



Investigation of Waterfowl Mortality
Eagle River Flats, Alaska

Submitted by:

Alaska Fish and Wildlife Research Center
and
National Wildlife Health Research Center

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I. Review of 1983-1985 Results

Carcasses of 60 birds, representing nine species, were submitted to the National Wildlife Health Research Center (NWHRC) for necropsy evaluation (Attachment 1). Pulmonary congestion and/or excess fluid was noted in about half of the birds examined, but gross necropsy results were largely non-contributory. No specimens tested positive for avian botulism and no bacterial or viral pathogens were isolated from tissues.

Because phosphorus is a component of smokes used on the Eagle River Flats, tissue phosphorus concentrations were determined for several birds submitted in 1983 and 1984. Mean phosphorus concentration in gastrointestinal tracts of four birds was slightly higher than in controls (1040 vs. 875 ppm wet wt.), but ranges overlapped. Interpretation of tissue phosphorus concentrations is difficult; Coburn et al. (1950) reported that control black ducks (Anas rubripes) had higher liver phosphorus levels than ducks poisoned with white phosphorus, but the reverse was true in kidney.

Concentrations of zinc, mercury, and magnesium in birds from the Eagle River Flats were within normal limits (Gasaway and Buss 1972, Nicholson 1981, Finley et al. 1979, Haarakangas et al. 1974, Custer et al. 1986). Lead was elevated in one bird, but typical lesions were near or below detection limits. Brain cholinesterase was checked in four birds collected in 1983; no inhibition was detected.

II. Proposed Field and Laboratory Research

A. Mortality Investigations of Wild Birds

To facilitate a rapid, accurate diagnosis it is necessary to obtain an adequate sample of specimens in good condition. Ideally, a combination of fresh and frozen specimens should be shipped to the NWHRC for evaluation. Sample size will depend on necropsy results and the level of testing required to reach a diagnosis. Multiple shipments may be required.

Laboratory testing will be heavily dependent on observations made during necropsy of specimens; our goal will be to determine cause of death and identify any predisposing factors. In addition, a sample of birds will be tested for viral and bacterial (including avian botulism) diseases. Liver, spleen, lung, kidney, and intestine will be collected for histopathological examination; toxicological analyses will be guided by

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necropsy observations and results of histopathology. Ducks will be collected from Knik Flats using non-toxic shot, and will provide control tissues for toxicologic analyses. Although components of most of the materials used on the Eagle River Flats are unknown at this time, previous laboratory work at the NWHRC indicates that further investigation of phosphorus as a potential toxicant is necessary. Liver, kidney, lung, and intestinal contents from a sample of carcasses will be analyzed for phosphorus. Decisions on further toxicologic analyses will be aided by the identification of potential toxicants occurring on the Eagle River Flats.

B. Sentinel Bird Investigations

Limited access and safety considerations dictate the need for supplemental techniques for collecting wild birds in the Eagle River Flats impact zone. Dead birds may not be recovered for several days or weeks because of their cryptic plumage and the dense vegetation cover. Potential redistribution of carcasses by tides may also preclude location of specimens suitable for laboratory analysis. Previous searches of the Eagle River Flats have resulted in collection of dead birds (waterfowl, shorebirds, passerines, and eagles), but have not permitted capture of sick individuals because they were capable of escaping field personnel.

The use of sentinel birds is an effective technique in site-specific studies of disease in waterfowl (Rocke and Brand 1987, Brand and Rocke 1987). Hand-reared birds confined in predator-proof enclosures permit accurate determination of the first case of mortality, mortality rates (number dying per number at risk), and site of exposure to disease or contaminant-causing agents.

To prevent the introduction of disease into wild populations, it is necessary to test sentinel birds before release. Cloacal swabs, serum, and whole blood will be collected from a sample of hand-reared mallard ducks (*Anas platyrhynchos*). Cloacal swabs and serum will be tested for evidence of duck plague virus, requiring about seven days at the NWHRC. Remaining serum will be frozen for potential future use and blood smears will be made from whole blood samples.

After ducks are tested, they will be enclosed in heavy wire mesh pens anchored with steel posts. The pen top will be covered with steel support wires and netting to prevent access by avian predators. Loafing and roosting sites will be constructed of logs and wooden platforms anchored to the substrate. Mallards released to the pens will be wing-clipped to prevent flight and will be marked with a numbered tarsus band for identification. Sentinel ducks will be maintained in each of three pens located in the safety zone (see map in General Study Plan), and in two pens situated at the EOD site and the ridge along the south side of the Eagle River Flats. Sentinel ducks will receive supplemental feed and grit on a daily basis. The pens will be searched daily and counts of all ducks will be recorded.

Behavior of sentinel birds will be monitored prior to and during U.S. Army field exercises by direct observation and remote photography. Time and activity budgets of ducks will be determined following techniques

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described by Ward et al. (1987). Behaviors of sentinel ducks exposed to field exercise activities will be compared to their behavior prior to these events. Clinical signs of disease or exposure to contaminants (such as wing droop, circling, etc.) will be noted. Dead or sick birds will be removed from the pens.

Blood samples will be collected from sentinel birds exhibiting clinical signs. Smears will be prepared and whole blood and serum will be stored frozen. Ducks will then be euthanized by cervical dislocation and handled as fresh carcasses. Necropsy and laboratory testing will be handled as for mortality investigations of wild birds described above. Sentinel ducks will be euthanized and burned or buried at the end of the study.

III. Protocol for Specimen Handling

Specimens referred to here would fall primarily into three categories: 1) fresh carcasses, 2) frozen carcasses, and 3) tissues for histopathology and toxicology. Handling procedures vary depending on the type of specimen involved. Fresh carcasses must reach the NWHRC within 24 hours of collection. Frozen carcasses can be stored until they can conveniently be shipped. Always call the NWHRC if any doubt exists about specimen handling.

A. Fresh carcasses

1. Label each carcass with a tag attached to a leg. Record date collected, location, species, collector, and any clinical signs observed.
2. Double-bag each carcass in plastic. Chill carcasses as soon as possible. Optimum situation would be to have a cooler with ice in vehicle.
3. Place double-bagged carcasses in styrofoam cooler lined with a large plastic bag. Include several frozen blue ice packs.
4. Close plastic bag and seal cooler lid with strapping tape. Tape specimen data sheet, including name and address of submitter and list of species in container, to the outside of the cooler lid.
5. Place cooler in a cardboard box, seal with strapping tape, and address to:

National Wildlife Health Research Center
6006 Schroeder Road
Madison, Wisconsin 53711

6. Write "Diagnostic Specimens - Wildlife" on outside of the cardboard box.
7. Ship on a carrier that will deliver the package to NWHRC within 24 hours. Possibilities include: Federal Express, DHL, Purolator, and air freight.

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8. Send no later in the week than Wednesday, unless prior arrangements have been made with NWHRC. Call NWHRC with name of carrier used, the estimated date of arrival, and airbill number.

B. Frozen carcasses

1. Same as above.
2. Freeze carcass as soon as possible.
- 3-8. Same as above.

C. Tissues for Histopathology and Toxicology

Only after requested by NWHRC and after receiving complete instructions; refer to NWHRC Disease Manual. When removing tissues from euthanized birds, it is important to standardize the length of time between death and tissue removal, since liver weights can vary 20-40%, depending on the amount of time elapsed between death and removal of the organ (Franson 1984). This has significant implications for results of residue analysis expressed in terms of wet organ weight.

1. Wearing rubber gloves, carefully open carcass according to NWHRC Disease Manual instructions.
2. Using a sharp scalpel blade, place slices (no more than 3-4 mm thick) of liver, spleen, lung, and kidney in 10% neutral buffered formalin. Volume of formalin should be at least 10 times volume of tissue. Use pint canning jars, etc. Place remainder of liver and kidney in acid-washed jars and freeze (toxicology samples).
3. After three days of fixation, tissue can be transferred to smaller jars or whirl-pack bags containing a small amount of formalin. Label with date collected, location, collector, and clinical signs observed.
4. Pack fixed tissue carefully to guarantee against leakage; ship via U.S. Postal Express Mail or First Class, after checking on regulations for formalin.
5. Ship labeled jars of frozen tissue as frozen carcasses above.
6. Call the NWHRC with shipping information.

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Attachment 1. Necropsy summary of specimens collected on Eagle River Flats, Alaska, 1983-1985.

1983 - National Wildlife Health Research Center (NWHRC) Cases 3410, 3427

Carcasses Received: 22

Shoveler (1)	Green-winged Teal (8)
Mallard (6)	Pintail (5)
Wigeon (2)	

Gross Necropsy Results:

Pulmonary congestion and/or excess fluid in lungs of 11. Several specimens in poor post mortem condition. Necropsy results largely non-contributory.

<u>NWHRC Lab Tests:</u>	<u># Tested</u>	<u>Results</u>
Avian botulism	11	Negative
Bacterial isolation	9	Non-contributory
Viral isolation	2	Negative
Histopathology	5	Pulmonary congestion (2) Mild enteritis (1) Non-contributory (2)
Methemoglobin	2	18.5%, 47.8%
Metals		Mean (range) ppm dry wt
Phosphorus, GI Tract	4	3672 (1730 - 8500)
Phosphorus, Lung	1	6200 ppm dry wt

Other Lab Tests:

Four (4) brains to Patuxent Wildlife Research Center (PWRC) for cholinesterase, all negative inhibition. Various tissues from 8 birds sent to PWRC on 10/5/83 for analysis (primarily nitrate, phosphorus; some metals) - no results received as of 2/1/88.

Diagnoses:

Undetermined (11)
Gunshot (1)
Pulmonary congestion, edema, enteritis (1)
Unsuitable for examination (3)
Saved for future use (6) - discarded 11/5/85

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Attachment 1 - Continued

1984 - National Wildlife Health Research Center (NWHRC) Case 3986

Carcasses Received: 31

Pintail (10)	Green-winged Teal (9)
Mallard (9)	Snipe (3)

Gross Necropsy Results:

Pulmonary congestion and/or excess fluid in lungs of 17. Several specimens in poor post mortem condition. Necropsy results largely non-contributory.

NWHRC Lab Tests:

	<u># Tested</u>	<u>Results</u>
Avian botulism	21	Negative
Bacterial isolation	9	Non-contributory
Viral isolation	8	Negative
Histopathology	8	Pulmonary congestion
Parasitology	1	Coccidia
<u>Metals</u>		
		Mean (range) ppm wet wt
Phosphorus, GI Tract	4	1040 (920 - 1190)
Phosphorus, Controls	4	875 (750 - 900)
Phosphorus, Liver	6	2647 (1661 - 3280)
Phosphorus, Kidney	6	2938 (2690 - 3114)
Zinc, Liver	6	21.0 (15.9 - 27.3)
Zinc, Kidney	6	14.0 (13.3 - 15.1)
Magnesium, Liver	6	135.2 (75.8 - 182.6)
Magnesium, Kidney	6	168.9 (143.1 - 209.5)
Arsenic, Liver	6	Less than 0.24
Arsenic, Kidney	6	Less than 1.16
Mercury, Liver	6	Less than 0.01
Mercury, Kidney	6	Less than 0.04

Other Lab Tests:

Tissue for histopathology sent to Col. Callis, Aberdeen Proving Ground, for evaluation on 3/29/85 - no results received as of 2/1/88.

Diagnoses:

Undetermined (24)
 Unsuitable for examination (2)
 Saved for future use (5) - discarded 11/5/85

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Attachment 1 - Continued

1985 - National Wildlife Health Research Center (NWHRC) Cases 5616, 5617

Carcasses Received: 7

Green-winged Teal	(2)	Shoveler	(1)
Long-billed dowitcher	(1)	Tundra swan	(1)
Bald eagle	(2)		

Gross Necropsy Results:

Non-contributory

NWHRC Lab Tests:

<u>NWHRC Lab Tests:</u>	<u># Tested</u>	<u>Results</u>
Avian botulism	4	Negative
Bacterial isolation	1	Non-contributory
Histopathology	6	Non-contributory Nephrosis (2 eagles)

Other Lab Tests:

Tissues from two bald eagles sent to PWRC for contaminants analysis on 8/26/85
- no results received as of 2/1/88.

Diagnoses:

Undetermined	(5)
Nephrosis	(2)