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Body Composition, Parasite Loads, and Blood Parameters of Spring-migrating Lesser Scaup in the Upper Midwest

Final Report

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<u>STUDY 1</u>: Body Composition, Parasite Loads, and Blood Parameters of Spring-migrating Lesser Scaup (*Aythya affinis*) in the Upper Midwest

Objectives:

- 1. Examine intestinal helminth parasite loads in up to 120 spring-migrating female Lesser Scaup;
- **2.** Examine body composition (i.e., determination of protein, lipid carbohydrate, and moisture content of carcasses), packed cell volume (PCV), white blood cell (WBC) differentials, and plasma metabolites; and,
- **3.** Compare parasite loads, body composition, and blood parameters with other metrics of Lesser Scaup health (e.g., fecal corticosterone, hepatic elements, etc.) and measures of wetland quality.

Activities

Specimen Collection, Preparation, and Assays

A total of 130 female Lesser Scaup (Aythya affinis; LESC) were collected during the periods of 10 February 2014 - 20 April 2014 and 11 March 2015 - 12 April 2015 as part of a larger study examining the ecology of spring migrating LESC in the middle latitudes of the Mississippi Flyway. Sixty hens were collected during the first field season and 70 were obtained during the second year of the project (Table 1). The study area included traditionally important stopover wetland sites along the Mississippi and Illinois rivers, and reservoirs in south-central Illinois. The study area was delineated into 4 distinct regions for comparison: Southern Illinois (SI; Rend Lake), the Illinois River Valley (IRV; Fowler Lake, Fuller Lake, Godar, Swan Lake, Babb's Slough, Big Lake, Carlson Unit at Anderson Lake State Fish and Wildlife Area, Chain Lake, Chautauqua National Wildlife Refuge, Emiquon Preserve, Hennepin & Hopper Lakes, Woodford County SFWA, and Worley Lake]), the Central Mississippi River Valley (CMRV; pools 19 and 12), and the Upper Mississippi River Valley (UMRV; pools 9 and 7) (Fig. 1). Foraging LESC were identified and approached using a camouflaged layout blind equipped with an electric trolling motor. Female scaup were collected via shotgun using nontoxic shot, and a GPS point was taken at each collection location. A 2-ml blood sample was taken using a cardiac puncture technique, and the sample was placed in an ethylenediaminetetraacetic acid (EDTA)-treated blood tube and

centrifuged for 10 minutes at 1500 rpm. The plasma was transferred to another vial and flash frozen in liquid nitrogen for subsequent blood metabolite analyses. Furthermore, whole blood was used to make two permanently fixed blood smears for white blood cell (WBC) differentials and identification of blood borne parasites, and whole blood was obtained using a capillary tube and centrifuged to determine PCV for each individual bird. A 2-ml fecal sample was also obtained and flash frozen for subsequent tests of the stress hormone corticosterone. Biometric data was then taken for each hen, including fresh and eviscerated mass, body length, wing chord, keel length, tarsus length, culmen length, and tail length. The body cavity was then accessed while utilizing sterile techniques and the right liver lobe of each bird was removed and frozen in marked plastic specimen bags. The upper portion of the alimentary canal (esophagus to distal gizzard opening) was injected with 10% buffered neutral formalin (BNF) and stored in 10% BNF for a concurrent study on diving duck diets (Hagy et al. 2015). The remaining portion of the alimentary canal (proximal duodenum to distal cloaca) was removed from the body cavity, injected with 70% ethanol (EtOH) and stored in 70% EtOH for parasitic community analysis in the lab. The remaining portions of the carcass were then tagged with a unique identification number, placed in a plastic bag, and frozen until proximate analysis could take place in the laboratory.

Carcasses were later plucked and, after removal of the bill and feet, were initially ground in a Beem Giant EFS 5-10 grinding mill with 1/8" plate. The resulting material was flash frozen with liquid nitrogen, ground to a powder using a Waring Commercial Blender CB15T, and re-frozen for later determination of ash, crude protein, lipid, and moisture content via proximate analysis conducted by the University of Illinois Urbana-Champaign's Meat Science Laboratory.

Tissue and blood samples collected during the spring of 2014 have been processed, including WBC differentials, plasma metabolite assays, and scaup body composition analyses. The presence of haemosporidian parasites was assessed both visually on blood smears and molecularly via polymerase

chain reaction (PCR) procedures. Tissue samples obtained during the 2015 field season are currently in the process of being analyzed, and results should be returned to us in the next segment.

To date, the intestines and ceca of 93 scaup have been dissected and examined for helminth infections. In the lab, intestines were cut longitudinally, and the ingesta was scraped, concentrated, and washed using a modified sedimentation technique described by Pritchard (1982). A 10% subsample of ingesta was removed, and absolute counts of helminthes were conducted. The helminths removed from the subsample were stored in 70% EtOH. Parasitologists Dr. Mike Kinsella from the HelmWest Laboratory and Dr. Rebecca Cole of the U.S. Geological Survey National Wildlife Health Center have been advising and confirming identifications of many of the recovered parasites. Identification priority has been placed on species known to be pathogenic to waterfowl (i.e., the trematodes *Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus*). These species of epizootic significance are exotic trematodes mediated by the invasive faucet snail (*Bithynia tentaculata*). These trematodes are responsible for the annual mortality events of LESC and American coots (*Fulica americana*) in many areas of the Upper Mississippi River Valley (Cole and Friend 1999). Additional identification is currently in progress with regards to the remaining birds, and permanent mounts of recovered helminth specimens are currently being created for deposition to the United States Parasite Collection (USDA) as voucher specimens.

Results

Changes in PCV, a measure of anemia or abnormal red blood cell volume percent, can be caused by a number of disorders of hemoglobin synthesis, fluid maintenance, and erythropoiesis, among others. PCV was lowest in LESC from UMRV (Table 2, Fig. 2). PCV did not differ between years, although it was lower in ducks collected from UMRV in 2014 as compared to 2015.

The percent of WBC that were lymphocytes and heterophils varied by region, consequently the heterophil/lymphocyte (H/L) ratio also varied considerably (Table 3, Fig. 3). Ducks collected at CMRV had lower ratio of heterophils to lymphocytes. Increased lymphocytes can result from acute viral infection, chronic bacterial infection, hematologic or inflammatory disorders, and other disease. Reduced heterophil counts could result from these same conditions with different etiologies. Changes in H/L ratios can be caused by a variety of chronic external (e.g., social, environment) and internal (parasitic infection, injury) stressors. Monocytes are WBCs specific to fighting foreign infections in the body, and altered proportions of such may be indicative of helminth infections and/or overall scaup health. A decrease in monocytes can be caused by hematologic disorders (e.g., aplastic anemia), whereas increased monocytes can result from a variety of causes (e.g., stress, infection, inflammation, etc.). Monocyte levels differed by region and were greater on average in ducks from IRV than in those from UMRV. Neither % eosinophils nor basophils varied by region.

Examination of plasma metabolites revealed that triglycerides (TRIG), blood urea nitrogen (BUN), and bilirubin (BIL) concentrations differed by region (Table 4, Fig. 4). Triglycerides are an important lipid storage mechanism in birds, and increases in circulating TRIG reflect accumulation (i.e., deposition) of lipids. Plasma TRIG were much greater, albeit more variable, in LESC from SI; TRIG concentrations were lowest, and BHB highest, in ducks from UMRV in 2014. The mean BUN concentration in ducks from IRV was less than in those from SI and UMRV. BUN is a measure of urea nitrogen in blood and small fluctuations of BUN could signal differences in dietary protein or hydration level. Lack of an association between BUN and PCV suggested that dehydration was not the cause of differences in BUN levels. BIL concentrations were greatest, on average, in ducks from SI than the other regions. Blood bilirubin (BIL) is a bile pigment, and increased BIL can signal liver or biliary dysfunction or breakdown of RBCs. In the absence of liver disease, increased BIL could be caused by increased hemolysis or

fasting and low caloric intake, while lowered BIL could signify oxidative stress. We observed significant associations between several blood metabolites; further evaluation of these relationships will occur in the next segment.

Mass (excluding SI, for which there were no 2014 samples) was marginally greater in 2015 (Table 5, Fig. 5), primarily due to the influence of UMRV, as mass of ducks collected in that region increased between 2014 and 2015. Mass varied among regions, and was greater in ducks from CMRV than in those from SI. When mass was adjusted for a structural size (wing chord), ducks from IRV were lighter in weight than those from CMRV. Carcasses of ducks from UMRV had less % lipid than those from IRV (Table 6, Fig. 6). Lipid proportion declined between years, driven primarily by the decrease in ducks from IRV.

Currently, 44 species of helminths representing 29 genera and 4 orders have been identified from 60 LESC collected in 2014 (Table 7); frequency of occurrence by species ranged from 2% to 83%. The number of individual helminths by species varied greatly among ducks and across regions, and fourteen taxa occurred in fewer than 3 of the 4 regions. Preliminary analyses suggested that helminth assemblages change with latitude, with scaup from northerly areas displaying proportionally fewer cestodes and more trematodes (Fig. 7). The highly pathogenic *Sphaeridiotrema pseudoglobulus* was observed only in LESC from Mississippi River pools 7, 8, 9 and 12, with a prevalence of 100% (17/17 ducks). Another highly pathogenic trematode, *Cyathocotyle bushiensis*, occurred in 3 of 17 (18%) LESC from pools 7, 8, 9 and 12.

Summary

The abundance and relative composition of helminth communities in the lower gut of LESC differed among regions. Generally, total helminths and % trematodes increased and % cestodes decreased

with increasing latitude. Measures of scaup health differed by region, and generally decreased with increasing latitude. Perturbations in health parameters that can be associated with infectious disease or other health insult (e.g., PCV, increased lymphocytes, reduced heterophil/lymphocyte ratios, reduced monocytes) reflected the geographic pattern of infection with the highly pathogenic trematode Sphaeridiotrema pseudoglobulus. However some exceptions were apparent and may reflect the combined effects of healthy and infected birds. For example, CMRV included LESC from Mississippi River pool 19, considered high quality habitat, and pool 12, where ducks were infected with S. pseudoglobulus. Patterns of variation in measures of condition were less clear. For example, fresh mass of LESC increased with latitude, with the exception of ducks from UMRV in 2014. This was reflected in lower carcass lipids, triglycerides and NEFA and increased BHB in that sample. Ducks collected from IRV were in poorer condition in 2015 than 2014, judging by a decline in carcass lipids without a concomitant decrease in mass. These results suggest interactions between annual wetland conditions (i.e., food availability) and infectious disease agents (highly-pathogenic intestinal helminths). In the sequel, we intend to further explore these patterns and interrelationships with the addition of the outstanding 2015 LESC health data, helminth community analyses, and incorporation of wetland quality indices.

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Prospectus:

- 1) Complete enumeration and identification of helminths in 2015 specimens.
- 2) Add remaining health parameter data (e.g., WBC differentials, carcass composition, etc.) from 2015 specimens to database as becomes available.
- 3) Perform comprehensive data analysis, presentation and interpretation.

Respectfully Submitted,

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Table 1. Number of female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest during 2014 and 2015 for this study. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys, and Southern Illinois (SI).

Region	Dates of	Collection
	10 February - 20 April 2014	11 March - 12 April 2015
UMRV	12	26
CMRV	10	12
IRV	38	22
SI^*	0	10
Total	60	70

Lesser Scaup were not collected from SI during 2014.

Table 2. Mean (± standard deviation), 95% confidence interval and minimum-maximum packed cell volume (PCV) in Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest during 2014 and 2015. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys and Southern Illinois (SI).

			PCV		
Year	Region*	n	Mean <u>+</u> sd	95% CI	Min-Max
	UMRV	12	39.2 ± 12.2	31.5 - 47.0	18.2-60.9
2014	CMRV	10	53.1 ± 4.4	50.0 - 56.3	43.1-58.8
	IRV	38	51.0 ± 7.5	48.5 - 53.5	36.7-63.0
	UMRV	26	46.7 ± 10.8	42.3 - 51.1	21.2-67.3
2015	CMRV	11	50.4 ± 8.4	44.8 - 56.1	40.5-67.9
	IRV	22	47.1 ± 10.2	42.6 - 51.6	32.7-68.9
	SI	10	45.7 ± 13.0	33.4 - 52.0	25.6-67.2

* One outlier omitted, 13.3 CMRV 2015

** for region F3,124= 3.72, P= 0.013; for year F1, 124= 0.02, P= 0.88; for region x year, F4, 124= 3.2, P= 0.016

*** Tukey's HSD Region, UMRV<CMRV=IRV=SIL

**** Tukey's HSD UMRV<CMRV=IRV=SIL in 2014

Table 3. White blood cell differentials (%) in female Lesser Scaup (*Aythya affinis*) collected from 3 regions of the upper Midwest during 2014. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys.

		Lymph	ocytes	Hetero	ophils	HL F	Ratio	Monoc	cytes	Eosino	phils	Basop	ohils
Region	n	$Mean \pm sd$	95% CI	Mean \pm sd	95% CI	Mean \pm sd	95% CI	Mean \pm sd	95% CI	Mean \pm sd	95% CI	$Mean \pm sd$	95% CI
		(min-max)		(min-max)		(min-max)		(min-max)		(min-max)		(min-max)	
UMRV	11	64.3 ± 9.7	57.7 - 70.8	30.4 ± 8.0	25.0 - 35.7	49.5 ± 18.4	37.2 - 61.9	0.5 ± 0.9	0.0 - 1.2	0.8 ± 1.1	0.1 - 1.5	4.0 ± 6.0	0.0 - 8.0
		(53 – 81)		(17 - 43)		(21 - 81)		(0 - 3)		(0 - 3)		(0 - 21)	
CMRV	10	78.7 ± 8.7	72.5 - 84.9	$16.8\pm\ 6.8$	12.0 - 21.6	22.4 ± 11.4	14.2 - 30.6	1.7 ± 1.8	0.4 - 3.0	1.1 ± 1.4	0.1 - 2.1	1.7 ± 2.8	0.0 - 3.7
		(65 – 90)		(9 - 27)		(10 - 42)		(0-6)		(0 - 4)		(0 - 8)	
IRV	36	62.6 ± 15.0	57.5 - 67.6	25.8 ± 12.1	21.7 - 29.8	47.0 ± 31.4	36.3 - 57.6	2.5 ± 2.5	1.6 - 3.3	1.4 ± 1.5	0.8 - 1.9	5.7 ± 4.9	4.0 - 7.3
		(36–95)		(5 - 50)		(5 – 139)		(0 - 8)		(0 - 6)		(0 – 19)	

*for region: LYMPHS F2,54= 5.9 P< 0.01, Tukey's HSD CMRV>UMRV=IRV; HETERO F2, 54 = 4.4, P= 0.02, Tukey's HSD CMRV>UMRV=IRV; MONO F2, 54= 3.5, p= 0.04, Tukey's HSD UMRV<IRV

Table 4. Blood plasma metabolites in female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest during 2014 and 2015. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys, and Southern Illinois (SI).

			BHB (n	nmol/L)	TRIG (r	ng/dL)	NEFA (mEq/L)	BUN (m	ng/dL)	GLU (n	ng/dL)	ALB (g	g/dL)	BIL (n	ng/dL)
Year	Region	n*	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI
	UMRV	12	0.72 ± 0.31	0.52 - 0.92	199 ± 85	146 - 253	0.41 ± 0.29	0.22 - 0.59	4.3 ± 1.4	3.4 - 5.1	160 ± 43	133 - 188	1.3 ± 0.4	1.0 - 1.5	0.20 ± 0.06	0.16 - 0.24
2014	CMRV	10	0.65 ± 0.31	0.43 - 0.87	244 ± 67	197 - 292	0.93 ± 0.90	0.24 - 1.62	5.5 ± 2.0	3.8 - 6.7	177 ± 45	146 - 210	1.4 ± 0.3	1.2 - 1.6	0.21 ± 0.07	0.16 - 0.26
	IRV	36	0.67 ± 0.34	0.55 - 0.78	239 ± 111	201 - 277	0.73 ± 0.23	0.65 - 0.81	4.1 ± 1.3	3.6 - 4.4	173 ± 38	160 - 186	1.6 ± 0.3	1.5 - 1.7	0.28 ± 0.11	0.24 - 0.32
			BHB (n	nmol/L)	TRIG (r	ng/dL)	NEFA (mEq/L)	BUN (m	ng/dL)	GLU (n	ng/dL)	ALB (g	g/dL)	BIL (n	ng/dL)
			$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI
	UMRV	25	0.56 ± 0.24	0.46 - 0.66	251 ± 113	205 - 298	1.24 ± 1.06	0.80 - 1.68	5.5 ± 1.4	4.9 - 6.1	160 ± 59	145 - 196	1.5 ± 0.5	1.3 - 1.6	0.35 ± 0.20	0.26 - 0.43
2015	CMRV	12	0.58 ± 0.28	0.41 - 0.76	289 ± 133	205 - 374	0.93 ± 0.24	0.78 - 1.08	4.7 ± 1.7	3.6 - 5.7	175 ± 64	134 - 216	1.7 ± 0.5	1.3 - 2.0	0.43 ± 0.25	0.27 - 0.58
2015	IRV	22	0.63 ± 0.29	0.51 - 0.76	243 ± 88	204 - 282	1.09 ± 0.45	0.89 - 1.29	4.6 ± 1.3	4.0 - 5.1	168 ± 53	145 - 192	1.5 ± 0.2	1.4 - 1.6	0.31 ± 0.13	0.25 - 0.37
	SI	9	0.86 ± 0.27	0.65 - 1.07	353 ± 223	167 - 539	1.07 ± 0.14	0.97 - 1.17	5.9 ± 2.2	4.1 – 7.7	156 ± 62	108 - 204	1.8 ± 0.6	1.3 - 2.2	0.39 ± 0.16	0.25 - 0.53

Plasma metabolites are beta-hydroxybutyrate (BHB), triglycerides (TRIG), non-esterified fatty acid (NEFA), blood urea nitrogen (BUN), glucose (GLU), albumin (ALB), and bilirubin (BIL).

BHB IRV 2014 n= 34; BUN and ALB IRV 2014 n= 35; BUN SIL 2015 n= 8; ALB UMRV 2014 n= 11, 2015 n= 24;

*Outliers omitted NEFA 9.546 CMRV 2014, n= 9; TRIG 1.234 SIL 2015, n= 8; BIL UMRV 2015 1.6, n= 24; BIL SIL 2015 2.9, n= 8

			Fresh Ma	ass (g)*	Eviscerated	Mass (g)*	Body Leng	gth (mm)	Keel (n	nm)
Year	Region	n	Mean \pm sd	95% CI	Mean \pm sd	95% CI	Mean \pm sd	95% CI	Mean \pm sd	95% CI
	UMRV	12	657 ± 68	614 - 700	500 ± 60	462 - 538	405 ± 10	399 - 412	79 ± 2	78 - 80
2014	CMRV	10	725 ± 72	673 - 777	$566\ \pm 64$	520 - 612	404 ± 12	396 - 413	83 ± 4	80 - 85
	IRV	38	683 ± 77	658 - 709	543 ± 71	520 - 566	410 ± 8	407 - 413	83 ± 4	82 - 84
	UMRV	26	725 ± 70	697 - 753	557 ± 60	533 - 581	409 ± 7	406 - 412	82 ± 3	80 - 83
2015	CMRV	12	724 ± 71	679 - 769	576 ± 61	537 - 615	402 ± 7	398 - 407	81 ± 2	80 - 82
2015	IRV	22	680 ± 54	656 - 704	538 ± 53	515 - 562	406 ± 11	401 - 411	81 ± 3	80 - 82
	SI	10	655 ± 75	601 - 709	526 ± 53	488 - 564	405 ± 6	400 - 409	86 ± 6	82 - 90
			Wing Cho	ord (mm)	Tail Length (mm)		Culmen (mm)		Tarsus (mm)	
Year	Region	n	Mean \pm sd	95% CI	Mean \pm sd	95% CI	Mean \pm sd	95% CI	Mean \pm sd	95% CI
	UMRV	12	189 ± 5	186 - 192	51 ± 4	48 - 54	40 ± 1	39 - 40	38 ± 2	37 - 40
2014	CMRV	10	189 ± 5	186 - 193	49 ± 3	47 - 51	40 ± 2	39 - 41	39 ± 3	37 - 40
	IRV	38	192 ± 5	190 - 194	$51\ \pm 4$	50 - 52	40 ± 2	40 - 41	41 ± 2	40 - 41
	UMRV	26	193 ± 5	192 - 195	52 ± 3	51 - 53	40 ± 1	39 - 40	41 ± 2	41 - 42
2015	CMRV	12	193 ± 3	192 - 195	51 ± 4	48 - 53	40 ± 2	39 - 41	42 ± 2	40 - 43
2013	IRV	22	192 ± 5	190 - 194	51 ± 3	50 - 52	40 ± 3	39 - 42	41 ± 3	40 - 43
	SI	10	192 ± 3	190 - 194	52 ± 2	51 - 53	39 ± 2	37 - 40	40 ± 1	39 - 41

Table 5. Morphometric measurements of female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest during 2014 and 2015. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys and Southern Illinois (SI).

* Fresh mass was obtained immediately after collection and prior to necropsy. Eviscerated mass is the measurement of the bird after the removal of the alimentary canal and right lobe of liver.

** pooled mass by year (excluding SI) t116=-1.81, p=0.08; UMRV mass by year t22=-2.9, p<0.01

***ANOVA mass by region F3, 126= 3.0, P= 0.03, Tukey's HSD SI<CMRV p= 0.05

****ANCOVA adjusting mass for structural size (wing chord) region F2, 113= 3.2, P= 0.04; Tukey's HSD IRV<CMRV

Table 6. Means (± standard deviation) and 95% confidence intervals of percent body composition obtained via proximate analysis of female Lesser Scaup (*Aythya affinis*) collected from 3 regions of the upper Midwest during 2014 and 2015. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys.

			Prote	Protein*		Lipid As		*	Moisture	
Year	Region [†]	n‡	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI	$Mean \pm SD$	95% CI
	UMRV	12	17.4 ± 1.6	16.4 - 18.4	11.3 ± 6.1	7.4 - 15.1	5.1 ± 0.8	4.6 - 5.5	63.6 ± 4.2	60.9 - 66.3
2014	CMRV	10	17.0 ± 1.0	16.3 - 17.8	17.0 ± 5.6	13.0 - 21.0	4.9 ± 0.5	4.6 - 5.3	59.1 ± 5.0	55.5 - 62.6
	IRV	37	17.6 ± 1.3	17.1 - 18.0	16.9 ± 5.5	15.1 - 18.8	4.9 ± 0.7	4.7 - 5.2	58.3 ± 4.0	56.9 - 59.6
					Mean \pm SD	95% CI			Mean \pm SD	95% CI
	UMRV	26			13.2 ± 4.7	11.3 - 15.2			64.1 ± 3.5	62.7 - 65.5
2015	CMRV	12			15.2 ± 5.9	11.4 - 18.9			61.9 ± 4.0	59.4 - 64.5
	IRV	22			13.3 ± 4.7	11.2 - 15.4			63.3 ± 4.6	61.2 - 65.3

*Protein and ash analysis are currently being conducted in the laboratory for specimens collected during 2015. † Proximate analysis was not conducted on 2015 specimens collected in SI due to budget constraints. ‡ Values found to be beyond 3 standard deviations were determined to be extreme outliers and were not considered for analysis

** ANOVA for lipid by region F2, 113= 4.2, P= 0.017, Tukey's HSD= UMRV<IRV=CMRV

*** Lipid % by year t₁₁₂ 2.1, P= 0.04

	Number (%)		Number (%)
Таха	Ducks Infected	Таха	Ducks Infected
Trematodes		Cestodes	
Cyathocotyle bushiensis	3 (5)	Fimbriariodes intermedia	5 (8)
Sphaeridiotrema pseudoglobulus	15 (25)	Fimbriaria fasciolaris	20 (33)
Apatemon gracilis	16 (27)	Dicranotaenia coronula	21 (35)
A. burti	5 (8)	Echinocotyle rosseteri	18 (30)
Cotylurus cornutus	1 (2)	Hymenolepis megalops	2 (3)
C. flabelliformis	9 (32)	H. pusilla	47 (78)
C. gallinulae hebraicus	6 (10)	H. spinocirrosa	50 (83)
<i>Cotylurus</i> sp.	12 (20)	H. tuvensis	13 (22)
Diplostomum phoxini	7 (12)	Hymenolepis sp.	28 (47)
Echinoparyphium recurvatum	48 (80)	Retinometra macrocanthos	18 (30)
Echinostoma trivolvis	22 (37)	Diorchis sp. 1	19 (32)
Psilochasmus oxyurus	22 (37)	Diorchis sp. 2	10 (17)
Zygocotyle lunata	25 (42)	Sobolevicanthus sp.	4 (7)
Notocotylus sp.	7 (12)	Anatinella sp.	1 (2)
Maritrema sp. 1	5 (8)	Juvenile	1 (2)
Maritrema sp. 2	2 (3)		
Plenosoma minimum	7 (12)		
Microphallus pygmaeus	5 (8)		
M. oblonga	8 (13)	Nematodes	
Microphallus sp.	16 (27)	Capillaria anatis	35 (58)
Prosthogonimus anatinus	2 (3)	C. obsignata	20 (33)
Paramonostomum parvum	2 (3)	C. spinulosa	1 (2)
		Capillaria sp.	12 (20)
Acanthocephalans		Strongyloides sp.	15 (25)
Corynosoma constrictum	1 (2)		
Polymorphus marilis	3 (5)		

Table 7. Helminth species recovered from the small and large intestines and ceca of 60 female Lesser Scaup (*Aythya affinis*) collected from 3 regions of the upper Midwest during 2014. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys.

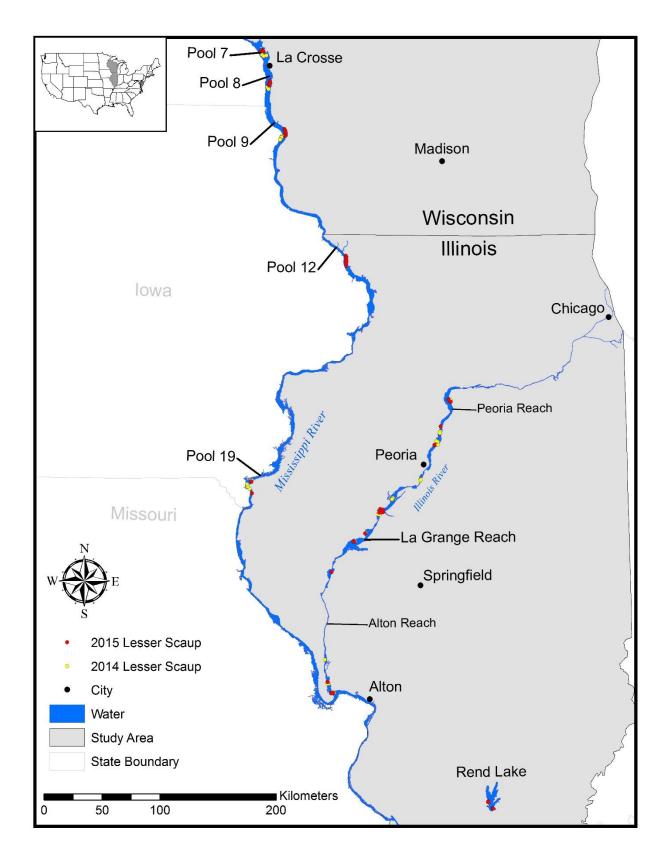


Fig. 1. Map showing Lesser Scaup collection locations in Illinois and Wisconsin.

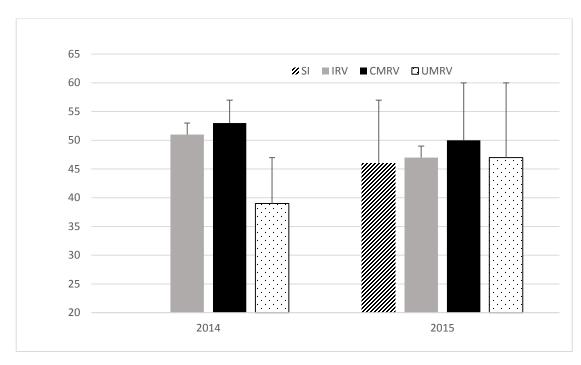


Figure 2. Mean + sd packed cell volume (PCV) by region of collection during 2014 and 2015. Regions were Upper Mississippi (UMRV), Central Mississippi (CMRV, and Illinois (IRV) River valleys, and southern Illinois (SI). Data were not available for SI in 2014.

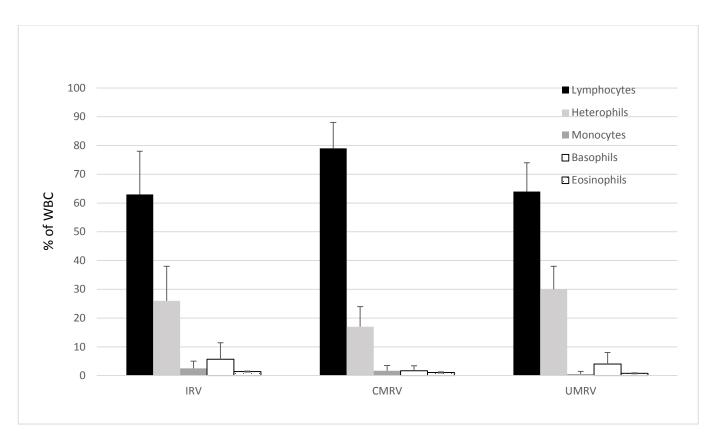


Fig. 3. Mean + sd % white blood cells by type of Lesser Scaup (*Aythya affinis*) from the upper Midwest by region of collection. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys.

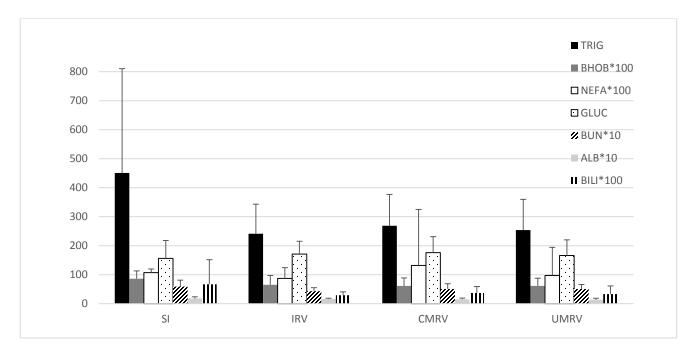


Fig. 4. Mean + sd plasma metabolites of Lesser Scaup (*Aythya affinis*) from the upper Midwest by region of collection, 2014 and 2015. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys and Southern Illinois (SI). Plasma metabolites are beta-hydroxybutyrate (BHB), triglycerides (TRIG), non-esterified fatty acid (NEFA), blood urea nitrogen (BUN), glucose (GLU), albumin (ALB), and bilirubin (BIL). Units are: TRIG mg/dL; BHB mmol/L; NEFA mEq/L; GLUC BUN and BILI mg/dL; ALB g/dL

** ANOVA by region: TRIG F3,122= 6.7, p<0.01, SI< IRV=UMRV=CMRV; BUN F3,120= 4.5, p<0.01, IRV<SI, IRV~<UMRV; BIL F3, 122= 4.3, p<0.05, SI>IRV=CMRV=UMRV

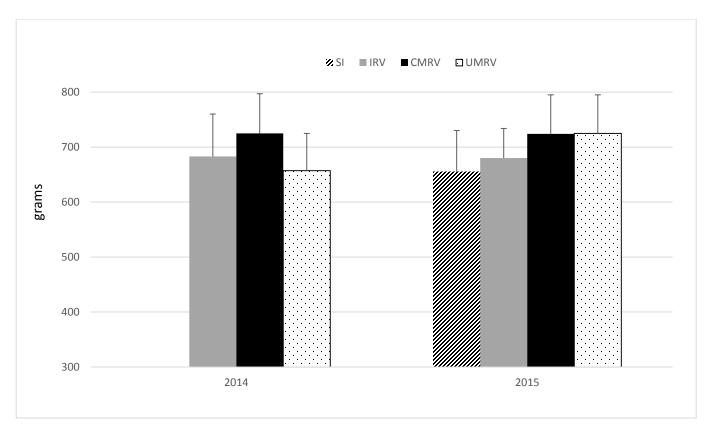


Fig. 5. Mean + sd body mass of Lesser Scaup (*Aythya affinis*) from the upper Midwest by region and year of collection. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys and Southern Illinois (SI). Data were not available for SI for 2014.

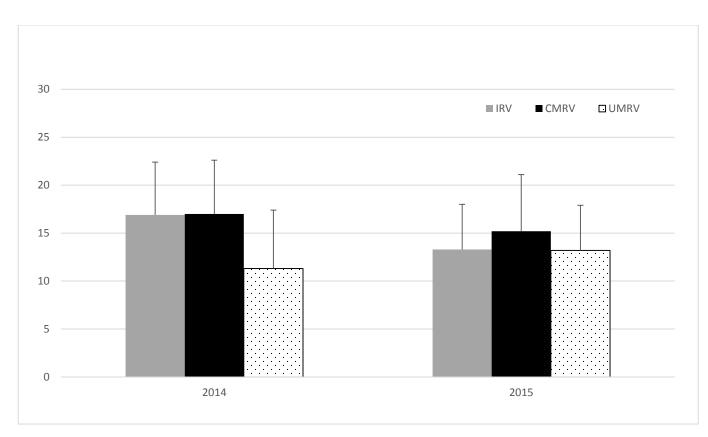


Fig. 6. Mean + sd carcass lipid (%) of Lesser Scaup (*Aythya affinis*) from the upper Midwest by region and year of collection. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), Illinois (IRV) River valleys.

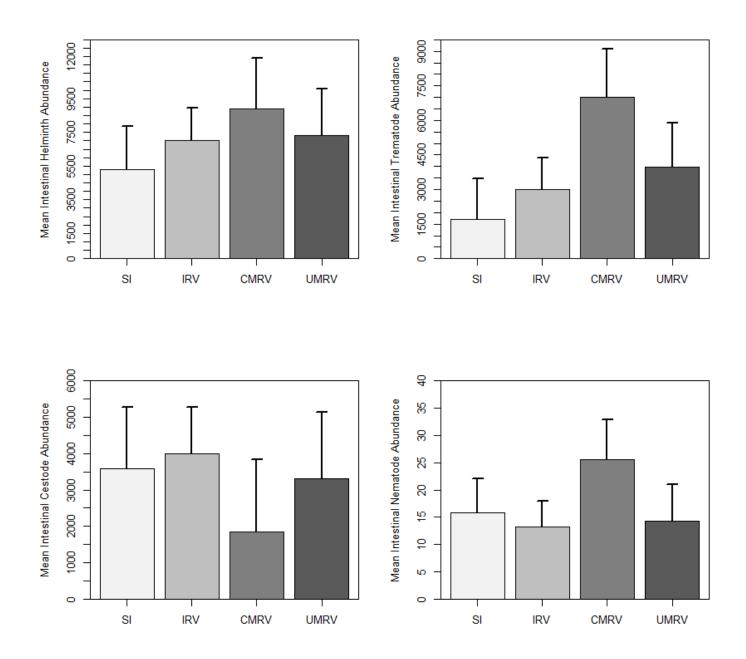


Fig. 7. Mean + sd (clockwise from upper left) total helminths, trematodes, nematodes and cestodes in Lesser Scaup collected during spring of 2014 from the upper Midwest by region. Regions were: Upper Mississippi (UMRV), Central Mississippi (CMRV), and Illinois (IRV) River valleys, and Southern Illinois (SI).