

Lucille and Bill Stickel: A Personal Perspective

By Nancy C. Coon

The Early Years

In late 1966, my husband, Richard, and I moved to the Washington, D.C., area, where he had been assigned to the National Naval Medical Center as a Medical Service Corps officer. Thinking that there might be something for me to do on the Washington Mall, I went to the Civil Service Commission and talked to a nice lady named Anna Berozowski. Ms. Berozowski told me that she knew a woman at the Patuxent Wildlife Research Center (Patuxent) in Laurel, MD, who occasionally came down to look through applications. She said she would call to see whether Dr. Lucille Stickel might be interested in talking to me. A few days later, Dr. Stickel did indeed call me, and invited me for a visit. I remember well my tour of Patuxent in the Stickels' Pontiac Tempest convertible. I was hired as a junior biologist working directly with Dr. Stickel. That was the beginning of a 40-year relationship with Dr. Lucille and her husband, Mr. William Stickel. Through the years, I came to know both of them very well. Their profound dedication to their work often made it difficult for others to understand them, particularly Dr. Stickel. She would forever be regarded by some as unapproachable, but by others as compassionate and friendly. She was a pioneer, and a person of immense achievement. When she began her research, it was rare for a woman even to participate in science, much less to triumph to such a degree.

In March 1998, Richard and I had a conversation with Lucille in which she shared with us some details about her early years. She said that her maternal grandfather was a successful lumberman and merchant in Michigan, having emigrated from Canada. He owned land near Alpena, including a lakeside property on which the family had cottages even at that time. Lucille was born in Hillman, MI, in 1915. It was there that her love for all natural things began as she roamed the fields, woods, and lakeside. Lucille's father died during the influenza epidemic that followed World War I, and her mother had a difficult time. Then the Great Depression (hereinafter Depression) came, and the family lost everything.

Lucille was fortunate enough to attend Eastern Michigan University, but worked 30 hours per week while taking a full academic load. After she graduated from college, she taught for 1 year at Ypsilanti, MI. In her view, she did well teaching biology, but she did not enjoy teaching math. She decided that



Lucille and Bill Stickel, Nanjemoy River, MD, 1952. Photo by Francis M. Uhler, Patuxent Research Refuge.

she was not destined to be a teacher and made the decision to return to school at the University of Michigan. She was told that women would have difficulty obtaining jobs and would not be hired before men unless they had top grades, so that was her goal, and she succeeded. Lucille received her master's degree in biology in 1938, and then began working toward her Ph.D. A duplication issue arose when, in her literature review, she came across a paper that essentially reported on her research topic (the embryology of an insect). Consequently, Lucille, not one who was easily discouraged or deterred, began searching for a new research topic.

Bill Stickel was born in Terre Haute, IN, in 1912. He attended Indiana State University for 2 years before transferring to the University of Michigan, where he met Lucille. He graduated with Bachelor of Science (1934) and Master of Science (1935) degrees in zoology/botany. He then continued research at the University of Michigan until 1939.

Bill accepted a position as a wildlife biologist with the Civil Service Commission in Washington, D.C., in 1940. He transferred to Patuxent in 1941, and Lucille joined him there. It was at Patuxent that Lucille selected a new research topic, one centered at Patuxent. In 1941, Bill and Lucille were married and thus became lifelong research partners, and stalwart supporters of each other.



Bill and Lucille Stickel on vacation along Steinhatchee River, Taylor County, FL, December 26, 1950. Photo by Francis M. Uhler, Patuxent Research Refuge.

A Beginning at the Patuxent Wildlife Research Center

During the early years at Patuxent, Lucille was offered several positions, including one as an editor, but declined each of them, stating that the men with families recovering from the Depression needed the paying jobs more than she did. In 1943, after spending time as a volunteer, Lucille accepted a position as a junior biologist, beginning a long and illustrious career that helped pave the way not only for women in science, but also for the field of environmental pollution research.

In the early 1940s, Lucille began studying the common box turtle (*Terrapene carolina*) at Patuxent. Perhaps it was only a “folklore story,” but some staff members were told that it was the Stickels’ walks in the Patuxent woods with their dogs and Lucille’s love for mushrooms that caused her to begin recording her observations and, subsequently, marking box turtles. After 1 or 2 years of data collection on box turtles, Lucille sent a partial manuscript to the University of Michigan asking them to consider box turtles as her new research topic. The University of Michigan approved her request, so she continued her box turtle research and received her Ph.D. in 1949. Lucille’s research on box turtle populations at Patuxent spanned several decades, as did her work with her husband, Bill, on black rat snakes (*Elaphe obsoleta obsoleta*). The

common box turtle work continues today, and is remarkable as a study of a wildlife species that has continued for decades. In recognition of her pioneering work, the Box Turtle Conservation Workshop Committee established the Lucille F. Stickel Box Turtle Research Award to contribute to the survival of wild box turtle populations.

Dr. Stickel’s interest in plants and animals extended far beyond her well-known research interest in contaminants. She published six papers in the *Journal of Mammalogy* about populations of small mammals, especially the estimation of home range size. Her scientific work distinguishes her as a member of a small but notable group of women who made important early contributions to the field of mammalogy.

During World War II, Bill was on military furlough from June 1943 to December 1945, serving in the U.S. Army’s 38th Malaria Survey Unit in New Guinea and the Philippines. Not surprisingly, while there, Bill collected reptiles and amphibians, which he donated to the U.S. National Museum. His animal collections included several new species, including one new frog species named for him (*Kaloula conjuncta stickeli*). Lucille told Richard and me that when he returned to the United States, Bill spent some time in a military hospital near Asheville, NC. The hospital stay may have influenced the Stickels’ selection of a retirement home in western North Carolina. Bill returned to Patuxent in 1945 and resumed his research.

The Prime Years at Patuxent

Throughout their long careers, the Stickels dedicated their lives to the field of wildlife toxicology and played a major role in the development of the worldwide recognition of Patuxent as an eminent research institution. They were also deeply interested in its varied habitats, and were often seen on weekends picking up litter and pruning a few trees and shrubs.

From 1952 to 1959, Bill was the editor of "Wildlife Review," which provided professional access to current research developments in the field of wildlife biology. Over the years, he answered many letters of inquiry to Patuxent, providing his unique insights in language that was readily understood. He also gave many tours of Patuxent to visiting dignitaries and the interested public.

Dr. Stickel published her first contaminant paper in 1946, reporting the results of a field study of a mouse population in an area treated with DDT. At that early date, virtually nothing was known about the harmful effects of pesticides on wildlife. Pioneering research by the Stickels and their colleagues formed much of the basis for Rachel Carson's groundbreaking 1962 book, "Silent Spring," which alerted the world to the dangers of pesticides (Carson, 1962).

In the early 1960s, biologists did not know conclusively the cause of population declines in several species of birds that were feeding high on the food chain. Eventually, in 1969, scientists at Patuxent published a paper linking dichlorodiphenyldichloroethylene (DDE), a metabolite of dichlorodiphenyltrichloroethane (DDT), to eggshell thinning in birds, which, in turn, resulted in reduced population recruitment (Heath and others, 1969). The Stickels' concern with the toxic effects of environmental contaminants, especially pesticides and heavy metals, continued throughout their lives. Their research on the use of diagnostic tissue residues of contaminants represents one of the major accomplishments in the history of wildlife toxicology. They demonstrated that the concentrations of pesticides in the brains of dead birds could be used to determine whether those chemicals were responsible for their deaths. With Dr. Stickel's leadership, Patuxent scientists provided the laboratory proof that chemicals were directly related to population declines in many bird populations, including brown pelicans (*Pelecanus occidentalis*) (Blus and others, 1977) and bald eagles (*Haliaeetus leucocephalus*) (Wiemeyer and others, 1993).

In 1968, Dr. Stickel received a Federal Woman of the Year award. She also received the U.S. Department of the Interior Distinguished Service Award. She was the first and only woman to date (2016) who received the Wildlife Society's Aldo Leopold Memorial Award in recognition of her "distinguished service to wildlife conservation," a distinction she received in 1974. Dr. Stickel also was the first woman to direct a major Federal fish and wildlife laboratory, serving as Patuxent's director from 1973 until 1981. Throughout the years, she was recognized as the "first lady" of the U.S. Fish and Wildlife Service, a mantle she wore with humility, but also with grace and charm.

Retirement

The Stickels remained at Patuxent, living in modest government housing, until their retirement, with a combined total of 81 years of government service, in March 1982. They retired to the mountains near Franklin, NC, where they spent many happy years identifying the flora and fauna on their property and the surrounding area, caring for their varied collection of dogs, and supporting local land conservation efforts.

Lucille's interest was in ferns and fungi, two that were difficult to study. Bill collected many plants, worked cooperatively with Western Carolina University in Cullowhee, NC, and added many species to plant distribution records for Macon County, NC, where they lived. Not surprisingly, the Stickels set up a laboratory in the lower level of their home to facilitate their work.

Lucille often inquired about the status of people they had worked with at Patuxent. Bill, on the other hand, did not participate in these discussions and stated that he wished to remember Patuxent and its staff as they were when he and Lucille left. They did not return to Patuxent during retirement.

Bill Stickel died on February 11, 1996, after a lingering illness. For many years, Bill and Lucille had hiked in the mountains on and near their property, drawing detailed maps and observing and recording interesting plants and animals. Lucille continued to hike even when Bill was no longer able to do so, leaving detailed maps of her travels with his caregivers. Eventually Lucille and her dog, Sharlie, moved to a villa in a retirement community in Asheville, NC.

Even after all the intervening years, Dr. Stickel's profound influence on the field of contaminants research remains. The approximately 40 research scientists she hired at Patuxent have published more than 1,000 scientific papers, chaired



Thanksgiving dinner at the Stickels' home at the Patuxent Wildlife Research Center, Laurel, MD, 1951 (from left to right: Bill, Lucille, Clark Webster, Lois Horn, Fran Uhler, and Helen Webster). Photo by Francis M. Uhler, Patuxent Research Refuge.

many symposia, and authored many books in the biological sciences. Several of these scientists have gone on to leadership roles in the U.S. Fish and Wildlife Service, the U.S. Geological Survey, universities, and private industry. As a testament to her continued influence and the respect with which she was regarded, two groups of research scientists she selected, mentored, and inspired visited her at her home in Asheville in late 2006. That 2006 visit was our last visit with her, and she died in Asheville on February 22, 2007 (Coon and Perry, 2007).

Mrs. Lilian Linduska shared some thoughts with me after hearing of Lucille's death. She and her husband, Dr. Joseph Linduska, lived at Patuxent in the 1940s. Lilian's memories are of "a warm and attractive and caring friend. She and Bill loved dogs and always had one or two. She was also a great hostess and party giver. Some of her recipes are still in my files marked with a star indicating they are especially good." I am also fortunate to have some of Lucille Stickel's recipes.

On November 15, 1998, more than 50 years after her first publication on contaminants appeared, the Society of Environmental Toxicology and Chemistry, at its annual meeting in Charlotte, NC, announced that it would present its prestigious Rachel Carson Award to Dr. Lucille F. Stickel. That award is further evidence of the continuing importance of her many contributions to the field of wildlife toxicology.

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Lucille and Bill Stickel at a picnic table during a farewell party at the Patuxent Wildlife Research Center, Laurel, MD, 1982. Photo by W. James Fleming, U.S. Fish and Wildlife Service.

Lead Poisoning Studies and Shooting Tests with Soft-Iron Shot

By Jerry R. Longcore and Ralph Andrews

Background

Lead poisoning in vertebrates was first reported in Germany in 1842 (von Fuchs, 1842). Waterfowl deaths caused by ingesting toxic lead pellets deposited in wetlands across the United States have been recorded since 1874 (Phillips and Lincoln, 1930). Early reports of lead poisoning in waterfowl were made by Bowles (1908), McAtee (1908), and Wetmore (1919), among others. One proposed remedy was the use of a form of “disintegrable” lead shot—that is, shot made from lead-magnesium alloys (Green and Dowdell, 1936; Dowdell and Green, 1937). Jordan and Bellrose (1950) tested Lubaloy (copper-coated lead) pellets, a lead-tin-phosphorus alloy, a lead-magnesium alloy, and a lead-calcium alloy for toxicity in Pekin ducks, but none of these showed promise under test conditions. Jordan and Bellrose (1950) also tested the components of commercial shot (lead, arsenic, and antimony) and determined that lead was the sole cause of lead poisoning. They tested the effects of the aquatic plant coontail (*Ceratophyllum demersum*) in the diet of lead-dosed ducks and reported a beneficial effect. Elder (1950) measured hunting pressure in waterfowl in Delta Marsh, Manitoba, Canada, with a portable x-ray machine, and noted high percentages (22–49 percent) of juvenile mallard (*Anas platyrhynchos*), Northern pintail (*Anas acuta*), and redhead (*Aythya americana*) ducks with ingested shot.

Bellrose (1959) comprehensively documented the extent of lead-shot pellets deposited by hunters in wetlands across the four flyways and then ingested by waterfowl and found in their gizzards. The Mississippi Flyway Council Planning Committee (1965, unpub. report) brought attention to the unintentional deaths of waterfowl throughout the flyway and advocated action. A year later, Baker (1966) reported on the industrial status of lead shot pellet substitutes that were far from being perfected, and was not optimistic about a substitute, because lead is so well suited for making shot. The continuing decline in duck numbers, however, prompted administrators of the Bureau of Sport Fisheries and Wildlife (part of the U.S. Department of the Interior [DOI]) to join with industry, represented by the Sporting Arms and Ammunition Manufacturers' Institute (SAAMI), to renew efforts to find or develop a nontoxic shot to replace lead shot in waterfowl hunting.

In November 1966, SAAMI obtained proposals from three private research firms and then awarded a \$100,000, 2-year contract to the Illinois Institute of Technology-Research Institute (IIT-RI) to develop a suitable substitute shot. Research biologists at the Patuxent Wildlife Research Center (Patuxent) evaluated each candidate shot for toxicity to ducks. Through a cooperative agreement (U.S. Department of the Interior, 1966), industry was tasked with testing the shot for ballistics.

Search for Nontoxic Substitute Shot

The challenge for IIT-RI was to find a nontoxic material that was at least as dense as iron (steel), soft enough to avoid scratching or blowing out the choke of shotgun barrels, and available at a reasonable cost (that is, less than two times the price of lead). IIT-RI used three approaches to address this challenge (Andrews and Longcore, 1969). First, researchers would seek to find a biochemical additive, an organic compound with the ability to hinder the formation of soluble lead salts in a duck's gizzard that could be added to powdered lead. This compound would then be extruded in wire form and cold headed (that is, the wire would be altered through force with a series of tools and dies) into shot. A second approach was to develop iron-lead composites in a thermoplastic binder. Low-carbon iron powder would be mixed with lead powder (to increase density), then coated with thermoplastic and extruded in wire form. The third approach was to develop a soft-iron shot by heating the iron to high temperatures to anneal commercial low-carbon steel wire, a process that produces wire that has an extremely coarse grain size and a low carbon content. During the first year of the contract, IIT-RI screened and bench tested many organic compounds and determined that a metallic ion-sequestering compound, ethylenediaminetetraacetic acid (EDTA), and the amino acid creatinine were the most promising. Attempts to extrude powdered lead into wire after the addition of small amounts of these compounds failed because the resulting wire was too brittle for use in fabricating shot. Similarly, the iron-lead thermoplastic mixtures were unsatisfactory because the flow properties of available thermoplastics were inadequate. The possibility of developing a soft-iron shot improved after a commercial

low-carbon steel wire that cost only about 10 cents per pound was located. It was believed by the industry that annealing this low-carbon wire in wet hydrogen at 1,600 degrees Fahrenheit would produce a material soft enough for use in fabricating a suitable shot.

Evaluation of Proposed Substitutes at Patuxent Wildlife Research Center

In the summer of 1964, Jerry Longcore was hired as a biological technician in Patuxent's Section of Wetland Ecology to assist Frank McGilvrey in waterfowl studies. Longcore's appointment ended in 1965, but he returned in 1966 at the request of Section Leader John Sincock to assist as a coinvestigator with Ralph Andrews on the lead poisoning project.

Initial testing of potential substitutes for lead shot at Patuxent began in 1965. Locke and others (1966) documented the formation of acid-fast intranuclear inclusion bodies in kidneys of ducks exposed to lead. These inclusion bodies were an accurate marker of lead intoxication in ducks. Irby and others (1967) reported that plastic-coated lead pellets were just as toxic as the lead standard (96 percent mortality); a lead-magnesium alloy was one-half as toxic (54–63 percent mortality); and iron, zinc-coated iron, and copper were slightly toxic (0–12 percent mortality). A second batch of candidate materials (tin-lead alloy, zinc, nickel, Teflon-coated steel, and tin), all in shot form, was used to dose male mallards in a 30-day test (Grandy and others, 1968). The tin-lead alloy caused 27 percent mortality of test mallards; the zinc caused 20 percent mortality. No mortality was observed with nickel, Teflon-coated steel, or pure tin.

Dosing of mallard ducks with proposed substitute shot types followed a standard protocol (Longcore and others, 1974a). Most tests were conducted during 1967–69 in late fall through early spring, when ducks in wetlands in the wild are most typically exposed to spent shot. Replicates (3–5) of a five-duck group were given eight number (no.) 6-size pellets of a proposed substitute shot; and at the same time, replicates were dosed with eight no. 6-size commercial shot as a toxicity standard. For each toxicity test, control mallards (6–16) were maintained. The test diet was whole corn; test duration was 40 days to evaluate shot retention and duck survival. Lead shot coated with nickel to various thicknesses reduced short-term mortality by one-half, but only delayed mortality until the nickel eroded. Combining tin with nickel did not reduce mortality (80 percent) because tin-nickel coating eroded, exposing ducks to lead. Steel shot plated with lead to increase density caused 95 percent mortality, whereas a thinner layer caused 60 percent mortality. Mortality of mallards dosed with two different shot types formed with lead powder and a mucilage type or a polyvinyl acetate water-soluble binder (73 percent) was not different from that of those dosed with the lead-shot test standard (that is, eight no. 6 lead shot) (87 percent). Mortality of mallards dosed with a 1.4-gram (0.05-ounce [oz]) piece

of wire containing either 1 or 2 percent creatinine or EDTA ranged from 75 to 90 percent, and was not different from mortality associated with the lead standard (70 percent).

The toxicity test results indicated that if a shot contained lead, the grinding action of the gizzard and acidic gastric juices usually would ultimately expose the lead and result in mortality. In 1933, one of the leading manufacturers of shotgun shells obtained a patent that claimed the addition of only 0.3 to 1.0 percent of phosphor-tin would render lead shot harmless to waterfowl (Jackson, 1933). The patent claimed "... actual experiments with the alloy upon wild ducks have shown it to be harmless." A quantity of this shot was obtained from the company and compared with standard lead shot. All 15 ducks in both groups on a corn diet died, but those dosed with the reputedly nontoxic shot died, on average, 4 days sooner than those dosed with commercial lead shot (Longcore, Andrews, and others, 1974). Finley and Dieter (1978) tested shot formed by combining lead with iron powder, referred to as "sintered" shot, in various amounts. Mortality was greater in ducks dosed with commercial lead shot than in those dosed with the lead-iron shot with a comparable amount of lead. Ingestion of two no. 4 lead-iron shot (0.004 oz of lead) caused slight weight loss and 5 percent mortality, but 45 percent of



Impaction of proventriculus caused by ingested lead shot in the mallard on the left. Photo by Fred B. Samson, Bureau of Sport Fisheries and Wildlife.

ducks dosed with five lead-iron shot died. Other candidate materials were not considered further for reasons of cost, malleability, production limitations, or low density with expected poor ballistics performance.

In addition to proposed substitute shot evaluation, effects of commercial lead shot were tested among adult and juvenile male and female mallards with no difference in mortality (90–100 percent) among sex and age groups. No differences in mortality (93–100 percent) were detected among male and female game-farm mallards, wild mallards, or male American black ducks (*Anas rubripes*). Rattner and others (1989) dosed game-farm mallards, pen-reared black ducks, and wild black ducks with one no. 4 lead shot and fed the ducks pelleted feed. After 14 days, these ducks were redosed with two or four additional no. 4 lead shot. On the basis of all measures of lead toxicity (that is, mortality, weight change, delta-aminolevulinic acid dehydratase activity, and protoporphyrin concentration), black ducks and mallards were considered equally tolerant of lead. Longcore and Andrews (1974) noted, however, that commercial duck pellet feed seems to ameliorate the toxic effects of lead. In contrast, a single no. 4 commercial shot killed 18 to 20 percent of either male or female yearling mallards on a corn diet during a 40-day test (Longcore and others, 1974a). Because Godin (1967) and others reported possible beneficial effects of oyster-shell grit in lead-poisoned ducks, we retested specifically to determine shot retention by fluoroscopy. We raised 50 grit-free mallards by transferring ducklings from brooders to wire-floored pens at 3 weeks of age and never exposed them to grit. Mortality of yearling, lead-dosed (five shot, no. 6 size) mallards offered oyster shell, quartz grit, or no grit was reduced in mallards fed oyster shell (only 4 of 12 died) compared with those on quartz grit (9 of 12 died) or no grit (12 of 12 died). Survival was related to the number of shot retained more than 14 days and to the associated degree of erosion of the shot pellets (Longcore and others, 1974a).

Because foods eaten by ducks may mitigate the effects of ingested lead pellets, Andrews, Longcore, and others initiated a study in January 1967 to clarify earlier work (Jordan, 1952). Jordan and Bellrose (1950) reported that of 80 mallards dosed with five no. 4 or no. 10 lead shot, only 5 ducks died (6.2 percent). We dosed 150 male mallards with either three or eight no. 6 lead shot and held birds on one of five diets—commercial duck pellets, whole corn, cracked corn, mixed small grains, or no food—for 40 days (Ralph Andrews and others, U.S. Fish and Wildlife Service, written commun., 1967). Seventy-five undosed male mallards also were held on the various diets to clarify the effect of diet on shot retention, shot erosion, production of acid-fast intranuclear inclusion bodies in kidneys (Locke and others, 1966), and mortality. We also monitored weight changes related to diet. On each of the grain diets, mortality was 80 percent for those groups of ducks on the eight lead-shot dose, whereas mortality was similar (20–30 percent) for ducks on each of the grain diets and the three lead-shot dose. In contrast, only two ducks on the commercial duck pellet diet and dosed with eight lead shot died, and none of the ducks dosed with three lead shot died. We fluoroscoped

surviving ducks on grain diets and determined that they lived because they voided shot before much of the lead could be eroded. Ducks fed commercial duck pellets, however, retained shot as readily as those on the grain diets, and the lead was rapidly eroded in their gizzard, but they did not show signs of poisoning. These data indicate that substances in the duck pellets may combine chemically with lead ions in the digestive tract and protect the ducks from poisoning. A follow-up study documented the efficacy of duck pellets. In late February 1967, each of 50 male mallards was dosed with eight no. 6 lead shot. Twenty were given a diet of whole corn, 10 were given corn meal, 10 were given duck pellets, and 10 were provided with mats of the aquatic vegetation (that is, water-starwort [*Callitriche* sp.]) in their water tanks. After 1 week, 10 of the ducks on whole corn were switched to a diet of duck pellets. Mortality rates recorded were 100 percent on the whole corn diet, 70 percent on the corn-meal diet, 0 percent on duck pellets, 40 percent on corn followed by duck pellets, and 40 percent on the aquatic vegetation. The *Callitriche* did not provide sufficient nutrients; therefore, duck pellets were supplied after 1 week for this group. We concluded that softness of the duck pellets was not the beneficial property and that this aquatic plant did not alleviate poisoning, but that duck pellets lessened the effects of ingesting lead even after signs of lead poisoning were evident. Lead is readily stored in bone and can be detected in many tissues, blood, and organs of organisms exposed to lead. The concentration of lead residues in tissue seems clearly diagnostic of acute lead poisoning in the mallard duck and was determined to equal or exceed 3 parts per million (ppm) in the brain, 6 to 20 ppm in the kidney, 6 to 20 ppm in the liver, and 10 ppm in clotted blood from the heart (Longcore and others, 1974b).

Evaluation of the Killing Efficiency of Lead and Iron Shot

The lack of emergence of any proposed shot type except iron shot as an alternative after all of the testing led to the big question: Does iron shot have adequate ballistics to effectively kill ducks at reasonable distances? Earlier, Bellrose (1959) had tested an annealed iron shot produced by Olin Mathieson Corporation (Clayton, MO) and determined that it performed almost as well as lead shot at distances of as much as 50 yards (yd) (Andrews and Longcore, 1969). The Mississippi Flyway Council Planning Committee (1965, unpub. report) reported on a comparative field test in which no. 2 iron shot killed ducks as effectively as no. 4 lead shot at a range of 40 yd, and resulted in fewer crippled ducks. Several studies documented that when lead shot was used, many ducks were crippled and not brought to bag, and that crippled ducks may recover (Tiemeier, 1941; Trautman, 1943; Whitlock and Miller, 1947; McGinnes and Beck, 1953; Kirby and others, 1981) and may even be harvested later. Bellrose (1953) stated that unretrieved kill was approximately 24 percent of total mallard kill and



Tom Whittendale, Jr., and Jerry Longcore, U.S. Fish and Wildlife Service, weighing a duck used in the lead-shot study, Patuxent Wildlife Research Center, Laurel, MD, 1967. Photo by Fred B. Samson, Bureau of Sport Fisheries and Wildlife.

that only a small percentage of the ducks knocked down, but unretrieved, would actually recover. Because many uncontrolled variables were associated with field tests of shot loads, SAAMI and Patuxent agreed to cooperatively develop a shooting rig that would allow the operators to choose variables independently. The following paragraph from Andrews and Longcore (1969) describes the shooting facility.

“A unique duck-transport device was engineered by the ammunition industry and constructed at the Patuxent Wildlife Research Center. This automated shooting device moved a tethered, wing-flapping duck across a point where the mounted, pre-aimed gun fired a ‘perfect’ shot....A close simulation of a free-flying duck, passing a shooting position, was achieved. The shotgun was mounted on a movable wooden ‘horse’ and triggered by a solenoid activated through a micro-switch. Other micro-switches braked the carriage on forward and return trips.” [A glitch emerged in the braking system as the carriage went over the end of the track. Longcore observed the repeatable malfunction and deduced that the clutch-brake unit required a keyway in the shaft. Industry engineers, although skeptical, agreed to send a new shaft with keyway and key and, once installed, it worked well.] “...A movable control

box for the entire facility was positioned beside the gun mount. Sighting stakes were erected for each shooting distance so that the gun could be accurately aimed prior to each shot. Standard 30-inch targets were shot to locate center of patterns and determine positions of sighting stakes. The targets were also used to assure that ducks were centered in the pattern prior to each day of shooting.”

Supplies for the test were provided by SAAMI. We used a 12-gauge pump shotgun with a full choke and 30-inch (in.) barrel. Because iron shot could potentially affect the choke, which could in turn affect test results, additional barrels were used after a preset number of rounds had been fired through a barrel. The shot types tested were 2.75-in., 1.25-ounce loads of commercial no. 4 lead shot, and no. 6 lead shot as standards for comparison. SAAMI supplied 1,000 pounds of no. 4 soft-iron shot and loaded rounds with slow-burning ball powder for maximum muzzle velocity. The standard iron load was 1 ounce of shot that contained 180 pellets, which was identical to the 180 pellets in a no. 4 lead load. The load of iron shot was encased in a polyethylene liner to further protect gun barrels.

Three thousand game-farm mallards were maintained in fenced impoundments at Patuxent in 1967. Keeping them fed daily was taxing. We received help in maintaining the ducks in



Jerry Longcore and Tom Whittendale, Jr., U.S. Fish and Wildlife Service, readying the target to test the shot pattern in the lead-shot study, Patuxent Wildlife Research Center, Laurel, MD, 1967. Photo by Fred B. Samson, Bureau of Sport Fisheries and Wildlife.

an unexpected way. Serendipitous circumstances led Lorenzo King, a Washington, D.C., taxi driver, to become a biological technician and to participate in the shooting test. One day in Washington, D.C., John Gottschalk, Director of the Bureau of Sport Fisheries and Wildlife, hailed a taxi and was picked up by King. Gottschalk noticed a copy of an outdoor sporting magazine in the back seat of the taxi and questioned King about his interest in the outdoors and wildlife. King indicated that he was very interested, and subsequently applied for and was offered a job in the Section of Wetland Ecology at Patuxent, where he became part of the shooting-test crew.

Robert G. Heath, Patuxent's resident statistician, used a split-plot statistical design to analyze the resulting data. Shooting distance made up whole plots, and combinations of shot type and sex of ducks, arranged factorially, made up subplots. Shot loads were patterned on a 30-in.-diameter circle for each distance before shooting to ensure the gun was centered for a "perfect" shot. For any given combination of shot type and

distance, groups of five ducks, either male or female, were shot in random sequence. Shot patterns were obtained after a shooting day to ensure the gun and carriage were performing as required. Initial tests started in March 1968 were at 30, 40, and 50 yd, but because all shot types were effective at 30 yd, we replaced the 30-yd range with a 60-yd range and finished the tests in June 1968. Later, during November–December, we tested the effectiveness of shot loads at 45, 55, and 65 yd. The basic testing was done by firing at the broadside of the passing duck, but 300 additional ducks were shot from a nearly head-on direction at 40 and 50 yd for all shot types. Because of a keen interest in degree of crippling among shot types, we had finite kill categories: "instant kill" (< [less than] 1 minute [min]), "death in 1–5 min," "death within 5 min to 1 day," and "death within 1–10 days." After each day of shooting, all dead ducks were weighed and examined for broken bones before they were stored in a freezer. Live ducks were kept on food and water for 10 days. Throughout most of this work, Tom Whittendale, Jr., was a valuable colleague and provided excellent support as the biological technician on the project. Ducks that were still alive after 10 days were euthanized with carbon monoxide, weighed, and fluoroscoped for embedded shot; a sample of 630 ducks was defeathered to count entrance and exit wounds. This task, like most tasks associated with this study, was somber. Every day, the empathy for the test ducks was etched in the faces of the crew. Although these longevity categories could not translate to field conditions, they were an objective way to compare effectiveness of shot types and inform about potential crippling losses.

The statistical examination of the shooting-test data by analysis of variance did not reveal differences ($P = 0.05$) between no. 4 lead and no. 4 iron shot in numbers of ducks



Tom Whittendale, Jr., and Jerry Longcore, U.S. Fish and Wildlife Service, placing a duck on the transport cart during the lead-shot study at the Patuxent Wildlife Research Center, Laurel, MD, 1967. Photo by Fred B. Samson, Bureau of Sport Fisheries and Wildlife.



Gun firing automatically when the transport cart carrying the duck hits the micro-switch during the lead-shot study at the Patuxent Wildlife Research Center, Laurel, MD, 1967. Photo by Fred B. Samson, Bureau of Sport Fisheries and Wildlife.

“probably bagged” or numbers of “crippled and lost” ducks. No difference in vulnerability was detected between males and females. Shooting distance was the only highly significant ($P = 0.01$) variable related to percentages of ducks “probably bagged.” The no. 6 lead load, however, was slightly more effective ($P = 0.05$) than either of the no. 4 loads (180 pellets), most likely because of the greater number of pellets (300) in the no. 6 lead load.

When Winchester-Western decided to conduct its own shooting test in November 1972–March 1973, Dr. Charles Loveless (Assistant Director of Research, U.S. Fish and Wildlife Service [USFWS]) sent Longcore to East Alton, IL, to be the official observer. A duck transport facility, similar to that used at Patuxent but 100 feet long and with more amenities (for example, Plexiglas windows in the shed for the rig operators), had been constructed at Nilo Farms, Brighton, IL. One morning a black limousine arrived at the facility where Ed Kozicky and John Madson (Winchester-Western employees) and Jerry Longcore were preparing to operate the rig. John Olin and Nathaniel Reed (Assistant Secretary of Fish and Wildlife and Parks) emerged from the vehicle and were introduced.

After some explanations, it was time to demonstrate how the facility worked. We caught and tethered a mallard on the carriage; Mr. Kozicky loaded the shotgun and, when all was ready, he hit the switch. As the carriage crossed the firing point, the presighted gun fired and the load of shot killed the duck instantly, revealing the lethality of a nontoxic steel shot that could replace toxic lead. Mr. Olin inquired if the shot was lead shot and Mr. Kozicky replied that it was not; it was steel. Secretary Reed looked at Longcore and nodded, acknowledging the performance of steel shot. Although this was an impressive demonstration of the lethality of iron shot, Winchester-Western interpreted shotshell efficiency to be the ratio of the number of birds bagged to the number crippled (Kozicky and Madson, 1973). All of the ducks (2,400) used in the Nilo Farms test were sent to the University of Wisconsin, Madison, where Cochrane (1976) performed a detailed examination of the carcasses and the shooting-test results. Also, he compared results of the Nilo Farms test with those of Andrews and Longcore (1969) and concluded that the Nilo Farms no. 4 lead shot performed more effectively than the Patuxent no. 4 lead shot or the no. 4 steel shot. This result was not unexpected because of the greater weight and number of pellets in the Nilo Farms no. 4 lead load (that is, 1.5 oz of shot, 2.75-in. Winchester-Western Super-X, XX magnum shell with 198 pellets) compared with the Patuxent no. 4 lead load (that is, 1.25 oz of shot with 180 pellets [10 percent fewer]), which was a less robust load (Kozicky and Madson, 1973). Furthermore, the Nilo Farms no. 4 steel load (that is, 1.13 oz of shot with 214 pellets) was also a superior load compared with the Patuxent no. 4 steel load (that is, 1.0 oz of shot with 180 pellets). In addition, the Nilo Farms no. 4 lead load contained “GreX” (granulated, high-density polyethylene) that filled the interstitial spaces between pellets, thereby helping to maintain pellet sphericity (Lowry, 1973), which improved pattern density (the number of pellets in a 30-in.-diameter circle) from 75 to 88 percent (a 14.8-percent increase) (Cochrane, 1976). The Nilo Farms no. 4 steel load also contained GreX, which resulted in a pattern density of 83 percent, in contrast to a pattern density of 70 percent for the Patuxent no. 4 steel shot load (Kozicky and Madson, 1973). The Nilo Farms no. 4 lead and steel loads were expected to perform better than Patuxent shotshell loads because the Nilo Farms shells had more pellets per load and, therefore, a greater pattern density, and a duck’s fate is determined by the number of pellets that strike it (Cochrane, 1976). Criteria used to designate bagged, crippled, and surviving ducks were defined more specifically. Kozicky and Madson (1973) maintained that the only true measure of shotshell efficiency as it relates to field conditions is the ratio of “birds bagged to birds crippled.” Despite the greater weight and number of pellets in the no. 4 lead and steel loads used in the Nilo Farms test compared to those used in the Patuxent test, many results were the same—no difference between sex and age groups; in broken bones within the categories of bagged, crippled, and survivor; in capacity to break wing or leg bones; in healing rates of bones; and in mean number of entrance wounds. Numbers of entrance wounds and embedded

shot were inversely correlated with distance for all shot types. Crippling rates per 100 mallards for the Nilo Farms no. 4 lead and no. 4 steel loads were inconsistent on the basis of the data of Kozicky and Madson (1973) and depicted in Cochrane (1976, fig. 3). At 50 and 60 yd, the crippling rate of no. 4 steel slightly exceeded that of no. 4 lead, but at 70 and 80 yd, the crippling rate of no. 4 lead substantially exceeded that of no. 4 steel. The anomaly is that at 40 yd, the Nilo Farms no. 4 steel had a crippling rate of approximately 20 percent, whereas the no. 4 lead had a rate of approximately 7 percent as estimated from Cochrane (1976, fig. 3). This anomaly is not fully explained, but Lowry (1973) attributed better performance of no. 4 steel shot in the Patuxent test compared to that of the commercial no. 4 steel shot in the Nilo Farms test to difference in average temperature during shooting—66.5 degrees Fahrenheit (°F) at Patuxent and 36.5 °F at Nilo Farms. At the shooting preserve of the Max McGraw Foundation, Nicklaus (1976) tested no. 4 lead, no. 6 lead, and no. 4 steel on flying mallards released from towers and found no difference in crippling rates between lead and steel. The number of body shot in these flighted ducks did not differ between ducks shot with lead and those shot with steel, and was not statistically different from numbers of embedded shot found in wild populations (Bellrose, 1953).

In an Olin Corporation news item, Madson and Kozicky (n.d.) released the results of the Nilo Farms shooting test of lead and steel shot and attempted to estimate crippling loss for steel shot. They calculated an estimate based on the average annual bag of ducks as 10.6 million during 1955–71 with lead shot; then, if crippling loss is 20 percent, about 2.1 million more ducks are lost as cripples caused by lead shot. Applying the Nilo Farms data to a bag of 10.6 million ducks per season, they estimated the use of iron shot would increase crippling losses by 3 million ducks annually. John P. Rogers (USFWS, Migratory Bird Management Office), however, prepared a dichotomous key of what happened when a duck was fired on and examined 5-min kills for both lead and iron shot used in the Nilo Farms test and in the Patuxent shooting test. His interpretation of the average percentage of ducks not retrieved for all ranges (weighted—that is, 75 percent of all shots 45 yd or fewer) was 2.25 for lead and 6.1 for steel. Therefore, the weighted average was a 16.6-percent increase in unretrieved ducks with steel, resulting in a change from 2.1 million unretrieved ducks to 2.45 million unretrieved ducks—an increase of 350,000 ducks, not 3 million.

With a desire to move forward in implementing a ban on the use of lead shot over wetlands, Robert I. Smith and Longcore were assigned the task of drafting the initial Environmental Impact Statement in 1974 regarding the proposed use of steel shot for hunting waterfowl in the United States (U.S. Fish and Wildlife Service, 1974). The basement of Snowden Hall at Patuxent was the refuge where Longcore spent about 2 months reading documents and drafting sections of the Environmental Impact Statement, which was about 0.5 in. thick. The final Supplemental Environment Impact Statement for Hunting Migratory Birds in the United States increased the thickness of

the document to about 2.5 in. by 1986 (U.S. Fish and Wildlife Service, 1986).

The Patuxent shooting tests (Andrews and Longcore, 1969) clearly established the premise that a nontoxic substitute (that is, soft iron, or steel as tagged by its detractors) for lead shot could be developed. The stream of events that followed to implement steel-shot regulations are documented in Friend and others (2009). In 1978, Senator Ted Stevens of Alaska amended the DOI appropriations bill so that the USFWS could not enforce use of nontoxic shot without State approval. In Maine, for example, Longcore was directed to collaborate with the Maine Department of Inland Fisheries and Wildlife to sample duck gizzards and sediments in Merrymeeting Bay to determine whether nontoxic shot was necessary (Longcore and others, 1982). Incidence of ingested lead shot (5.9–8.1 percent) in the gizzards of black ducks from the bay during 1976–80 exceeded the action threshold (5 percent).

Although steel shot was clearly capable of killing ducks, hunters complained about the higher cost of shells and the presumed higher rate of crippling, and their impression was that steel shot was ineffective. It soon became evident that hunters were having difficulty adjusting to the steel shot loads with ballistic characteristics (a smaller, but denser shot pattern; shorter shot string; the need to adjust aiming point as distance increased) different from those of lead shot. Hunters would shoot at a duck, miss the duck, and blame it on the shot load. Poor performance by hunters, in reality, was the result of their inexperience with an unfamiliar product (Tom Roster, Cooperative Nontoxic Shot Education Program, Klamath Falls, OR, oral commun., 1996). Tom Roster, an independent ballistic consultant, author, and mathematician, was also an avid waterfowl hunter who took an interest in the controversy. He conducted many steel-shot shooting clinics, including “participatory” shooting events for hunters; these educational efforts furthered the acceptance by hunters of switching to steel shot or a future nontoxic shot. Necessity was the mother of invention; ammunition manufacturers needed to respond to meet the demand for improved nontoxic shot loads (Taylor, 2011). To evaluate newly developed substitute shot types for toxicity, however, the USFWS needed a protocol to thoroughly test candidate substitutes following standard procedures. This was a timely effort, as the Final Supplemental Environmental Impact Statement for Hunting Migratory Birds was being published in 1986 and steel shot was the only nontoxic shot approved for hunting migratory birds. Ammunition companies, however, were gearing up to seek alternatives to steel shot. In just a few days in 1985, Patuxent scientists Susan D. Haseltine and Barnett A. Rattner (U.S. Fish and Wildlife Service, written commun., 1985) drafted a set of testing protocols for determining toxicity of candidate shot types to waterfowl, which was recast to the format of the Federal Register and published by Morehouse (1986). This early, amended set of protocols appeared annually for about 10 years in the Code of Federal Regulations (Morehouse and Rattner, 1996). As use of other elements and compounds emerged in shot development, Dr. Rattner took the initiative

not only to expand the guidelines for testing candidate shot (or coatings) on waterfowl, but to include tests covering effects on other aquatic fauna and flora. This ecosystem-oriented, tiered testing protocol was presented at the Fifteenth Annual Meeting of the Society of Environmental Toxicology and Chemistry (Rattner and Morehouse, 1994). After several lengthy delays, a final rule for the testing protocol was published (Perry and others, 1997). Rattner continued to advise the USFWS on testing guidelines and proposed nontoxic shot for approximately 20 years.

Waterfowl ammunition has evolved with the use of higher velocity steel-shot loads, the development of hexagonal shot for more pellets per payload, and the substitution of loads composed of a blend of steel and tungsten shot, tungsten-iron alloy, tungsten-polymer, tungsten-iron-nickel alloy, and bismuth alloy shot (Sanderson and others, 1997a, 1997b). Implementation of nontoxic shot has progressed from initial regulations on seven National Wildlife Refuges in 1972, to increased regulation in 1985, and to mandatory use of nontoxic shot for waterfowl hunting in the United States in 1991 (Friend and others, 2009). Canada converted to nontoxic shot in 1999 (Taylor, 2011). Longcore recalls that, while expressing concern about how the public would react to the shooting of captive ducks, a high-ranking DOI administrator suggested that the steel shot should have been tested with bags of gelatin.

This approach, however, would have been inadequate because of the need to objectively determine the lethality of steel shot and to evaluate its effects on crippling of waterfowl. The emotional effects on the crew of this difficult study were mitigated by the expected conversion to nontoxic shot that ultimately would prevent thousands of migratory waterfowl and scavenging raptors from being poisoned by lead, which causes many birds to starve before dying. Throughout the long process of seeking a nontoxic substitute for lead shot, many State wildlife agencies and nonprofit organizations, especially the National Wildlife Federation, supported Patuxent's efforts and advocated for conversion to nontoxic shot for waterfowl hunting. The ultimate conversion resulted from a broad collaboration of Federal and State agencies, industry, and private citizens, whose persistent efforts greatly reduced the waterfowl lead-poisoning issue.

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Tom Roster, contract ballistics specialist, U.S. Department of the Interior, instructing hunters on techniques for shooting with nontoxic shot, Montana, 1994. Photo by Jeff Herbert, Montana Fish, Wildlife, and Parks Commission.

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Patuxent Researchers Tackle Heavy Metal Poisoning in Wildlife

By Gary H. Heinz

An Early Memory

When I first arrived at the Patuxent Wildlife Research Center (Patuxent) in Laurel, MD, in 1969, I saw a device that resembled a small railroad track out in a field. When I asked what it was, I was told it was a trolley on which tethered game-farm mallard ducks (*Anas platyrhynchos*) were shuttled in front of a shotgun. Everything was automated: when the duck crossed a certain point, a perfectly aimed shotgun was fired. Jerry Longcore and his supervisor, Ralph Andrews, U.S. Fish and Wildlife Service (USFWS) biologists at the time, were comparing the killing efficiency of iron shotgun pellets and traditional lead pellets (Andrews and Longcore, 1969). They found that, at typical shooting distances, the iron shot were perfectly capable of killing a duck. At the time, such an experiment, in which more than 2,000 ducks were being sent in front of a shotgun to be killed—and some only badly wounded—did not raise any questions in my mind. Today, if such an experiment were to be proposed and submitted to our Animal Care and Use Committee, I strongly doubt it would be approved; however, back then, it was approved and the results of this scientific “duck killing experiment” were critical in saving millions of ducks and other waterfowl from dying each year from lead-shot poisoning.

Jerry Longcore was only one of many hard-working and dedicated research scientists who proved that lead was killing millions of waterfowl each year when the birds inadvertently swallowed the shot from the bottoms of marshes across the country. They not only showed that it was the swallowing of lead shotgun pellets that killed the birds, they also paved the way for the eventual banning of lead shot from waterfowl hunting.

Purpose and Scope

First, I am not attempting to review the entire history of Patuxent’s research on heavy metals. That could be a book in itself. I want to tell a short story that is not mired in all the heavy metals that were studied at Patuxent and all the publications that resulted from those studies. This story is as much

about the people who studied heavy metals like lead as it is about the findings from their studies.

In this chapter, I share two often unappreciated observations: (1) it takes a surprisingly large group of dedicated researchers many years to bring about a change such as the banning of lead shot; and (2) even when one problem with a particular heavy metal, like lead shot, has been solved, different problems with the same heavy metal commonly surface down the road, and their solution can require an equally great effort.

Why Focus on Lead?

Why am I writing about lead contamination? First, I have personally been involved with some studies on lead, so I am familiar with lead toxicity. Second, studies on lead represent some of Patuxent’s most important contributions to solving contaminant problems, and success stories are what contaminant research is supposed to be all about.

For many years, I lived in a house on the Patuxent Research Refuge (Patuxent’s original name). One day my son, Brian, who was about 10 years old at the time, was out with two of his neighbor friends, Nate and Ben, exploring the marshes and ponds that dot Patuxent. They found a dead Canada goose (*Branta canadensis*) and brought it up to our house, asking me why I thought it had died. To give them a lesson in biology, I got a knife and I opened the goose. Everything looked normal. It had lots of fat. I did not see any injuries. I began to identify for them all the internal organs—here is the heart, here is the liver, the lungs, and so forth. Here is the gizzard. So they asked, “What is the gizzard for? Why is it so big? Do all animals have one?” “Well,” I said, “I will open it up and tell you.”

As I slit through the muscular wall of the gizzard, the metal of the knife made a strange sound, like metal scraping against metal. Inside was the normal assortment of sand-sized to small-gravel-sized grit. To my surprise, however, mixed in with that grit was a total of 518 shotgun pellets, plus a half-dozen small lead fragments. Some of these pellets were rusted and, suspecting they were steel shot, I used a magnet

and determined that 55 of the shot were, in fact, steel, but the remaining 463 were very small lead pellets—about size 9—that might be used to shoot clay pigeons at a trap and skeet range. I guessed that this bird had been feeding at such a range and had picked up the shot, mistaking them for the grit it was seeking.

None of the shot had been eroded out of the normal, round shape into the flattened, disk-shaped pieces one typically finds in a bird that has survived long enough to have its gizzard grind away at the pellets. The several small lead fragments I found were probably pieces of lead shot that were created by collisions of the shot with other shot as they exited the shotgun barrel or as they collided with the clay pigeons they hit. The lack of erosion of the lead pellets, plus the fact that this goose had not progressed through the typical lead-poisoning phase of weight loss, suggested to me that the dose of lead shot likely was so massive that the bird had died of rapid, acute poisoning.

Holly Obrecht, our refuge biologist, told me he had found many dead geese that year, all with lead pellets in them. Holly sampled the mud on the bottoms of local marshes and visited local shooting ranges, trying to find the place where these geese had picked up their pellets, but he could never locate the source of all this lead shot. To my knowledge, all these years later, no one has ever found it.

Lead-Shot Research

Long before my son and his friends found the dead Canada goose, Patuxent researchers knew the same thing: ingesting lead pellets, even a few, can kill a bird. In 1951, Dr. Don Coburn and his coworkers published a paper in “The Journal of Wildlife Management” (Coburn and others, 1951) describing the toxicity of lead to mallards. A laboratory building at Patuxent was later named after Coburn and, for several decades, Coburn Laboratory was used for the study of the effects of lead and many other contaminants on birds.

In the 1960s, Lou Locke, the Patuxent veterinarian, and his coworkers George Bagley and H.D. Irby reported on the histopathological effects of ingested lead shot on mallards, leading the way in showing how to identify lead poisoning in dead birds (Locke and others, 1966; Locke and others, 1967). Lou was fun to be around and seemed to be at his happiest when he was examining a dead bird to determine what had killed it. In the late 1960s, interest rose in finding a metal that could be formed into shotgun pellets and was not toxic to waterfowl. Soft-iron pellets (later called “steel shot”) were determined to be satisfactory, as discussed above (Andrews and Longcore, 1969). In one Patuxent study, nine different types of shotgun pellets were compared for their toxicity to mallards (Irby and others, 1967). Simply coating lead pellets with plastic did nothing to reduce their toxicity, as the plastic was ground off in the gizzard; iron and copper shot, however, were nontoxic. Patuxent biologists also discovered that mourning doves (*Zenaida macroura*) could be exposed to lead shot,

presumably mistaking them for grit (Locke and Bagley, 1967); therefore, other birds in addition to waterfowl were at risk.

In the 1970s, Patuxent scientists continued the research on lead-shot poisoning of birds. Wildlife biologist Mack Finley and physiologist Mike Dieter determined that merely mixing iron with the ballistically superior lead to make shotgun pellets did not completely resolve the poisoning problem (Finley and Dieter, 1978). Finley and Dieter joined with Lou Locke to show that lead-shot poisoning could be diagnosed by measuring an enzyme (delta-aminolevulinic acid dehydratase, or ALAD) in the blood of ducks (Finley and others, 1976). At about the same time, Patuxent scientists were in the field, determining the number of waterfowl being exposed to lead shot (White and Stendell, 1977; Stendell and others, 1979). Don White was a “no-nonsense” wildlife biologist who completed a study and promptly published it, then completed another study and published it; he was efficient and hard working. Rey Stendell went on to become a laboratory director at another U.S. Fish and Wildlife Service Research Center. An unusually large number of Patuxent scientists—I can think of nine off the top of my head—went on to become laboratory directors. I am not sure what that means; personally, I believe it indicates that Patuxent was a good training ground for future leaders, but perhaps there are other interpretations as well.

Biologists who were not in the contaminants program, but who did important work on lead shot, were frequently at Patuxent. For example, Joe Artmann and Woody Martin were never in the contaminants program and I do not think they did any other contaminant research, but they discovered that the sora rail (*Porzana carolina*) was another species that was ingesting lead shot in marshes (Artmann and Martin, 1975).

In the 1980s, Barnett Rattner and his colleagues determined that wild American black ducks (*Anas rubripes*) seemed to be more sensitive to lead poisoning than were game-farm mallards (Rattner and others, 1989). Barnett was a highly



Joe Artmann (left) and Woody Martin, U.S. Fish and Wildlife Service, on rail study in Patuxent River marshes, Maryland, 1976. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.



Tundra swans killed by lead in sediments of Coeur d'Alene River, ID, 1991. Photo by Dan Audet, U.S. Fish and Wildlife Service.

trained physiologist who blended his academic skills with an expanding interest in wildlife biology—a transition similar to the paths that many wildlife biologists took toward a career in wildlife toxicology. Such blending of talents and interests was common in those days, when few professionals were actually academically trained in what is now called ecotoxicology. In his office, Barnett has a picture of a double-crested cormorant (*Phalacrocorax auritus*) he shot for contaminant analysis; not bad for a physiologist. In 2012, Barnett started his term as president of the Society of Environmental Toxicology and Chemistry, the largest professional society in the world dedicated to studying the effects of environmental contaminants on wildlife. This was a great honor for him and for Patuxent.

In one example of how Patuxent scientists with various academic backgrounds joined forces to study lead-shot poisoning, Chris Franson, a veterinarian at Patuxent, teamed up with Mike Haramis and Matt Perry, both wildlife field biologists, and John Moore, a chemist, to measure protoporphyrin (a precursor to hemoglobin in the blood) to reveal how many canvasbacks (*Aythya valisineria*) had been exposed to lead shot (Franson and others, 1986). Hank Pattee, who was an avid duck hunter and wholeheartedly embraced the transition to steel shot, demonstrated that predatory birds such as bald eagles (*Haliaeetus leucocephalus*) could be poisoned by eating lead-poisoned ducks (Pattee and Hennes, 1983).

The studies mentioned above are only a fraction of the work done at Patuxent to verify the threat of lead pellets to birds. It was not until 1991 that lead shot was banned for waterfowl hunting in the United States. From the first Patuxent study back in 1951 by Don Coburn, it had taken 40 years of dedicated research, not only by Patuxent scientists but also by a legion of other scientists, to gather enough convincing information to ban lead shot. However, the decades of work were well spent, as millions of waterfowl and other birds were spared death caused by ingestion of lead shot. As the development of nontoxic substitute shot expanded beyond the iron

shot tested years earlier by Jerry Longcore, Barnett Rattner at Patuxent was designated as the scientist who would review the toxicity data generated for each of these proposed substitutes, making sure they would not pose a risk to birds.

So, With Lead Shot Banned, We Have Solved the Lead Problem, Right?

Unfortunately, no! Although lead shot was banned for waterfowl hunting in this country, there was no way to ban lead itself. The first problem with lead not associated with lead shot that came to the attention of Patuxent scientists was the emission of lead from leaded gasoline. Could lead from this source get into wildlife? To determine whether lead from vehicle emissions was getting into wildlife, Chris Grue, Dave Hoffman, and Nelson Beyer measured lead concentrations in the tissues of European starlings (*Sturnus vulgaris*) nesting near heavily used roads and in starlings nesting next to little-used roads at Patuxent. Lead concentrations were several times higher in starlings living near the heavily used roads, but reproductive success was not different (Grue and others, 1986). With the phasing out of leaded gasoline between 1975 and 1986, lead from gasoline ceased to be a source of lead in wildlife.

In the 1990s, Patuxent scientists began studying still another dangerous source of lead—mining operations. This work initially focused on lead contamination of the Coeur d'Alene River in Idaho. Each year, about 150 tundra swans (*Cygnus columbianus*) with lead poisoning would be found in the Coeur d'Alene River Basin. More than a century of mining operations left the sediments in much of the Coeur d'Alene River contaminated with lead. At first, lead was suspected to have moved up the food chain, as many contaminants do kill birds this way; however, studies with ospreys (*Pandion haliaetus*) by Chuck Henny and Larry Blus at Patuxent's Corvallis, OR, field station demonstrated that lead was not moving up the food chain (Henny and others, 1991). Henny was a field biologist with a remarkable ability to detect previously unrecognized contaminant problems. Blus had already made his own mark decades earlier, demonstrating that dichlorodiphenyldichloroethylene (DDE), the metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), thinned the eggshells of brown pelicans (*Pelecanus occidentalis*).

Follow-up fieldwork by Henny and Blus strongly indicated that the tundra swans were getting their lethal dose of lead because they ingested some lead-contaminated sediment along with food they had gleaned off the bottom of marshes (Blus and others, 1991). To prove that the ingestion of lead-contaminated sediments was poisoning waterfowl at the Coeur d'Alene River, however, a series of controlled laboratory studies was needed. As is usually the case with contaminant problems affecting wildlife, a coordinated combination of field and laboratory studies is needed to fully understand the processes at work.

Back at Patuxent headquarters, a series of controlled feeding studies was conducted in which Coeur d'Alene River sediment was mixed into waterfowl diets at rates comparable to the sediment ingestion rates of wild birds. These studies proved that sediments collected from the Coeur d'Alene River contained enough lead to poison mallards, Canada geese, and mute swans (*Cygnus olor*); the mute swan served as a surrogate for the tundra swan (Heinz and others, 1999; Hoffman and others, 2000; Day and others, 2003).

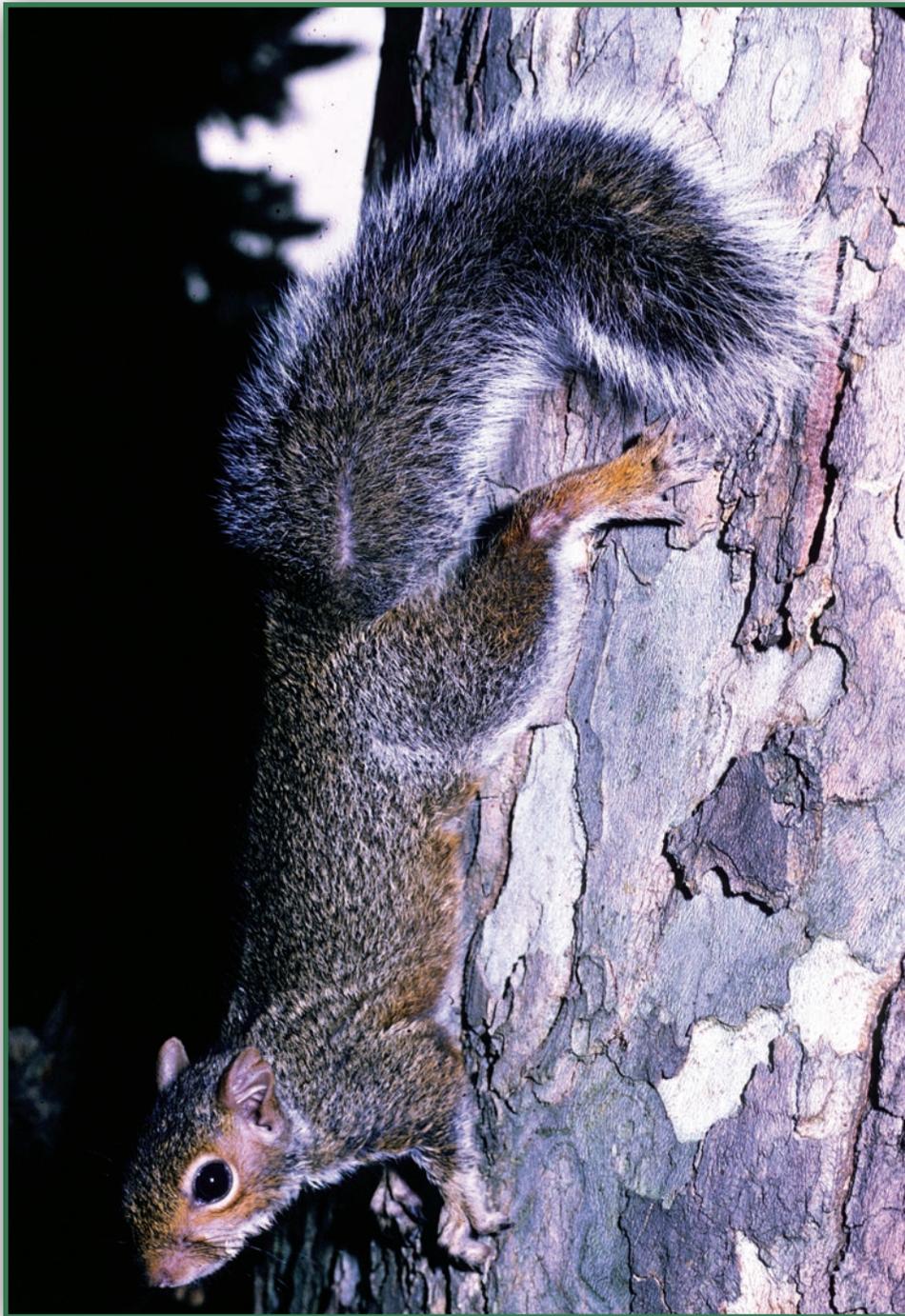
The studies Patuxent scientists carried out on lead poisoning in Idaho were part of a Natural Resource Damage Assessment (NRDA) by the U.S. Department of the Interior (DOI). A NRDA is a legal process the DOI established to determine the degree of restoration needed to compensate the public for harm to natural resources because of the release of a hazardous substance into the environment. A court settlement was reached in the case of the mining companies that had released lead-contaminated sediments into the Coeur d'Alene River in Idaho. Approximately \$370 million was awarded to clean up the Coeur d'Alene River Basin. This large court settlement validated the years of field and laboratory research carried out by Patuxent scientists and scientists from the USFWS. It is this kind of success story about contaminant research that gives scientists at Patuxent a great deal of pride, whether the success resulted from our contributions to the banning of lead shotgun pellets or led to the cleanup of a lead-contaminated river.

I mentioned at the outset of this chapter that it commonly takes a large and dedicated staff of researchers many years to bring about the resolution of a contaminant issue. This was clearly true of the various forms of lead contamination we studied over many decades at Patuxent. No one can be sure that some other source of lead contamination will not arise in the future that presents an equal research challenge. As I reflect on those "railroad tracks" I first saw in 1969—the tracks on which all those mallards were sent to be shot—I realize that Patuxent scientists of all kinds and with different training were up to the task of determining just what the contaminant issue was and how it might be solved. I feel privileged to have known them.

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Gray squirrel in sycamore tree, Patuxent Research Refuge, Laurel, MD, 1973, Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.

Role of Raptors in Contaminant Research at Patuxent

By Charles J. Henny

Introduction

This chapter reviews the history of and approaches used in studies focused on the effects of contaminants on raptors and raptor populations at the Patuxent Wildlife Research Center (Patuxent) in Laurel, MD. Worldwide raptor declines following World War II were unprecedented and resulted in a sequence of major efforts at Patuxent to understand their cause(s). The peregrine falcon (*Falco peregrinus*), bald eagle (*Haliaeetus leucocephalus*), and osprey (*Pandion haliaeetus*) were the species of most concern in North America. Laboratory and field studies at Patuxent complemented each other and yielded timely results of national and international importance, including some findings published in the journals “Science” and “Nature.”

Concern about contaminant effects on wildlife populations came to the forefront during the years immediately following World War II. This concern was worldwide and not limited to one taxonomic group or to personnel and

investigations at Patuxent. Contaminant studies of raptors were only part of the story, but this review, with minor exceptions, is limited to raptor studies and the role Patuxent played in this research. Indeed, many important nonraptor contaminant studies done at Patuxent, as well as raptor studies conducted elsewhere, are not mentioned here. For other reviews of contaminant-wildlife issues in the 1950s and 1960s, the reader is referred to “Silent Spring” by Rachel Carson (1962), “Pesticides and the Living Landscape” by Robert Rudd (1964), and “Return of the Peregrine: A North American Saga of Tenacity and Teamwork” by Tom Cade and Bill Burnham (Cade and Burnham, 2003).

Early Years (Pre-1960)

Before 1960, few raptor studies were conducted at Patuxent or by personnel stationed there. The notable exception is the long-term red-shouldered hawk (*Buteo lineatus*) study



Chuck Henny, U.S. Fish and Wildlife Service, with young red-shouldered hawk at Patuxent Wildlife Research Center, Laurel, MD, 1972. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.

along the Patuxent River flood plain, initiated by Bob Stewart in 1943 (Stewart, 1949) and continued by Henny and others (1973) and Martin (2004). The study, which continues today (2016), was designed to improve understanding of habitat requirements, population densities, reproductive rates, and food habits, although a few eggs were analyzed for contaminants recently. Contaminant levels in eggs were generally low in the early 1970s, but habitat loss resulted in a long-term population decline of 78 percent from 1971 to 2002. Other studies prior to 1960 involved the development of techniques for live-trapping hawks and owls (Stewart and others, 1945) and the reporting of hawk migration count data (Robbins, 1950, 1956).

Raptor Pesticide Studies (1960–64)

The early 1960s brought the issues of raptor population declines and pesticides together. Earlier, James DeWitt (1956) at Patuxent had reported that pheasants and quail exposed to several pesticides in controlled laboratory conditions laid fewer eggs and produced fewer chicks than birds not exposed to pesticides, a finding that caused considerable concern among conservationists. Robbins (1960) evaluated the status of the bald eagle in summer 1959 by compiling information from the U.S. Fish and Wildlife Service (USFWS), State organizations, National Audubon Society, and private individuals. Charles Broley's 20-year bald eagle dataset from the west coast of Florida was particularly alarming (Broley, 1958); it showed a 50- to 90-percent population decline, with markedly decreasing productivity rates after 1946. Broley (1958) and Robbins (1960) pointed out that Maurice Broun's fall migration count data from Hawk Mountain Sanctuary in Pennsylvania showed that during 1935–40, 38 percent of the bald eagles migrating over the sanctuary were in immature plumage, but during the last 6 years of the study (1953–58), the percentage of immatures was only 21 percent. The percentage was especially low (10 percent) during 1957–58.

DeWitt and Buckley (1962), in an interim report, noted that definitive proof of the cause(s) of the bald eagle population declines and lowered reproductive success was lacking, although it was postulated that prolonged and continued exposure to dichlorodiphenyltrichloroethane (DDT) and related pesticides might have been responsible. Bald eagles were trapped in Alaska in 1961 and 1962, brought into captivity at Patuxent, and fed various diets of DDT. These 1961–62 feeding experiments demonstrated that DDT could kill bald eagles. By 1963, Patuxent had obtained 54 dead bald eagles and 5 unhatched eggs. All but one bird (from Alaska) contained detectable DDT residues (Buckley and DeWitt, 1963). Buckley and DeWitt (1963) concluded (1) wild eagles carry body burdens of DDT, but they were uncertain whether burdens in wild eagles were sufficiently high to be detrimental; and (2) all eggs analyzed contained DDT residues, indicating that some DDT was transferred to the egg, but they were uncertain whether DDT levels measured in the eggs affected hatching.

Peregrine Falcon Conference (1965)

The Peregrine Falcon Conference held in Madison, WI, in 1965 (Hickey, 1969) was a landmark event. Joe Hickey (University of Wisconsin) had organized a 1964 repeat of his 1939–40 Peregrine Falcon Survey in the Eastern United States (east of the Mississippi River). Hickey's (1942) data plus data from several additional sites yielded 209 perceived "valid" eyries, but Berger and others (1969), who checked 133 sites, found no occupied peregrine falcon eyries in 1964. The surveyors realized the impossibility of thoroughly covering the survey area, but emphasized that the species, if not extirpated in the United States east of the Mississippi River, was drastically reduced. Ratcliffe (1969) noted a sequence of peregrine falcon population declines in Great Britain that included egg breakage, egg-hatching failure, death of young, and failure of adults to lay eggs, which preceded actual desertion of the territory. Eggs from 14 peregrine falcons all contained residues of DDT/ dichlorodiphenyldichloroethylene (DDE, a metabolite of DDT), benzene hexachloride (BHC), dieldrin, and heptachlor epoxide, and Derek Ratcliffe, chief scientist, Nature Conservancy Council, United Kingdom, argued that concentrations in some were sufficient to account for sublethal effects leading to reduced breeding success. Similar population declines and low productivity were reported for ospreys in Connecticut (Peterson, 1969) and Michigan (Postupalsky, 1969).

John Buckley, former director of Patuxent, led a roundtable discussion on "pesticides as possible factors affecting raptor populations" with Joe Hickey, Ian Prestt, Lucille Stickel, and Bill Stickel. The primary focus was to review "what we know" and to identify "what we do not know" in 1965. Lucille and Bill Stickel took an active role in the discussions (Hickey, 1969) and listed several tentative conclusions: (1) birds may have normal or near-normal reproductive success despite relatively high DDT residues in eggs (this was later recognized to occur with insensitive species); (2) there is no evidence that a few parts per million (ppm) of DDT in eggs causes reproductive trouble; (3) chlorinated hydrocarbon dosages that clearly reduce avian reproduction are, with possible exceptions, not far below those that will kill some birds if continued; (4) long-term intake of small doses is far more lethal than once thought; (5) declines in avian reproductive success with insecticidal dosages are almost always partial, are typically small, and are rarely eliminative; and (6) it is not characteristic of DDT, dieldrin, or most other chlorinated hydrocarbons to kill birds or to block reproduction without leaving residues that are substantial in relation to the toxicity of the chemicals involved—for example, DDT levels representing serious damage to birds will be well above 2 or 3 ppm of total residues. The Stickels noted that it was necessary to deal with these questions because there was still a strong tendency to attach much importance to low DDT residues, or pesticides in general, when we could have been missing the real causes of the population declines.

Early Patuxent field studies with DDT at application rates of 2 to 5 pounds per acre (Hotchkiss and Pough, 1946; Stewart and others, 1946; Robbins and Stewart, 1949; Robbins and others, 1951; Mitchell and others, 1953) resulted in mixed findings regarding effects on passerine bird populations. The Stickels at the round-table discussion further noted that although pesticides kill wildlife and may cause population declines, many other factors—for example, disease and metals, such as mercury and lead—do so as well. They concluded that more work was required in the study of behavioral effects and combinations of pesticides, and more wild species needed to be tested because sensitivity to contaminants differs greatly among species.

Regarding procedural matters, Lucille Stickel noted a serious bias when eggs were collected for residue analysis (especially failed eggs from nests) and suggested using the volume of the egg in its shell as an adjustment for moisture loss. This suggestion was first mentioned in 1965 (Stickel and others, 1965); a detailed paper (Stickel and others, 1973) was published 8 years later. Without the adjustment, residue concentrations on a wet-weight basis were inflated, commonly by 50 percent or more. Some researchers today (2016) still make this mistake when reporting egg residues. Lucille believed the only way to verify lethal concentrations of contaminants in hawks was experimentally—that is, feed the birds a diet that contains the pesticide of concern while maintaining suitable controls. She also pointed out that birds that died during the lab experiments with DDT and DDE had brain concentrations of the same magnitude whether they died immediately or after months on clean food. Because concentrations in other tissues were highly variable, Patuxent recommended that, for diagnostic purposes, brain concentrations be used to establish the cause of death from chlorinated hydrocarbons (Stickel and others, 1969; Stickel and others, 1970). The Stickels downplayed the importance of egg breakage at this time (1965), and noted that it was not uncommon in captivity, even with birds not on dose.

Rapid Increase in Contaminant Studies (1966–90)

The pesticide-eagle studies at Patuxent by Buckley and DeWitt (1963) mentioned earlier were updated at the North American Wildlife and Natural Resources Conference by Stickel and others (1966), who concluded that (1) pesticide residue transfer from adult to egg is well known; (2) the quantity of residues that may indicate an adverse effect on hatching and survival is far from clear (but they noted that quantities of DDE, DDT, and dichlorodiphenyldichloroethane (DDD) in eagle eggs so far reported are much lower than those reported in gull and pheasant eggs that hatched or were alive, which provides little basis for suspecting that DDT in eggs prevented hatching); (3) exposure of eagles to DDT and

dieldrin is nationwide; (4) at least an occasional eagle obtains enough dieldrin, and perhaps DDT, to place it at risk; and (5) most eagles that die in the United States today die of causes other than pesticide poisoning. Finally, the important question of sublethal effects on behavior, particularly parental behavior, could not yet be answered. Future research plans at Patuxent were also mentioned; they included (1) continue monitoring eagle eggs and adults for pesticide residues, (2) extend analyses to some of the more important heavy metals, (3) begin food-chain investigations specific to eagles, and (4) improve understanding of residues in eggs and tissues (Patuxent already had established a colony of American kestrels [*Falco sparverius*] to test for reproductive effects with a raptor).

Additional Patuxent field data on raptor populations were reported when Schmid (1966) compared the number of successful osprey nests and young banded per successful nest in parts of Cape May County, NJ, in 1937, 1938, and 1939 with numbers observed in 1963. This one-trip visit at banding time, of course, did not include those nests that failed, although the number of successful nests had decreased dramatically (perhaps by 60–70 percent) by 1963. Schmid concluded that possible explanations might be diminishing food supply, contaminants in the food chain, or a growing frequency of disturbance and persecution. Subsequent Patuxent field studies emphasized a much more detailed approach, which included methods for separating several of the possible factors that could cause population declines (for example, see the section below titled “Osprey” for a description of the osprey egg transfer study between Connecticut and Maryland).

At the time of the Peregrine Falcon Conference in 1965, eggshell thinning was not yet known; only the alarming and rapid declines of the peregrine and their unusual behaviors were recognized at many locations. No conclusions had yet been reached regarding the cause(s) of the declines at the 1965 conference. Egg breakage was mentioned as one of the many factors that needed to be considered in evaluating worldwide peregrine population declines. Derek Ratcliffe left the conference with egg breakage on his mind and then talked with Desmond Nethersole-Thompson—a long-time friend of his, a field biologist, and an early egg collector—who suggested that Ratcliffe look at eggs in collections. Desmond’s suggestion was critical and led to the first understanding that eggshells themselves were affected along with, of course, the females that laid those eggs. Ratcliffe devised an eggshell “thickness index” because he could not directly measure eggshell thickness; the oologists who collected the eggs and removed the contents of the eggs prided themselves on making a very small hole in the eggshell. Ratcliffe reported his astounding results that eggshells were now thinner than in the past to Joe Hickey, professor at the University of Wisconsin, even before he went to press in “Nature.” Ratcliffe and Hickey were friends and talked on the phone often. Dan Anderson, a graduate student working with Hickey, was immediately sent to many museums in the United States to measure eggshell thickness with a modified micrometer that would fit through the tiny holes the egg

collectors made (Daniel Anderson, University of California, Davis, oral commun., 2012). Hickey, whose long association with Patuxent dated back to his early studies of banding data from the Bird Banding Laboratory (Hickey, 1952), obtained USFWS funding for the eggshell-thickness project through Patuxent and Lucille Stickel.

Thus, Ratcliffe (1967), while investigating the peregrine falcon and sparrow hawk (*Accipiter nisus*) in the United Kingdom, noted a relation between decreases in eggshell weights, decreases in sizes of breeding populations, and exposure of populations of these species to persistent organic insecticides. Hickey and Anderson (1968) reported similar findings for North American species the next year. The observation was that eggshell thinning in archived samples of raptor eggs and



Young eaglet in captive study at Patuxent Wildlife Research Center, Laurel, MD, 1970s. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.



Brown pelican egg without eggshell, 1970s. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.

other species had occurred over a critical time, namely the period coincident with the post-World War II introductions of organochlorine (OC) pesticides and radioactive contamination; this was an important discovery. The next step involved a hypothesis that DDT (later discovered to be DDE) was the major cause of eggshell thinning and that this eggshell thinning was related to population declines through reduced reproductive success. The critical step was the testing for effects of DDT and dieldrin on eggshell thinning and reproduction of American kestrels at Patuxent under controlled laboratory conditions (Porter and Wiemeyer, 1969; Wiemeyer and Porter, 1970). These controlled studies showed the same pattern of reproductive failure and reduced eggshell thickness that appeared in several raptor populations in the United States and Western Europe. This early laboratory work was completed under the direction of Lucille Stickel, whose leadership and insight, in addition to her command of the necessary resources for critical experiments, made it possible.

Eggshell thickness was an increasingly important factor and was studied intensively under controlled conditions at Patuxent. Thus, much of the work at Patuxent followed general concepts developed by the Stickels prior to 1965, with slight modifications—for example, eggshell thinning came to be considered much more important than had been anticipated in earlier years. One outcome of the discovery of eggshell thinning was the realization that in studying other contaminants it was beneficial also to study not only mortality, but also the subtle, insidious effects of the contaminants. Many of the people employed at Patuxent after 1965 were hired specifically to conduct various types of studies to complete missing parts of the contaminant story, not only for raptors, but also for all wildlife. Later studies extended to groups of contaminants other than OCs, including organophosphates (OPs), carbamates, anticoagulants, mercury, lead, selenium, fluoride, cadmium, flame retardants, chlorophenoxy herbicides, perfluorinated acids, and sulfates, as well as combinations of chemicals.

Laboratory Studies

The laboratory studies at Patuxent with American kestrels (table 1), eastern screech owls (*Otus asio*), and common barn owls (*Tyto alba*) (table 2) were many and involved many contaminants and endpoints. The compilation of reproductive-success and egg-residue data in a series of papers on a nest-by-nest basis in the laboratory and field (see Blus [1984] for the sample egg approach) was used to estimate the proportion of a population adversely affected by various contaminants—for example, the percentage of eggs containing concentrations greater than a perceived critical level for reproductive effects (table 3). The critical residue concentration information was especially useful to the field biologist who was trying to interpret observed local contaminant concentrations.

Table 1. Patuxent Wildlife Research Center laboratory studies on contaminants in American kestrels, 1969–2011.

[DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; PCB, polychlorinated biphenyl; anti-ChE, anti-cholinesterase; PBDE, polybrominated diphenyl ether; OP, organophosphate; OC, organochlorine]

Contaminant(s)	Year(s) studied
Dieldrin + DDT	1969
Dieldrin + DDT, DDE	1970
DDE	1970
DDE	1972
Lead	1980
Parathion	1982
Oil	1982
Lead	1983
Lead	1984
Lead	1984
Methyl parathion, fenvalerate	1984
Lead	1985
Lead	1985
DDE, DDT + dieldrin	1986
Paraquat	1987
Lead + OCs	1989
Dicofol (kelthane)	1990
Diphenyl ether herbicides	1991
Aroclor 1248	1991
Four anti-ChEs	1991
PCB 126	1996
White phosphorus	1997
Aroclor 1248	1998
Planar PCBs	1998
OPs, carbamates	1998
Dicofol (kelthane)	2001
Aroclor 1242	2002
PBDEs	2005
Methylmercury	2007
Methylmercury	2009
PBDEs	2009
Methylmercury	2010, 2011
Diphacinone	2011

Table 2. Patuxent Wildlife Research Center laboratory studies on contaminants in eastern screech owl (*Otus asio*) and common barn owl (*Tyto alba*), 1972–98.

[DDE, dichlorodiphenyldichloroethylene; anti-ChE, anti-cholinesterase; OP, organophosphate]

Species	Contaminant(s)	Year studied
Eastern screech owl	DDE	1972
Eastern screech owl	Aroclor 1248	1980
Common barn owl	Famphur	1980
Common barn owl	Six anti-coagulants	1980
Eastern screech owl	Endrin	1982
Common barn owl	DDE, dieldrin	1983
Eastern screech owl	Fluoride	1985
Eastern screech owl	Fluoride	1988
Eastern screech owl	Dicofol (kelthane)	1989
Eastern screech owl	Four anti-ChEs	1991
Eastern screech owl	Selenium	1996
Eastern screech owl	OPs, carbamates	1998



Matt Perry banding young American kestrel at Patuxent Wildlife Research Center, Laurel, MD, 1975. Photo by U.S. Fish and Wildlife Service.

Table 3. Patuxent Wildlife Research Center studies¹ to determine the effect of contaminant residue concentrations in eggs on productivity of various raptor species using the sample egg technique.

[DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; PCB, polychlorinated biphenyl; OP, organophosphate; OC, organochlorine; Hg, mercury]

Species	Contaminant(s)	Author (Year)
American kestrel	Heptachlor epoxide	Henny and others (1983)
Bald eagle	OCs, PCBs, Hg	Wiemeyer and others (1984)
American kestrel	DDE, DDT, dieldrin	Wiemeyer and others (1986)
Osprey	OCs, PCBs, Hg	Wiemeyer and others (1988)
Bald eagle	OCs, PCBs, Hg	Wiemeyer and others (1993)

¹The collection and analysis of an egg from a series of nests for these studies provided an approach to evaluate the percentage of individuals in a wild population whose reproduction was adversely affected by various contaminants.



John Maistrelli, U.S. Fish and Wildlife Service, transferring adult eagle at Patuxent Wildlife Research Center, Laurel, MD, 1976. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.

Field Studies and Monitoring

The status of several raptor species and various raptor populations was not well known in the late 1960s and 1970s. The concern focused strongly on the peregrine falcon, bald eagle, and osprey, all of which were prominently mentioned at the 1965 Peregrine Falcon Conference. In the mid-1960s, the peregrine falcon was already extirpated or nearly extirpated in the Eastern United States, and reintroduction was in progress. Given the devastating population declines in the Eastern United States, contaminant studies on peregrines at Patuxent focused on monitoring northern latitude breeding populations that migrated along the Atlantic Coast and the Texas coast by sampling blood to determine contaminant trends from 1978–2004 (see Henny and others, 1982; Henny and others, 2009). The nationwide bald eagle population had declined substantially and the species was very sensitive to disturbance at the nest; however, unhatched eggs and dead eagles were analyzed routinely at Patuxent. Therefore, the osprey became the obvious candidate for intensive field studies because it could be studied more easily in the wild.

Osprey

During the Peregrine Falcon Conference, exceptionally poor productivity or declining osprey numbers were reported for Long Island, NY; Connecticut; New Jersey; Rhode Island; Maine; Massachusetts; Wisconsin; and Michigan. Most localized studies that followed the conference included an evaluation of (1) reproductive success, (2) changes in population numbers over time (although few series with more than a decade of data were available), (3) contaminant residues in some eggs and fish, and (4) eggshell thickness. Because

reproduction was the apparent “weak link” in the life cycle, the number of young fledged per nesting pair was considered of primary importance (table 4).

Structural modeling based on survival-rate estimates from banding data and life-history characteristics (funded by the Migratory Bird Populations Station [MBPS] at Patuxent) (Henny and Wight, 1969; Henny and others, 1970) was used to estimate a recruitment standard (0.95–1.30 young per nesting pair) needed to maintain a stable osprey population. At that time, most osprey populations were producing at what was considered extremely low rates, although the normal (or standard) rate was unknown. Observed production rates were compared to the standard rate, which was later lowered to 0.80 young per nesting pair on the basis of a comparison between the observed population response and the projected population response determined by using the model (Spitzer and others, 1983).

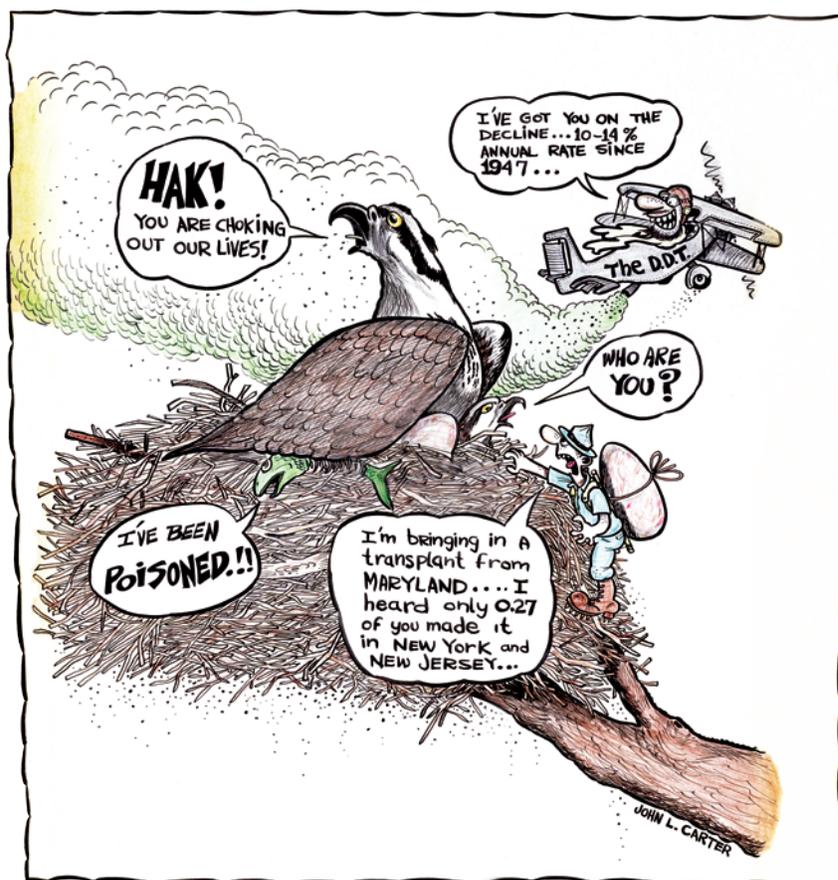
In 1968 and 1969, osprey eggs were exchanged between Connecticut (low reproduction) and Maryland (higher reproduction) nests to test the hypothesis that the decline in reproductive success of Connecticut ospreys was caused by something external to the eggs (Wiemeyer and others, 1975)—for example, recall the concerns mentioned above about food supply, persecution, and human disturbance in the 1960s. A cartoon of the era representing the egg exchange study is shown in figure 1. Incubation of Connecticut osprey eggs by Maryland ospreys did not improve the hatching rate. Maryland eggs incubated by Connecticut ospreys hatched at their normal rate. The results of the exchanges and associated observations indicate that the most probable cause of the poor reproduction in Connecticut ospreys was related to contamination of eggs—namely eggshell thinning and embryo mortality, and not to subtle behavioral effects on the incubating parents. Henny and Van Velzen (1972) found that ospreys from New York, New Jersey, and Maryland shared the same general wintering

Table 4. A, Breeding population changes and, B, productivity of ospreys along the North Atlantic Coast of the United States, 1945–75.

[Modified from Henny (1977); see Henny (1977) for more information, including citations for publications; >, greater than; NA, not available]

A. Breeding population changes of ospreys						
Location	Number of occupied nests					
	Pre-1945	1960	1965	1970	1975	
Gardiner's Island, New York	300	100	70	38	31	
Connecticut River, Connecticut	200	71	13	4	1	
Rhode Island	130	> 60	23	7	8	
Total	630	> 231	106	49	40	
Observed annual rate change (percent)	-6.5	-14.4	-14.3	-14.3	-4.0	

B. Productivity of ospreys						
Location	Number of young fledged per occupied nest					
	1950–52	1953–57	1958–62	1963–67	1968–72	1973–75
Gardiner's Island, New York	1.19	0.83	0.75	0.16	0.53	0.68
Connecticut River, Connecticut	NA	0.37	0.23	0.33	0.25	0.00
Rhode Island	NA	NA	0.27	0.40	0.61	1.00
Total	1.19	0.65	0.47	0.23	0.52	0.73


Figure 1. A cartoon of the early 1970s, which depicts the Patuxent Wildlife Research Center osprey egg exchange. (Cartoon by John L. Carter, a friend of the author, and used with his permission.)

grounds, which implied that local breeding success of an osprey population depended on environmental conditions in the breeding area. Their hypothesis was supported by the results of the egg exchanges, including the differences in OC residues in fish from the two osprey breeding areas. Average eggshell thickness of osprey eggs collected from Connecticut had declined 18 percent from pre-1947 norms, whereas that of eggs from Maryland had declined 10 percent (Wiemeyer and others, 1975).

In the late 1960s and early 1970s, the vision of the USFWS, including the MBPS located at Patuxent, was broadening to species other than migratory game species. Planes and experienced pilots were available for aerial surveys of non-game species if they did not conflict with breeding and wintering grounds waterfowl surveys. Therefore, the first USFWS aerial survey of nesting ospreys was conducted in Chesapeake Bay in 1973 (Henny and others, 1974). The survey was conducted much like a breeding ground survey of waterfowl with a double-sampling approach (both an overall air survey covering the entire area and ground surveys in a portion of the larger aerial survey area); this approach provided a total population estimate and its associated variance. Before the survey, both Chan Robbins (long-time Patuxent ornithologist) and Alexander Wetmore (long-time Smithsonian Institution ornithologist) believed that about 200 to 400 pairs of ospreys were nesting in Chesapeake Bay (Chandler Robbins, U.S. Fish and Wildlife Service, oral commun., 1973; Alexander Wetmore, Smithsonian Institution, oral commun., 1973), but the survey results indicated the estimated population in 1973 to be 1,450 pairs (Henny and others, 1974).

The Chesapeake Bay study was extended to the coastal Carolinas the next year (1974), and was followed in 1975 by studies of coastal New Jersey, Delaware, Maryland, and Virginia (Henny and Noltemeier, 1975; Henny and others, 1977). Similar osprey surveys were conducted in northern California in 1975, Oregon in 1976, and coastal northwestern Mexico (Baja California, Gulf of California, Sonora, and Sinaloa) in 1977 (Henny and others, 1978a, 1978b; Henny and Anderson, 1979). These surveys provided base values for future population comparisons.

Upon my return to Patuxent following the Carolina survey in 1974, I mentioned to Fran Uhler (long-time Patuxent biologist) that 38 pairs of ospreys were nesting in relatively short bald cypress trees (*Taxodium distichum*) in the lake at Mattamuskeet National Wildlife Refuge. Fran explained that the lake had been drained in 1914 to convert it to farmland, but the plan was later abandoned. Consequently, the lakebed was dry in 1928 when he surveyed it as a possible refuge site. In 1934, the U.S. Government acquired the land and a refuge was established. Increment borings of the cypress trees used as nest sites in 1974 placed their ages at 30 to 40 years, which corresponds to the period shortly after the land was acquired and reflooding began (Fran Uhler, U.S. Fish and Wildlife Service, oral commun., 1974).

By 1975, osprey populations had been studied at many locations and a review of research, management, and status of

the osprey in North America was presented at the First World Birds of Prey Conference in Vienna, Austria (Henny, 1977). Declining populations and low productivity were apparent at many locations, but with an indication that productivity was improving. Later, Wiemeyer and others (1988) reported that 15 percent and 20 percent eggshell thinning of osprey eggs was associated with 4.2 and 8.7 ppm wet weight (ww) DDE, respectively. Lincer (1975) reported that no North American raptor population that exhibited 18 percent or more eggshell thinning was able to maintain a stable population. In later years, the percentage of eggs with greater than 4.2 and greater than 8 ppm DDE ww (the latter value more closely approximating 18 percent thinning) was used to evaluate DDE effects on osprey reproduction. Reproduction rate information (based on nests with one egg randomly collected and chemically analyzed) further supported these classifications of contaminant effects (Henny and others, 2004). Wiemeyer and others (1975) suspected that dieldrin may have increased the mortality rate of adult ospreys in Connecticut, and reported a lethal concentration in the brain of an adult male that died in 1967. Another adult osprey in South Carolina was believed to have been poisoned by dieldrin in 1970 (Wiemeyer and others, 1980). None of 29 dead ospreys evaluated (1964–73) died of DDE poisoning. The Connecticut population appeared to decline more rapidly (from 71 pairs in 1960 to 31 pairs in 1961) than reproductive failure alone would predict; however, this precipitous decline may be at least partly explained, as suggested by Henny and Ogden (1970), by catastrophic mortality associated with the occurrence of the worst hurricane in decades (Donna) in September 1960, during the osprey's fall migration.

By 1981, a nationwide osprey nesting population estimate resulted in a count of approximately 8,000 pairs (Henny, 1983). Another nationwide population estimate, made in 1994, showed a 77.5-percent increase (to about 14,200 pairs; Houghton and Ryman, 1997), and a similar survey in 2001 indicated an approximate 25-percent increase (about 16,000–19,000 pairs; Poole and others, 2002). The initial survey in northwestern Mexico in 1977 (810 pairs) was followed by others during 1992–93 (1,362 pairs) and 2006 (1,343 pairs) (Henny and others, 2008). The increase in osprey eggshell thickness following the 1972 ban of DDT in the United States was reported in a study with a large series of 238 eggs collected in the Pacific Northwest from 1973 to 2008 (fig. 2; Henny and others, 2010).

Many of the OC pesticide, polychlorinated biphenyl (PCB), dioxin, and furan concentrations in osprey eggs decreased by the end of the 20th century; the decrease in residues resulted in limited or no adverse effects on populations, except in a few localized areas (Henny and others, 2010). Thus, the osprey, now with large, widely distributed populations again (at lakes, rivers, bays, and estuaries), provides a means of evaluating emerging contaminants with limited potential for confounding effects from the “legacy” group of contaminants (the “old” OCs). Newer contaminants, such as polybrominated diphenyl ethers (PBDEs), are widely used as flame retardants in thermoplastics, textiles, polyurethane

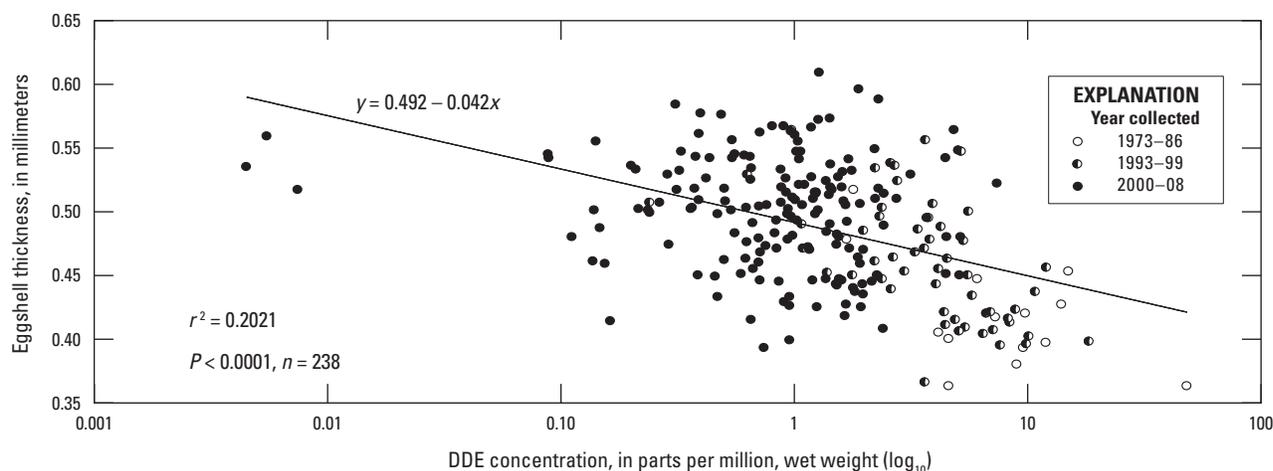


Figure 2. Semilogarithmic relation between dichlorodiphenyldichloroethylene (DDE) concentration in the egg and eggshell thickness in osprey eggs collected from the Pacific Northwest (Oregon, Washington, Idaho), 1972–2008. (Modified from Henny and others, 2010)

foams, and electronic circuitry. PBDEs have been reported in osprey eggs from Delaware, Maryland, Virginia, Oregon, and Washington (Rattner and others, 2004; Toschik and others, 2005; Henny and others, 2009a). In contrast to the legacy contaminants, PBDEs have increased in the biota since the 1970s, and, as concentrations increased above 1 ppm ww, there was some evidence of reduced osprey productivity (Henny and others, 2009a). More recently, wastewater-treatment-plant discharge, a known source of PBDEs (and also an indication of human population size at a location), was added to stream discharge (both converted to millions of gallons per day) in a novel approach (namely an approximate dilution index) to relate concentrations of waterborne contaminants to levels of these contaminants that reach osprey eggs (Henny and others, 2011). This simple approach improved understanding of the spatial patterns of the contaminants observed in osprey eggs. Other emerging contaminants found in osprey eggs since 2000 included perfluorinated acids and sulfonate compounds in the Eastern United States (Rattner and others, 2004; Toschik and others, 2005) and the chlorophenoxy herbicide DCPA (trade name Dacthal®) and the fungicide chlorothalonil in Puget Sound, WA (Chu and others, 2007). The osprey has played the role of a worldwide “sentinel species” for contaminant investigations. The species characteristics that make it so useful for this purpose were recently reviewed by Grove and others (2009).

Bald Eagle

Tissues from field-collected bald eagles and eggs were analyzed for pesticide residue content at Patuxent as part of the National Pesticide Monitoring Program; the first eagle

carcass collected was obtained in 1960 (Coon and others, 1970). A limited number of carcasses were available for earlier years, but substantial numbers (692 carcasses) became available from 1966 to 1981 and routinely were analyzed for a series of contaminants; each report included a diagnosis for cause of death. Reports that included the raw data were published regularly by Patuxent scientists. A review of these Patuxent bald eagle data (see Peakall [1996], which includes diagnostic criteria developed at Patuxent) indicated that OC insecticides, especially the cyclodienes like dieldrin, killed eagles. The percentage of bald eagle deaths reported in the Patuxent literature and attributed to dieldrin poisoning decreased after 1970 (that is, 13 percent, 1966–70; 6.5 percent, 1971–74; 3.0 percent, 1975–77; and 1.7 percent, 1978–81). The use of dieldrin plus aldrin (which is metabolized to dieldrin) peaked in the United States in 1966 and 1967, and was banned by the U.S. Environmental Protection Agency for nearly all purposes in 1974 (Nisbet, 1988). Other causes of death from contaminants in bald eagles that were reported in papers published by Patuxent scientists and reviewed by Peakall (1996) included lead, thallium in poisoned bait, DDE and metabolites, and perhaps PCBs and endrin.

Because bald eagles are sensitive to human visits early in the nesting cycle, most bald eagle eggs were collected after the nest failed. This practice is in contrast to the random collection of fresh eggs from the osprey, which is more tolerant of human activity at its nest early in the nesting cycle. Bald eagle eggs were collected in 14 states from 1969 to 1979 (Wiemeyer and others, 1984) and 15 states from 1980 to 1984 (Wiemeyer and others, 1993). Bald eagle productivity appeared normal when eggs contained less than 3.6 ppm ww DDE, but decreased at higher concentrations. The largest series of eggs was collected in Wisconsin, Maine, Maryland, and Virginia; DDE residues declined substantially from 1969 to 1984 in all four states.

Uniqueness of the Patuxent Approach

Patuxent could conduct controlled laboratory studies, had a large chemistry section to measure contaminant levels, and had several field stations pursuing investigations throughout the United States; these characteristics resulted in a robust combined approach to studying contaminant issues and provided a critical number of personnel. The field stations often provided initial leads on which contaminants to test further in the laboratory. A good example in 1977 was Warbex (famphur), an OP, used on cattle as a pour-on for warble fly control. Black-billed magpies (*Pica pica*) in Oregon were reportedly dying nearby following a topical famphur application. The dead magpies were collected and frozen by an Oregon Department of Fish and Wildlife biologist in LaGrande, who also happened to be a raptor rehabilitator. During a weekend when he was gone, his wife ran out of food for the great horned owl (*Bubo virginianus*) they were rehabilitating. She found a magpie in the freezer and fed it to the owl. The owl immediately died. The story was relayed to me at the Pacific Northwest field station in Corvallis, OR, and a memo was sent to Patuxent. A laboratory study of common barn owls fed famphur-exposed quail showed significant cholinesterase (ChE) inhibition. Hill and Mendenhall (1980) concluded that owls could succumb to secondary OP poisoning. Then, in 1982, my colleagues and I conducted a field study that followed the recommended famphur pour-on treatment of 535 head of cattle at seven ranches in Washington (Henny and others, 1985). Famphur persisted on cow hair for more than 100 days, and magpies started dying on the day of treatment. A red-tailed hawk (*Buteo jamaicensis*) died 8 to 10 days after cattle treatment, and another was found sick 11 to 15 days

after treatment (both had severe ChE inhibition); the dead hawk had eaten a magpie. From March 1984 to March 1985, other raptors were tested for famphur and fenthion poisoning at Patuxent. The list of deaths attributed to famphur or fenthion included nine bald eagles in four states, three red-tailed hawks in two states, and one great horned owl. The eagles and hawks had scavenged cattle carcasses or eaten magpies, European starlings (*Sturnus vulgaris*), or brown-headed cowbirds (*Molothrus ater*) (Franson and others, 1985; Henny and others, 1987). Before 1982, only two bald eagles had been checked at Patuxent for anti-ChE exposure (both negative), but many cases remained open (no cause of death determined).

Some Final Thoughts

The number of papers authored by Patuxent scientists from 1945 to 2010 dealing with raptors and contaminants (142 papers) and raptor population numbers and status (58 papers) peaked in the 1980s and declined rather dramatically in later years, after Patuxent lost many of its field stations following a 1993 reorganization of the USFWS. Publication of raptor contaminant studies at Patuxent started in the early 1960s; rapidly increased in the 1970s and 1980s, when the status and future of many raptor species were of great concern; then decreased in later years (fig. 3). Many field station personnel stayed in close contact with their Patuxent colleagues and shared information, although their publications were counted elsewhere. To address important issues, the Patuxent approach involved methods development, combining laboratory and field studies, using the scientific method/experimental approach (asking questions and formulating hypotheses),

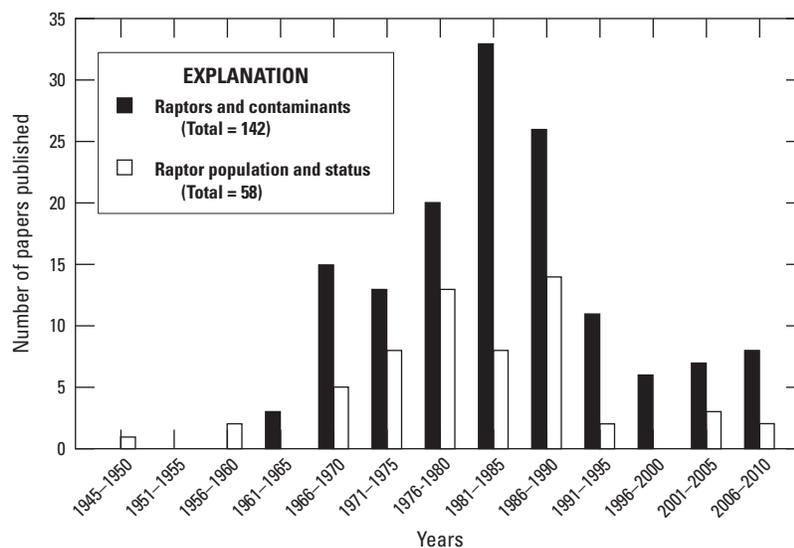


Figure 3. Summary of publications dealing with raptors and contaminants and raptor population and status, Patuxent Wildlife Research Center, Laurel, MD, 1945–2010.

developing forensic ecotoxicology approaches, and solving problems systematically. A unique combination of personnel was assembled to address important issues of the time. It was a joy to work with them and in the atmosphere at Patuxent during those critical years.

Acknowledgments

A list of all raptor publications originating from Patuxent Wildlife Research Center (Patuxent), with complete citations, is available from the Patuxent library, Laurel, MD (<http://www.pwrc.usgs.gov/library/>). Lynda J. Garrett, Patuxent librarian, graciously provided me with the list. I thank scientists Dan Anderson (University of California, Davis) and Gary H. Heinz (Patuxent), who made valuable comments on the first draft that improved the final document.

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Research on Amphibians and Reptiles at Patuxent: A Synopsis

By Donald W. Sparling

Patuxent Wildlife Research Center (Patuxent) in Laurel, MD, has a long history of research on amphibians and reptiles, beginning long before such research became commonplace. A survey of the Patuxent bibliography revealed 385 papers, books, or book chapters written or cowritten by Patuxent scientists from 1942 to 2015. Patuxent scientists are authors on 231 publications on amphibians, 226 publications on reptiles, and 36 publications that included both classes.

These papers cover a wide range of topics, including contaminants, systematics, general ecology, sampling techniques, and disease (fig. 1). Notably, papers on amphibians and reptiles are nearly equal in number, in contrast to the literature as a whole, where papers on amphibians far outnumber papers on reptiles.

The oldest paper in the Patuxent bibliography on herpetofauna was published by biologist William Stickel in 1942 (Stickel, 1942). Bill's wildlife career at Patuxent in the early 1940s was only partially interrupted when he was drafted into the Army during World War II. While in New Guinea and the Philippines, he found time to pursue his biological interests. He collected a variety of specimens, several of which were

later found to be new to science. A species of lizard (*Sphenomorphus stickeli*) from New Guinea (Loveridge, 1948) and a frog (*Kaloula stickeli*) from the Philippines (Inger, 1954) were named in his honor (Stickel, 1996; Perry, 2007, p. 259). Patuxent biologist Francis Uhler was also active in providing information about snakes in the 1940s (Uhler, 1944). Uhler also conducted a food habits study with snakes in the George Washington National Forest in Virginia just prior to coming to Patuxent (Uhler and others, 1939).

Mr. Stickel and his wife, Dr. Lucille Stickel, were prolific writers in the 1940s and 1950s. Dr. Stickel published several papers on the ecology and movements of eastern box turtles (*Terrapene carolina*), and all of her research was done at Patuxent (Stickel, 1950, 1978). Her study on box turtles was continued for approximately 60 years by several biologists and is a highly cited classic (Hall and others, 1999). Patuxent biologist Paula Henry did the most recent field work on this subject, and published reviews of the work in 2003 (Henry, 2003).

Research on herpetofauna was at a low during the 1960s and 1970s, but increased dramatically during the 1980s, primarily because of the work of three Patuxent scientists, Thomas H. Fritts, Russell J. Hall, and Robert P. Reynolds. Tom Fritts was a herpetologist at the Museum of Natural History, Washington, D.C., one of the field stations of Patuxent. He and Gordon Rodda wrote extensively on the invasive brown tree snake (*Boiga irregularis*), its effects on native populations of birds on Guam, and its threat to other Pacific Islands (Fritts, 1988; Rodda and others, 1991). Fritts also studied sea turtles and published papers on their distribution, ecology, and exposure to contaminants (Fritts, 1981).

Russ Hall was a major contributor of herpetofauna research during the 1980s, and an early pioneer in the area of amphibian and reptile ecotoxicology. Studies were published on the effects and uptake of pesticides, polychlorobiphenyls (PCBs), metals, polycyclic aromatic hydrocarbons (PAHs), and organochlorines on anurans (frogs and toads), salamanders, lizards, sea turtles, and other reptiles (Hall, 1988; Hall and Coon, 1988; Hall and Henry, 1992). Another contaminant study on herpetofauna was conducted by Peter Albers, who studied survival of spotted salamander (*Ambystoma maculatum*) eggs in temporary woodland ponds (Albers and Prouty,

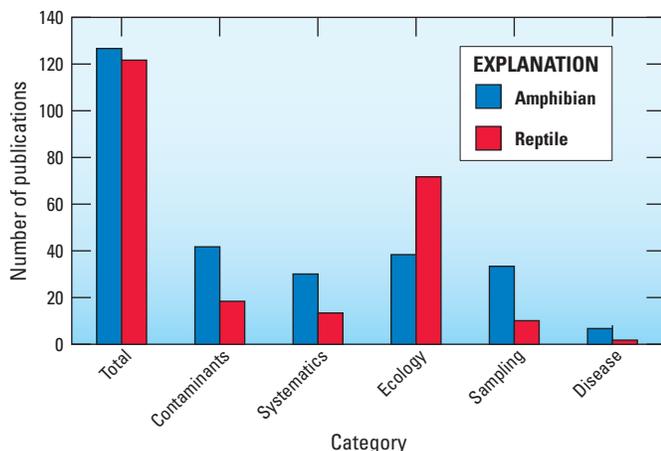


Figure 1. Number of publications on amphibians and reptiles authored by Patuxent Wildlife Research Center scientists, 1942–2011, by category.

1987) and contaminants in snapping turtles (*Chelydra serpentina*) in a tidal wetland (Albers and others, 1986). Patuxent researcher Gary H. Heinz studied contaminant levels in alligators (*Alligator mississippiensis*) in Florida (Heinz and others, 1991) and in snakes in Lake Michigan (Heinz and others, 1980).

During the 1990s, the National Biological Survey (later the National Biological Service) (NBS) was formed from research entities within the U.S. Department of the Interior (DOI), and vertebrate biologists from the National Museum of Natural History of the Smithsonian Institution joined the Patuxent staff. This collaboration resulted in an extensive series of publications on the distribution and systematics of amphibians and reptiles under the Patuxent banner. Patuxent researcher Roy McDiarmid published three books on tadpoles in collaboration with Ronald Altig (McDiarmid and Altig, 1999; Altig and McDiarmid, 2015; Altig and others, 1998). McDiarmid also published several descriptions of amphibian taxa as peer-reviewed articles or book chapters, and with other Patuxent scientists published the widely used references for the inventory and monitoring of amphibian (Heyer and others, 1994) and reptile (McDiarmid and others, 2012) biodiversity. As a coauthor with colleagues, McDiarmid also wrote a monograph on the history of herpetologists and herpetology in the DOI (Lovich and others, 2012).

A colleague of McDiarmid, Bob Reynolds, station leader of the Patuxent Biological Survey Unit at the National Museum of Natural History, conducted surveys of amphibians and reptiles throughout northern South America in Bolivia, Ecuador, Guyana, and Peru. In 2005, he collaborated with Tom Hollowell on a checklist of the terrestrial vertebrates of the Guyana Shield (Hollowell and Reynolds, 2005); more recently, he collaborated on a monograph on the amphibians and reptiles of Guyana (Cole and others, 2013). In addition, he published a number of regional herpetological surveys throughout Guyana (MacCulloch and others, 2007; MacCulloch and Reynolds, 2012; Reynolds and MacCulloch, 2012; MacCulloch and Reynolds, 2013). Reynolds also published peer-reviewed descriptions of new species for four amphibians and three snakes (Reynolds and Foster, 1992; Wynn and others, 2012).

Patuxent biologist Matthew Perry monitored amphibians and reptiles with pitfall and funnel traps set along drift fences on mitigated forested wetlands (fig. 2). These studies revealed that the wood frog (*Rana sylvatica*) was the only amphibian species found in reference forested wetlands, but not in adjacent mitigated sites (Perry and others, 1996, 2001). Perry also monitored amphibians and reptiles to evaluate five habitat management practices on a powerline right-of-way (Perry and others, 1997).



Figure 2. Pitfall and funnel traps along drift fence to capture amphibians and reptiles sampled by Brian Eyler, U.S. Geological Survey, as part of forested wetland mitigation study, 1996. Photo by Matthew C. Perry, U.S. Geological Survey.

During the 2000s, research, especially on amphibians, expanded into multiple areas because of the publication of papers resulting from the U.S. Geological Survey (USGS) Amphibian Research and Monitoring Initiative (ARMI), the Patuxent North American Amphibian Monitoring Program (NAAMP), and other ongoing studies. The ARMI was started in 2000, after the NBS became part of the U.S. Geological Survey (Muths and others, 2005). Its mission was to monitor amphibian populations and investigate probable causes of amphibian declines (Corn and others, 2005). Robin Jung, the first Northeast ARMI coordinator, collaborated with biostatisticians at Patuxent to improve methods of surveying and sampling amphibians. Patuxent biologists Larissa Bailey and Evan Grant subsequently led the Northeast ARMI program at Patuxent. Some examples of ARMI research include vernal pool egg mass counts and the study of potential climate change effects on the endangered Shenandoah salamanders (*Plethodon shenandoah*) in Virginia (Jung and others, 2005).

The NAAMP, initiated by Patuxent biologist Sam Droege, later was led by Patuxent biologist Linda Weir, who was followed by Evan Grant. It is a large-scale monitoring program consisting of more than 500 volunteers in more than 20 states collecting data to assess frog population trends (Weir and Mossman, 2005). This book chapter by Weir and Mossman describes the NAAMP protocol and partnership. Droege was also instrumental in developing Frogwatch USA, a citizen-based science program for people to monitor their backyard pond or neighborhood wetland. The program was transferred to the National Wildlife Federation and is now (2016) coordinated by the Association of Zoos and Aquariums (accessed May 21, 2015, at <https://www.aza.org/frogwatch/>). Additional key USGS scientists in this area included Jim Hines, Bill Kendall, Jim Nichols, Andy Royle, and John Sauer (MacKenzie and others, 2002).

Investigations on contaminants continued during the 2000s with my work, which focused on effects of pesticides on

amphibians in the Sierra Nevada of California, and the effects of variety of pesticides, acidification, metals, perchlorate, and sediment-borne lead on amphibians (Sparling and others, 2000; Linder and others, 2003a; Linder and others, 2003b). Patuxent biologist Mark Melancon focused on biomarkers. Patuxent scientists J. Michael Meyers, Jeff Hatfield, Robin Jung, Priya Nanjappa, and Jerry Longcore studied the conservation of amphibians and reptiles (Hatfield and others, 2004; Whiting and others, 2004, Jung and others, 2005). Longcore and others (2006) surveyed anurans in the northeastern United States to determine the distribution of chytridiomycosis, a lethal disease in some species of amphibians.

Productivity at Patuxent in terms of publications on amphibians and reptiles has generally increased since the 1950s (fig. 3) and continues to increase. Approximately 20 new papers were published during the first 18 months of 2010–11; at that rate, more than 125 papers would be published during the decade from 2010–19. The collaborators on these projects number more than 100, and contributions have been received from colleagues from all over the United States and several foreign countries.

Major Contributions of Research by Patuxent Scientists

Over the years, research on amphibians and reptiles has focused on natural history, contaminants, systematics, sampling methodology, distribution, and conservation. Major contributions of Patuxent scientists to research on amphibians and reptiles include—

- New and improved methodologies to survey and accurately estimate the size of amphibian and reptile populations;

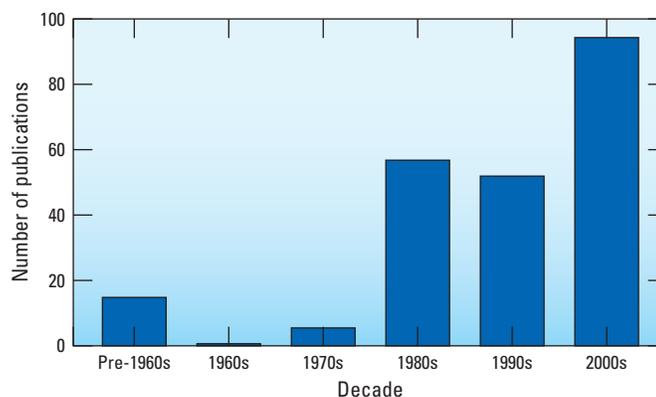


Figure 3. Number of publications on amphibians and reptiles published by Patuxent Wildlife Research Center scientists, by decade.

- Information on the accumulation and effects of many environmental contaminants, including mercury, organophosphate pesticides, lead, perchlorate, ammonium, toxaphene, endrin, PCBs, and methoxychlor, as well as multiple stressors, on amphibians;
- Increased knowledge about the effects of PAHs, dicofol, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), organochlorines, aldrin, petroleum, organophosphate pesticides, and mercury on reptiles;
- North American Amphibian Monitoring Program, developed by using the Breeding Bird Survey as a model;
- Information on the ecology, distribution, and conservation needs of many species of amphibians and reptiles;
- Early warning about and studies of the invasive brown tree snake before and during its devastation of avifauna on Guam;
- Input to and guidance on the formation of the Amphibian Research and Monitoring Initiative;
- Important research and conservation guidelines for endangered sea turtles;
- Assistance in the creation of Frogwatch USA, a citizen-based program to monitor amphibians; and
- A more than 60-year-long investigation of the population dynamics of eastern box turtles, one of the longest studies ever conducted on a single species.

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Chesapeake Bay Contaminant Studies by Patuxent Wildlife Research Center Scientists

By Barnett A. Rattner

Chesapeake Bay is the largest estuary in the United States (165,000 square kilometers [63,700 square miles]). In the 1600s, the bay “teemed with life,” and forests covered 95 percent of the watershed (Alliance for the Chesapeake Bay, 1998, unpub. fact sheet). The arrival of European settlers was accompanied by the clearing of forests and gradual conversion of wetlands to agriculture and urban centers, and, in the centuries that followed, the ability of the watershed to keep contaminants from reaching the bay and its tributaries diminished (Baldwin and others, 2012). In addition to the loss of habitat, overharvesting of living resources, as well as agricultural, industrial, and urban activities, have had major effects on invertebrate, fish, and wildlife populations residing in the bay and its watershed.

During its 75-year history, staff of the Patuxent Research Refuge and its successor research entities (Patuxent) and affiliates have contributed greatly to our present-day understanding of Chesapeake Bay. Our contaminant biologists (today more commonly referred to as wildlife toxicologists or ecotoxicologists) have conducted innumerable laboratory and controlled exposure investigations (for example, egg injection studies involving developing embryos in incubators, young and adult wildlife in cages or pens), field monitoring, and hypothesis-driven studies. By using a combined laboratory/pen-field approach, Patuxent scientists have elucidated both the direct and indirect effects of environmental contaminants on wildlife. Notably, some of the first studies of the effects of dichlorodiphenyltrichloroethane (DDT) on wild birds and mammals were conducted in forests at Patuxent or surrounding areas within the bay watershed, and their place in the history of wildlife toxicology is well established (for example, studies by Robert Stewart, Lucille Stickel, Chandler Robbins, Clarence Cottam, and others as described in an historical review by Rattner [2009]). Although the geographic scope of nearly all our studies is broad, this chapter is an historical perspective of Patuxent research efforts that specifically examined contaminant exposure and resulting effects on Chesapeake Bay wildlife and their supporting habitat.

Early Years

Through interviews with retirees who worked at Aberdeen Proving Ground, the U.S. Army facility in Aberdeen, MD, it was learned that an unknown quantity of white phosphorus munitions is said to be buried offshore (that is, a barge containing munitions may have been purposefully or accidentally sunk) in the vicinity of Black Point (upper Chesapeake Bay near U.S. Army Aberdeen Proving Ground) between 1922 and 1925 (John Paul and John Wrobel, U.S. Army Garrison, Aberdeen Proving Ground, MD, oral commun., 2012). In addition, large segments of open water in this region had been used for decades as an ordnance impact area. Undoubtedly, ignited white phosphorus from artillery rounds was extinguished upon entering the bay. In 1933, the so-called burial site was disturbed by a hurricane. Resuspended white phosphorus may have been responsible for the large waterfowl kill that followed (ducks were said to have “turned pink and died”)

(see the U.S. Environmental Protection Agency Superfund Record of Decision [U.S. Environmental Protection Agency, 1991]). In 1939, Secretary of the Interior Harold Ickes designated parts of the site as a Migratory Waterfowl Closed Area for waterfowl hunting. These events and subsequent waterfowl die-offs may have been the impetus for a study of white phosphorus toxicity in mallards (*Anas platyrhynchos*) and American black ducks (hereafter black ducks) (*Anas rubripes*) (Coburn and others, 1950). In one of the first studies with captive waterfowl at Patuxent, survival of, and hematologic and histopathological responses to, acute and chronic exposure regimens were examined. Remarkably, tissue phosphorus concentrations in control and treated birds were determined and compared by using inferential statistical methods. Don Coburn and coworkers (1950) evaluated phosphorus concentrations in redhead ducks (*Aythya americana*) collected from northern Chesapeake Bay that were suspected to have died from phosphorus poisoning and concluded “it appears probable” the

birds had been killed by ingestion of elemental phosphorus. This issue reemerged and was scientifically revisited following frequent waterfowl die-offs at a military firing range on the Eagle River Flats, AK, from 1980 to the mid-1990s (reviewed by Sparling, 2003). The U.S. Army has since banned the firing of white phosphorus rounds over the wetlands at Eagle River Flats (U.S. Department of Defense, 2007).

1960s

Following the publication of “Silent Spring” (Carson, 1962), a National Pesticide Monitoring Program was initiated in response to public concern (Johnson and others, 1967). The Bureau of Sport Fisheries and Wildlife of the U.S. Fish and Wildlife Service (USFWS), including staff at Patuxent, collected starlings (*Sturnus vulgaris*) (initially at 44 locations, then at 110 locations), obtained hunter-collected mallard and black duck wings from nearly every state in the continental United States (organized into the four North American Flyways), and was sent dead golden eagles (*Aquila chrysaetos*) and bald eagles (*Haliaeetus leucocephalus*) from many locations across the country (Johnson and others, 1967). Samples initially were analyzed for organochlorine pesticides. Many Patuxent scientists contributed to the nationwide monitoring of starlings and duck wings in the decades that followed. The suite of analytes was expanded to include polychlorinated biphenyls (PCBs) and metals, and results were chronicled in special USFWS reports and scientific journal publications (Schmitt and Bunck, 1995). Because these monitoring

schemes focused on nationwide trends over time, little information can be derived with respect to comparisons of contaminant concentrations between states or within a particular estuary, such as Chesapeake Bay.

Examination of data obtained from 69 moribund or dead bald eagles collected in 25 states from 1966 to 1968 included one specimen from the Chesapeake Bay region. That eagle contained a dieldrin concentration of 4.3 micrograms per gram ($\mu\text{g/g}$) brain tissue on a wet weight (ww) basis, which may have contributed to its death (Mulhern and others, 1970), and another suspected dieldrin poisoning ($11 \mu\text{g/g ww}$) was documented in 1970 (Belisle and others, 1972). Regionally focused studies also were conducted during this period. For example, a survey of organochlorine pesticide residues in black duck eggs was conducted in 1964 (Reichel and Addy, 1968). The dataset indicated that eggs from the Chesapeake Bay region contained lower concentrations of dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), and dieldrin than samples from New Jersey, New York, Massachusetts, and other North Atlantic States.

Perhaps one of the most classic avian contaminant field studies of this era entailed the exchange of osprey (*Pandion haliaetus*) eggs from nests on the lower Potomac River, MD, with eggs from Old Lyme, Niantic, Trumbull Airport, and Mason’s Island in Connecticut by Stan Wiemeyer, Paul Spitzer, and others (Wiemeyer and others, 1975). The results of this highly cited study indicate that the most probable cause of poor reproduction in Connecticut ospreys was DDE, dieldrin, and PCB contamination, of both fish consumed and eggs laid by ospreys.



Young ospreys in nest on the James River, VA, 2012. Photo by Rebecca S. Lazarus, U.S. Geological Survey.

1970s

During the 1970s, Patuxent scientists devoted much effort to generating basic toxicological data on pesticides, industrial compounds, and metals through controlled-exposure studies with penned or caged birds (for example, Northern bobwhite [*Colinus virginianus*], Japanese quail [*Coturnix japonica*], mourning doves [*Zenaida macroura*], waterfowl, raptors, and black-crowned night-herons [*Nycticorax nycticorax*]). In addition, many regional or national contaminant-monitoring efforts included Chesapeake wildlife and, in some instances, research studies focused directly on the bay.

A study of ospreys on the Potomac River in 1970 and 1971 revealed low reproductive success, and indicated that the effects of pesticides and other contaminants could be investigated as a possible cause (Wiemeyer, 1971, 1977). Osprey eggs collected from 1970 to 1978 contained about 3 µg/g DDE ww; other organochlorine pesticides were present at much lower concentrations, yet PCBs were present at concentrations as high as 20 µg/g ww (Wiemeyer and others, 1988). In some instances, eggshell thinning approached levels (greater than 15 percent) that are associated with breakage and reduced reproductive success. Using aerial surveys by Patuxent scientists Chuck Henny, Vern Stotts, and others, Henny and others (1974) estimated the Chesapeake Bay osprey population to be about 1,450 nesting pairs in 1973; however, only 7 of these nesting pairs were observed in the northwestern part of the bay, and only 2 nesting pairs were observed on the James River and nearby tributaries. These results triggered several osprey ecotoxicological investigations in the subsequent decades.

During this era, Chesapeake Bay bald eagle eggs that failed to hatch contained greater concentrations of organochlorine pesticides than those from 13 other states (Wiemeyer and others, 1984), and eggshells were significantly thinner (-11 percent in Maryland and -18 percent in Virginia) than museum samples collected before the introduction of DDT (before 1946). In a continuation of this sampling effort, DDE concentrations in eagle eggs had declined by 1980–84, although shell thickness still averaged 13 to 14 percent less than pre-1946 values (Wiemeyer and others, 1993).

At about the same time (1972–73), eggs of barn owls (*Tyto alba*) were collected from offshore duck blinds on the lower Potomac River (Klaas and others, 1978). Eggshell thickness was determined to be inversely related to DDE, DDD, and dieldrin concentrations, and reproduction (1.7 young per clutch) was slightly less than that necessary to maintain population stability. In a long-term study of red-shouldered hawk (*Buteo lineatus*) populations in the Patuxent River Valley (1943–71), Henny and coworkers (Henny and others, 1973) suggested that concentrations of DDT, its metabolites, and dieldrin may have caused as much as a 9-percent decrease in eggshell thickness in samples collected in 1971, although it was unlikely that such exposure was having detrimental effects on hawk populations in this region.

As part of an eastern United States sampling effort in 1972 and 1973, concentrations of DDE and PCBs in eggs collected from great blue herons (*Ardea herodias*) and cattle egrets (*Bubulcus ibis*) nesting in the Potomac River were determined to be low to moderate compared to those in eggs collected from nests in other regions (Ohlendorf and others, 1979). In a highly cited publication, Patuxent scientist Harry



Banded young ospreys on nest in Chesapeake Bay, 2012. Photo by Rebecca S. Lazarus, U.S. Geological Survey.

Ohlendorf and others (1978) described contaminants in black-crowned night herons (*Nycticorax nycticorax*) and determined that eggs in Chincoteague, VA, in the Chesapeake Bay region (but not the bay proper) contained low to moderate concentrations of chlorinated hydrocarbons and metals; no evidence of substantial shell thinning was observed. This colony was used as a reference site for many studies in subsequent decades. In 1978, black duck eggs were collected along the Atlantic Flyway, and concentrations of DDE, PCBs, and mercury were determined to be the lowest in the Chesapeake Bay region (Pennsylvania, Maryland, Virginia) compared to more northerly locations extending into Nova Scotia (Haseltine and others, 1980).

Pesticide residues in carcass and brain, and metals in liver and kidney, were quantified in a subset of 15 ospreys found dead in the Chesapeake Bay region (1964–73; Wiemeyer and others, 1980). Large concentrations of organochlorine pesticides and PCBs were detected in a few individuals (for example, greater than 40 µg/g ww in brain or carcass for DDE and PCBs), although the extreme values for mercury and lead in these Chesapeake Bay region samples were generally lower than values for samples from New York, New Jersey, Ohio, and Florida. Other geographically broad-scale efforts to monitor carcasses and tissues that included samples from the Chesapeake Bay drainage area used American woodcock (*Scolopax minor*) (Clark and McLane, 1974), mourning doves (Kreitzer, 1974), and herons (Ohlendorf and others, 1981). Concentrations of chlorinated hydrocarbons and mercury in these samples were generally moderate compared to concentrations in samples from other regions in the United States. One possible exception was dieldrin, which was implicated in several poisonings of bald eagles in the Chesapeake Bay region in the 1960s and 1970s (Cromartie and others, 1975; Prouty and others, 1977). Furthermore, of 27 herons found dead in the Chesapeake Bay region from 1966 to 1978, 3 great blue herons and 2 cattle egrets contained dieldrin residues that probably contributed to their deaths (Ohlendorf and others, 1981).

To investigate the potential role of contaminants in declining populations of canvasbacks (*Aythya valisineria*), blood samples were collected from birds trapped at two Chesapeake Bay locations (Westmoreland State Park in Virginia and Cove Point in Maryland) by Patuxent scientists Mike Dieter and Matt Perry from 1972 to 1974 (Dieter and others, 1976). Abnormal enzyme activity was detected in about 20 percent of the samples; plasma aspartate aminotransferase activity was positively correlated with PCB and DDE concentrations in blood, and whole blood delta-aminolevulinic acid dehydratase (ALAD) activity was inversely related to lead concentrations in blood. This was the first published report describing the use of ALAD inhibition as a biomarker of lead exposure in wildlife, and its use in lead studies and Natural Resource Damage Assessments continues to the present day (2016). In a related study, canvasback carcass and tissue samples collected in 1973, 1975, and 1976 were analyzed for chlorinated hydrocarbons and several metals (cadmium, chromium, copper, lead,

mercury, and zinc); for most individuals, concentrations were less than levels known to cause adverse effects (White and others, 1979).

One of many necropsy case reports described by Patuxent scientist Lou Locke involved a moribund tundra swan (*Cygnus columbianus*) collected on the bank of Seneca Creek in Essex, MD (Locke and Young, 1973); this case report is particularly important because it may have been the first paper attributing lead poisoning in a swan to ingestion of fishing tackle. Patuxent veterinarian Jim Carpenter contributed to another case report that described an immature bald eagle recovered from western Maryland that died during treatment and rehabilitation attempts (Jacobson and others, 1977). Lead concentrations in tissues were elevated (liver, 22.9 µg/g; kidney, 12.3 µg/g), but most remarkable were the radiograph and necropsy of the gizzard, which contained 20 lead pellets.



Pair of canvasbacks used in contaminant study, Chesapeake Bay, 1976. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.



Oiled canvasback in Chesapeake Bay, 1978. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.



Tidal salt marsh in Chesapeake Bay, 1978. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.

During this period, Patuxent contaminant studies in mammals were limited. Patuxent scientist Don Clark and coworkers (Clark and Krynitsky, 1978; Clark and Lamont, 1976; Clark and Prouty, 1976) documented DDE and PCB concentrations in several species of bats (big brown bat [*Eptesicus fuscus*], little brown bat [*Myotis lucifugus*], eastern pipistrelle [*Pipistrellus subflavus*]) captured in Maryland and West Virginia. The many purposes of these studies included comparing placental transfer and sensitivity of fetuses to these compounds. In a review published decades later (Clark and Shore, 2001), concentrations of chlorinated contaminants in these bats from the Chesapeake Bay region were moderate to low compared to those in bats from other locations in the United States. Notably, whole-body concentrations of lead in big brown and little brown bats exceeded concentrations determined in meadow voles (*Microtus pennsylvanicus*), white-footed mice (*Peromyscus leucopus*), and short-tailed shrews (*Blarina brevicauda*) in the Chesapeake Bay region, but were comparable to levels measured in bats collected at mining sites (Clark, 1979).

Just after the close of the decade, Harry Ohlendorf (1981) summarized organochlorine contaminant data for birds collected in Chesapeake Bay at the Forty-Sixth North American Wildlife and Natural Resources Conference in Washington, D.C., in 1981. Ohlendorf pointed out that although organochlorine compounds were still present in eggs and tissues, the production of the two compounds of greatest concern, DDT and dieldrin, had been banned for use in the United States, and the manufacture and use of other organochlorine pesticides and

industrial compounds had declined (for example, production of Kepone in Hopewell, VA; this compound had contaminated much of the James River [Huggett and Bender, 1980], and was suspended, and sales of PCBs had been restricted). In closing, Ohlendorf states "...it appears that the impact of these chemicals in the future should be much less than in the past 35 years. In the Chesapeake Bay attention should be focused on fish-eating birds, primarily bald eagles and ospreys, but it is unlikely that organochlorines will present a serious threat to these species, or others of the Chesapeake Bay region" (Ohlendorf, 1981).

1980s

Unlike the decline in DDE concentrations observed in bald eagle eggs in the Chesapeake Bay area, a similar trend for DDE in osprey eggs collected at the Glenn L. Martin National Wildlife Refuge, Chesapeake Bay, in 1986 was not statistically supported, and the concentrations present were reported to be large enough to cause a 10-percent eggshell thinning (Audet and others, 1992). At that particular location, PCB concentrations appeared to have declined, and other DDT metabolites and dieldrin were not detected. Results of additional studies of osprey carcasses from Chesapeake Bay (1975–82) revealed that concentrations of some organochlorine compounds had declined substantially (Wiemeyer and others, 1987). Interestingly, the mercury concentration of 21 $\mu\text{g/g}$ ww in the liver of

one of the dead ospreys might have contributed to the death of this bird, which was killed when it was struck by a motor vehicle (Wiemeyer and others, 1987).

Departing from the long-standing focus of Patuxent biologists on wildlife, Jim Fleming and coworkers (Fleming and others, 1988) led a series of studies of submerged aquatic vegetation (SAV). Water-quality problems and storms in the bay had long been identified as the most likely causes of dramatic declines in the abundance of SAV, and its loss adversely affected other biota throughout the bay. The toxicity of the widely used herbicide atrazine was tested using sago pondweed (*Potamogeton pectinatus*) grown in sterile cultures (axenic conditions) and in buckets (nonaxenic conditions). At concentrations of 1,000 micrograms per liter, atrazine impaired growth of plants in both axenic and nonaxenic conditions. This bioassay system showed considerable promise for effluent screening and testing in Chesapeake Bay.

In 1987 and 1988, Patuxent scientist Keith Miles collected samples of sediment, composites of various invertebrates, and clams (*Macoma* spp.) in Baltimore Harbor, MD, where large numbers of waterfowl had been observed to feed and rest (Miles and Tome, 1997). These samples were analyzed for 20 metals and metalloids, and concentrations of many elements were greater in invertebrates than in sediment. At some locations, concentrations of aluminum, boron, chromium, mercury, lead, and selenium exceeded toxic thresholds, and it was suggested that individual birds using some of the study areas might be adversely affected, although probably not at the population level. In a companion waterfowl study, concentrations of metals and metalloids were measured in livers of dabbling and diving ducks collected from Baltimore Harbor by Patuxent scientist Mike Tome. Lead concentrations exceeded the 2- $\mu\text{g/g}$ ww threshold for subclinical poisoning in some mallards, black ducks, and scaup (*Aythya* spp.), but mercury, cadmium, and selenium levels were generally well below toxicity thresholds (U.S. Geological Survey, 2014; Rattner and McGowan, 2007).



Contamination in Baltimore Harbor, MD, 1973. Photo by Matthew C. Perry, U.S. Fish and Wildlife Service.

Toward the end of the decade, Patuxent scientists Ohlendorf and Fleming (1988) undertook a comparison of Chesapeake Bay and San Francisco Bay waterbird contaminant data collected. Based on field and laboratory studies, the authors concluded that the concentrations of some trace elements and organochlorine compounds in avian tissues and their food items could evoke adverse effects. In Chesapeake Bay, elevated concentrations of cadmium and lead in seaducks, lead in dabbling waterfowl, and DDE in ospreys and bald eagles were of concern, whereas major issues in San Francisco Bay included selenium, cadmium, and mercury in waterfowl and PCBs and DDE in shorebirds and herons. Ohlendorf and Fleming (1988) outlined a research- and information-needs strategy, but ultimately their plan was only partially pursued as a result of funding limitations and shifts in research priorities.

1990s

Patuxent staff members became increasingly involved with the Chesapeake Bay Program, a consortium of Federal, State, and nonprofit agencies and organizations working toward bay restoration. With the passage of the 1987 Chesapeake Bay Agreement, we began to serve on committees and collaborate with other scientists to develop guidelines for the protection of habitat, water quality, and living resources in Chesapeake Bay. At about this time, many of our researchers developed long-lasting collaborations with contaminant biologists (operational staff) at the Chesapeake Bay field office of the USFWS that continue to this day (2016). Gary H. Heinz and Stan Wiemeyer prepared a chapter titled “Effects of Contaminants on Birds” (Heinz and Wiemeyer, 1991) in the frequently cited compendium “Habitat Requirements for Chesapeake Bay Living Resources: A Report from the Chesapeake Bay Living Resources Task Force” (Funderburk and others, 1991). They describe the history of contaminant effects on birds of Chesapeake Bay and point out that the banning of the most harmful organochlorine pesticides and the replacement of lead shot with steel shot has reduced poisoning and reproductive problems. Nevertheless, they suggest that contaminants such as cadmium, petroleum (oil), and industrial chemicals could adversely affect avian species.

A small nesting colony of black-crowned night herons became established in the Baltimore Harbor area in 1979 and had grown to 300 nests by 1990, constituting the largest colony of this species in Maryland. Remarkably, the foraging habits of this colony were concentrated in this highly industrialized area (Erwin and others, 1991), one of three U.S. Environmental Protection Agency-designated Chesapeake Bay Regions of Concern. The colony was popularized in an article by Patuxent scientist Mike Erwin titled “Industrial Strength Herons,” which appeared in “Maryland Magazine” (Erwin and others, 1990). In 1991, Mark Melancon began a study examining contaminant exposure and hepatic cytochrome P450 induction (a biochemical biomarker of polyhalogenated hydrocarbon exposure) in pipping embryos and nestlings from

the Baltimore Harbor heron colony and at a colony in Rock Creek Park in Washington, D.C. (Rattner and others, 1997). Cytochrome P450-associated monooxygenase enzymes were induced more than fivefold in pipping embryos from Baltimore Harbor and to a smaller degree in those from Rock Creek Park, and concentrations of organochlorine contaminants in pipping embryos and nestlings were markedly elevated compared to those collected from the Chincoteague Bay reference site. The concentration of some PCB congeners (numbers 77 and 126) actually exceeded values observed in this species in the Great Lakes and appear to have been partly responsible for cytochrome P450 induction (Rattner and others, 1997).

These results were the impetus for testing the hypothesis that PCBs might be leading to the declining size of the Baltimore Harbor heron colony. In a follow-up study conducted in 1998, USFWS Chesapeake Bay field office biologist Pete McGowan and I determined that the heron colony had moved about 2 kilometers (1.2 miles) south to Fort Carroll, a mid-19th-century military structure built by Robert E. Lee before the Civil War. In a large-scale study, concentrations of 12 arylhydrocarbon receptor-active PCB congeners and dioxin-related toxic equivalents were more than 35 times greater in sampled eggs from Baltimore Harbor than in those from the reference site in southern Chesapeake Bay (Holland Island). Seventy-four percent of the nests produced at least one chick, and productivity (2.05 young per nest) was adequate to maintain a stable population (Rattner and others, 2001). No significant relation was found between hatching, fledging, or overall reproductive success and concentrations of PCBs and toxic equivalents. The authors concluded that contaminants were not having a dramatic effect on reproduction in the Baltimore Harbor heronry. In the years that followed, the numbers of black-crowned night herons at Fort Carroll continued to decrease to a mere 17 pairs in 2008 (D.F. Brinker, Maryland Department of Natural Resources, oral commun., 2008), and this now-mixed waterbird colony was dominated by double-crested cormorants (*Phalacrocorax auritus*), herring gulls (*Larus argentatus*), and cattle egrets.

Led by colleagues of the USFWS Chesapeake Bay field office, Mark Melancon and Dave Hoffman of Patuxent assisted with a study of potential contaminant effects in great blue herons nesting at Mason Neck National Wildlife Refuge on the banks of the Potomac River in Lorton, VA, in 1997 (Johnson and others, 2001). Eggs were collected and artificially incubated, and biochemical (cytochrome P450 and measures of oxidative stress) and eggshell-thickness measurements did not differ from those for the Coaches Island reference site. Results indicated that great blue herons at Mason Neck, the largest great blue heron colony in Virginia, were probably not being adversely affected by polyhalogenated contaminants.

As part of a study examining potential endocrine disruptive effects of PCBs, Patuxent scientist John French reported that common tern (*Sterna hirundo*) eggs from South Sand Point (off Barren Island in Maryland and Virginia) in 1994 contained relatively low levels of Aroclor 1260 (0.44–1.50 $\mu\text{g/g}$ ww) (U.S. Geological Survey, 2014; Rattner

and McGowan, 2007). As part of this effort, eggs also were collected from Bodkin Island, MD, in 1997 and contained less than 10 micrograms total PCB per gram lipid; Bodkin Island served as a comparative reference site for the more contaminated samples from Ram Island in Buzzards Bay, MA (French and others, 2001). There was no evidence that the concentrations of steroid hormones that were maternally deposited in eggs were affected by contaminant exposure.

Tree swallow (*Tachycineta bicolor*) eggs and nestlings were collected from the Patuxent River, a tributary to the middle Chesapeake Bay, to serve as a reference for PCB-contaminated sites in Indiana, New York, and Pennsylvania (Yorks, 1999). As expected, total PCB concentrations in samples from the Patuxent reference site (eggs, 0.69 $\mu\text{g/g}$ ww; nestling carcass, 0.29 $\mu\text{g/g}$ ww) were much lower than those in samples from the PCB-contaminated sites (eggs, 0.94–4.6 $\mu\text{g/g}$ ww; nestling carcass, 0.17–18.5 $\mu\text{g/g}$ ww).

Following an avian cholera outbreak in 1994, 41 long-tailed duck (*Clangula hyemalis*) carcasses were collected throughout Chesapeake Bay (Mashima and others, 1998). Liver and kidney cadmium concentrations were greater in birds that succumbed to cholera than in apparently healthy birds collected during 1985–87. The authors suggested that cadmium may have contributed to cholera susceptibility in these ducks, and concentrations of lead, mercury, and selenium in tissues were probably too low to evoke immunotoxicity. The authors also indicated that weight loss owing to cholera could have concentrated metals in tissues.

As part of a series of studies examining tissue uptake of metals from ingested soil and sediment, Nelson Beyer, Dan Day, and other Patuxent colleagues collected mute swans (*Cygnus olor*) from several locations in Chesapeake Bay (for example, Bloodsworth Island, Horseheads Wetland Center, and Eastern Neck and Blackwater National Wildlife Refuges) (Beyer and others, 1998). Concentrations of metals in sediment were low, and concentrations in liver were considered to be at background levels for this species. Copper concentrations were remarkably high (as much as 1,200 milligrams per kilogram dry weight [dw]); apparently swans, in the absence of environmental contamination, can accumulate large quantities of copper in the liver, far more than other species of waterfowl. Although this study revealed little about the hazards posed by sediment to mute swans throughout the bay, it demonstrated the importance of sediment ingestion for the accumulation of lead in mute swans. Additional studies near Aberdeen Proving Ground indicated that hepatic lead, cadmium, copper, and selenium concentrations did not represent a toxic threat to the swans (Beyer and Day, 2004).

Some heavy metals can be incorporated into feathers at the time they are grown, and the sampling of feathers has gained some acceptance as a minimally invasive sublethal contaminant monitoring technique. My graduate student assistant, Nancy Golden, collected feathers from black-crowned night heron nestlings and determined lead concentrations in herons from Baltimore Harbor to average 0.32 $\mu\text{g/g}$ dw, which was greater than those in feathers collected from Chincoteague



Osprey on nest in Baltimore Harbor, MD, 2011. Photo by Rebecca S. Lazarus, U.S. Geological Survey.

Bay and Holland Island (less than or equal to $0.13 \mu\text{g/g dw}$) (Golden and others, 2003b). In a related study of lead-dosed heron nestlings, red blood cell ALAD activity was inversely related to lead concentrations in feathers (Golden and others, 2003a); thus, lead concentrations in feathers of some heron nestlings from Baltimore Harbor might be great enough to cause enzyme inhibition and impaired heme (porphyrin ring component of hemoglobin) synthesis.

Patuxent staff member Mark Melancon collaborated for several years with a team of scientists investigating contaminant exposure, pathological lesions, and cytochrome P450 induction in brown bullheads (*Ameiurus nebulosus*) collected from highly contaminated locations in the Chesapeake Bay area, including the Anacostia River near Washington, D.C., and Back River and Furnace Creek near Baltimore (Pinkney and others, 2001, 2004). These studies documented tumor prevalence; concentrations of DDT, PCBs, and various polynuclear aromatic hydrocarbons (PAHs); and cytochrome P450 induction. In some instances, tumor prevalence was associated with biliary PAH concentrations. Some of the skin tumors were rather grotesque and received considerable attention in the media.

2000s

With recovery and expansion of the Chesapeake osprey population in the 1990s, birds began nesting in some of the most contaminated sites in the bay, including Baltimore

Harbor, and the Anacostia and Elizabeth Rivers. In 2000 and 2001, a large-scale study was conducted in which osprey eggs were collected from nests in these Chesapeake Bay Regions of Concern and nearby tributaries (Rattner and others, 2004). Concentrations of DDE, dieldrin, and chlordane in eggs collected from the middle Potomac River in 2000 were less than half those observed in 1970s, and there were no effects on reproductive success when compared to the reference sites (South, West, and Rhode Rivers). However, shell thickness of eggs from the Anacostia River and middle Potomac River averaged 8.7 percent less than in the pre-DDT era, and more than half of these sampled eggs contained DDE at concentrations within the 95-percent confidence interval ($1.2\text{--}3.0 \mu\text{g/g ww}$) associated with 10-percent eggshell thinning. Compared to total PCB values reported in eggs collected in the 1970s and 1980s (Wiemeyer and others, 1988), concentrations in osprey eggs in the 2000 and 2001 samples had not declined. Notably, total PCBs in the reference area averaged more than $4 \mu\text{g/g ww}$, which alerted fisheries biologists to a potential hazard, eventually leading to a human-health fish consumption advisory for some species in the South River (Joseph Beaman, Maryland Department of the Environment, oral commun., 2002). Concentrations of toxicologically potent coplanar and semicoplanar PCB congeners were similar among study sites, and dioxin-like toxic equivalents were not unlike values reported for the Delaware Bay and the Great Lakes.

Several groups of emerging contaminants also were quantified in these osprey egg samples. Perhaps the most interesting group was the polybrominated diphenyl ethers

(PBDEs), which are flame retardants; concentrations approached 1 $\mu\text{g/g}$ ww, which were some of the greatest values reported in bird eggs at that time. Follow-up PBDE egg injection studies indicated that pipping and hatching success might be adversely affected at 1.8 $\mu\text{g/g}$ ww (McKernan and others, 2009). Perfluorinated surfactants also were detected in osprey eggs, although concentrations were well below adverse-effect levels. Alkylphenol and ethoxylate surfactants occasionally were detected in low nanogram-per-gram wet weight quantities, although effects of this putative endocrine disruptor in birds have yet (2016) to be definitively verified. Blood and feather samples also were collected from 40- to 45-day old osprey nestlings, and results of analyses indicated that concentrations of several heavy metals (cadmium, lead, mercury) were well below toxicity thresholds (Rattner and others, 2008).

A reevaluation of contaminant exposure, biomarker responses, and potential reproductive effects in ospreys nesting in several tributaries and in Regions of Concern was initiated in 2011. In this large-scale collaborative study, research trainee Rebecca Lazarus and I are examining food-web transfer of legacy-halogenated contaminants, pharmaceuticals, and personal care products in water, fish, and ospreys (Lazarus and others, 2010). Results for legacy contaminants in osprey eggs revealed that concentrations of DDE are below thresholds associated with eggshell thinning and total PBDE concentrations have declined by 40 percent in the past decade, although concentrations of total PCBs in eggs from Baltimore Harbor

and the Elizabeth River have remained unchanged (Lazarus and others, 2015). Of 23 pharmaceuticals measured in samples from the bay, 18 analytes were detected in water and 8 were detected in plasma from fish; only 1 of the 23 compounds (the antihypertensive diltiazem) was detected in nestling osprey plasma, but at concentrations well below the human therapeutic plasma concentration (Lazarus and others, 2014). Although there was some evidence of genetic damage in osprey nestlings from the most industrialized regions of the bay, overall findings document the continued recovery of the Chesapeake Bay osprey population (Lazarus and others, 2015).

Over the years, there have been many oil spills in Chesapeake Bay (about 500 incidents annually); fortunately, most have been small events. In 2000, a pipeline rupture released about 126,000 gallons of no. 2 and no. 6 fuel oil at the Potomac Electric Power Company Chalk Point Facility near Aquasco, MD. The spill spread to Swanson Creek, a tributary to the Patuxent River, and killed about 55 birds (principally waterfowl, ospreys, herons, gulls, and terns), and 109 oiled birds were collected for rehabilitation (Cardano, 2001; McGee and others, 2001). This event occurred in April and was coincident with nesting of many species. Patuxent biometrician Jeff Hatfield provided statistical assistance to Daniel Murphy and Craig Koppie of the USFWS Chesapeake Bay field office in evaluating reproductive success of great blue herons and osprey. Fortunately, nest success of herons and ospreys did not seem to be adversely affected by the spill (Cardano, 2001; McGee and others, 2001).



Barnett Rattner, U.S. Geological Survey (left), and Pete McGowan, U.S. Fish and Wildlife Service, approaching an osprey nest by boat in Chesapeake Bay, 2013. Photo by Reese F. Lukei, Jr., Center for Conservation Biology, College of William and Mary, Williamsburg, VA.

In 2001, reports of dead and dying waterbirds at the Poplar Island Complex, Kent Island, and Grasonville, MD, coincided with several harmful algal blooms (HABs). Most prominent was the mortality event at the Poplar Island Complex involving about 100 great blue herons. Results of necropsies performed by Patuxent veterinarian Glenn Olsen were consistent with steatitis (inflammation of adipose tissue), and microcystin toxins from cyanobacteria (*Anabaena* spp.) were detected in water samples and in tissues of dead herons. These HABs and the bird die-offs recurred in 2004 and 2005, and several hypotheses were developed (but remain untested) to examine the role of HABs and diet in steatitis and death of herons (Rattner and others, 2006).

As part of an interspecific study examining the comparative sensitivity of birds to PBDE, common tern eggs were collected from Poplar Island, MD, in 2010 (Rattner and others, 2013). Six eggs were chemically analyzed, and all were determined to contain low levels of organochlorine pesticides (less than 0.08 µg/g ww), total PCBs (less than 0.45 µg/g ww), and total PBDEs (less than 0.05 µg/g ww), indicating that eggs from this mid-Chesapeake Bay location could be used to study the commercial PBDE DE-71 formulation for embryotoxicity.

In their continued study of bullheads from many Chesapeake Bay tributaries, investigators examined tumor prevalence and biomarkers of genotoxicity (Pinkney and others, 2011). Natalie Karouna-Renier identified DNA adducts in liver tissue of bullheads collected from the South and Anacostia Rivers, although this endpoint did not seem to be associated with liver- or skin-tumor prevalence.

Rattner and McGowan (2007) reviewed the potential hazards of contemporary environmental contaminants to avifauna in the Chesapeake Bay estuary by using the Contaminants Exposure and Effects—Terrestrial Vertebrates database (U.S. Geological Survey, 2014). They identified several groups of contaminants (for example, dioxins, dibenzofurans, rodenticides, pharmaceuticals, personal care products) that have not been systematically examined and highlighted the need for toxicological evaluation of birds found dead, and perhaps an avian ecotoxicological monitoring program.

Conclusions

Patuxent scientists have studied environmental contaminants and contamination processes in Chesapeake Bay for decades. Our efforts have been intermittent, reflecting ever-changing research priorities, perceived needs of natural-resource managers, and fluctuating budgets. During the organochlorine pesticide era, Chesapeake Bay served as a convenient outdoor laboratory to monitor exposure and test hypotheses. In fact, this estuary provided remarkable evidence of “a great natural experiment,” a wonderful phrase first coined by Patuxent contaminant biologist Bill Stickel and passed on to me by my colleague Gary H. Heinz. After the use of certain organochlorine pesticides was restricted, residues in tissues of wildlife and in their foods declined, toxic effects

were abated, and, in some instances, wildlife populations (for example, osprey, bald eagle, peregrine falcon [*Falco peregrinus*]) recovered. With each successive decade, new chemicals and stressor interactions emerge that we must consider and evaluate. In the last several decades, Chesapeake Bay has been a source of plants and animals that can be used to study contaminant uptake, metabolism, clearance, and toxicity in our laboratories and animal holding facilities. We have contributed to the recovery of parts of the Chesapeake Bay ecosystem, but have come to the realization that it will never return to the condition that existed before the arrival of European settlers to the New World.

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