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CONCENTRATION OF CREATINE KINASE AND ASPARTATE AMINOTRANSFERASE IN THE BLOOD OF WILD MALLARDS FOLLOWING CAPTURE BY THREE METHODS FOR BANDING

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ABSTRACT: The concentration of the enzymes creatine kinase (CK) and aspartate aminotransferase (AST), that are released from damaged muscle, was measured in the blood of wild adult male mallards (*Anas platyrhynchos*) captured for banding in decoy and bait traps and by rocket net. The concentration of CK and to a lesser extent AST was elevated markedly in ducks captured by all methods, indicating muscle injury. The level of CK was significantly greater in ducks captured in decoy traps and rocket net than in those captured in bait traps. This elevation appeared to be related to the length of time the birds were restrained and to the method of restraint.

Key words: Mallard, *Anas platyrhynchos*, muscle, capture myopathy, banding, creatine kinase, aspartate aminotransferase.

INTRODUCTION

Capture myopathy (CM) is a well recognized complication in the capture of wild ungulates (Chalmers and Barrett, 1982), but it has received little attention in birds. It has been reported in greater flamingoes (*Phoenicopterus ruber roseus*) and lesser flamingoes (*Phoeniconaias minor*) (Young, 1967), a secretary bird (*Saggitarius serpentarius*) and ostriches (*Struthio camelus*) (Heerden, 1977), bar-tailed godwits (*Limosa lapponica*) (Minton, 1980), African crowned cranes (*Balearica rugelorum gibbericeps*) (Brannian et al., 1981), lesser snow geese (*Anser caerulescens caerulescens*) and Ross' geese (*Anser rossi*) (Wobeser, 1981), a sandhill crane (*Grus canadensis*) (Windingstad et al., 1983), Canada geese (*Branta canadensis*) (Wobeser and Howard, 1987) and in wild turkeys (*Meleagris gallopavo*) (Spraker et al., 1987). Undiagnosed mortality occurs during banding of birds, and waterfowl with severe incoordination following handling are observed occasionally. The latter condition in waterfowl is not considered part of "trapping mortality" and the fate of these birds after release is unknown. Some of these birds may suffer from CM. The exact pathogenesis of CM is still unclear, but it involves anaerobic metabolism during intense muscular activity. Lactic acid produced in muscle causes local and systematic acidosis resulting in the lesions and clinical signs of CM (Chalmers and Barrett, 1982). Low pH at the tissue level results in increased permeability of cell membranes and cell lysis, releasing intracellular enyzmes including creatine kinase (CK) and aspartate aminotransferase (AST) to the blood. Elevated concentrations of CK and AST in serum or plasma thus reflect damage to skeletal and cardiac muscle. Elevation of serum CK concentration appears to be the most sensitive and specific index of muscle damage in both mammals (Chalmers and Barrett, 1982) and birds (Franson et al., 1985). The objective of the present study was to measure the concentration of CK and AST in serum of wild adult male mallards (*Anas platyrhynchos*) captured for banding by three techniques (decoy trap, bait trap, rocket net).

MATERIALS AND METHODS

Blood samples were obtained from wild adult male mallards captured during routine banding operations in Saskatchewan of the Prairie Mi-

TABLE 1. Sequence of events in each of the three methods used to capture mallard ducks.

Bait trap	Decoy trap	Rocket net
Bird enters trap	Bird enters trap	Net is fired[a]
Handler touches cage[a]	Handler touches cage[a]	All birds are removed individually from net and placed in burlap sacks
Birds hazed into cage	Bird caught by hand, carried to shore for banding	After all birds removed from net, individual birds removed from sack for banding
Individual bird caught from carrying cage for banding		
	Bird banded	
	First blood sample taken[b]	
	Bird placed in handling cage for 1 hr	
	Second blood sample taken	
	Bird released	

[a,b] "Handling time."

gratory Bird Research Centre (Canadian Wildlife Service, Saskatoon, Saskatchewan, Canada S7N 0X4). Decoy trapping was done between 8 May and 4 June 1987 on shallow wetlands near St. Denis (52°13′N, 106°05′W). The traps consisted of a central wire-mesh compartment containing a live "decoy" female mallard and surrounded on each side by a 1.2 × 1.2-m wire-mesh compartment with a spring-loaded door. Male mallards became entrapped when they entered one of the compartments and triggered the door closure mechanism. Bait trapping was done between 12 and 21 August 1987 at Moose Mountain Lake (49°50′N, 102°50′W), using a 1 × 1-m wire-mesh cage with a funnel entrance. This type of trap was set in shallow water near duck loafing areas and was baited with barley. The actual time of entry of individual ducks into decoy and bait traps was unknown, but no bird was in a trap for >24 hr. In the case of these two methods "handling time" was recorded from the time the handler first touched the cage until a blood sample was taken immediately after banding. Ducks were captured by rocket net on three occasions between 15 and 19 August 1987 on Moose Mountain Lake using techniques described by Day et al. (1980). All birds were removed from under the net and held in burlap sacks prior to banding. In the case of rocket nets, handling time was considered to be from the instant of capture under the net until the bird was bled immediately following banding. The amount of time that individual birds spent under the net prior to being placed in a sack was not recorded; however, the maximum time that any bird within a group spent under the net was recorded. The sequence of events in each capture method is outlined in Table 1. Approximately 2 ml of blood was collected from the brachial vein of each duck immediately after banding using a 20-ga needle. Blood was allowed to drip from the hub of the needle into a 3-ml glass tube. Samples were immediately placed in a chilled cooler and allowed to clot. Serum was removed within 12 hr by centrifugation and frozen. Samples with hemolysis were discarded.

After collection of the initial blood sample most ducks were held in a 1 × 1.5-m poultry cage or similar sized wire cage at the collection site. At the end of 1 hr a second blood sample was collected from the contralateral brachial vein and the bird was released. The flight of each bird after release was observed and any abnormalities were recorded. Enzyme analyses were done in the Department of Veterinary Pathology (Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0), using an automated analyzer (DACOS Analyzer, Coulter Electronics of Canada, Ltd., Burlington, Ontario, Canada L7E 5J8) and the modified Oliver-Rosalki method for CK and the modified International Federation of Clinical Chemistry method for AST, as provided by the manufacturer (Coulter Electronics of Canada, Ltd.). The concentration of enyzmes and the handling time among the three capture methods (groups) were compared using one-way analysis of variance; when there was a significant difference among these groups, significance between means was tested by the method of least significant differ-

TABLE 2. Concentration of creatine kinase (CK) and aspartate aminotransferase (AST) in the serum of wild adult male mallards captured by three different techniques. Blood samples were taken from all birds at the time of banding and from most birds again 1 hr after banding.

Method of capture	Concentration of enzyme (IU/liter)											
	CK						AST					
	Banding			Banding + 1 hr			Banding			Banding + 1 hr		
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD
Bait trap	23	1,000[a]	651	23	3,208	2,298	23	43	33	23	93	48
Decoy trap	34	2,485[b]	2,869	23	3,899	3,990	30	59	33	23	106	59
Rocket net	31	2,692[b]	3,024	31	5,432	6,968	31	61	46	31	100	67

[a,b] Values within column followed by different superscripts are significantly different ($P \leq 0.05$).

ence. Differences in concentration of CK and AST in blood samples collected at the time of banding and 1 hr later from the same bird were compared using the paired t-test. Statistical significance was established at $P \leq 0.05$.

RESULTS

The average handling time for ducks captured in bait traps was 15.1 min [standard deviation (SD) = 8.9]; which was not significantly different from that for birds captured in decoy traps (18.9 min, SD = 13.2). The average time interval from capture under a rocket net until bleeding was 106.1 min (SD = 51.9), which was significantly different from the handling time for birds captured in either type of trap. Ducks captured in bait traps had a significantly lower concentration of CK in their serum at the time of banding than did ducks captured by either of the other two methods (Table 2). The average concentration of both CK and AST in blood samples taken 1 hr after banding was significantly greater and approximately double that taken at the time of banding in birds captured by all methods, but there was no significant difference among birds captured by different methods (Table 2). The concentration of AST both at the time of banding and in samples collected 1 hr later followed the same trend as that of CK, but the differences among capture methods were not significantly different.

The amount of time required to remove all birds from under the net and the average time from capture until banding of individual birds were considerably different among the three groups of ducks captured on different occasions by rocket net (Table 3). The concentration of CK in serum collected both at the time of banding and 1 hr later were significantly different among the three groups captured by rocket nets and appeared to be related to amount of time the birds were held prior to banding (Table 3). Thirteen (17%) of the 77 ducks in this study were judged to have weak flight or were unwilling to fly at the time of release (Table 4). The only significant difference found between birds with normal flight and those with abnormal flight was in the ducks captured in bait traps. In this group, birds with abnormal flight had a significantly greater concentration of CK in their serum at the time of banding than did the normal birds.

DISCUSSION

This study was designed to measure CK and AST concentrations in mallards captured under the actual field conditions used during banding of this species in Saskatchewan. The most important finding was that birds captured by all three methods had concentrations of these enzymes that were much higher than the normal or baseline values reported for a variety of waterfowl species (Table 5). It is unwise to compare the absolute concentration of enzymes found in birds in the present study with those reported by others, because of differences in sampling and analyses as well

TABLE 3. Relationship between time from capture until blood sampling and concentration of creatine kinase (CK) in serum of three groups of wild adult male mallards captured by rocket net.

Group	n	Time from capture until first bird in group banded (min)[a]	Time from capture until individual bird banded (min)		Concentration of CK (IU/liter)			
					Banding[b]		Banding + 1 hr[c]	
			$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
A	5	26	100	25	906[d]	615	980[d]	559
B	11	89	153	58	4,738[e]	3,856	9,447[e]	9,557
C	15	38	74	19	1,787[d]	1,887	4,075[d]	4,124

[a] Approximates the maximum amount of time any bird in group was restrained under rocket net.
[b] Birds bled immediately after handling.
[c] Birds held at capture site for 1 hr after initial blood sample taken and then rebled.
[d,e] Values within column followed by different superscripts are significantly different ($P \leq 0.05$).

as species and sex differences (Franson et al., 1985). However, comparison of relative values may be useful, particularly since many of the values reported in Table 5 were obtained using the same analytical method and in the same laboratory as in our study. It appears from published information that CK levels in normal captive waterfowl are usually in the range from about 50 to 200 IU/liter, while the concentrations reported for waterfowl with myopathy are >1,000 IU/liter. The concentrations found in ducks in this study were similar to or higher than those reported for birds with myopathy. Unfortunately, the correlation between serum CK and AST concentration and debilitating or fatal muscle injury is unknown for birds. In the present study there was no clear correlation between weak or abnormal flight and concentration of enzymes. In some cases, poor flight might have been the result of wet or deranged feathers, rather than muscle injury. In many cases the time from handling or capture until release may have been insufficient for the clinical signs of CM to be evident. Wobeser (1981) observed stiffness and inability to fly in geese captured by rocket nets 18 hr postcapture and Brannian et al. (1981) reported that three of four African crowned cranes that died of CM did not show clinical signs until 2 to 10 days following the restraint procedure. The cranes had been

TABLE 4. Comparison of average handling time and concentration of creatine kinase (CK) in the serum of adult mallards with abnormal flight at the time of release with those mallards judged to have normal flight.

Capture method	n	Handling time (min)		CK (IU/liter)			
				Banding		Banding + 1 hr	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Bait trap							
Normal flight	17	13.5	7.6	875*	474	2,814	2,110
Abnormal flight	6	19.5	10.0	1,353*	864	4,317	2,245
Decoy trap							
Normal flight	22	21.7	13.8	2,386	2,426	3,780	3,950
Abnormal flight	1	22.0	—[a]	1,133	—	6,506	—
Rocket net							
Normal flight	35	113	53	2,588	2,936	5,936	7,371
Abnormal flight	6	81	26	3,014	3,187	4,840	3,987

* Values are significantly different ($P \leq 0.05$).
[a] No data available.

restrained by wrapping with burlap for 1 to 6 hr. Four of 12 birds handled in this way died, including three of the four birds restrained for the full 6-hr period. Serum CK and AST levels in blood taken immediately after restraint ranged from 389 to 7,450 and 280 to 783 IU/liter, respectively. These compared with levels of 43 and 75 IU/liter (CK) and 259 and 265 IU/liter (AST) in two "normal" cranes.

In the present study there were significant differences in the concentration of CK, and hence in the amount of muscle injury, among birds captured by three commonly used trapping techniques. Factors such as differences in season when various methods are used and differences in the amount of time between capture and handling may confound this comparison to some degree. However, because these ducks were sampled under the actual conditions and methods used for banding, we believe the comparison to be relevant and valid, particularly for the comparison between bait trapping and rocket net which were done on the same lake and within the same 10 day time period. The lowest level of CK was found in birds captured in bait traps ($\bar{x}$ = 1,000 IU/liter), whereas the average level in birds captured by rocket nets and decoy trap were similar and more than double that concentration (2,962 IU/liter, 2,485 IU/liter, respectively). There might be several explanations for the difference. First, the "handling time" was shorter in birds captured in bait traps than by rocket net. Birds captured in bait traps were handled less than birds captured by the other methods. In the case of bait traps, the birds were hazed gently from the trap into a carrying cage and each bird was only caught and actually held at the immediate time of banding. In contrast, ducks caught in decoy traps were caught by hand in the trap, carried to shore (sometimes as far as 40 m) and held until banded. Birds captured under rocket nets were physically restrained by the net, handled when they were removed from the net, placed in holding sacks and then han-

TABLE 5. Concentration of creatine kinase (CK) and aspartate aminotransferase (AST) reported in the blood of waterfowl.

Species	n	CK (IU/liter)		AST (IU/liter)		Comment	Authority
		$\bar{x}$	Range	$\bar{x}$	Range		
Redhead (*Aythya americana*)	10	97	48–280	23	13–134	Normal, captive	Franson et al. (1985)
Canvasback (*Aythya valisneria*)	10	70	46–122	15	11–20	Normal, captive	Franson et al. (1985)
	14	—[a]	—	87	SD 52	Wild	Perry et al. (1986)
Mallard	4	—	—	13.5	SD 4.6	Normal, captive	Franson (1982)
	34	993.9	—	41.9	—	Captive	Driver (1981)
	10	1,486	SE 91	84	SE 2.0	Wild (bait trap)	Driver (1981)
Black duck (*Anas rubripes*)	10	265	SD 144.5	18.6	SD 8.2	Captive	Franson (1982)
Canada goose (*Branta canadensis*)	14	135.9	58–289	18.3	10–35	Captive	Wobeser (unpubl. data)[b]
Northern shoveler (*Anas clypeata*)	4	1,500	963–2,511	78	27–162	Wild, unable to fly, myopathy	Wobeser and Howard (1987)[b]
Ross goose (*Anser rossi*)	1	—	478–1,718	—	197–1,160	Capture myopathy	Wobeser (1981)[b]

[a] No data available.

[b] Analyses performed in the Department of Veterinary Pathology (Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

dled again when banded. Herding and confinement of flightless lesser snow geese (*Anser caerulescens caerulescens*) in large pens has been found to have little effect on serum CK and AST levels; however, restraint and handling associated with banding resulted in a rapid increase in the concentration of both enzymes in serum (D. J. Rainnie, pers. comm.). The degree of muscle injury in birds captured by rocket net seemed to be related to the time birds spent under the net (Table 4), rather than to the overall time from capture until banding. This suggests that physical restraint with subsequent straining and struggling may be more injurious than loose confinement in a trap, pen or cloth sack. Minton (1980) concluded that wading birds should be extracted from the net as rapidly as possible and placed in darkened holding cages where they can stand. Spraker et al. (1987) suggested that CM was more severe among wild turkeys captured by drop net than in those captured in bait traps or by alpha chloralose treated baits. Southern and Southern (1983) reported an association between time spent under a rocket net and postbanding recovery of ring-billed gulls (*Larus delawarensis*). They found that 88% of the first 100 birds handled after each net firing were observed subsequently in the colony, whereas only 60% of the birds that were handled later in each net capture were seen. The fate of those birds that were not seen subsequently was not reported.

Although this study was preliminary in nature, it indicates that a significant amount of muscle damage may occur in ducks during routine trapping and handling procedures. The occurrence of muscle injury could have serious implications for analyses such as the calculation of mortality/survival rates based on banding data. Further study of the occurrence and significance of muscle injury during banding is needed and should include refinement of baseline values, correlation of enzyme concentrations with functional and morphologic injuries, and determination of the fate of birds released with muscle injury. Some such studies could be done with captive birds, but it is very important to consider the difference in stress that may be experienced by captive and wild birds subjected to the same degree of handling.

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