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Using Pimephales promelas as an Indicator Species for the Presence of Endocrine Disruptors in Nebraskan Streams

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Disclaimer: The following thesis was produced in the Environmental Studies Program as a student senior capstone project.

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University of Nebraska-Lincoln, 2019

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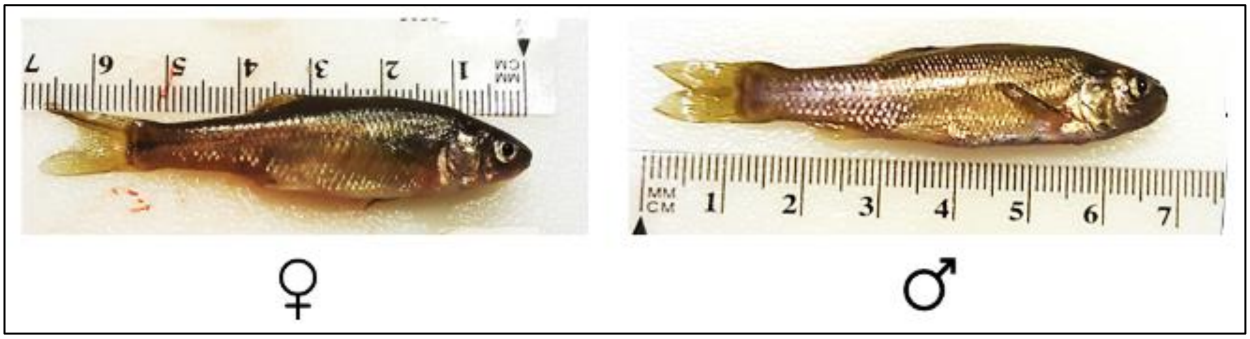
Abstract: Specific sites in Southeast and Southwest Nebraska were chosen to add to the collective knowledge on how cattle feed lot effluents continue to have significant effects downstream of their point source locations. Both locations were sampled via seining net in first: 40.241249 latitude, -95.616191 longitude (near Barada, Nebraska), and second: 41.117368 latitude, -100.794332 longitude (directly South of North Platte in the South Platte River). They were chosen not only for their proximity to differing livestock practices of pasture vs. feed lot, but also where locals fish for the best bait fish including but not limited to fathead minnows (*Pimephales promelas*). The research base for the effects of exposure to endocrine disrupting hormones on fathead minnows is vast, and they are considered a key species in determining the health of Nebraskan streams.

Purpose:

To determine if morphological changes in fathead minnow individuals during spawning season, and changes in male to female ratios within a population are indicators of proximity to cattle feedlots and androgen contaminant runoff.



Introduction:

The U.S. Food and Drug Administration has 25 approved cattle hormone implants used today. Of those, estradiol, progesterone, trenblone acetate, and progestogin melengestrol acetate are the main CFEs (cattle feedlot effluents) currently used in the United States and are the main compounds researched for their effects on downstream (Soto A.M. et al., 2004). These growth promoting compounds are excreted by cattle in major feedlot practices and are subsequently introduced into the aquatic environment. These endocrine disrupters (both androgens and estrogens) can wreak havoc on sensitive aquatic species. They alter hormone pathways that dictate development, spawning, regulate body function and metabolism, and also influence behavior and immunity. Although cattle growth-promotor background knowledge is extensive (from use by the industry since the 1950's), the fate and transport of these compounds in surface waters have only been studied for their environmental implications for the past 17 years (Kolok A.S. et al., 2008).

Pimephales promelas (the fathead minnow) was used as an indicator species because of its prevalence in streams throughout much of North America, its use as a classroom model organism, and the abundant research accumulated on this species in laboratory settings. The EPA uses this species as a biological model in aquatic toxicology to evaluate acute and chronic toxicity of chemicals/compounds in vertebrates. The changes seen in this aquatic species after exposure to CFEs range from differential gene expression, altered sexual characteristics, reproductive success, and changing sex ratios (Kolok & Sellin, 2008). The impacts of these contaminants on a population level need to be evaluated in order to develop credible ecological risk assessments for the future (Miller & Ankley, 2004). According to Kolok et al. in 2009, these mobile contaminants in waterways may have biological effects at very low concentrations and may also represent the greatest contribution of organic contaminants to surface waters. Ankley* G.T., D.L. (2006) states, "[*Pimephales promelas is*] an excellent model for addressing new challenges in aquatic toxicology, including identification of sensitive life-stages/endpoints for chemicals with differing modes/mechanisms of action, predicting population-level effects based on data collected from lower levels of biological organization, and exploring/understanding the emerging role of genomics in research and regulation."

Endocrine disrupting chemicals (EDCs) have mostly affected organisms in and associated with the aquatic environment. These effects are loosely identified with disruptions in the developmental process, abnormal behaviors, and alterations in reproductive capacity (Trudeau & Tyler, 2007). The Endocrine Society defines an EDC as an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action. Estradiol, testosterone, and trenblone acetate are the CFE related compounds with the most laboratory data, whereas progesterone and progestogin melengestrol acetate require further lab studies to determine how they continue or dissipate in the water column and also their effects on aquatic species. Multiple authors have argued that the period of sexual development in fish most sensitive to endocrine disrupting chemicals is that of sex differentiation (Holbech H. et al., 2006).

The objective of the study was to examine the effect of estrogens and androgens on the morphology of the spawning male and female fathead minnow. The sexual dimorphic characteristics are seen more easily during the breeding period of 2-3 months annually (Miller & Ankley, 2004). Thanks to fish farming and the need to control population growth it has been discovered that androgens generally defeminize females, while estrogens feminize males. Exposures to either during early developmental stages can skew the sex ratio of a population generating all male, or all female populations; and can also lead to intersex individuals (Holbech et al., 2006). The changes seen in the Fish Sexual Development Test confirm: sex ratios are a sensitive endpoint when detecting androgens; whereas even in adult fish vitellogenin (vtg) is an endpoint detecting estrogen exposure. The reason why vitellogenin is important is because it is a blood protein precursor and is generally formed by females in egg-yolk production, seeing a heightened increase in this protein is an endpoint for estrogen exposure in males because this is generally not associated with healthy male sexual development and was found studying the effects of high estrogen wastewater effluent in UK streams which led to the regulations in place today (Nichols K.M. et al., 1999). Testing these vtg levels and genetic material for sexual identification or confirmation of environmentally altered or intersex individuals was not available during this current study. The question posed relied on the assumption that the study of morphological features followed by dissection and measurement of sex organs would determine sex ratios, from which a pattern may arise to show correlation with other research on the effects of androgen CFEs among fathead minnows. The change in sex ratio of a developing population of fathead minnows exposed to CFEs is a very sensitive endpoint in the detection of androgenic chemicals (Holbech H. et al., 2006). If in comparing different regions of a watershed there are fluctuations in sex ratios and thus are unstable further study is warranted for they may be sensitive indicators of environmental hormonal effects (Kolok A.S. et al., 2009)

The two sampling locations were chosen based on land use and proximity to cattle feedlot effluent. Location 1 was a stream surrounded by both forest and agricultural fields producing corn and soybean crops with silty soil. This was in Richardson County where cattle are raised in pasture without growth-hormone implants, and pastures are relatively spread out among crop lands and forested areas. Also in this region is Indian Cave State Park which is a 3,000 acre state park. Location 2 consisted of the South Platte River in Lincoln County, near the city of North Platte. This area has some city runoff and is more sand than silt as far as soil type. The Factory Farm Map from the Food and Water Watch's Department of Agriculture Census highlighted this county as having a very high density of cattle feedlots upstream and down of the Location 2 sampling site. This line of reasoning from the Census map was also highlighted in Kolok A.S. et al. (2008), where it was reiterated feedlots represent a greater environmental risk than pasture fed by (1) having higher concentrations, and (2) high potency growth promoting compounds used to maximize feed efficiency, cattle growth, and carcass quality to optimize economic returns. Thus the sources of study which were chosen were both from a highly impacted site and a less impacted control site.

Materials and Methods:

Two locations were sampled: Location 1, a small stream in Barada, Nebraska which has runoff from only crop lands and dirt roads. Location 2, the South Platte River, runs through Lincoln County, an area of highly concentrated cattle feedlots. These locations are in separate watersheds so the data generated is not redundant and shared exposures were avoided (Kolok A.S. et al., 2009).

Location sampling consisted of recording latitude and longitude coordinates, county and location information, relative stream or river categorization, land use, soil type, and ecology. A water sample was taken from each site location and atmospheric temperature was recorded with the date and time of collection. A two-person seining net was utilized for collecting sample species; samples were deposited into a bait bucket equipped with a Bubble Box© portable air pump and stream water from that location.

The live minnows were transported less than 230 miles and any that did not survive were frozen upon arrival.

After sampling, each minnow was inspected, noting mucus presence; epithelial pad (using scale from Smith and Murphy, 1974), ovipositor, and tubercles; coloration distinctions; species; sex; possible abrasions, melanomas, deformations, and parasitic evidence. Each minnow had a corresponding site-specific number, and photographs were taken of each once pithed with a needle tool and laid on a clear metric ruler for scale. Measurements recorded via ruler include length in millimeters from snout to the tip of the dorsal caudal tail, girth in millimeters at the widest point, and weight in grams using a digital scale with accuracy up to a one hundredth of a gram. Dissection along with ovary and testes identification and parasite extraction used Field Necropsy for Students as a general guide, and previous experience in collegiate setting (Field Epidemiology CPBS-UNL). A stereoscope was utilized in dissection and borrowed from the UNL H.W. Manter Parasitology Lab. Ovaries and testes were identified using a visual diagram (Weber D.N. et al., 2013). Both male and female organs were subsequently weighed and recorded. Frozen specimens were similarly recorded and generally lacked mucosa and coloration data. The dissection of previously frozen specimens (done so upon death) generated less than desirable sexual organ elasticity making identification possible but more difficult.

Results:

The average sizes for both male and female fathead minnows were larger at the Barada Loccation 1 than the likely contaminated South Platte Location 2. The samples taken determined a population sex ratio to be 3:1 females to males respectively at Location 1. The testes size at Location 1 was much larger (0.02g) than that of males at Location 2 (0.001g), which also maintained a considerably higher parasite load than males from Location 1. Location 2 males also had confusing morphometrics in terms of what looked like the presence of an ovipositor in half of the identified males; 2/3 of these had epithelial pads as well. The range from each site was in fitting with the normal range of measurements recorded for the species, but location and population specific data was accumulated for future continuation of the study. Please see Figure 1 (page 8) for representative graph.

Discussion:

The range from each site fit the normal range of measurements from published literature for *P. promelas*; location and population-specific data were documented for future continuation of this study. Since different watersheds with differing stream volumes allow for smaller or larger minnows, these general measurements are within the norm and are inconclusive. The most interesting differences are seen in the ambiguous characteristics of the male fathead minnows of Location 2 (the Platte River). The males that had a slight swelling around where and ovipositor would be in female minnows was somewhat of an oddity and could be explained by the frozen nature of some of the samples but not all. It was postulated during dissection the possibly heightened vtg levels could be manifesting in a morphological way, but since the plasma was not tested there was only speculation. While water samples were taken, adequate water sampling methods were not utilized to their fullest due to lack of knowledge on the researcher's part. POCIS or polar organic chemical integrative samplers, allow the identification of most endocrine disrupting compounds in surface waters (Sellin M.K. et al., July 2009). Water temperature was taken, and water pH should have been but was not. The two locations had differing levels of precipitation before sampling and led to less sample locations than previously planned, as well as a larger gap between sampling than was originally anticipated.

Among aspects to change in future, intraocular distance was not recorded in this study and was determined to be one of the only varying changes of head morphometrics in Orlando et al.'s field study *Endocrine-Disrupting Effects of Cattle Feedlot Effluent on an Aquatic Sentinel Species, the Fathead Minnow* of 2004. Where intraocular distance was smaller in both males and females at the contaminated site vs. the reference site.

An assessment of current field studies indicate the presence of these compounds in runoff/river systems cannot predict the individual level or length of exposure, nor when in development these fish were exposed, which significantly affects toxin sensitivity of the fathead minnow. Even population scale changes similar to laboratory predictions do not provide the degree of causality of these metabolites (Miller & Ankley, 2004). Large deposition of these contaminants often follow precipitation events, and while sorption data indicates estrogens (ethinylestradiol) are highly sorped to soils, sediments alone do not appear to be a significant source for steroidogenic compounds in Nebraska's own Elkhorn River (Kolok A.S. et al., 2007). The type of soil plays a part in the transport of these materials in surface waters due to the organic matter content which leads to the bioavailability of these contaminants. And while a sample was not taken, observations and previous soil science courses allowed for the differing soil types to be noted. Also, all agrichemicals have some affinity for organic matter and interactions between metabolized steroids and pesticides are currently unknown (Kolok et al., 2009). So in order to further the study these factors must too be taken into account.

Conclusion:

This is one summer of research with a slightly flawed research plan. To capitalize on what was learned this should be the beginning of a 2-5 year study. Population-wise a carrying capacity for each location is necessary, if using the by volume approach of O'Neil 1979, would be 5.19-6.14 fish/m^3. Since the fathead minnow uses scramble competition, creating equal sharing of resources, survivorship and fecundity can be studied annually with the help of life table data readily available for this species.

Unfortunately streams world-wide are not necessarily pristine and may contain more contaminants and compounds than CFEs, but are still EDCs. Many chemicals can have multiple effects on physiological systems, so there is a need to develop chemical screens and use today's molecular approaches to aid future studies of how chemicals persist in nature (Trudeau V. & Tyler C., 2007). Much research on human waste effluent has spurred regulations and treatments of wastewater with the most successful being biological treatments in countries such as the UK, Canada, Germany, Sweden, Japan, and North America (Stevenson A. et al., 2003). It starts in your very own state however as Sellin M.K. et al., (January 2009) has proven: "These results confirm that WWTP (wastewater treatment plant) effluent contributes biologically significant levels of estrogens to Nebraska surface waters." The freshwater stream environment contains many species which use chemical cues to locate food, mates, avoid predators, and engage in social interactions especially in areas with low visibility (Corkum L.D. & Belanger R.M., 2007). We as humans are interfering with basic functions when our waste effluent, farming practices, livestock, and terraforming go un-researched.

Many varying factors besides feedlot runoff could affect sexual characteristics, such as precipitation in the area, parasite load, age, etc. Regardless of how much or little we know about these factors, there is limited information about site specific fish populations, and any data is important. What little research exists is based in lab settings rather than data collected in field environments (Scott A.P. & Ellis T., 2007). We however do have multiple sources of information regarding contaminants within U.S. streams and their levels (Kolpin D.W. et al., 2002), as well as proposals on how to mediate using regulatory measures from the sources of these pollutants (Hecker M. & Hollert H., 2011). As always, more data is needed to more fully examine environmental impacts of agricultural runoff as a whole. As Kolok A.S. et al., 2009 has stated: "Additional research is needed to clarify relationships between deployment sites and the occurance of these compounds in surface waters." It was in hopes of adding to the world of knowledge of high density livestock practices having an impact on Nebraska streams this study was embarked upon.

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Figure 1.

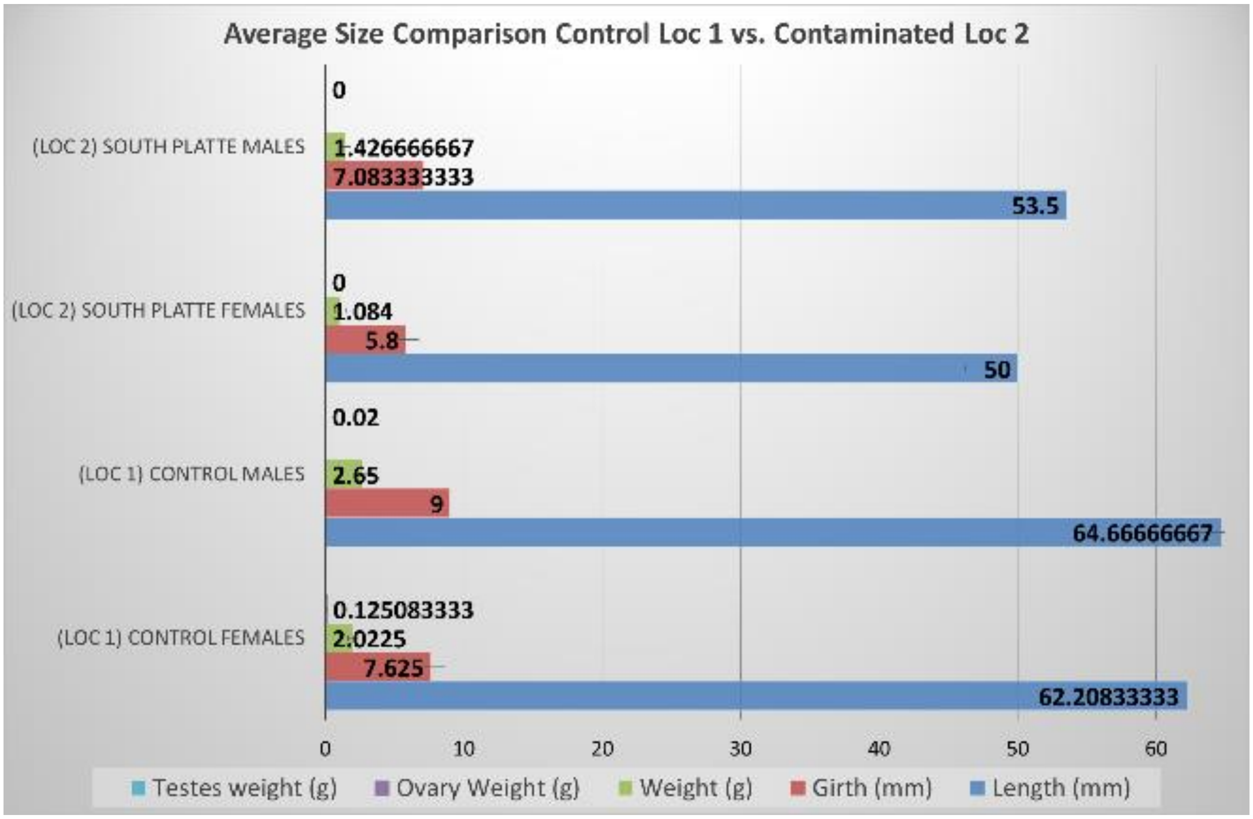

Figure 1. Average *Pimenphales promelas* sizes of each sex with regard to length, girth, weight, and sex organ weight. As compared to Loc 2, Loc 1 had larger average sizes and weights. Standard deviations for each measurement are represented with a bar.

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