Effects of fish size and feeding frequency on metabolism of juvenile walleye

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Department: Animal Ecology
Interdepartmental Major: Water Resources

Iowa State University
Ames, Iowa

1991

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GENERAL INTRODUCTION

Because the walleye (Stizostedion vitreum) is a highly prized sport and food fish, interest in the commercial production of walleye as a food fish has grown. There is limited supply of walleye flesh from natural sources but strong demand for this product. This fact has produced high market prices which are encouraging the development of walleye aquaculture. Production systems for walleye include shallow pond sites to rear fingerlings and tank culture systems to train pond-reared fish to formulated feed (Colesante et al. 1986). Once walleye fingerlings have been trained to formulated feed they can be reared to food-size in single-pass (once through) or recycle systems.

In single-pass aquaculture systems oxygen is replenished and waste products removed by continuous replacement of the culture water with well oxygenated fresh water. In water-reuse aquaculture, the culture water is reused after biological removal of ammonia and reaeration; with only small additions of fresh water (Piper et al. 1986). The critical components of reuse systems are those needed for addition of dissolved oxygen (DO) and conversion of ammonia (NH_3) to non-toxic nitrate. Effective procedures and apparatus for waste removal and oxygen addition are of the utmost importance to

obtain growth and to maintain fish health (Brett 1979; Colt and Armstrong 1981).

The advantages of water-reuse systems over single-pass systems include: economical maintenance of constant culture temperatures, lower overall water demand and greater flexibility in site selection (Luchetti and Gray 1988). However, before reuse systems can be rationally designed to rear walleye, information is needed concerning the oxygen consumption and waste excretion rates of walleye under intensive culture conditions, and how to equalize diel variations in fish metabolism by regulation of the feeding schedule. The intensive culture of walleye to near food-size in closed-loop water-reuse systems has previously been untested or unsuccessful, due principally to a lack of information concerning the physiological responses of walleyes to the intensive culture environment (Craig 1987; Cai and Summerfelt 1991).

Oxygen Consumption and Fish Respiration

The concentration of oxygen in water is inversely related to temperature and salinity and directly proportional to barometric pressure (Colt 1984). Dissolved oxygen in intensive culture systems is depleted by respiration of the cultured species and by biochemical oxidation (BOD) of metabolic waste products (Piper et al. 1986). In single-pass

rearing systems, continuous removal of feces and wasted feed from culture tanks by high exchange rates limits the relative effect of BOD on available oxygen, therefore, fish respiration is the major source of oxygen depletion in these systems. However, in water-reuse systems dissolved organics and solids which are not removed by simple filtration can produce substantial BOD. Water-reuse systems must be designed with considerations for both BOD and fish respiration.

When ambient DO concentrations are high (80-100% saturated) the external oxygen tension exceeds the internal oxygen tension of a fish, forming an oxygen gradient. Oxygen quickly diffuses across the gill tissues and into the blood (Randall and Daxboeck 1984). When environmetal oxygen concentrations are less than saturation, the oxygen gradient between blood in the fish's gill capillaries and water is greatly reduced and fish are unable to remove oxygen from the water, resulting in reduced growth and eventual death. Brett (1979) states that any reduction in oxygen concentration below saturation will result in reduced growth. Piper et al. (1986) suggest oxygen concentrations of > 5 mg L^{-1} for trout and > 3 mg L^{-1} for walleye fry are necessary for fish survival, while Westers (1979) suggests minimum allowable effluent levels of 5.0, 4.0 and 3.0 mg L^{-1} for cold-, cool- and warmwater fish, respectively.

Ammonia Excretion

Ammonia is the principal nitrogenous excretory product of fish, accounting for up to 90% of branchially excreted nitrogen (Smith 1929). Nitrogen may also be excreted as urea or trimethylamine oxide (Forster and Goldstein 1969). In some teleost species urea may account for 20% or more of the total nitrogen excreted (Wood 1958).

Ammonia is produced during protein digestion and amino acid catabolism. Deamination of amino acids produces ammonia, which if not excreted would become toxic to the fish (Schmidt-Nielsen 1983). While some ammonia passes through the kidney and is excreted in the urine, the major pathway of ammonia excretion is through the gills (Smith 1929). Smith (1929) reported that 6 to 10 times more nitrogen was excreted through the gills of common carp (Cyprinus carpio) and goldfish (Carassius auratus) than in the urine. Regardless of the source, water-reuse systems must be designed with considerations for all nitrogenous excretions.

Two forms of ammonia exist in aqueous solutions: unionized ammonia (NH_3-N), which is highly toxic to aquatic organisms, and ionized ammonia (NH_4^+-N) (Burrows 1964; Colt and Armstrong 1981; Thurston et al. 1979). The sum of NH_3-N and NH_4^+-N is referred to as total ammonia nitrogen (TAN). Analytical procedures for measurement of ammonia measure TAN (APHA et al. 1989). The equilibrium between NH_3 and NH_4^+ is

principally a function of the pH, temperature and ionic strength of the aqueous solution with NH_3 predominating at higher temperatures and pH levels (Thurston et al. 1979). NH_3-N concentrations can be calculated as a percentage of TAN based on ambient pH values and temperatures (Thurston et al. 1979).

Ammonia may cause hyperplasia of gill epithelium (Meade 1985; Burrows 1964), impaired oxygen carrying capacity of blood hemoglobin (Brockway 1950), impaired cerebral energy metabolism (Smart 1978) and acute kidney failure (Lloyd and Orr 1969). Colt and Armstrong (1981) report that in channel catfish (Ictalurus punctatus) and rainbow trout (Oncorynchus mykiss) significant growth reduction occurs at unionized ammonia concentrations of 0.05 to 0.20 mg L^{-1}.

Aeration and Ammonia Treatment Options

Maintaining adequate dissolved oxygen levels in intensive aquaculture systems is achieved by in-tank aeration, aeration of reuse water and/or continuous addition of well oxygenated fresh water (Lucchetti and Gray 1988; Piper et al. 1986). U-tube aerators, air injection or pure oxygen injection are all efficient ways of maintaining adequate dissolved oxygen levels in rearing water (Colt and Tchobanoglous 1981). Speece (1981) identifies 11 ways

dissolved oxygen concentrations can be maintained in reuse systems.

Ammonia can be removed from reuse systems by air stripping, biological nitrification and ion exchange (Lucchetti and Gray 1988). In the air stripping process, the pH of the water is raised to > 10 to convert all forms of ammonia to the unionized, gaseous phase, then the water is sprayed into the air in small droplets resulting in dissipation of the gaseous ammonia (Piper et al. 1986). Biological nitrification is accomplished in a two-step process using Nitrosomonas spp. and Nitrobacter spp. bacteria that convert NH_3 to harmless nitrate (NO_3^-). Ammonia may also be removed by passing reuse water through a column of natural zeolite, which removes ammonia by an ion exchange process (Lucchetti and Gray 1988).

Regardless of how aeration or ammonia removal is achieved, the sizing and subsequent operation of aeration and treatment apparatus is dependent upon the oxygen consumption and ammonia excretion rates of the cultured species. In addition, stocking densities must be calculated based on oxygen requirements and the ability of an aquaculture facility to meet those requirements (Willougby 1968).

Factors Affecting Oxygen Consumption and Ammonia Excretion

Although oxygen consumption has generally been used in determining metabolic rates (standard, routine and active), oxygen consumption and ammonia excretion rates are related (Table 1; Brett and Zala 1975; Cai and Summerfelt 1991; Elliott 1976; Fry 1957; Cai 1988). Ammonia excretion is a direct result of metabolism making it an appropriate measure of metabolism (Fry 1971; Kaushik and Dabrowski 1983).

The metabolic rate of a fish, measured by oxygen consumption or ammonia excretion (mg kg^{-1} h^{-1}), is affected by the physiological state of the animal (Fromm and Gillette 1968; Savitz 1969; Guerin-Ancey 1976), temperature (Forsberg 1989; Jobling 1981; Porter et al. 1987; Fry 1971; Cai and Summerfelt 1991), the digestible protein content of the diet (Lied and Braaten 1984; Ramnarine et al. 1987; Kaushik 1980) and body size (Brett 1965; Cai and Summerfelt 1991; Beamish 1964). The metabolic rates of walleye have been studied by Beamish and MacMahon (1988), Forsberg (1989) and Cai and Summerfelt (1991). These studies describe the effects of temperature, size and feeding strategy on the metabolic rates of walleye weighing less than 130 g.

Objectives

The objectives of this project were: 1) to assess the effects of size (mass) on the average, maximum and minimum

Table 1. The relationship of rates (mg kg^{-1} h^{-1}) of oxygen consumption and ammonia excretion reported in the literature

Metab. rate	Fish species	Mean wgt. (g)	Std. rate[a]	Rtn. rate[b]	Act. rate[c]	Source
oxygen	sockeye	29	168	274	370	Brett and
ammonia	salmon		7.27	14.5	35	Zala (1975)
ratio			23.1	18.9	10.6	
oxygen	walleye	4-6	---	293	333	Cai and
ammonia				21	26	Summerfelt
ratio				13.9	12.8	(1991)
oxygen	walleye	33-37	---	230	256	Cai and
ammonia				19	24	Summerfelt
ratio				12.1	10.7	(1991)
oxygen	walleye	57-66	---	240	267	Cai and
ammonia				14	18	Summerfelt
ratio				17.1	14.8	(1991)
oxygen	walleye	127-	76	158	172	Cai and
ammonia		133	0.9	12	14	Summerfelt
ratio			84.4	13.1	12.3	(1991)
oxygen	common	11-13	267	592	728	Cai (1988)
ammonia	carp		9.3	60.1	105	
ratio			28.7	9.9	6.9	

[a] Standard rates of metabolism defined as resting rates of unfed fish.

[b] Routine rates of metabolism defined as spontaneous rates of fed fish under normal conditions.

[c] Active rates of metabolism defined as active rates of fed fish forced to swim continously.

metabolic rates of walleye reared in a commercial production setting and 2) to determine the ammonia excretion and oxygen consumption rates and hourly variations in these rates as functions of feeding frequency of juvenile walleye (> 200 g) in an intensive culture environment. This information is essential in determining daily operating parameters and in sizing aquaculture equipment for commercial walleye production in intensive water-reuse systems.

Explanation of Thesis Format

This thesis has been produced in the alternate thesis format. A general introduction and summary are given; Sections I and II will be submitted for publication in scientific journals under the authorship of Timothy K. Yager and Robert C. Summerfelt. The style used in this thesis follows that of the Transactions of the American Fisheries Society.

SECTION I. EFFECTS OF SIZE AND FEEDING FREQUENCY ON METABOLISM OF JUVENILE WALLEYE

ABSTRACT

The effects of fish size and feeding frequency on mean rates of metabolism of juvenile walleye reared under intensive culture conditions are presented. Mean rates of oxygen consumption were significantly affected by fish size. For each gram increase in fish weight of fish ranging from 200 to 425 g, the rate of oxygen consumption declined by 0.2198 mg kg^{-1} h^{-1}. Mean rates of ammonia excretion, however, were not significantly affected by fish size, but declined by 0.0054 mg kg^{-1} h^{-1} for each gram increase in fish weight. Mean daily rates of oxygen consumption and ammonia excretion were not significantly affected by feeding frequency after the effects of fish size were removed from the analysis of variance. However, the variation in mean oxygen consumption was significantly affected by feeding frequency. The variance in mean oxygen consumption rates declined with increasing number of feedings per day.

INTRODUCTION

It is generally accepted that the metabolic rates of most vertebrates decline with increasing size. The relation of metabolism to body weight has been described by the equation:

$$M/W = aW^{b-1}$$

where M/W is the rate of metabolism (M) per kg of body weight (W)(Fry 1971). Expressed arithmetically, the relationship is curvilinear, thus, it has been the practice to fit a straight line to a logarithmic transformation of the data (Fry 1971; Cai and Summerfelt 1991):

$$\log (M/W) = \log a + (b-1)\log W.$$

Beamish (1964) found that weight-specific oxygen consumption rates of several fish species decreased with increasing weight. Brett (1965) observed a rapid decrease in oxygen consumption of sockeye salmon (Oncorynchus nerka) with increasing fish size. Kindschi et al. (1990) observed declines in oxygen consumption with increasing weight of two strains of trout. Cai and Summerfelt (1991) reported a decline in routine oxygen consumption of walleye with increasing weight from 4 to 133 g.

Jobling (1981) reported that weight-specific rates of nitrogenous excretions declined with increasing fish size in experiments on 5 to 90 g plaice (Pleuronectes platessa).

Porter et al. (1987) observed peak ammonia excretion rates declined as weight increased from 3 to 90 g in gilthead seabream (Sparus aurata).

There are few studies on the effect of feeding frequency on fish metabolism. Beamish and MacMahon (1988) reported no significant effect of feeding frequency on metabolism, but recommended adoption of a frequent feeding strategy to enhance food intake.

In many studies of fish metabolism, one to several (normally less than 10) fish are observed in specialized chambers (respirometers) and are often "starved" or fasted before measurements of metabolism are made (Savitz 1971; Tandler and Beamish 1981; Kaushik and Dabrowski 1983; Cai 1988). In studies of active metabolism, the fish are stimulated (electroshock, or fast current) to swim continuously during measurements of metabolism (Tandler and Beamish 1981; Cai 1988). Both of these type studies are of value to fish physiologists, but extrapolating results obtained on few fish under stressed conditions for the purpose of designing and operating intense, commercial operations is difficult. Studies which examine entire tanks of intensively reared fish under various conditions are needed for this purpose.

The objectives of this project were to assess the effects of fish size and feeding frequency on mean rates of

oxygen consumption and ammonia excretion of larger (> 200 g) walleye reared intensively. Fish were fed from two to fifteen times daily and metabolic rates were monitored during thirteen 24-h periods.

METHODS

Culture Conditions

Two groups of fish were used to assess the effects of size and feeding frequency on metabolism. Group 1 (GRP1) walleye averaged 313 ± 28 mm (total) and 339 ± 99 g on January 25, 1990. The mean condition factor (K = (weight in g X 10^5)/(length in mm)^3) of GRP1 fish on January 25 was 1.08 ± 0.17. Group 2 (GRP2) fish averaged 258 ± 30 mm (total) and 166 ± 58 g on May 17, 1990. The mean condition factor of GRP2 fish on May 17 was 0.92 ± 0.10.

Walleye were reared in round (155 cm diameter, 75 cm depth) fiberglass tanks with water volumes of about 1136 L. The culture tanks were supplied with dechlorinated tap water. Dechlorination was accomplished with two in-line high pressure tanks filled with granular activated carbon. The supply water was periodically checked for the presence of chloramines and free chlorine with test kits (HACH Company, Loveland, CO). No evidence of chlorine or chloramines was ever detected. Heated water was mixed with cold water to approximately 23^{o}C and degassed by passage through a column (0.61 m diameter by 4.88 m) packed with 8.5 cm polyethylene ballast rings (Glitsch, Inc., Dallas, TX).

Flow rates to the rearing tanks were monitored once per hour by computer (model AT286/16 PC; Gateway 2000, Sioux

City, SD) using FP 5800 flow transmitters (Omega, Inc., Stamford, CT) connected to a data acquistion and control system (Strawberry Tree^{TM}, Inc., Sunnyvale, CA). Flow rates averaged 11.9 $\pm$ 1.0 L min^{-1} (0.6 tank exchange h^{-1}) except during 24-hour diurnal studies when rates were increased to maintain in-tank DO concentrations of near 5 mg L^{-1}. In-tank DO levels always exceeded 4.9 mg L^{-1} and were normally at or near saturation (approximately 8.5 mg L^{-1} at 23^{o}C). Water temperatures were measured with a mercury thermometer (0.01^{o}C). In-tank pH values were measured with a combination pH electrode connected to a model 05669-20 pH-vision microcomputer (Cole-Parmer, Chicago, IL). Average water temperatures and pH values based on means of 1 daily observation were 23.2 $\pm$ 0.5^{o}C and 7.23 $\pm$ 0.5, respectively. Except during 24-hour studies, rearing tank water was continuously aerated through flexible membrane diffusers (Parkson Inc., Ft. Lauderdale, FL) supplied with compressed air.

Rearing tanks had a center drain and were largely self-cleaning, excess feed and feces was removed quickly. Tank walls and the center standpipe were scrubbed periodically to remove bacterial and fungal growth from these surfaces.

Culture room light was controlled to provide a 15 h-20 min light and 8 h-40 min dark photoperiod (0440 - 2000).

Light intensity at the water surface was 8.6 ± 2.4 lx. No feed was introduced during dark periods.

Feeding

Feed was dispensed with scraper feeders operated by time clock. Fish were fed 15 times daily in intervals varying in number and length (Table 1). Fish were fed BioDry 3000 or 1000 (BioProducts, Inc., Warrenton, OR) in the 6 or 8 mm pellet size at a constant ration of 2% of in-tank fish biomass per day. These are high protein (44.5%), semimoist (14.5%) feeds. The proximate compositions of BioDry 3000 and 1000 are the same except for a red pigment in BioDry 3000. Rations and food sizes were adjusted weekly to compensate for changes in fish biomass and size. Feeding rations for days 1 through 7, 8 through 14, and 15 through 21 were calculated from in-tank biomass estimates for days 4, 11 and 18, respectively. Estimates of fish size on these dates were derived from measurements of length and weight at 21-day intervals. A linear regression of length on the number of days in culture was used to estimate length on days 4, 11 and 18 of each 21-day growth interval. These length estimates were then applied to a log-log regression of weight on length to calculate approximate fish weight and in-tank biomass on days 4, 11 and 18.

Table 1. Feeding variables for GRP1 and GRP2 walleye

Dates	Number of feeding intervals	Time	Duration (h)
GRP1			
January 25 to April 4	2	05:00-06:30 17:00-18:30	1.5 h 1.5 h
April 5 to April 26	3	05:00-06:00 11:30-12:30 17:00-18:00	1.0 h 1.0 h 1.0 h
April 27 to May 17	15	05:00-19:00	hourly
GRP2			
June 29 to July 19	2	05:00-06:30 17:00-18:30	1.5 h 1.5 h
July 20 to August 9	3	05:00-06:00 11:30-12:30 17:00-18:00	1.0 h 1.0 h 1.0 h
August 10 to September 19	15	05:00-19:00	hourly

Daily Monitoring of Ammonia-N (TAN)

Both influent and effluent flows were sampled daily with the use of composite samplers (N-Con Systems Co., New Rochelle, NY), one sampler for each flow. This device consists of a small electrically driven pump connected to a timer. These samplers were used to obtain a composite sample throughout a daily interval. A 100 ml sample of water was collected once every 20 min for a 24 h period. Influent and effluent samples were collected separately in 18.9 l Nalgene^{TM} jugs. Approximately 10 ml of concentrated sulfuric acid (H_2SO_4) was added to the receiving jugs prior to sampling to lower the pH of composite samples to < 2.0. APHA et al. (1989) states that TAN samples preserved in this manner may be held for up to 28 days, but for best results should be analyzed within 7 days of collection. All samples were analyzed within 7 days of collection, generally in less than 4 days. Two 100 ml sub-samples were collected from each composite sample and were analyzed for TAN concentrations using an Orion model 95-12 ammonia selective electrode (APHA et al. 1989). The average daily in-tank TAN concentration was 0.54 ± 0.24 mg L^{-1}. The average un-ionized ammonia concentration, based on pH and temperature, over the entire study was 0.005 ± 0.009 mg L^{-1}, below concentrations considered to influence growth or well-being (Piper et al. 1986).

24-hour Feeding Trials

Thirteen 24-h diurnal monitorings of ammonia excretion and oxygen consumption were conducted, 7 with GRP1 fish and 6 with GRP2 fish. Three different feeding treatments were utilized: twice daily, three times daily and hourly for 15 hours (Table 2). A polyethylene cover was placed over the tank during each monitoring event to limit gaseous exchanges with the atmosphere. Light intensity below this cover was 20% lower (6.9 $\pm$ 1.3 lx) than under normal conditions (8.6 $\pm$ 2.4 lx). Supplemental in-tank aeration was not used during 24-h studies, but flow rates were increased to maintain effluent DO concentrations near 5 mg L^{-1}. Flow rates averaged 39.7 $\pm$ 0.6 L min^{-1} (2.1 tank exchanges h^{-1}) for monitorings 2 through 13. The average flow rate for monitoring 1 was 45.0 $\pm$ 0.4 L min^{-1} (2.4 tank exchanges h^{-1}).

Samples for effluent TAN and DO concentrations were collected every 30 min, but influent TAN and DO samples were collected every 2 h because their concentrations were consistent. Two replicate water samples of influent and effluent flows were collected to measure TAN and DO concentrations. These samples were collected from sample ports in the supply and drain pipes, near to, but not in the rearing tank itself. At the conclusion of some 24-hour runs, samples were collected from in-tank influent and effluent

Table 2. 24-hour trial dates and treatment variables

Trial dates (1990)	Number of feeding intervals	Time	Duration (h)
February 16 to 17	2	05:00-06:30	1.5 h
March 2 to 3		17:00-18:30	1.5 h
March 30 to 31			
April 11 to 12	3	05:00-06:00	1.0 h
April 18 to 19		11:30-12:30	1.0 h
		17:00-18:00	1.0 h
May 2 to 3	15	05:00-19:00	hourly
May 9 to 10			
July 5 to 6	2	05:00-06:30	1.5 h
July 18 to 19		17:00-18:30	1.5 h
July 25 to 26	3	05:00-06:00	1.0 h
August 2 to 3		11:30-12:30	1.0 h
		05:00-06:00	1.0 h
August 22 to 23	15	05:00-19:00	hourly
August 27 to 28			

sites and compared to sampling site data to verify the representativeness of sampling site locations. No significant differences (t-tests, $P > 0.05$) existed between sampling site and in-tank concentrations for DO and TAN (Table 3). DO samples were collected in 300 ml BOD bottles and analyzed using the Azide modification of the Winkler method (APHA et al. 1989). TAN samples were collected in 150 ml polyethylene bottles, acidified to $pH < 2.0$ and analyzed using the electrode method (APHA et al. 1989). Flow rates and effluent temperatures were recorded every 30 min. Effluent pH values were recorded every 60 min.

Fish Lengths and Weights

Measurements of total length (± 1 mm) and weight (± 1 g) were recorded on 30 to 60 fish at 21 (20-23) day intervals. To sample fish from the tanks, flow was stopped through the rearing tank and tricaine methansulfonate (Finquel^{TM}; Argent Chemical Laboratories, Redmond, WA) was added to produce a concentration of approximately 22 ppm, sufficient to sedate the fish. All fish in each tank were sedated to reduce oxygen debt which might occur if the fish were chased about the tank to collect them for full anesthesia. After 10 to 15 minutes induction time for the anesthesic, fish were randomnly netted from the tank and placed in a solution of 80 ppm Finquel^{TM} and 1% NaCl. This solution produced rapid and

Table 3. Comparisons of in-tank and sampling site concentrations of DO and TAN. Average influent and effluent samples were compared using Student's t-tests

	TAN (mg L^{-1})				DO (mg L^{-1})			
	influent		effluent		influent		effluent	
Date	in-tank	samp. site	in-tank	samp. site	in-tank	samp. site	in-tank	samp. site
4/26	0.038	0.044	0.266	0.266	7.65	7.58		
5/3	0.024	0.031	0.147	0.141	8.11	8.04	5.33	5.38
5/10	0.025	0.031	0.144	0.139			5.31	5.29
6/15	0.008	0.007	0.049	0.047	8.20	8.10	5.79	5.74
6/28	0.027	0.026	0.040	0.043	8.21	8.28	6.30	6.24
7/6			0.077	0.077	8.28	8.14	6.20	6.04
7/19	0.009	0.007	0.100	0.113	8.34	8.30		
7/26	0.011	0.012	0.165	0.165	8.12	8.13	5.66	5.57
8/3	0.007	0.008	0.127	0.127	8.07	8.05	6.20	6.18
8/23	0.007	0.007	0.137	0.143	7.93	8.01	5.15	5.16
8/28	0.010	0.012	0.175	0.169	8.19	8.09	5.23	5.14
Mean	0.017	0.019	0.130	0.130	8.11	8.07	5.57	5.53
SD	0.010	0.013	0.060	0.060	0.19	0.19	0.41	0.38
n =	10	10	11	11	10	10	9	9
t =	0.366		0.011		0.453		0.255	
P > t	0.73		0.99		0.67		0.82	

deep anesthesia in 3 to 5 min at which time fish could be handled without resistance. After weighing and measuring, anesthetized fish were placed in a recovery tank with a continuous flow of fresh water. Fish were returned to the rearing tank after a recovery interval of 20 to 30 minutes.

Biomass Estimates

Mean lengths and weights were calculated from fish measurements and plotted (Y-axis) with number of days in culture (X-axis) for GRP1 and GRP2 fish. These length/weight-day graphs showed a close-fit linear relationship existed for both length and weight to days in culture (Figure 1). Estimates of mean fish weight and ultimately in-tank biomass for monitoring events 1 through 13 and daily ammonia sampling were calculated from the linear regression equations of weight and number of days in culture (Table 4).

Mass-specific Metabolic Rates

For 24-hour studies of oxygen consumption and ammonia excretion and for daily ammonia excretion data, the difference in DO and TAN concentrations between the influent and effluent samples, taking into consideration the flow rates and fish biomass present in the rearing tank, was used as a measure of the rate of oxygen consumed or ammonia

Figure 1. Growth in length (top) and weight (bottom) of GRP1 (left) and GRP2 (right) walleye reared for 112 days (January 25 to May 17, 1990) and 126 days (May 17 to September 20, 1990), respectively. Growth rates were 0.26 mm d^{-1} and 0.86 g d^{-1} for GRP1 and 0.54 mm d^{-1} and 1.52 g d^{-1} for GRP2

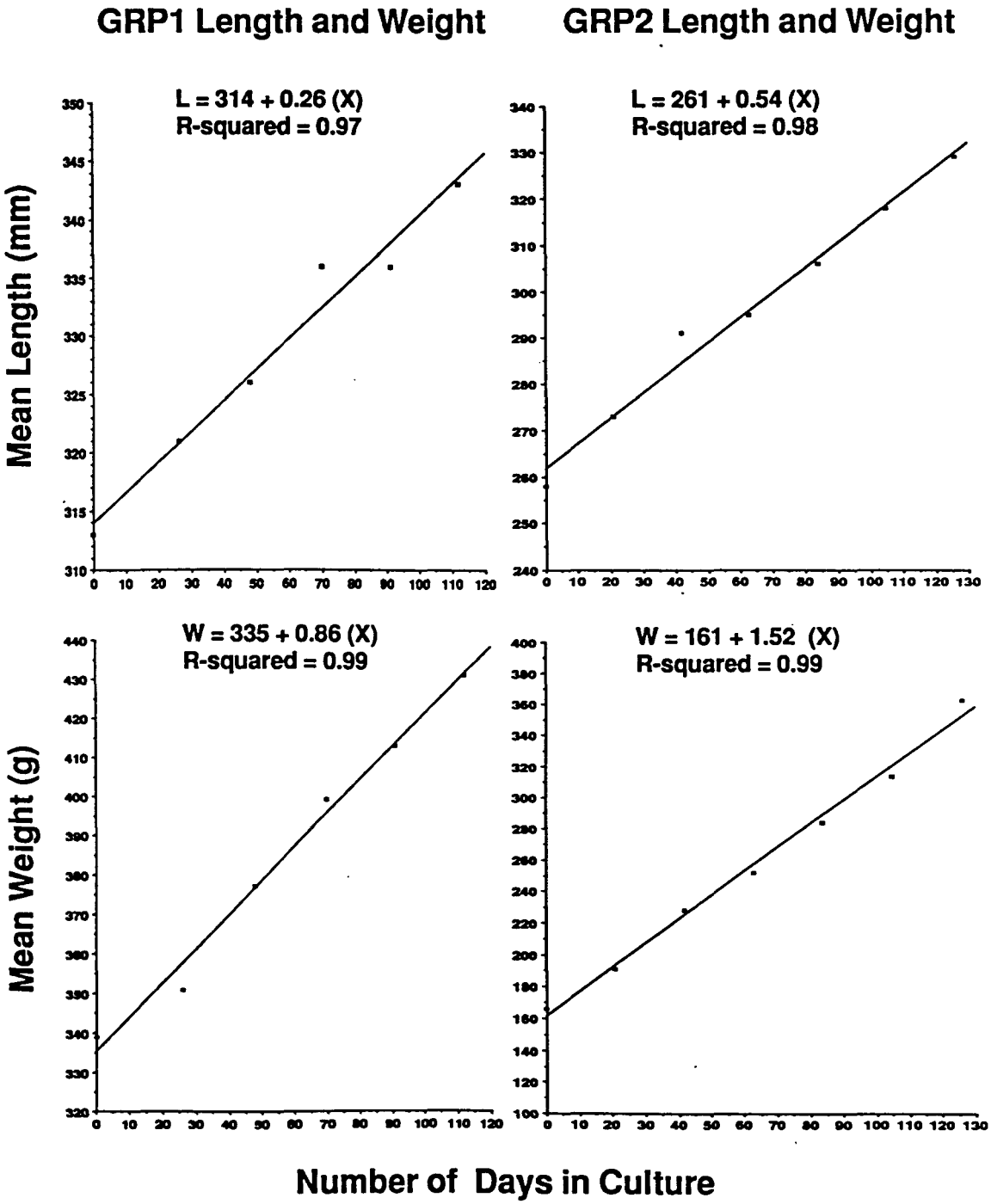

GRP1 Length and Weight
GRP2 Length and Weight
L = 314 + 0.26 (X)
R-squared = 0.97
L = 261 + 0.54 (X)
R-squared = 0.98
W = 335 + 0.86 (X)
R-squared = 0.99
W = 161 + 1.52 (X)
R-squared = 0.99
Mean Length (mm)
Mean Weight (g)
Number of Days in Culture

Table 4. Predicted weights and in-tank biomass of GRP1 and GRP2 walleye on days of 24-hour trials

Trial date	Days[b]	Mean weight (g)[a]	Fish density	
			number	biomass (kg)
GRP1				
February	16-17 (22)	354	120	42.5
March	2-3 (36)	366	119	43.6
March	30-31 (64)	390	114	44.5
April	11-12 (76)	401	120	48.1
April	18-19 (83)	407	120	48.8
May	2-3 (97)	419	120	50.2
May	9-10 (104)	425	120	51.0
GRP2				
July	5-6 (49)	235	135	31.8
July	18-19 (62)	255	134	34.2
July	25-26 (69)	266	134	35.6
August	2-3 (77)	278	133	37.0
August	22-23 (97)	309	131	40.4
August	27-28 (102)	316	131	41.4

[a] Mean weights were estimated from linear regression of weight on number of days in culture (Fig. 1, 2) for GRP1 and GRP2 walleye.

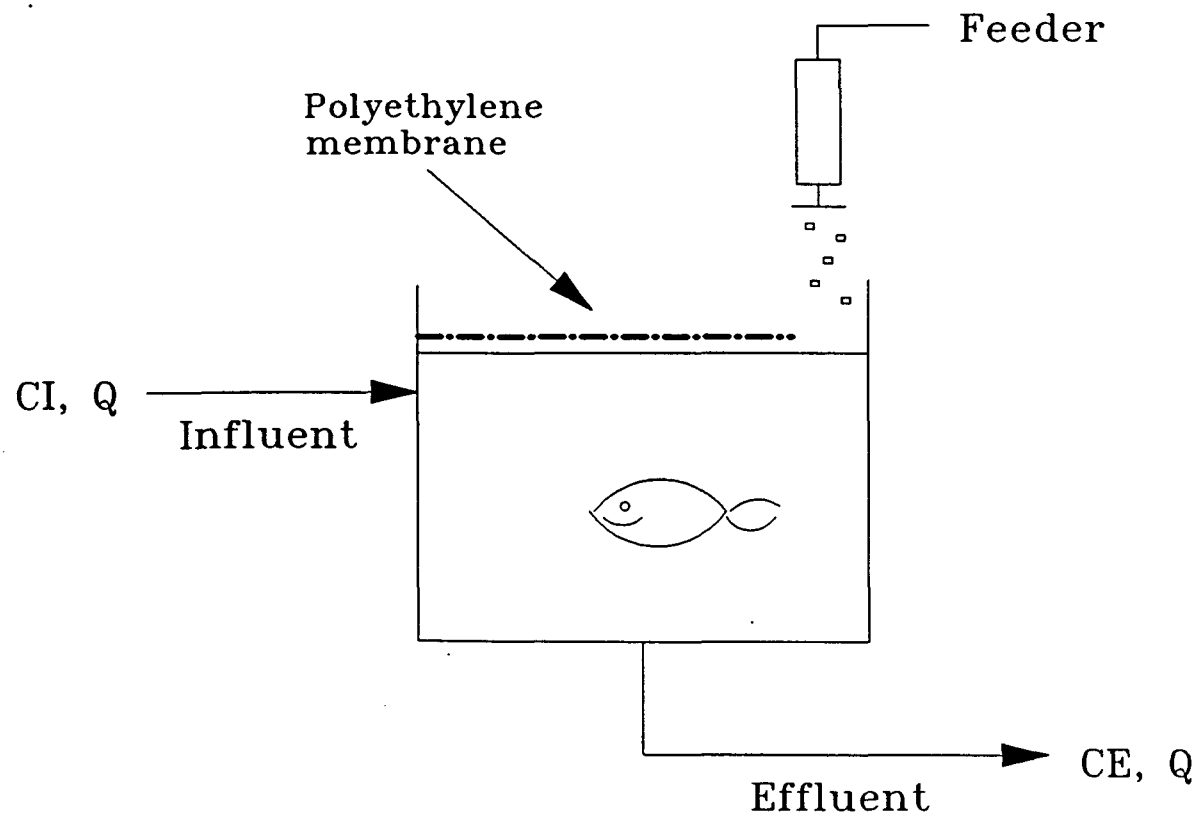
[b] Number of days in culture.

excreted per kg live weight (mg kg^{-1} h^{-1}; Figure 2). Estimates of ammonia excretion and oxygen consumption rates were related to the hourly (in the 24-h studies) and daily (for daily ammonia) trends. To facilitate comparison with other research results, a logarithmic transformation of metabolic rate data was performed and mean log oxygen consumption and mean log ammonia excretion rates were calculated for each 24-hour trial.

Statistical Analysis

A general linear models procedure (GLM; SAS 1982) was used to assess the effect of fish size (log weight) on oxygen consumption (log O_2 consumption) and ammonia excretion (log NH_3 excretion) over all feeding treatments. To compare mean oxygen consumption and mean ammonia excretion rates among different feeding treatments, fish size effects were removed by an analysis of covariance (ANCOVA; SAS 1982) with fish weight as the covariate. ANCOVAs on the variance in mean oxygen consumption rates and the variance in mean rates of ammonia excretion were also conducted with fish weight as the covariate. Analyses were performed on replicate trials of the 2, 3 and 15 feeding treatments conducted on both GRP1 and GRP2 fish.

Figure 2. Schematic of rearing tank used in oxygen consumption and ammonia excretion 24-hour trials. Mass balances of consumed oxygen and excreted ammonia were calculated from the differences between influent and effluent samples of DO and TAN, taking into consideration flow rates and in-tank fish biomass



MASS BALANCE EQUATION

$$MB = Q[(CE_{t1} + CE_{t2})/2 - CI_{t1}] (t2 - t1) + V(CE_{t1} - CE_{t2})$$

Where: MB = mass (mg) of oxygen consumed or ammonia excreted in the interval of study (30 min)

Q = flow rate (l/min)

CI = influent concentration (mg/l)

CE = effluent concentration (mg/l)

t = time (min)

V = tank volume (l)

RESULTS

Effects of Size

Oxygen consumption

Mean oxygen consumption (OC) rates for each 24-hour trial declined with increasing fish weight (Table 5). This relationship was rectilinear expressed either arithmetically (Figure 3) or on a log-log basis (Figure 4). Expressed arithmetically, the relationship of OC to weight, disregarding feeding treatments, was described by the equation:

$$OC = 228.4 - 0.2198 \text{ (fish weight)}$$ (Figure 3).

This relationship was highly significant ($F = 68.1$, $P < 0.01$), with weight accounting for 86% of the variability in OC. Expressed on a log-log basis, the relationship of OC to weight, disregarding feeding treatments, was described by the equation:

$$\log OC = 3.3496 - 0.4623 \text{ (log weight)}$$ (Figure 4).

This relationship was also highly significant ($F = 45.1$, $P < 0.01$) and accounted for 80% of the variability in the log rate of OC.

When analyzed separately, close fit linear relationships between OC and weight were also observed within the 2, 3 and 15 feeding treatments (Table 7). Weight accounted for 99%, 65% and 98% of the variability in OC in the 2, 3 and 15

Table 5. Oxygen consumption rates (mg kg^{-1} h^{-1}) for each feeding trial conducted on GRP1 and GRP2 walleye. Treatments are the number of feedings per day (ie. 2, 3 and 15)

Trial date	Mean wgt. (g)[a]	Tank biomass (kg)	No. of feedings	O_2 consumption max.	min.	mean ± SD[b]
GRP1						
Feb. 16-17	354	42.5	2	184	129	153 ± 14.1
Mar. 2-3	366	43.6	2	174	122	149 ± 11.9
Mar. 30-31	390	44.5	2	180	113	141 ± 15.3
Apr. 11-12	401	48.1	3	183	134	154 ± 12.0
Apr. 18-19	407	48.8	3	162	118	134 ± 9.8
May 2-3	419	50.2	15	153	110	130 ± 9.2
May 9-10	425	51.0	15	150	108	131 ± 8.4
GRP2						
July 5-6	236	31.8	2	213	150	178 ± 14.5
July 18-19	255	34.2	2	212	136	173 ± 18.1
July 25-26	266	35.6	3	198	126	161 ± 10.8
Aug. 2-3	278	37.0	3	181	140	165 ± 10.6
Aug. 22-23	308	40.4	15	185	140	163 ± 10.7
Aug. 27-28	316	41.4	15	187	143	166 ± 8.8

[a] Mean weights estimated from relationship of average weight to days in culture (Figures 1 and 2).

[b] Means are derived from 48 half-hour measures of oxygen consumption rates over a 24-hour interval.

Figure 3. Relationship of oxygen consumption to fish weight for 24-h feeding trials conducted on GRP1 and GRP2 walleye

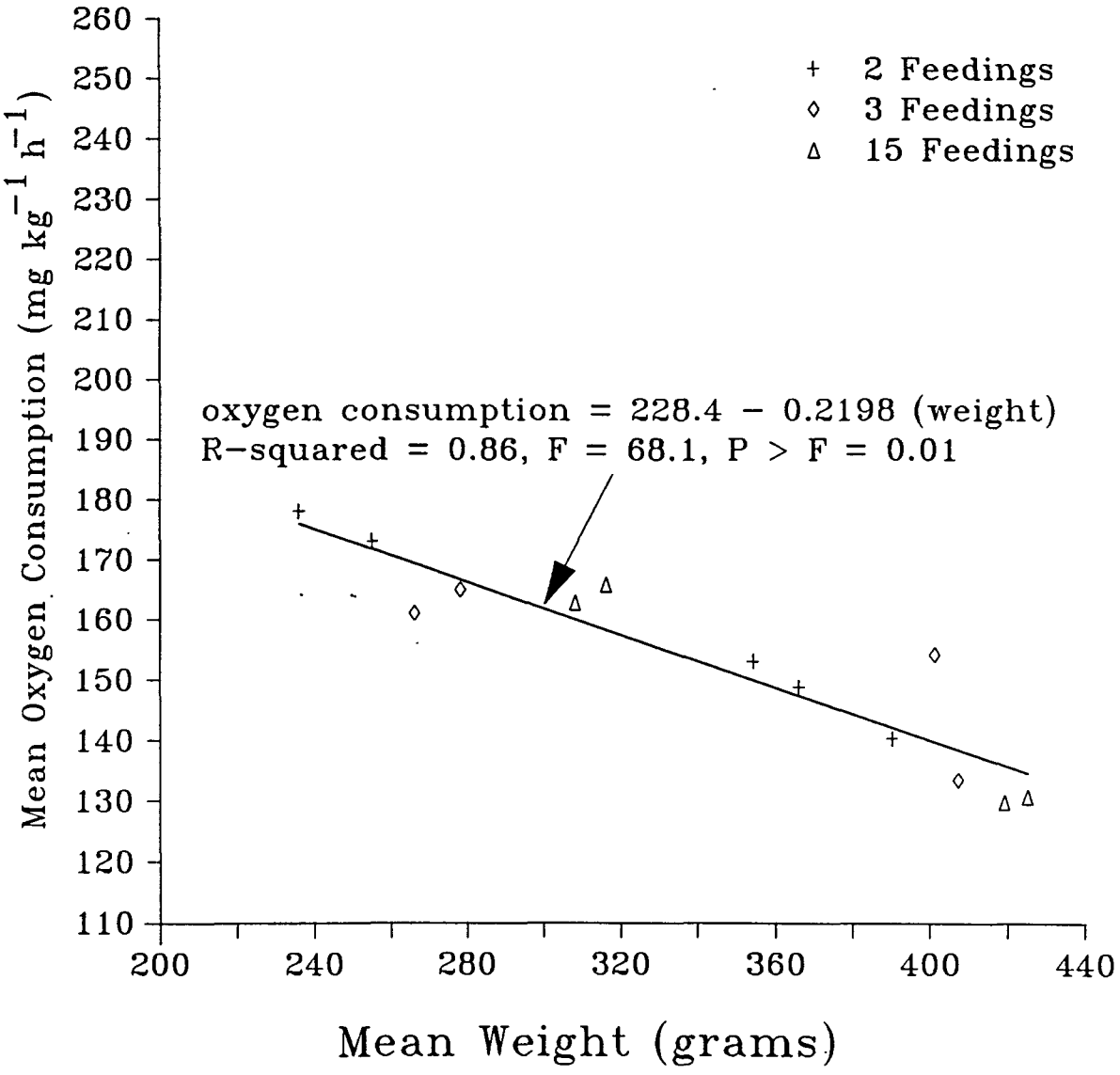
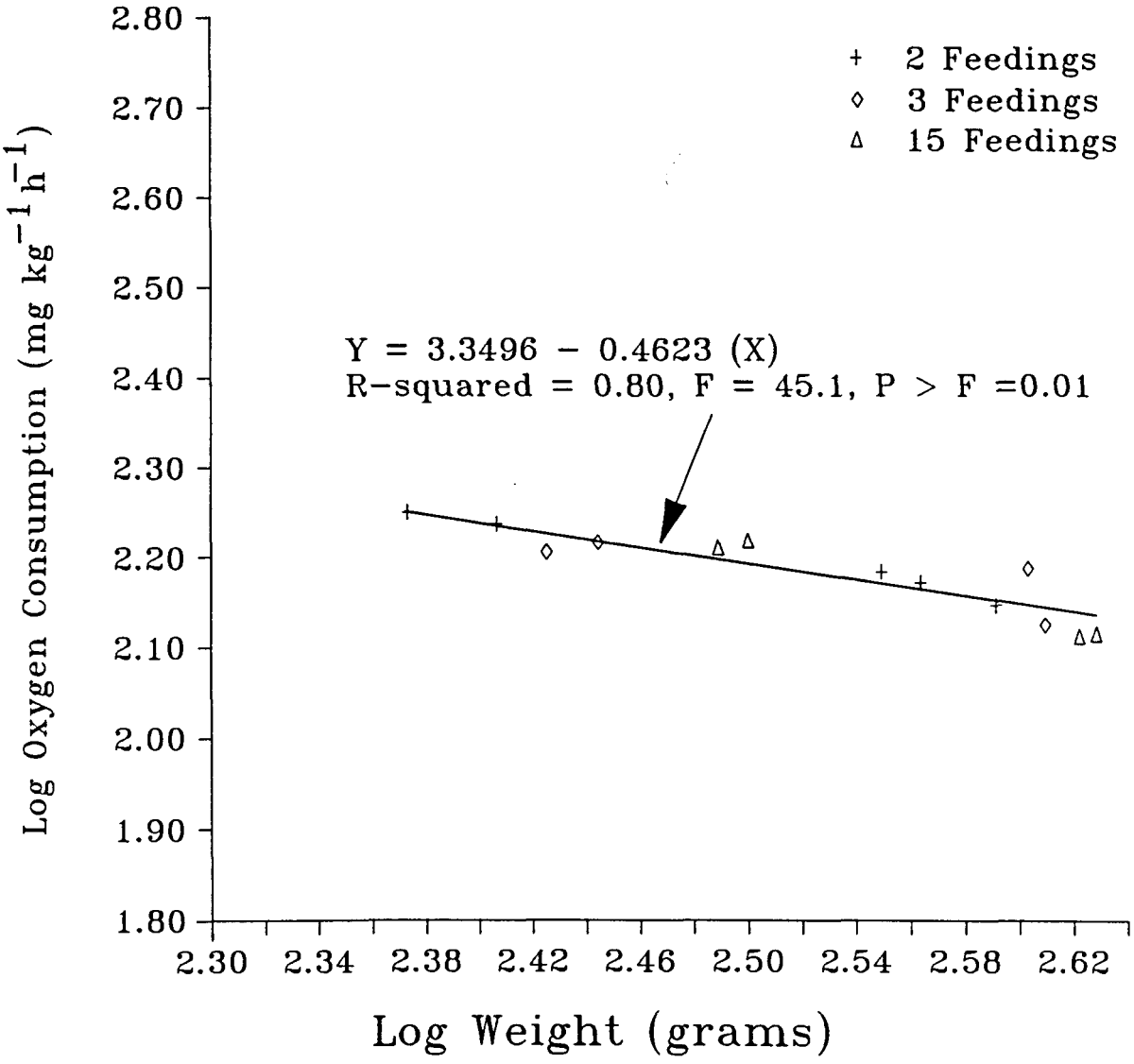

260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
Mean Oxygen Consumption (mg kg^{-1} h^{-1})
+ 2 Feedings
◊ 3 Feedings
Δ 15 Feedings
oxygen consumption = 228.4 − 0.2198 (weight)
R−squared = 0.86, F = 68.1, P > F = 0.01
200
240
280
320
360
400
440
Mean Weight (grams)

Figure 4. Relationship of the log of oxygen consumption (Y) to the log of fish weight (X) for 24-h feeding trials conducted on GRP1 and GRP2 walleye


2.80
2.70
2.60
2.50
2.40
2.30
2.20
2.10
2.00
1.90
1.80
Log Oxygen Consumption (mg kg^{-1} h^{-1})
+ 2 Feedings
◊ 3 Feedings
Δ 15 Feedings
Y = 3.3496 − 0.4623 (X)
R-squared = 0.80, F = 45.1, P > F =0.01
2.30 2.34 2.38 2.42 2.46 2.50 2.54 2.58 2.62
Log Weight (grams)

feeding treatments, respectively. The mean X-intercept and slope values for the 2, 3 and 15 feeding treatments were equal to 231.4 and -0.2265, respectively (Table 7).

Within the 2, 3 and 15 feeding treatments, log rates of OC were inversely related to log weights. Weight accounted for 97%, 57% and 98% of the variability in the mean log rates of OC in the 2, 3 and 15 feeding treatments, respectively (Table 8). The regression coefficients of the mean log rate of OC to mean log weight in the 2 and 15 feeding treatments were highly significant ($P<0.01$). The regression coefficient of the mean log rate of OC to mean log weight in the 3 feeding treatment was not significant ($P>0.05$). Averaged across treatments, the relationship of the log rate of OC to log weight was described by the equation:

$$\log OC = 3.4529 - 0.5016 \text{ (log weight)(Table 8).}$$

Ammonia excretion

Unlike the inverse size-metabolic rate relationship for oxygen consumption, mean ammonia excretion (AE) rates showed no distinct decline with increasing weight (Table 6). Neither the arithmetic (Figure 5) nor the log-log (Figure 6) expressions of the relationship between AE and weight were significant ($P > 0.05$). Expressed arithmetically, the relationship between AE and weight, disregarding feedng treatments, was described by the equation:

$$AE = 8.37 - 0.0054 \text{ (fish weight)(Figure 5).}$$

Table 6. Ammonia excretion rates (mg kg^{-1} h^{-1}) for each feeding trial conducted on GRP1 and GRP2 walleye. Treatments are the number of feedings per day (ie. 2, 3 and 15)

Trial date	Mean wgt. (g)[a]	Tank biomass (kg)	No. of feed-ings	NH_3 excretion max.	min.	mean ± SD[b]
GRP1						
Feb. 16-17	354	42.5	2	10.8	5.4	7.8 ± 1.8
Mar. 2-3	366	43.6	2			
Mar. 30-31	390	44.5	2	8.8	4.3	6.5 ± 1.0
Apr. 11-12	401	48.1	3	10.9	5.8	8.0 ± 1.1
Apr. 18-19	407	48.8	3	13.6	2.2	5.2 ± 2.4
May 2-3	419	50.2	15	8.5	2.5	5.2 ± 1.3
May 9-10	425	51.0	15	6.5	1.3	4.4 ± 1.2
GRP2						
July 5-6	236	31.8	2	8.4	2.7	5.7 ± 1.2
July 18-19	255	34.2	2	7.8	2.6	5.0 ± 1.6
July 25-26	266	35.6	3	11.8	5.5	8.4 ± 1.8
Aug. 2-3	278	37.0	3	10.6	3.5	7.2 ± 1.9
Aug. 22-23	308	40.4	15	9.7	3.7	7.4 ± 1.7
Aug. 27-28	316	41.4	15	9.6	4.6	7.7 ± 1.6

[a] Mean weights estimated from the relationship of average weight to days in culture (Figures 1 and 2).

[b] Means are derived from 48 half-hour measures of ammonia excretion rates over a 24-hour interval.

Figure 5. Relationship of ammonia excretion to fish weight for 24-h feeding trials conducted on GRP1 and GRP2 walleye

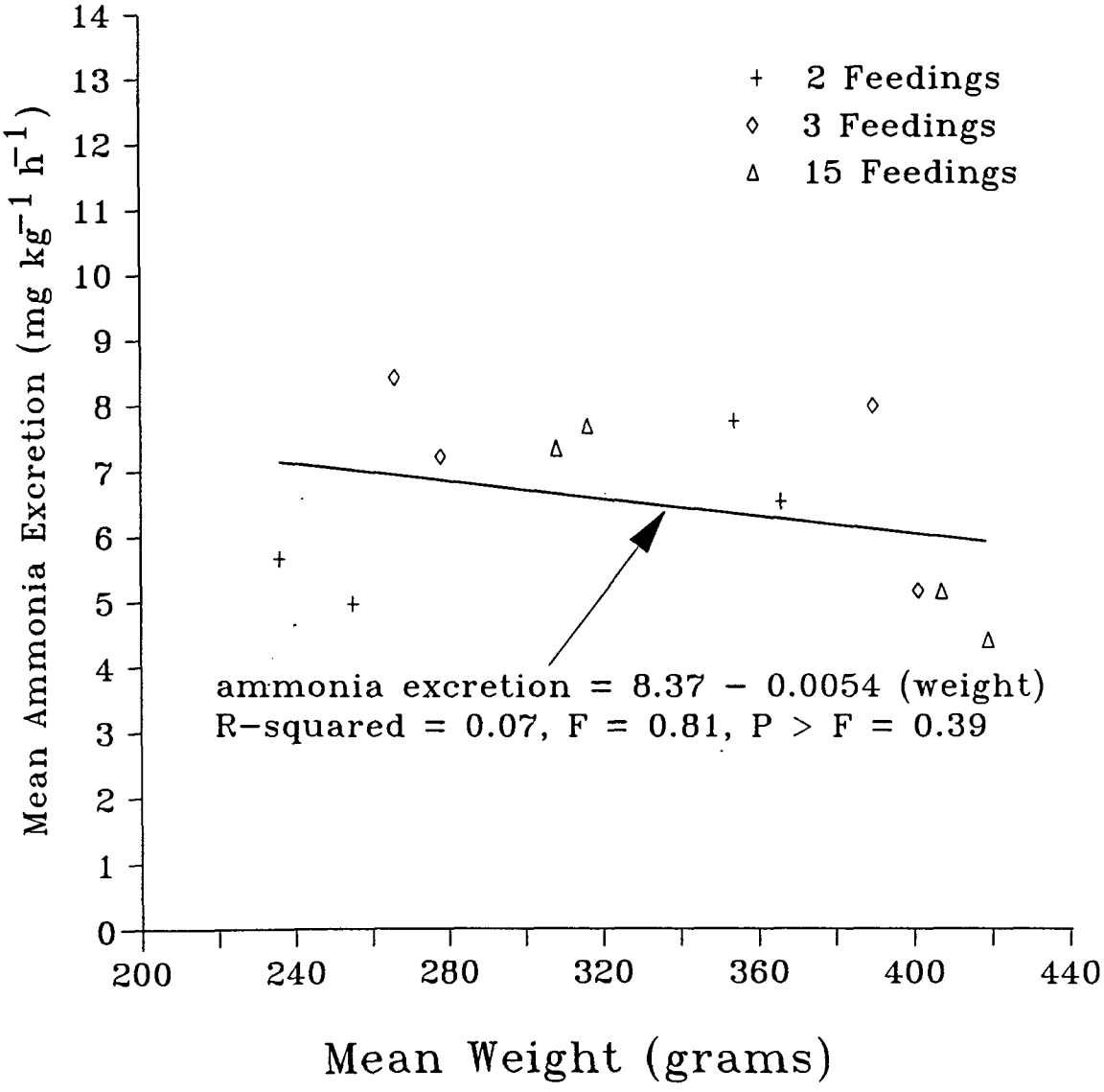

Mean Ammonia Excretion (mg kg^{-1} h^{-1})
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
200
240
280
320
360
400
440
Mean Weight (grams)
+ 2 Feedings
◊ 3 Feedings
Δ 15 Feedings
ammonia excretion = 8.37 − 0.0054 (weight)
R−squared = 0.07, F = 0.81, P > F = 0.39

Figure 6. Relationship of the log of ammonia excretion (Y) to the log of fish weight (X) for 24-h feeding trials conducted on GRP1 and GRP2 walleye

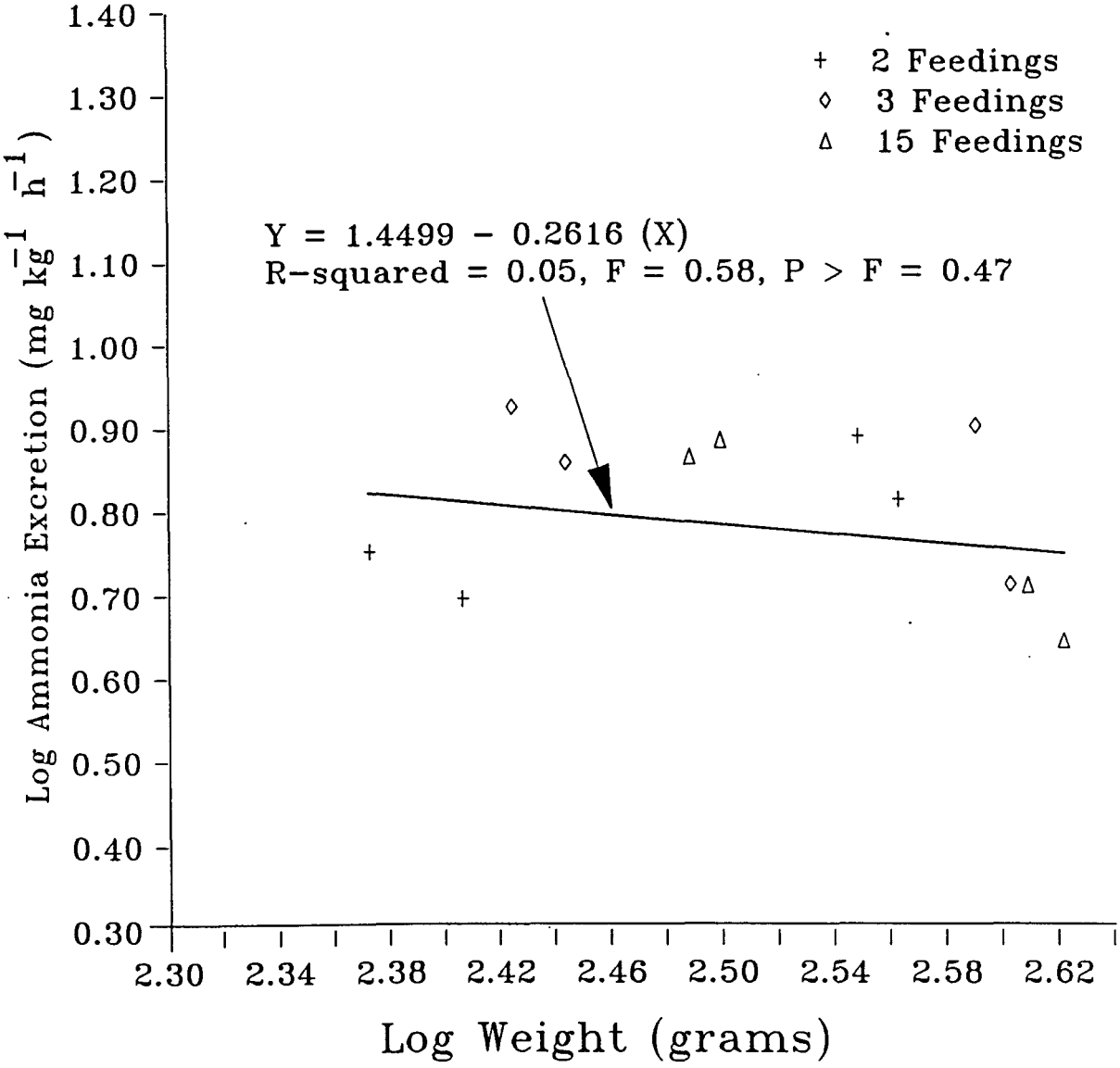

1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
Log Ammonia Excretion (mg kg^{-1} h^{-1})
+ 2 Feedings
◊ 3 Feedings
Δ 15 Feedings
Y = 1.4499 - 0.2616 (X)
R-squared = 0.05, F = 0.58, P > F = 0.47
2.30
2.34
2.38
2.42
2.46
2.50
2.54
2.58
2.62
Log Weight (grams)

Table 7. Regression equations of mean metabolic rates (mg kg^{-1} h^{-1}) for oxygen consumption and ammonia excretion related to mean fish weight within each feeding treatment. Feeding trials on both GRP1 and GRP2 fish were used in the analysis of data

Treatment (no. of feedings)	Metab. rate	X-intercept	Slope	R-square	F value	P >F
2	oxygen consump.	231.7	-0.2277	0.99	376	0.01
3	oxygen consump.	202.6	-0.1453	0.65	3.71	0.19
15	oxygen consump.	260.0	-0.3066	0.98	120	0.01
AVG.	oxygen consump.	231.4	-0.2265	****	****	****
2	ammonia excret.	2.624	0.0117	0.53	2.31	0.27
3	ammonia excret.	10.56	-0.0099	0.28	0.80	0.47
15	ammonia excret.	15.35	-0.0250	0.96	45.2	0.02
AVG.	ammonia excret.	9.51	-0.0077	****	****	****

Table 8. Regression equations of the mean log rates of metabolism (mg kg^{-1} h^{-1}) for oxygen consumption and ammonia excretion related to the mean log of fish weight within each feeding treatment. Feeding trials on both GRP1 and GRP2 fish were used in the analysis of data

Treatment (no. of feedings)	Log metab. rate	X-intercept	Slope	R-square	F value	P >F
2	oxygen consump.	3.2687	-0.4294	0.97	93.2	0.01
3	oxygen consump.	2.9800	-0.3153	0.57	2.60	0.25
15	oxygen consump.	4.1099	-0.7602	0.98	93.8	0.01
AVG.	oxygen consump.	3.4529	-0.5016	****	****	****
2	ammonia excret.	-0.867	0.6631	0.62	3.21	0.22
3	ammonia excret.	2.3030	-0.5829	0.27	0.75	0.48
15	ammonia excret.	4.7680	-1.5657	0.93	25.6	0.04
AVG.	ammonia excret.	2.068	-0.4952	****	****	****

The calculated F-statistic for this equation was 0.81 ($P > 0.39$) with weight accounting for only 7% of the variability in AE. Expressed on a log-log basis, the relationship between AE and weight was described by the equation:

$$\log AE = 1.4499 - 0.2616 (\log \text{weight})$$ (Figure 6).

The regression coefficient of this equation was not significant ($P > 0.05$) and accounted for 5% of the variability in AE (Figure 6).

Among the 2, 3 and 15 feeding treatments only the 15 feeding treatment had an arithmetic relationship of AE to weight which was significant ($F = 45.2$; $P < 0.02$; Table 7). Averaged across treatments the X-intercept and slope were equal to 9.51 and -0.0077, respectively.

Among the 2, 3 and 15 feeding treatments, the only significant ($P < 0.04$) relationship of the mean log rate of AE to mean log weight was the 15 feeding treatment relationship. Mean log weight accounted for 62%, 27% and 93% of the variability in mean log AE in the 2, 3 and 15 feeding treatments, respectively (Table 8). Averaged across feeding treatments the relationship between the log rate of AE and log weight was described by the equation:

$$\log AE = 2.068 - 0.4952 (\log \text{weight})$$ (Table 8).

Daily ammonia excretion

While the mean log rate of AE was not significantly affected by log weight within and across the 24-hour trials, the log of daily AE derived from daily composite sampling declined with increasing log weight (Figure 7) and was described by the equation:

log daily AE = 2.1684 - 0.4605 (log weight)(Figure 7).

The regression coefficient of this equation was highly significant ($F = 34.1$, $P < 0.01$) and accounted for 30% of the variability in log daily AE.

Effects of Feeding Schedule

Oxygen consumption

In feeding trials conducted on GRP1 and GRP2 walleye, mean OC rates for the 2, 3 and 15 feeding treatments were equal to 159, 154, and 147 mg kg^{-1} h^{-1}, respectively (Table 9). After the effect of fish size was removed by ANCOVA, treatment means were not significantly different ($P > 0.05$; Table 10). However, an ANCOVA conducted on the mean variance in OC between treatments showed significant differences (Table 10). After the effect of fish size was removed, the mean variance in OC declined with increasing feeding frequency. Differences in OC and the variance in OC were not significant between GRP1 and GRP2 fish ($P > 0.05$; Table 10).

Figure 7. Relationship of the log of daily ammonia excretion to the log of fish weight

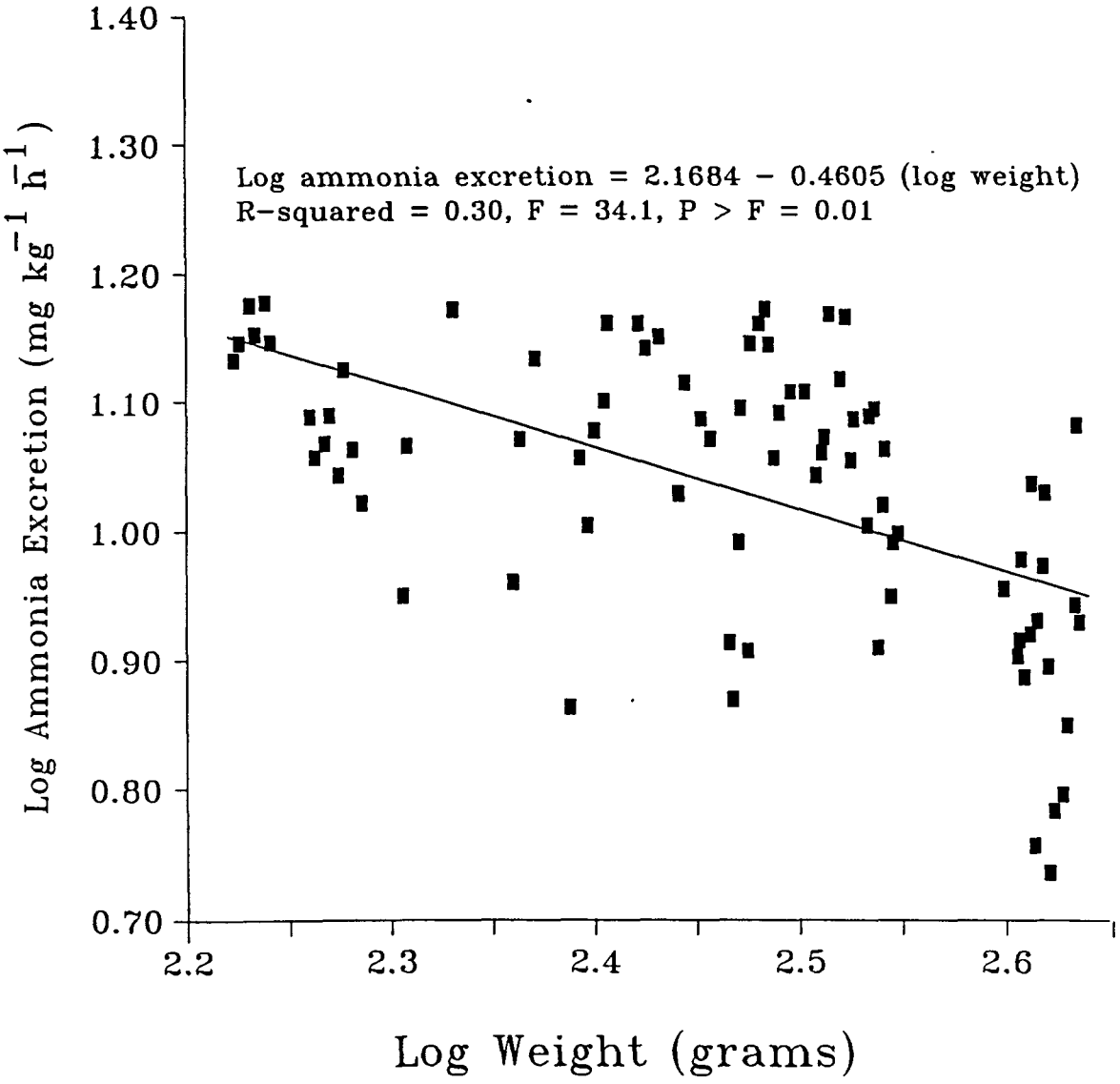

Log ammonia excretion = 2.1684 - 0.4605 (log weight)
R-squared = 0.30, F = 34.1, P > F = 0.01
Log Ammonia Excretion (mg kg-1 h-1)
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
2.2
2.3
2.4
2.5
2.6
Log Weight (grams)

Table 9. Means (± SD) of oxygen consumption and ammonia excretion rates for each 24-hour trial. Trials on both GRP1 and GRP2 walleye are included in this table

N[a]	Trial 1	2	3	4	5	Treatment means	(s^2)[b]
			(mg O_2 kg^{-1} h^{-1})				
2	153 ± 14.1	149 ± 11.9	141 ± 15.3	178 ± 14.5	173 ± 18.1	159 ± 16.1	222 ± 67.9
3	154 ± 12.0	134 ± 9.8	161 ± 10.8	165 ± 10.6		154 ± 14.0	117 ± 19.9
15	130 ± 9.2	131 ± 8.4	163 ± 10.7	166 ± 8.8		147 ± 19.8	86.8 ± 19.3
			(mg NH_3 kg^{-1} h^{-1})				
2	7.8 ± 1.8		6.5 ± 1.0	5.7 ± 1.2	5.0 ± 1.6	6.2 ± 1.2	2.1 ± 1.0
3	8.0 ± 1.1	5.2 ± 2.4	8.4 ± 1.8	7.2 ± 1.9		7.2 ± 1.4	3.5 ± 1.9
15	5.2 ± 1.3	4.4 ± 1.2	7.4 ± 1.7	7.7 ± 1.6		6.2 ± 1.6	2.1 ± 0.7

[a] Treatment (number of feedings per day).

[b] Mean variance (S^2), averaged across treatment trials.

Table 10. Mean rates, mg kg^{-1} h^{-1}, (± SE) of oxygen consumption and ammonia excretion and mean variances in rates of walleye under 3 different feeding treatments. Trials on both GRP1 and GRP2 walleye are included in this analysis

	Least squares means[a]							
Number of feedings (no. of trials)	oxygen consumption		ammonia excretion		variance in oxygen consumption		variance in ammonia excretion	
2 (n = 5)[b]	158 ± 7.2		5.9 ± 1.5		271 ± 43.6		2.1 ± 1.4	
3 (n = 4)	153 ± 3.6		7.2 ± 0.8		120 ± 21.6		3.5 ± 0.7	
15 (n = 4)	150 ± 6.5		6.6 ± 1.5		48.6 ± 39.2		2.1 ± 1.4	
	F	P >F	F	P >F	F	P >F	F	P >F
SIZE	52.2	0.01	0.66	0.44	6.23	0.04	0.11	0.75
FEEDING	0.05	0.95	0.58	0.59	10.2	0.01	1.19	0.36
GROUP	0.32	0.59	0.03	0.88	1.88	0.21	0.00	0.95
ANCOVA	12.6	0.01	0.46	0.76	7.12	0.01	0.62	0.66

[a] Least Squares means were obtained from the analysis of covariance with the effect of weight on oxygen consumption and ammonia excretion removed.

[b] Only 4 replicates of the 2 feeding treatment were analyzed for effects on ammonia excretion, n = 4.

Ammonia excretion

The effect of feeding schedule on AE was not significant. Mean AE rates were 6.2, 7.2 and 6.2 mg kg^{-1} h^{-1} for the 2, 3 and 15 feeding treatments, respectively (Table 9). The ANCOVA on these rates, with fish weight as the covariate, indicated no significant differences existed between treatment means ($P > 0.05$; Table 10). Unlike the variance in oxygen consumption, the mean variances in AE rates were not significantly different between treatments after the effects of fish size had been removed (Table 10). Significant differences in AE and the variance in AE were not detected between mean rates for GRP1 and GRP2 walleye.

DISCUSSION

Effects of size

Oxygen consumption rates varied inversely with weight. Many investigators have observed this effect and it is a generally accepted principle that metabolism declines with increasing size (Schmidt-Nielsen 1975). Brett (1965) reported rapid decreases in oxygen consumption of sockeye salmon (Oncorhyncus nerka) with increasing fish weight. Cai and Summerfelt (1991) observed declines in oxygen consumption with increasing weight when data were plotted on a logarithmic basis. Normally, the arithmetic relationship between oxygen consumption and weight is curvilinear when plotted over a wide range of fish sizes. In the present study, the range in fish weights was small (204-425 g), resulting in a strong linear relationship of oxygen consumption to weight when plotted on an arithmetic scale and there was no improvement in the R-squared value using a log-log relationship. The slope of the arithmetic relationship between fish weight and oxygen consumption rates for fish from 236 g and 425 g was equal to -0.2198. This means that for each 1 gram increase in fish weight, a resulting reduction in oxygen consumption of 0.22 mg kg^{-1} h^{-1} will result.

Because of the exponential nature of the arithmetic

relationship between metabolism and weight, researchers normally perform logarithmic transformations of data to obtain linear relationships on logarithmic scales. In the present study the slope of the logarithmic regression line between log 236 g and log 425 g equalled -0.4623. Cai and Summerfelt (1991) observed regression slopes of -0.1403 at 20°C and -0.2153 at 25°C of 4 to 133 g walleye. Fry (1971) stated that the slope of the logarithmic regression line normally lies between -0.5 and 0.

The relationship between size and ammonia excretion rate was less apparent, rates declined with increasing weight but the regression coefficients were not significant. The slope of the arithmetic regression between 236 g and 425 g was equal to -0.0054, indicating a 0.005 decrease in ammonia excretion rates per g increase in fish weight. The regression coefficient of the logarithmically transformed data equalled -0.2616. Jobling (1981) reported a logarithmic regression slope of ammonia excretion to weight of -0.33 for young plaice. Cai and Summerfelt (1991) reported regression slopes of -0.3716 at 25°C and -0.1523 at 20°C.

Effects of feeding frequency

No significant differences in mean oxygen consumption and ammonia excretion rates were detected among feeding treatments. Beamish and MacMahon (1988) also reported no

effect of feeding schedule on mean oxygen consumption.

The mean variations in oxygen consumption and ammonia excretion rates under different feeding schedules, however, did show significant differences. In general, the variance in oxygen consumption declined with increasing frequency of feeding, while the variance in ammonia excretion was not significantly affected by different feeding schedules.

Aquaculturists must be aware of the effects of feeding and size on metabolism and must manage their facilities to adjust for rapid, large increases in oxygen consumption or ammonia excretion associated with feeding. Porter et al. (1987) states that from a practical point of view it is important to determine diurnal patterns of ammonia excretion by fish of different sizes in order to establish an optimum feeding regime. Beamish and MacMahon (1988) suggest that a frequent feeding schedule will improve feed intake and growth. The findings of the current study indicate that a frequent feeding schedule will reduce the variability in metabolism, thus avoiding large fluctuations in oxygen consumption and ammonia excretion.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Iowa Department of Economic Development to the Engineering Research Institute of Iowa State University.

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SECTION II. PATTERNS OF SPECIFIC DYNAMIC ACTION IN JUVENILE WALLEYE UNDER FOUR DIFFERENT FEEDING FREQUENCIES

ABSTRACT

The effects of feeding frequency on daily patterns of metabolism of juvenile walleye reared under intensive culture conditions are reported. Rates of oxygen consumption and ammonia excretion were highly responsive to feeding, increasing rapidly after the start of feeding and peaking 2 to 5 hours thereafter. Maximum rates were 12-30% higher than mean rates for oxygen consumption and 35-162% higher than mean rates for ammonia excretion. Mean oxygen consumption rates in 3-h blocks varied significantly when fish were fed twice daily, but not when fed three or fifteen times daily. Ammonia excretion rates varied significantly when fish were fed once daily, but not when fed two, three or fifteen times. To reduce variability in rates of oxygen consumption and ammonia excretion managers should feed frequently throughout the course of a day.

INTRODUCTION

A positive relationship exists between feeding and metabolism. Jobling (1981) reported an immediate increase in oxygen consumption and nitrogenous excretions of plaice (Pleuronectes platessa) after feeding. Kausch (1968) reported that oxygen consumption and metabolism of common carp (Cyprinus carpio) increased after feeding. Meade (1985) reported that the daily feeding schedule or distribution of food has a major effect on peak (in-tank) concentrations of ammonia. A strong pulse of ammonia excretion developed 4 to 4.5 h after feeding fingerling sockeye salmon (Oncorynchus nerka)(Brett and Zala 1975) and gilthead seabream (Sparus aurata)(Porter et al. 1987). Lied and Braaten (1984) observed maximal ammonia excretion rates 5 to 6 h after feeding 256 to 482 g Atlantic cod (Gadus morhua). Ammonia excretion was closely related to activity and oxygen consumption within 4 h of feeding 29 to 70 g rainbow (Oncorynchus mykiss)(Rychly and Marina 1977) and small brown trout (Salmo trutta)(Elliott 1976). Kindschi et al. (1990) observed two periods of maximum oxygen consumption in trout, during the first feeding and following the last feeding.

The increase in metabolism after feeding has been termed apparent Specific Dynamic Action (SDA; Tandler and Beamish 1981) or Apparent Heat Increment (AHI; Beamish and MacMahon

1988). In strictest terms, SDA refers to the heat increment resulting from the many biochemical reactions which occur following the ingestion of a meal (Rubner 1902). However, measurement of heat production and separating energy associated with heat increment from that used in the mechanical aquisition, digestion and absorption of food is difficult, therefore, oxygen consumption and ammonia excretion are used as indirect measures of apparent SDA (Beamish 1974).

Recognizing and managing the changes in metabolism associated with feeding is crucial to maintaining optimal conditions in production facilities. Elimination of large fluctuations in the culture environment should be the goal of any facility. Metcalf and Eddy, Inc. (1979) state that biological treatment is enhanced when "shock" loadings of ammonia are eliminated or minimized. Stabilization of ammonia inputs to nitrification apparatus and maintenance of constant in-tank concentrations of dissolved oxygen will improve operating efficiency. Procedures must be defined which produce these conditions.

In this study, variations in oxygen consumption and ammonia excretion rates were compared across the course of a 24-h period. Juvenile walleye (> 200 g) were fed once to several times (15) a day and metabolic rates were monitored to examine patterns of SDA in relation to feeding and to

determine which feeding schedule produced the least variation in daily metabolic rates.

METHODS

Culture Conditions

Two groups of fish were used to assess the effects of feeding frequency on SDA. Group 1 (GRP1) walleye averaged 313 ± 28 mm (total) and averaged 339 ± 99 g on January 25, 1990. The mean condition factor ($K = (\text{weight in g} \times 10^5)/(\text{length in mm})^3$) of GRP1 fish on January 25 was 1.08 ± 0.17. Group 2 (GRP2) fish averaged 258 ± 30 mm (total) and averaged 166 ± 58 g on May 17, 1990. The mean condition factor of GRP2 fish on May 17 was 0.92 ± 0.10.

Walleye were reared in round (155 cm diameter, 75 cm depth) fiberglass tanks with water volumes of about 1136 L. The culture tanks were supplied with dechlorinated tap water. Dechlorination was accomplished with two in-line high pressure tanks filled with granular activated carbon. The supply water was periodically checked for the presence of chloramines and free chlorine with test kits (HACH Company, Loveland, CO). No evidence of chlorine or chloramines was ever detected. Heated water was mixed with cold water to approximately 23°C and degassed by passage through a column (0.61 m diameter by 4.88 m) packed with 8.5 cm polyethylene ballast rings (Glitsch, Inc., Dallas, TX).

Flow rates to the rearing tanks were monitored once per hour by computer (model AT286/16 PC; Gateway 2000, Sioux

City, SD) using FP 5800 flow transmitters (Omega, Inc., Stamford, CT) connected to a data acquistion and control system (Strawberry Tree^{TM}, Inc., Sunnyvale, CA). Flow rates averaged 11.9 $\pm$ 1.0 L min^{-1} (0.6 tank exchange h^{-1}) except during 24-hour diurnal studies when rates were increased to maintain in-tank DO concentrations of near 5 mg L^{-1}. In-tank DO levels always exceeded 4.9 mg L^{-1} and were normally at or near saturation (approximately 8.5 mg L^{-1} at 23^{o}C). Water temperatures were measured with a mercury thermometer (0.01^{o}C). In-tank pH values were measured with a combination pH electrode connected to a model 05669-20 pH-vision microcomputer (Cole-Parmer, Chicago, IL). Average water temperatures and pH values based on means of 1 daily observation were 23.2 $\pm$ 0.5^{o}C and 7.23 $\pm$ 0.5, respectively. Except during 24-hour studies, rearing tank water was continuously aerated through flexible membrane diffusers (Parkson Inc., Ft. Lauderdale, FL) supplied with compressed air.

Rearing tanks had a center drain and were largely self-cleaning, excess feed and feces was removed quickly. Tank walls and the center standpipe were scrubbed periodically to remove bacterial and fungal growth from these surfaces.

Culture room light was controlled to provide a 15 h-20 min light and 8 h-40 min dark photoperiod (0440 - 2000).

Light intensity at the water surface was 8.6 ± 2.4 lx. No feed was introduced during dark periods.

Feeding

Feed was dispensed with scraper feeders operated by time clock. Fish were fed 15 times daily in intervals varying in number and length (Table 1). Fish were fed BioDry 3000 or 1000 (BioProducts, Inc., Warrenton, OR) in the 6 or 8 mm pellet size at a constant ration of 2% of in-tank fish biomass per day. These are high protein (44.5%), semimoist (14.5%) feeds. The proximate compositions of BioDry 3000 and BioDry 1000 are the same except for a red pigment in BioDry 300. Rations and food sizes were adjusted weekly to compensate for changes in fish biomass and size. Feeding rations for days 1 through 7, 8 through 14, and 15 through 21 were calculated from in-tank biomass estimates for days 4, 11 and 18, respectively. Estimates of fish size on these dates were derived from measurements of length and weight at 21-day intervals. A linear regression of length on the number of days in culture was used to estimate length on days 4, 11 and 18 of each 21-day growth interval. These length estimates were then applied to a log-log regression of weight on length to calculate approximate fish weight and in-tank biomass on days 4, 11 and 18.

Table 1. Feeding variables for GRP1 and GRP2 walleye

Dates	Number of feeding intervals	Time	Duration (h)
GRP1			
January 25 to April 4	2	05:00-06:30 17:00-18:30	1.5 h 1.5 h
April 5 to April 26	3	05:00-06:00 11:30-12:30 17:00-18:00	1.0 h 1.0 h 1.0 h
April 27 to May 17	15	05:00-19:00	hourly
GRP2			
May 18 to June 28	1	11:00-13:30	2.5 h
June 29 to July 19	2	05:00-06:30 17:00-18:30	1.5 h 1.5 h
July 20 to August 9	3	05:00-06:00 11:30-12:30 17:00-18:00	1.0 h 1.0 h 1.0 h
August 10 to September 19	15	05:00-19:00	hourly

24-hour Feeding Trials

Fifteen 24-h diurnal monitorings of ammonia excretion and oxygen consumption were conducted, 7 with GRP1 fish and 8 with GRP2 fish. Four different feeding treatments were utilized: once daily, twice daily, three times daily and hourly for 15 hours (Table 2). A polyethylene cover was placed over the tank during each monitoring event to limit gaseous exchanges with the atmosphere. Light intensity below this cover was 20% lower (6.9 $\pm$ 1.3 lx) than under normal conditions (8.6 $\pm$ 2.4 lx). Supplemental in-tank aeration was not used during 24-h studies, but flow rates were increased to maintain effluent DO concentrations near 5 mg L^{-1}. Flow rates averaged 39.7 $\pm$ 0.6 L min^{-1} (2.1 tank exchanges h^{-1}) for monitorings 2 through 15. The average flow rate for monitoring 1 was 45.0 $\pm$ 0.4 L min^{-1} (2.4 tank exchanges h^{-1}). Samples for effluent TAN and DO concentrations were collected every 30 min, but influent TAN and DO samples were collected every 2 h because their concentrations were consistent. Two replicate water samples of influent and effluent flows were collected to measure TAN and DO concentrations. These samples were collected from sample ports in the supply and drain pipes, near to, but not in the rearing tank itself. At the conclusion of some 24-hour runs, samples were collected from in-tank influent and effluent sites and compared to sampling site data to verify

Table 2. 24-hour trial dates and treatment variables

Trial dates (1990)	Number of feeding intervals	Time	Duration (h)
February 16 to 17	2	05:00-06:30	1.5 h
March 2 to 3		17:00-18:30	1.5 h
March 30 to 31			
April 11 to 12	3	05:00-06:00	1.0 h
April 18 to 19		11:30-12:30	1.0 h
		17:00-18:00	1.0 h
May 2 to 3	15	05:00-19:00	hourly
May 9 to 10			
June 14 to 15	1	11:00-13:30	2.5 h
June 27 to 28			
July 5 to 6	2	05:00-06:30	1.5 h
July 18 to 19		17:00-18:30	1.5 h
July 25 to 26	3	05:00-06:00	1.0 h
August 2 to 3		11:30-12:30	1.0 h
		05:00-06:00	1.0 h
August 22 to 23	15	05:00-19:00	hourly
August 27 to 28			

the representativeness of sampling site locations. No significant differences (t-tests, $P > 0.05$) existed between sampling site and in-tank concentrations for DO and TAN (Table 3). DO samples were collected in 300 ml BOD bottles and analyzed using the Azide modification of the Winkler method (APHA et al. 1989). TAN samples were collected in 150 ml polyethylene bottles, acidified to $pH < 2.0$ and analyzed using the electrode method (APHA et al. 1989). Flow rates and effluent temperatures were recorded every 30 min. Effluent pH values were recorded every 60 min.

Biomass Estimates

Measurements of mean fish weights were made about every 21 d and plotted against the number of days in culture. Estimates of mean fish weight and ultimately in-tank biomass for monitoring events 1 through 15 were calculated from the linear regression equation of weight and number of days in culture.

Mass-specific Metabolic Rates

For 24-hour studies of oxygen consumption and ammonia excretion, the difference in DO and TAN concentrations between the influent and effluent samples, taking into consideration the flow rates and fish biomass present in the rearing tank, was used as a measure of the rate

Table 3. Comparisons of in-tank and sampling site concentrations of DO and TAN. Average influent and effluent samples were compared using Student's t-tests

	TAN (mg L^{-1})				DO (mg L^{-1})			
	influent		effluent		influent		effluent	
Date	in-tank	samp. site	in-tank	samp. site	in-tank	samp. site	in-tank	samp. site
4/26	0.038	0.044	0.266	0.266	7.65	7.58		
5/3	0.024	0.031	0.147	0.141	8.11	8.04	5.33	5.38
5/10	0.025	0.031	0.144	0.139			5.31	5.29
6/15	0.008	0.007	0.049	0.047	8.20	8.10	5.79	5.74
6/28	0.027	0.026	0.040	0.043	8.21	8.28	6.30	6.24
7/6			0.077	0.077	8.28	8.14	6.20	6.04
7/19	0.009	0.007	0.100	0.113	8.34	8.30		
7/26	0.011	0.012	0.165	0.165	8.12	8.13	5.66	5.57
8/3	0.007	0.008	0.127	0.127	8.07	8.05	6.20	6.18
8/23	0.007	0.007	0.137	0.143	7.93	8.01	5.15	5.16
8/28	0.010	0.012	0.175	0.169	8.19	8.09	5.23	5.14
Mean	0.017	0.019	0.130	0.130	8.11	8.07	5.57	5.53
SD	0.010	0.013	0.060	0.060	0.19	0.19	0.41	0.38
n =	10	10	11	11	10	10	9	9
t =	0.366		0.011		0.453		0.255	
P > t	0.73		0.99		0.67		0.82	

(mg kg^{-1} h^{-1}) of oxygen consumed or ammonia excreted per kg live weight (Figure 1). Estimates of ammonia excretion and oxygen consumption rates were calculated and related to the hourly trends.

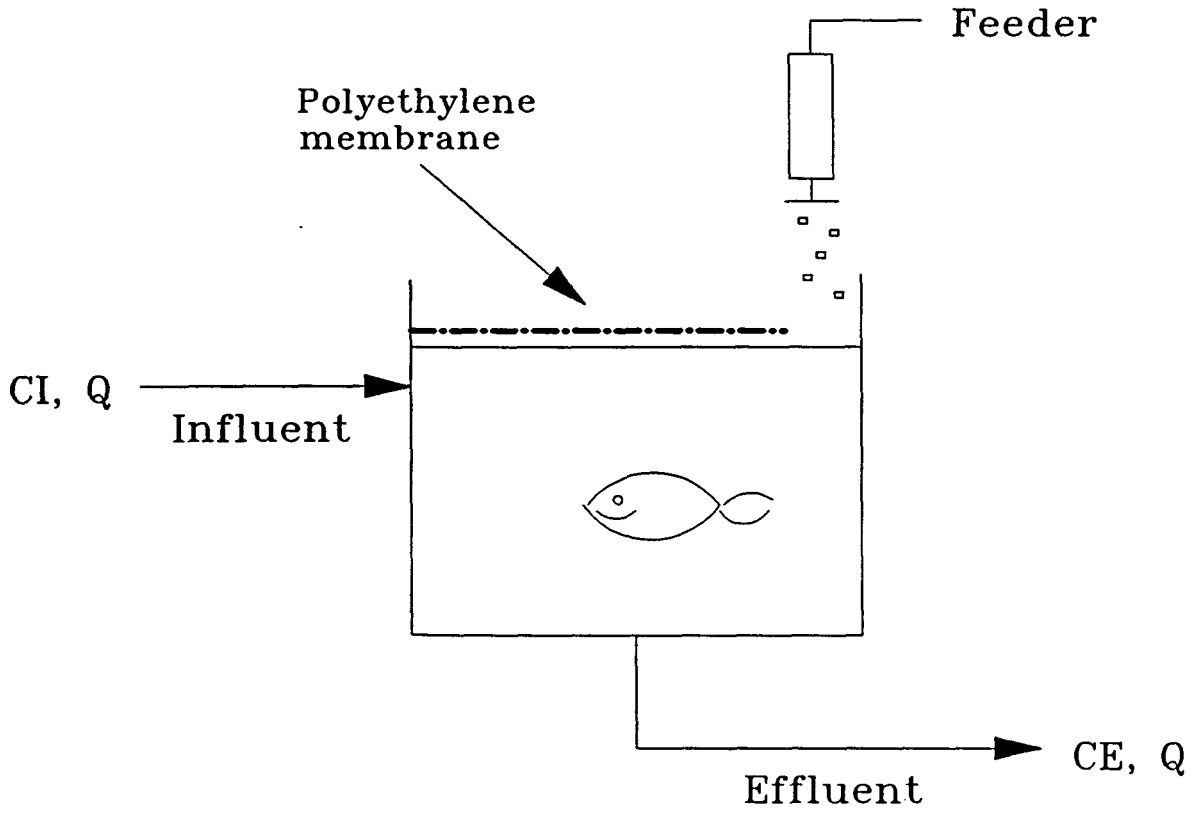
Statistical Analysis

The effects of feeding frequency on SDA were analyzed by dividing each 24-hour study into 3-hour blocks to represent pre- and post-feeding intervals. Mean oxygen consumption and ammonia excretion rates for each 3-hour block within 24-h trials with similar feeding treatments were analyzed by ANOVA. To remove the effects of fish weight from these analyses all metabolic rates were adjusted to a standard weight ie., the mean weight of GRP1 and GRP2 fish combined (340 g). For oxygen consumption rates a regression coefficient of -0.2198 was used to adjust rates to the standard weight. For ammonia excretion rates a regression coefficient of -0.0054 was used. These coefficients were determined from plots of oxygen consumption and ammonia excretion rates versus fish weight. For example, oxygen consumption for a 354 g walleye averaged 153 mg kg^{-1} h^{-1}, the following procedure would be used to adjust this rate to the standard weight of 340 g:

adjusted rate = 153 mg kg^{-1} h^{-1} - (-0.2198)(354 g - 340 g).

= 149.9 mg kg^{-1} h^{-1}.

Figure 1. Schematic of rearing tank used in oxygen consumption and ammonia excretion 24-hour trials. Mass balances of consumed oxygen and excreted ammonia were calculated from the differences between influent and effluent samples of DO and TAN, taking into consideration flow rates and in-tank fish biomass



MASS BALANCE EQUATION

$$MB = Q[(CE_{t1} + CE_{t2})/2 - CI_{t1}] (t2 - t1) + V(CE_{t1} - CE_{t2})$$

Where: MB = mass (mg) of oxygen consumed or ammonia excreted in the interval of study (30 min)

Q = flow rate (l/min)

CI = influent concentration (mg/l)

CE = effluent concentration (mg/l)

t = time (min)

V = tank volume (l)

RESULTS

Patterns of SDA

Oxygen consumption and ammonia excretion rates were highly responsive to feeding and parallel patterns of SDA were apparent between metabolic rates (Figures 2-5).

One feeding

When fed once daily, oxygen consumption (OC) rates increased rapidly after the start of feeding, reaching maximum levels in 3.5-4 h (Figure 2). Maximum OC rates were 12.5-15.9% greater than mean rates and 38.5-43.1% greater than minimum rates (Table 4). OC rates remained elevated for 18-19 h after the end of feeding (Figure 2). Similarly, ammonia excretion (AE) rates peaked 4.0-4.5 h after the start of feeding and remained above pre-feeding rates for 13-18 h after the end of feeding. Maximum AE rates were 300-600% higher than minimum rates.

Two feedings

When fed twice daily, OC rates peaked 3.5-4.5 h after both the first and second feeding (Figure 3). Maximum OC rates were 17-28% greater than mean daily rates and were always observed in either the 3.5-4.5 h after the first feeding or the 3.5-4.5 h following the second feeding (Table 5; Figure 3). OC rates remained elevated above pre-feeding levels for 7-8 h following the end of each feeding interval.

AE rates peaked 3.5-4.0 h after the start of each

Figure 2. Relationship of oxygen consumption and ammonia excretion rates to time of day for each 24-hour trial of the 1 feeding per day treatment conducted on GRP2 walleye

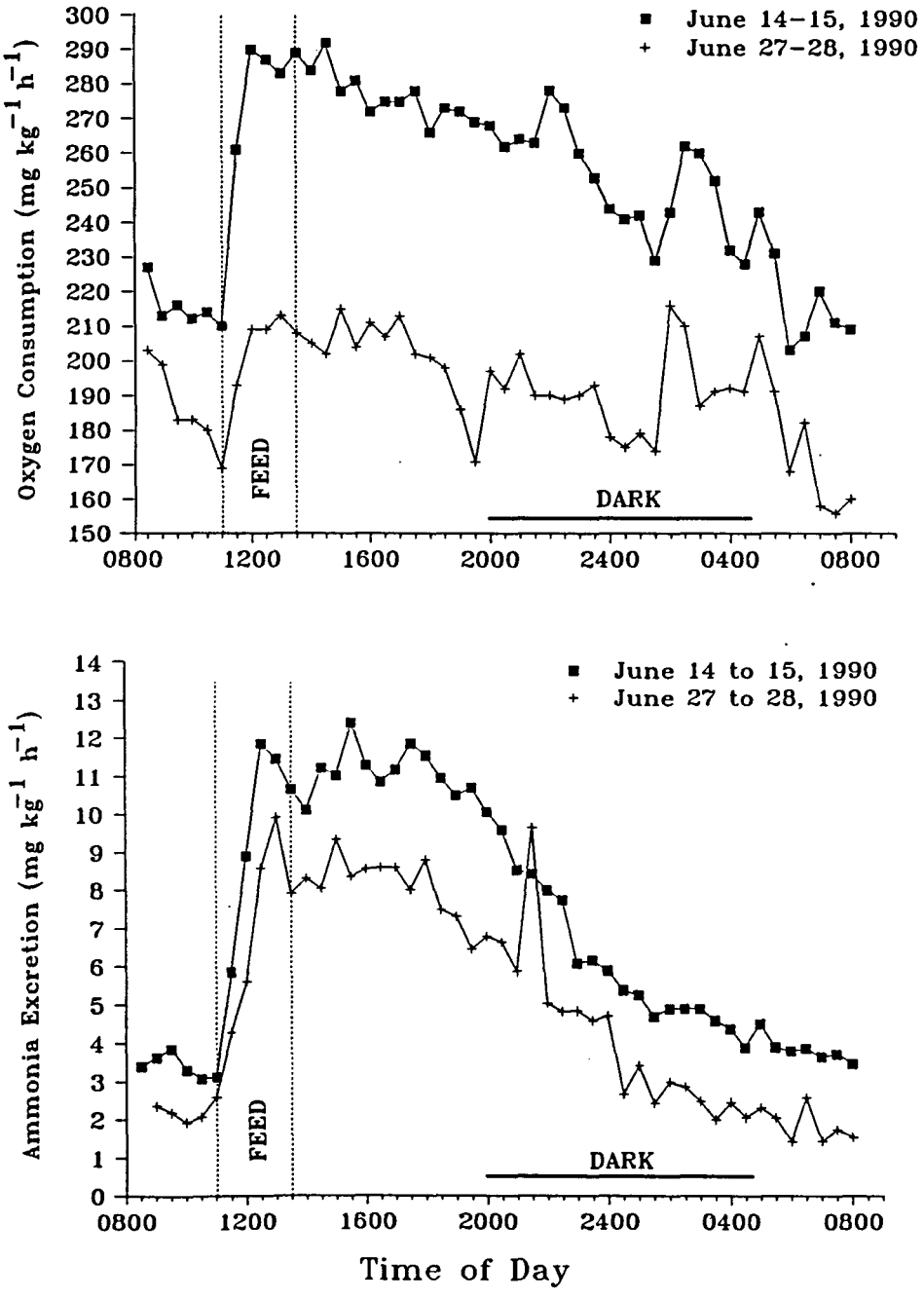

June 14–15, 1990
June 27–28, 1990
Oxygen Consumption (mg kg^{-1} h^{-1})
300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
FEED
DARK
0800
1200
1600
2000
2400
0400
0800
June 14 to 15, 1990
June 27 to 28, 1990
Ammonia Excretion (mg kg^{-1} h^{-1})
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
FEED
DARK
0800
1200
1600
2000
2400
0400
0800
Time of Day

Table 4. Mean, maximum and minimum oxygen consumption and ammonia excretion rates for each trial of the one feeding per day treatment

Trial	Metabolic rate			Increase in max. from min. (%)	Increase in max. from mean (%)
	Mean	Maximum	Minimum		
	(mg O_2 kg^{-1} h^{-1})				
1	252	292	204	43.1	15.9
2	192	216	156	38.5	12.5
	(mg NH_3 kg^{-1} h^{-1})				
1	7.1	12.4	3.1	300	74.6
2	5.0	9.9	1.4	607	98.0

Figure 3. Relationship of oxygen consumption and ammonia excretion rates to time of day for each 24-hour trial of the 2 feedings per day treatment conducted on GRP1 and GRP2 walleye

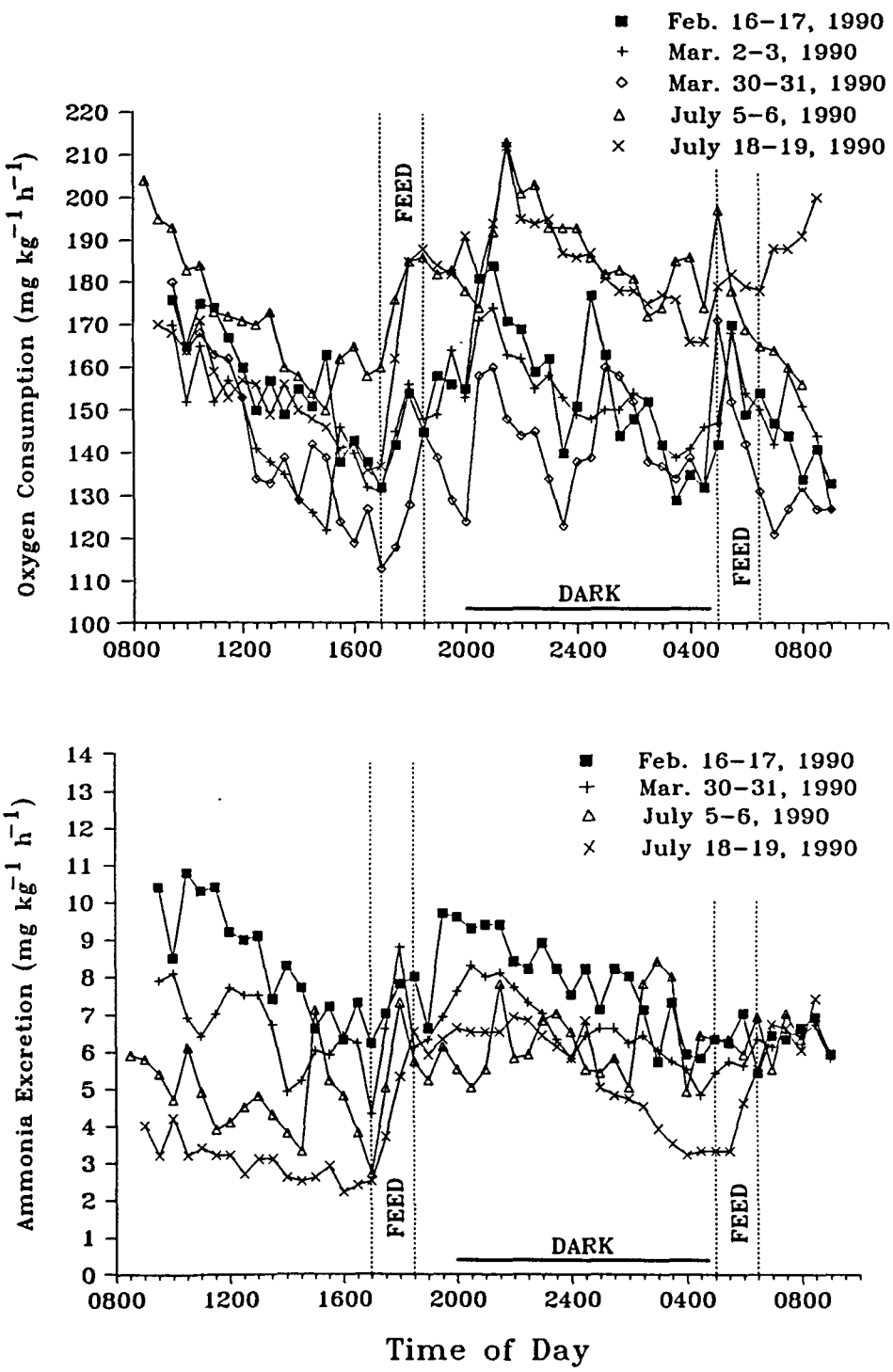

Feb. 16–17, 1990
Mar. 2–3, 1990
Mar. 30–31, 1990
July 5–6, 1990
July 18–19, 1990
Oxygen Consumption (mg kg^{-1} h^{-1})
220
210
200
190
180
170
160
150
140
130
120
110
100
FEED
FEED
DARK
0800
1200
1600
2000
2400
0400
0800
Feb. 16–17, 1990
Mar. 30–31, 1990
July 5–6, 1990
July 18–19, 1990
Ammonia Excretion (mg kg^{-1} h^{-1})
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
FEED
FEED
DARK
0800
1200
1600
2000
2400
0400
0800
Time of Day

Table 5. Mean, maximum and minimum oxygen consumption and ammonia excretion rates for each trial of the two feedings per day treatment

Trial	Metabolic rate			Increase in max. from min. (%)	Increase in max. from mean (%)
	Mean	Maximum	Minimum		
	($mg\ O_2\ kg^{-1}\ h^{-1}$)				
1	153	184	129	42.6	20.3
2	149	174	122	42.6	16.8
3	141	180	113	59.3	27.7
4	178	213	150	42.0	19.7
5	173	212	136	55.9	22.5
	($mg\ NH_3\ kg^{-1}\ h^{-1}$)				
1	7.8	10.8	5.4	100	38.5
2	6.5	8.8	4.3	105	35.4
3	5.7	8.4	2.7	211	47.4
4	5.0	7.8	2.6	200	56.0

feeding interval (Figure 3). Maximum rates were 100-211% higher than minimum rates and 35-56% higher than mean rates (Table 5).

Three feedings

When fed 3 times daily, OC rates peaked rapidly following feeding, normally reaching maximum rates in 30 min to 1.5 h after the start of feeding (Figure 4). Maximum OC rates were 10-23% greater than mean daily rates and 29.3-57.1% higher than minimum rates (Table 6). Peaking shortly after the end of feeding, OC rates declined prior to the start of the next feeding interval. However, OC rates prior to the second and third daily feeding periods were never as low as rates prior to the first daily feeding period (Figure 4).

AE rates increased rapidly in the 1.0-2.5 h following the start of each feeding (Figure 4). However, maximum daily rates were not observed until after the end of the third feeding of the day. Maximum AE rates were 36-162% greater than mean rates and up to 518% greater than minimum rates (Table 6).

Multiple feedings

Under the multiple feeding schedule, OC rates rapidly increased after the start of feeding and maximum rates occurred from 1.0-2.0 h after the start of feeding (Figure 5). OC rates remained elevated for several hours

Figure 4. Relationship of oxygen consumption and ammonia excretion rates to time of day for each 24-hour trial of the 3 feedings per day treatment conducted on GRP1 and GRP2 walleye

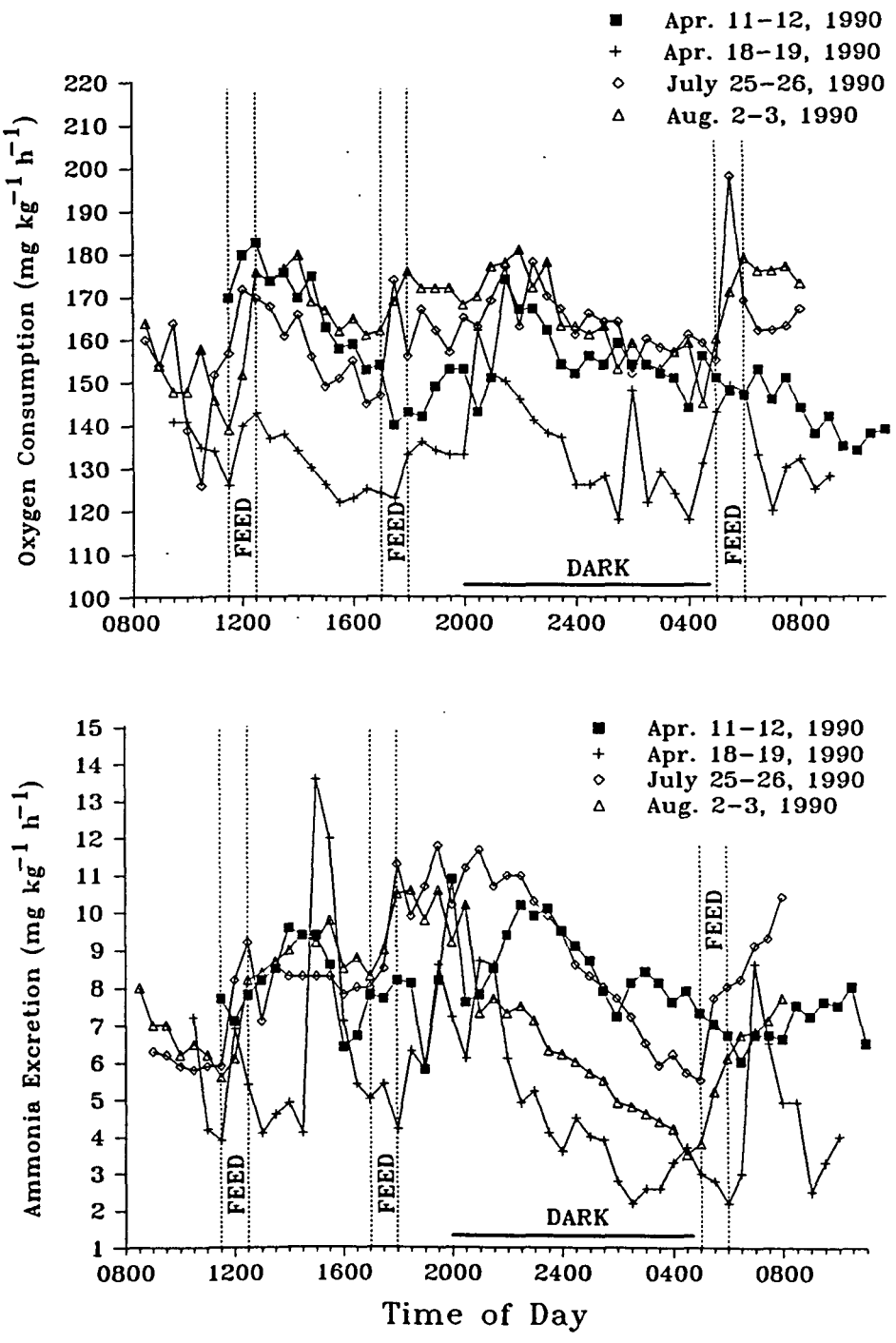

Apr. 11–12, 1990
Apr. 18–19, 1990
July 25–26, 1990
Aug. 2–3, 1990
Oxygen Consumption (mg kg^{-1} h^{-1})
220
210
200
190
180
170
160
150
140
130
120
110
100
FEED
FEED
FEED
DARK
0800
1200
1600
2000
2400
0400
0800
Apr. 11–12, 1990
Apr. 18–19, 1990
July 25–26, 1990
Aug. 2–3, 1990
Ammonia Excretion (mg kg^{-1} h^{-1})
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
FEED
FEED
FEED
DARK
0800
1200
1600
2000
2400
0400
0800
Time of Day

Table 6. Mean, maximum and minimum oxygen consumption and ammonia excretion rates for each trial of the three feedings per day treatment

Trial	Metabolic rate			Increase in max. from min. (%)	Increase in max. from mean (%)
	Mean	Maximum	Minimum		
	($mg\ O_2\ kg^{-1}\ h^{-1}$)				
1	154	183	134	36.6	18.8
2	134	162	118	37.3	20.9
3	161	198	126	57.1	23.0
4	165	181	140	29.3	9.7
	($mg\ NH_3\ kg^{-1}\ h^{-1}$)				
1	8.0	10.9	5.8	87.9	36.3
2	5.2	13.6	2.2	518	162
3	8.4	11.8	5.5	115	40.5
4	7.2	10.6	3.5	203	47.2

Figure 5. Relationship of oxygen consumption and ammonia excretion rates to time of day for each 24-hour trial of the 15 feedings per day treatment conducted on GRP1 and GRP2 walleye

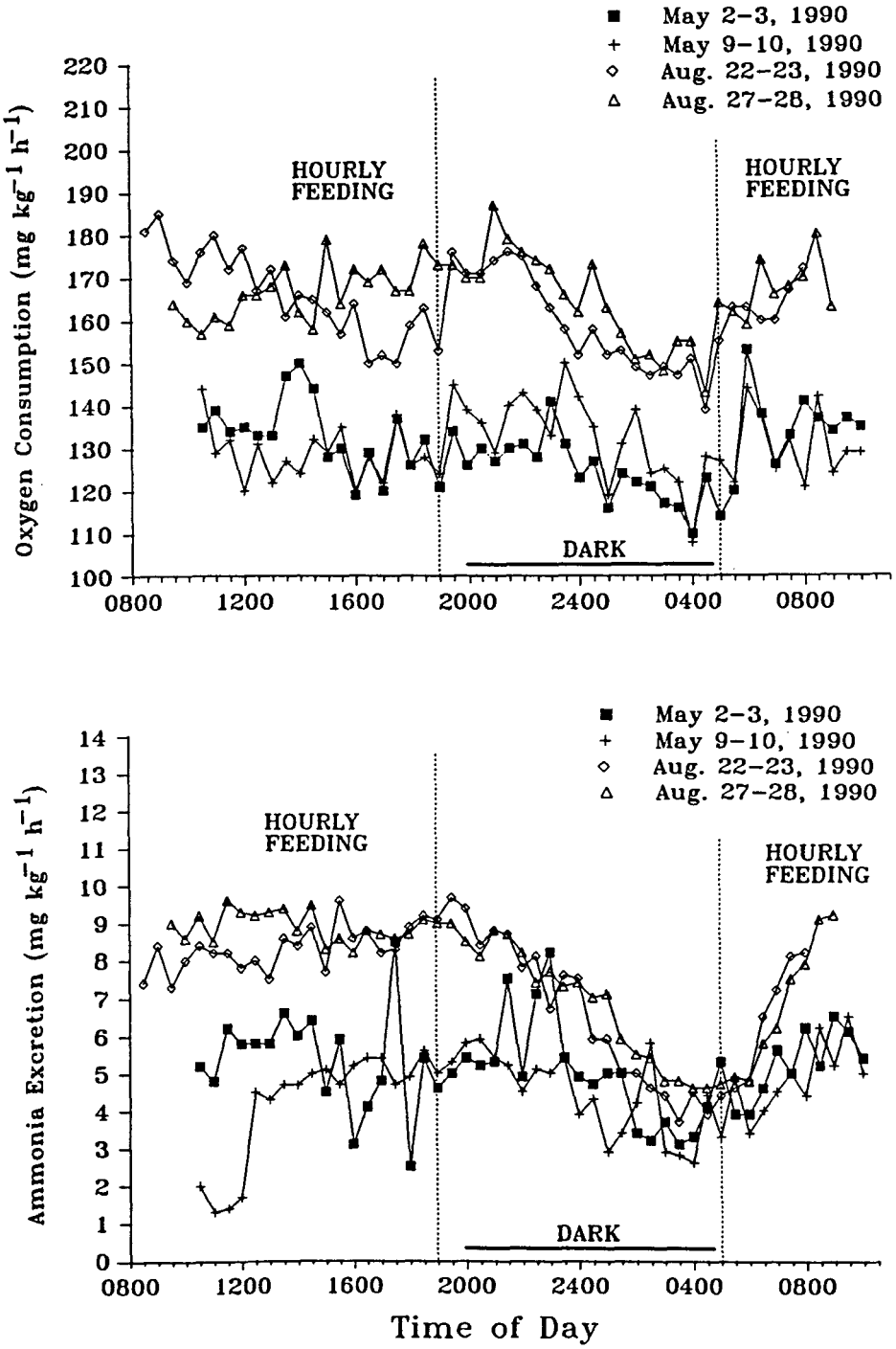

May 2-3, 1990
May 9-10, 1990
Aug. 22-23, 1990
Aug. 27-28, 1990
HOURLY FEEDING
HOURLY FEEDING
DARK
Oxygen Consumption (mg kg^{-1} h^{-1})
Ammonia Excretion (mg kg^{-1} h^{-1})
0800
1200
1600
2000
2400
0400
0800
Time of Day

Table 7. Mean, maximum and minimum oxygen consumption and ammonia excretion rates for each trial of the fifteen feedings per day treatment

Trial	Metabolic rate			Increase in max. from min. (%)	Increase in max. from mean (%)
	Mean	Maximum	Minimum		
	($mg\ O_2\ kg^{-1}\ h^{-1}$)				
1	130	153	110	39.1	17.7
2	131	150	108	38.9	14.5
3	163	185	140	32.1	13.5
4	166	187	143	30.8	12.7
	($mg\ NH_3\ kg^{-1}\ h^{-1}$)				
1	5.2	8.5	2.5	240	63.5
2	4.4	6.5	1.3	400	47.7
3	7.4	9.7	3.7	162	31.1
4	7.7	9.6	4.6	109	24.7

after the end of feeding before declining to pre-feeding levels. Maximum OC rates were 12.7-17.7% greater than mean rates (Table 7).

AE rates normally peaked shortly after the start of feeding, usually in 1.5-3.0 h (Figure 5). Maximum AE rates were 31.1-63.5% greater than mean daily rates (Table 7). As with oxygen consumption, AE remained elevated for several hours after the end of feeding.

ANOVA on 3-h Mean Metabolic Rates

One feeding

The ANOVA conducted on 3-hour mean rates of OC of GRP2 walleye fed once daily revealed no significant differences in 3-hour mean rates. However, 3-hour mean AE rates did vary significantly. The maximum 3-hour mean rate of 9.3 mg kg^{-1} h^{-1} occurred in the second 3-hour interval following the start of feeding (Table 8). This rate was 323% greater than the pre-feeding 3-hour mean of 2.2 mg kg^{-1} h^{-1}.

Two feedings

In the analysis of weight adjusted metabolic rates, significant differences in 3-hour mean OC rates were found (Table 9). Mean rates in the second 3-hour interval following the start of each feeding period were 15 to 35 mg kg^{-1} h^{-1} greater than 3-hour pre-feeding rates. Mean OC rates were highly responsive to feeding (Figure 6).

Figure 6. Relationships of oxygen consumption and ammonia excretion rates to time of day for replicate trials of the 1, 2, 3 and 15 feedings treatments. Data points are means of replicate feeding trials of each treatment conducted on GRP1/GRP2 combined after adjustment to a standard weight of 340 g

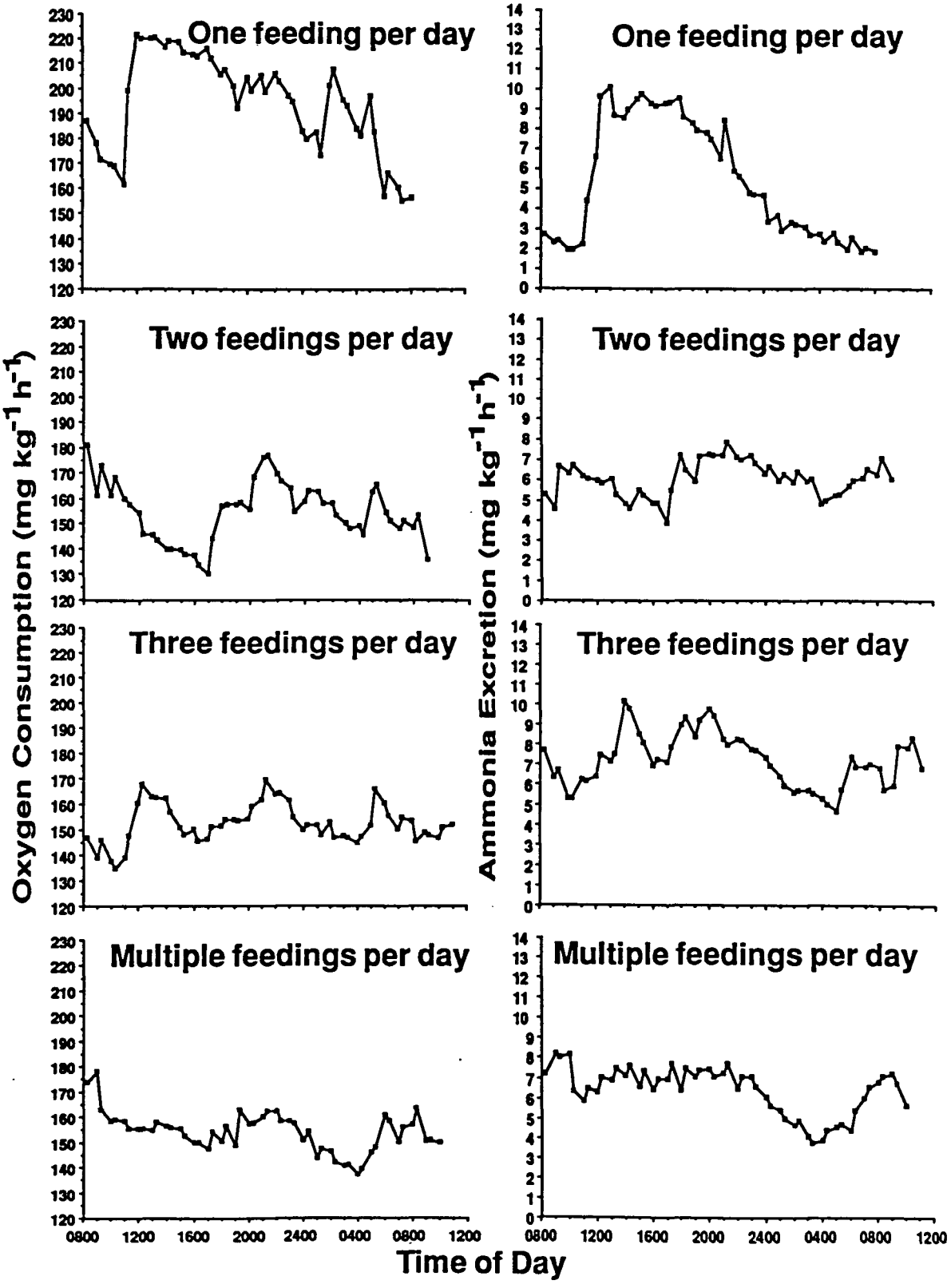

One feeding per day
One feeding per day
Two feedings per day
Two feedings per day
Three feedings per day
Three feedings per day
Multiple feedings per day
Multiple feedings per day
Oxygen Consumption (mg kg-1 h-1)
Ammonia Excretion (mg kg-1 h-1)
0800 1200 1600 2000 2400 0400 0800 1200
Time of Day

Table 8. Metabolic rates (mg kg^{-1} h^{-1}) of walleye fed once daily. Oxygen consumption (OC) and ammonia excretion (AE) rates are adjusted to rates for a 340 g fish. Values are 3-hour means (± SD) averaged for two replicate trials on GRP2 fish for the 1 daily feeding treatment

3-hour mean oxygen consumption and ammonia excretion rates with 1 feeding per day								
		Post-feed[b]						
Metab. rate	Pre-feed[a]	1	2	3	4	5	6	7
OC[c]	173 ± 17.7	216 ± 51.0	216 ± 46.7	204 ± 52.7	201 ± 49.9	186 ± 36.9	193 ± 32.5	163 ± 28.5
AE[d]	2.2 ± 0.7	8.0 ± 1.6	9.3 ± 1.8	8.6 ± 2.4	6.5 ± 1.3	3.8 ± 1.3	2.8 ± 1.4	2.1 ± 1.3

[a] 3-hour period prior to feeding.

[b] Successive 3-hour periods following the start of feeding.

[c] $F = 0.43$, $P > F = 0.86$.

[d] $F = 7.50$, $P > F = 0.01$.

Table 9. Metabolic rates (mg kg^{-1} h^{-1}) of walleye fed twice daily. Oxygen consumption (OC) and ammonia excretion (AE) rates are adjusted to rates for a 340 g fish. Values are 3-hour means (± SD) averaged for five replicate trials, three on GRP1 fish and two on GRP2 fish, for the 2 daily feedings treatment

	3-hour mean oxygen consumption and ammonia excretion rates with 2 feedings per day							
		Post-feed[b]				Post-feed		
Metabolic rate	Pre-feed[a]	1	2	3	Pre-feed	1	2	3
OC[c]	152 ± 5.9	153 ± 9.7	167 ± 12.8	148 ± 9.1	136 ± 8.7	156 ± 8.1	170 ± 6.7	159 ± 3.8
AE[d,e]	5.6 ± 1.4	6.0 ± 0.5	6.5 ± 2.9	5.7 ± 2.9	4.8 ± 2.1	6.6 ± 1.4	7.3 ± 1.6	6.3 ± 1.3

[a] Prefeeding means are averages of 3-hour periods prior to feedings.

[b] Post-feeding means are averages of successive 3-hour periods following the start of feedings.

[c] $F = 8.07$, $P > F = 0.01$

[d] $F = 0.60$, $P > F = 0.75$

[e] Only 4 replicates were analyzed for ammonia excretion.

While 3-hour mean OC rates varied singificantly throughout the day, AE rates did not. No significant differences were observed between pre- and post-feeding 3-hour mean rates (Table 9). However, increases in 3-hour mean AE rates of > 52% were observed between pre-feeding and post-feeding means. Like OC rates, 3-hour mean AE rates were the greatest in the second 3-hour interval following the start of feeding.

Three feedings

Weight adjusted metabolic rates of walleye fed three times daily did not vary significantly across the course of a day (Table 10). Mean OC rates were consistently lowest during pre-feeding periods and they increased by less than 15% in the 3-hour intervals following the start of feeding (Table 10). AE rates climbed steadily as the day progressed (Figure 6) but differences between pre- and post-feeding 3-hour means were not significant. However, the 3-hour mean rate reported in the first 3-hour interval following the last feeding (8.9 mg kg^{-1} h^{-1}) was 67% greater than the first 3-hour mean pre-feeding rate (5.3 mg kg^{-1} h^{-1}).

Multiple feedings

Mean 3-hour metabolic rates of walleye fed 15 times daily did not vary significantly. Both OC and AE rates were lowest in the 3-hour interval prior to feeding (Table 11). Maximum 3-hour mean OC and AE rates occurred in the first

Table 10. Metabolic rates (mg kg^{-1} h^{-1}) of walleye fed three times daily. Oxygen consumption (OC) and ammonia excretion (AE) rates are adjusted to rates for a 340 g fish. Values are 3-hour means (± SD) averaged for four replicate trials, two on GRP1 fish and two on GRP2 fish, for the 3 daily feedings treatment

	3-hour mean oxygen consumption and ammonia excretion rates with 3 feedings per day							
		Post-feed[b]		Post-feed		Post-feed		
Metabolic rate	Pre-feed[a]	1	Pre-feed	1	Pre-feed	1	2	3
OC[c]	148 ± 11.0	157 ± 5.4	142 ± 9.6	163 ± 18.2	150 ± 16.9	153 ± 6.7	163 ± 8.2	152 ± 11.0
AE[d]	5.3 ± 2.2	6.8 ± 1.2	6.3 ± 0.9	7.7 ± 0.8	7.9 ± 0.9	8.9 ± 1.1	8.3 ± 2.1	6.7 ± 2.5

[a] Prefeeding means are averages of 3-hour periods prior to feedings.

[b] Post-feeding means are averages of successive 3-hour periods following the start of feedings.

[c] $F = 1.58$, $P > F = 0.19$

[d] $F = 2.20$, $P > F = 0.07$

Table 11. Metabolic rates (mg kg^{-1} h^{-1}) of walleye fed 15 times daily. Oxygen consumption (OC) and ammonia excretion (AE) rates are adjusted to rates for a 340 g fish. Values are 3-hour means (± SD) averaged for four replicate trials, two on GRP1 fish and two on GRP2 fish, for the 15 daily feedings treatment

	3-hour mean oxygen consumption and ammonia excretion rates with 15 feedings per day							
		Hourly feeding					Post-feed[b]	
Metabolic rate	Pre-feed[a]	1	2	3	4	5	1	2
OC[c]	141 ± 5.7	155 ± 5.7	159 ± 9.1	157 ± 7.9	155 ± 6.7	152 ± 9.7	161 ± 10.7	154 ± 7.5
AE[d]	4.3 ± 0.3	5.7 ± 0.7	7.2 ± 1.3	6.5 ± 2.6	7.1 ± 1.7	7.1 ± 1.8	7.2 ± 1.5	6.3 ± 1.0

[a] Prefeeding means are averages of 3-hour periods prior to feedings.

[b] Post-feeding means are averages of successive 3-hour periods following the end of feedings.

[c] $F = 2.21$, $P > F = 0.07$

[d] $F = 1.80$, $P > F = 0.13$

3-hour interval following the end of feeding (Table 11).

DISCUSSION

SDA results in oxygen consumption rates up to 100% pre-feeding levels and increases of 500% for pre-feeding ammonia excretion rates (Brett and Zala 1975). Cai and Summerfelt (1991) oberved increases of 10% in oxygen consumption and 30% in ammonia excretion after one daily feeding. In the present study, increases between minimum and maximum levels ranged between 38-60% for oxygen consumption and between 87-600% for ammonia excretion. Significant increases in pre-feeding oxygen consumption occurred 3-4.5 hours after feeding when fish were fed once or twice each day, but not when fed more than twice each day. Increases in ammonia excretion after feeding were significant when fed once per day, but not when more than one feeding per day occurred. Ramnarine et al. (1987) reported a cumulative effect of feeding on ammonia excretion. Ammonia excretion rates progressively increased throughout the day when fish were fed more than one time daily. This effect was also observed in the present study. Ammonia excretion rates progressively increased when fish were fed 3 and 15 times daily, peaking sometime after the end of feeding.

These findings indicate that aquaculturists must be aware of the effects of feeding on metabolism and must manage their facilities to adjust for rapid, large increases in

oxygen consumption or ammonia excretion associated with feeding. Porter et al. (1987) state that from a practical point of view it is important to determine diurnal patterns of ammonia excretion in order to establish optimum feeding regimes. Beamish and MacMahon (1988) recommend a frequent feeding schedule to enhance food intake. The findings of the current study indicate that a frequent feeding schedule will reduce the variability in oxygen consumption and ammonia excretion rates, thus improving the abilities of hatchery operators to maintain a constant rearing environment.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Iowa Department of Economic Development to the Engineering Research Institute of Iowa State University.

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SUMMARY AND DISCUSSION

The effects of size and feeding frequency on metabolic rates have important impacts on how an aquaculture facility is designed and managed. Operators must adjust their daily feeding schedules to meet the demands of the cultured species. System carrying capacities must be adjusted for changes in oxygen consumption and ammonia excretion associated with increasing fish size. Oxygen consumption rates declined at the rate of 0.22 mg kg^{-1} h^{-1} per kg increase in fish size. Ammonia excretion rates declined at the rate of 0.005 mg kg^{-1} h^{-1} per kg increase in fish size.

Feeding frequency had little effect on mean daily rates of metabolism. However, the variation in mean metabolism was affected by the feeding schedule. Ideally, the goal of any culturist is to maintain a constant rearing environment. Comparing pre- and post-feeding metabolic rates in 3-hour time blocks within each treatment type indicated that when fish were fed once or twice daily significant increases in either oxygen consumption or ammonia excretion rates or both occurred following feeding, although mean daily rates did not vary significantly between treatment types. The variation observed in metabolism under the 1 and 2 daily feeding treatments should be avoided. However, some advantages in terms of fish growth may be achieved by feeding less

frequently. If meals are equally divided into several similar sized feedings, the more aggressive individuals will consume a larger percentage of the food, whereas, if food is introduced in mass quantities, the aggressive individuals become rapidly satiated allowing less aggressive fish to feed. Mass feeding would promote growth of the entire group rather than strictly the more aggressive fish. Subjectively, this effect was observed. The 21-day measurements of fish weight showed a progressively increasing variation in average fish size with the largest variations in size occurring when fish were fed more frequently. Unfortunately, individual growth rates were not monitored, giving little indication of how individual fish were growing. Further research needs to be conducted on the effects of feeding frequency on growth.

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ACKNOWLEDGMENTS

I would like to thank Dr. E. Robert Baumann for the opportunity to conduct this research and for his constant support, both financial and moral. I am also grateful to Dr. Robert Summerfelt, my academic advisor, for his critical review and advice on this thesis. Ms. Donna Lutz, Mr. Kelly Buchman, Mr. Steven Summerfelt, Mr. Jon Petersen and Mr. Greg Sindt are deservedly acknowledged for their assistance in fish weighing, data collection during the 24-h monitoring events and analysis of dissolved oxygen and ammonia samples collected during these events. Thank you all.

This study was supported in part by a grant from the Iowa Department of Economic Development to the Engineering Research Institute.