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LARVAL LAKE STURGEON (*ACIPENSER FULVESCENS*) ACCEPTANCE OF FORMULATED DIETS BASED ON MATERNAL LINEAGE

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We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Biology Aquatic Science Concentration.

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ABSTRACT

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Lake Sturgeon (*Acipenser fulvescens*) populations declined throughout the Great Lakes and Mississippi River basins during the late 19th and early 20th centuries due to overexploitation and habitat degradation. Aquaculture of Lake Sturgeon can be expensive where brine shrimp, blood worms, and krill (traditional diets) are fed. Formulated diets, a cheaper option, were fed to sturgeon over a 120-day period to determine acceptance rates across maternal lineages while comparing survival, growth, and body condition to traditional diets. Eggs were collected from the Wolf River and transported to Genoa National Fish Hatchery. Survival was recorded daily, and subsamples were measured to total length (±1 mm) and weighed (±0.001 g) biweekly. A Kaplan Meier analysis determined that mortality distribution was significantly different for all females and diets. A two-way ANOVA revealed significantly higher survival for traditional diets on day 120 ($\chi^2 = 3886$, df = 5, $P < 0.001$). A repeated measures ANOVA revealed both total length ($F_{1,11} = 62.2$, $P < 0.001$) and body condition ($F_{2,11} = 4.37$, $P = 0.04$) to be significantly higher for traditional diets. These formulated diets provide further evidence indicating maternal lineages were similar in all parameters and Lake Sturgeon will survive and grow once acceptance has occurred.

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INTRODUCTION

Conservation plans for aquatic organisms utilize management tools such as habitat rehabilitation, invasive species removal, and the propagation of individuals to maximize the probability of species persistence. Aquaculture is the production of individuals through propagation and is practiced at state and federal hatcheries for sportfish management and the conservation of native species. Aquaculture helps maintain populations and restore numerous 'at-risk' native fish species throughout their native ranges, including Lake Sturgeon (*Acipenser fulvescens*, Aloisi et al. 2006).

Lake Sturgeon were once the most widely distributed sturgeon species in North America and historically supported subsistence and valuable commercial fisheries throughout the Great Lakes region and the Mississippi River basin (Haxton and Cano 2016). Populations throughout their native range declined during the late 19th and early 20th centuries due to overexploitation, river fragmentation, and sediment pollution from the logging industry (Kline et al. 2009; Reid et al. 2013). Currently, Lake Sturgeon are of conservation concern throughout their native range and are 'under review' for federal protection in the United States (Williams et al. 1989; Haxton and Cano 2016). Populations exist in 23 U.S. states but are considered to be stable in only Wisconsin and Michigan (Williams et al. 1989; Galarowicz 2003; Holst and Zollweg-Horan 2018). Recovery plans have been established to restore the Lake Sturgeon populations throughout their historic range by means of captive propagation, stocking, targeted dam removals, and spawning habitat restoration (Kempinger 1996; Holst and Zollweg-Horan 2018). Propagation of the Lake Sturgeon began in the early 1980s (Wang et al. 1973; Buddington 1985; Ćeskleba et al. 1985). Subsequent research has established standard fertilization and egg disinfection techniques, and improved egg rearing methods to maximize Lake Sturgeon production and post-stocking survival (Alderdice and Velsen 1978; Piper et al. 1982; Kempinger 1996; Bouchard III and Aloisi 2002; Runstrom et al. 2002; Eckes et al. 2015). Observations of Lake Sturgeon polygamy during spawning were used to develop the mating schemes used for production (Kapuscinski et al. 1996; Aloisi et al. 2006). To maintain similar genetic contributions of males and females in wild mating groups five males are crossed with one female for propagation (Kapuscinski et al. 1996; Aloisi et al. 2006). Suitable (traditional) feeding regimes have been established to maximize growth and survival, while maintaining accepted mating schemes, but are relatively expensive compared to diets fed to many other hatchery fishes (Bruch and Binkowski 2002; Bruch et al. 2007; Chiotti et al. 2008; Kline et al. 2009). The high cost of Lake Sturgeon diets necessitates further research of alternative feeding regimes to maximize sturgeon production and benefit conservation stockings (Ćeskleba et al. 1985; DiLauro et al. 1998; Lee et al. 2018).

The established (i.e., traditional) feeding regime originates from observations of sturgeon feeding on zooplankton during early larval stages before consuming invertebrates (Aloisi et al. 2006; Kline et al. 2009). The traditional diet consists of brine shrimp (*Artemia* nauplii), bloodworms (Chironomidae), and krill (Euphausiidae) and often results in survival rates between 90-95% (Aloisi et al. 2006). Lake Sturgeon fed this diet grow to ~180mm during the typical five-month growing season (May-September), a size at which post-stocking survival is estimated to be 80% (Baker and Scribner 2017; Aloisi et al. 2019). Although successful in rearing the species to desired sizes, the traditional feeding regime is 10x the expense of widely available formulated diets (Aloisi et al. 2006). Research to identify potential alternative feeding regimes that minimize costs while maintaining high larval survival and growth rates would benefit Lake Sturgeon conservation efforts (Aloisi et al. 2006; Baker and Scribner 2017; Holst and Zollweg-Horan 2018).

Alternative feeding regimes, such as commercially available formulated diets have proven effective for propagating Shortnose Sturgeon (*Acipenser brevirostrum*) and White Sturgeon (*Acipenser transmontanus*, Moreau and Dabrowski 1996; Hung et al. 1997; Ware et al. 2006). However, past attempts to use formulated diets to lower production costs for Lake Sturgeon have been unsuccessful due to low survival rates (0-20%, DiLauro et al. 1998; Deng et al. 2003; Bauman et al. 2016; Valentine et al. 2017; Lee et al. 2018). These studies utilized direct feeding and different transitional periods from the traditional food items to formulated feed which likely accounted for the inconsistent acceptance rates observed (DiLauro et al. 1998; Bauman et al. 2015). However, when formulated feed have been accepted, researchers have observed increased growth rates and improved body conditions relative to traditionally fed groups (Lee et al. 2018; Yang et al. 2019).

Although formulated feeding regimes are a viable alternative for closely related sturgeon species and have shown promise for Lake Sturgeon growth and body condition, they have not yet resulted in suitable survival to meet conservation stocking needs (DiLauro et al. 1998; Deng et al. 2003; Lee et al. 2014, 2018). However, studies have shown that once Lake Sturgeon have accepted formulated diets their survival rates can be similar to traditional diets (Lee et al. 2018). Therefore, the survival, growth, and relative body condition of larval Lake Sturgeon fed traditional and formulated diets was compared across three maternal lineages to refine future propagation standards. The availability of an alternative feeding regime that utilizes formulated diets and yields similar survival rates, growth rates, and body conditions to traditional diets would reduce costs of propagation efforts by about $19,295 per 10,000 larval Lake Sturgeon. The equal survival of progeny along with suitable survival, growth and body condition would allow hatchery facilities to increase propagation efforts of the Lake Sturgeon at a lowered cost.

METHODS

Gamete Collection

Lake Sturgeon eggs and milt were collected during spawning events at the Shawano dam on the Wolf River near Shawano, Wisconsin (Figure 1). Approximately 30mL of milt was collected from a single male and ~500mL of eggs were collected from three separate females to represent distinct maternal lineages. Fertilized eggs were transported to Genoa National Fish Hatchery (U.S. Fish and Wildlife Service, Genoa, Wisconsin) for rearing (Figure 1). Each set of progenies was reared separately to ensure adequate survival of the three distinct maternal lineages prior to the start of the study. Egg fertilization, incubation, and rearing followed standard operating procedures for the Genoa National Fish Hatchery (Aloisi et al. 2006). During the first week of the rearing period, a 500ppm 35% hydrogen peroxide treatment was administered each day for a 15-minute period to reduce infection and help remove dead or infected eggs (Bouchard III and Aloisi 2002; Aloisi et al. 2006).

Hatching and Exogenous Feeding

At seven days post fertilization, eggs began to hatch and were placed on hatching screens in six treatment specific tanks due to density constraints. Hatching finished at 10 days post-fertilization and the larval Lake Sturgeon were introduced to 18 study tanks (~0.40m^3, Figure 2). The progeny from each of the three females were randomly assigned to six study tanks, of which three were fed the formulated diet and three were fed the traditional diet (*N* = 18 total tanks). A total of 400 larval Lake Sturgeons were introduced to each study tank. Lake Sturgeon were reared in a shared flow-through system (~100 turnovers/day), using surface hatchery pond water for the duration of the 120-day study (May 23, 2022 – September 20, 2022). All larval Lake Sturgeon were introduced to brine shrimp 24-48 hours prior to exogenous feeding. Once exogenous feeding was observed (15 days post hatch, dph), tanks were switched to the randomly assigned diet treatments (Fajfer et al. 1999; Aloisi et al. 2006; Bauman et al. 2015).

Throughout the study period, dissolved oxygen (mg/L), total gas saturation (total pressure), and pH were measured daily using a YSI professional plus and Saturometer (Model SM1). Water temperature (°C) was measured every 15 minutes using a HOBO temperature tracker (Onset HOBO pendant temp, Model UA 001 64). The number of mortalities in each tank was recorded daily and Lake Sturgeon total length (±1 mm) and weight (±0.001 g) were measured every other week starting at 71dph. Larval sturgeons were not measured before this time due to fish handling constraints and mortality risk. During each sample, twenty individuals were randomly selected from each tank, dried, measured to total length, and weighed using an Intel-Lab Balance (0-100g; model: PM 100) to adjust feeding rates, describe growth, and estimate relative condition factor (K_n). Mean total length and relative condition factor (K_n) were estimated at each time interval to describe sturgeon growth and body condition for each treatment group.

Feeding Regimes

Six treatments were used throughout the study: female 1 + traditional diet, female 1 + formulated diet, female 2 + traditional, female 2 + formulated, female 3 + traditional, female 3 + formulated (Figure 2). Traditional diets were fed three times per day at a minimum rate of 15% wet body weight (WBW) and increased to satiation to ensure availability of feed to individuals (Aloisi et al. 2006). Traditionally fed sturgeon first received brine shrimp (*Artemia* nauplii, 5% ash, 1% moisture, 62% protein, 11% fat) for four weeks, then bloodworms (Chironomidae, 1.85% ash, 93.3% moisture, 2.86% protein, 0.19% fat) for eight weeks and finally krill (Euphausiidae, 1.82% ash, 85.4% moisture, 12% protein, 1.54% fat) for the remaining six weeks (DiLauro et al. 1998; Aloisi et al. 2006, Table 1). Alternatively, the sturgeon assigned the formulated diet treatment were fed at a minimum rate of 10% WBW and up to satiation to ensure availability of feed to individuals. The formulated diet consisted of brine shrimp (*Artemia* nauplii), Otohime (50% protein, 10% fat, 16% ash, 6.5% moisture), and Skretting trout feed (52% protein, 16% fat, Aloisi et al. 2019). To ensure a homogenous mixture and break surface tension, all formulated feeds were mixed in water prior to feeding. Sturgeons were fed ~60mL of brine shrimp (~3 million individuals) for the first week and then transitioned to Otohime B1 (250-360µm, Table 1). At week two, sturgeons were fed a 50:50 ratio of Otohime B1 to brine shrimp (~30mL of brine shrimp and ~6g of Otohime B1). Only Otohime B1 was fed during week three. During week four, 4g of Otohime B1 and 4g of Otohime B2 (360-650µm) were mixed, and at the start of week five only 8g of Otohime B2 was fed. At week six, larvae were fed a 50:50 mixture of Otohime B2 and Skretting trout feed #1, and in week seven, sturgeons were only fed Skretting trout feed #0 (Table 1). During the eighth week, sturgeons were fed a 50:50 mixture of Skretting trout feed #1 and Skretting trout feed 1.6mm and were then fed only Skretting trout feed 1.6mm for the remaining study period (Table 1). At all times, sufficient feed was ensured to be available in all study tanks.

Table 1. Feeding regimes of Lake Sturgeon (*Acipenser fulvescens*) from three different maternal lineages over a 120-day study period.

	Traditional Diet			Formulated Diet				
Week 1.6mm	Brine shrimp	Blood worms	Krill	Brine shrimp	Otohime B1	Otohime B2	Skretting #1	Skretting
1	60mL			60mL				
2	60mL			30mL	6g			
3	60mL				8g			
4	60mL				4g	4g		
5	30mL	30mL				8g		
6		60mL				4g	4g	
7		Satiation					½ Satiation	½ Satiation
8		Satiation						Satiation
9		Satiation						Satiation
10		Satiation						Satiation
11		Satiation						Satiation
12		Satiation						Satiation
13		½ Satiation	½ Satiation					Satiation
14			Satiation					Satiation
15			Satiation					Satiation
16			Satiation					Satiation
17			Satiation					Satiation

Zooplankton Availability

Zooplankton assemblages were described in the surface pond water as a source for initialization of feeding on zooplankton. Zooplankton samples were collected each day from three random tanks during the first four weeks of feeding to determine their availability. A 102mm funnel with 100µm mesh was placed under the tank input until the standard 10L was sampled (Sipaúba-Tavares et al. 2008; Afia et al. 2018). Each 10L sample was rinsed into a 153mm funnel over a 500mL sample bottle containing 4% formalin for preservation (Sipaúba-Tavares et al. 2008; Afia et al. 2018). Zooplankton samples were then filtered and rinsed into a petri dish and placed into a zooplankton counting chamber. All zooplankton were counted among five categories: Copepods (Copepoda), Nauplius (Artemiidae), Cladocerans (Diplostraca), Rotifers (Rotifera), and Amphipods (Amphipoda). Relative abundance (taxa per 10L) of each taxonomic group and richness was recorded to describe changes to zooplankton assemblage through time.

Data Analysis

Analysis was conducted using the six previously described lineages and diet treatment groups. A Kaplan-Meier survival analysis was used to describe Lake Sturgeon survival among treatment groups throughout the study period. A Wilcoxon chi-square was used to analyze cumulative survivorship among treatments where all sturgeon that survived to day 120 were right censored. A Cox proportional hazards regression was used to determine the effects of diet and lineage on the risk of death of sturgeon larvae. Additionally, a two-way ANOVA was conducted to determine the effects of maternal lineage and diet on the final survival rates of Lake Sturgeon at day 120. A TukeyHSD was used to determine any significant differences in mean survival. Repeated measures

ANOVA was used to evaluate differences in Lake Sturgeon total length and relative condition factor throughout the study period based on maternal group and diet. All data analysis was conducted using the R program (version 4.1.1). Significance for all statistical tests was ascribed at $\alpha = 0.05$.

RESULTS

Temperature (20.24 ± 0.02 °C), pH (8.31 ± 0.03), and dissolved oxygen (9.85 ± 0.21 mg/L) were relatively stable through time and consistently within accepted rearing conditions for Lake Sturgeon (Figure 3). Total gas saturation exceeded 100% at times during the study period (101.2 ± 0.28%), but never reached lethal levels for sturgeon larvae (Counihan et al. 1998).

Zooplankton were present in the propagation tanks during onset of exogenous feeding and throughout the first four weeks of the study (Figure 4). Zooplankton numbers were extirpated out based on sample counts and were determined to have an average of 1.7 million zooplankton present throughout each day with a single day peak of 4.3 million whereas brine shrimp are fed out at 9 million *Artemia* nauplii per day. Based on this data the zooplankton were relatively rare when compared to the abundance of brine shrimp provided to each tank. There were substantial increases to the relative abundances of both *Artemia* nauplii and cladocerans, but these varied through time (Figure 4). *Artemia* nauplii were most abundant (~1500 organisms/10L) at day 8 and then their abundances decreased at day 14 of the experiment (Figure 4). Cladocerans were most abundant on day 17 (~1650 organisms/10L) and then tapered off slowly starting on day 21 of the experiment (Figure 4). Rotifers and copepods were present throughout the onset of exogenous feeding (~200 organisms/10L) and amphipods were rarely captured (Figure

4). Lake Sturgeon, from all maternal lineages, were observed feeding on formulated and traditional diets during the periods in which zooplankton were present.

Survival Analysis

At 55dph, water flow loss occurred in one tank and resulted in 78 mortalities. These individuals were removed from survival analyses. Throughout the 120-day study, survival was substantially higher for sturgeon fed traditional diets than sturgeon fed formulated diets ($\chi^2 = 3886$, df = 5, $P < 0.001$). However, all maternal lineages within both treatments had significantly different survivorship curves throughout the duration of the study ($P < 0.001$, Figure 5). A cox regression revealed a hazard ratio of 1.9 for maternal lineages and 8.1 for diet treatment, indicating that those fed the formulated diets were 8.1 times more likely to perish throughout the study (Figure 5). Sturgeon fed formulated diets also had a 76.3% - 93.0% chance to reach pre-transition to formulated diets and 16.2% - 50.5% chance to reach post-transition to formulated diets. Whereas sturgeon fed traditional diets had a 91.5% - 95.5% chance to reach pre-transition and 71.2% – 89.8% chance of reaching post-transition. On day 120, maternal lineages within formulated diets ranged in survival from 0.5% - 17.4% and traditional diets ranged from 70.4% - 89.3% and were not significantly different within each treatment ($F_{2,2} = 0.57$, $P > 0.05$, Figure 6).

Sturgeon Length Comparisons

A repeated measures ANOVA determined that all Lake Sturgeon grew during the study ($F_{1.5,16.7} = 239.0$, $P < 0.001$) and stockable sturgeon (>180 mm) were produced by feeding traditional (n = 1,327) and formulated diets (n = 33). Formulated diets had 11 ±

1% of sturgeon reach stockable size and sturgeon fed traditional diets had 49 ± 2% reach stockable size (Table 2, Figure 7). Traditional diets resulted in significantly larger sturgeon than formulated diets through time ($F_{1,11} = 62.2$, $P < 0.001$, Table 2, Figure 7); however, mean length did not vary among maternal lineages ($F_{2,11} = 2.0$, $P = 0.28$, Figure 7). The effect of diet varied through time ($F_{1.5,16.8} = 8.9$, $P = 0.004$), whereas the effect of maternal lineage did not vary through time ($F_{3,16.8} = 1.8$, $P = 0.18$). The interaction between diet and maternal lineage was not significant ($F_{2,11} = 2.8$, $P = 0.76$).

Sturgeon Relative Condition Factor

Above and below average body conditions were observed across maternal lineages for sturgeon fed both traditional and formulated diets during the 120-day study (Figure 8). Maternal lineages from traditional diets resulted in significantly higher body condition than maternal lineages from formulated diets ($F_{2,11} = 4.37$, $P = 0.04$, Figure 8). Sturgeon fed the traditional diet had similar body condition to sturgeon fed formulated diets through time ($F_{1,12} = 0.03$, $P = 0.86$, Figure 8) and within diets body condition did not differ across maternal lineages ($F_{2,11} = 1.64$, $P = 0.24$, Figure 8). The effect of diet did not vary through time ($F_{2,22.3} = 0.42$, $P = 0.66$, Figure 8) and the effect of maternal lineage did not differ through time ($F_{4.1,22.3} = 1.24$, $P = 0.32$, Figure 8). The interaction between diet and maternal lineage through time was not significant ($F_{4.1,22.3} = 1.10$, $P = 0.39$, Figure 8). Body conditions were significantly higher for traditional female 1 ($F_{1,34} = 6.51$, $P = 0.02$, Figure 9) and female 2 ($F_{1,34} = 10.9$, $P = 0.002$, Figure 8). However, traditional female 3 was not significantly higher than formulated female 3 ($F_{1,28} = 0.01$, $P = 0.92$, Figure 8). Progeny of female 1 and 2 fed traditional diets have better body

Table 2. Lake Sturgeon maternal lineages and number of stockable fish from 1,200 initial fish ± standard error and the percent of the final number of fish that were stockable (total length ≥18cm) after the 120-day study.

Diet	Female	Mean Length ± SE (mm)	Stockable ± SE	Stockable (%)
Formulated	1	139.07 ± 2.27	25 ± 1.70	16.02 ± 0.68
Formulated	2	139.95 ± 0.41	7 ± 0.44	5.69 ± 0.10
Formulated	3	96.78 ± 12.58	1 ± 0.58	5.00 ± 0.13
Traditional	1	179.78 ± 0.33	570 ± 2.60	57.00 ± 1.97
Traditional	2	185.80 ± 0.25	518 ± 3.30	64.27 ± 2.64
Traditional	3	168.73 ± 0.12	239 ± 1.55	25.98 ± 0.79

condition, but progeny of female 3 did not have a significantly better body condition than female 3 fed formulated diets (Figure 8).

DISCUSSION

The establishment of an alternative formulated diet, with comparable survival to traditional diets, would benefit conservation of Lake Sturgeon through reduced feed costs and labor. The alternative feeding regime proposed herein utilized cheaper formulated diets, but resulted in lower survival, slower growth, and generally worse body condition than the traditional diets. All sturgeon treatments had different distributions of mortality throughout the duration of the study, but sturgeon fed traditional diets had higher survival than those fed formulated diets. Maternal lineages within each diet did not have different survival on day 120. Thus, the progeny of all female sturgeons were equally likely to survive to the end of the growing period suggesting that formulated diet acceptance did not significantly impact maternal lineage. Although the traditional diets resulted in a higher survival rate, and greater growth and better body condition, the feed cost was nearly 10x that of formulated diets.

Survival

Mortality observed during the study was largely attributed to starvation, but intervention was required for disease and water loss at 44 dph. All Lake Sturgeon were treated with a 1% salt treatment for 15 minutes due to a unicellular slime mold infection in two tanks. After an initial 1% salt treatment, a second 1.5% salt treatment was conducted seven days later, and the slime mold subsided. In this study, an accepted diet of brine shrimp, bloodworms, and krill resulted in higher survival than an experimental formulated diet of brine shrimp, Otohime, and Skretting trout feed. The transition from brine shrimp to Otohime was consistent with transitional steps used by Ware et al. (2006). Unlike this study, Ware et al. (2006) found survival to not significantly differ compared to traditional diets when provided to Shovelnose Sturgeon. Similarly, when provided a salmonid based diet, White Sturgeon growth rates increased with increasing protein and lipid content (Hung et al. 1997). However, Valentine et al. (2017) found when Lake Sturgeon were provided frozen or live *Artemia* they had higher survival (98.3 ± 2.9%) than larvae fed only trout diets (19.0 ± 8.1%), after transitioning from *Artemia.* DiLauro et al. (1998) also found that when Lake Sturgeon were fed *Artemia* for two-weeks, followed by formulated diets, they were smaller and weighed less than those fed traditional diets. The findings of DiLauro et al. (1998) align with this study where sturgeon fed formulated diets have shorter total lengths and worse body conditions. However, unlike this study, DiLauro et al. (1998) observed similar survival rates for traditional and formulated diets (58.3 - 67.4%) indicating that a 2-week transitional period from *Artemia* to formulated diets may allow for greater acceptance. Another study by Lee (2018) used Lake Sturgeon that were co-fed *Artemia* and Otohime and then gradually transitioned to only formulated feed for the first 6 weeks and showed no mortality during each of the one-week trials. Thus, DiLauro et al. (1998) and Lee et al. (2018) both provide evidence that Lake Sturgeon can feed on *Artemia* and Otohime and grow well, while maintaining similar survival rates. To better understand the critical feed transition period, we fed sturgeon brine shrimp to initialize feeding, similar to DiLauro et al. (1998) and Valentine et al. (2017), and transitioned them to Otohime, used by Lee et al. (2018), and finally transitioned to Skretting trout feed. Survival estimates indicate that the low survival of formulated diets may be due to a critical transition period where chance of survival drops from 76.3% - 93.0% on day 25 of exogenous feeding to 16.2% - 50.5% on day 45 of exogenous feeding.

Length and Body Condition

Lake Sturgeon fed traditional diets were on average 1.5x larger and had better body condition than individuals fed formulated diets throughout the study. Similarly, sturgeon fed live *Artemia* were significantly larger than those fed frozen *Artemia* or formulated diets during past research efforts (DiLauro et al. 1998; Valentine et al. 2017). However, after 6 weeks of sturgeon being co-fed *Artemia* and Otohime, Lee et al. (2018) observed more percent weight gain in formulated diets (69.3 ± 1.5%) than traditional diets (58.3 ± 4.0%). Due to increased cost of a co-feeding regime consisting of brine shrimp and Otohime, sturgeon were transitioned off of brine shrimp, after a week, to lower the duration of feeding the more expensive brine shrimp. This transition resulted in similar length and body conditions found in DiLauro et al. (1998) and Valentine et al. (2017) than in Lee et al. (2018).

Lake Sturgeon have been shown to accept formulated diets at relatively low rates but can grow rapidly upon acceptance (DiLauro et al. 1998; Valentine et al. 2017; Lee et al. 2018). In this study, growth and body condition differed between the sturgeon fed the traditional and formulated diets, but stockable (~180mm) individuals were observed in both treatments at the end of the 120-day study period (Baker and Scribner 2017). Although relatively few individuals survived, these stockable individuals are evidence that Lake Sturgeon are able to consume formulated feed and grow to desirable sizes for hatchery facilities. By utilizing an extended co-feeding regime of *Artemia* and Otohime, as described by Lee et al. (2018), future studies may identify increased survival, higher growth, and high body condition of larval Lake Sturgeon.

Maternal Lineages

To maximize genetic contributions in production and limit impacts on wild strains it is important that hatchery practices eliminate activities that may alter genetic diversity (Schreier et al. 2012; Aloisi et al. 2019). Lake Sturgeon are broadcast spawners that release many eggs to be fertilized by a large number of males (Kapuscinski et al. 1996). During these spawning events, about five males will fertilize the eggs of a single female (Kapuscinski et al. 1996). Maternal lineages were not significantly different within diets on day 120 but were significantly different across diets for survival and growth. Body condition was similar within female three across traditional and formulated diets. This similarity may be due to differences in survival. caused by starvation, of individuals that had not readily accepted the formulated diet. Lake Sturgeon of female three that accepted the formulated diets survived and grew to stockable sizes and a body condition that was similar to individuals fed the traditional diet. Although maternal lineages showed evidence of equal representation and limited evidence of artificial selection of progeny, paternal lineage may need to be explored to further understand acceptance based on accepted mating schemes. Formulated diets may be an alternative to traditional diets by maintaining equal representation of Lake Sturgeon maternal lineages and supporting growth to desirable sizes.

Applications and Implications

Larval Lake Sturgeon fed the experimental formulated diets had reduced survival, generally slower growth, and were in worse condition than those fed traditional diets throughout the course of the study. Although formulated diets resulted in fewer fish that were generally smaller, some sturgeon accepted the formulated diet and grew readily. Maternal lineages used in this study also indicate limited risk of artificial selection of progeny. Based on these results of lowered survival, propagation can be altered to reach stocking requests by increasing egg take, where formulated diets would ideally provide a cost reduction of 10x that of traditional diets without accounting for additional facility costs.

The high-cost traditional diet consists of brine shrimp costing $109.03/kg, bloodworms costing $13.18/kg and krill costing $4.35/kg. Alternatively, while feeding the formulated diets the cost was relatively low where brine shrimp was $109.03/kg, Otohime was $21.89/kg, and Skretting trout feed was $3.08/kg for #0 and #1 and $1.85/kg for 1.6mm. Based on this study to it costs about $4.59 to produce a stockable sturgeon fed traditional diets and about $3.73 for a stockable sturgeon fed formulated diets. However, if all fish that survived were of ideal stockable size the cost of an individual fed the formulated diets would be about $0.26 ($2,580.15/10,000 individuals) and the cost for an individual fed the traditional diet to be about $2.19 ($21,875.70/10,000 individuals). Therefore, to meet a stocking number of 5,000 sturgeon it requires a starting population of about 60,241 individuals or ~1,200mL of eggs for formulated and about 6,605 individuals or ~132mL of eggs for traditional, with survival rates similar to the study. Without accounting for additional facility costs it would cost about $1,290.08 to feed formulated diets compared to $10,937.85 for traditional diets. This ideal reduction of cost of formulated diets and equal survival of maternal lineages could offset the lowered survival and growth by increasing egg take. This ideal scenario would allow hatchery managers, with a restricted budget, to produce stockable sturgeon at a fraction of the cost.

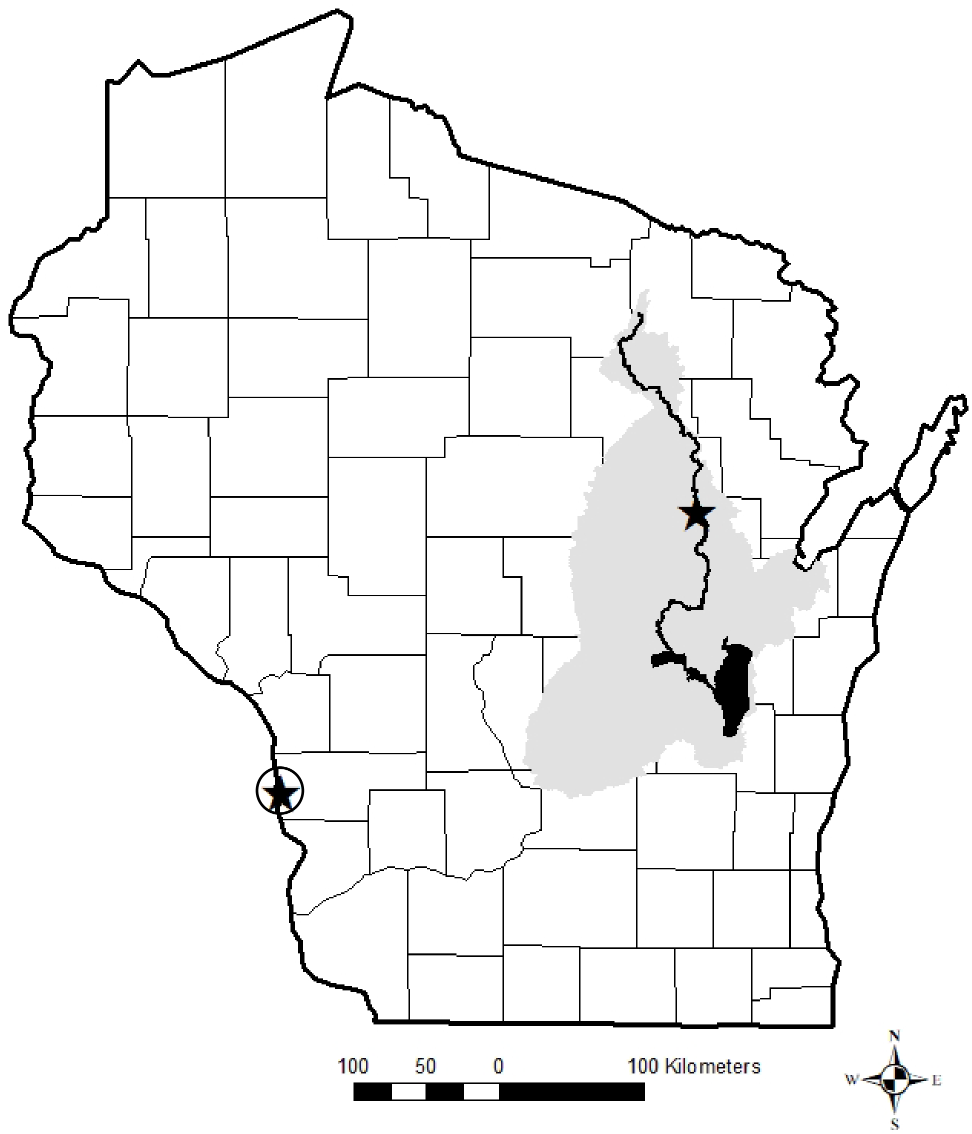

Figure 1. Map of Wisconsin indicating the spawning location of the Wolf River at the Shawano Dam in Shawano, WI (star) and rearing location, the Genoa National Fish Hatchery, (circled star) of Lake Sturgeon (*Acipenser fulvescens*) over the course of the 120-day study.

T: Traditional F: Formulated

F_1: Female 1 F_2: Female 2 F_3: Female 3

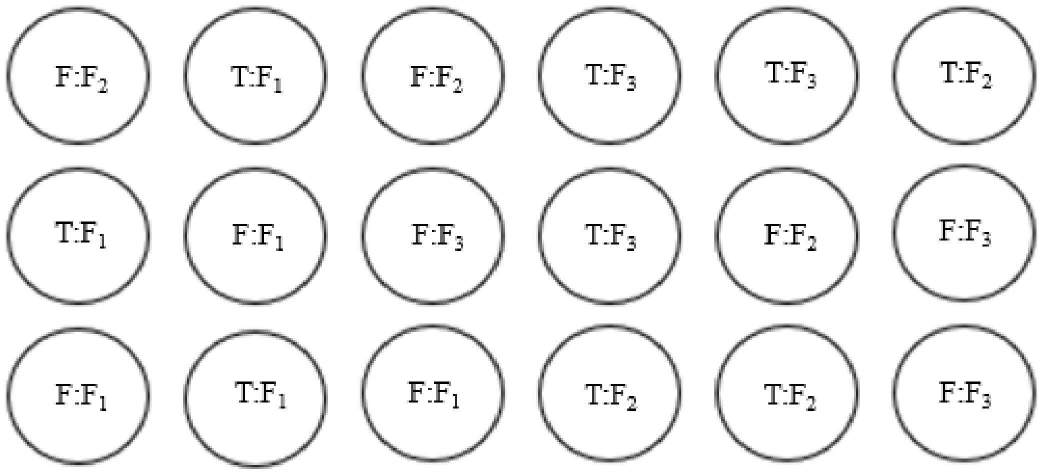

Figure 2. The randomized experimental set up of 18 tanks (~0.40m^3) used for the larval Lake Sturgeon (*Acipenser fulvescens*) feed study comparing traditional and formulated diets at the Genoa National Fish Hatchery (n = 400 per tank).

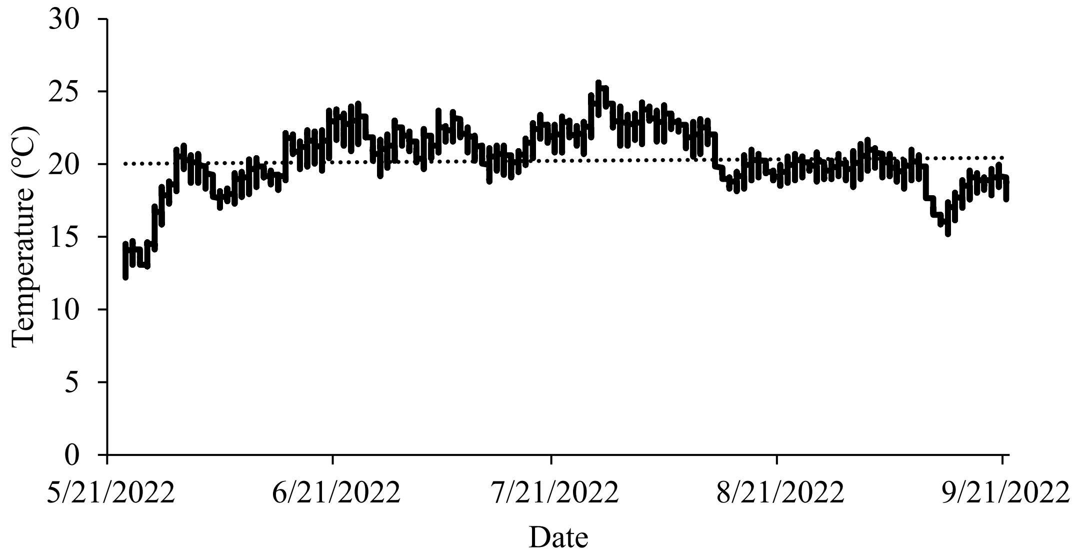

Figure 3. The water temperature (°C) of the Lake Sturgeon (*Acipenser fulvescens*) study system measured at 15-minute intervals throughout the 120-day study where the dotted line represents the average temperature.

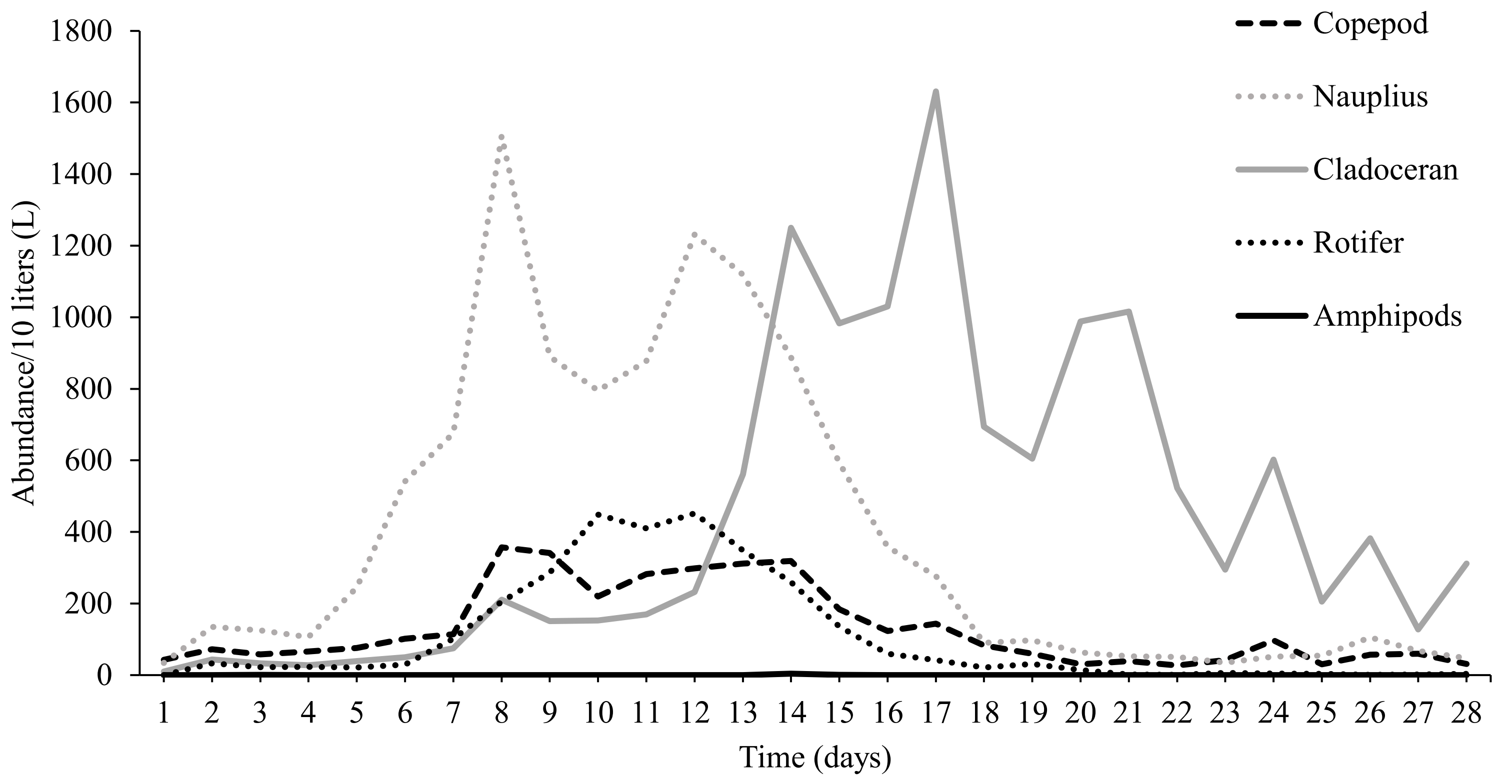

Figure 4. The abundance of zooplankton (>100µm, Copepods, Nauplius, Cladocerans, Rotifers and Amphipods) found within Lake Sturgeon (*Acipenser fulvescens*) rearing tanks in 10 liters of water during the first 28 days of the 120-day study.

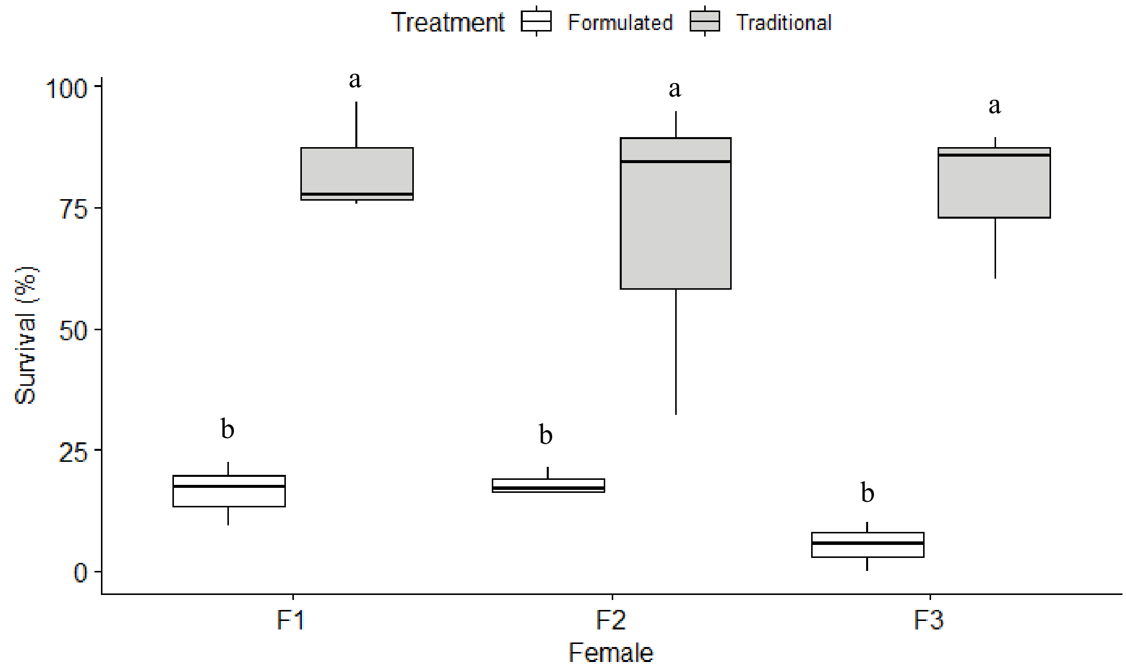

Figure 5. The final survival (%) of Lake Sturgeon across three females and two diets over a 120-day study period including TukeyHSD analysis where “a” are similar and “b” are similar.

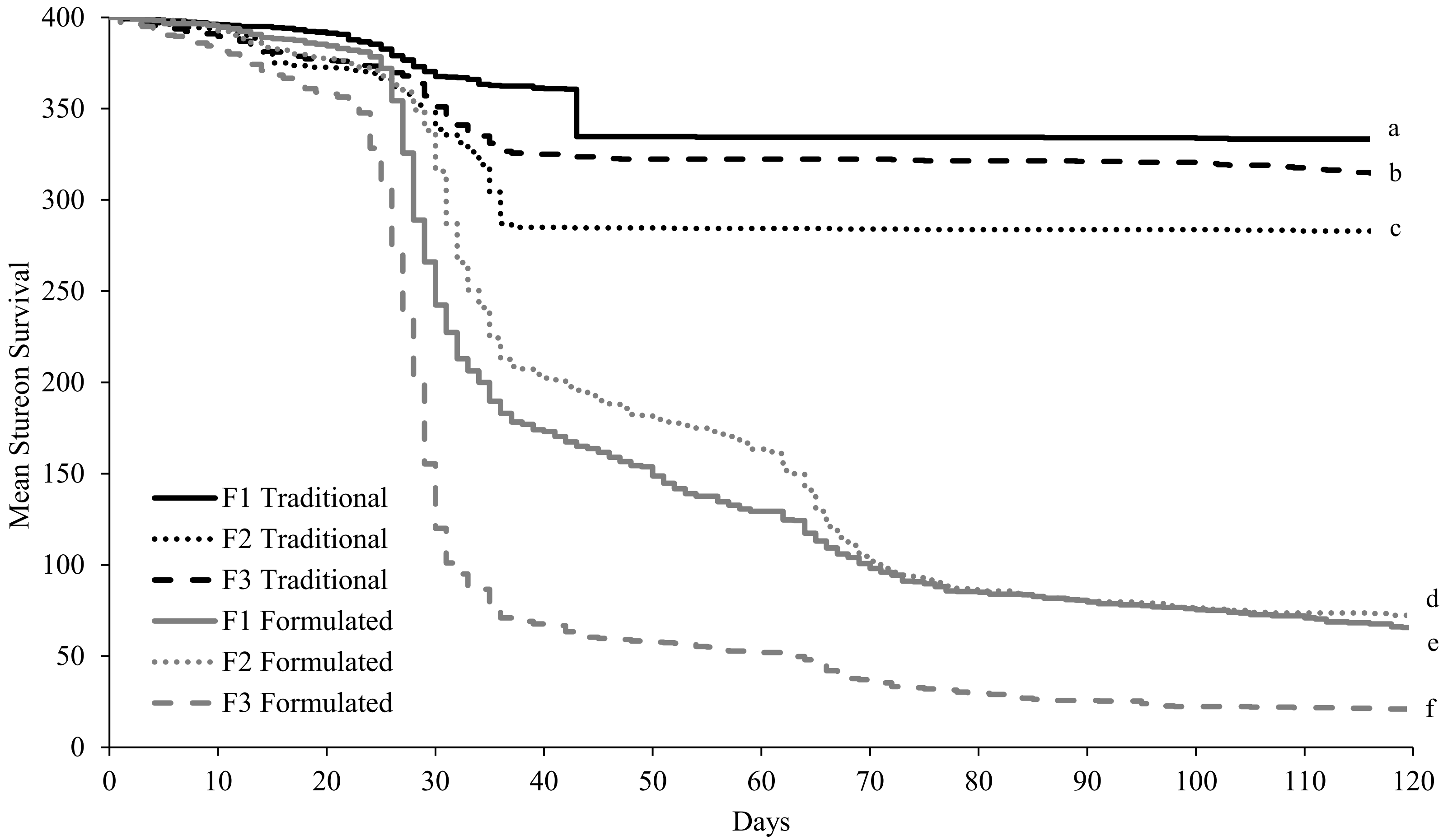

Figure 6. The mean survival of Lake Sturgeon (*Acipenser fulvescens*) over a 120-day study period by female and treatment type where a Kaplan Meier analysis revealed a significant difference in distribution of mortality of all females and treatments and is indicated by letters (P <0.001).

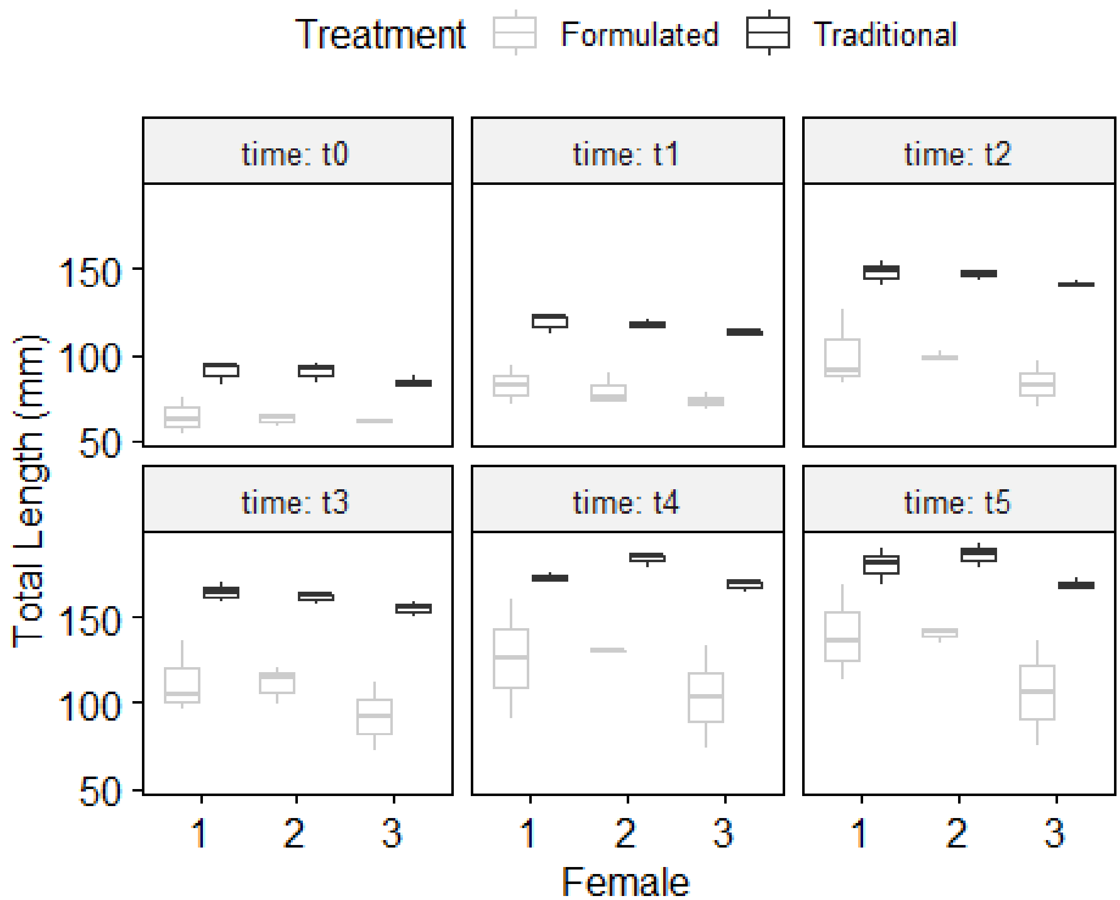

Figure 7. Boxplot of Lake Sturgeon (*Acipenser fulvescens*) total length (mm) of progeny of Female 1, Female 2, and Female 3 at six different time measurement intervals across formulated and traditional diets analyzed via repeated measures ANOVA. Determination of traditional diets to be significantly higher in total length than formulated diets ($F_{1,11}$ = 62.2, P <0.001) and females within each diet were similar ($F_{2,11}$ = 2.0, P =0.28).

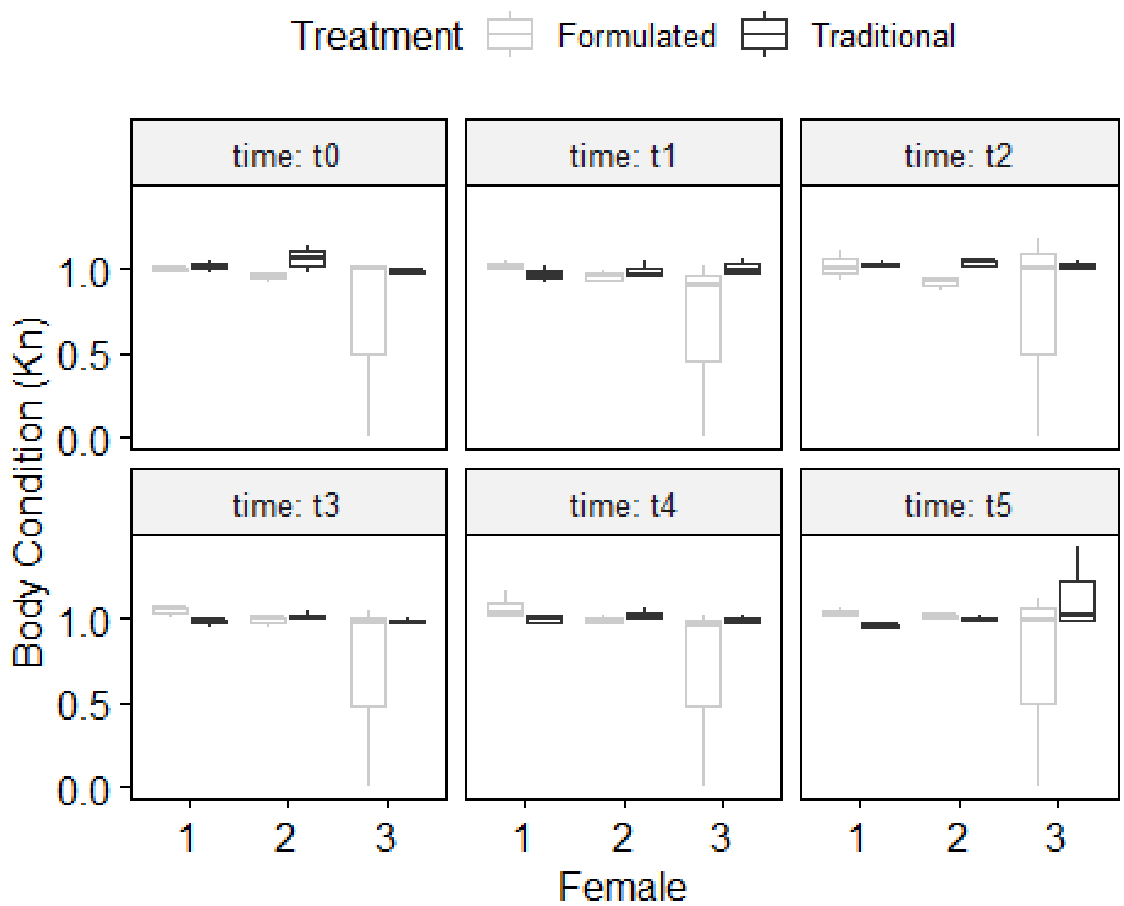

Figure 8. Boxplot of Lake Sturgeon (*Acipenser fulvescens*) body condition (K_n) across Female 1, Female 2, and Female 3 at six different time measurement intervals across traditional diets and formulated diets analyzed via repeated measures ANOVA. Determination of Lake Sturgeon maternal lineages fed traditional diets to be significantly higher in body condition than maternal lineages fed formulated diets ($F_{2,11} = 4.37$, $P = 0.04$, Figure 8).

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