The Progressive Fish-Culturist 57:199–205, 1995

Ammonia Excretion by Rainbow Trout over a 24-Hour Period at Two Densities during Oxygen Injection

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Abstract.—Ammonia and dissolved oxygen (DO) concentrations were measured at six times over a 24-h period in 1992 and 1993 in raceways containing two densities of rainbow trout (*Oncorhynchus mykiss*). Ammonia production was highly variable, with peak concentrations 136–490% higher than the lowest concentrations, which usually occurred just before dawn. The peaks were generally observed during daylight, but could not be accurately predicted by feeding time or time of day. Ammonia concentrations observed in this study were compared with levels predicted by published models or summarized in other studies. The ratios of ammonia concentrations in higher-density raceways to the mean ammonia concentration of the two control raceways ranged from 1.54 to 2.15, which were similar to the ratios of fish density (1.75 to 2.58; high density/mean control density), indicating that ammonia production, on a per fish basis, was the same at both densities. Ammonia should be measured several times during the day for greatest accuracy. One measurement should be taken prior to the first morning feeding, when ammonia concentrations are lowest. Concentrations of DO at the lower end of the raceways showed a diel variation; the greatest oxygen consumption occurred at feeding time (in anticipation of feeding) or during the first hour after feeding. The differences between the highest and lowest DO values were greater (133–329%) in raceways with higher densities of fish. The greater fluctuation in higher-density raceways underscored the importance of choosing the appropriate time (within the first hour after feeding) to monitor DO in order to maintain adequate oxygen flows.

Oxygen injection technology has recently allowed fish to be reared at higher densities than previously possible (Colt and Watten 1988), and this increases the possibility of lethal or sublethal effects of ammonia on fish. Un-ionized ammonia is toxic to fish, although the reported threshold concentration varies, depending on factors such as salinity, pH, and dissolved oxygen (DO) concentration (see reviews by Meade 1985; Randall and Wright 1987). Sublethal concentrations of ammonia can be deleterious to fish, influencing fish behavior (Woltering et al. 1978) and causing gill lesions (Smith and Piper 1975; Lang et al. 1987).

Measurement of ammonia concentrations for monitoring hatchery populations is complicated by the diel variations of ammonia concentration. Although the diel fluctuations of ammonia and oxygen consumption have been studied, results have been mixed. Rychly and Marina (1977) found some peaks in ammonia excretion in fed fish in a static system, but the timing and magnitude of those peaks were highly variable. Ewing et al. (1993) also reported highly variable data. Gunther et al. (1981) found that ammonia concentrations in a recycled-water system were roughly cyclic, fitting a Fourier series form, with peaks in the evening and troughs in the early morning. Brett and Zala (1975) measured the ammonia excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) and found that ammonia concentration peaked about 4–4.5 h after feeding and dropped to a daily low between 0200 and 0800 hours. Oxygen consumption peaked just before and during the first hour after feeding (Brett and Zala 1975). The present study was conducted, in part, to further explore the diel dynamics of ammonia and oxygen in oxygen-supplemented raceways with two densities of rainbow trout (*Oncorhynchus mykiss*).

Schuett (1933) observed that oxygen consumption in fish held singly was greater than that of fish held in groups. Parker (1973) also noted this phenomenon, which he attributed to an interaction of a calming effect and a possible hydrodynamic effect. Based on this research, it was hypothesized that at the higher densities of this study, lower metabolic rates and lower ammonia production rates would be observed. This phenomenon, if true, would permit higher densities of fish in systems where ammonia might be a limiting factor.

This study also compared observed ammonia levels with those predicted by published models. Accurate models are useful for production predictions, particularly in oxygen-injection systems where oxygen may not be the limiting factor.

Methods

The ammonia tests were conducted as part of an overall study evaluating the effects of rearing rainbow trout in raceways with supplemental oxygen and at higher than normal densities (Miller et al. 1995, this issue). The overall study was conducted with two lots of Sand Creek strain rainbow trout, one reared in 1992 and the other in 1993. In both 1992 and 1993, two of the raceways were stocked with 12,000 fish each and two others with 3,000 fish each. These population figures were calculated from the mean fish weight of three samples and the total biomass. Mortality records were maintained daily. Each raceway received supplemental oxygen from a low-head oxygen injection unit (Watten and Boyd 1990). In 1992, oxygen flows were kept constant at the upper end of all four raceways; oxygen flows to the injection unit during the rearing period were increased equally among raceways. In 1993, oxygen flows were manipulated throughout the rearing period (April–November 1993) to maintain an equal concentration of oxygen leaving the raceways. During the 24-h tests, the oxygen flow was constant.

The 24-h tests were conducted on 8–9 May and 2–3 October 1992 and on 24–25 and 26–27 August and 16–17 and 18–19 November 1993. Ammonia and oxygen concentrations were monitored hourly from 0800 to 2000 hours, and every 2 h thereafter until 0600 hours on the following day. Additional samples were taken at 0700 and 0800 hours. In 1993, the hours between 2400 and 0600 were not sampled because previous testing showed that ammonia concentrations declined linearly during this period. Extrapolation between samples taken at 2400 hours and just before dawn would thus be representative of ammonia concentrations during the period.

In 1992, densities at the time of the May sample averaged 7.98 and 27.08 kg/m^3 (density indexes of 0.10 and 0.36; Piper et al. 1982) in the control and higher density raceways. In the October sample, mean densities were 18.6 and 68.2 kg/m^3 (density indexes of 0.16 and 0.65). In the August and November 1993 sampling, densities were 43.2 and 50.0 kg/m^3 (density indexes of 0.49 and 0.55) in the higher density raceways and 17.3 and 27.9 kg/m^3 (density indexes of 0.15 and 0.19) in the controls. The mean weights of the fish (all raceways combined) were 17 and 50 g in May and October 1992 and 38 and 65 g in August and November 1993.

Fish were hand fed between 0800 and 0835, 1200 and 1215, and 1645 and 1715 hours, either immediately before or after the sample for that hour was taken. Equal amounts of commercial pelletized feed (at 1–2% of body weight) were weighed out into separate containers for each of the three daily feedings. Feed samples were analyzed for protein content in October 1992, and August and November 1993. The percent protein in the feed was consistently 41–42%.

To simulate the typical hatchery routine, the raceways were cleaned immediately after the 0900-hour sample. Care was taken to exclude unnecessary foot traffic from the vicinity of the raceways. When taking samples, the raceways were approached from the lower end, giving the upper portion a wide berth to avoid triggering a feeding frenzy.

Dissolved oxygen concentrations were measured at the lower end of the raceways with a polarographic probe (YSI Inc., Yellow Springs, Ohio), calibrated with replicate Winkler titrations (APHA et al. 1989). Ammonia concentrations were determined by the Nesslerization method (APHA et al. 1989), with six standard concentrations used for the standard curve. The water samples were preserved by adding sulfuric acid and then stored in a refrigerator until they were neutralized with sodium hydroxide and brought to room temperature prior to analysis. Standards and reagent blanks used in the assay were similarly treated.

The well water supplied to each of the raceways had total hardness of 222 mg/L as $CaCO_3$, total alkalinity of 205 mg/L as $CaCO_3$, pH of 7.6, and temperature of 17°C. Water flows were maintained at 291 L/min throughout the study. The calculated water exchange rate of the raceway was 1.85 replacements/h.

The ammonia concentrations found in this study were compared with concentrations predicted by models or summarized in other studies. For the mean ammonia concentration of this study, an average of all the samples in the 24-h period was used. To avoid a weighting bias in the calculation of the mean, data for all hours without measured values were calculated by averaging the values of the previous and subsequent hours. Oxygen consumption for input into Liao's (1974) model was based upon data collected by Miller et al. (1995). For inputs into Paulson's (1980) rainbow trout regression model, the nitrogen content of the feed was assumed to be 8.7% (Paulson 1980), and all feed fed was assumed to have been consumed.

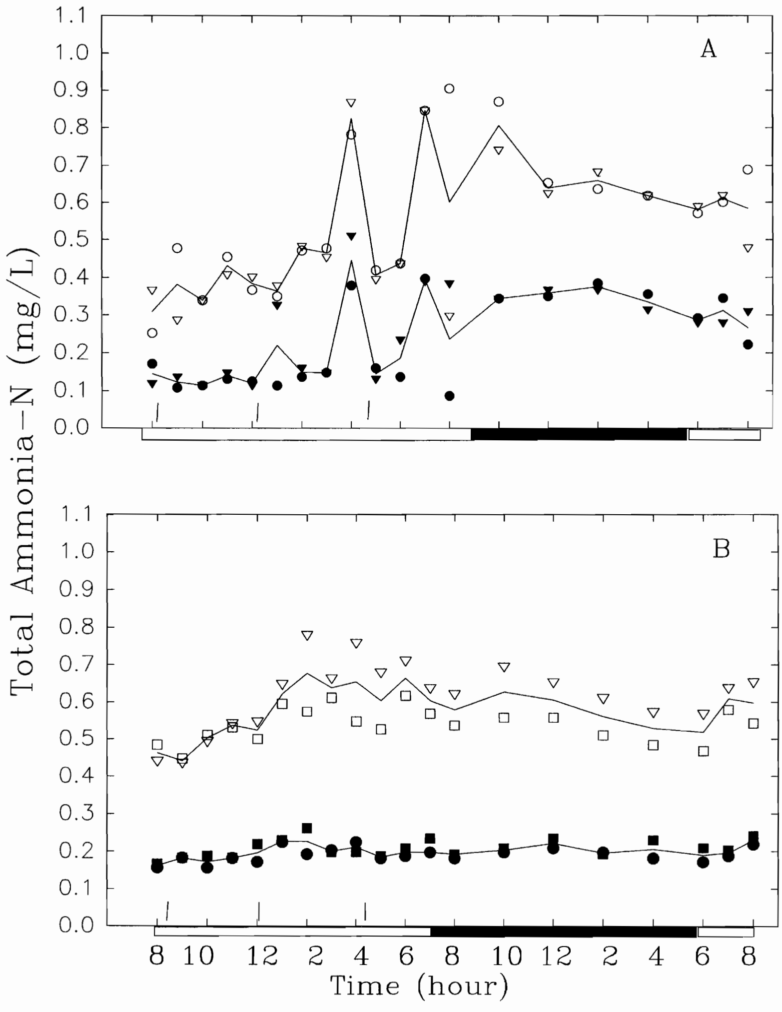

FIGURE 1.—Total ammonia nitrogen concentrations measured over a 24-h period (starting at 0800 hours) at the lower end of raceways containing a control density (solid circles and triangles) or a higher density (open circles and triangles) of rainbow trout. The lines represent means of two replicates. Measurements were made in 1992 on **(A)** 8–9 May and **(B)** 2–3 October. The vertical lines above the *x*-axis indicate the three feeding times; the solid dark line indicates hours of darkness.

Results and Discussion

Ammonia concentrations measured over a 24-h period were highly variable, both in 1992 (Figure 1) and 1993 (Figure 2). Peak concentrations were 136–490% higher than the lowest concentrations, which usually occurred just before dawn. The peaks were generally observed during daylight, but could not be accurately predicted by feeding time or time of day. This variability agrees with the findings of Rychly and Marina (1977) and Ewing et al. (1993), who observed no diel pattern. The patterns described by Gunther et al. (1981) and Brett and Zala (1975) were not observed in this study. Because Gunther et al. (1981) presented the means for a 3-d period, the intertank and among-day variations were not shown. Unfortunately for fish culturists interested in monitoring ammonia, no peak time was predictable from the data. Since peak concentrations can be five times the lowest ammonia concentrations, measurement of ammonia for culture purposes should be made several times during the day for greater accuracy. For greater consistency in measurements over days or weeks, sampling at dawn or prior to the first feeding is recommended.

As expected, higher ammonia concentrations

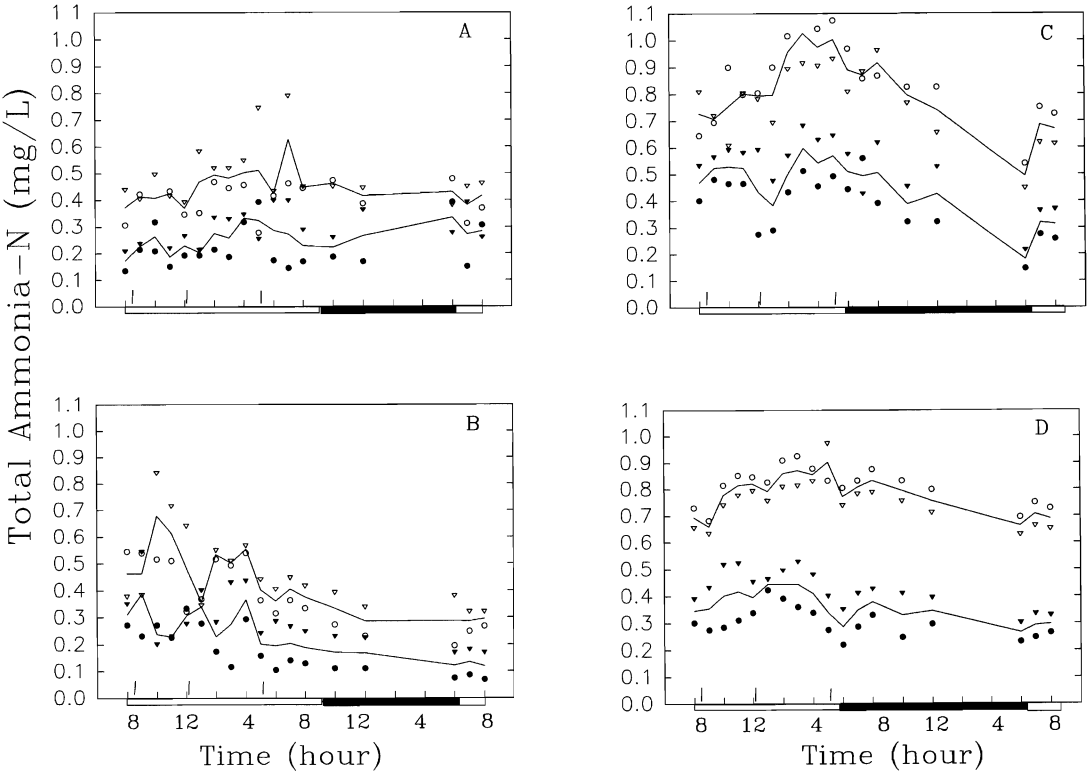

FIGURE 2.—Total ammonia nitrogen concentrations measured over a 24-h period (starting at 0800 hours) at the lower end of raceways containing a control density (solid circles and triangles) or a higher density (open circles and triangles) of rainbow trout. The lines represent means of two replicates. Measurements were made in 1993 on **(A)** 24–25 August, **(B)** 26–27 August, **(C)** 16–17 November, and **(D)** 18–19 November. The vertical lines above the *x*-axis indicate the three feeding times; the solid dark line indicates hours of darkness.

were noted in the effluent of raceways with the higher density of fish, but higher densities did not lower the ammonia production on a per-fish basis. The ratio of ammonia in higher density raceways to a mean of the two control densities ranged from 1.54 to 2.15, and was similar in both August and November 1993 samples. These ratios were similar to the fish density ratios (1.75 to 2.58; high density to mean control density).

Several models have been developed to predict ammonia concentrations in aquatic systems. Willoughby et al. (1972) estimated ammonia production based upon the amount of feed fed and the water flow. Paulson (1980) developed a regression model of ammonia excretion that took into account the effects of temperature, the amount of nitrogen in the feed fed, and the weight of the fish. Liao (1974) estimated ammonia production based on oxygen consumption. With the oxygen consumption data presented by Miller et al. (1995), ammonia production estimated by Liao's method (1974) was only 10–33% of observed ammonia.

Meade (1985) summarized the efforts of five researchers who predicted daily ammonia production based upon the amount of feed fed each day; the reported values ranged from 20 to 78.5 g total ammonia/kg feed. In this study, the daily values ranged from 35 to 69 g/kg feed in the higher density raceways, and from 39 to 125 g/kg feed in controls. The ranges given by Meade (1985) were converted to total nitrogen (μg/L) and are presented as maximum and minimum values in Table 1. With the exception of two means that exceeded the range, all the observed ammonia concentrations fell within this range.

Comparison of other predicted concentrations (Willoughby et al. 1972; Paulson 1980) with observed levels of ammonia in this study are also presented in Table 1. The concentrations predicted by Paulson (1980) ranged from 36 to 642% of

TABLE 1.—Comparison of total ammonia nitrogen between concentrations observed in this study (24-h mean) and predicted by Willoughby et al. (1972), Meade (1985), and Paulson (1980).

			Ammonia nitrogen (μg/L)				
					Meade		
Date	Unit	Number of fish	This study	Willoughby	Minimum	Maximum	Paulson
8 May 1992	1	11,960	589	272	170	667	170
	2	2,994	245	71	44	174	44
	3	11,979	537	272	170	667	170
	4	2,922	277	71	44	174	43
2 Oct 1992	1	11,855	535	424	265	1,039	449
	2	2,975	191	99	62	244	106
	3	11,846	620	506	315	1,238	496
	4	2,903	209	103	64	251	117
24 Aug 1993	1	11,726	415	272	170	665	219
	2	2,886	240	103	64	251	89
	3	11,848	472	374	234	917	296
	4	2,940	298	188	118	462	246
26 Aug 1993	1	11,726	340	272	170	665	219
	2	2,886	152	103	64	251	89
	3	11,848	442	374	234	917	296
	4	2,940	262	188	118	462	246
16 Nov 1993	1	11,574	801	667	416	1,634	676
	2	2,870	343	229	143	560	360
	3	9,560	714	640	399	1,566	766
	4	2,932	474	330	206	808	1,126
18 Nov 1993	1	11,574	794	667	416	1,634	676
	2	2,870	316	229	143	560	360
	3	9,560	702	640	399	1,566	766
	4	2,932	403	330	206	808	1,126

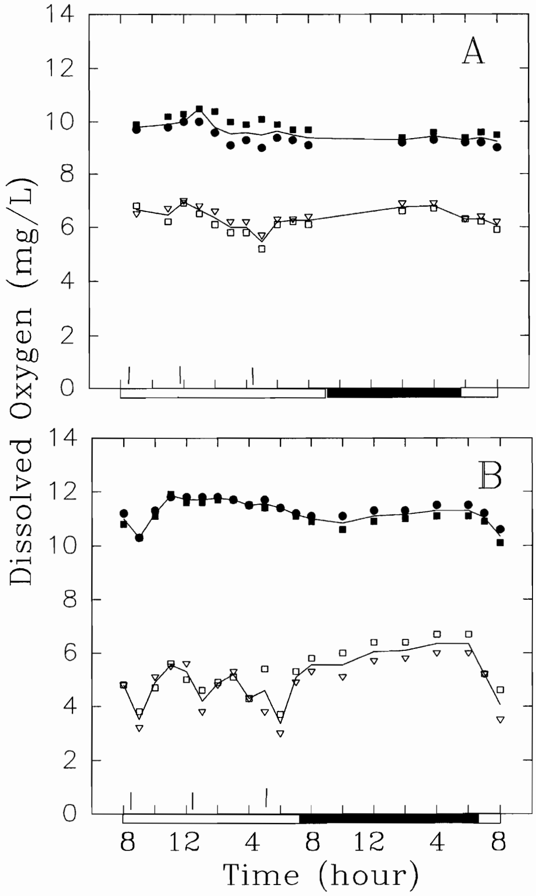

FIGURE 3.—Dissolved oxygen concentrations measured over a 24-h period (starting at 0800 hours) at the lower end of raceways containing a control density (solid circles and triangles) or a higher density (open circles and triangles) of rainbow trout. The lines represent means of two replicates. Measurements were made in 1992 on **(A)** 8–9 May and **(B)** 2–3 October. The vertical lines above the *x*-axis indicate the feeding times; the solid dark line indicates hours of darkness.

observed ammonia concentrations. The discrepancy was greatest in the May 1992 samples when fish were only 17 g in size. The other samples were fairly close to Paulson's predictions. Ammonia production predicted by the formula presented in Willoughby et al. (1972) was 26–91% of that observed. The difference was more pronounced in control raceways than in raceways with higher densities. Differences may be due to differences in the protein content of the diet used in the two studies. A positive relationship between the percentage of protein in the diet and ammonia production has been observed (Goldstein and Forster 1970; Jayaram and Beamish 1992; Li and Lovell 1992).

Concentrations of DO at the lower end of the raceways varied in a diel pattern, similar to that reported by Brett and Zala (1975); the greatest oxygen consumption occurred at feeding or during the first hour afterwards (Figures 3, 4). Oxygen consumption increased in anticipation of feeding in several instances. The low DO after feeding was not observed in November 1993 samples taken after the evening feed. This is probably because darkness had a greater effect upon oxygen consumption than feeding and digestion just prior to dark. The difference between the highest and lowest DO values was generally greater (by 133–329%) in raceways with higher densities of fish. The greater fluctuation in higher density raceways underscored the importance of choosing the appropriate time to monitor DO in order to maintain adequate oxygen flows.

The effects of oxygen supersaturation on ammonia concentrations could not be tested because a control raceway without supplemental oxygen

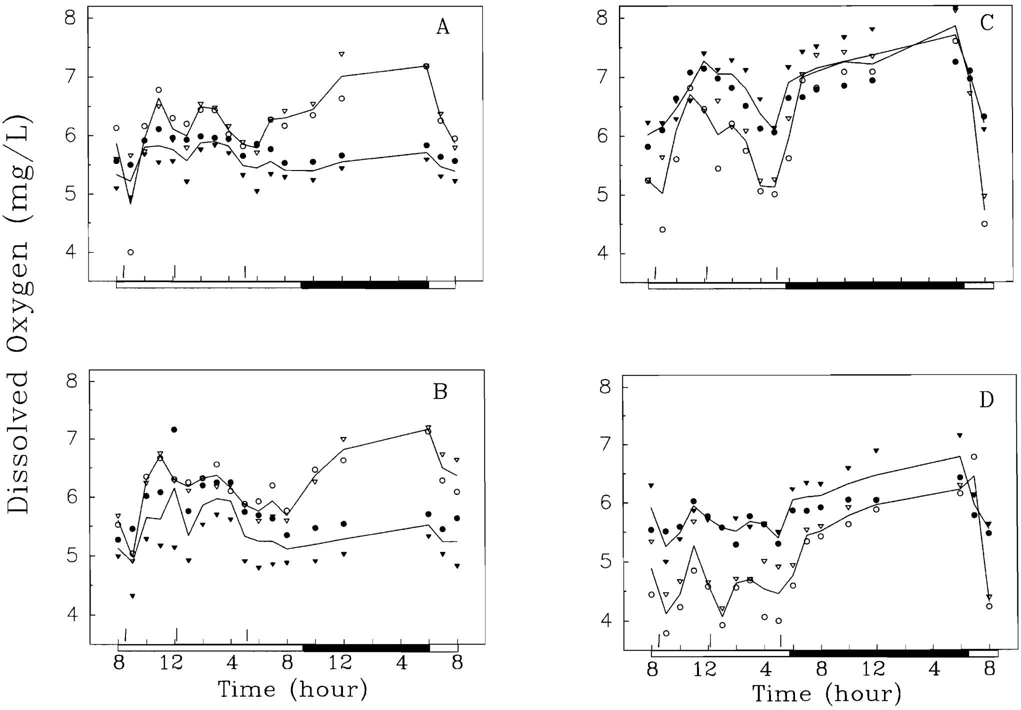

FIGURE 4.—Dissolved oxygen concentrations measured over a 24-h period at the lower end of raceways containing a control density (solid circles and triangles) or a higher density (open circles and triangles) of rainbow trout. The lines represent means of two replicates. Measurements were made in 1993 on **(A)** 24–25 August, **(B)** 26–27 August, **(C)** 16–17 November, and **(D)** 18–19 November. The vertical lines above the *x*-axis indicate the feeding times; the solid dark line indicates hours of darkness.

injection was not included in this experiment. However, ammonia production was similar to that summarized by Meade (1985) for systems without supplemental oxygen. Hanna et al. (1991) added ammonium chloride to water supersaturated with oxygen, but did not observe any differences in the response of fish (humoral immunity, weight gain, feed conversion, condition factor, or survival) relative to control fish in water with dissolved oxygen below saturation.

In summary, density had no effect upon ammonia production per fish. The ammonia production model by Paulson (1980) was the most accurate for predicting observed ammonia levels, probably because factors such as nitrogen consumption, size, and temperature were accounted for. Predictions by the model of Willoughby et al. (1972) underestimated ammonia, but it was more accurate than the oxygen model of Liao (1974), which also underestimated ammonia concentrations. Because of diel variation, DO should be measured within the first hour after feeding to avoid mortality due to hypoxia. Ammonia should be measured at several times during the day for greater accuracy. One of these measurements should be prior to the first feeding in the morning when ammonia concentrations are lowest.

Acknowledgments

We thank T. Miles, D. Routledge, B. Burningham, and D. Alplanalp for the fish husbandry. We thank M. Ashcroft and A. Salar of the Utah Department of Agriculture for analysis of the fish feed. We gratefully acknowledge R. Goede, R. Lee, J. Valentine, and C. Wilson for their review of the manuscript. This research was supported by the Utah Division of Wildlife Resources and the Federal Aid in Sport Fish Restoration program, project F-53-R.

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