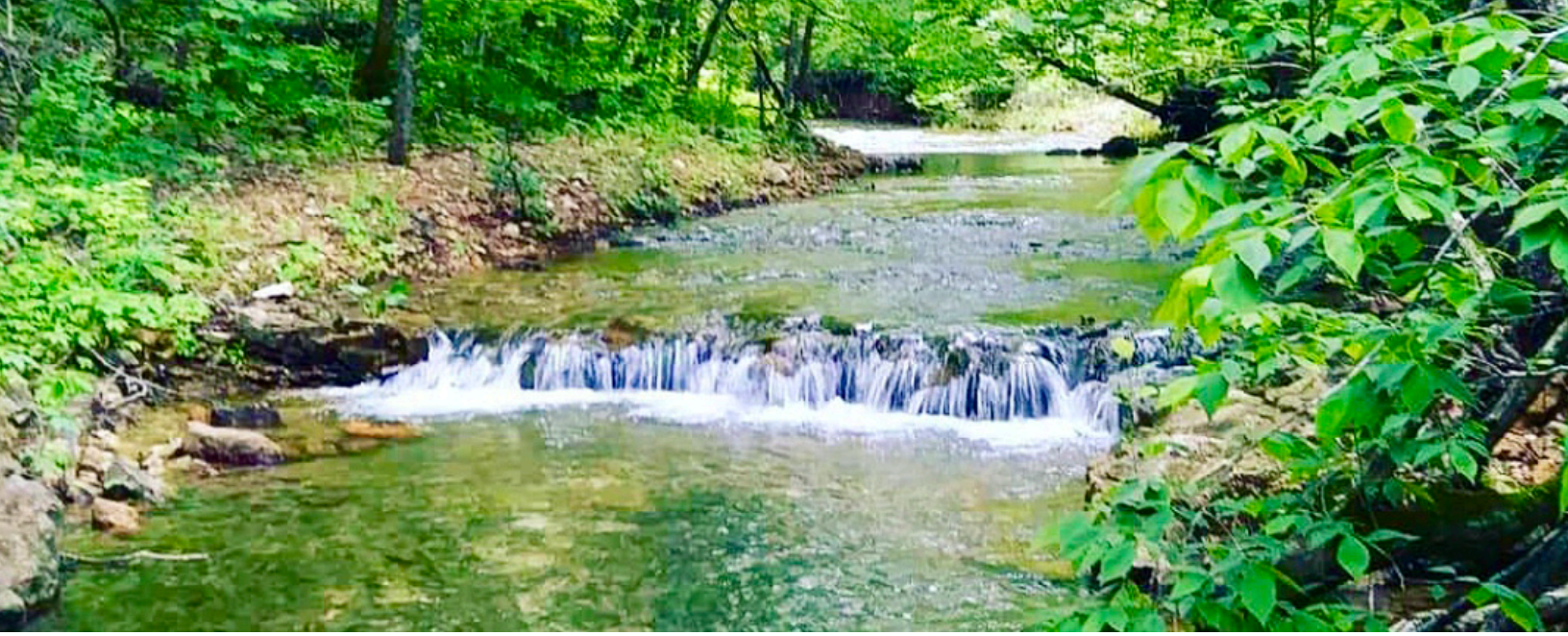

Image caption: Diles Creek. Photo courtesy of Allyn Dodd.

Assessing Water Quality and Biological Impacts of Nonpoint Source Pollution in the Eleven Point and Lower Black River Watersheds

Allyn Dodd[1]*, Maryline Bossus[1], Allison Mundy[2], Linda Fowler[2], Jordan Webb[2], Olivia Echols[2], and Erik Pollock[3]

[1]Assistant Professor, Natural Sciences Division, Lyon College, Batesville, Arkansas 72501; [2]Student, Natural Sciences Division, Lyon College, Batesville, Arkansas 72501; [3]Laboratory Manager, Stable Isotope Laboratory, University of Arkansas, Fayetteville, Arkansas, 72701
*Corresponding author, dodda@asmsa.org

Abstract: Poultry and livestock agriculture continue to expand in Northeast Arkansas, increasing the potential for nutrient enrichment and ecological degradation in critical waterways in the Mississippi Alluvial Plain. We sampled twelve tributaries of the Eleven Point and Black rivers from June 2019 to February 2020 to determine if relationships existed between poultry and livestock agriculture and water quality, periphyton abundance, presence of algal toxins, dominant water sources, and invertebrate community structure and physiology. We found no significant relationships between animal agriculture and stream nutrient levels, though preliminary results from summer 2019 suggested a positive relationship between poultry house density and phosphorus concentrations. Fewer pollution-intolerant macroinvertebrate taxa were found in tributaries closer to poultry farm operations. Our findings suggest that streams near poultry farming operations are in need of targeted mitigation to prevent further declines in sensitive invertebrate taxa. While the mechanism inducing these declines is unclear, our continued work in these watersheds may shed light on the specific habitat metrics or pollutants responsible. Additionally, the positive relationship between poultry house density and phosphorus concentrations during summer 2019 suggests a seasonal component to the impact of poultry farming on instream nutrients. These efforts to determine the impact of animal agriculture are critical as poultry agriculture continues to expand in Arkansas, potentially impacting water quality and biological condition throughout the Mississippi Alluvial Plain.

Key Points:

- We found no relationship between nutrients and animal agriculture over nine months of data collection.
- Preliminary analysis in summer 2019 revealed a positive relationship between subwatershed poultry house density and instream phosphorus concentrations, suggesting temporal variation in poultry impacts.
- Relative abundance of sensitive EPT taxa declined with increasing proximity to poultry houses.
- High nitrogen and phosphorus do not appear to impact Na^+/K^+-ATPase function in osmoregulation in a keystone crayfish species.

Introduction

Arkansas is currently the nation's second greatest producer of poultry (USDA, 2018), with poultry farming for food production expanding eastward in the state and throughout the Mississippi Alluvial Plain. The encroachment of new poultry agriculture has been especially noticeable in the Eleven Point River (EPR) and Lower Black River (LBR) watersheds; approximately 400 acres of land in these watersheds have been permitted for the construction of new poultry operations since 2016. Additionally, poultry processing facilities have been constructed or upgraded in nearby municipalities to keep pace with growing production. Currently, over half of land in the LBR and one-third of land in the EPR drainages in northeastern Arkansas is utilized for poultry or livestock production (ADEQ, 2020).

The Arkansas Natural Resource Council (ANRC) has identified two nonpoint source pollution concerns in northeastern Arkansas: nutrient loading and sedimentation from animal agriculture (ANRC, 2014). Increases in animal agriculture can affect nearby surface waters by introducing runoff high in phosphorus and nitrogen, increasing the potential for harmful algal blooms and leading to declines in invertebrate communities (Lombardo, 2000; ANRC, 2014). While the 2014 Arkansas Water Plan lists "improving water quality through nonpoint source management" as a priority issue, and poultry production continues to grow in the region (ANRC, 2014), there is currently no detailed monitoring of water quality and biological condition in the EPR and LBR drainages.

Our objective was to determine whether relationships exist between nearby animal agriculture and water quality, algal abundance, invertebrate community structure and crayfish physiology in both the EPR and LBR watersheds in Randolph, Sharp, Lawrence, and Independence counties. We also sought to determine dominant water sources, which can affect the magnitude of nonpoint source impacts. We predicted that nutrient and total suspended solid concentrations would exhibit positive relationships with subwatershed poultry house density, subwatershed pastoral land use, and flow path distance to poultry farms. Algal biomass was also expected to increase with agricultural land use metrics. Sources of channel discharge throughout both drainages were expected to be dominated by groundwater. We hypothesized that macroinvertebrate communities in the upstream portions of the EPR drainage, which had fewer surrounding poultry farms, would contain greater amounts of intolerant taxa than habitats near the EPR outlet or throughout the LBR drainage. Additionally, physiological mechanisms such as osmoregulation and respiration of invertebrate bioindicators were predicted to be negatively impacted by pollution from nearby agricultural land. Indeed, elevated nitrogen in the environment is dangerous for aquatic organisms, as it stimulates growth of nitrifying bacteria which convert it to highly toxic ammonia. Ammonia is known to disrupt ionoregulatory function in crustaceans by increasing ion permeability (Spaargaren, 1990), and exposure to ammonia is lethal at relatively low doses (Weihrauch et al., 2004). Crustaceans excrete excess ammonia mainly using their gills and antennal glands. The ammonia excretion rates are correlated with sodium absorption which is regulated by the activity of the pump Na^+/K^+-ATPase (NKA; Weihrauch et al., 2004). Increase in osmoregulation mechanisms in gills is also known to affect respiration ability in aquatic organisms (Ouattara et al., 2009). Therefore, we also assessed the impact of high nutrient concentrations in the environment on the expression and localization of NKA in a crustacean.

Methods

This study took place in 12 sites within the Eleven Point River and Lower Black River drainages (Figure 1). Sites were selected along a land use gradient of animal agriculture (e.g., pastoral livestock, poultry houses within a subwatershed, and flow path distance to poultry operations). Water quality grab sampling, in situ measurements, and periphyton sampling took place during base flow conditions monthly from June 2019 through February 2020. One storm sampling event was completed in June 2019. Total organic carbon (TOC) was determined using a Shimadzu TOC-L analyzer (Shimadzu Corporation, Kyoto, Japan). Nitrate (NO_3^-) concentrations were determined using cadmium reduction. Filtered samples were subjected to the ascorbic acid method to determine soluble reactive phosphorus (SRP) concentrations. Total suspended solids were determined by vacuum filtration of samples and determining weight of previously suspended particles on pre-weighed filters (APHA, 2005). Trace elements (Al, As, Ba, Be, B, Cd, Cs, Co, Cr, Cu, Fe, K, Li, Lu, Mn, Hg, Mo, P, Ni, Pb, Sm, Se, Ti, U, V and Zn) of source (groundwater and precipitation) and stream water samples were measured with an inductively-coupled plasma mass spectrometer (Thermo Fisher Scientific, Waltham, MA), then end-member mixing analysis was used to calculate water source mixing ratios (Rueedi et al., 2005). We collected samples for microcystin analysis on two sampling dates with highest stream temperatures that were predicted to facilitate algal toxin presence (i.e., July and August).

Water temperature, dissolved oxygen, pH, and specific conductance were measured in the field using a handheld multiparameter probe. Periphyton from benthic rocks was collected throughout each reach to determine algal biomass by ethanol extraction of chlorophyll a. Invertebrates were collected at three riffles within each stream reach in June of 2019 and identified to genus or subfamily (for Chironomidae) to determine invertebrate community structure and diversity.

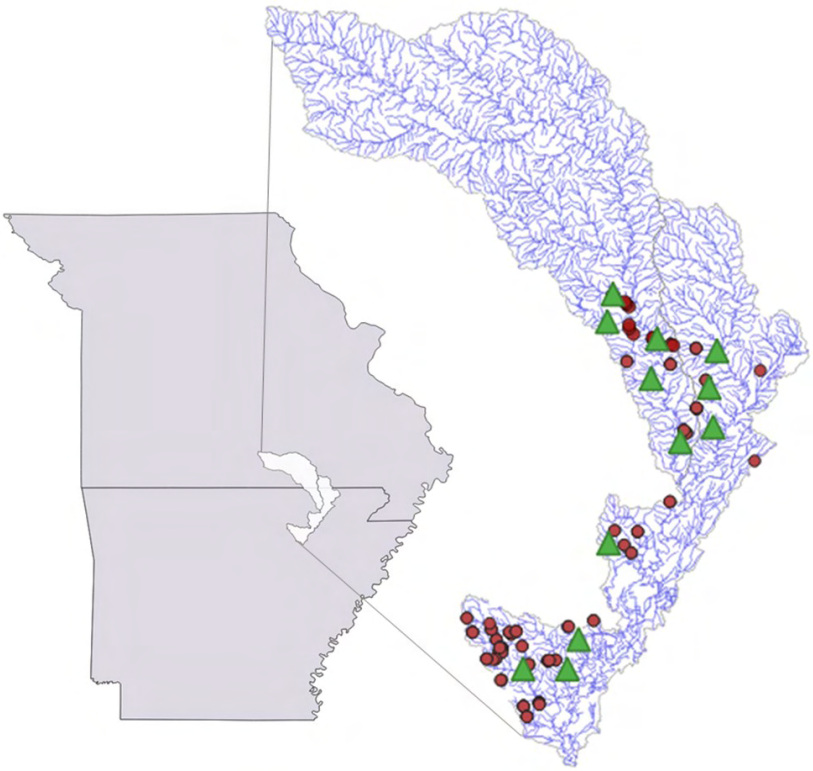

Figure 1: Map of study sites showing target watersheds. Poultry farm operations are shown as red circles while study sites are marked by green triangles.

Faxonius ozarkae specimens were sampled at each site when available in June 2019 and labeled as from polluted or non-polluted sites depending on the results from our nutrient analyses. Prior to sampling, organisms were ice-anesthetized and quickly decapitated. The three main osmoregulatory organs (gills, antennal gland, and intestine) were sampled for histology and molecular analysis. Organs from three individuals per creek were fixed using Bouin liquid (24hrs), washed with 70% alcohol and processed following the protocol from Bossus et al. (2013). Primary monoclonal mouse NKA antibody (8 µg/mL) raised against the α-5 subunit of the chicken NKA (IgG α5,Developmental Studies Hybridoma Bank; University of Iowa, USA) was used to detect NKA. The secondary antibody used at 10 µg/mL was AlexaFluor® 594 (shown in red or green) donkey anti-mouse (InvitrogenTM, Carlsbad, CA). Negative control slides were made without primary antibody and showed no staining (not shown). Slides were mounted in anti-bleaching mounting medium with dapi and visualized using a fluorescent Leica microscope and its LAS software. Organs from 10 individuals per creek were collected for molecular analysis. RNA extraction and purity check were performed following Bossus et al. (2015). The cDNA of NKA and elongation factor 1 alpha (EF1a, used as reference gene) were first amplified by PCR using Taq 2x Master mix (NEB, Ipswich, MA) and degenerate primers (Table 1) designed based on the nBLAST alignments of the gene of interest on NCBI website from several species (Table 2). PCR products were purified using Wizard® Plus SV Minipreps DNA Purification System (Promega, Madison, WI), ligated to a pXT vector (kindly provided by Dr. A. Beeser, Lyon College) using the Blunt/TA ligase master mix (NEB), and cloned into E. coli. Vectors with inserted fragments of interest were purified using PureYieldTM Plasmid Miniprep System (Promega) and sequenced by Genewiz (South Plainfild, NJ). Partial sequences for both NKA and EF1a were submitted to GenBank. Specific primers were designed using Primer3 (Table 1). cDNA synthesis and qRT-PCR followed protocol by Bossus et al. (2015) and a BioRad MiniOpticon Real-Time PCR System (Hercules, CA).

Water quality and biological data were analyzed utilizing linear regression to identify relationships between instream variables and agricultural metrics (i.e. density of subwatershed poultry houses, pastoral land use, and flow path distance from poultry operations to study streams). We employed an information theoretic approach (Burnham and Anderson, 1998) and multiple linear regression to determine what instream variables explained the greatest amount of variation in periphyton abundance and macroinvertebrate diversity across study sites. qRT-PCR data were analyzed using one-way ANOVA to compare variance of expression in transcripts between areas with low versus high nitrogen and phosphorus concentrations. Data are reported as mean + standard error.

Table 1: List of degenerated (d) primers used for cloning and sequencing (CS) and primers used for qPCR

Primer name	Nucleotide sequences (from 5' to 3')	Use
EF1a-dF1	TCGACGYDGGCCTGTTGC	CS
EF1a-dF2	AGATYGAGCGCAAGARTGG	CS
EF1a-dR1	AYTTKGGCTCDGTGCTGTCC	CS
EF1a-dR2	TCCYTGGCDGGRTCRTTCTT	CS
EF1a-F	CCCATCTCTGGCTTTAATGG	qPCR
EF1a-R	CGAAGGGGCTTGTCTGTAGG	qPCR
NKA-F6	ATGGCCTATGGTCAGATTGG	CS
NKA-dR6	GGAAGGCAGCCAGTGTRG	CS
NKA-dR7	CGRCGACACTCRTCRTAMAC	CS
NKA-F	CGTGAACAGTGGGACTCAAA	qPCR
NKA-R	AATGGAGTTACGTCGGGTCTTGC	qPCR

Table 2: List of species and GenBank Accession number of sequences used to generate degenerated primers in order to sequence NKA and EF1a

Species name	GenBank Accession number	Gene
H. sapiens	NM_000701.8	
Xenopus laevis	BC125976.1	
Anguilla marmorata	KP161606.1	NKA
Macrobrachium nipponense	MH378774.1	
Eriocheir sinensis	KC691291.1	
Portunus trituberculatus	KU361820.1	
Procambarus fallax	LC035460.1	
Drosophila melanogaster	NM_001299393.1	EF1a
Eriocheir sinensis	KY356884.1	
Penaeus monodon	MG775229.1	

Results and Discussion

We found no significant relationships between average measures of physicochemical variables at base or storm flow and subwatershed poultry house density, pastoral land use, or flow path distance (Table 3). However, we did find that the percentage of streamflow coming from groundwater was negatively related to surrounding poultry and pastoral agriculture ($R^2 = 0.70$, $p = 0.02$). This reveals that among the twelve streams in this study, those with more animal agriculture in the surrounding watershed receive greater inputs from interflow than groundwater intrusion. Streams dominated by interflow would be expected to have elevated nutrient concentrations from greater animal agriculture in the surrounding watershed, but we did not find evidence of enrichment over the year.

However, preliminary data analysis in summer 2019 showed that, after removing an outlier (Hubble Creek) with low poultry house density but high P levels, there was a positive relationship between subwatershed poultry house density and stream phosphorus concentrations ($R^2 = 0.84$, $p = 0.001$) (Figure 2). Storm samples taken in summer did not reveal relationships between instream nutrients (nitrogen and phosphorus) and surrounding poultry house density or flow path distance. Additionally, removing Hubble Creek from the regression with nine months of data did not yield a relationship. It may be that enrichment in these systems is a seasonal phenomenon, and future work in the study area will provide insight into whether phosphorus enrichment is a consistent concern during the summer months.

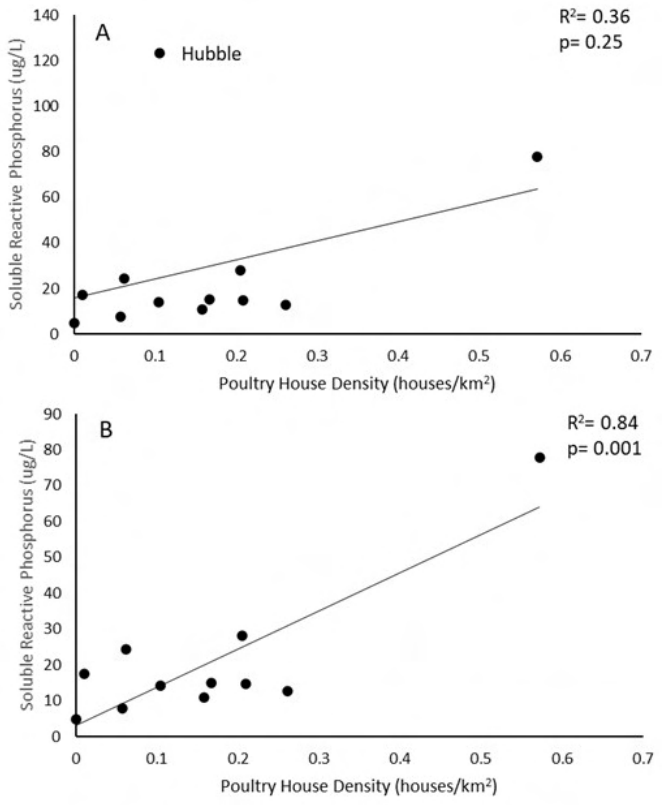

Figure 2: Relationship between summer SRP concentrations and subwatershed poultry house density with outlier due to poultry waste being stored close to the stream (Hubble) (A). Panel (B) shows relationship with outlier removed.

Soluble reactive phosphorus varied from 5 to 52 µg/L at base flow, with maximum P concentrations found in Hubble Creek, a stream with moderate surrounding poultry density but storage of poultry wastes near the stream (Dodd, personal observation). Hubble Creek had markedly greater P levels than other streams; 35 µg/L was the second-highest concentration found in the study. Phosphorus concentrations were fairly low at storm flow; however, Hubble Creek P levels were exceedingly high during storm sampling, at 186 µg/L SRP. This nutrient spike was likely caused by runoff from a nearby pile of poultry waste (within two hundred meters of the stream) washing into the channel. Measures of physicochemical variables from each stream at base flow can be found in Table 4. Table 5 shows nutrient and sediment concentrations from storm samples collected in June of 2019.

Algal biomass was not related to poultry house density, pastoral land use, and flow path distance to poultry opera-

Table 3: Results of multiple linear regression analyses between stream physicochemical variables and agricultural metrics (subwatershed poultry farm density, flow path distance from poultry operations to stream, and percent pastoral land use). Asterisks denote significant relationships.

Dependent Variable	Independent Variables	R^2	p
Total Phosphorus	Poultry Density + Percent Pasture + Flow Path Distance (for all dependent variables)	0.16	0.70
Soluble Reactive Phosphorus		0.11	0.81
Total Nitrogen		0.30	0.40
Nitrate		0.39	0.24
Total Organic Carbon		0.24	0.50
TSS		0.38	0.25

Table 4: Mean values of instream physicochemical variables and agricultural metrics at base flow. Mill-11 Pt. denote Mill Creek in the Eleven Point River watershed near Dalton, AR. Mill-Black denotes a different Mill Creek in the Lower Black watershed in Pocahontas, AR. * indicates the creeks from which F. ozarkae were sampled from, based on availability and when large enough for dissection.

Site	Poultry House Density (km^2)	Percent Pasture	Flow Path Distance (m)	Discharge (m^3/s)	Percent Groundwater	Total P (ug/L)	SRP (ug/L)	Total N (mg/L)	NO_3^- (mg/L)	TOC (mg/L)	TSS (mg/L)	Temperature (°C)	Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
Curia*	0.26	30.10	3017.00	3.00	47.03	16.4	5.86	0.98	0.65	2.27	16.80	13.74	240.43	6.94	7.56
Cypress*	0.21	26.90	1694.00	0.37	72.77	44.1	23.20	2.59	0.98	3.46	26.05	12.91	366.57	7.05	7.62
Diles*	0.00	26.70	6201.00	0.06	78.95	20.9	17.42	0.92	0.51	2.97	11.10	13.62	396.83	8.30	7.73
Dota*	0.57	42.90	1060.00	0.93	14.29	76.7	36.52	2.02	1.31	3.37	10.81	14.28	80.00	6.50	6.83
Eassis*	0.17	30.10	871.00	0.36	82.16	22.1	12.98	1.91	0.63	2.62	27.43	12.81	412.57	7.48	7.89
Hubble	0.11	32.40	707.00	0.34	58.67	114.2	51.91	1.39	0.83	8.10	23.53	13.69	297.50	6.86	7.82
Knotts*	0.06	30.20	1548.00	0.13	63.64	37.1	5.03	0.92	0.91	8.50	12.34	13.24	321.86	6.62	7.70
Lick	0.21	37.30	390.00	0.04	19.74	27.7	15.40	2.00	1.15	4.78	11.49	13.82	106.71	6.46	7.03
Mill-11 Pt.	0.00	42.30	996.00	1.74	58.30	13.4	10.04	2.17	1.75	2.52	24.91	13.50	403.14	8.09	7.95
Mill-Black*	0.16	51.60	1915.00	0.27	80.23	36.7	22.93	2.23	1.31	9.56	11.90	14.21	295.67	7.56	7.57
Tennessee*	0.06	35.20	163.00	1.01	73.50	57.9	35.68	2.60	1.33	3.22	41.63	13.69	370.14	6.99	7.64
Upshaw*	0.10	28.20	531.00	0.20	85.71	16.5	10.92	1.42	1.09	1.87	20.14	12.83	430.00	7.79	7.72

Table 5: Mean values of soluble reactive phosphorus (SRP), nitrate (NO_3^-), and total suspended solids (TSS) from storm samples collected in June 2019.

Site	Storm SRP (ug/L)	Storm NO_3^- (mg/L)	Storm TSS (mg/L)
Curia	8.83	1.44	54.25
Cypress	26.50	3.38	31.29
Diles	19.42	1.31	56.50
Dota	38.84	2.21	4.60
Eassis	17.07	2.61	57.75
Hubble	238.59	2.97	71.75
Knotts	80.26	2.08	46.25
Lick	19.18	2.33	5.29
Mill-11 Pt.	40.42	3.57	44.25
Mill-Black	62.18	3.84	215.25
Tennessee	92.84	3.57	50.50
Upshaw	16.31	1.71	79.50

tions ($R^2 = 0.38$, $p = 0.25$) (Table 6). Algal biomass was also not related to any other instream variable, including phosphorus and nitrogen concentrations. Chlorophyll a was low across most sites (Table 7), and lowest at Hubble Creek (1.9 μg Chl a/cm^2), though algae were potentially constrained by noticeable shading of the stream channel, as nutrient limitation was unlikely given the high phosphate and nitrate concentrations found at that site.

The percentage of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa decreased when poultry operations were closer to a stream ($p = 0.01$) (Figure 3), confirming our hypothesis that poultry agriculture would reduce the presence of sensitive invertebrates. Proximity to poultry agriculture appears to be negatively impacting sensitive taxa, even though we found no discernible mechanistic relationship between instream variables and physicochemical conditions. In the future, we will be measuring substrate size and embeddedness in these streams as we continue sampling to determine whether poultry houses are influencing habitat metrics rather than nutrient and sediment inputs. EPT taxa richness was greatest in Diles Creek (73%), the stream with the lowest subwatershed poultry house density and greatest distance to poultry operations. EPT richness was lowest in Upshaw Creek (5%), which was in a subwatershed with low poultry house density but was directly adjacent to a poultry farm.

We found no relationships between Shannon's diversity index, macroinvertebrate abundance, and any instream variable or surrounding animal agriculture (Shannon's: $R^2 = 0.24$, $p = 0.67$; Abundance: $R^2 = 0.13$, $p = 0.77$) (Table 6). Shannon's diversity varied from 0.87 to 1.95, with the lowest diversity as well as the lowest abundance of invertebrates found at Hubble Creek. The benthos of Hubble Creek is dominated by sand with only small patches of cobbles in addition to the high nutrient levels and nearby application of poultry waste. The habitat itself was not conducive to colonization, but it was difficult for us to determine whether habitat or poultry waste runoff was the dominant metric keeping macroinvertebrate diversity and abundance low, or if perhaps some combination of the two factors were discouraging macroinvertebrate colonization and/or secondary production. Future sampling events and collection of habitat data may provide greater insight into the primary impact affecting macroinvertebrates at Hubble Creek. Surprisingly, abundance was greatest at Upshaw, which was next to a poultry farm; Upshaw had the lowest amount of EPT taxa, and most of invertebrates found there were chironomids.

Table 6: Results of multiple linear regression analyses between algal biomass, macroinvertebrate indices (Shannon's diversity, abundance, and percent EPT taxa), and their respective candidate models. Asterisks denote significant models. Italicized independent variables under a candidate model show individual p-values for each model parameter to illustrate primary drivers explaining variation in the dependent variable.

Dependent Variable	Independent Variables	R^2	p
Chlorophyll a	Poultry Density + Percent Pasture + Flow Path Distance	0.38	0.25
	SRP + NO3- + TSS + TOC + Discharge	0.77	0.06
Shannon's Diversity	Poultry Density + Percent Pasture + Flow Path Distance	0.67	0.24
	SRP + NO3- + TSS + TOC + Discharge	0.51	0.4
	Chlorophyll a	0.09	0.66
Abundance	Poultry Density + Percent Pasture + Flow Path Distance	0.13	0.77
	SRP + NO3- + TSS + TOC + Discharge	0.42	0.55
	Chlorophyll a	0.15	0.22
Percent EPT Taxa	Poultry Density + Percent Pasture + Flow Path Distance	0.65	0.03*
	Poultry Density		0.75
	Percent Pasture		0.71
	Flow Path Distance		0.01*
	SRP + NO3- + TSS + TOC + Discharge	0.55	0.33
	Chlorophyll a	0.04	0.54

Table 7: Mean values of algal biomass sampled from June 2019 to February 2020. Microcystin was measured in July and August 2019. Macroinvertebrates were sampled in June 2019.

Site	Microcystin (ug/L)	Chlorophyll a (ug/cm^2)	Shannon's Diversity	Abundance	Percent EPT Taxa
Curia	0.026	4.25	1.87	261	55.29
Cypress	0.016	3.30	1.75	184	6.82
Diles	0.029	3.16	1.68	852	73.40
Dota	0.009	4.39	1.72	254	23.56
Eassis	0.022	4.89	1.86	533	47.52
Hubble	0.020	1.91	0.87	33	30.05
Knotts	0.023	2.39	1.77	486	26.20
Lick	0.018	3.26	1.59	336	6.95
Mill-11 Pt.	0.015	11.40	1.74	696	21.95
Mill-Black	0.035	1.93	1.95	346	42.80
Tennessee	0.049	4.23	1.16	875	5.98
Upshaw	0.035	6.33	1.20	2530	4.71

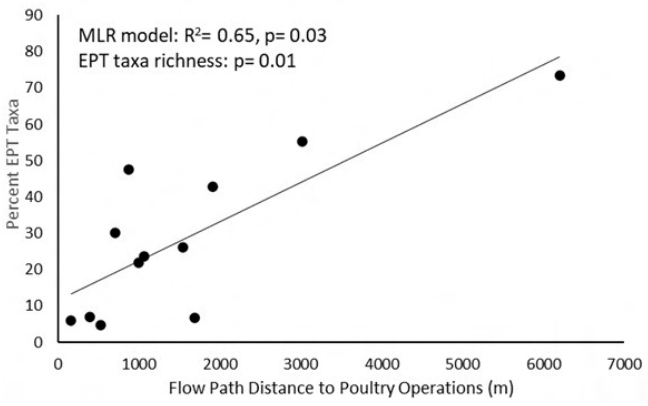

Figure 3: Percent EPT taxa versus flow path distance in meters from poultry operations. Omnibus multiple linear regression statistics and percent EPT taxa richness significance within the MLR model are shown at top left.

Relative abundance of taxa compared to poultry house density and flow path distance to poultry are shown in Figure 4.

The most common crayfish observed in the area was F. ozarkae (personal observation). This species was sampled from the creeks indicated by * in Table 4. Three creeks from which crayfish samples were analyzed first were selected based on summer N and P level data: Diles Creek was our non-polluted creek; Tennessee and Mill-Black creeks were our polluted samples. The partial sequence of NKA in F. ozarkae showed a significantly high level of identity (99.35%) with another crayfish Procambarus clarkii (QDE54942.1) and at least 96.75% similar to several other decapods, indicating a high level of conservation of this gene in this Order. The transcript expression of NKA in gills and intestine (Figure 5) shows no significant difference among the 3 creeks (p = 0.77 in gills and p = 0.55 in intestine). Results in the antennal gland are in progress. These results are consistent with the similar localization and observed staining intensity of NKA in gills, intestine and antennal gland in crayfish from Diles, Mill-Black, and Tennessee creeks (Figure 6). In the gills, about half of the lamellae had NKA localized in the cells' basolateral membrane indicating their role in osmoregulation, while the other half showed little to no NKA, suggesting rather a role in gas exchange (Figure 6, A,A'). NKA basolateral localization was also found in the enterocytes

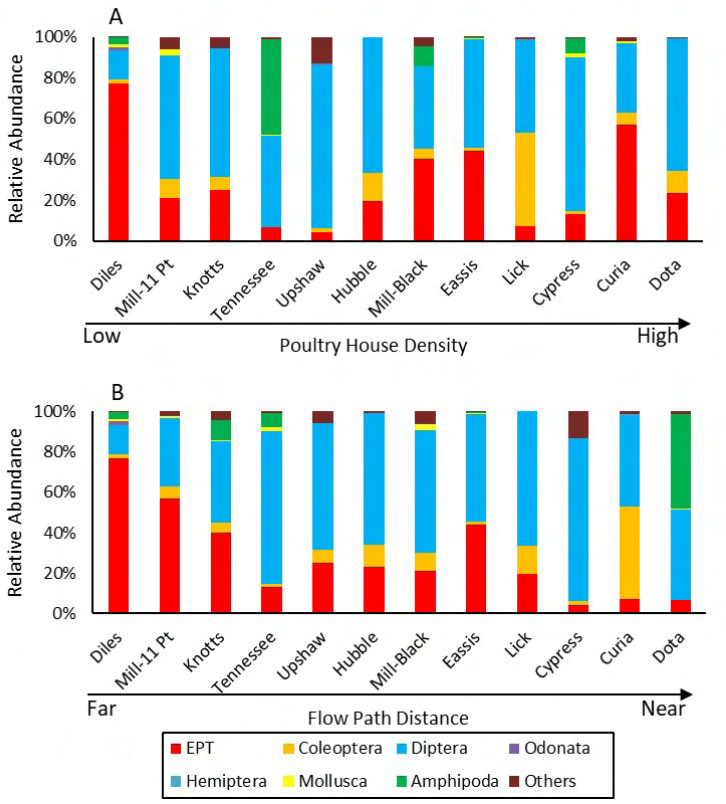

Figure 4. Relative abundance of macroinvertebrate taxa from low to high poultry house density (a) and low to high flow path distance from poultry operations (b).

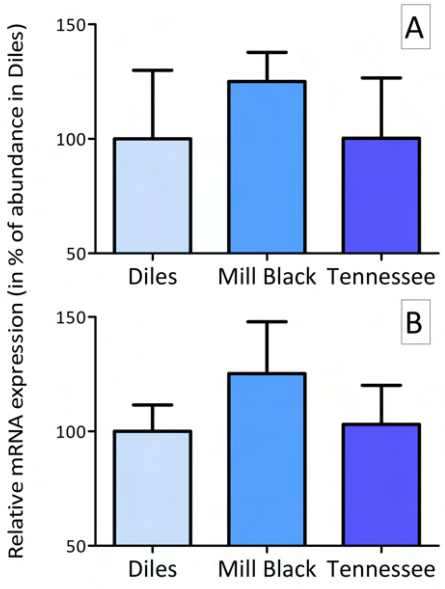

Figure 5: NKA transcript abundance in gills and intestine from the Ozark crayfish in non-polluted creek (Diles) and two polluted creeks (Mill-Black and Tennessee). The expression of NKA is expressed in % of NKA abundance in Diles. Bars represent the mean value + s.e.m. (N = 6 to 9). Expression levels were normalized against a reference gene, EF1a. No significant difference in expression between creeks was found (p = 0.7732 in gills and p = 0.5469 in intestine).

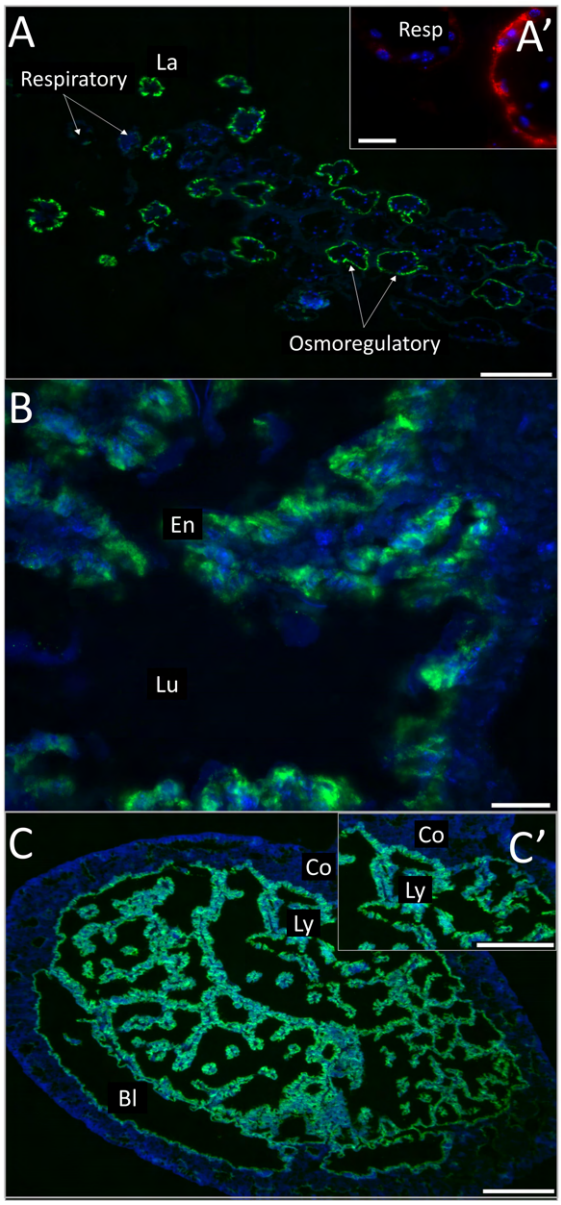

Figure 6: Cross sections of gill (A,A'), intestine (B) and antennal gland (C,C') from F. ozarkae immunostained with monoclonal NKA antibody (green or red) and nuclei stained with dapi. The gill apparatus consists of lamellae (La) made of a thin layer of respiratory (Respiration/Resp) or osmoregulatory (osmoregulation) epithelium, covered with a thin cuticle. The osmoregulatory lamellae (A,A') and the enterocytes of the intestine (B) showed a basolateral NKA immunostaining. The antennal gland (C,C') was stained with NKA antibody in the basolateral membrane of the cells forming the epithelium of the labyrinth (Ly) and bladder (Bl) but absent in the coelomosac (Co). Immunostaining and morphology were similar in all three organs of organisms sampled from polluted and non-polluted creeks (N = 3). Lu: lumen of the intestine. Scales: 100 µm in A, C and C', 20 µm in A' and B'.

(Figure 6, B) and the cells forming the epithelium of the labyrinth and bladder in the antennal gland (Figure 6, C,C'), but was absent in the coelomosac. No change in morphology of those organs was observed between crayfish of non- and polluted creeks. The lack of significant differences in organ morphology and NKA expression and localization in osmoregulatory organs indicates that neither respiration nor osmoregulation seem to be impacted by an increase in nitrogen and phosphorus at the levels detected in our study. However, to confirm this result, we will look at potential differences in NKA activity levels as well as the impact on expression of the other proteins involved in ammonia excretion (Na^+/NH_4^+ exchanger, Na^+/H^+-antiport, V-Type H^+-ATPase).

Conclusions

Animal agriculture is affecting sensitive macroinvertebrate taxa in tributaries of the Lower Black and Eleven Point Rivers despite having no significant relationships with nutrient and sediment concentrations. While we found evidence of poultry farms increasing phosphorus in the summer of 2019, no effect was detected over the rest of the study. Sensitive EPT taxa are not as abundant in streams with nearby chicken houses, though we are still working to determine the mechanism by which intolerant taxa are being excluded. No effect of pollution was shown on NKA expression which might indicate that the observed nutrient concentrations found do not affect crayfish osmoregulation. These findings benefit Arkansas water resource managers by revealing a decline in sensitive invertebrates, which are often considered sentinel species, in streams with nearby poultry operations. This research assists the USGS in addressing pressing water issues by demonstrating that proximity to farming operations is a critical consideration in the conservation of water quality and macroinvertebrate communities.

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