COMPARISON OF TWO ALFALFA FERTILIZERS USED FOR WALLEYE FINGERLING PRODUCTION IN LINED PONDS

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ABSTRACT

Physiochemical variables, zooplankton densities, and walleye (*Sander vitreus*) fingerling production and food habits were compared in 0.8-ha, lined ponds provided alfalfa (*Medicago* spp.) as either ground hay (hay) or meal. Each fertilizer treatment was used in 4 ponds and a standard stocking density of 312,500 fry/ha was used in all ponds. Mean total Kjeldahl nitrogen (TKN) was significantly greater at two of four sampling dates in ponds fertilized with meal compared to those fertilized with hay. TKN increased in both treatments as the interval progressed while total phosphorus decreased. Dissolved oxygen was generally lower in the meal treatment compared to the hay treatment, but remained within an acceptable range (>5 mg/L) in both treatments. Pond water pH was significantly greater in the hay treatment, compared to the meal treatment, on two sampling dates. No significant differences were found among zooplankton densities at any sampling date. Walleye grew similarly until the end of the interval when individual walleye in the meal treatment were significantly longer (3 mm) than those in the hay treated ponds. Walleye survival was not different between alfalfa types, while yield was greater in the meal treatment. These results suggest that nitrogen limited production in the hay treatment.

Keywords

walleye, pond fertilization, fingerling production, lined ponds

INTRODUCTION

Alfalfa (*Medicago* spp.) products are commonly used as organic fertilizers at hatcheries that produce percid fingerlings (Summerfelt et al. 1993; Harding and Summerfelt 1993; Rogge et al. 2003). The decomposition of alfalfa by microbes serves as an immediate food source for zooplankton (Barkoh and Rabeni 1990), releases inorganic nutrients that promote phytoplankton growth that also support zooplankton (Qin and Culver 1996), and provides fine, particulate matter which nourishes benthic invertebrates (Hilsenhoff 2001). These characteristics are important because both zooplankton and benthic invertebrate larvae are reported to be the primary prey sources utilized by walleye (*Sander vitreus*) in drainable culture ponds (Fox and Flowers 1990; Rogge et al. 2003).

Previous studies have compared walleye fingerling production between fertilizer treatments. Harding and Summerfelt (1993) compared alfalfa pellets and soybean meal applied at similar total nitrogen, but differing biomass, and observed no differences in yield, size, or number at harvest. Interestingly, neither treatment resulted in differences in water quality or production compared to the control that did not receive any fertilizer. However, the next year, production was 45% greater in ponds that received alfalfa pellets compared to unfertilized ponds, highlighting that production can be improved with fertilizer use. Other studies have also shown a clear effect of fertilizer regimen on walleye production. Fox et al. (1989) found that a constant application of soybean meal (36 g/m^3/wk) significantly increased size, survival, and biomass compared to a progressively reduced strategy (32 to 0 g/m^3/wk). Culver et al. (1993) implemented an inorganic fertilization regimen once a week that raised the concentration of inorganic nitrogen (nitrate and ammonia) and phosphate concentrations to 600 µg/L as N and 30 µg/L as P. Percid fingerling survival was 77% greater in ponds fertilized with the aforementioned inorganics compared to inorganics combined with alfalfa meal. While previous studies have incorporated alfalfa products as a treatment in their comparisons, few, if any, have compared walleye production between alfalfa products. The objectives of this study were to compare physiochemical indices, zooplankton densities, and walleye production (growth, survival, and yield) and food habits in lined ponds provided alfalfa as either ground hay or meal.

METHODS

This study was conducted at Blue Dog Lake State Fish Hatchery, near Waubay, South Dakota, in the summer of 2011. Alfalfa as either ground hay (hay; grown on site) or meal (purchased from Labolt Farmers Elevator, Waubay, SD) were each provided to four, lined ponds (0.8 ha) that had been filled with unfiltered Blue Dog Lake water. The historical procedure for fertilization at Blue Dog Hatchery was followed for the ponds that received hay. This involved filling a loader bucket on a tractor and dumping the contents into a pond. A full bucket was assumed to be 113.6 kg of hay, which had been verified previously. However, the actual weights of hay used for this study were not verified. Meal fertilizer was purchased in bags weighing 22.7 kg and was manually poured into the ponds. Initial fertilization involved applying a standard amount of 227.3 kg of alfalfa (hay or meal), as well as 22.7 kg of yeast to all ponds on May 16, 17 or 18. Supplemental fertilization (113.6 kg) involved only alfalfa (hay or meal) and was administered twice at approximately 10-d intervals (May 27 and June 6). Walleye fry (1-3 d old) were enumerated by volumetric displacement (Piper et al. 1982) at a constant of 220 walleye fry/mL and were stocked at a standard rate of 312,500/ha in all ponds. Culture duration was 33 days for all meal ponds and either 33 or 34 days for hay ponds. The hay treatment ponds were filled and initially fertilized two or three days prior to stocking, while the timeline between initial fertilization and stocking in all meal ponds was one to five days. Variation (i.e., one to five days) in the timeline between filling and stocking is caused by the short timeline (three to five days) when large numbers of fry were available, and the longer timeline (ten days) that it takes to get all of the ponds filled at Blue Dog Hatchery. Thus, the timeline between filling and stocking generally averages three days for all ponds (Broughton et al. 2009).

Total nitrogen and phosphorus content of the hay was analyzed at Olson Biochemistry Laboratories at South Dakota State University, and percent protein (17%) of the feed label was used to determine nitrogen and phosphorus content of the meal fertilizer (Hubbell 1989). Later analysis of the alfalfa meal product (17% protein) occurred at South Dakota Agricultural Laboratories during 2015 and verified that the nitrogen and phosphorus values presented in Hubbel (1989) were accurate (within 0.1% for nitrogen and 0.02% for phosphorus). Nitrogen and phosphorus content of the yeast was not determined.

Physiochemical variables and zooplankton were measured weekly (May 24 and June 1, 6, and 14) beginning at 0830 h and ending by 1200 h. All physiochemical variables were collected or measured from the drain structure of the pond where the water depth is 1.5 m. Water samples were collected from 0.3 m below the surface. Total phosphorus (TP) and total Kjeldahl nitrogen (TKN) samples were frozen and sent to Olson Biochemistry Laboratories for determination (APHA et al. 1998), while ammonia as nitrogen (mg/L; Hach Method 8155, salicylate) and alkalinity (mg/L as $CaCO_3$; Hach Method 8203) were determined at Blue Dog Hatchery on the day of collection following procedures in Hach (1997). To determine chlorophyll a concentration (µg/L), I first filtered water samples through a 0.7-µm GF/F Whatman filter on the day of collection. The volume of filtered water was recorded, and filters were frozen. At a later date, filters were placed in alkalized acetone (90%) to extract chlorophyll a, and the concentration was measured with a TD-700 fluorometer (Turner Designs, Sunnyvale, CA). Dissolved oxygen and temperature were measured using a YSI Model 55 meter (YSI, Inc., Yellow Springs, OH) at three locations in the water column (subsurface, 1-m depth, and substrate) and an average was calculated. pH was determined with an Oakton 6 Acorn Series meter (Oakton Instruments, Vernon Hills, IL) only at the subsurface of each pond. Procedures for zooplankton sampling and quantification were the same as in Ward et al. (2011).

For each pond, walleye were collected at two dates (June 7 and 10) by seining (1.6 mm bar mesh), and total length measurements were obtained from 20 fish. Gastrointestinal tracts were removed from ten fish, and zooplankton and chironomids (head capsules) were identified and enumerated. At harvest (June 21 through 24), 65 fish were measured from each pond for the final total length estimate, and food habits were similarly quantified in ten fish. To estimate the number of fish harvested from each pond, I counted and weighed together a minimum of 250 fish to estimate the number of walleye per kilogram. Then, the total kilograms of walleye harvested from each pond was multiplied by the fish/kg estimate to determine the number of walleye harvested. Finally, the number harvested was divided by 250,000 (initial stocking estimate) and multiplied by 100 to determine percent survival for each pond.

Independent, two sample t-tests were used to test for significant differences in water quality, zooplankton, and walleye food habits and production between fertilizer types. Significance was set a priori at 0.05.

RESULTS

Walleye Growth, Survival, Yield and Food Habits. There was no significant differences in growth (t = 0.24-0.83, df = 6, P = 0.44-0.81) until the end of the interval when individual fish in the meal treatment were 3 mm longer than those in the hay treatment (t = 4.36, df = 6, P < 0.01; Figure 1). Survival was not different (t = 0.89, df = 6, P = 0.41), but yield was 23 kg/ha greater in the meal treatment compared to the hay treatment (t = 4.64, df = 6, P < 0.01; Table 1). Food habit comparisons revealed no differences between the number of zooplankton (t = 0.04-1.72, df = 6, P = 0.14-0.97) or chironomids (t = 0.90-1.86, df = 6, P = 0.11-0.40) present within digestive tracts on June 10 or at harvest (Table 2).

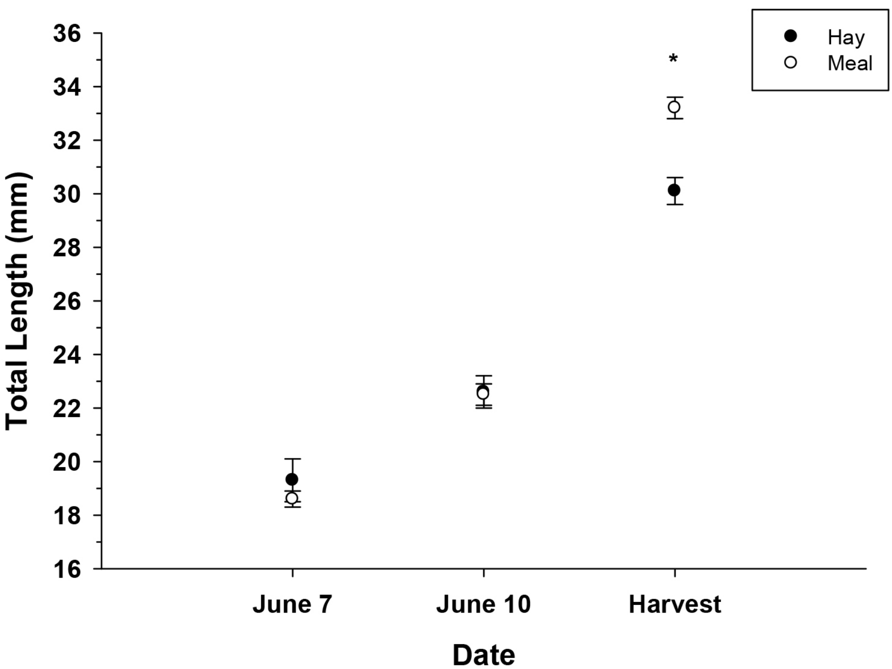

Figure 1. Mean (±SE) walleye total length (mm) at three dates in lined ponds fertilized with alfalfa as either hay or meal. An asterisk indicates a significant difference (P < 0.05) in total length between fertilizer treatments (n = 4).

Table 1. Mean (±SE) survival (%) and yield (kg/ha) for walleye raised in lined ponds fertilized with alfalfa as hay or meal. An asterisk in the meal row indicates a significant difference (P < 0.05) between treatments (n = 4).

Treatment	Survival	Yield
Hay	63 (4)	45 (4)
Meal	67 (1)	68 (3)*

Table 2. Mean (±SE) number of zooplankton and larval chironomids (number of head capsules) per walleye digestive tract in lined ponds fertilized with alfalfa as hay or meal. No significant differences were found between fertilizer types (P > 0.05) (n = 4).

	Number of Zooplankton/Walleye		Number of Chironomids/Walleye	
Date	Hay	Meal	Hay	Meal
June 10	9.3 (2.2)	9.2 (0.7)	1.2 (0.5)	2.7 (1.6)
Harvest	3.1 (1.7)	0.2 (0.2)	4.7 (1.1)	1.8 (1.2)

Fertilizer and Physiochemical Indices. As applied, estimated nitrogen content of the alfalfa types was the same (2.7%), while phosphorus content was slightly higher in the hay treatment (meal = 0.23%; hay = 0.28%). No significant differences were found between treatments for water temperature (t = 0.11-0.89, df = 4-6, P = 0.42-0.92), alkalinity (t = 0.09-1.46, df = 4-6, P = 0.19-0.93), or chlorophyll-a comparisons (t = 0.03-2.54, df = 4-6, P = 0.06-0.97) at any sampling date (Table 3). Chlorophyll-a remained low until the final sampling date when an increase was observed. Alkalinity decreased between May 24 and June 1 and then remained stable. A substantial increase in water temperature was noted on June 6, which corresponded to the lowest dissolved oxygen in both treatments (hay = 6.0; meal = 5.4). Dissolved oxygen was significantly lower in the meal treatment on June 1 (t = 2.59, df = 6, P = 0.04), while pH was significantly greater in the hay treatment on May 24 (t = 9.48, df = 4, $P < 0.01$) and June 6 (t = 5.22, df = 6, $P < 0.01$). TP was not significantly different at any sampling date (t = 0.39-0.98, df = 4-6, P = 0.38-0.70), and a decreasing trend over time was observed in both treatments. In contrast, TKN and ammonia as nitrogen values tended to increase over time. In the meal treatment, TKN was significantly greater on May 24 (t = 3.81, df = 4, P = 0.02) and June 14 (t = 5.18, df = 6, $P < 0.01$) compared to the hay treatment. Ammonia as nitrogen was never significantly different (t = 0.00-2.04, df = 4-6, P = 0.09-1.00) between treatments, but mean values were higher in the meal treatment at the last two sampling dates (Table 3).

Zooplankton. No significant differences were detected between treatments for total zooplankton (t = 0.22-0.95, df = 4-6, P = 0.38-0.84; Figure 2A), rotifer (t = 0.01-0.95, df 4-6, P = 0.38-0.99; Figure 2B) copepod (t = 0.11-1.6, df = 4-6, P = 0.18-0.92; Figure 2C), or cladocera densities (t = 0.79-1.82, df = 4-6, P = 0.12-0.48; Figure 2D). Overall zooplankton, rotifer, and copepod densities were initially high and then declined until June 14 when an increase was observed while mean cladocera density began low and tended to increase.

Table 3. Mean (±SE) physiochemical indices measured on four dates in lined ponds fertilized with alfalfa as hay or meal. For a given date, an asterisk indicates a significant difference (P < 0.05). Each treatment received equal replication per sampling date, but the number of replicates varied between three and four across dates. Temp = temperature, °C; DO = dissolved oxygen, mg/L; Alkal = alkalinity, mg/L $CaCO_3$; Chl-a = chlorophyll a, µg/L; TKN = total Kjeldahl nitrogen, mg/L; TP = total phosphorus, mg/L; NH_3-N = ammonia as nitrogen, mg/L.

	Temp		**DO**		**pH**		**Alkal.**	
Date	Hay	Meal	Hay	Meal	Hay	Meal	Hay	Meal
May 24	15.7 (0.2)	16.0 (0.3)	7.8 (0.2)	7.7 (0.1)	8.6 (<0.1)	8.4 (<0.1)*	122 (11)	135 (4)
June 1	14.0 (0.4)	13.9 (0.3)	8.1 (0.1)	7.8 (0.1)*	8.5 (0.1)	8.5 (0.1)	107 (9)	108 (5)
June 6	23.3 (0.3)	23.6 (0.3)	6.0 (0.2)	5.4 (0.3)	8.3 (<0.1)	8.1 (<0.1)*	103 (3)	110 (3)
June 14	18.8 (0.1)	18.9 (0.1)	7.7 (0.7)	7.6 (0.2)	8.4 (0.1)	8.2 (<0.1)	98 (4)	102 (5)

	Chl-a		**TKN**		**NH_3-N**		**TP**	
Date	Hay	Meal	Hay	Meal	Hay	Meal	Hay	Meal
May 24	0.2 (<0.1)	0.3 (<0.1)	0.640 (0.013)	0.777 (0.033)*	0.013 (0.009)	0.013 (0.009)	0.047 (0.005)	0.052 (0.004)
June 1	0.1 (<0.1)	0.6 (0.4)	0.808 (0.091)	0.824 (0.033)	0.050 (0.007)	0.040 (0.021)	0.029 (0.003)	0.035 (0.005)
June 6	0.2 (<0.1)	0.2 (0.1)	0.792 (0.043)	1.132 (0.144)	0.110 (0.028)	0.167 (0.044)	0.029 (0.001)	0.034 (0.005)
June 14	1.2 (0.2)	1.2 (0.2)	0.959 (0.027)	1.263 (0.052)*	0.113 (0.038)	0.218 (0.109)	0.016 (0.006)	0.013 (0.003)

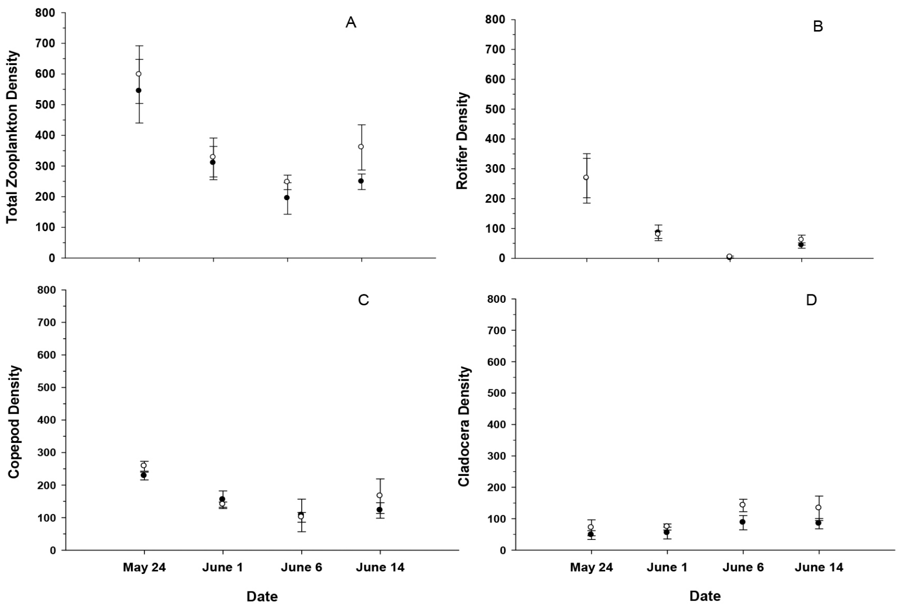

Figure 2. Mean (±SE) total zooplankton, rotifer, copepod, and cladocera density (number of individuals/L) in lined ponds fertilized with alfalfa as either hay (filled) or meal (unfilled). Each treatment received equal replication per sampling date, but the number of replicates varied between three and four per comparison. No significant differences were found between fertilizer treatments.

DISCUSSION

The positive results on walleye yield from the meal fertilizer in this study are likely due to increased nitrogen levels, which have been previously documented. Harding and Summerfelt (1993) measured differences in average total ammonia nitrogen in unfertilized earthen ponds between 1990 and 1991. Walleye yield was an additional 32 kg/ha in 1990 when average total ammonia nitrogen concentration increased from 0.14 mg/L to 0.53 mg/l. At Rathbun Hatchery in Iowa, Rogge et al. (2003) documented that nitrogen was the limiting nutrient in plastic-lined ponds that were used to raise walleyes. More recent research at this hatchery suggested that increasing nitrogen inputs from 20.2 to 34.8 kg/ha via organic fertilizers will allow for the use of higher stocking densities and an increased number of fingerlings (additional 44,000/ha) that still attained an aggressive size goal of 0.57 g/walleye (Johnson and Esser 2012). The authors did note that ponds that received higher nitrogen inputs produced elevated unionized ammonia levels (0.3 mg/L), however no mortality was observed. Helal (1990) showed that bottom-up effects of inorganic nitrogen to phosphorus ratios can affect energy transfer to walleye in earthen ponds through autotrophic food webs. Specifically, weekly additions of inorganic fertilizer that raised concentration ratios in ponds to 20 nitrogen : 1 phosphorus reduced the bust ponds (<10% percid survival) from 47% to <10% at three Ohio hatcheries (Culver et al. 1993). This ratio has since been adjusted to 60 nitrogen : 1 phosphorus and has been implemented for saugeye (female walleye × male Sauger (*Sander canadensis*)) production at Ohio hatcheries (Jacob and Culver 2010). The large amount of nitrogen required relative to phosphorus supports the contention that nitrogen most likely limits production.

Identifying how the increased nitrogen levels in the meal treatment produced the increased production is more challenging. No differences were found among chlorophyll-a or zooplankton densities in this study which suggests that either benthic prey sources were responsible for the increased walleye production or the relationship between phytoplankton, zooplankton and fish biomass within the pond was changing rapidly and precluded detection of differences. For instance, primary production may have been higher in the meal treatment, but increased consumption by zooplankton may explain the lack of differences in chlorophyll-a. In this scenario, increased consumption of zooplankton by walleye may similarly explain the lack of differences in zooplankton densities between treatments. Culver et al. (1992) described the complex relationships between these trophic levels as not being in an equilibrium state. They suggested that pond yields could be increased through increasing percid stocking density to reduce the zooplankton population, thereby promoting zooplankton reproduction rather than overgrazing on algae that results in zooplankton competition and mortality. Thus, it seems possible that the higher nitrogen levels in the meal treatment fueled the increased production through autotrophic pathways, and no differences were detected in chlorophyll-a concentrations or zooplankton density because of increased rates of consumption of subsequently higher trophic levels.

The higher nitrogen levels in the meal treatment may have also simply coincided with an increased rate of decomposition that appeared to occur in the meal treatment. Significantly lower pH values (i.e., higher carbon dioxide) and consistently lower dissolved oxygen concentrations, as well as significantly greater TKN levels corroborate a faster rate of decomposition and mineralization was occurring in the meal treatment. Fertilizer particle size is suggested to affect decomposition rate (Barkoh and Rabeni 1990) but was not measured in this study. Both zooplankton and chironomids could have benefited from the increased decomposition of the meal fertilizer through heterotrophic pathways and produced the increased walleye production also. Aquatic insects (mostly chironomids) have been found to account for 45% to 90% of walleye diets by weight during the latter half of the pond interval at White Lake Fish Culture Station in Ontario (Fox and Flowers 1990). Chironomid densities were not estimated in this study, but according to the food habit samples there was no difference in the number of chironomids or zooplankton in walleye digestive tracts on June 10 or at harvest which captures the timeframe when growth was increased in the meal treatment (Figure 1). The prey resource and mechanism responsible for the increased production of walleye in the meal treatment is not known.

In summary, both types of alfalfa produced largely similar and suitable water quality parameters, zooplankton densities, and walleye survival, but meal was superior in terms of walleye size and subsequent pond yield. The exact mechanism was not determined, but higher TKN levels in the meal treatment suggest that nitrogen limited production in the hay treatment. To test this finding, I suggest that a future study compare walleye production between fertilizer treatments with differing nitrogen inputs.

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