method 3051, was utilized to dissolve sample

homogenates into solution for total metals analysis. Samples were digested into solution in six microwave batches. Results for most metals were obtained by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using Scandium, Niobium, Lanthanum, and Thorium as internal standards. Mercury results were obtained by Atomic Fluorescence. Quality control (QC) samples were prepared in each digestion batch in the same manner as embryo samples. Batch QC measures included digested reagent blanks, digested duplicates, analytical duplicates, matrix spikes, analytical spikes, and Standard Reference Materials (SRMs) traceable to NIST. NIST Bovine Liver SRM #1577b was prepared with the first three digestion batches and Dogfish Muscle, DORM-2, from the National Research Council Canada, was prepared with the last three digestion batches.

A sub-sample of each ground specimen was removed and extracted with organic solvents. The solvent extracts were concentrated, evaluated for lipid content and taken through two clean-up steps. During these clean-up procedures, the majority of the lipids were removed and the analytes of interest were separated into two fractions based on their chemical properties as well as the polarity of the fractionation solvents.

The appropriate fraction was analyzed for either PCBs or chlorinated pesticides using GC-ECD, which is selective for halogenated compounds. Sample fractions were then evaluated using GC-MS to confirm results obtained by GC-ECD. In some cases the GC-MS was able to confirm the presence of analytes that were not picked up on the GC-ECD due to interferences. Additionally, GC-MS was used to differentiate and quantitate some of those analytes that co-eluted on the GC-ECD. In some instances, interferences associated with the ECD for BZ#74, BZ#138&163, BZ#14, BZ#65, BZ#155 and p'p'-DDD made it necessary to report results from the GC/MS confirmation analysis. Interferences associated with the ECD analysis were also observed for the co-eluting analytes, Heptachlor epoxide and Oxychlordane. In some instances, a value for Heptachlor epoxide could not be calculated since the quantitation is based upon both ECD and GC/MS results.

Upon ECD evaluation it was noted that the embryos from all three sites contained unidentified peaks; however, the embryo samples from the Illinois sites contained larger amounts of these unidentified peaks. A mass spectral library search was used to determine that these compounds matched mass spectra correlating to tetrachlorobiphenyls, pentachlorobiphenyls, hexachlorobiphenyls, heptachlorobiphenyls, and octochlorobiphenyls. In addition, another non-target peak was identified to match mass spectra associated with a chlordane compound other than gamma-chlordane or alpha chlordane.

QC samples were processed and evaluated with each extraction batch. QC samples included reagent blanks, duplicate spiked samples, and Standard Reference Material (SRM) NIST 2978, Mussel Tissue (Organic Contaminants-Raritan Bay, New Jersey). Additionally, surrogate analytes were added to all blanks, samples, and QC samples prior to extraction. The calculated recovery was used to assess accuracy and was an indication as to how well analytes would recover, if present, in the presence of the sample matrix. There were no corrections or adjustments to sample results based on surrogate recovery. Analytical spikes were prepared from post-extracted sample solutions. The recovery of analytes from this preparation was used to evaluate the accuracy of sample results as well.

Concentrations less than the lower reporting limit were entered as ½ the lower reporting limit.

Analytes in samples with no instrument response were entered as 0.0. Means were not compared statistically if concentrations in < 60% of cases were not detectable. Normality was assessed using the Kolmogorov-Smirnov statistic with Lilliefors Significance Correction, the skewness statistic, and visual examination of frequency histograms. Levene's test was used to assess homogeneity of variance. Subsequently all organic and inorganic analytes were lg10-transformed prior to statistical analysis. Means were compared via ANOVA with pairwise comparisons using the Bonferroni correction. Relationships among variables were examined using simple correlation and linear regression analysis. P-values of ≤ the alpha level of 0.05 were considered significant. Alpha levels were adjusted (0.05/# of tests) for multiple one-way ANOVAs and simple correlations to account for non-independence among parameters, e.g., metal concentrations in the same egg.

Results

Quality Control -Elemental Analysis

Overall, the quality control for elemental determination in samples was acceptable. For As, Cd, Co, Pb, Hg, Rb, Se and Tl, digestion blanks were absent of contamination, duplicates were reproducible and accuracy parameters (spikes and Standard Reference Materials) recovered well. Duplicates and accuracy parameters for Cu, Mn, and Zn were acceptable as well. Sporadic contamination of Mn and Zn was observed in the digestion blanks while copper was consistently present in a narrow range in all five batch blanks. However, contamination levels observed for these elements in blanks were extremely low relative to sample concentration levels. Thus, sample results for As, Cd, Co, Pb, Hg, Rb, Tl, Mn, Cu and Zn pose an uncertainty of no more than ± 20% or the detection limit, whichever is greater.

All of six matrix spikes recovered poorly for Ag (22-39%) and five out of the six matrix spikes recovered poorly for antimony (29-63%). The most probable cause for this is precipitation of metal species, i.e. silver chloride. However, this did not affect our interpretation of either Ag or antimony in samples because both elements were spiked at fairly high levels (~8 mg/kg) where they will likely to precipitate, while both elements were either not observed or only present at low concentrations in the samples. In addition, Ag recovered reasonably well at low levels (0.04 mg/kg) in the standard reference material. Both elements also recovered well in analytical spikes confirming that low-level results for these elements are reliable. Ba, Cr, and Ni were also observed in a majority of digestion blanks at concentrations that are high relative to those found in the samples. While duplicates generally reproduced well and accuracy parameters recovered, sample results for these elements are likely biased high by amounts similar to that found in the blanks.

Quality Control- Organic Compounds

The reagent blanks were generally free of low-level contamination, duplicates were reproducible and analytes, based on surrogate, spike, and SRM results, recovered well. Reagent blanks were very clean with low-level contamination occurring in only two instances. Congener BZ#153 was observed in several batches but at levels that were very low compared to sample results. Congener BZ#74 was observed in one batch at concentrations that were similar to those of four

of eight samples in that batch. Three surrogates, BZ#s14, 65, and 155, were evaluated in each blank, sample, and QC sample. The mean recovery of surrogates BZ#14, 65, and 155 in the samples was 63%, 64%, and 65%, respectively. The RSD for these surrogates was 15%, 21%, and 21%, respectively. With three exceptions the overall mean spiked recovery for PCB congeners was good, with a range of 70%-85%. BZ#74 recovered at 96% and BZ#200 recovered at 101%. Incomplete resolution of BZ#200 from the subsequent analyte peak may have contributed to the elevated recovery result. The elevated recovery result of BZ#74 may have been due to unknown co-eluting compounds which were observed upon GC-MS evaluation. Congener BZ#153 had an overall recovery of 66%. The relative standard deviation (RSD) for recoveries was less than 23% with four exceptions; BZ#s 18, 74, 153, and 200 resulted in RSDs greater than 30%. The analytical spike recovery was excellent; the overall percent recovery for all congeners ranged from 86%-111%. The overall reproducibility for all congeners was good with RPDs of less than or equal to 25%. The mean percent recovery of congeners in the SRM was acceptable with recoveries ranging from 79%-129% with four exceptions. Two mean recovery results were low, with BZ#180 recovering at 43% and BZ#31&28 recovering at 44%. Two mean recoveries were high, with BZ#105 recovering at 175% and BZ#77&110 recovering at 146%. Because the SRM was not the same matrix as the samples, the values obtained for the SRM were not weighted as heavily as results from other QC parameters when interpreting uncertainty of reported sample results. Based upon the evaluation of the QC samples, the reported results are estimated to be $\pm$ 30% of the true value with two exceptions. Congeners BZ#200 and BZ#74 are estimated to be $\pm$ 50% of the true value.

The QC parameters for the chlorinated insecticides heptachlor epoxide, oxychlordane, alpha-chlordane, gamma-chlordane, dieldrin, transnonachlor, 4,4'DDD, and 4,4'DDE were acceptable. The reagent blanks were generally free of low-level contamination, duplicates were reproducible and analytes, based on surrogate and spike results, recovered well. QC results for Lindane, Heptachlor, and 4,4'-DDT were poor due to low recovery (<12%) or absence of analyte recovery, in the spiked samples. Reagent blanks were very clean, with only sporadic, low-level contamination. Low-level 4,4'-DDE contamination was seen in some of the blanks but was extremely low in relation to the sample results. Transnonachlor contamination was seen at <0.7 ng/g levels in one batch. In another batch, reagent blank-2 had transnonachlor contamination of 1 ng/g. Low-level 4,4'-DDT was observed in one batch at concentrations <0.7 ng/g.

The surrogate used to evaluate the chlorinated insecticides was tetrachloro-m-xylene (TCMX). The mean recovery of TCMX in the samples was 51% with an RSD of 10%. Overall recovery of heptachlor epoxide, oxychlordane, alpha-chlordane, gamma-chlordane, dieldrin, transnonachlor, and 4,4'DDD in the spiked samples ranged from 58%-84% with RSDs less than or equal to 23%. The recovery of 4,4'-DDE was higher, with a mean of 99% and an RSD of 54% as was 4,4'-DDD; recovering at 134%. Lindane, heptachlor, and 4,4'-DDT had mean recoveries of 7%, 11%, and 4%, respectively. Recovery of these three analytes was acceptable in the silica gel clean-up QC sample and the spiked reagent blank, the latter of which was taken through the entire process along with the samples. Since the spiked reagent blank, void of sample matrix, was acceptable, a sample matrix effect was suspected. Analytical spike recoveries were acceptable for all analytes with recovery ranging from 77%-111%. Except for lindane, heptachlor, and 4,4'-DDT, reproducibility was very good with RPDs less than or equal to 30%. Three analytes, alpha-

chlordane, gamma-chlordane, and 4,4'-DDD, recovered from SRMs at about 60%. Trans-nonachlor recovered at 99% with dieldrin and 4,4-DDE recovering greater than 100%. Only one result could be obtained for heptachlor epoxide and oxychlordane recovered at 166%. The recovery of 4,4'-DDT in one SRM sample resulted in a value of 42%.

Based upon the evaluation of the QC samples, the reported results for heptachlor epoxide, oxychlordane, alpha-chlordane, gamma-chlordane, transnonachlor, and 4,4'-DDD are estimated to be $\pm$ 40% of the true value. The reported results for dieldrin are estimated to be $\pm$ 15% of the true value with the estimate uncertainty for 4,4'-DDE at $\pm$ 50%. Due to the poor recovery for lindane, heptachlor, and 4,4'-DDT, the uncertainty for these values is high.

Limits of Detection

Detection limits for PCB congeners ranged from 0.1 to 17 (BZ#18, 200/201) ppb, though were more typically 0.3 to 2 ng/g. Detection limits for organochlorine pesticides ranged from 0.2 to 1.0 ng/g. Detection limits for elements were as follows: for Ag, Ba, Cd, Co, Cr, Cu, Mn, Pb, Rb 0.05 mg/kg; Se, Tl 0.5 mg/kg; Zn 1 mg/kg; Sb 3 mg/kg; Hg 0.001 mg/kg.

Contaminants in BCNH Embryos

PCBs

Twenty-seven of 31 congeners (includes individuals of co-eluting pairs) were detected; BZ 5, 8, 18, and 33 were not detected in any of the specimens. With the exception of BZ 31 (55% of embryos), 77 (65%), and 200/201 (50%), the remaining congeners were present in all of the IL samples. BZ 44, 49, and 70 were detected only in embryos from IL; BZ 110 and 200/201 were each detected, with the exception of IL samples, in one sample from VA. All of the individual PCBs detected were present in much higher concentrations in embryos from IL (Fig. 1); consequently $\sum$PCBs were significantly greater in IL samples (Fig. 2; $F= 61.9$, $P< 0.001$). In general, the higher molecular weight congeners were present in the highest concentrations in IL samples (Fig. 1).

Organochlorine Pesticides

With exception of g-Chlordane, Hepatchlor, and Lindane, the organochlorine pesticides were detected in samples from all three locations (Fig. 3). DDE and transnonachlor were most frequently detected overall. Residues of 10 organochlorine pesticides or breakdown products were detected in IL BCNH embryos; lindane was not detected in IL specimens.

Most organochlorine pesticides were present in very low concentrations (Fig. 4). Mean DDE concentrations were much greater in embryos from IL (Fig. 5; $F= 31.2$, $P< 0.001$). The highest DDE concentration, 18,000 ppb (14,940 ppb adjusted for moisture loss) in an embryo from MN, was much higher than the next highest concentration observed, 5,200 ppb in a specimen from IL.

Elements

As (DL< 1.0), Cd (<0.05), antimony (< 3.0), and thallium (<0.5) were not detectable in any of the embryos. Ag was detected (>0.05) in 16 samples albeit at low concentrations (maximum= 0.23

ppm). Mean concentrations of Co, Cu, Hg, Ni, Rb, Se, and Zn were significantly different among sites ($F= 6.1$ to 18.6, $P< 0.0045$). Post-hoc testing revealed that mean Co and Se concentrations in IL samples were significantly greater than in both MN and VA embryos (Fig. 6-7). Specimens from IL also had greater Cu and Zn concentrations than MN samples, and Ni levels than VA embryos. Embryos from MN had higher Rb concentrations than both IL and VA, whereas those from VA had greater Hg concentrations than samples from MN.

Biomarkers of Contaminant Exposure

BCNH embryos from IL evinced greater mean EROD ($F= 6.6$, $P< 0.01$) and BROD ($F= 5.9$, $P< 0.01$) induction, when compared to those from MN and VA (Fig. 8). Lipid-normalized $\sum$PCB concentrations were correlated with EROD ($r= 0.65$, $P<0.001$) and BROD ($r=0.61$, $P<0.001$). The total burden of PCB 153 (present in the greatest concentrations) in embryos was negatively correlated with mass of the embryos ($r= -0.31$, $P<0.02$).

Lipid-normalized DDE concentrations were negatively associated with eggshell thickness ($r= -0.37$, $P< 0.01$). There was a significant association between variation in cellular DNA content and $\sum$PCB, DDE, transnonachlor, Co, and Se concentrations ($F= 4.1$, $P= 0.004$, $R^2= 0.35$). Testing of regression coefficients revealed that only DDE contributed significantly to R^2 ($t= 3.8$, $P< 0.001$). Moreover, DDE was the only independent variable which, after partialling out the influence of the others, was moderately correlated with variation in cellular DNA content ($r_{yx....}= 0.52$).

We did not detect significant differences in measures of oxidative stress among colonies ($P> 0.10$). Ba, Cr, Mn, Pb, Rb, and Se were significantly correlated with one or more measures of oxidative stress ($r= -0.31$ to 0.42, $P= 0.005$ to 0.4); however, after adjusting alpha $0.05/17= 0.003$ to account for multiple omnibus comparisons, none of these correlations were significant.

Only GSSG was associated with the elements we examined in combination ($F= 2.09$, $P= 0.05$, $R^2= 0.42$). After partialling out variance shared with other predictor variables, only Ba contributed significantly to the variance in GSSG accounted for by the model ($t= 2.55$, $P= 0.016$, $r_{yx....}= 0.41$). However, based on eigenvalues and condition indicies, as well as simple correlations among elements ($r= -0.26$ to 0.62, $P\leq 0.05$), there was a great deal of collinearity among element concentrations in embryos.

Discussion

BCNH embryos from the Lake Calumet, Chicago, IL, colony had greater exposure to PCBs, DDE and other organochlorine pesticides (most notably dieldrin, heptachlor {expoxide} and trannonachlor), Co, and Se than those from colonies in northwestern MN and coastal VA. The colony located at Chincoteague National Wildlife Refuge, VA, has been the subject of much study, and BCNH nesting there have had low levels of organic and elemental contaminants (Rattner et al. 1994, 1996, 1997). Agassiz NWR (AgNWR) is located in the aspen parklands of nw MN, an intensively-farmed area with a low human population density. Although waterbirds

from AgNWR were previously reported to have greatly elevated concentrations of toxic elements, i.e., Cd, Cr, and Hg (Burger and Gochfeld 1996), recent studies have indicated that these elements were not elevated to toxic levels (Custer and Custer 2004). This was consistent with the our analysis of BCNH eggs from AgNWR.

With a few exceptions, PCB and DDE concentrations in Lake Calumet, IL, BCNH eggs approached or exceeded those reported in recent studies, including for colonies in urban port/industrial areas (Table 1). However, concentrations in IL specimens did not approach the highest mean or maximum values reported, i.e., were not among the most highly-contaminated colonies.

EROD and BROD are cytochrome P450 monooxygenase proteins involved in the biotransformation of organic molecules. Thus, they can provide a measure of the extent of exposure to halogenated and aromatic compounds, including PAHs, coplanar PCBs, dioxins, and furans. In addition, in the proces of detoxification these P450s can in turn activate procarcinogens and produce toxic reactive metabolites and free radicals (Stegeman et al. 1992). In our study $\sum$PCBs were associated with EROD and BROD induction, a relationship which has been documented previously (e.g., Brunstrom 1989; Brunstrom et al. 1990; Bosveld et al. 1992). Consequently, activities were considerably higher in IL eggs, which had much greater exposure to PCBs.

We did not measure PAH concentrations under the assumption that, as there would be negligible, if any, exposure after egg formation, PAHs would be metabolized by the embryo. We did not measure levels of dioxins and furans, which undoubtedly occur in some of the BCNH foraging locations in the south Chicago region (e.g., Scaup using Indiana Ship Canal, Custer et al. 2000). *However, dioxins and furans may not contribute substantially to observed TEQs in fish-eating birds outside of environments contaminated by effluents from the bleached-paper industry* (**help here- I read this somewhere but now cant find the cite**).

Weathering, diet, metabolic differences between species, and presence of different mixtures can influence PCB exposure and accumulation and subsequently, PCB congener profiles. Profiles for BCNH embryos from IL suggested exposure to the more persistent, bioaccumulative higher-molecular weight congeners, as opposed to the less-persistent, episodic ones. Congeners BZ#136/163, 153, and 180 contributed an average of 45-64% of the $\sum$PCB concentration in IL embryos. This is consistent with 55-64% contribution observed in serum of humans regularly consuming fish from Lake Michigan (Humphrey et al. 2000).

Congener BZ#77, second only to BZ#126 in terms of toxicity (Hoffman et al. 1998a), was detected in 13 of 20 IL specimens, in concentrations ranging from 0.03 to 6.0 ppb. Hoffman et al. (1998a) reported an LD50 of 2.6 ppb and LOEL (including EROD induction and altered glutathione metabolism) of 1.2 ppb for BZ#77 in chicken eggs. Nine of 13 IL embryos with detectable concentrations of BZ#77 exceeded this lower effects concentration; four of these samples exceeded 2.6 ppb. Reduced hatching success and increased incidence of malformations and edema were observed in chickens dosed with 6 ppb BZ#77, our maximum observed value. However, chickens and other gallinaceous birds are more sensitive to PCBs than other avian groups including fish-eating birds such as gulls and terns (Brunstrom et al. 1989; Bosveld and Van den Berg 1994; Hoffman et al. 1998a), thus these effects levels are likely conservative for BCNH. In contrast to the planar congeners, the nonplanar PCBs are much less toxic. For

example, the LD50 for BZ#153 in chickens, the predominant congener in our sample, was 14,000 ppb (Hoffman et al. 1998a); the highest concentration we observed was 1,113 ppb.

The association between DDE and eggshell thinning has been well documented, including in BCNH (Custer et al. 1983; Ewen et al. 1984; Henny et al. 1984; Ohlendorf et al. 1988). Rather than complete reproductive failure at some threshold as in many avian species, BCNH experience a decline in successful nests with increasing DDE levels, with some nests producing young even at high concentrations (Henny et al. 1984). Eggshell thinning was greatest, and clutch size and productivity began to decrease, at DDE concentrations above 8 ppm (Henny et al. 1984). The maximum concentration we observed in IL specimens was below, whereas one specimen from MN was well above, this threshold.

Chromosomal damage may occur through exposure to genotoxic pollutants acting as mutatgens, resulting in cells with altered DNA content (**cite**). A combination of selected analytes were associated with variation in cell nuclear DNA content. DDE contributed most to the variation accounted for in coefficient of variation in cellular DNA content, and has been shown to be mutagenic in previous studies (Clive et al. 1979; Valencia et al. 1985; Thies et al. 1996; Yanez et al. 2004).

A threshold for reproductive effects, including deformities and reduced hatching, of 3 ppm ww Se in eggs has been established (Heinz 1996). However, BCNH displayed no reduction in hatching of fertile eggs at 3 ppm ww Se in eggs, although sublethal effects were noted (Smith et al. 1988). The maximum value observed in the IL sample in this study was 9.90 dw, or approximately 1.98 ppm ww (based on 80% moisture content of embryos). Thus it would appear that BCNH foraging in the south Chicago region are not at risk of reproductive effects stemming from Se in eggs; however more sensitive species could be negatively affected at this level. It is important to recognize that the form of Se present is important determinant of toxicity, with selenomethionine being highly toxic to birds; we did not differentiate Se species in this study.

Background Cr concentrations typically average less than 0.5 µg/g dry weight (Burger 1994). Our geometric mean concentrations were higher than this in all three colonies. Cr as CrO_3 was more teratogenic to ducks than either Cd or Pb (Kertesz and Fancsi 2003); injection of 5 ng Cr03 in mallard eggs, although not lethal to early embryos, did increase the malformation, preincubation mortality, and hatching rates. By comparison, the embryo in our sample with the highest Cr concentration contained 15,000 ng Cr. However, as in Se, the form of Cr present is an important factor in determining toxicity, with hexavalent forms (Cr+VI) being the most toxic; we measured total Cr present in the embryos.

The maximum Hg concentrations (converted to ww) observed in embryos from IL (0.44 mg/kg) and MN (0.32 mg/kg) were below those associated with reproductive effects (0.5 to 2.0 ppm ww, Thompson 1996; LOEL 0.74 ppm ww, Heinz and Hoffman 2003). However, Hg concentrations in 3 VA specimens exceeded the lower observed effects level of 0.5 ppm ww.

Hepatic oxidized glutathione (GSSG) activities in this study were an order of magnitude greater than reported for BCNH from the Delaware Bay (Rattner et al. 2000); concentrations of most elements common to both studies were not dramaticllay higher in the current study (Table 2). However, mean and/or maximum concentrations of Ba, Cu, Hg, Ni, Pb, and Se were considerably greater in our study. GSSG activity was associated with the combination of elements we examined. Elements such as Hg (Hoffman et al. 1998b; Hoffman and Heinz 1998)

and Se (Hoffman et al. 1998b; Hoffman and Heinz 1998; Hoffman 2002) can induce oxidative stress in waterbirds. In fact, the pattern of mean Hg concentrations among locations reflected that of GSSG activities, i.e., highest in VA embryos, followed by IL and then MN. Hoffman (2002) reviewed the impacts of Se exposure and oxidative stress in aquatic birds; embryonic or early post-hatch Se-mediated oxidative stress was associated with malformations and reduced growth. Embryos are particularly susceptible to oxidative stress, mediated by reactive oxygen species, which can result in the oxidation of DNA, protein, and lipid. While excess Se can induce oxidative stress, at some level it can also be antagonisic, alleviating the effects of Hg exposure on glutathione metabolism **(cites)**. Embryos from IL had the highest Se concentrations, and in fact GSSG activities were similar to those from MN, which had the lowest Hg levels.

Conclusions

BCNH embryos from the Lake Calumet, Chicago, IL, colony had greater exposure to PCBs, DDE and other organochlorine pesticides, Co, and Se than those from colonies in northwestern MN and coastal VA. Contaminant levels in IL embryos were consistent with some colonies in industrialized urban areas, though were not among the highest reported.

$\sum$PCB concentrations were associated with EROD/BROD induction, and activities of these Cytochrome P450 monooxygenase proteins were greatly increased in IL embryos, compared to reference colonies. $\sum$PCBs, DDE, transnonachlor, Co, and Se in combination were associated with variation in cellular DNA content; DDE contributed most to this relationship. Measures of oxidative stress did not differ among colonies, though increased GSSG activities were associated with concentrations of the suite of elements examined in this study.

Concentrations of BZ#77, one of the more toxic PCB congeners, in some IL specimens exceeded lower observed effects levels established for chickens. The concentrations of Se in eggs of BCNH from IL approached published effects thresholds. BCNH are relatively resistant to the effects of contaminant exposure; although there was increased exposure in this colony we did not observed any dramatic effects on the health of the embryos. In addition, Levengood et al. (2005) determined that reproductive parameters in 2002 and 2003 were typical for this species. Contaminants at these levels in eggs may be of concern in piscivorous waterbird species that more sensitive to contaminant exposure than BCNH.

Acknowledgements

Special thanks to the following for their assistance and expertise: Paul Dummer, Craig Beckman, and Craig Meeusen, USGS; Gary Huschle, USFWS; Robin Holmlund, WMRC; Margaret Kurcz, Walter Marcisz, INHS.

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Figure 1. Arithmetic mean concentrations (+ sd) of selected PCB congeners in Black-Crowned Night-Heron embryos from Illinois (n= 20), Minnesota (n= 20), and Virginia (n= 19).

Figure 2. Arithmetic mean (+sd) ∑PCB concentrations (sum of measured congeners) in Black-Crowned Night-Heron embryos from Illinois (n= 20), Minnesota (n=20), and Virginia (n= 19).

Figure 3. Organochlorine pesticide detection rates in Black-Crowned Night-Heron embryos from Illinois (n= 20), Minnesota (n= 20), and Virginia (n= 19).

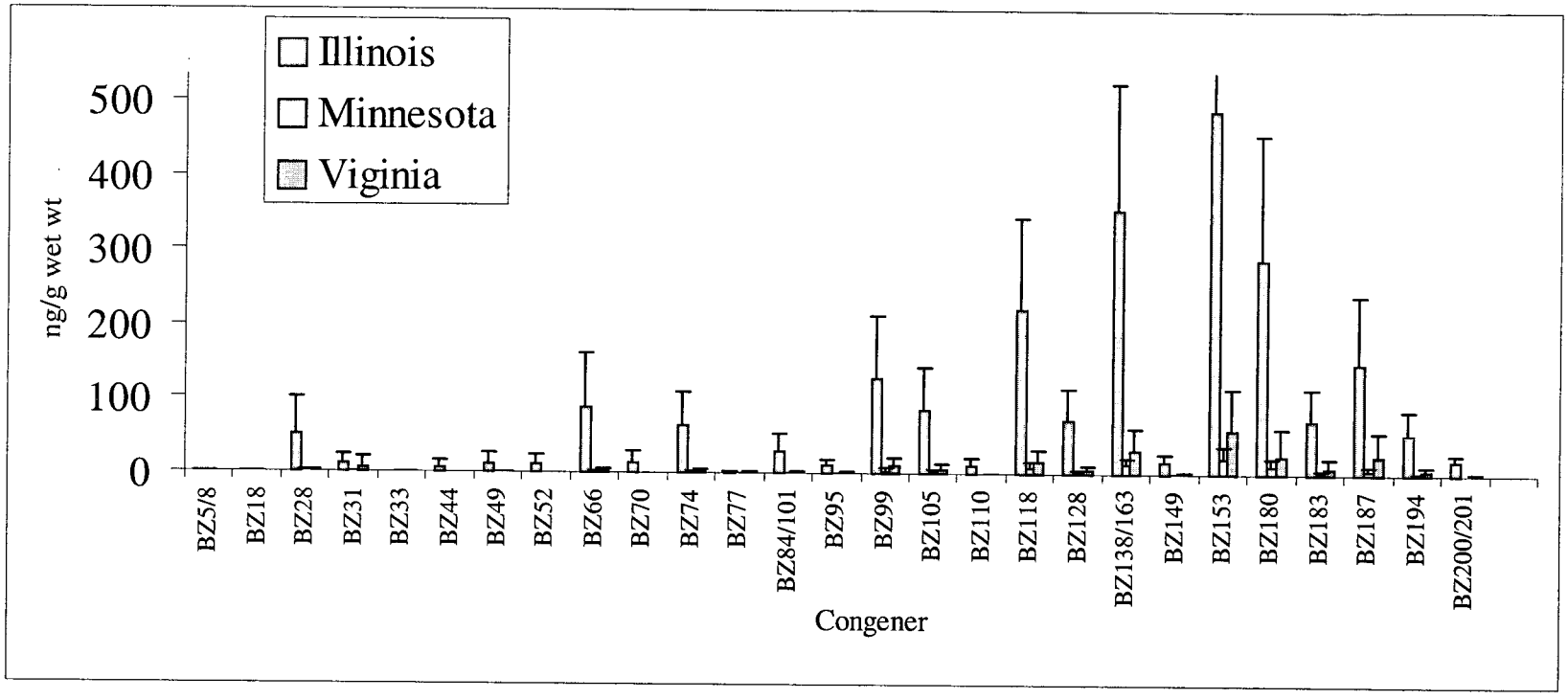
Figure 4. Arithmetic mean concentrations (+ sd) of organochlorine pesticides in Black-Crowned Night-Heron embryos from Illinois (n= 20), Minnesota (n= 20), and Virginia (n= 19).

Figure 5. Arithmetic mean (+ sd) DDE concentrations in Black-Crowned Night-Heron embryos from Illinois (n= 20), Minnesota (n= 20), and Virginia (n= 18); one outlier (14,940 ppb) from Minnesota omitted.

Figure 6. Arithmetic mean concentrations (+ sd) of elements in Black-Crowned Night-Heron embryos from Illinois (n= 20), Minnesota (n= 20), and Virginia (n= 19).

Figure 7 . Arithmetic mean concentrations (+ sd) of elements in Black-Crowned Night-Heron embryos from Illinois (n= 20), Minnesota (n= 20), and Virginia (n= 19).

Figure 8. Physiological biomarkers (arithmetic mean + sd) of contaminant exposure in Black-Crowned Night-Heron embryos from Illinois, Minnesota, and Virginia. Units were as follows: EROD, BROD pmol/min/mg; GSH, TSH, PBSH μmol/g; GSSG, TBARS nmol/g; GSSG/GSH %; DNA HPCV; shell thickness mm x 10. With the exception of shell thickness, sample sizes for Illinois, Minnesota, and Virgina were 14 or 15, 14, and 15 to 17, respectively. Shell thickness was determined for the full complement of 59 specimens.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Minnesota
Viginia
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400
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200
100
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ng/g wet wt
BZ5/8
BZ18
BZ28
BZ31
BZ33
BZ44
BZ49
BZ52
BZ66
BZ70
BZ74
BZ77
BZ84/101
BZ95
BZ99
BZ105
BZ110
BZ118
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BZ149
BZ153
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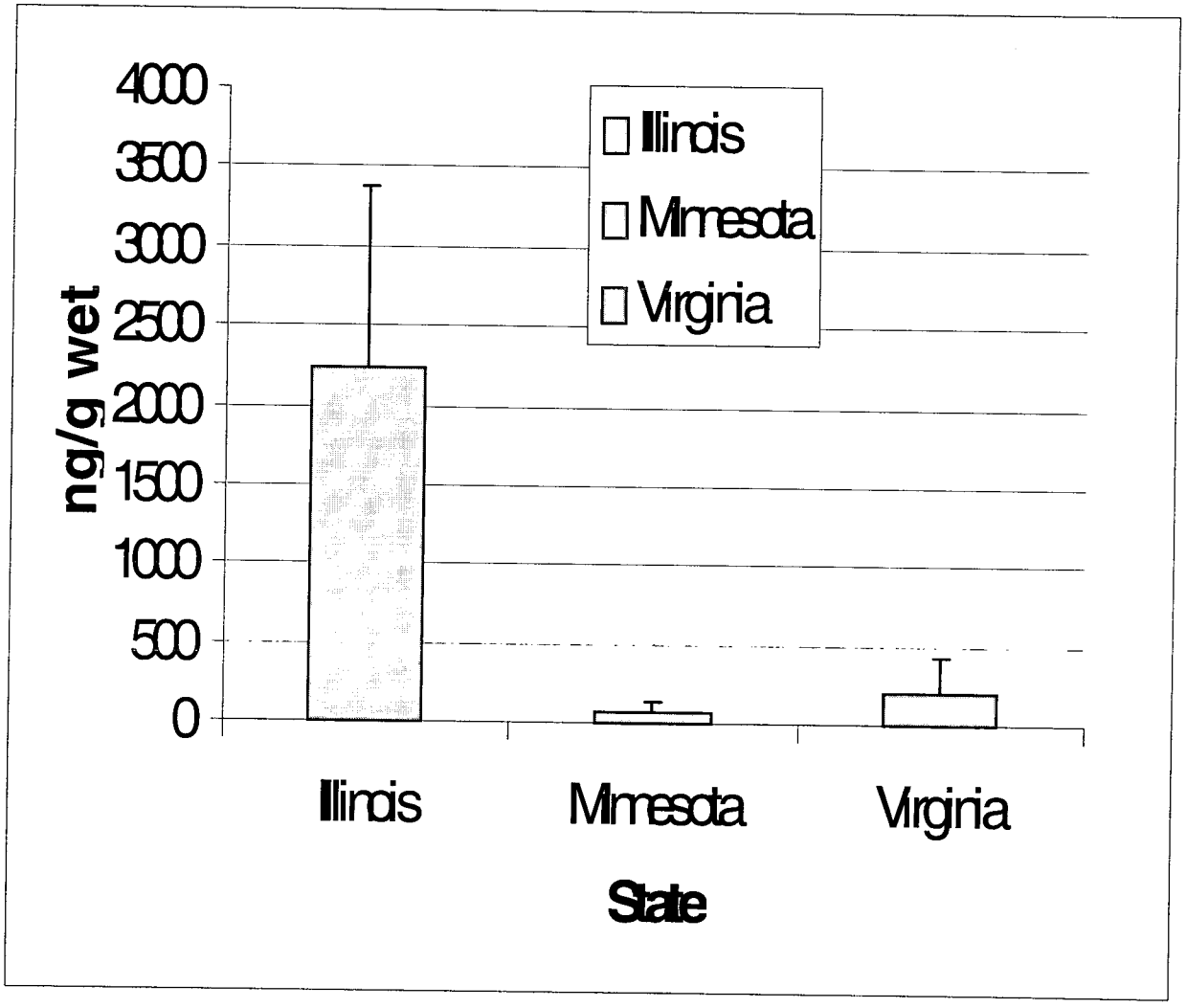
BZ183
BZ187
BZ194
BZ200/201
Congener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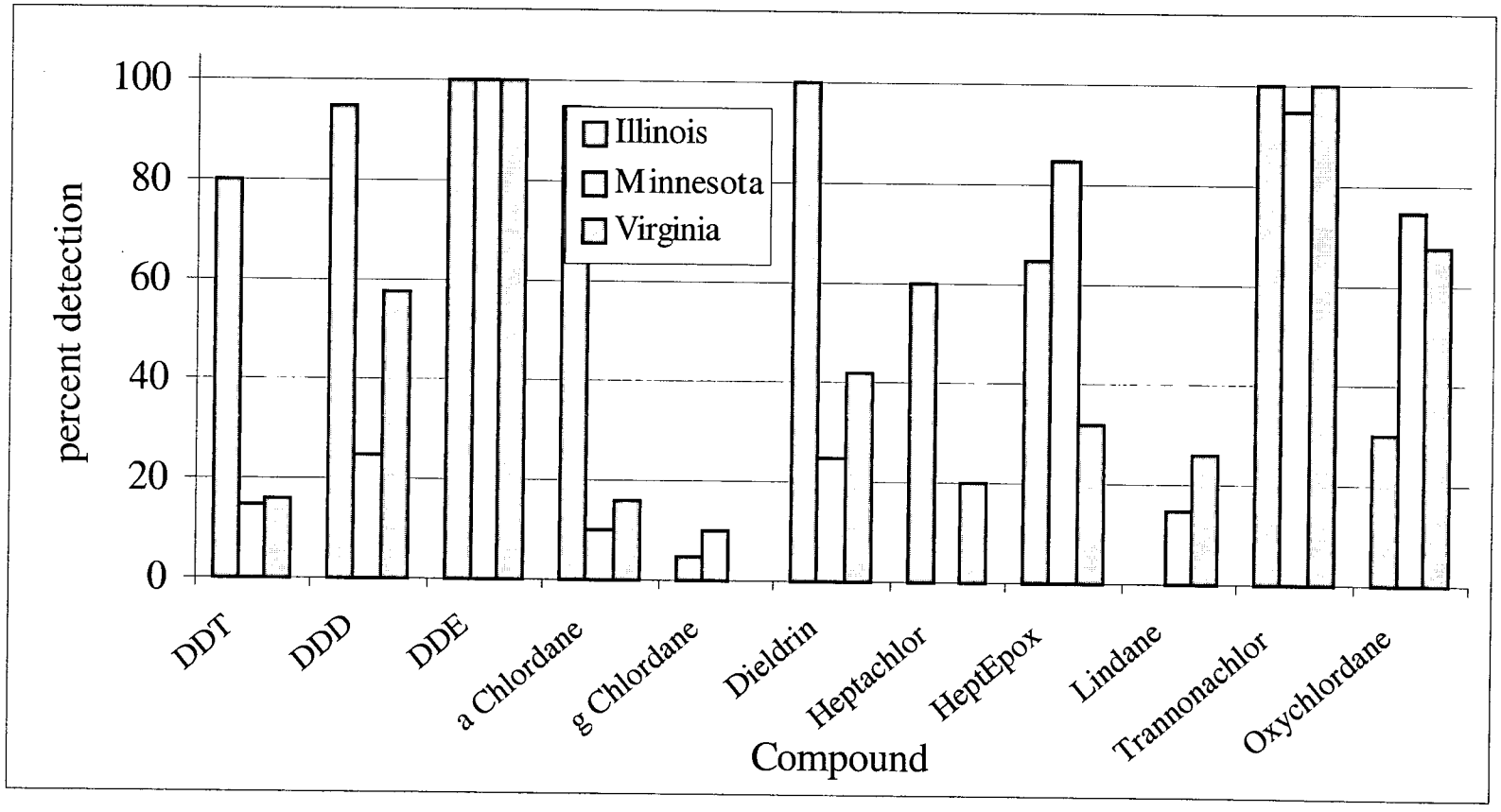
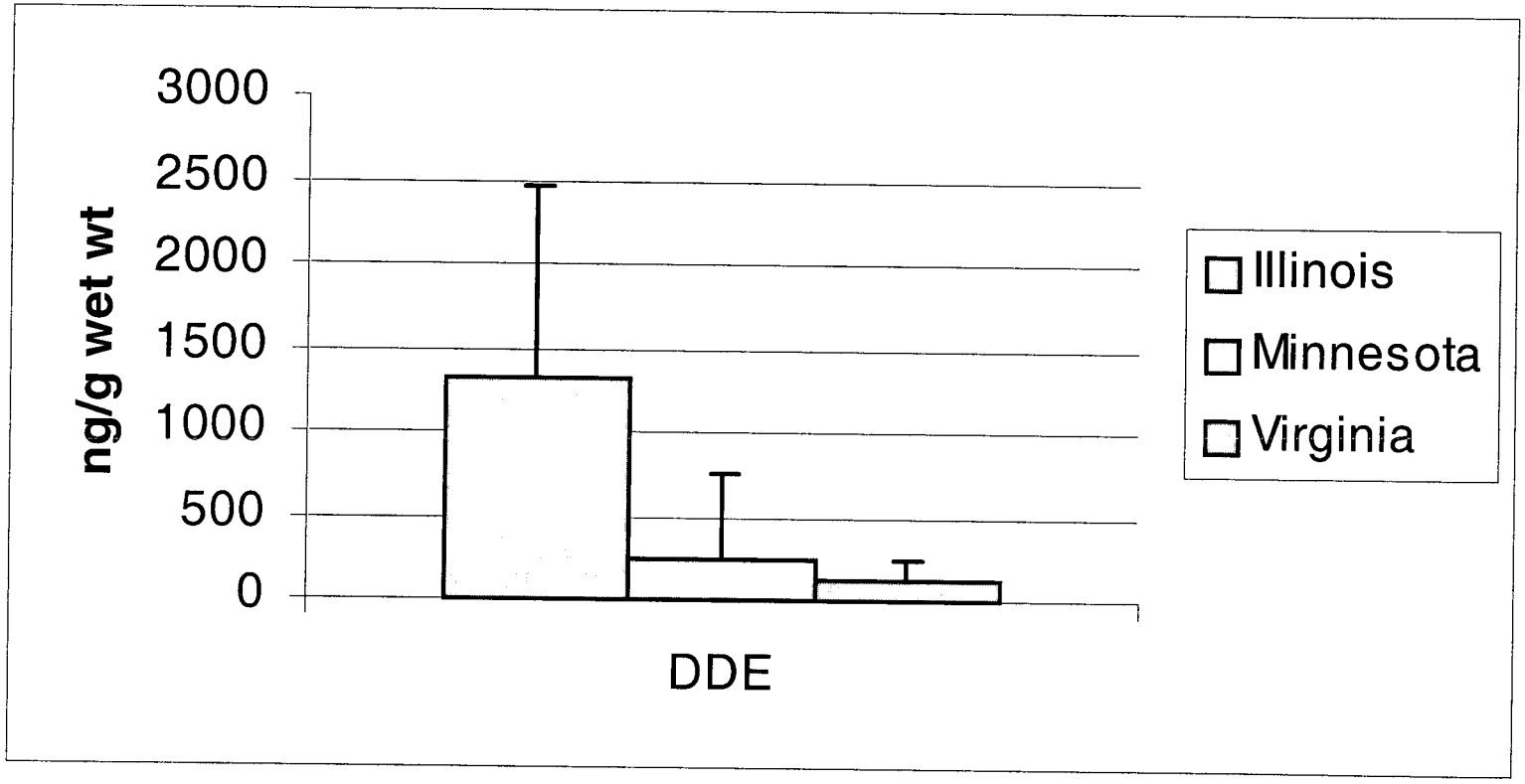
3500
3000
2500
2000
1500
1000
500
0
ng/g wet
Illinois
Minnesota
Virginia
Illinois
Minnesota
Virginia
State


100
80
60
40
20
0
percent detection
Illinois
Minnesota
Virginia
DDT
DDD
DDE
a Chlordane
g Chlordane
Dieldrin
Heptachlor
HeptEpox
Lindane
Trannonachlor
Oxychlordane
Compound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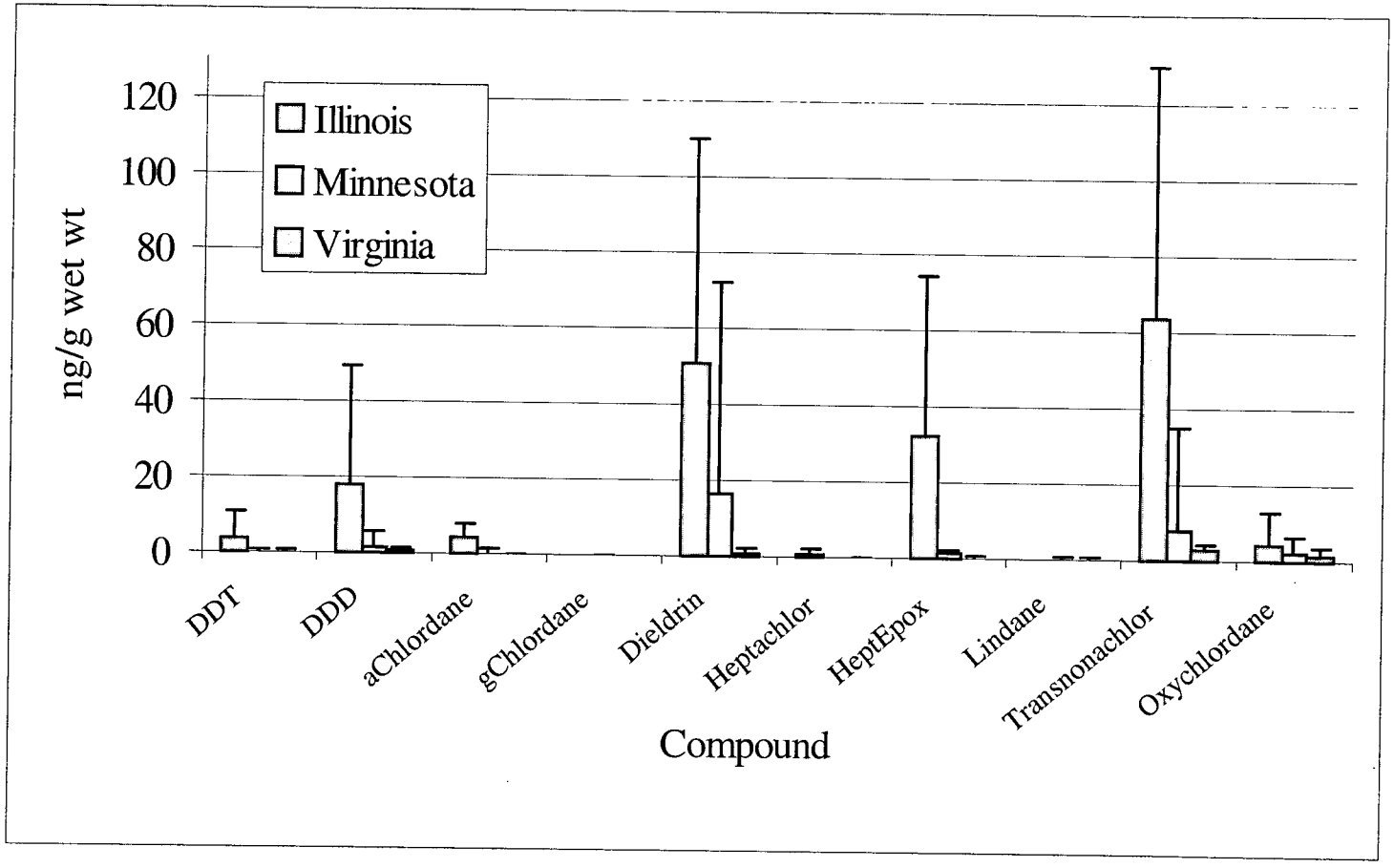
ng/g wet wt
DDE
Illinois
Minnesota
Virginia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80
60
40
20
0
ng/g wet wt
Illinois
Minnesota
Virginia
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DDD
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HeptEpox
Lindane
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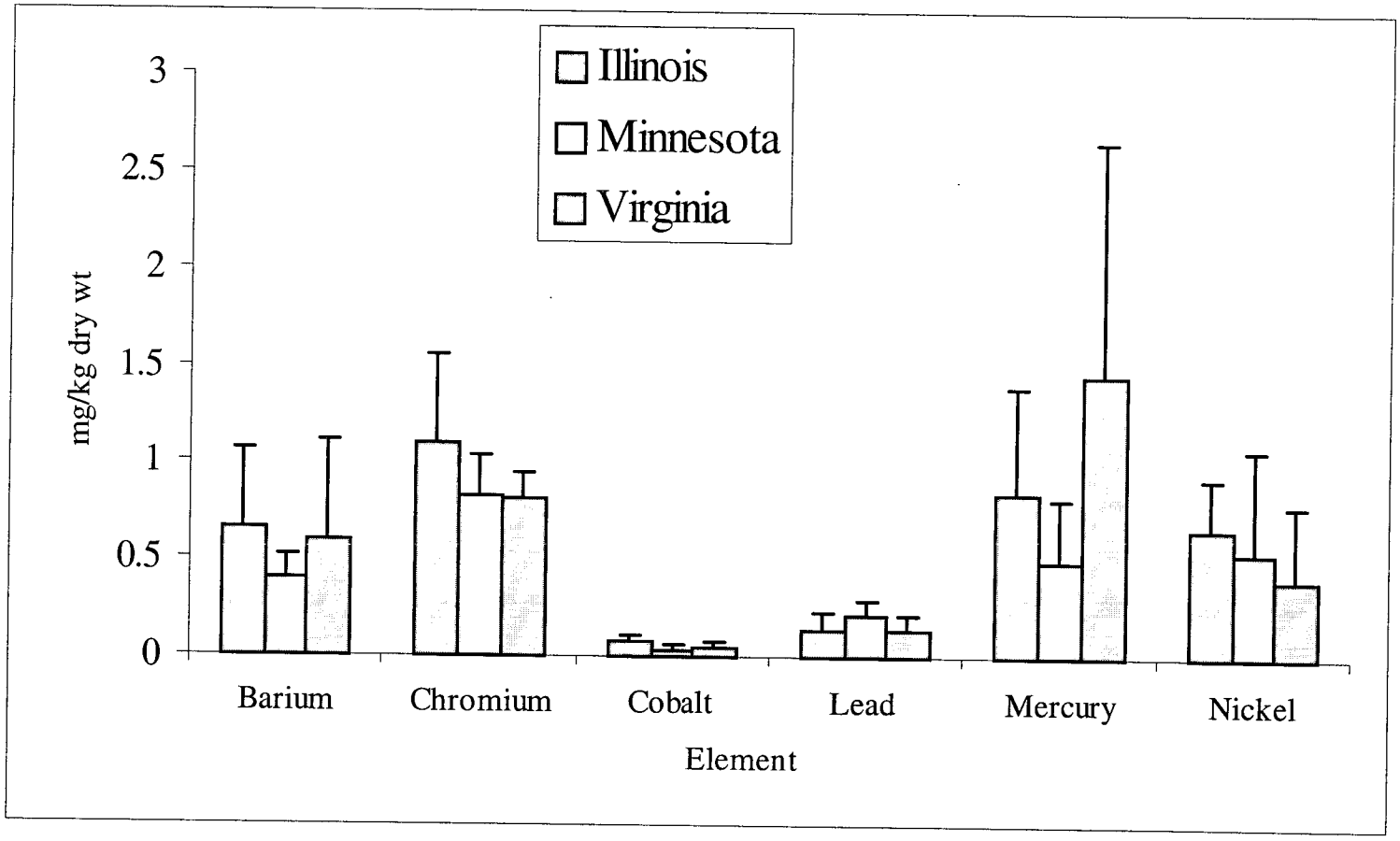
Oxychlordane
Compound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Minnesota
Virginia
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1
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0
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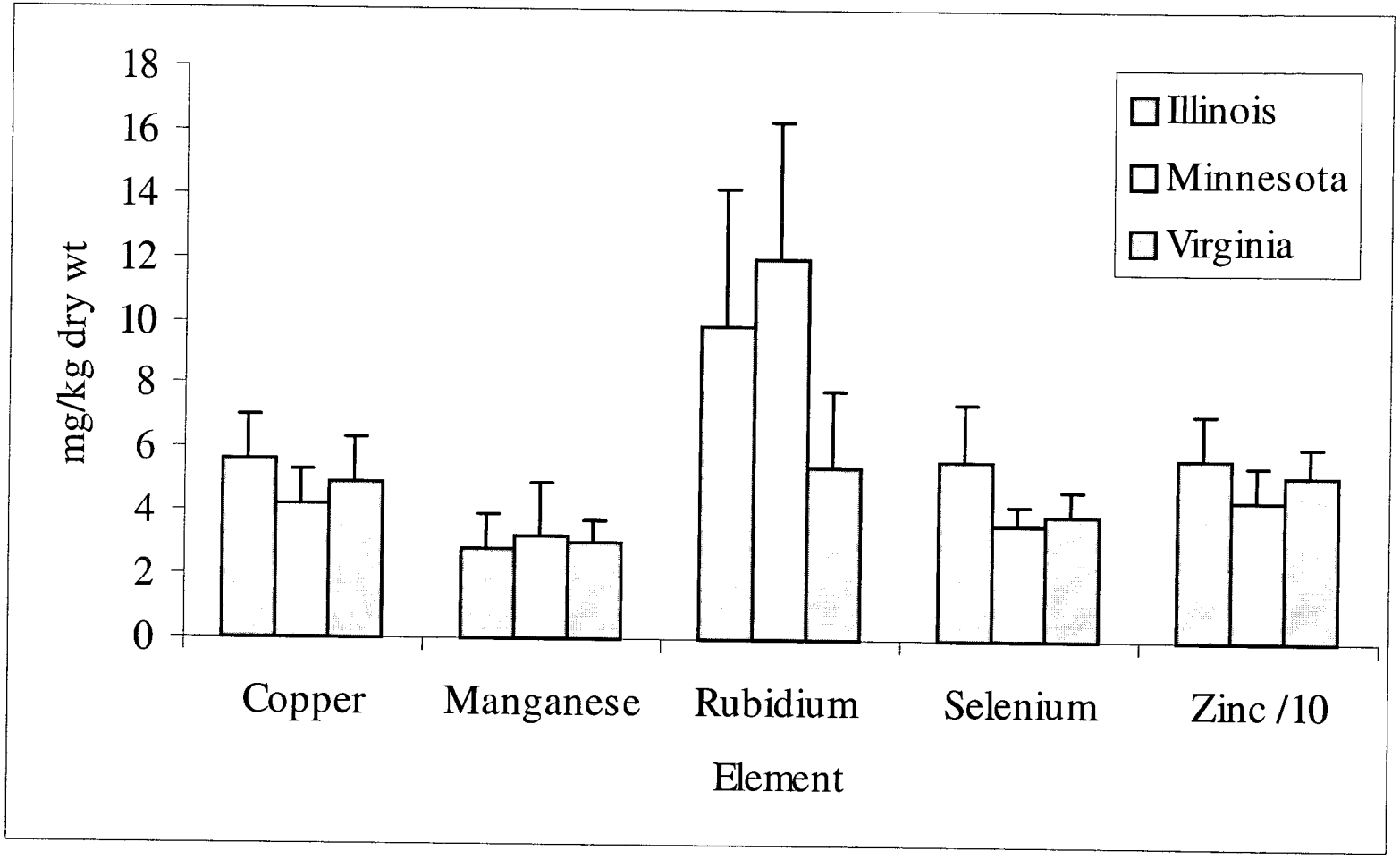
Chromium
Cobalt
Lead
Mercury
Nickel
Element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14
12
10
8
6
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mg/kg dry wt
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Minnesota
Virginia
Copper
Manganese
Rubidium
Selenium
Zinc /10
Element

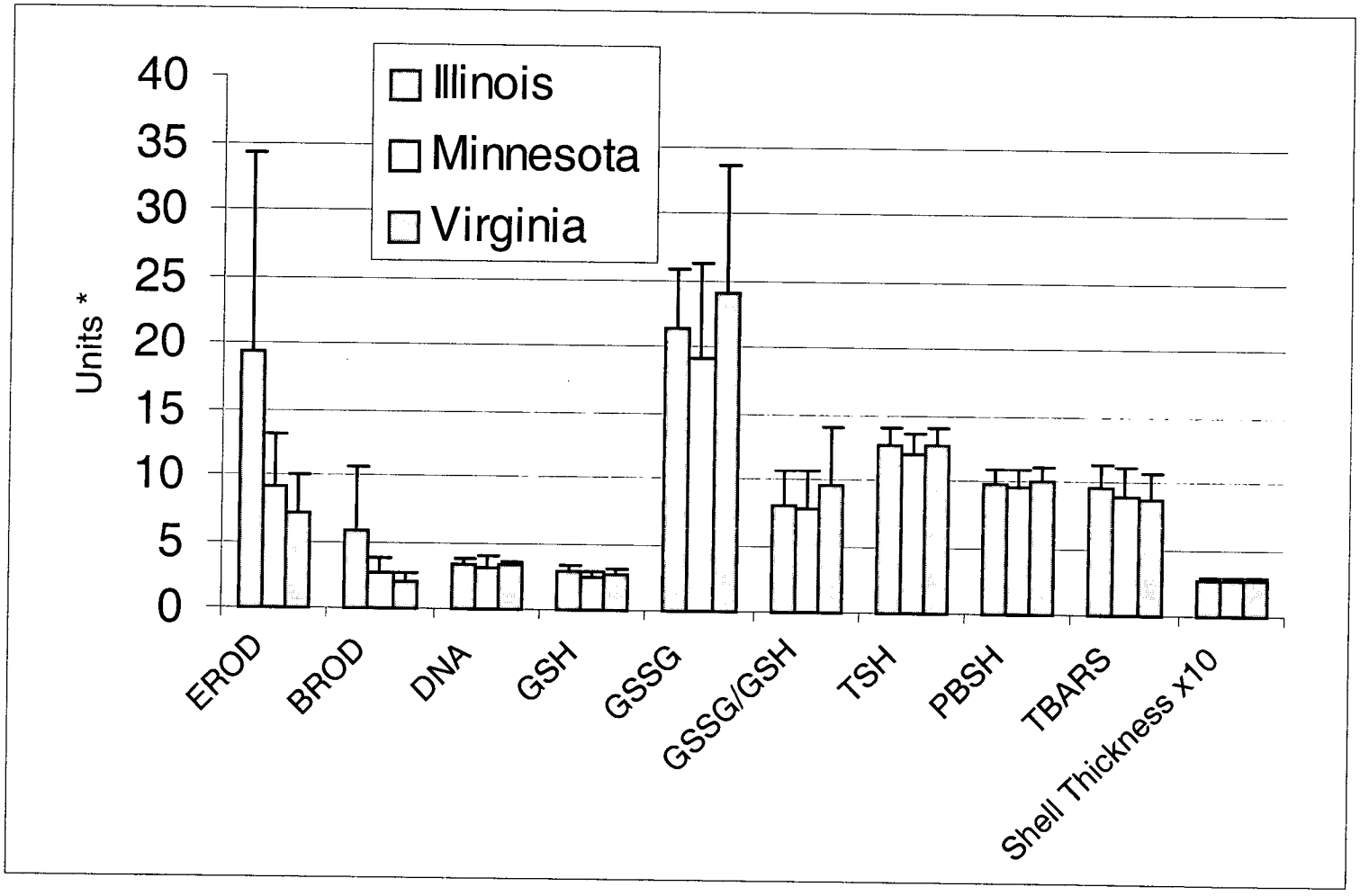
Illinois
Minnesota
Virginia
Units *
40
35
30
25
20
15
10
5
0
EROD
BROD
DNA
GSH
GSSG
GSSG/GSH
TSH
PBSH
TBARS
Shell Thickness x10

Table 1. Comparisons of concentrations of selected organic contaminants in Black-Crowned Night- Heron embryos from south Chicago, IL, with those observed in previous studies of this species.

Analyte	Geometric mean & (maximum) concentrations IL	Reported concentrations[1]	Locale	Source
DDE ng/g	1029 (5188)	380/540 (2200)	Delaware Bay	Rattner et al. 2000
		230/1390/1590/ (22000)	Chesapeake Bay area colonies	Rattner et al. 1997
		1070 (2600)	Green Bay	Rattner et al. 1996
		59/199 (1293)	Chesapeake Bay	Rattner et al. 2001
		NR (4600)	OR and WA	Blus et al. 1997
		401 (12,900)	Galveston Bay	Frank et al. 2001
		14 (47)	northern Italy	Fasola et al. 1998
BZ# 77	NR (6.0)	NR (0.6)	Delaware Bay	Rattner et al. 2000
		0.51/5.0/5.0 (23)	Chesapeake Bay area colonies	Rattner et al. 1997
		NR/0.14 (2.2)	Chesapeake Bay	Rattner et al. 2001
BZ# 105	73 (250)	16/23 (150)	Delaware Bay	Rattner et al. 2000
		5.6/83/146 (1,045)	Chesapeake Bay area colonies	Rattner et al. 1997
		5.7/53 (753)	Chesapeake bay	Rattner et al. 2001
BZ# 118 ng/g	118 (520)	64/82 (520)	Delaware Bay	Rattner et al. 2000
		10.2/214/288 (939)	Chesapeake Bay area colonies	Rattner et al. 1997
		17/132 (1602)	Chesapeake Bay	Rattner et al. 2001
		146 (424)	Galveston Bay	Frank et al. 2001

NR= not reported

Table 2. Comparisons of concentrations of elements in Black-Crowned Night-Heron embryos from south Chicago, IL, with those observed in previous studies of this species.

Analyte	Geometric mean and (maximum) concentrations this study	Rattner et al. 2000 Delaware Bay	Hothem et al. 1995 San Francisco Bay
Antimony	ND	NR	NR
Arsenic	ND	NR	NR
Barium	0.45, 2.4	0.22, 0.59	ND-1.9, NR
Cadmium	ND	NR	NR
Chromium	0.86, 2.3	4.6, 16.3	ND-1.0, NR
Cobalt	0.05, 0.14	NR	NR
Copper	4.7, 8.8	3.6, 4.40	5.0-6.4, NR
Iron	77.9, 108	104, 195	87.4-110, NR
Lead	0.14, 0.33	NR, 0.17	NR
Manganese	2.7, 7.3	NR	2.3-4.8, NR
Mercury	0.64, 4.6	0.60, 2.2	0.50-2.2, 5.9
Nickel	0.48, 1.5	NR, 0.46	NR
Rubidium	8.0, 25.0	NR	NR
Selenium	4.2, 9.9	4.2, 6.0	2.8-5.7, 9.7
Silver	NR, 0.23	NR	NR
Thallium	ND	NR	NR
Zinc	49.6, 81.0	64.22, 75.1	40.6-53.9, NR

ND= not detected, NR= not reported