



Effect of diet on body composition and physical ability of intensively cultured Walleye fingerlings
by Jay Jeffrey Pravecek

A thesis submitted in partial fulfillment of the requirements for the degree Of Master of Science in Fish
and Wildlife Management

Montana State University

© Copyright by Jay Jeffrey Pravecek (1995)

Abstract:

Hatcheries are under increased scrutiny due to the unpredictable performance of hatchery-reared fish after release into the wild. If hatcheries are to continue as an important tool for fish management, evaluation of hatchery-reared fish will continue to be an important aspect of fish culture. This study evaluated the effect of dietary lipids on the body composition and physical ability of walleye fingerlings. Diets provided lipids at five levels (3.3, 7.9, 12.2, 19.2, and 22.3%) and fish were fed for 18 weeks. The percentage of body fat, protein, and moisture were affected ($P < 0.05$) by diet. Dietary lipid levels also affected both weight gain and length change ($P < 0.05$) but not condition factor. The weight gains of fat and protein were related linearly to dietary lipids ($P < 0.05$). Dietary lipid levels did not affect blood protein or hematocrit levels. Testing the effect of body fat level on the physical ability of walleye fingerlings showed thermal tolerance and swimming force were affected by body fat levels ($P < 0.05$) while oxygen tolerance and swimming stamina were not. Survival over 103d of starvation was not significantly affected by body fat content. Body fat, protein, and moisture were significantly different between dietary treatment groups before 103d of starvation ($P < 0.05$) but not after. Data suggest a diet containing 12.2% fat maintains acceptable growth in hatchery-reared walleye and maximizes thermal tolerance and swimming stamina. These factors may increase their chance at survival after release into the wild. Further research is necessary to directly evaluate the effect of body fat on post-release survival.

EFFECT OF DIET ON BODY COMPOSITION AND PHYSICAL
ABILITY OF INTENSIVELY CULTURED WALLEYE FINGERLINGS.

by

Jay Jeffrey Pravecek

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 1995

N378
P8915

ii

APPROVAL
of a thesis submitted by
Jay Jeffrey Pravecek

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

8/14/95
Date

Robert D. White
Chairperson, Graduate Committee

Approved for the Major Department

Date

ERW
Head, Major Department

Approved for the College of Graduate Studies

8/27/95
Date

B. Brown
Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature

Jay Pravecek

Date

7-10-95

ACKNOWLEDGMENTS

I would like to thank all the people at the Fish Technology Center for advice, encouragement, guidance, and support during all aspects of my graduate work. Without their help, none of this would have been possible. Special thanks goes to Dr. Frederic Barrows for his advice and enthusiasm during this study. Unit leader, Dr. Robert White deserves special recognition for his advice and support. I would also like to thank Drs. Thomas McMahon and Harold Picton for reviewing this manuscript and for their input into this study. This project was completed through the Walleye Diet Development Project, and I would like to thank all the cooperators for their support.

For all her encouragement and help with deadlines and paperwork, I would like to extend my gratitude to Dee Topp. Thanks are also due to Chris Downs, Sue Ireland, and Eric Reiland for their friendship and assistance. For encouraging me to pursue this degree and supporting me through the process, I would like to thank Lara Trandahl. Finally, I would like to thank my parents and my grandma "4" for the important role they played throughout my education.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	ix
ABSTRACT	xi
INTRODUCTION	1
METHODS	6
Objective 1: Effect of diet on body composition. .	6
Fish culture.	6
Diets	7
Data collection.	9
Proximate analysis.	10
Crude fat	11
Crude protein	11
Moisture	12
Ash	12
Objective 2: Effect of body composition on physical	
ability	13
Thermal and hypoxia challenge	13
Apparatus.	13
Testing procedure	15
Sustained swimming.	16
Apparatus.	16
Testing procedure	18
Burst Force	19
Apparatus	19
Testing procedure	21
Starvation	22
Statistics	22
RESULTS	23
Objective 1: Effect of diet on body composition. .	23
Body composition	23
Wet weight	23
Dry weight	23
Growth indices	30
Blood chemistry.	34
Objective 2: Effect of body composition on physical	
ability	35

TABLE OF CONTENTS (continued)

	Page
Thermal tolerance	35
Oxygen tolerance	37
Sustained swimming.	39
Burst force	39
Prolonged starvation	43
DISCUSSION	45
Objective 1: Effect of diet on body composition. .	45
Body composition	45
Growth indices	46
Objective 2: Effect of body composition on physical ability.	48
Thermal tolerance	48
Oxygen tolerance	49
Sustained Swimming.	50
Burst force	51
Starvation	52
LITERATURE CITED	56
APPENDICES	61
Appendix A--Periodic water quality measurements taken during the walleye fingerling diet trial. .	62
Appendix B--Results of proximate analysis tests performed on walleye fingerlings fed six levels of dietary fat. Tests include pretrial data. . .	64
Appendix C--Results of proximate analysis tests performed on experimental diets from walleye fingerling diet trial.	66

LIST OF TABLES

Table		Page
1.	Composition of experimental diets fed to walleye fingerlings. L = low; M = medium; H = high crude fat.	8
2.	Effect of dietary lipids on body composition of walleye fingerlings on a wet-weight basis. Probabilities are those of greater F-values (ANOVA). (mean + SD)	24
3.	Effect of dietary lipids on body composition of walleye fingerlings on a dry-weight basis. Probabilities are those of greater F-values (ANOVA). (mean + SD)	27
4.	Effect of dietary lipids levels on weight gain, length change, and condition factor of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (mean + SD)	30
5.	Effects of dietary lipids on type of weight gain of walleye fingerlings. Probabilities are those of a greater F-value (ANOVA). (mean + SD)	32
6.	Effect of the level of dietary lipid on blood protein and hematocrit level of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (mean + SD).	34
7.	Arrangement of fat groups of walleye fingerlings by percent body fat.	36
8.	Effect of body fat level on thermal and oxygen tolerance of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (mean + SD).	37
9.	Effect of body fat level on swimming stamina and burst force of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (means + SD)	40

LIST OF TABLES (continued)

Table		Page
10.	Correlation coefficient and regression equation for walleye fingerlings subjected to burst force tests.	42
11.	Comparison of walleye fingerling burst force (g/cm) in equal size categories.	42
12.	Effects of 103 d of starvation on survival and compositional weight loss of walleye fingerlings fed varying levels of dietary lipids. Probabilities are those of greater F-values (ANOVA). (means + SD)	43
13.	Body compositional changes following 103 days of starvation for walleye fingerlings fed diets containing varying levels of lipids. Probabilities are those of greater F-values (ANOVA). (mean + SD).	44
14.	Periodic water quality measurements taken during the walleye fingerling diet trial	63
15.	Results of proximate analysis tests performed on walleye fingerlings fed six levels of dietary fat. Tests include pretrial data.	65
16.	Results of proximate analysis tests performed on experimental diets from walleye fingerling body composition trial	67

LIST OF FIGURES

Figure		Page
1.	Schematic illustration of thermal and oxygen challenge unit. A = oxygen and temperature monitors; B = challenge container; C = recirculation tank; D = emersion heaters; E = nitrogen bottle; F = pump; G = water filter; H = water quality monitoring tank.	14
2.	Schematic illustration of apparatus used for determination of swimming stamina. A = flow meter; B = bypass valve; C = baffle system; D = inlet; E = outlet; F = screen with electric grid; G = pump; H = swimming lane; I = headbox.	17
3.	Schematic illustration of apparatus used for determination of burst force of walleye. A = aluminum tank; B = laboratory scale; C = 15 cm diameter PVC cut in 1/2; D = size 4 barbless hook; E = electrical band; F = 6-volt lantern battery.	20
4.	Effect of dietary lipids on body fat of walleye fingerlings ($r = 0.99$).	25
5.	Effect of dietary lipids on body moisture of walleye fingerlings ($r = -0.95$)	25
6.	Relationship between body fat and moisture of walleye fingerlings on a wet-weight basis ($r = -0.99$).	26
7.	Effect of dietary lipids on dry fat content of walleye fingerlings ($r = 0.99$).	28
8.	Effect of dietary lipids on dry protein content of walleye fingerlings ($r = -0.98$).	28
9.	Relationship between fat content and protein content of walleye fingerlings on a dry-weight basis ($r = -0.99$).	29

LIST OF FIGURES (continued)

Figure		Page
10.	Weight gain (g) of walleye fingerlings fed varying levels of dietary lipids for 18 weeks ($r = 0.88$).	31
11.	Effect of dietary lipids on the length gain of walleye fingerlings after 18 weeks ($r = 0.93$)	31
12.	Effect of dietary lipids on body fat gained of walleye fingerlings ($r = 0.98$).	33
13.	Effect of dietary lipids on protein gain of walleye fingerlings ($r = 0.87$).	33
14.	Effect of body fat level on thermal tolerance of walleye fingerlings ($r = -0.82$). See table 7 for an explanation of fat groups.	38
15.	Relationship between length of walleye and burst force ($r = 0.68$).	41

ABSTRACT

Hatcheries are under increased scrutiny due to the unpredictable performance of hatchery-reared fish after release into the wild. If hatcheries are to continue as an important tool for fish management, evaluation of hatchery-reared fish will continue to be an important aspect of fish culture. This study evaluated the effect of dietary lipids on the body composition and physical ability of walleye fingerlings. Diets provided lipids at five levels (3.3, 7.9, 12.2, 19.2, and 22.3%) and fish were fed for 18 weeks. The percentage of body fat, protein, and moisture were affected ($P < 0.05$) by diet. Dietary lipid levels also affected both weight gain and length change ($P < 0.05$) but not condition factor. The weight gains of fat and protein were related linearly to dietary lipids ($P < 0.05$). Dietary lipid levels did not affect blood protein or hematocrit levels. Testing the effect of body fat level on the physical ability of walleye fingerlings showed thermal tolerance and swimming force were affected by body fat levels ($P < 0.05$) while oxygen tolerance and swimming stamina were not. Survival over 103d of starvation was not significantly affected by body fat content. Body fat, protein, and moisture were significantly different between dietary treatment groups before 103d of starvation ($P < 0.05$) but not after. Data suggest a diet containing 12.2% fat maintains acceptable growth in hatchery-reared walleye and maximizes thermal tolerance and swimming stamina. These factors may increase their chance at survival after release into the wild. Further research is necessary to directly evaluate the effect of body fat on post-release survival.

INTRODUCTION

During the past decades, hatcheries have come under increased scrutiny due to the unpredictable performance of the hatchery-reared fish (Hilborn 1992; White 1992). Reasons for poor survival after release into the wild possibly include: inability to compete for food and territory (Miller 1958); domestication of broodstocks resulting in genetically inferior fish not suited for the natural environment (Vincent 1960); and, lower stamina causing increased predation and decreased foraging ability (Green 1964; Shustov and Shchurov 1988). In a comparison of hatchery-reared and wild brown trout (Salmo trutta), Bachman (1984) attributed mortality of the hatchery-reared fish to excessive energy expenditure. This energy expenditure is expressed through frequent movement, inefficient use of feeding areas, and increased aggressive behavior. Ersbak and Haase (1983) showed that the nutritional condition at the time of release can affect the survival of hatchery-reared brook trout (Salvelinus fontinalis). These fish were unable to capture enough food to satisfy the high metabolism established in the hatchery. Hatcheries are an important mechanism in establishing and maintaining valuable

fisheries. As an example, striped bass (Morone saxatilis) are now being released in 456 reservoirs in 36 states (Parker 1986). Walleye (Stizostedion vitreum) culture is also a success, with 43 states now maintaining size records (Anonymous 1993). If hatcheries are to continue as a tool for fish management, evaluation of hatchery-reared fish will continue to be an important aspect of fish culture.

Walleye fry have been released from hatcheries for the past 120 years. By the early 1980's, the number of walleye being released throughout North America was close to one billion (Conover 1986). The growing popularity of walleye as a sportfish makes it a likely candidate for expanded introduction. Although they have been stocked for over 100 years, the success of new introductions and stocking has been highly variable (Bennett and McArthur 1990). Colesante et al. (1986) reported that improvements in intensive walleye culture are necessary because many hatcheries do not have extensive culture ponds. Also, pond culture of walleye often results in unpredictable production. Recently, improvements have been made in the areas of nutrition and culture techniques for walleye fry (Barrows et al. 1993). These improvements increase survival and make intensive culture of walleye more practical.

Nutrition is an important aspect of fish survival within the hatchery as well as the wild. Because cultured fish are reared in a relatively small area, details of their

requirements for growth and health are important to the fish culturist. Development of a feeding program requires attention to several factors including nutrient requirements, environmental conditions, and stress (Piper et al. 1982). Body composition at the time of release is a result of the feeding program and may influence the fishes survival in the wild. Hatchery-reared fish usually show a higher lipid content compared to wild counterparts (Vincent 1960; Thompson et al. 1991). Burrows (1969) demonstrated that higher lipid reserves in fall chinook salmon (Oncorhynchus tshawytscha) can increase survival. The evaluation of nutrition with respect to physical ability would be a valuable tool to improve the quality of walleye produced in the hatchery. An understanding of how a fish may react under certain physical constraints is important to fishery managers.

Performance tests are used in determining fish tolerance to certain conditions which may be harmful to them. These tests are based on three assumptions: 1) a large stress load is harmful and weakens fish physically; 2) the effect of multiple stressors is additive; and, 3) the reduced capacity of individual fish may impair the population as a whole (Wedemeyer et al. 1984).

The effects of stressors may be monitored with the use of thermal and hypoxia performance tests. These tests determine whether the temperature or oxygen range for fish

has been narrowed. Wedemeyer et al. (1984) found these tests to be practical when determining physiological changes. Hoar and Dorchester (1949) were able to detect changes in the thermal tolerance in goldfish (Carasius auratus) by altering the type of lipids fed in the diet. Hoar and Cottle (1952) reported changes in the heat tolerance of goldfish by modifying the degree of saturation of lipids in the diet.

A second type of performance test is the determination of swimming stamina. Swimming stamina tests have been used to show differences in physical ability between domestic and wild stocks of fish. Green (1964) and Miller (1958) found domestic stocks of rainbow trout (Oncorhynchus mykiss) and brook trout are not as strong as wild fish in the natural environment. Shustov and Shchurov (1988) found wild Atlantic salmon (Salmo salar), as measured by swimming force, were two times stronger than their hatchery-reared counterparts. Horak (1972), however, was unable to detect a relationship between swimming stamina of hatchery-reared rainbow trout and post-stocking survival.

Researchers have also used starvation studies to detect differences in survival of young fish. Oliver et al. (1979) showed that larger smallmouth bass (Micropterus dolomieu) survive better than smaller members of the same year class. Thompson et al. (1991) reported similar results for young Colorado squawfish (Ptychocheilus lucius).

Performance studies have centered on trout and salmon for two reasons; first, their enormous popularity and economic importance and second, destruction of their natural habitat with the building of dams and diversions across the United States. While reservoirs have destroyed habitat for salmonids, they have opened up millions of acres of water for other sportfish such as walleye.

Evaluation of hatchery-reared walleye with respect to performance is required to determine culture practices which optimize survival in the wild. To date, no information is available to document the effects of body composition on the performance of walleye.

The objectives of this project were:

- 1) To evaluate the effect of diet on body composition of intensively reared walleye fingerlings.
- 2) To evaluate the effect of body composition on the physical ability of walleye fingerlings using accepted laboratory testing procedures.

METHODS

Objective 1: Effect of diet on body composition

Fish culture

Pond-reared walleye fingerlings were obtained from the Miles City State Fish Hatchery, Montana. Fish had been fed Biodiet, a commercial dry diet, for 2 months prior to the experiment. Twenty-four hours after arriving at the Bozeman Fish Technology Center, Montana, 100 fish (initial weight, $5.2 \text{ g} \pm 0.1 \text{ g}$) were randomly assigned to one of 24, 120 L tanks. Fish were fed 3 mm Biodiet for a 3-week acclimation period during which mortalities were counted and replaced. Feed was dispensed by Ziegler 12 h belt feeders (3 p.m. to 3 a.m.) to reduce disturbance. Feed amounts were 5% of the tank weight adjusted every 3 weeks. Lighting was controlled automatically to approximate a 12 h light/dark cycle. At the beginning and ending of each 12 h period, light was gradually intensified or reduced over 90 min allowing fish to acclimate. Waste feed and feces were drained daily. Twice monthly throughout the study, fish were subjected to a 1% salt bath for 15 min to alleviate handling stress.

Water was received from two sources. The first was a

well with a constant temperature of 19.8°C. Water was delivered via 5 cm diameter, poly-vinyl-chloride (pvc) pipe which ran approximately 300 m along the ground. Weekly water temperature analysis showed weather was influencing temperatures (Appendix A). Average water temperatures dropped from 19.8°C on September 16 to 17.3°C on November 18. On the tenth week of the trial, water was switched to a warm spring (20.3°C). All water was degassed in a 20 cm diameter, 1 m-long PVC pipe filled with plastic media. Water flow into the tanks was adjusted to 5.5 L/min resulting in three exchanges of water per hour. On a weekly basis, water quality was assessed by using a Sweeny gas saturometer Model DS1-A (Sweeny Co., CT) and a YSI Model 50 dissolved oxygen meter (Yellow Springs Instrument Co., OH). Parameters measured included: temperature, total dissolved gases, delta pressure, oxygen (mg/L), oxygen (%saturation), and nitrogen (%saturation). All water quality data are reported in Appendix A.

Diets

Diets were formulated to provide five different levels of dietary lipids (Table 1). Diets were maintained isocaloric by replacing fish oil and lecithin with dextrin and cellulfil (cellulose) as the lipid level was reduced. The general procedure for preparing diets was to mix all non-variable ingredients in a large mixer as a base mix

Table 1. Composition of experimental diets fed to walleye fingerlings. L = low; M = medium; H = high crude fat.

Ingredient	Diet(g/100 g)				
	L	LM	M	MH	H
Herring meal	23.4	23.4	23.4	23.4	23.4
Wheat gluten	27.0	27.0	27.0	27.0	27.0
Wheat flour	11.5	11.5	11.5	11.5	11.5
Corn gluten meal	8.1	8.1	8.1	8.1	8.1
Blood meal	4.2	4.2	4.2	4.2	4.2
Fish oil	2.0	4.3	6.5	8.8	11.0
Lecithin	0.0	2.5	5.0	7.5	10.0
Cellulfil	9.0	6.8	4.5	2.5	0.0
Dextrin	10.0	7.5	5.0	2.3	0.0
Finstim ¹	1.5	1.5	1.5	1.5	1.5
TIC 515 ²	2.0	2.0	2.0	2.0	2.0
Choline chloride	0.5	0.5	0.5	0.5	0.5
Vitamin C	0.2	0.2	0.2	0.2	0.2
Trace minerals ³	0.1	0.1	0.1	0.1	0.1
Vitamin premix ⁴	0.6	0.6	0.6	0.6	0.6

Proximate composition

Crude protein	52.1	52.0	51.0	51.1	51.3
Crude fat	3.3	7.9	12.2	19.2	22.3
Moisture	10.1	8.8	8.5	8.4	8.2
Ash	2.2	3.1	3.4	3.6	4.0

¹ Feeding stimulant

² Binder, TIC Gums Inc., New Jersey

³ Supplied the following per kg of dry ingredients: vitamin A, 10,000 international units (IU); vitamin D₃, 720 IU; vitamin E, 530 IU; vitamin B₁₂, 30 ug; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamine mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; folic acid, 13 mg; menadione sodium bisulfite, 25 mg; biotin, 1 mg; niacin 330 mg. The carrier was wheat midlings.

⁴ Contributed in mg/kg diet: zinc, 100; manganese, 70; iron, 3; copper, 2; and iodine, 1.

and then divide the base mix into five equal units. Each smaller unit was placed in a smaller mixer and the remaining ingredients added. Tap water was then added to produce the proper consistency among all diets to facilitate pelleting. Feed was formed into a 3 mm or 4 mm pellet using a California pellet mill and dried for 24 h in a forced air drier. Walleye grower 9015 was fed as a reference diet. Each of the six diets was randomly assigned to four of the 24 tanks after the 3 week acclimation period and fed for 18 weeks.

Data collection

After the 3 week acclimation period, a sample of five fish per tank ($n = 120$) was pooled and frozen for proximate analysis. The remaining fish from each tank were anesthetized in a mild solution of MS-222 (Tricaine Methanesulfonate) and weighed in water as a group to the nearest gram. Ten fish were randomly selected after weighing, and measured to the nearest millimeter. This procedure was repeated every 3 weeks to monitor growth and adjust feeding rates. At the end of 18 weeks, five fish were collected from each tank for proximate analysis. Before freezing, each fish was weighed to the nearest gram and measured to the nearest millimeter to determine condition factor.

After 18 weeks of feeding the experimental diets, two

fish were randomly selected from each tank for histological and blood chemistry examination. Blood samples were taken from the two fish by removing the caudal peduncle and drawing blood into a heparinized micro-hematocrit capillary tube. Immediately following, the blood was centrifuged in a Model MB International Micro-capillary centrifuge (IEC Boston, MA) for 3 min. The tube was placed in an IEC International Micro-capillary reader (IEC Boston, MA) and the percentage of blood hematocrit was determined. To determine the level of protein in the blood, the serum was separated from the hematocrit by breaking the capillary tube. A drop of serum was spread onto a Model 100/B refractometer (National Co. Baltimore, MD) and protein levels read as grams/deciliter (g/dL). The fish were then preserved in formalin for histological examination by Elizabeth MacConnell, histologist at the Fish Technology Center.

Proximate analysis

All samples were subjected to proximate composition according to methods specified by the Association of Official Analytical Chemists (AOAC 1984). Each fish sample was mixed with an equal weight of distilled water and homogenized in a homogenizer (Tekmar Mod T-50). For each sample, fat and protein analysis was performed in triplicate while moisture and ash analysis was performed in duplicate.

Crude fat

Approximately 50% of the fish homogenate was poured into a crucible and dried at 100°C for 24 h. Feed was ground to less than 1 mm and dried. After drying, approximately 1 g of sample was weighed into a porous cellulose thimble. The thimble was placed into a Tecator model Soxtec 1040 fat extraction unit (Tecator Co., Hoganas, Sweden) and reflux extracted with purified diethyl ether for 90 min. Extracted fat was collected in a clean aluminum flask below the thimble. The flask was weighed before and after boiling to determine the amount of fat in the sample.

Crude protein

Protein content was determined by placing a 1 g sample of the tissue homogenate on nitrogen-free filter paper into a Kjeldahl test tube containing 15 mL of H_2SO_4 (sulfuric acid). Two reagent tablets (3.9 g each) consisting of 89.70% K_2SO_4 (potassium sulphate) and 10.30% CuSO_4 (cupric sulphate) were added per Kjeldahl tube. The sample was digested in a Kjelttec 1015 digester to completion. The sample was cooled to room temperature and 100 mL of distilled water added to bring the solution up to an adequate volume for distillation. Nitrogen as NH_3 was determined by distillation and titration in a Kjelttec Auto 1030 Analyzer (Tecator Co. Hoganas, Sweden).

Moisture

A 2 g sample of the homogenate was weighed into a petri dish and dried for 24 h at 100°C in a conventional drying oven. The sample was reweighed and moisture calculated from the difference between the initial and final weights.

Ash

The sample used for moisture analysis was placed into a Thermolyne muffle furnace Type 48000 (Thermolyne Co., Debuque, IO) at 600°C for 2 h. After cooling to room temperature, the sample was weighed and ash weight calculated from the difference between the initial and final weights.

Objective 2: Effect of body composition on physical ability.**Thermal and hypoxia challenge****Apparatus**

This system was designed and built by William Lellis and Montie Peterson at the Haggerman Field Station, ID. (Figure 1). The unit consists of eight, 12 L cylindrical plexiglass containers with lids. The containers drain to a 130 L fiberglass collection tank which is sealed except for small holes for gas exchange. Inside the collection tank are three, 200 watt AREA quartz immersion heaters used for the thermal trials. Each heater is individually operable for adjustable heating rates. Water is recirculated via 1/6 horsepower electric pump. Before water reenters containers, it passes through an Aquanetics 220 canister filter containing zeolite. For thermal trials, water levels are maintained by individual internal standpipes. For hypoxia trials, water levels are maintained by an external standpipe located in the fiberglass tank.

Dissolved oxygen was monitored with a YSI model 59 oxygen meter (Yellow Springs Instruments, OH). Gas pressure was monitored with a Sweeny model DS1-A gas satumeter (Sweeny Co., CT) and temperature with an Omega RDT digital thermometer with a type 100w30 fast response element.

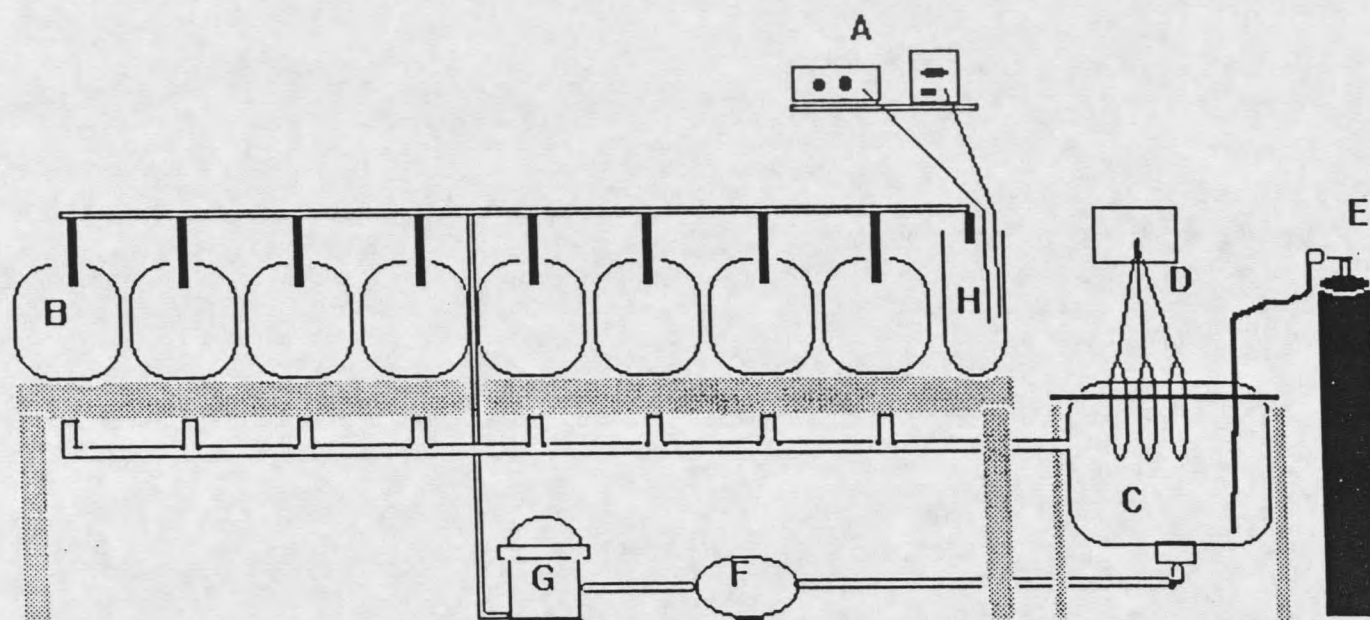


Figure 1. Schematic illustration of thermal and oxygen challenge unit.
 A = oxygen and temperature monitors; B = challenge container;
 C = recirculation tank; D = emersion heaters; E = nitrogen bottle;
 F = pump; G = water filter; H = water quality monitoring tank.

Testing procedure

Thermal challenge. A randomized block design was utilized to represent each diet treatment during each test. A total of eight tests were conducted.

Water was supplied to the challenge containers at the same temperature as experimental rearing tanks and flow was maintained at 5 L/min. Oxygen was maintained near 100% saturation by bubbling atmospheric air through a diffusion stone located in the collection tank. Six fish were selected at random from each tank, weighed as a group to the nearest gram, and placed in the container.

Heaters were turned on to raise the temperature of the water at a constant rate of 0.16 C/min. Temperature was recorded every 1/2 h to monitor rate of increase. Critical thermal maxima was determined as the temperature when each fish lost equilibrium and touched the bottom of the container. Once the endpoint was attained, fish were returned to fresh water of initial acclimation temperature.

Hypoxia challenge. Procedure for this test was identical to the thermal challenge with the exception that temperature was maintained a constant 20°C and oxygen was depleted by diffusing nitrogen gas through a diffusion stone located in the collection tank. Minimum oxygen tolerance was recorded as mg/L of oxygen in challenge containers when each fish lost equilibrium and touched the bottom of the

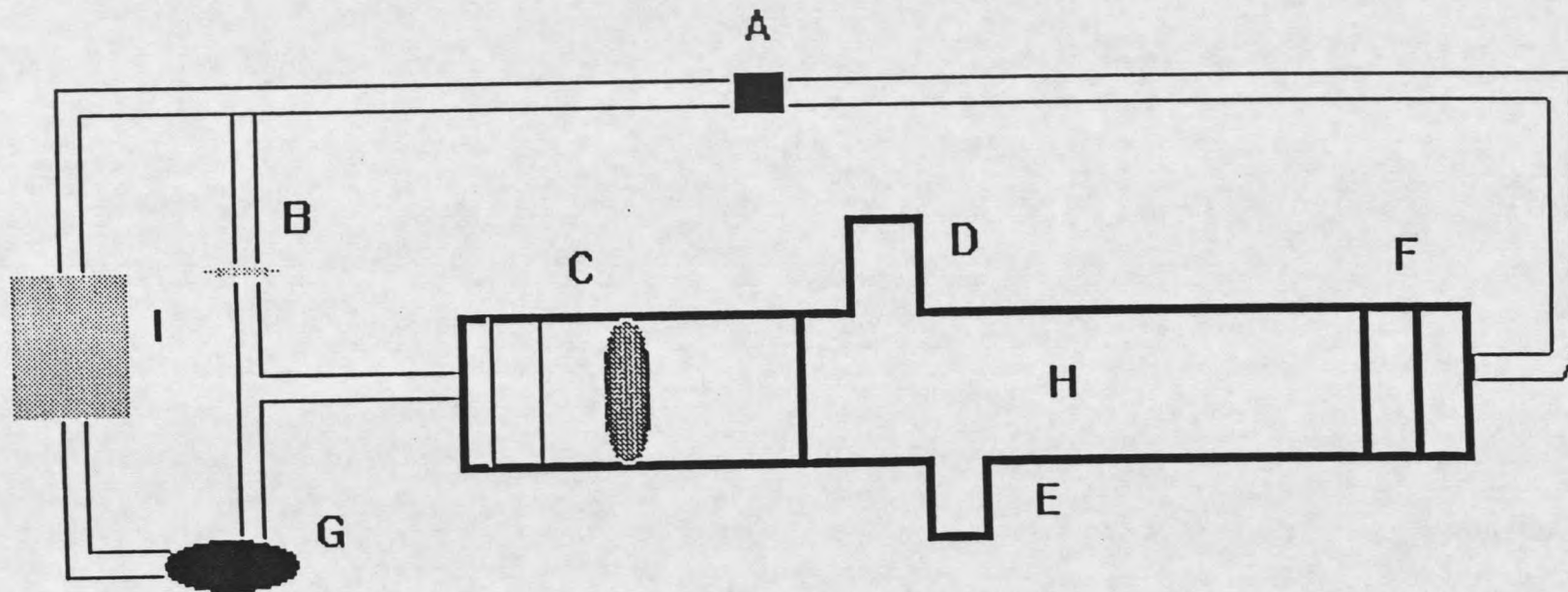
tank. Fish were then removed and placed in oxygenated water.

Sustained swimming

Apparatus

A stamina tunnel (Figure 2) was designed to measure differences between groups of fish and not for exact determination of swimming speed. Velocity in the tunnel was controlled by either diverting water through a bypass line (for low velocity) or through the actual test chamber (for higher velocities) and was monitored by a paddlewheel type flowmeter. Velocities were exact and reproducible. Water flow was calculated at 30 cm/sec by timing neutrally buoyant particles passing through the tunnel. Water was recirculated using two, 200 L/min electric pumps.

The stamina tunnel consisted of two, 1.5 m lengths of 20 cm clear plexiglass connected by two PVC T-connectors. The outlet for one T-connector faced up and one faced down to introduce and remove the fish. Metal plates were installed to maintain laminar flow within the tunnel and prevent fish from resting in the open area of the T-connectors. Two screens confined fish within the testing portion of the tunnel.



17

Figure 2. Schematic illustration of apparatus used for determination of swimming stamina. A = flow meter; B = bypass valve; C = baffle system; D = inlet; E = outlet; F = screen with electric grid; G = pump; H = swimming lane; I = headbox.

Turbulence was dissipated by two perforated sheets of plexiglass placed perpendicular to the flow and a 24 cm-long "tube-within-a-tube" baffle. This baffle was constructed from four PVC pipes of consecutively smaller size placed one inside of the other and suspended by wire. The water continued through the test chamber and returned to the headbox via 12 cm PVC pipe and a 0.75 m-long aeration tower. At this point, fresh water was added to the system to maintain high water quality. The fresh water also provided the cooler water necessary for constant temperatures.

An electrical fish barrier was installed at the downstream end of the tunnel to encourage fish to swim in the designated area. The barrier consisted of a 6-volt lantern battery connected to a metal band located 10cm in front of the tail screen. Fish were also encouraged to swim in the front portion of the tunnel by covering it with black plastic. The uncovered portion received light from a 150 watt floodlamp.

Testing procedure

To avoid variation between each test in the tunnel, six fish from three diets were tested at one time. Each group was freeze-branded 24 h before testing with a unique pattern distinguishable through the clear plexiglass tunnel. The test was conducted twice.

To begin the test, fish were combined into a 20 L bucket and poured into the inlet. Immediately after the fish were added, pumps were turned on and water diverted through the bypass line until the unit was filled. Because the return line was above the level of the tunnel, it was necessary to seal the tunnel before it was completely full. Once the unit was filled, the bypass line was closed to divert all water through the tunnel for the remainder of the test.

The fish were required to swim for 200 min as described by Brett (1967). A fish was considered exhausted as it entered the electrical field and was unable to return to the testing portion of the tunnel. All fish remained in the chamber until the trial was completed.

Burst force

Apparatus

A device for measuring burst force was built which consisted of a 45 cm section of 15 cm diameter PVC pipe cut in half lengthwise and suspended in a 30 cm x 30 cm x 120 cm aluminum rearing tank (Figure 3). Attached at the upstream end of the rearing tank was a Homs Model 200 g laboratory scale with a maximum indicator pin (Douglas Homs Co., CA). The scale was designed for use in a horizontal position and calibrated to $200\text{ g} \pm 2\text{ g}$. A 38 cm piece of 3 Kg test monofilament fishing line was attached to the scale and a

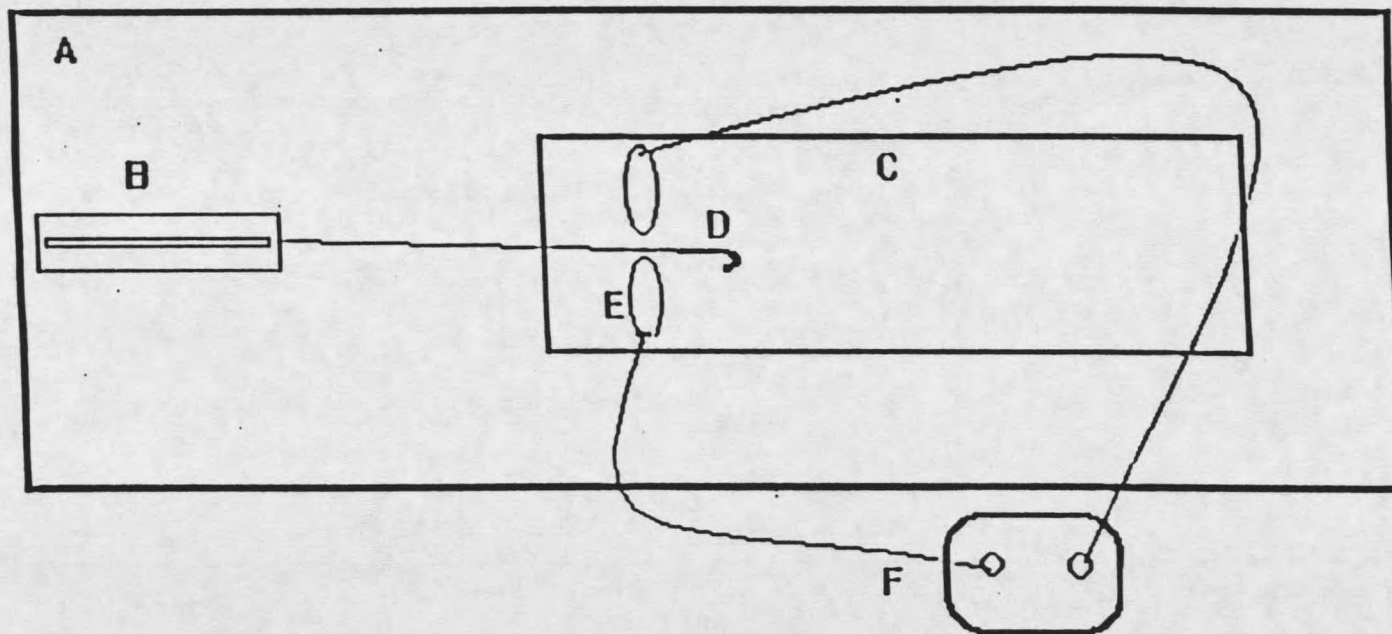


Figure 3. Schematic illustration of apparatus used for determination of burst force of walleye. A = aluminum tank; B = laboratory scale; C = 15 cm diameter PVC cut in 1/2; D = size 4 barbless hook; E = electrical band; F = 6-volt lantern battery.

size 4 barbless fishing hook was attached to the opposite end.

An electric band was placed on the PVC pipe at the end closest to the scale. The band was attached to a 6-volt lantern battery and provided the stimulus for maximum escape response. All tests were conducted in freshwater at a temperature equal to rearing temperatures (21°C).

Testing procedure

A randomized block design was utilized for this experiment. Ten fish were randomly chosen from each experimental tank. To reduce the stress associated with handling, two trials were conducted, with five fish handled during each trial. Individual fish were removed from a 5 L holding tank. Each fish was immediately attached to the hook just behind the second dorsal fin and below the vertebrae and allowed to swim for 15-20 sec. Maximum registered force in grams was recorded. The fish was then measured to the nearest millimeter and placed in a 3% saltwater solution for up to 10 min. Data recorded included length of fish in millimeters and maximum registered force in grams. Observations during preliminary trials indicated that one fish from each test group was reluctant to swim in the prescribed space. The fish with the lowest force from each group of five was dropped from the analysis.

Starvation

A test was conducted to determine the effects of prolonged starvation on survival and changes in body composition of walleye fingerlings. Only the L, M, and H diets (Table 1) were tested due to space constraints. Each diet was divided into two replicates. The fish were not fed for a period of 103 d after which all remaining fish were euthanized, counted, weighed, and frozen for proximate analysis. Water temperature was held constant at 20°C. Data recorded included: mortality, body composition at the start of the test, and body composition at the end of the test.

Statistics

Data were analyzed using the Statistical Analysis System (SAS 1986). The general linear model procedure was used to test for main effects and interactions.

RESULTS

Objective 1: The effect of diet on body composition.Body compositionWet weight

Body fat levels were affected by the level of dietary lipids ($P < 0.001$; Table 2), increasing linearly as dietary lipids increased ($r = 0.99$; $Y = 3.72 + 0.20X$; Figure 4). Body moisture was also affected by dietary lipid levels ($P = < 0.001$; Table 2; Figure 5), but decreased linearly as dietary lipids increased ($r = -0.95$; $Y = 74.77 + -0.18X$; Figure 5) and was related to body fat level ($r = -0.99$; $Y = 78.4 + -0.96X$; Figure 6). Body protein ($P = 0.09$) and ash ($P = 0.52$) were not affected by the level of dietary lipids (Table 2). A complete list of proximate analysis results by treatment and tank is given in Appendix B.

Dry weight

On a dry-weight basis, body fat was also affected by dietary lipids ($P = < 0.001$; Table 3), increasing linearly as dietary lipid increases ($r = 0.99$; $Y = 15.8 + 0.58X$;

Table 2. Effect of dietary lipids on body composition of walleye fingerlings on a wet-weight basis. Probabilities are those of greater F-values (ANOVA). (mean + SD)

Dietary lipids (%)	Body composition							
	Moisture		Fat		Protein		Ash	
	%	P	%	P	%	P	%	P
		0.001		0.001		0.09		0.52
3.3	73.7(0.8)		4.6(1.1)		17.1(0.1)		3.5(1.0)	
7.9	74.0(1.1)		5.0(0.6)		16.9(0.4)		3.1(0.4)	
12.2	72.3(0.8)		6.2(0.6)		17.6(0.1)		3.2(0.1)	
19.2	71.6(1.1)		7.3(0.8)		17.2(0.6)		2.9(0.2)	
22.3	70.3(0.3)		8.4(0.5)		17.4(0.3)		3.2(0.3)	
19.5 ¹	73.8(1.3)		4.1(1.2)		17.0(0.3)		4.0(0.4)	

¹ Reference diet

Figure 7). In contrast to wet-weight calculations, dry protein was inversely related ($r = -0.98$; $Y = 71.06 + -2.1X$; Figure 8) to, and significantly influenced by the level of dietary lipids ($P < 0.001$; Table 3). There was also an inverse relationship between the level of dry fat and dry protein in the body ($r = -0.99$; $Y = 81.81 + -0.73X$; Figure 9). Ash was not significantly affected by dietary lipids ($P = 0.15$; Table 3).

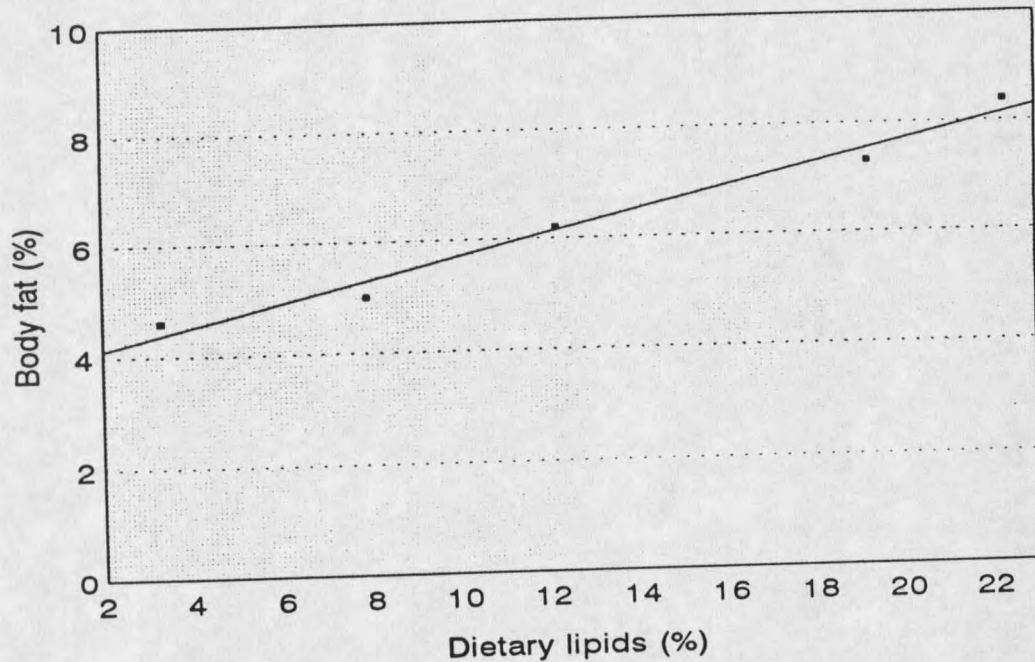


Figure 4. Effect of dietary lipids on body fat of walleye fingerlings ($r = 0.99$).

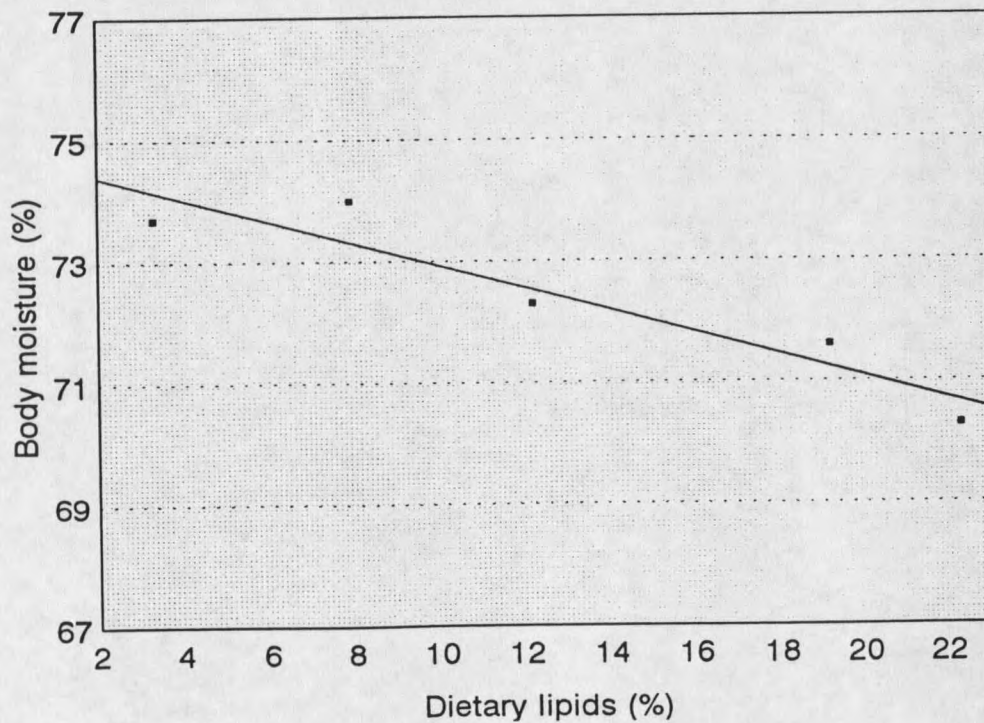


Figure 5. Effect of dietary lipids on body moisture of walleye fingerlings ($r = -0.95$).

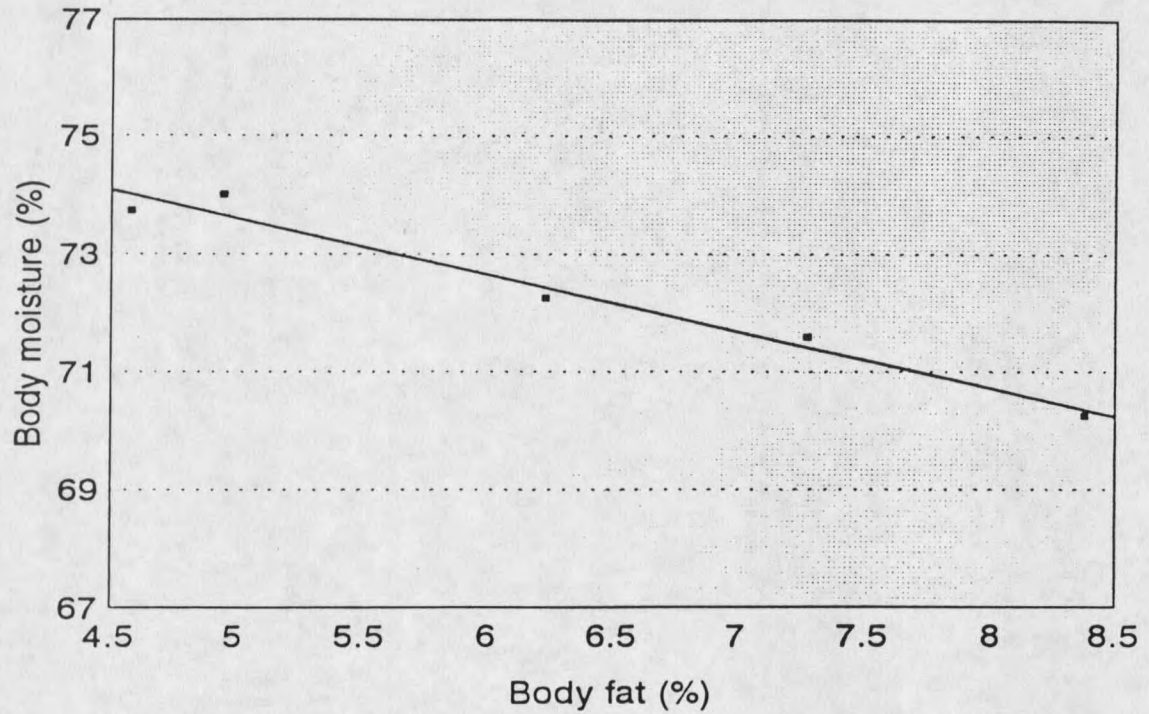


Figure 6. Relationship between body fat and moisture of walleye fingerlings ($r = -0.99$).

Table 3. Effect of dietary lipids on body composition of walleye fingerlings on dry-weight basis. Probabilities are those of greater F-values (ANOVA). (mean + SD)

Dietary lipids (%)	Body composition					
	Fat		Protein		Ash	
	%	P	%	P	%	P
		<0.001		<0.001		0.15
3.3	18.1(3.6)		68.0(3.3)		13.9(3.7)	
7.9	19.8(2.1)		67.8(0.6)		12.4(1.5)	
12.2	23.0(1.6)		65.3(1.0)		11.7(0.7)	
19.2	26.7(2.5)		62.9(1.5)		10.5(1.0)	
22.3	29.0(1.4)		60.1(0.9)		10.9(1.0)	
19.5 ¹	16.1(4.3)		68.0(2.5)		15.9(2.0)	

¹ Reference diet

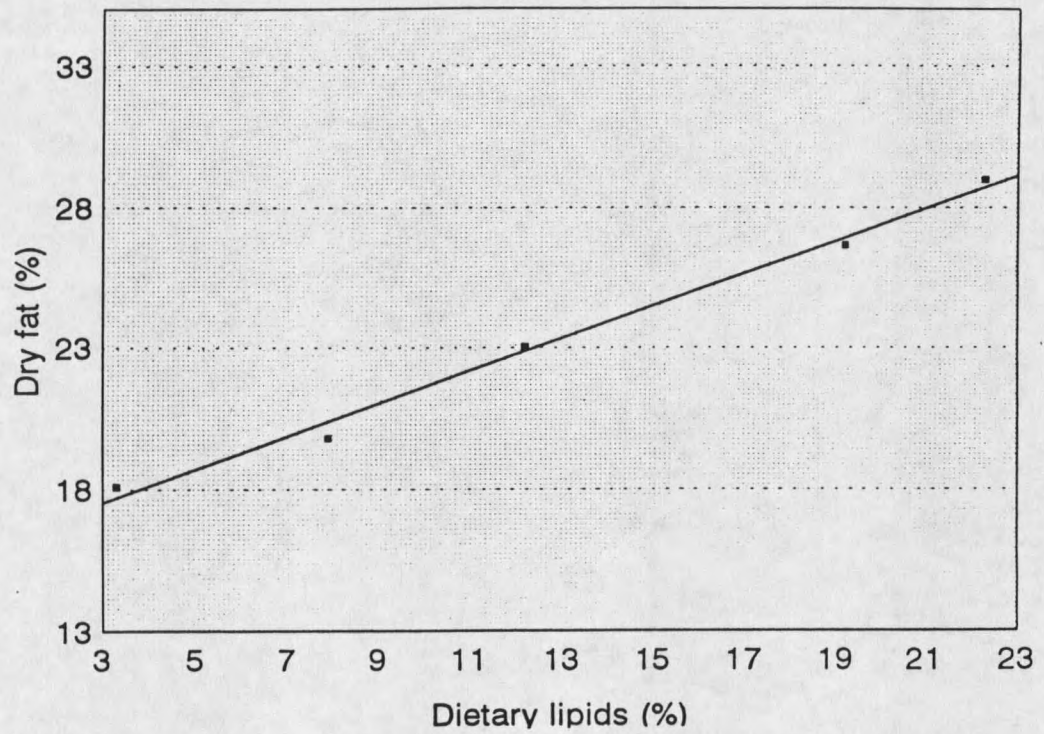


Figure 7. Effect of dietary lipids on dry fat content of walleye fingerlings ($r = 0.99$).

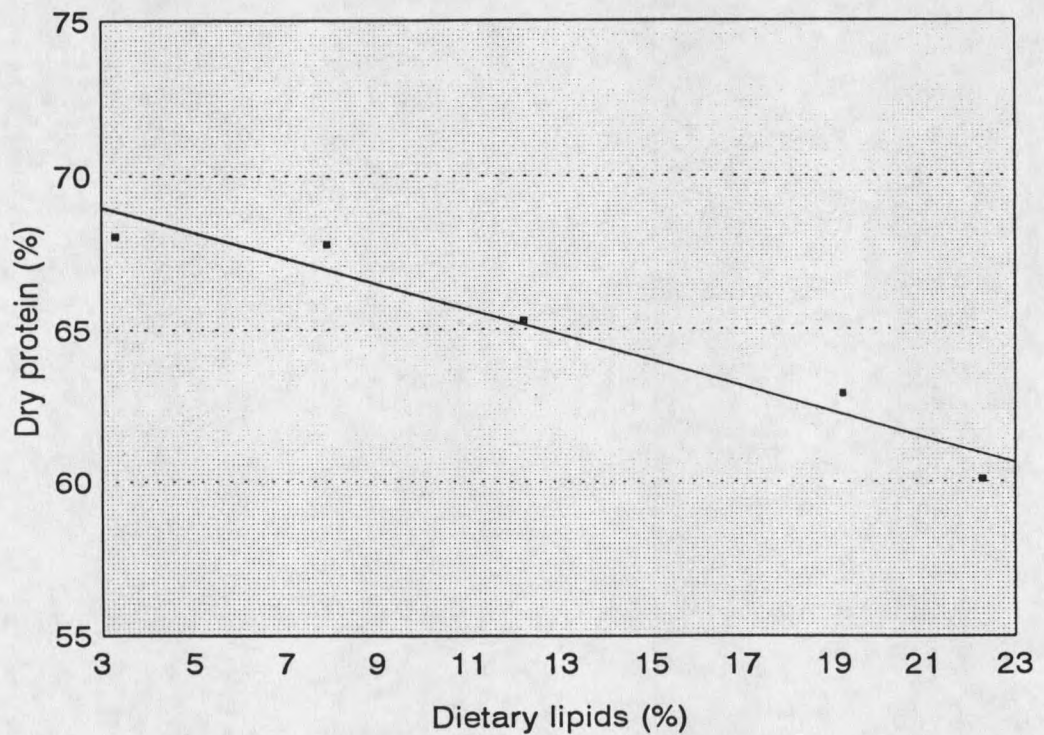


Figure 8. Effect of dietary lipids on dry protein content of walleye fingerlings ($r = -0.98$).

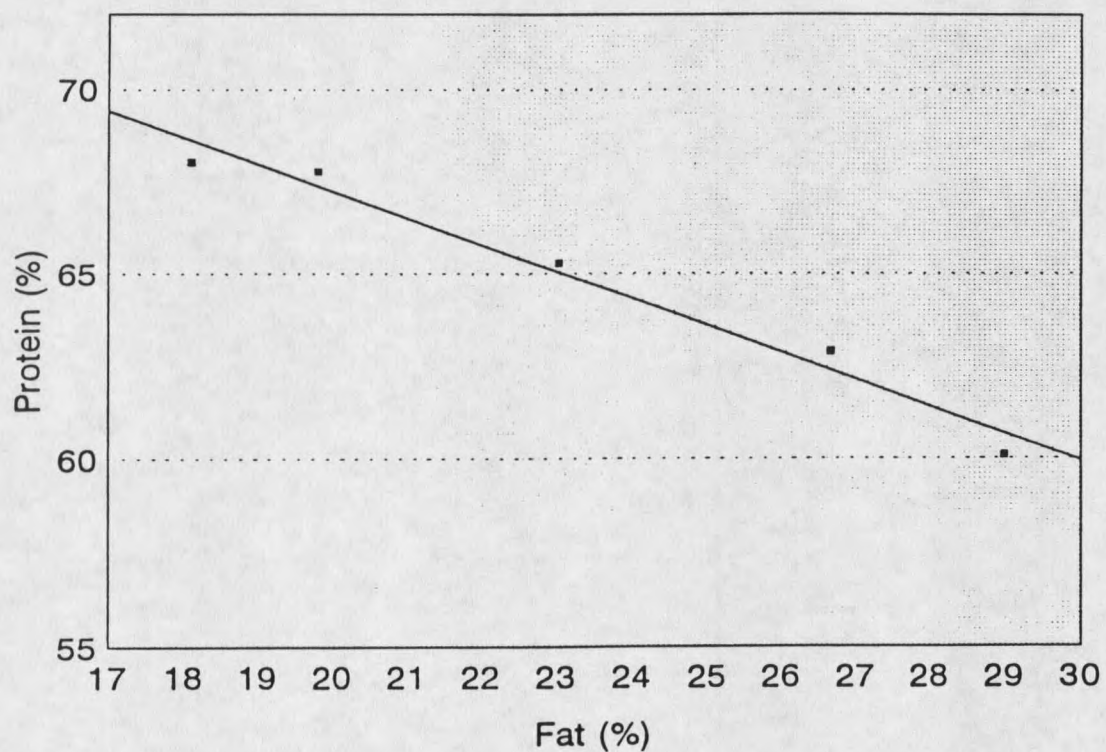


Figure 9. Relationship between fat content and protein content of walleye fingerlings on a dry-weight basis ($r = -0.99$).

Table 4. Effect of dietary lipid levels on weight gain, length change, and condition factor of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (mean + SD)

Dietary lipid (%)	Weight gain		Length change		Condition factor ¹	
	g	P	mm	P	value	P
		0.03		0.04		0.71
3.3	22.0(6.9)		45.5(14.7)		0.966(0.071)	
7.9	22.1(11.6)		41.0(17.4)		0.990(0.021)	
12.2	32.3(4.4)		51.8(9.5)		1.003(0.052)	
19.2	33.1(4.5)		62.5(8.0)		1.008(0.044)	
22.3	33.7(2.9)		63.5(4.0)		0.980(0.018)	
19.5 ²	27.9(2.1)		58.5(4.8)		0.933(0.083)	

¹ Formula for condition factor = $W/L^3 \times 100,000$

² Reference diet

Growth indices

Weight gain was affected by the level of dietary lipid ($P = 0.03$; Table 4; Figure 10) and the relationship was linear ($r = 0.88$; $Y = 206.85 + 6.91X$; Figure 10). The linear ($r = 0.93$) increase in length was also related to dietary lipid ($Y = 37.46 + 1.19X$; $P = 0.06$; Table 4; Figure 11). Dietary lipids, however, did not affect condition factor ($P = 0.71$; Table 4).

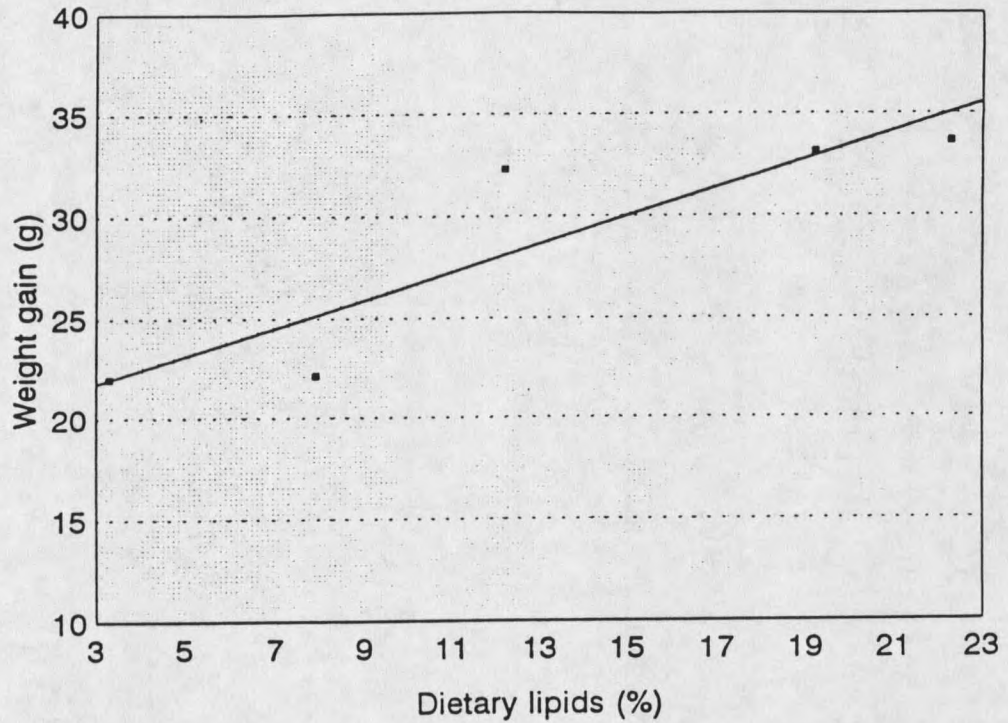


Figure 10. Weight gain (g) of walleye fingerlings fed varying levels of dietary lipids for 18 weeks ($r = 0.88$).

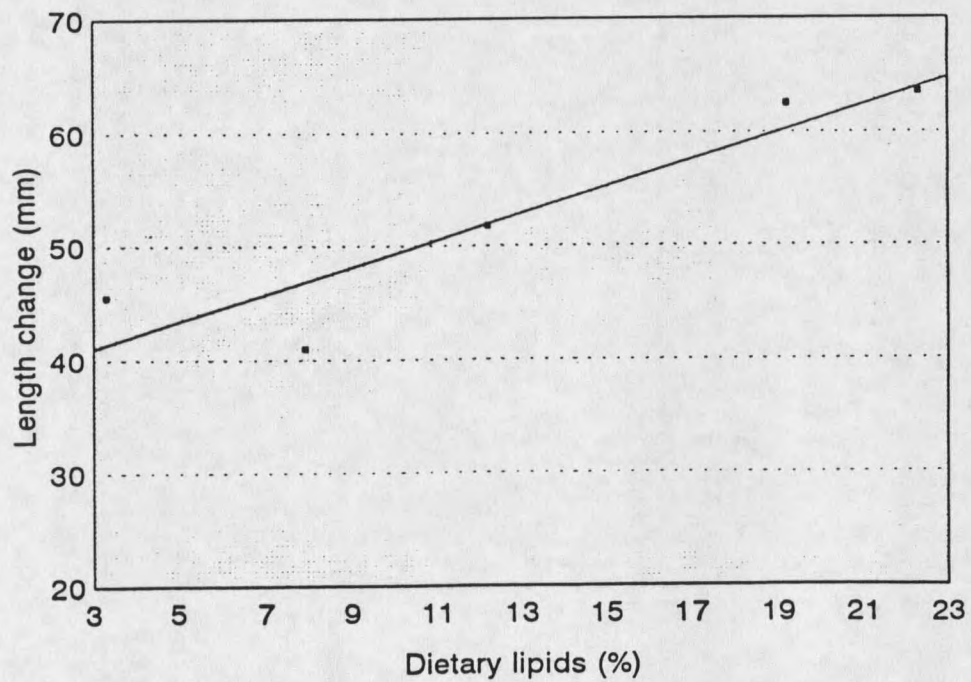


Figure 11. Effect of dietary lipids on the length gain of walleye fingerlings after 18 weeks ($r = 0.93$).

Table 5. Effects of dietary lipids on weight gain of walleye fingerlings. Probabilities are those of a greater F-value (ANOVA). (mean + SD)

Dietary lipid(%)	Weight Gain							
	Fat		Protein		Moisture		Ash	
	g	P	g	P	g	P	g	P
	<0.01		0.03		0.09		0.02	
3.3	1.2(0.7)		3.8(1.2)		16.0(5.1)		0.7(0.2)	
7.9	1.3(0.8)		3.8(2.0)		16.1(8.4)		0.6(0.3)	
12.2	2.3(0.4)		5.8(0.8)		22.9(3.2)		1.0(0.1)	
19.2	2.8(0.6)		5.7(0.7)		23.2(2.8)		0.9(0.1)	
22.3	3.3(0.1)		5.9(0.5)		23.0(2.1)		1.0(0.2)	
19.5 ¹	1.2(0.5)		4.8(0.4)		20.3(1.6)		1.2(0.2)	

¹ Reference Diet

Dietary lipids had a significant effect on the amount of body fat gained ($P = <0.01$; Table 5). The response was strongly linear ($r = 0.98$; $Y = 0.69 + 0.11X$; Figure 12). Grams of protein and ash gained were also affected by dietary lipid with a substantial increase between 7.9% and 12.2% dietary lipid and leveling off at higher levels (Table 5; Figure 14). Walleye fed the reference diet gained protein at a level intermediate to that of the 7.9% and

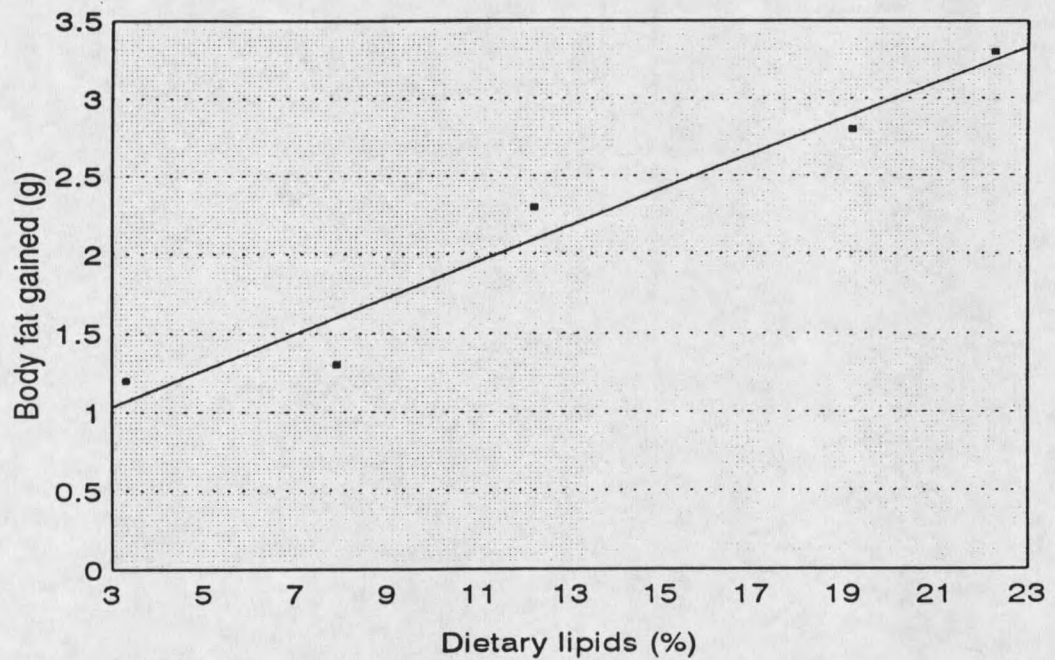


Figure 12. Effect of dietary lipids on body fat gained of walleye fingerlings ($r = 0.98$).

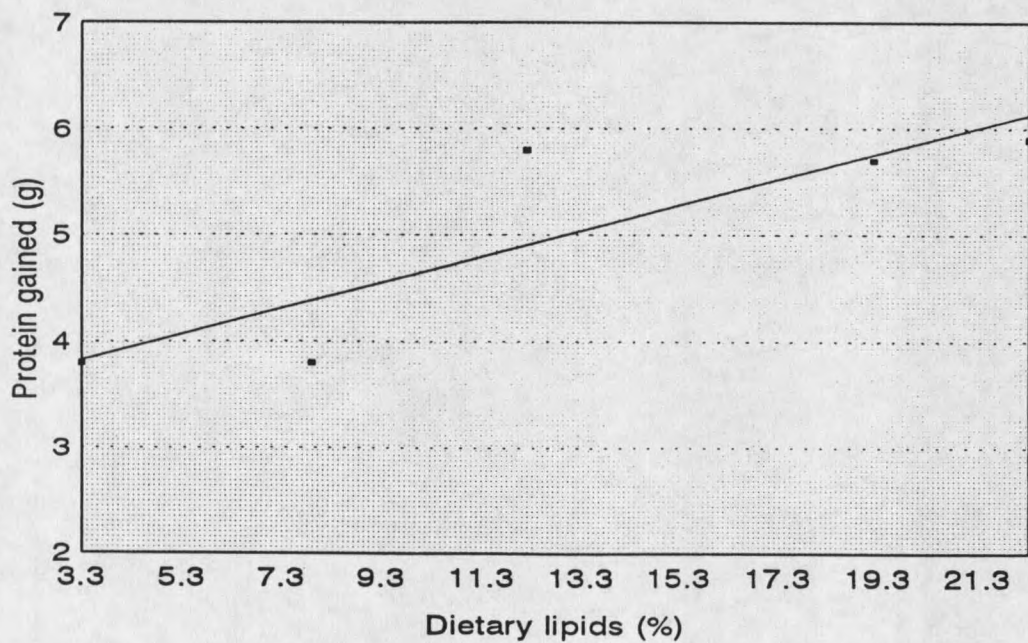


Figure 13. Effect of dietary lipids on protein gain of walleye fingerlings ($r = 0.87$).

Table 6. Effect of the level of dietary lipid on blood protein and hematocrit level of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (mean + SD)

Dietary lipid (%)	Blood protein		Blood hematocrit	
	g/dL	P	%	P
		0.10		0.59
3.3	7.53 (2.65)		37.25 (5.50)	
7.8	9.68 (0.63)		41.38 (6.94)	
12.2	9.83 (0.66)		42.38 (5.54)	
19.2	9.63 (0.54)		42.88 (5.75)	
22.3	9.93 (0.72)		42.63 (3.09)	
19.5 ¹	8.13 (0.85)		40.75 (3.01)	

¹ Reference diet

12.2% dietary lipid diets. The gain of body moisture was not significantly affected by dietary lipid ($P = 0.09$; Table 5).

Blood chemistry

The level of protein in the blood following 18 weeks of feeding experimental diets ranged from 7.53 g/dL (grams/deciliter) to 9.93 g/dL and was not significantly affected by dietary lipid ($P = 0.10$; Table 6). The percentage of blood hematocrit ranged from 37.25% to 42.88% and was not significantly affected by dietary lipid ($P = 0.59$; Table 6).

Objective 2: Effect of body composition on physical ability.

Body composition of walleye fingerlings varied among tanks within diet groups. Because the objective of this portion of the study was to test for effects of fat level in fingerling walleye, tanks having fish with similar levels of percent body fat were grouped (Table 7).

Thermal tolerance

As temperature began to rise, fingerling walleye swam in a group, a behavior not normally seen in rearing tanks. The darkened coloration of fish associated with routine handling also faded as water temperatures rose. As temperature approached upper tolerance levels, fish began to swim spastically in a vertical position. Eventually, fish lost equilibrium and were removed from the test system. Fish recovered immediately upon removal from the system and mortality was minimal. No records, however, were kept of post treatment mortality. Fat level in the body had a significant effect on the thermal tolerance of walleye ($P = 0.04$; Table 8). Fat group LM (5.6%) had the highest thermal

Table 7. Arrangement of fat groups of walleye fingerlings by percent body fat.

Fat group	Diet	Tank	%Body fat	X% fat for group
L	L	4	4.1	4.3
	L	13	4.1	
	L	18	3.9	
	LM	16	4.6	
	LM	19	4.6	
LM	LM	10	5.6	5.5
	LM	23	5.3	
	M	3	5.6	
M	L	5	6.2	6.3
	M	2	6.0	
	M	14	6.4	
	MH	15	6.5	
MH	M	20	6.9	7.3
	MH	9	7.3	
	MH	12	7.0	
	H	8	7.8	
H	MH	7	8.3	8.7
	H	6	8.8	
	H	11	8.7	
	H	24	8.2	
R	R	1	4.4	4.1
	R	17	5.3	
	R	21	4.1	
	R	22	2.4	

Table 8. Effect of body fat level on thermal and oxygen tolerance of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (means + SD)

Fat group(%)	Thermal tolerance		Oxygen tolerance	
	Temperature (C)	P	Oxygen (ppm)	P
		0.04		0.34
L(4.3)	33.18(0.06)		1.29(0.10)	
LM(5.5)	33.23(0.13)		1.18(0.13)	
M(6.3)	33.22(0.16)		1.17(0.06)	
MH(7.3)	33.06(0.11)		1.18(0.14)	
H(8.7)	32.99(0.12)		1.18(0.06)	
R(4.1)	32.98(0.15)		1.19(0.12)	

resistance of 33.23°C. Thermal tolerance decreased with higher or lower body fat. Fat group H (8.7%) had the lowest thermal tolerance of 32.99°C (Figure 14).

Oxygen tolerance

Throughout the trials, fingerling walleye exhibited certain behaviors not observed in the rearing tanks. Behavior in the rearing tanks was characterized by very little activity. The behaviors observed during hypoxia trials include: exploratory behavior while oxygen dropped to 3 parts per million (ppm), intensified exploring and muscle

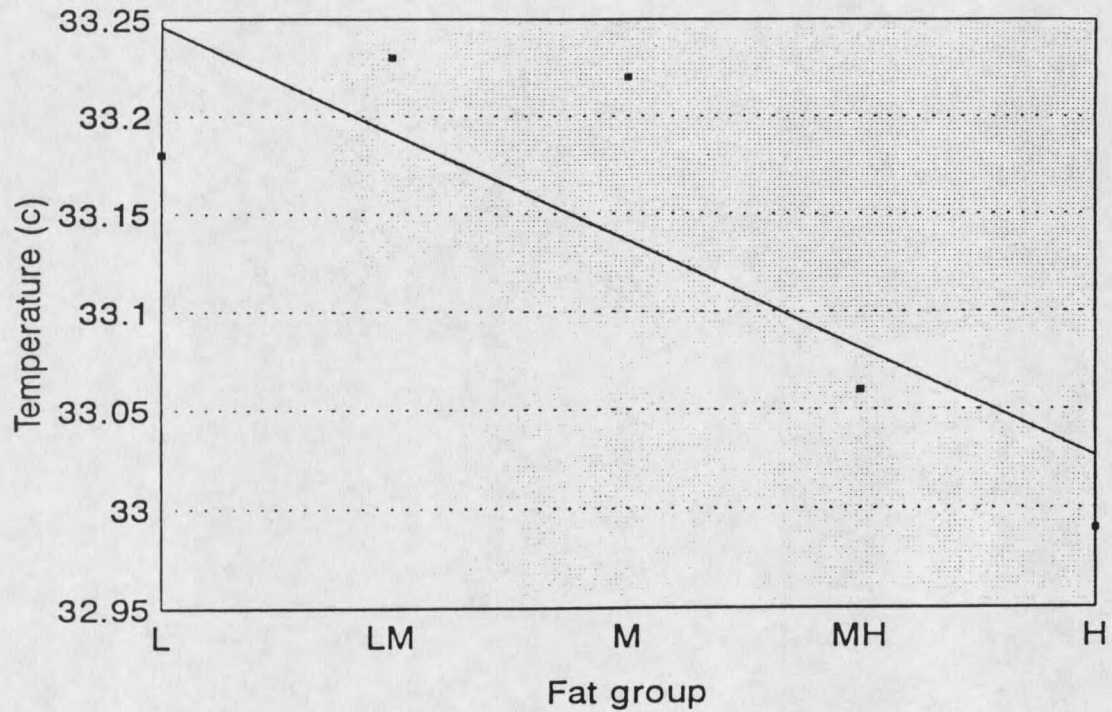


Figure 14. Effect of body fat level on thermal tolerance of walleye fingerlings ($r = -0.82$). See table 7 for an explanation of fat groups.

spasms characterized by burst and coast swimming at 2 ppm oxygen, and ending with cessation of exploratory behavior and severe spasms characterized by sustained high speed swimming around the tank at levels less than 1.5 ppm oxygen. Fish removed from the anoxic water and placed into fresh water recovered quickly and no mortality occurred.

Fat level in the body had no effect on the oxygen tolerance of walleye fingerlings ($P = 0.34$; Table 8). With the exception of fat group L (4.3%), all groups lost equilibrium at or near 1.18 ppm.

Sustained swimming

Although some fish became fatigued, swimming stamina was not significantly affected by body fat level ($P = 0.46$; Table 9). No fish were able to escape after turning sideways on the effluent screen and briefly shocked. At the end of both trials, fish still swimming were observed crowding near the head screen of the tunnel. All freeze-brand marks were easily observed during the trials.

Burst force

Body fat level significantly affected burst force of walleye fingerlings ($P < 0.01$; Table 9). The fat group with 5.5% body fat (LM) had the highest burst force while the fat group with the lowest body fat (4.3%) had the lowest burst force, although considerable variation occurred.

Table 9. Effect of body fat level on swimming stamina and burst force of walleye fingerlings. Probabilities are those of greater F-values (ANOVA). (means + SD)

Fat group(%)	Stamina		Burst force	
	SS ¹	P	g/cm	P
		0.46		<0.01
L(4.3)	75		1.46(0.45)	
LM(5.5)	100		2.53(0.09)	
M(6.3)	NR ²		2.06(0.34)	
MH(7.3)	80		2.09(0.37)	
H(8.7)	75		1.99(0.11)	

¹ SS = % fish still swimming after 3 h

² NR = This fat level was not represented in the trial

Using the regression equation for each body fat group (Table 10), burst force of walleye fingerlings of equal size was compared between body fat groups (Table 11). Fat group LM (5.5%) had the highest force in the 140 mm through 170 mm size classes. Fat group M (6.3%) had slightly higher force in the 180 mm size class and fat group H (8.7%) had the lowest force for all size categories. I also examined the relationship between burst force and fish length irrespective of fat group, using the 188 fish tested in burst force trials. The correlation coefficient was 0.68 (Table 10; Figure 15; $Y = -77.81 + 0.69X$).

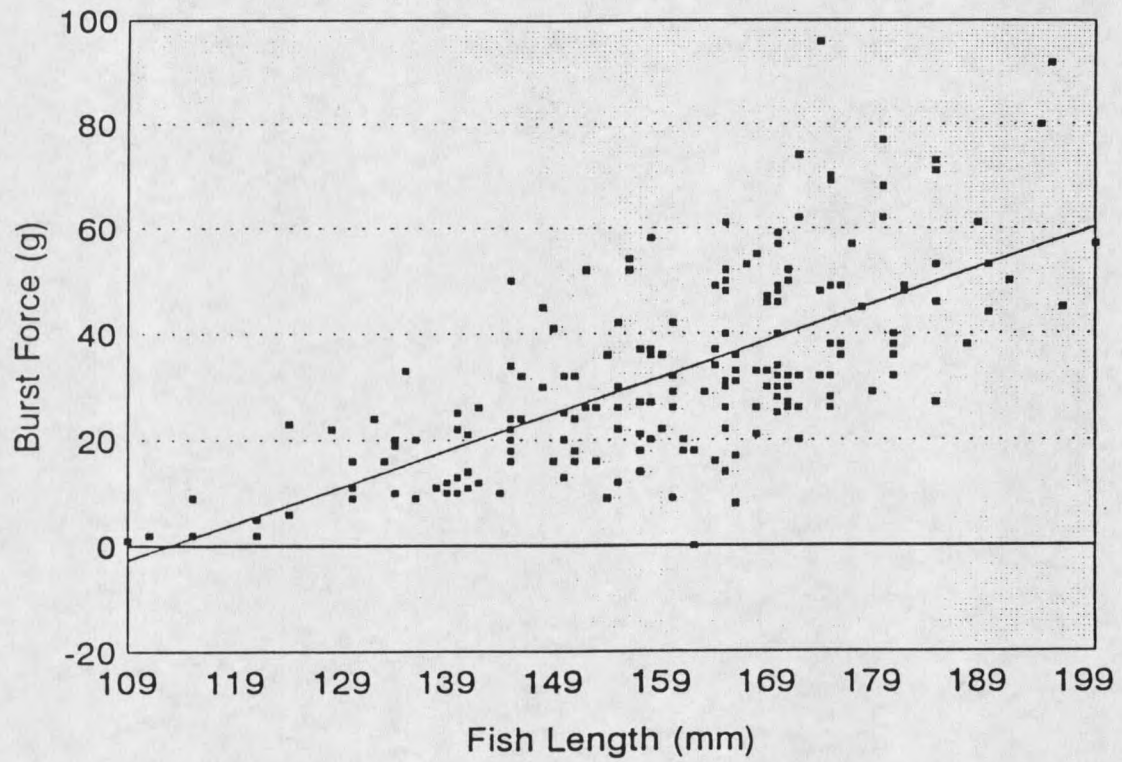


Figure 15. Relationship between length of walleye and burst force ($r = 0.68$).

Table 10. Correlation coefficient and regression equation for walleye fingerlings subjected to burst force tests.

Experimental group ¹	N	Correlation coefficient between fish length and swimming force	Equation X=length(mm) Y=force(g)
L	36	0.73	$Y = -68.99 + 0.63X$
LM	24	0.43	$Y = -38.77 + 0.49X$
M	32	0.72	$Y = -101.43 + 0.84X$
MH	32	0.73	$Y = -110.84 + 0.87X$
H	30	0.57	$Y = -101.74 + 0.80X$
R	32	0.69	$Y = -66.85 + 0.63X$
Force/length	188	0.68	$Y = -77.81 + 0.69X$

Table 11. Comparison of walleye fingerling burst force (g/cm) in equal size categories.

Fat group(%)	Length (mm)				
	140	150	160	170	180
L(4.3)	1.37	1.70	1.99	2.24	2.47
LM(5.5)	2.13	2.32	2.48	2.62	2.75
M(6.3)	1.16	1.64	2.06	2.43	2.77
MH(7.3)	0.78	1.31	1.77	2.18	2.54
H(8.7)	0.73	1.22	1.64	2.02	2.34
R(4.5)	1.53	1.84	2.12	2.37	2.59

¹ See table 7 for an explanation of fat groups.

Table 12. Effects of 103 d of starvation on survival and compositional weight loss of walleye fingerlings fed varying levels of dietary lipids. Probabilities are those of greater F-values (ANOVA). (means + SD)

Dietary lipid (%)	Body composition									
	Survival		Fat		Protein		Moisture		Ash	
	%	P	% Lost	P	% Lost	P	% Lost	P	% Lost	P
	0.30		0.50		0.74		0.09		0.67	
3.3	41(19)		83.5(18.7)		29.9(11.0)		12.7(4.3)		+4.6(19.4)	
12.2	67(16)		95.0(3.3)		32.4(9.1)		5.9(2.5)		8.0(3.0)	
22.3	75(39)		96.9(1.7)		36.7(1.8)		16.5(1.9)		+9.9(27.0)	

Prolonged starvation

After 103 d of starvation, survival ranged from 41.5% to 75.0%, but was not significantly affected ($P = 0.30$; Table 12) by body fat level. The loss of body fat, protein, moisture, and ash was also not different between groups (Table 12). The loss of body fat ranged from 83.5% to 96.9%, protein from 29.9% to 36.7%, and ash from 8% lost to 9.9% gained. Body fat, protein, and moisture were significantly different between the three groups ($P = 0.02$, 0.04, and 0.03; Table 13) at the start of the experiment. At the end of the test, body fat did not differ significantly between groups ($P = 0.79$). Fat levels among

Table 13. Body compositional changes following 103 days of starvation for walleye fingerlings fed diets containing varying levels of lipids. Probabilities are those of greater F-values (ANOVA). (mean + SD)

Dietary lipid (%)	Body composition ¹					
	Fat		Protein		Moisture	
	g	P	g	P	g	P
Beginning		0.02		0.04		0.03
3.3	1.4(0.2)		5.2(0.2)		22.2(0.6)	
12.2	2.7(0.3)		7.6(0.2)		31.0(0.4)	
22.3	3.5(0.4)		7.3(0.9)		29.3(3.1)	
End		0.79		0.10		0.03
3.3	0.2(0.2)		3.6(0.5)		19.4(0.5)	
12.2	0.1(0.1)		5.1(0.5)		29.2(0.4)	
22.3	0.1(0.1)		4.6(0.4)		24.5(3.1)	

¹ Ash weights were not significantly different at the start or end of this trial and are not reported.

the groups dropped to 0.1 g/fish for the medium and high fat levels while the lowest fat level retained the most fat (0.2 g/fish) (Table 13).

DISCUSSION

Objective 1: The effect of diet on body composition.**Body composition**

Both wet-weight and dry-weight analyses showed that body fat increased linearly as dietary fat increased from 3.3% to 22.3%. These results agree with Reinitz et al. (1978) for rainbow trout, Dupree et al. (1979) for channel catfish, and Millikin (1983) for striped bass. Conversely, body moisture decreased linearly as body fat increased. A similar relationship was reported by Millikin (1983) for striped bass and Barrows et al. (1988) for walleye.

Dry protein decreased linearly in relation to dietary fat. A similar relationship was found between body fat and body protein. Satia (1974) and Millikin (1983) reported similar findings for rainbow trout and striped bass, respectively.

Expression of proximate composition on a dry matter basis can often lead to erroneous conclusions on the effects of different treatments (Shearer 1994). Shearer (1994) recommends that results concerning proximate composition of fish be reported on a wet weight basis. In the present study, dry matter composition suggests that increasing body

fat levels leads to decreased body protein (Table 3). This would lead to the conclusion that lower dietary lipids can increase whole body protein. Analysis of wet weight data shows that this is not the case (Table 2).

Growth indices

The greatest weight gain in walleye fingerlings was in the group fed 22.3% dietary lipids and the response to increasing dietary lipids was linear. Other authors have reported similar results depending on the level of dietary protein. Garling and Wilson (1976) reported linear growth associated with dietary lipids and up to 28% dietary protein. Higher levels of protein resulted in a quadratic growth pattern for channel catfish fingerlings. For rainbow trout fingerlings, Lee and Putman (1973) found linear growth with 8%, 16%, and 24% dietary lipids and up to 44% dietary protein. A protein level of 53% resulted in a quadratic growth pattern. Dietary protein (53%) and lipid (24%) levels associated with decreased growth rate were higher than in my study. Although increasing dietary lipids resulted in a linear pattern of growth for walleye fingerlings, growth rates decreased at dietary lipid levels above 12.2%.

In the present study, the greatest gain in length of walleye fingerlings occurred at the 22.3% dietary lipid level. Reinitz et al. (1973) reported similar results for

rainbow trout. They observed linear increases in length associated with dietary lipids and 30% or 40% protein.

Dietary lipids had no effect on condition factor. Barrows et al. (1988) reported similar findings while studying effects of protein and energy levels on walleye fingerlings. They point out that because both weight gain and length change were affected by protein and energy levels, no change in condition factor would be expected.

The level of dietary fat also affects the composition of the weight gained. Increasing dietary fat from 3.3% to 22.3% resulted in a linear increase of body fat gained. When fed 3.3% to 7.9% dietary lipids walleyes gained 3.8 g of protein. Feeding diets containing 12.2% dietary lipids resulted in a gain of 5.8 g of protein. However, further increases in lipids did not result in significant increases in protein gain. The grams of ash gained increased in fish fed diets containing 3.3% to 12.2% lipids and then leveled off with further increases in lipids. My results suggest that between 8% and 12% dietary lipids is optimum for growth. At this level, protein previously used for basal metabolism is available for growth. If the nutritional goal for cultured walleye is to maximize growth by protein deposition while maintaining adequate fat stores, 12.2% dietary lipids combined with 51% dietary protein would meet that goal. Increasing dietary lipids beyond 12.2% would result in less efficient growth.

Objective 2: The effect of body composition on physical ability.

Thermal tolerance

The observed effect of body fat level on thermal tolerance of walleye fingerlings has not previously been reported. Thermal tolerance was significantly affected by the level of body fat with fish containing 5.5% body fat having a higher temperature tolerance than those with more or less body fat (Figure 14). Apparently, there is a slight physiological advantage to having moderate levels of body fat when subjected to thermal stress. However, the small difference among body fat levels in temperature tolerance makes it difficult to assess which physiological mechanism is responsible. Weatherley (1963) reported severe histological changes in the interrenal tissue of perch following thermal tolerance tests. He suggested that these tissues in fish are equivalent to the adrenocortical tissue in mammals and the response of fish to thermal stress is similar to the General Adaptation Syndrome put forth by Selye in 1950 for mammals. One possible explanation for the observed increase in temperature tolerance of fish with moderate levels of body fat is that these levels resulted in better developed interrenal tissue which allowed fish to

cope with thermal stress more efficiently. Hoar and Cottle (1952) were unable to show a relationship between thermal resistance of goldfish and the melting point of dietary fats, revealing the complexity of dietary effects on thermal tolerance. Future studies that utilize thermal tolerance as an indication of dietary effects should include an investigation into the histological changes as well as the body composition of the fish in order to better understand this complex relationship.

Oxygen tolerance

Oxygen tolerance was not significantly affected by body fat levels. With the exception of group L (4.3% fat), all fingerling walleye body fat levels tested (Table 8) had a minimum oxygen requirement of 1.18 ppm. Decreased oxygen tolerance is the result of pathological changes that impact the oxygen transport system (Wedemeyer et al. 1984). Histological examination of walleye fingerlings showed little or no damage to gill tissue (Elizabeth MacConnell, pers. comm.). The higher growth rates of walleye fed higher dietary lipids should result in increased oxygen demand and lower tolerance for oxygen depleted water. A possible explanation for why this was not observed is the rate at which oxygen was depleted in the test chamber during the trial. Using similar methods, Mathews and Maness (1979)

found the plains minnow (Hybognathus placitus) and three Notropis spp. able to survive nearly 2 h in water with only 1.2-1.5 ppm oxygen. During my study, oxygen dropped below 1.2 ppm in less than 40 min. Future studies using these methods should consider a rate of depletion closer to that of the sealed jar method which is approximately 5-8 h (Wedemeyer et al. 1990).

Sustained swimming

Swimming stamina was not significantly affected by body fat levels in this study. Lack of detectable differences in stamina between fish reared under varying culture techniques (diet) is inconsistent with Bams (1967), who detected differences in fish incubated by various hatchery methods; and Leon (1968) who was able to detect differences in fish exercised in the hatchery versus unexercised fish. However, Horak (1972) was unable to predict survival of rainbow trout according to the associated swimming stamina.

Sustained swimming speeds of 3-4 body lengths per second (Lg/s) have been documented for larval perch and walleye. The maximum speed in the tunnel during my trial corresponded to only 2 Lg/s, much slower than the 5 Lg/s suggested by Wedemeyer et al. (1990). The slow speed of the tunnel was probably responsible for the low number of fish fatigued during the trial.

Burst force

When viewing burst force relative to body fat content, walleye fingerlings with 5.5% body fat had the highest burst force. Greater or lesser body fat resulted in decreased burst force. Green (1964) found wild brook trout to have greater swimming force than cultured brook trout. In a similar study, Shustov and Shchurov (1988) found that different rearing conditions affected the burst force of Atlantic salmon. Wild fish reared in the natural environment had higher burst force than cultured fish reared in a raceway. Further, fish cultured in a raceway had higher burst force than fish cultured in a pond.

Using the regression equation for each body fat group, burst force of equal length walleye varied with body fat content. Fish up to 170 mm with a body fat level of 5.5% produced the greatest force. For walleye between 170-180 mm, the highest burst force was associated with a slightly higher level of fat, 6.3%. A possible explanation for this difference is the lower metabolic rate of larger fish. Thus, larger walleye may need slightly more body fat to maintain a healthy body composition. This analysis also showed that too little or too much body fat can decrease the burst force of walleye fingerlings.

The relationship between burst force and length of walleye has not previously been reported. Shustov and Shchurov (1988) and Nashimoto (1980) report a similar

relationship for Atlantic salmon and rainbow trout. Combining experimental fat groups to determine the force/length relationship led to expected variability among size classes. The large variability is also characteristic of wild fish. Shustov and Shchurov (1988) found wild Atlantic salmon had greater variability of swimming force than did domesticated fish.

Burst force may be an indicator of a fishes overall ability to escape from predators and capture food effectively. In this experiment, the level of body fat affected the burst force of walleye. A walleye fingerling about to be released into the wild would probably not benefit from excess body fat. Excess body fat may decrease burst force resulting in a greater chance of being captured by predators and decreasing the ability of the fish to forage effectively.

Starvation

Survival over 103 d of starvation was not significantly affected by body fat content in walleye fingerlings. However, the trend during this study suggested the fish with the lowest body fat levels may have died, had the trial continued. Wicker and Johnson (1987) were unable to relate body fat level to first year survival of largemouth bass. Thompson et al. (1991) determined that fat level affects

overwinter survival of Colorado squawfish. However, these authors used different sized fish rather than fish with different fat levels. Oliver and Holeyton (1979) also found that fat levels affected overwintering mortality using different sized fish. Their study was 129 d longer than mine. Oliver and Holeyton (1979) and Thompson et al. (1991) offer the explanation of larger fish having lower metabolism per gram of body weight than smaller fish, sparing larger fish from expending energy for foraging and cell building during difficult winter months.

Fat content was significantly different at the start of the trial but not at the end. This study found fat levels as low as 0.97% in starving walleye fingerlings. Thompson et al. (1991) reported minimum values of 3-6% fat in dying Colorado squawfish. Wicker and Johnson (1987) found fat levels of 2.8% in overwintering largemouth bass. Oliver and Holeyton (1979) found fat levels above 6% in dying smallmouth bass. All of the authors decreased temperatures to simulate overwintering conditions. Due to laboratory constraints, water temperatures in my trial remained at 21°C.

Fish fed higher levels of dietary fat had faster growth rates. It is hypothesized that the fish with higher fat content maintained a higher growth rate throughout the study, depleting fat reserves and breaking down protein faster than fish with a lower fat content. This is supported by trends in the proportional weight loss where

low fat fish lost less fat and protein than walleye fingerlings with higher fat contents.

Protein and moisture were also lost in starved walleye fingerlings. Changes in body composition other than fat were not found in the literature. Reimers (1963) stated that after prolonged starvation, rainbow trout will begin to break down body protein in order to maintain normal nutrition for basal metabolism. Walleye fingerlings challenged with starvation broke down body protein although fish previously fed a diet with 12.2% fat maintained higher protein levels. Higher protein content may be an advantage to fish when environmental conditions become favorable again. Retaining more muscle tissue may allow fish to avoid predators and capture more food items as food availability increases in the spring, resulting in a better chance at recovery and survival.

Moisture content of fingerling walleye was significantly different between diet groups throughout the trial. As body fat decreased, body moisture increased. A curious aspect of body moisture was the relatively low loss of moisture in the fish fed 12.2% dietary lipids. These fish also retained more protein. It is possible that these fish were better adapted nutritionally to handle starvation. No literature relating moisture level to prolonged starvation was found.

Ash weights did not vary significantly at the start or

end of this trial. No ash weight changes in relation to starvation were found in the literature.

After combining all data from this experiment, it appears that dietary lipid levels of 12.2% are adequate for walleye fingerlings faced with starvation after release. This level maintained good survival with moderate fat and protein retention. It is not known if fish will resume feeding and recover successfully from the effects of prolonged starvation. Further tests are needed to assess the effects of decreased water temperature and resumed or continued feeding on survival of walleye fingerlings with various fat contents.

LITERATURE CITED

LITERATURE CITED

Anonymous. 1993. Fisheries 18:35.

Bachman, R. A. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Transactions of the American Fisheries Society 113:1-32.

Bams, R. A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. Fisheries Research Board of Canada 24:1117-1153.

Barrows, F. T., J. L. Sell, and J. G. Nickum. 1988. Effects of dietary protein and energy levels on weight gains, body composition, and RNA:DNA ratios of walleye fingerlings. Progressive Fish-Culturist 50:211-218.

Barrows, F. T., R. E. Zitsow, and G. A. Kindschi. 1993. Effects of surface water spray, diet, and phase feeding on swim bladder inflation, survival, and cost of production of intensively reared larval walleyes. Progressive Fish-Culturist 55:224-228.

Bennett, D. H. and T. J. McArthur. 1990. Predicting success of walleye stocking programs in the United States and Canada. Fisheries 15:19-23.

Burrows, R. 1969. The influence of quality on adult salmon survival. Transactions of the American Fisheries Society. 98:777-784.

Colesante, R. T., N. B. Youmans, and B. Ziolkoski. 1986. Intensive culture of walleye fry with live food and formulated diets. Progressive Fish-Culturist 48:33-37.

Conover, M. C. 1986. Stocking cool-water species to meet management needs. Pages 18-30 in R. H. Stroud, Editor. Fish culture in fisheries management. American Fisheries Society, Maryland.

- Dupree, H. K., E. J. Gauglitz Jr., A. S. Hall, and C. R. Houle. 1979. Effects of dietary lipids on the growth and acceptability (flavor) of channel catfish (Ictalurus punctatus). Pages 87-103 in J. E. Halver and K. Tiews, editors. Finfish nutrition and fishfeed technology, volume 2. Heenemann, Berlin, Federal Republic of Germany.
- Ersbak, K. and B. L. Haase. 1983. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. North American Journal of Fisheries Management 3:142-151.
- Garling, D. L. Jr., and R. P. Wilson. 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, Ictalurus punctatus. Journal of Nutrition 106:1368-1375.
- Green, D. M. 1964. A comparison of stamina of brook trout from wild and domestic parents. Transactions of the American Fisheries Society 93:96-100.
- Hilborn, R. 1992. Hatcheries and the future of salmon of the northwest. Fisheries 17:5-8.
- Hoar, W. S. and J. E. C. Dorchester. 1949. The effect of dietary fat on the heat tolerance of goldfish (Carassius auratus). Canadian Journal of Research 27:85-91.
- Hoar, W. S. and M. K. Cottle. 1952. Dietary fat and temperature tolerance of goldfish. Canadian Journal of Zoology 30:41-48.
- Horak, D. L. 1972. Survival of hatchery-reared rainbow trout (Salmo gairdneri) in relation to stamina tunnel ratings. Journal of the Fisheries Research Board of Canada 29:1005-1009.
- Houde, E. D. 1969. Sustained swimming ability of larvae of walleye (Stizostedion vitreum) and yellow perch (Perca flavescens). Journal of the Fisheries Research Board of Canada 26:1647-1659.
- Lee, D. J., and G. B. Putman. 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. Journal of Nutrition 103:916-922.
- Leon, K. A. 1986. Effect of exercise on feed consumption, growth, food conversion, and stamina of brook trout. The Progressive Fish-Culturist 48:43-46.

- MacConnell, E. Personal Communication. July, 1994.
- Mathews, W. J. and J. D. Maness. 1979. Critical thermal maxima, oxygen tolerances and success of cyprinid fishes in a southwestern river. *The American Midland Naturalist* 102:374-377.
- Miller, R. B. 1958. The role of competition in the mortality of hatchery trout. *Journal of the Fisheries Research Board of Canada* 15:27-45.
- Millikin, M. R. 1983. Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. *Transaction of the American Fisheries Society* 112:185-193.
- Nashimoto, K. 1980. The maximum swimming force of rainbow trout. *Bulletin of the Japanese Society of Scientific Fisheries* 46:949-954.
- Oliver, J. D., G. F. Holeton, and K. E. Chua. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperature. *Transactions of the American Fisheries Society* 108:130-136.
- Olla, B. L. and M. Davis. 1989. The role of learning and stress in predator avoidance of hatchery-reared coho salmon (Oncorhynchus kisutch) juveniles. *Aquaculture* 76:209-214.
- Parker, N. C. 1986. Physical and biological factors influencing growth and survival of stocked fish. Pages 235-242 in R. H. Stroud, Editor. *Fish culture in fisheries management*. American Fisheries Society, Maryland.
- Phillips, A. M., D. R. Brockway, F. E. Lovelace, and H. A. Podoliak. 1957. A chemical comparison of hatchery and wild brook trout. *Progressive Fish-Culturist* 19:19-25.
- Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. *Fish hatchery management*. U.S. Fish and Wildlife Service, Washington, D.C.
- Reimers, N. 1963. Body condition, water temperature, and over-winter survival of hatchery-reared trout in Convict Creek, California. *Transactions of the American Fisheries Society* 92:39-46.

- Reinitz, G. L., L. E. Orme, C. A. Lemm, and F. N. Hitzel. 1978. Influence of varying lipid concentrations with two protein concentrations in diets for rainbow trout (Salmo gairdneri). Transactions of the American Fisheries Society 107:751-754.
- Satia, B. P. 1974. Quantitative protein requirements of rainbow trout. Progressive Fish-Culturist 36:80-85.
- Shearer, K. D. 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119:63-88.
- Shustov, Y. A. and I. L. Shchurov. 1988. Quantitative estimation of stamina of wild and hatchery-reared Atlantic salmon (Salmo salar L.). Aquaculture 71:81-87.
- Thompson, J. M., E. P. Bergersen, C. A. Carlson, and L. R. Kaeding. 1991. Role of size, condition, and lipid content in the overwinter survival of age-0 Colorado squawfish. Transactions of the American Fisheries Society 120:346-353.
- Vincent, R. E. 1960. Some influences of domestication upon three stocks of brook trout (Salvelinus fontinalis M.). Transaction of the American Fisheries Society 89:35-52.
- Weatherley, A. H. 1963. Thermal stress and interrenal tissue in the perch Perca fluviatilis (Linnaeus). Zoological Society of London 141:527-561.
- Wedemeyer, G. A., B. A. Barton, and D. J. McLeay. 1990. Stress and acclimation. Pages 451-490 in C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.
- Wedemeyer, G. A., D. J. McLeay, and C. P. Goodyear. 1984. Assessing the tolerance of fish and fish populations to environmental stress: the problems and methods of monitoring. Pages 164-186 in V. W. Cairns, P. V. Hudson, and J.O. Nriagu, editors. Contaminant effect on fisheries. Wiley, Toronto.
- White, R. J. 1992. Why wild fish matter: a biologist's view. Trout, Summer 1992.
- Wicker A. M. and W. E. Johnson. 1987. Relationships among fat content, condition factor, and first-year survival of Florida largemouth bass. Transactions of the American Fisheries Society 116:264-271.

APPENDICES.

APPENDIX A
Water Quality Measurements

Table 14. Periodic water quality measurements taken during the walleye fingerling diet trial.

Date	Temp (°C)	Total Gases (%)	Delta P (mm)	Bar (mm)	O ₂ (mg/L)	O ₂ (%sat)	N ₂ (%sat)
9-16-93	19.8	102.8	18	641	7.5	97.8	104.3
9-24	20.1	102.9	19	642	7.5	98.4	104.3
9-30	19.6	102.5	17	636	7.5	98.4	104.0
10-7	19.0	102.5	16	638	7.6	98.1	103.8
10-15	18.7	102.3	15	636	7.7	99.1	103.3
10-20	18.3	102.3	16	646	7.9	99.3	103.5
10-28	18.1	102.0	14	639	7.9	100.0	102.9
11-4	17.8	104.7	30	644	8.0	99.8	106.1
11-12	17.6	107.2	46	633	8.0	101.1	109.2
11-18	17.3	100.1	2	638	8.1	100.9	100.2
11-25	20.3	100.0	0	644	7.5	98.5	100.4
12-2	20.7	101.3	9	640	7.5	99.9	101.9
12-9	20.7	101.5	10	637	7.4	99.1	102.3
12-16	20.6	102.1	14	638	7.4	98.7	103.2
12-31	21.6	100.0	0	636	7.3	99.7	100.1
1-6-94	21.4	101.9	12	639	7.4	100.1	102.5
1-16	21.8	101.4	9	638	7.4	101.1	101.6
1-20	22.2	101.2	8	644	7.3	99.6	101.8
2-8	22.4	102.0	15	631	7.3	102.0	102.6

APPENDIX B
Walleye Fingerling Proximate Analysis Results

Table 15. Results of proximate analysis tests performed on walleye fingerlings fed six levels of dietary fat. Tests include pretrial data.

Diet ²	Tank	Composition ¹			
		Fat	Moisture	Protein	Ash
Pretrial	All ³	3.2	76.3	16.5	3.4
Post trial					
L	4	4.1	74.7	17.1	3.0
	5	6.2	73.2	17.2	3.1
	13	4.1	74.0	17.2	4.1
	18	3.9	73.0	16.9	5.0
LM	10	5.6	73.6	17.3	2.9
	16	4.3	73.7	17.1	3.6
	19	4.6	75.6	16.4	3.1
	23	5.3	73.1	16.9	2.8
M	2	6.0	72.4	17.5	3.2
	3	5.6	73.3	17.6	3.3
	14	6.4	71.7	17.7	3.0
	20	6.9	71.6	17.7	3.1
MH	7	8.3	70.1	18.0	2.5
	9	7.3	71.9	17.0	2.9
	12	7.0	71.5	18.0	3.0
	15	6.5	72.2	16.8	3.0
H	6	8.8	70.0	17.1	2.9
	8	7.8	70.3	17.2	3.5
	11	8.7	70.1	17.4	3.2
	24	8.2	70.6	17.7	3.0
R	1	4.4	74.4	16.6	3.6
	17	5.3	72.0	17.1	3.9
	21	4.1	73.6	17.4	3.9
	22	2.4	75.1	17.0	4.5

¹ Expressed as a % of wet weight.

² See table 1 for an explanation of diet codes.

³ Pretrial sample consisted of 5 fish per tank (n = 120).

APPENDIX C
Diet Proximate Analysis Results

Table 16. Results of proximate analysis tests performed on experimental diets from walleye fingerling body composition trial.

Diet ²	Composition ¹			
	Fat	Moisture	Protein	Ash
L	3.3	10.1	52.1	2.2
LM	7.9	8.8	52.0	3.1
M	12.2	8.5	51.0	3.4
MH	19.2	8.4	51.1	3.6
H	22.3	8.2	51.3	4.0
R	19.5	6.2	46.0	8.3

¹ Expressed as a % of wet weight.

² See table 1 for an explanation of diet codes.



HOUCHEN
BINDERY LTD
UTICA/OMAHA
NE.