

## BLOOD LEAD CONCENTRATIONS IN MALLARDS FROM DELEVAN AND COLUSA NATIONAL WILDLIFE REFUGES<sup>1</sup>

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**Blood samples were taken from 181 (108 adult drakes and 73 individuals of mixed age and sex) mallards, *Anas platyrhynchos*, from Colusa and Delevan National Wildlife Refuges during late winter and summer of 1987. The percentage of birds with elevated lead concentrations was 28.7 for late winter and 16.4 for late summer. For summer trapped birds, a significantly greater proportion of males than females contained elevated lead levels. These findings indicate that lead poisoning may be a year-round event in certain areas of the Sacramento Valley.**

### INTRODUCTION

Lead poisoning in waterfowl, resulting from ingestion of lead shot, is an issue that has polarized both biologists and the hunting public for many years. It is one of the major diseases of wild waterfowl (Friend 1985) with annual losses estimated to be as high as 2-3 percent of the continent's population (Bellrose 1959). Although large outbreaks have been documented, it is believed that most losses occur as isolated cases (Sanderson and Bellrose 1986; NWHRC, unpubl. data). Lead poisoning is usually a chronic, debilitating disease, requiring 3 weeks or longer from ingestion of lead shot to death. Epizootics become apparent when local predators cannot consume sick and dead birds fast enough to prevent a visible accumulation of sick or dead birds.

Most studies on lead pellet ingestion and lead poisoning mortality are conducted during or just after the waterfowl hunting season (Bellrose 1959; NWHRC, unpubl. data). Few studies have been conducted during the summer or early fall. We provide information on blood lead concentrations in wild mallards, *Anas platyrhynchos*, in California's Sacramento Valley during late winter and summer.

<sup>1</sup> Accepted for publication March 1990.

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### STUDY AREA

Samples were collected at Delevan and Colusa National Wildlife Refuges (NWRs), in Glenn and Colusa counties of California. Both refuges are part of the Sacramento NWR complex and are administered by the U.S. Fish and Wildlife Service. Delevan and Colusa NWRs are comprised of 2280 and 1635 ha, respectively, of seasonally-flooded marsh (60%), watergrass fields, *Echinochloa cruzgalli*, (10%), permanent ponds (5-7%), rice (<2%) and uplands (20%). Each area is managed primarily for fall migrant and wintering waterfowl. Approximately 40% of each area is open to waterfowl hunting. Non-toxic (steel) shot has been required since the fall of 1986.

### METHODS

All birds used in the study were captured opportunistically and thus were not a random sample of mallards from the study area. In addition, most birds sampled were captured on previously hunted areas of the refuge (hunted since 1962).

During February 1987, 108 drake mallards were captured with baited funnel traps. These birds were captured for use as sentinels in a study of botulism on Sacramento NWR. Birds were bled within 24 hours of capture and placed in holding pens where they were fed a combination of rice and scratch grains. Pens were checked daily for sick and dead birds, and cause of death was determined by necropsy and supporting diagnostic laboratory studies at the U.S. Fish and Wildlife Service, National Wildlife Health Research Center (NWHRC) Madison, Wisconsin.

Blood samples were similarly collected from 73 mallards of both sexes trapped from July through early September, 1987. These birds were banded with U.S. Fish and Wildlife Service leg bands, to insure that recaptured birds would not be bled a second time and were immediately released.

Using 21 gauge needles and plastic syringes, blood samples (1.0-2.0 ml) were drawn from the jugular vein of all birds, placed in heparinized glass tubes and frozen for later analysis at NWHRC. Blood lead concentrations were determined with a Perkin-Elmer HGA-400 graphite furnace coupled to a Perkin-Elmer Model 2380 atomic absorption spectrophotometer set at a wavelength of 283.3 nm (Fernandes and Hilligoss 1982). Blood lead concentrations of 0.2-0.5 ppm were considered to be elevated and  $\geq 0.5$  ppm were considered within the range known to be toxic to waterfowl (Friend 1985). Lead poisoning diagnoses in sick and dead birds was based on pathology and a toxic concentration of lead in liver ( $\geq 8.0$  ppm, wet weight) (Friend 1985).

For summer trapped birds, Chi square analysis was used to test for differences in the numbers of males and females with elevated blood lead concentrations ( $> 0.2$  ppm; age groupings were consolidated to yield cell expected values of at least 5).

### RESULTS

Of the 108 wild drake mallards captured in February, 15 (14%) had blood lead concentrations greater than 0.5 ppm, 16 (15%) had concentrations ranging from 0.2 to 0.5 ppm, and 77 (71%) were below 0.2 ppm (background concentrations). Within 20 days of capture, 12 birds had died, including 8 of 15

birds (53%) that had blood lead concentrations exceeding 0.5 ppm when initially captured; 2 of 16 birds (12%) with concentrations ranging from 0.2 to 0.5 ppm; and 2 of 77 birds (3%) below 0.2 ppm. Lead poisoning was diagnosed as the cause of death in all 12 birds. Ten of the 12 lead poisoned birds had lead pellets in their gizzards at the time of death (ranging from 1 to 52 pellets). Two of the 12 also had lesions associated with avian cholera, and *Pasteurella multocida* was isolated from their livers. One additional death occurred from among the 77 birds with background lead concentrations. Cause of death was diagnosed as emaciation suspected as a result of parasitism by *Echinuria uncinata*.

Of the 73 birds trapped during the summer, 12 (16%) had elevated or toxic blood lead concentrations. Elevated concentrations were detected in 15% (2 of 13) of birds trapped in July, 11% (5 of 44) in August and 31% (5 of 16) in September (Table 1). A significantly greater proportion of males than females contained elevated concentrations of lead ( $X^2 = 6.62, p < 0.05$ ) (Table 2).

TABLE 1. Distribution of Blood Lead Concentrations of 73 Mallards from Colusa and Delevan National Wildlife Refuges Captured During July, August, and September 1987.

Blood Lead Concentration	Percentage of Total		
	July	August	September
Background ( $\leq 0.2$ ppm)	85 (11)	89 (39)	69 (11)
Elevated (0.2-0.5 ppm)	15 (2)	9 (4)	12 (2)
Toxic ( $\geq 0.5$ ppm)		2 (1)	19 (3)

TABLE 2. Blood Lead Concentrations by Sex for 71 Mallards Captured During July, August and September 1987 on Colusa and Delevan NWRs

Blood Lead Concentration	Percentage of Total	
	Males	Females
Elevated ( $> 0.2$ ppm)	26 (8)	7 (3)
Background ( $< 0.2$ ppm)	70 (19)	93 (41)

A significantly greater proportion of males had elevated lead concentrations than females ( $X^2 = 6.62, p < 0.05$ ).

## DISCUSSION

The most sensitive method of determining lead exposure in live waterfowl is the measurement of blood lead concentrations (Anderson and Havera 1985). Dieter (1979) demonstrated that signs of lead poisoning in canvasbacks, *Aythya valisineria*, appeared at blood lead concentrations of 0.2 ppm. At lead concentrations above 0.5 ppm, 12% of canvasbacks exhibited reduced activity of delta-aminolevulinic acid dehydratase (ALAD), a key enzyme in the hemoglobin biosynthetic pathway. Reduced ALAD activity in the brain causes severe biochemical lesions and cerebellar damage (Dieter and Finley 1979). The resulting motor disfunction coupled with other pathologic effects of lead poisoning such as anemia, impaction, and tissue degeneration can eventually lead to death.

Two male mallards trapped in late winter with background lead concentrations ( $< 0.2$  ppm) that later died of lead poisoning, could have ingested lead pellets shortly before or on the day of capture. One lead pellet was found in the gizzard of each bird at necropsy, and thus the lead was not yet detectable in the

blood. The mortality rates of the birds held in captivity do not necessarily reflect rates of lead poisoning mortally in free-ranging populations. Nonetheless, the high lead exposure rate is indicative that lead poisoning is a problem to waterfowl in these areas.

The percentage of adult male mallards trapped in September with elevated or toxic blood lead concentrations (27%) was nearly as high as that of males trapped in February (29%). This was unexpected because most cases of lead poisoning are reported during the winter (Bellrose 1959; NWHRC, unpubl. data). The availability of lead shot is thought to be correlated with the amount of shot deposited on an area during the fall hunting season. However, the heavy clay soils of the Sacramento NWR complex prevent lead pellets from settling into the sediments. Thus, pellets are available to birds on these wetlands year-round. High lead shot ingestion rates have been reported prior to hunting season in other studies. Zwank et al. (1985) found that lead ingestion rates of mallards and northern pintails, *Anas acuta*, were higher before, rather than after, the hunting season on 2 waterfowl wintering areas in Louisiana. Lead pellet ingestion and lead poisoning mortality rates were also higher in the fall rather than in the winter in a study conducted at the Sacramento NWR using sentinel mallards confined to a heavily hunted wetland (NWHRC, unpubl. data). Moreover, lead poisoning in the summer and fall may not be easily observed because summer resident populations are sparse, and sick birds or carcasses do not persist for long in the environment.

The difference in proportions of males and females with elevated blood lead concentrations could be due to differences in feeding habits. Male and female mallards are known to molt at different times (Bellrose 1976). During August and September, males have generally finished molting flight feathers, whereas females are just entering the molt (adults only). These differences in physiologic condition associated with molt might affect their diet (Heitmeyer 1985). Diets high in calcium and protein have been found to mitigate the effects of lead shot ingestion (Sanderson and Bellrose 1986). In addition, females have been shown to be less susceptible to lead poisoning during the breeding season. Increased mobilization of calcium from bones for egg laying and a high metabolic rate apparently decrease the absorption of lead (Finley and Dieter 1978).

Recent conversion to non-toxic shot will not result in immediate major reductions of lead poisoning in certain habitats. The heavy clay soils of the Sacramento Valley apparently reduce settling of lead shot into sediment beyond the reach of feeding waterfowl. Surveys for pellets in wetland sediments at the Sacramento NWR in 1987 revealed densities of up to 900,000 pellets per acre in the top 10 cm (NWHRC, unpubl. data). Although the use of steel shot has been enforced on the National Wildlife Refuges of the Sacramento Valley since the fall of 1986, lead poisoning in waterfowl continues to be documented (NWHRC, unpubl. data).

## ACKNOWLEDGMENTS

Sincere thanks are extended to M. Smith, National Wildlife Health Research Center for lead analysis, and E. Collins and P. O'Halloran for permission to collect samples. F. Weekley, S. Shaeffer, C. Franson, L. Locke, and M. Friend provided helpful editorial comments and H. Berg provided statistical advice.

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*Calif. Fish and Game* 76(3): 137-145 1990

## VIRULENCE OF FOUR ISOLATES OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN SALMONID FISHES AND COMPARATIVE REPLICATION IN SALMONID FISH CELL LINES<sup>1</sup>

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**The virulence of low-passage isolates of infectious hematopoietic necrosis virus (IHNV) obtained from diverse geographic locations was compared in juvenile chinook, *Oncorhynchus tshawytscha*, sockeye (kokanee), *O. nerka*, and coho, *O. kisutch*, salmon, and rainbow (steelhead) trout, *O. mykiss*. All isolates tested were pathogenic for sockeye salmon and trout, but two isolates were comparatively avirulent for chinook salmon. Hybrids of IHNV-resistant coho salmon and susceptible trout appeared to be resistant; however, infection of coho salmon with IHNV was demonstrated. The infectivity and replication of the IHNV isolates were compared in cell lines derived from chinook, coho, and sockeye salmon. Infective dose assays, growth curves, and efficiency of plaquing comparisons showed that the host preference of IHNV isolates could be demonstrated at the cellular level.**

### INTRODUCTION

Infectious hematopoietic necrosis virus (IHNV) is a highly destructive pathogen of juvenile salmonid fishes, while adult fish act as asymptomatic carriers (Wingfield and Chan 1970). The virus affects different host species throughout its range on the Pacific Coast of North America. In Alaska, hatchery-reared sockeye salmon, *Oncorhynchus nerka*, have experienced severe outbreaks (Grischowsky and Amend 1976). In California, chinook salmon, *O. tshawytscha*, have been killed by the virus. (Wingfield and Chan 1970). Resident and anadromous (steelhead) rainbow trout, *O. mykiss*, are also susceptible to IHNV, but coho salmon, *O. kisutch*, are considered resistant (Pilcher and Fryer 1980).

In Oregon, epizootics of IHNV have occurred in juvenile steelhead trout at the Round Butte Hatchery on the Deschutes River. Juvenile chinook salmon reared at the same facility have not been affected. However, outbreaks of IHNV have occurred in juvenile chinook salmon reared at the Elk River Hatchery on the Oregon coast (Mulcahy et al. 1980). We compared the virulence of virus isolates from the Round Butte and the Elk River hatcheries, Oregon, and one each from an Alaska and California hatchery to determine if the Elk River and California isolates were unique in their ability to kill juvenile chinook salmon.

<sup>1</sup> Accepted for publication April 1990.

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