ABSTRACT

Richard F. Bauer, Jr. A BIOENERGETIC STUDY OF METABOLISM AND NUTRITION IN EGGS AND LARVAL STRIPED BASS (MORONE SAXATILIS). (Under the direction of Charles W. O'Rear) Department of Biology, May 1972.

The purpose of this study was to develop a bioenergetic equation which can be used for striped bass eggs and larva in evaluating their metabolic and nutritional requirements. The total energy content of the embryo and yolk was measured as calories for several developmental periods. Energy utilizations of metabolism and waste excretion were also determined for various stages using oxygen consumption and ammonia excretion, respectively. Energy content and energy utilization were related using a balanced equation for four developmental stages and graphically in relation to overall development. All of the equations were found to balance with only a small error which was attributed to inadequate oxygen consumption data. These results support the use of the chosen bioenergetic equation for striped bass eggs and larva. The total energy content was found to decrease through development due to yolk resorption, while energy used for metabolism and lost through excretion both increased with development. These general trends found for striped bass have also been found for other species of fish eggs and larva.

A BIOENERGETIC STUDY OF METABOLISM AND NUTRITION IN EGGS AND LARVAL STRIPED BASS (MORONE SAXATILIS)

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

by

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May 1972

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ACKNOWLEDGEMENT

I wish to show my sincere appreciation to Dr. Charles W. O'Rear for his advice and criticism. I would also like to express gratitude to Dr. Alice S. Scott and Dr. William J. Smith for their assistance during this study.

Eric A. Slaughter should be recognized for his help in the ammonia concentration determination. I would like to thank J. C. Fuller, Chief of Fish and Game, and J. Bayless, Fisheries Biologist, of the South Carolina Wildlife Resources Department for the use of hatchery facilities and in supplying the striped bass eggs and larva.

Equipment and supplies for this study were provided by the Department of Biology East Carolina University and some funds were obtained through Sea Grant No. GH 103 University of North Carolina.

DEDICATED TO MY WIFE, MARTHA, FOR HER UNDERSTANDING, LOVE, AND HELP WITHOUT WHICH THIS STUDY WOULD NOT HAVE BEEN POSSIBLE.

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INTRODUCTION

The efficiency with which any organism utilizes energy for the processes of life is best understood through an investigation of that organism's energy metabolism. An effective means of studying food energies and the energy requirements of metabolism is with a bioenergetic assessment. The total energy flow and the degree of energy utilization can be determined by integrating the energy values for food consumed, waste products, metabolism, and growth into a bioenergetic equation (Davis and Warren, 1968). Although bioenergetic techniques have been established for domestic animals (Brody, 1945) and considerable work has been done with adult fish (Fry, 1969; Krueger et. al., 1968; Warren and Davis, 1967) information is lacking in the area of fish eggs and fish larva metabolism. The eggs and larva of fish are expected to have a distinct pattern of energy flow due to the unique physiology and metabolic demands of the organism. With changes in physiology during development the energy requirements, as well as, the energy food source are altered (Bagenal and Baum, 1968; Blaxter, 1969).

Specifically, this investigation is concerned with the energy budget of eggs and larva of striped bass (Morone saxatilis) (Walbaum). The objective is to develop an equation representing the energy budget of striped bass eggs and larva.

REVIEW OF LITERATURE

Bioenergetics

Bioenergetic equations previously developed for fish have been reviewed by Warren and Davis (1967). They refined Ivlev's (1939) and Winberg's (1956) equations and developed an overall energy budget equation.

$$C = F + U + \Delta B + R$$

$$R = Rs + Rd + Ra$$

The original equation of Warren and Davis (1967) was altered slightly in a later publication by Davis and Warren (1968) and is reviewed in Appendix 1. Of the original food consumed (C) only a portion is absorbed by the alimentary canal. The undigested materials pass out as fecal waste. The total energy lost as waste (F) also includes nitrogenous wastes or metabolic waste (U) (Appendix 1). Harris (1966) has prepared an energy utilization scheme for domestic animals (Appendix 2) and he points out two fractions in both fecal and urinary energy. In addition to the energy of direct food origin, waste products also contain materials of body origin or metabolic origin. Intestinal mucosa, digestive enzymes, and materials in the urine other than nitrogenous waste are maintenance energy products found in waste. Gaseous products of digestion are included under waste products by Harris (1966), but are significant for ruminants and not fish. Energy is also freed as heat through deamination and other conversion processes and is termed specific dynamic action (SDA) (Warren and Davis, 1967). Heat of fermentation and nutrient metabolism in Harris' scheme (1966) represent the same utilization and loss of energy associated with food consumption. SDA only represents a portion of the total energy

needed for food consumption (Rd). The remainder of the energy is obtained from the net energy fraction and is used for digestion, assimilation, movement and deposition (Appendix 1). The net energy is the energy available to the organism for physiological processes and can be broken down into the energy fixed in the growth process (ΔB) and the energy of metabolism (R). Harris (1966) divides net energy into production energy or energy storage and maintenance energy. Many of the subdivisions under these headings apply only to endothermic, domestic animals (Appendix 2). Warren and Davis (1967) include energy released for activity (Ra), standard metabolicm (Rs) and all food handling (Rd) under metabolic energy (R). Standard metabolism is the metabolic rate of an unfed fish when projected to zero activity level, as defined by Warren and Davis (1967). Phillips (1969) states that standard metabolism for fish is approximately equivalent to basal metabolism when estimated by oxygen consumption.

Krueger et. al. (1968) have presented an energy balance equation for adult fish which is similar to the bioenergetic equation of Warren and Davis (1967) (Appendix 3). The organism (E_o) and food ration (E_f) represent unoxidized energy components at time zero and have an energy content equivalent to their heat of combustion. After a time interval "t", the energy will be in the form of heat energy and unoxidized energy. Heat energy is metabolic and appears as a result of basal metabolism (H_{basal}), food handling costs (H_{SDA}) and metabolism of exercise ($H_{activity}$). Waste products ($E_{excretion}$) make up part of the unoxidized portion with the remainder fixed as growth or the energy content of the organism after the time interval (E_t).

Development of the Eggs and Larva

Bagenal and Baum (1968) and Blaxter (1969) have pointed out various developing stages and the physiological features of these stages generally found in fish. The egg first undergoes hardening of the chorion after fertilization with a reduction in water permeability. Living eggs appear translucent, while dead ones appear white. At hatching the chorion becomes softened due to utilization of nutrient material in that membrane via the perivitelline fluid. The hatched larva or prolarva is characterized by a prominent yolk sac, absence of a mouth and jaws, and the primitive conditions of a primordial fin fold, notochord, pronephric kidney and straight gut. Although the heart is functioning, circulation is poor and respiration is cutaneous. With the complete absorption of the yolk, development of the gut, functioning of the mouth, and the presence of a medial fin, skeleton, and bronchial respiration, the postlarval stage is obtained.

An extensive study by Mansueti (1958) gives the details and time sequences in the early development of striped bass at 17° C. The eggs are described as spherical, non-adhesive, and buoyant with a clear chorion. Water hardening occurs from one to two hours after fertilization and is characterized by swelling due to the uptake of water. After formation of the perivitelline space, swelling stops and the chorion becomes impermeable. The 16 to 32 blastomere stage predominates at this time; also, the yolk sac appears green and a large oil globule is present. After 24 hours the embryo is well differentiated and hatching occurs between 36 to 48 hours. At hatching the larva has the developmental characteristics described by Blaxter (1969). Mansueti (1958) points out random movement

as being positively phototrophic although the larvae settle to the bottom despite swimming activity. At 72 hours after fertilization the yolk sac and oil globule are still present; thus, the fish still remains in the prolarval stage. The postlarval stage is fully attained at 240 hours with complete yolk absorption and the development of dorsal, anal, and caudal fins. The developmental stages of Mansueti (1958) were all determined at 17.0° C. Since the rate of development is related to temperature, as pointed out by Bagenal and Baum (1969) and Blaxter (1969), the time sequences are dependent on that temperature.

MATERIALS AND METHODS

Design of the Experiment

A bioenergetic equation was formulated from equations previously established for fish studies, but it uses terms which are applicable to early development. Each of the following symbols are measurable separately:

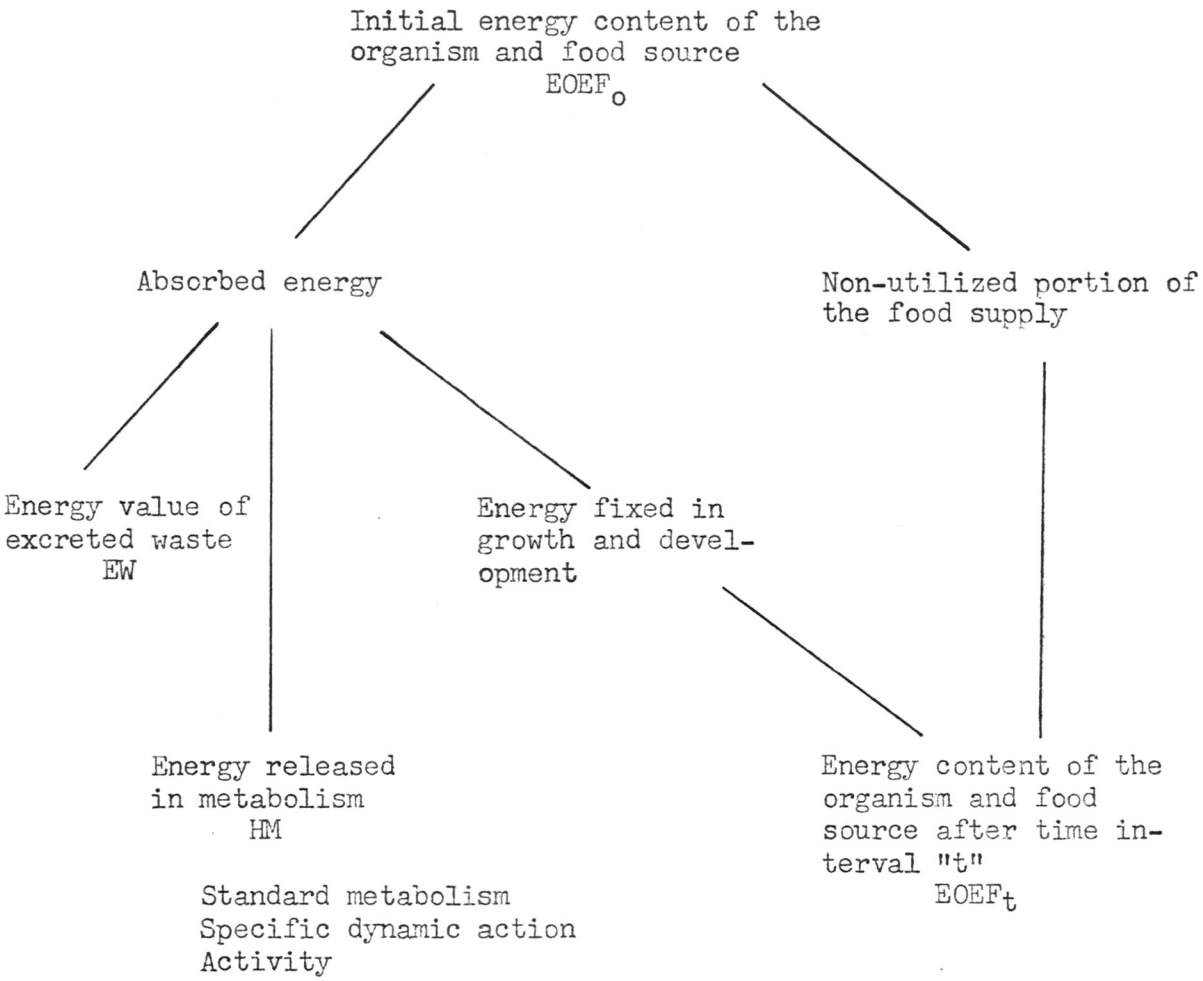
$$EOEF_o = EOEF_t + EW + HM$$

The components of the equation are initial total energy ($EOEF_o$), final energy ($EOEF_t$), waste (EW) and metabolism (HM) and their relationship to an energy flow is shown in Figure 1. The equation allows comparisons between energy budgets of each stage measured. This equation is developed from and can be compared to the equations of Davis and Warren (1968) (Appendix 1) and Krueger et. al. (1968) (Appendix 3).

The energy content of the organism at time zero and time "t" as well as the food consumed are shown as separate values in Krueger's et. al. (1968) scheme. Growth is expressed as the difference between initial and final energies of the organism and is best evaluated by direct calorimetry according to both Krueger et. al. (1968) and Davis and Warren (1968). Heat of combustion values should also be used to determine the energy of the food ration. Since the organism and the food source or yolk are difficult to separate in eggs and prolarva of striped bass, a single value is used to represent both (EOEF). Growth and food consumption are continuous processes in development, and a food source is always present in the early stages. To account for this, the energy portions of the organism and food supply are measured at the beginning ($EOEF_o$) and end ($EOEF_t$)

FIGURE 1: EXPERIMENTAL SCHEME

$$EOEF_o = EOEF_t + EW + HM$$



An energy equation and distribution scheme for striped bass during early development.

of the time interval. The difference in energies between these two values would be due to both net energy fixed as new cells and food consumption (Figure 1). Although the food source becomes external in the postlarva, it can be combined with the organism's energy in a single term if the contents of the digestive system is known. Mansueti (1958) demonstrates that invertebrate food, such as brine shrimp eggs, can be easily observed in the intestine. The composition of the food source varies from yolk, which can be utilized directly in eggs and prolarva, to brine shrimp eggs, which must first undergo digestion in postlarva. This variability in food composition and means of consumption have an important effect on energy values of waste and metabolism.

In the absence of a complete alimentary canal in the egg and prolarva, all food energy is derived directly from the yolk. As a result, the waste product fraction contains no fecal energy. All waste energy in these early stages is a result of nitrogen metabolism (EW). The yolk is mostly composed of protein according to Blaxter (1969) who reports unfertilized, rainbow trout eggs as being 70% protein, 23.3% fat and less than 1% carbohydrate. If protein is used for metabolic energy, 16% of its energy will be lost as nitrogen and excreted, as estimated by Phillips (1969). When considering these figures, nitrogen waste could represent a considerable energy value in a bioenergetic equation for developing fish. Winberg (1956) and Davis and Warren (1968) argue that nitrogenous wastes such as ammonia can be disregarded due to their low energy value. Krueger et. al. (1968) on the other hand, stress the importance of an extensive evaluation of urinary losses which includes calorie determination for ammonia. Since ammonia is the chief end product of nitrogen metab-

olism with other products found only in traces according to Forester and Goldstein (1969), it was used to measure energy losses of waste (EW) in eggs and prolarva. In the postlarval stages the major component of waste energy is not ammonia, but faeces. Digestion is incomplete and occurs only in the section of the intestine near the anus as described by Blaxter (1969); thus, fecal energy losses are present and may be high. Phillips (1969) gives the average protein content of invertebrate food as 11.5%, which is considerably lower than the percent protein in the yolk. The portion of the waste energy composed of ammonia would be lower in the postlarva. Ammonia determination represented all waste energy (EW) and the equation and methods fit only the eggs and prolarva. The energy of waste (EW) for postlarva would contain a large fecal energy value and a nitrogenous waste portion. Ammonia determinations would thus be only an indicator of total waste (EW) when fecal energy is not measured.

Energy utilization includes the energy released in nutrient conversion processes including deamination. SDA results in heat loss and is part of the metabolic energy term (HM). Krueger et. al. (1968) review several methods for measuring SDA which comprehensively include a starvation experiment. SDA is not separated as one of the energy portions of metabolism because starvation in eggs and prolarva would be impractical and fatal. Food handling cost other than SDA, such as movement and assimilation, (Davis and Warren, 1968) do not occur in early development since energy is absorbed directly from the yolk by the circulatory system. The high protein content of the yolk would result in a great deal of deamination and SDA heat loss as a result of protein metabolism. In the postlarva the SDA value would be less significant in the heat of

metabolism term since less protein is available for deamination in the food source, but other nutrient cost such as digestion are present in this stage. Standard metabolism and energy released through activity are the remaining portions of metabolism (HM). Fry (1969) discusses types of metabolism and its measurement by previous workers. The heat of standard metabolism from the reactions which yield the energy needed to maintain the organism. Active metabolism includes the energy used for exercise and can be obtained at various activity levels induced by flow rate variations. With standard metabolism calculated from zero activity each of the terms making up the energy of metabolism can be given a separate value according to Davis and Warren (1968) and Fry (1969). As with the heat of nutrition energy or SDA, the other metabolism components cannot be separated in eggs and larva. Activity is zero in the egg and the metabolism term (HM) represents standard metabolism and SDA energies. In the prolarva and postlarva activity is random and cannot be manipulated due to lack of development. Metabolism (HM) represents the energies of SDA, standard metabolism, and random activity for the later developmental stages. The procedures for measuring metabolism are outlined by both Davis and Warren (1968) and Krueger et. al. (1968), and they involve measurements of heat production, oxygen consumption, or estimations from composition or heat of combustion data. Oxygen consumption rates were used to determine the energy of metabolism (HM) for striped bass. Beamish and Dickie (1969) discuss oxygen consumption methods and point out that in a closed system only extremes in oxygen and carbon dioxide concentrates effect oxygen consumption, but that temperature is a critical consideration in consumption rates.

Animals

Striped bass (Morone saxatilis) eggs and larva were obtained from the South Carolina Wildlife Resources Department hatching facility at Moncks Corner, South Carolina in the spring of 1971 and 1972. Eggs and sperm were obtained from fish caught in the Tail Race Canal. Modified McDonald hatching jars were used for egg development and larva were reared in aquaria. Water temperature fluctuated between 14° C. and 19° C. resulting in developmental sequences followed closely to the time periods given by Mansueti (1958).

Experimental Procedures

Each component of the bioenergetic equation was determined separately for each of the developmental stages tested. A complete bioenergetic evaluation was made on four developmental periods; this included measurements of initial and final energy (EOEF), rate of excretion (EW) and rate of metabolism (HM). These time intervals were: 2 to 24 hour old eggs marked by embryo differentiation; 24 to 48 hour old prehatching eggs; 56 to 72 hour old early prolarva; and 7 to 12 day old postlarva. The first three stages have an endogenous food source in that all energy is obtained from the yolk. The last stage or postlarval fish were fed on brine shrimp eggs and the presence of food in the intestine could be detected visually. In addition to the initial energy of the organism and food for each of the above intervals, total energy was also measured for unfertilized eggs.

The total energy content of the organism and the food (EOEF) was determined from their heat of combustion in a Phillipson oxygen, microbomb

calorimeter (Gentry and Wiegert Instruments, 1007 Owens Street, Aiken, South Carolina, 29801). Samples for each of the stages were frozen at -20° C., transported on dry ice and stored at -20° C. until needed. Caloric values were made on a dry weight basis as outlined by Cummins and Wuycheck (1971). Samples were dried at 98° C. for 48 hours in an oven, cooled in a desiccator for 2 hours and weighed. Two trials were made on each of the stages collected and calories calculated according to the instructions provided with the calorimeter by Gentry and Wiegert Instruments. Corrections were made for ash and residue. The energy content of the organism and food (EOEF) was reported as calories per individual organism, after conversion from a dry weight basis.

Dry weight determinations were also made to find the weight of one organism in each of the developmental stages. Counted samples were collected and oven dried as described above.

The energy lost as waste (EW) and the energy of metabolism (HM) were measured at the hatchery as change in ammonia and oxygen concentration in a water sample. Golterman's (1969) colorimetric method for ammonia determination (Page 61) was used to measure waste excreted by eggs or larva. Oxygen consumption was determined using a Clark type, polarographic oxygen probe (YSI 5331), a biological oxygen monitor (YSI 53), a standard bath assembly (YSI 5301), and a laboratory recorder (YSI 80) provided by the Yellow Springs Instrument Company (Yellow Springs, Ohio, 45387). The electrode method of measuring dissolved oxygen is discussed by Golterman (1969) (Page 131). Several organisms from one of the stages to be measured were placed in a 50 ml beaker containing water and fitted with an oxygen probe. A magnetic stirrer was mounted to the probe, and the

organisms were held away from the stirrer and to the bottom of the chamber with a copper mesh screen. Prior to the experimental run a water sample was frozen and later used to determine initial ammonia concentration. The initial oxygen content of the water was found using the Winkler method described by Golterman (1969) (Page 128), this value was used for calibration of the electrode. Temperature, numbers of organisms, duration of the trial, and initial concentrations of ammonia and oxygen varied between runs although temperature was maintained during each run. Sample volume and the current due to the stirring unit were held constant. The oxygen consumption rate was measured during the run with a probe as the fish removed oxygen from the sealed container. Ammonia accumulation was found by freezing a water sample for later analysis, at the same time the number of organisms used for the particular run was counted. Several oxygen consumption and ammonia excretion runs were made for each of the developmental stages. The changes in concentrations of ammonia and oxygen were adjusted for the organism number, sample volume, duration of run, and initial concentration. The results were recorded as milligrams of ammonia excreted per individual organism per hour and milligrams of oxygen consumed per individual organism per hour. The energy of waste (EW) and metabolism (HM) needs to be converted to calories in order to be applied to the energy balance equation. In Krueger's et. al. (1968) discussion on urinary nitrogen losses, they state that on combustion of 1 gram of protein 160 milligrams of ammonia nitrogen is possible and has a caloric equivalent to 804 calories. From this a caloric coefficient would be 5.025 calories per milligram of ammonia excreted. Krueger et. al (1968) also give the oxycaloric coefficient of 3.36 calories per milli-

gram of oxygen at a respiratory quotient of 0.8.

RESULTS AND DISCUSSION

Part I

Dry Weights and Total Energy Content

The dry weight values for the eggs and larva are given in Table 1 and Figure 2. The results demonstrate a continuous loss in weight, due to yolk resorption. The increase in weight from before fertilization to the 2 hour old egg can be explained in part by a large variation in the individual samples of the 2 hour old eggs. The 168th to 288th hour period is marked by complete yolk resorption and external feeding and is termed the "critical period" by Laurence (1969) since body tissue may be used for energy resulting in death if feeding is not successful. The continual loss in weight is thus due to both yolk resorption followed by some body tissue losses.

Total energy in calories of the egg and larva is summarized in Table 1 and Figure 2. Laurence (1969) in his investigation on largemouth bass larva determined an average calorie value for both embryo tissue and yolk. He then applied these values to the proportion of embryo and yolk weights to find energy content for each developmental stage. As a result a gradual decrease in energy was noted. In contrast, the values for striped bass as shown in Table 1 and Figure 2 indicate a sharp decrease in the prehatching energy. This can be substantiated when considering utilization sequences of protein, fat, and carbohydrate. Smith (1952) and Hollett and Hayes (1946) found a period of fat and carbohydrate resorption for about 10 days prior to hatching in rainbow trout and salmon, respectively. Additional energy from the chorion is being used for nu-

TABLE 1

Dry Weight and Total Energy

Determinations for Striped Bass

Eggs and Larva

Age after fertilization (hour)	A Dry weight of sample (mg)	B Number of individuals per sample	C* Mean dry weight (mg/individual)	D Calorie value (cal/mg)	E** Mean calorie value (cal/individual)
0	54.65 54.45 56.45	200	275.92 ± 2.86	8.270 8.462	2.308 ± 0.026
2	32.50 25.75 27.50	100	285.83 ± 20.00	8.635 8.305	2.421 ± 0.047
24	49.05 48.00 46.99	200	240.07 ± 2.97	8.994 9.382	2.206 ± 0.047
48	40.40 41.67 41.00	200	204.45 ± 1.30	7.047 7.478	1.485 ± 0.044
56				7.137 6.941	1.439 ± 0.020
72	40.10 42.11 40.10	200	203.85 ± 3.36	7.152 7.052	1.441 ± 0.011
168	27.80 27.70 28.10	200	139.33 ± 0.60	6.356 7.067	0.960 ± 0.074
288	8.48	74	114.60	8.625 8.917	1.005 ± 0.017

* Column A divided by column B and expressed as $\bar{X} \pm$ S. E.

** Column C multiplied by column D and expressed as $\bar{X} \pm$ S. E.

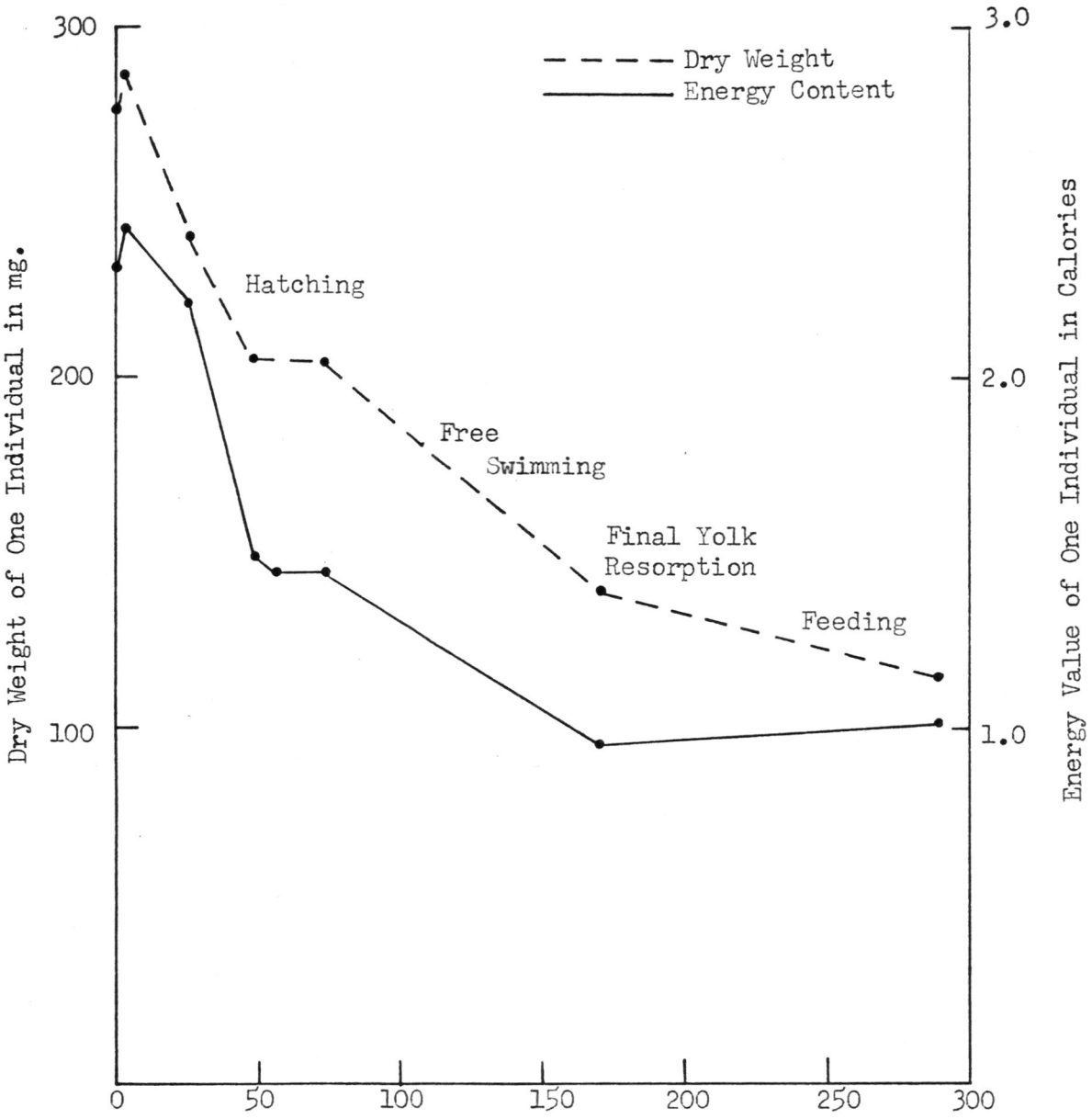
FIGURE 2

Change in Dry Weights and

Total Energy Content with

Development in Striped Bass

Eggs and Larva



trition at this time in striped bass and rainbow trout (Manseuti, 1958; Smith, 1947). The decrease in energy utilization during hatching and in the early prolarva could partially be due to low fat resorption if striped bass have the utilization sequence found in rainbow trout and salmon. The absence of a rapid drop in the hatching egg energy due to loss of the chorion can be explained by much of its decomposition before hatching and to lack of information during this stage. The increase in energy during the "critical period" is caused by external feeding on brine shrimp eggs in the 288 hour old postlarva (Table 1 and Figure 2).

Part II

Energy Utilization

The data obtained for oxygen consumption of eggs and larva is presented in Table 2. The standard error is large for these values and indicates variability between samples in oxygen consumption. Laurence (1969) used oxygen consumption as a measure of metabolism while Smith (1947) used heat production and both these authors found fluctuations throughout development in metabolic energy utilization. The effect of temperature within a range of 3° C. (16° C. to 19° C.) appears to have no relationship to the extremes in oxygen consumption values found. A trend of increased metabolic rates can be detected from the data, which agrees with the relationships found by Laurence (1969) and Smith (1947) using largemouth bass and salmon respectively. The calorie values in Table 2 indicate total energy utilization for metabolism at any one particular time interval.

Ammonia excretion rates are found in Table 3 and are very close to

TABLE 2

Oxygen Consumption Rates and

Energy of Metabolism Determinations

for Striped Bass Eggs and Larva

Age after fertilization (hours)	A Oxygen consumption (mg/individual per hour)	B Temperature (°C.)	C* Mean calorie value (cal/individual per hour X 1000)	D Energy equation time interval (hours)	E** Energy of metabolism (cal/individual X 100)
24	1.148 1.208 2.172 0.170 1.984	17.0 17.0 17.0 17.0 17.0	4.50 ± 1.20	2-24	9.90
48	0.406 0.561 0.507	19.0 18.0 18.0	1.70 ± 0.20	24-48	4.08
72	0.436 0.726 0.806 1.066 1.471	16.0 16.0 16.5 17.5 17.5	3.00 ± 0.60	56-72	4.80
288	3.226 3.225 2.559 2.293 1.525	17.0 17.0 17.0 17.0 16.0	8.60 ± 1.10	168-288	10.32

* Column A multiplied by 3.36 cal/mg and expressed as $\bar{X} \pm$ S. E. Sample size averaged 70 and duration of determination averaged 40 minutes.

** Column C multiplied by the time interval given in column D.

TABLE 3

Ammonia Excretion Rates and

Energy of Waste Determinations

for Striped Bass Eggs and Larva

Age after fertilization (hours)	A* Ammonia excretion (mg/ individual per hour X 100)	B Temperature (°C.)	C** Mean calorie value (cal/ individual per hour X 10,000)	D Energy equation time interval (hours)	E Energy of waste (cal/individual X 1000)
24	3.08 6.17 3.46 4.95 2.65	19.0 19.0 19.0 19.0 19.0	2.04 ± 0.33	2-24	4.5
48	5.75 5.97 4.96	19.0 18.0 18.0	2.80 ± 0.17	24-48	6.2
72	4.71 5.53 7.67 8.96 9.71	16.0 16.0 16.5 17.5 17.5	3.70 ± 0.48	56-72	8.9
288	22.3 21.4 19.1 17.7 11.8	17.0 17.0 17.0 17.0 16.0	9.27 ± 0.90	168-288	14.8

* Values determined by E. Slaughter in a thesis presented to ECU.

** Column A multiplied by 5.025 cal/mg and expressed as X ± S. E. Sample size averaged 70 and duration of determination averaged 40 minutes.

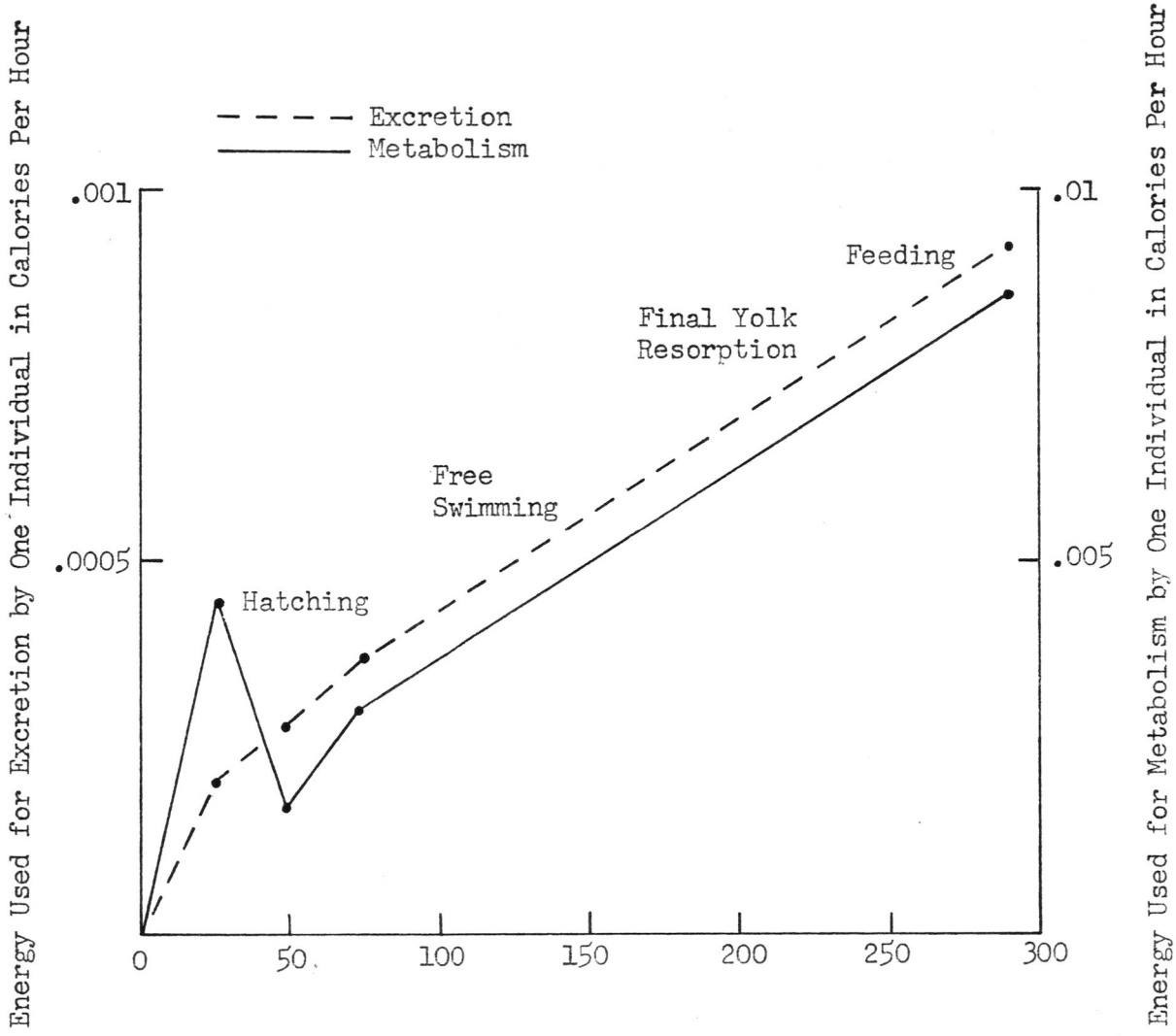
FIGURE 3

Change in Metabolic Rates

and Excretion Rates with

Development in Striped Bass

Eggs and Larva



the values obtained by Smith (1947) for rainbow trout with both sets of data showing an increase in nitrogen waste during development (Figure 3). Smith (1947) did find some fluctuation after hatching due to release of amino-nitrogen which was not apparent for striped bass. These nitrogen waste components are unable to diffuse through the chorion and thus appear after the chorion is broken. The absence of a peak in Figure 3 after 48 hours is probably due to insufficient data. Ammonia excretion was used for total nitrogenous waste since urea is found in negligible quantities (Smith, 1947). Table 3 shows the total waste energy lost for each interval tested. Table 4 summarizes the total energy expended for metabolism and waste from fertilization to the 288 hour postlarva and sums the energy used for all processes other than growth.

Part III

Relationship of Energy Content to Energy Utilization

With the measurements of energy content and energy requirements being measured independently for eggs and larva, they can be compared both graphically (Figure 4) and by using a bioenergetic equation (Figure 1). Figure 4 compares the total energy content change of the embryo and yolk (Table 1) to energy utilization (Table 4) for each of the stages of development. The balancing of an energy equation requires values for total energy of the embryo and yolk (EOEF) (Table 1), energy utilized in metabolism (HM) (Table 2) and energy lost as waste (EW) (Table 3).

The decrease in energy in the egg should be correlated with an increase in energy utilization. Metabolic rates and waste excretion rates are high as expected but only for early egg development. Extensive

TABLE 4

Accumulated Energy Expenditure

of Metabolism and Waste for

Striped Bass Larva and Eggs

Time interval after fertilization (hours)	A* Total energy of metabolism (cal/individual X 100)	B** Total energy of waste (cal/individual X 100)	C*** Sum of energy expended (cal/individual)
24	10.80	0.49	0.1129
48	14.88	1.16	0.1604
72	22.08	2.03	0.2417
288	187.84	22.05	2.0989

* From column C Table 2 multiplied by time interval.

** From column C Table 3 multiplied by time interval.

*** Column A plus B.

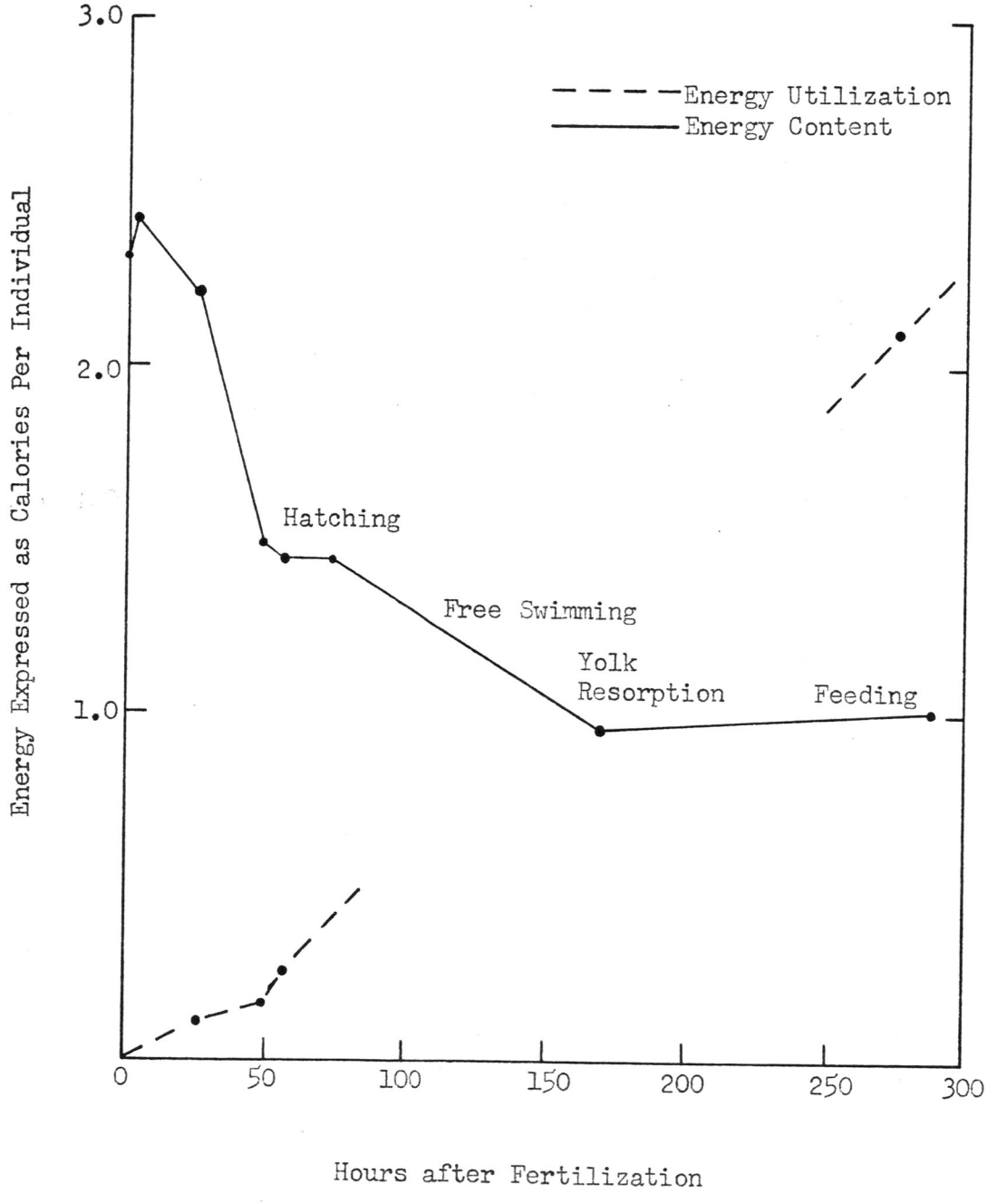
FIGURE 4

Change in Total Energy Content

and Total Energy Utilization

with Development in Striped Bass

Eggs and Larva



protein resorption found in rainbow trout eggs (Smith, 1952) may be occuring in striped bass since the waste product of protein metabolism, ammonia, was measured in high concentrations. Energy needed for the formation of new cells may be supplied by protein at this time. These findings are consistent with the occurrence of rapid cell division of early egg development. Using the bioenergetic equation given in Figure 1 with a time interval of 2 to 24 hours the following values were found:

$$EOEF_2 = EOEF_{24} + EW_2 + HM_{24}$$

$$2.421 = 2.206 + .0045 + .0990$$

$$2.421 = 2.310$$

An error of 0.111 is a result of fluctuation in oxygen consumption rates (Table 2). In the prehatching egg, the energy content continues to decrease although metabolic energy decreases and nitrogen excretion slows down (Figure 3). This inconsistancy is primarily due to inaccuracy in metabolic data. Since oxygen consumption has been shown to fluctuate, the metabolic value for the 48 hour old egg may be unusually low. This rate value has been projected over the entire interval from 24 to 48 hours and if atypical, these results could show an energy imbalance. A second possibility may result in a decreased energy curve without increased energy utilization. If fats and carbohydrates are being used for energy at this time as is believed, these high energy components of the yolk may be converted to protein and used for growth. This would account for both a decrease in nitrogen, since ammonia is needed for this conversion, and a decrease in energy content without energy losses of metabolism. Unpublished work by Slaughter (1972) on striped bass larva indicates a sharp increase in the protein content of the prehatching egg

which supports the conversion of fats and carbohydrates to cellular protein. Low protein utilization has been demonstrated for salmon (Hollett and Hayes, 1946) and trout (Smith, 1952) and for striped bass protein may too be fulfilling only a portion of the energy needs, as indicated by the low nitrogen excretion. A balanced equation for the prehatching egg, 24 to 48 hours, indicates a large error of 0.734 due to the possibilities discussed.

$$EOEF_{24} = EOEF_{48} + EW_{48} + HM_{48}$$
$$2.206 = 1.485 + .0062 + .0408$$
$$2.206 = 1.532$$

Hatching occurs between 48 and 56 hours after fertilization and energy losses at this time are due to shedding of the chorion, metabolic energy for activity in addition to other metabolism, and waste energy.

The early prolarval stage is characterized by a plateau on the energy content curve indicating an extremely low utilization of energy. This inactive period is also reflected in the absence of weight change (Figure 2). Although metabolism is low an increase in rate was measured and results in an error of 0.059 in the following energy balance:

$$EOEF_{56} = EOEF_{72} + EW_{72} + HM_{72}$$
$$1.439 = 1.441 + .0089 + .0480$$
$$1.439 = 1.498$$

Laurence (1969) found that largemouth bass prolarva demonstrated a severe decrease in oxygen consumption after hatching. These findings and the discrepancy between energy content and energy utilization for striped bass, suggests the error is due to incomplete metabolism data. Studies with largemouth bass, rainbow trout, and salmon prolarva show a period

of rapid growth of the embryo which is very likely the condition in striped bass. A decrease in energy content in the late prolarva is associated with high metabolism and excretion rates and possibly fat resorption. Hollett and Hayes (1946) found a second period of fat metabolism at the mid-prolarva stage for salmon. The larva are actively swimming and if fat resorption occurs in striped bass at this time it may be fulfilling a metabolic energy need. Protein resorption continues to increase after the egg stage and reaches its maximum utilization rate in the late prolarva in salmon (Hollett and Hayes, 1946) and rainbow trout (Smith, 1952). This occurrence in striped bass is indicated by a nitrogen excretion increase, thus protein may be used for most of the metabolic and growth energy needs of the late striped bass postlarva. At the time of yolk sac resorption, which occurs at approximately 240 hours after fertilization (Manseuti, 1958), energy demands are extremely high and eventually become greater than the energy content value. At this point the larva enters the "critical period" and must successfully begin feeding. Since energy utilization values have been projected over a large range, 72 hours to 288 hours, the position of the "critical period" cannot be determined from the data obtained (Figure 4). In a bioenergetic assessment of the postlarva, the value for metabolism may be incorrect due to the small amount of data for this long time interval (Table 4) and the occurrence of oxygen consumption fluctuations (Table 2). Waste energy (EW) includes only nitrogen excretion but in the postlarva fecal wastes are present; therefore, the energy utilization value composed of metabolic and waste energies are thus only approximations. An error of 0.163 could be accounted for exclusively by inaccuracy in

metabolism and waste energy values. This would indicate that feeding during the 168 hour to 288 hour postlarva was very low. The energy supplied by brine shrimp eggs if present appears as part of the error value.

$$EOEF_{168} + EF_{external} = EOEF_{288} + EW_{288} + HM_{288}$$

$$0.960 + EF_{external} = 1.005 + 0.0148 + 0.1032$$

$$0.960 + EF_{external} = 1.123$$

SUMMARY AND CONCLUSIONS

1. Developing striped bass demonstrate a decrease in energy content, as measured by dry weight change and total caloric energy, from the fertilized egg to the postlarva.

2. Metabolism continues to increase throughout development although it may fluctuate considerably, as measured by oxygen consumption rates.

3. Nitrogen excretion also exhibits a continual increase in striped bass development when determined from ammonia concentrations.

4. The rapid energy decrease in the eggs of striped bass is in part due to high metabolism and waste energies and possible fat resorption for growth.

5. The absence of energy content change in the early prolarva is due to low metabolic energy utilization.

6. Gradual energy decrease in the remainder of the prolarva stage results from swimming activity and a corresponding high metabolic and excretion energy value.

7. The postlarva exhibits a small increase in energy content with energy for growth, metabolism and excretion supplied by an external food source.

RECOMMENDATIONS

1. A complete investigation of metabolism and excretion should be conducted, including measurements on a greater number of developmental stages especially during the postlarval period.

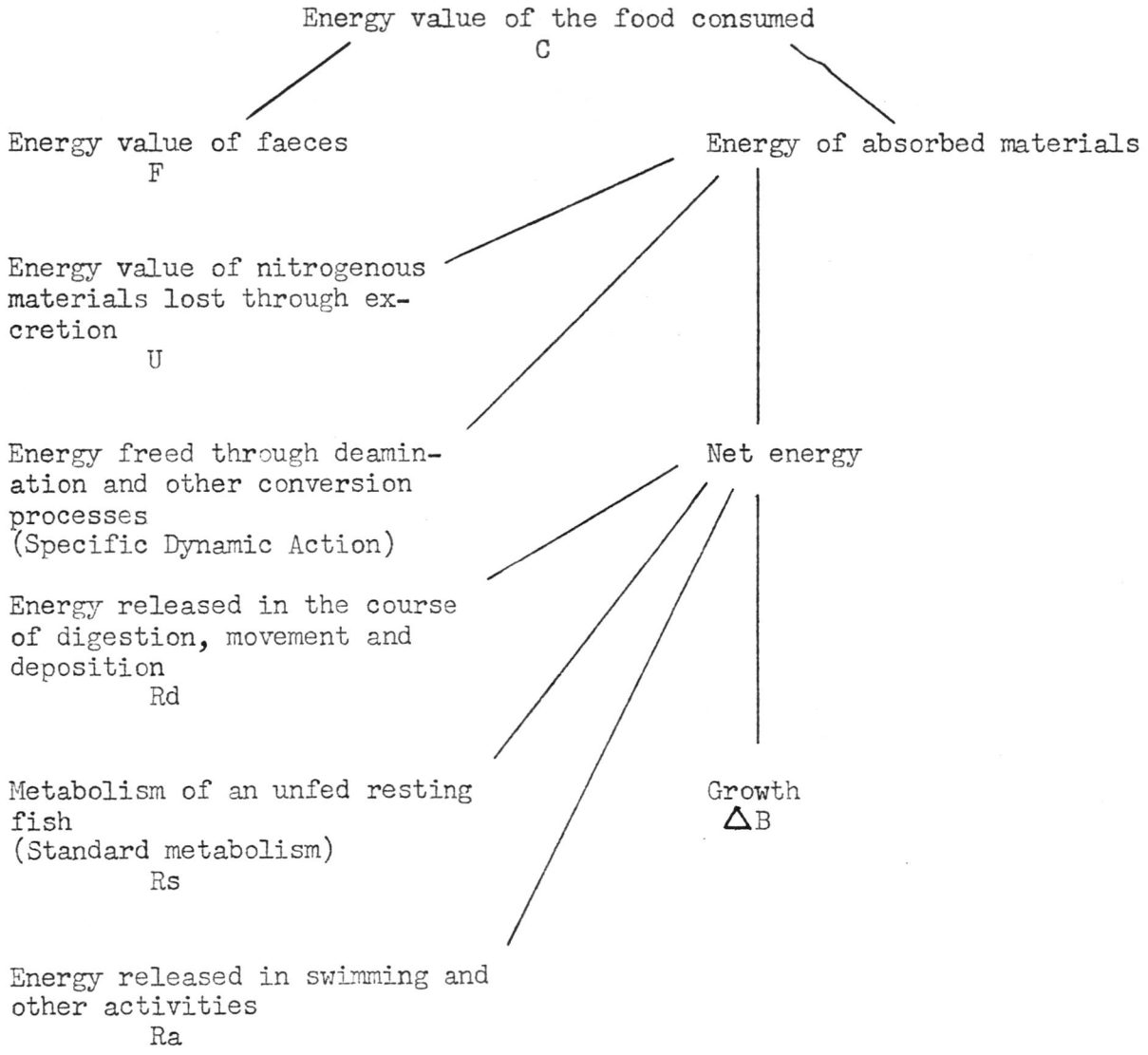
2. The "critical period" and external feeding for striped bass during the postlarval stage should be studied in relation to energy content of the organism to determine the possibility of an energy deficiency.

3. A study on the energy utilization sequence of protein, fat, and carbohydrate and the compostion of the embryo and yolk of those three components would clarify the observations of energy content changes and sources of energy presented in this research.

APPENDIX 1: ENERGY DIAGRAM

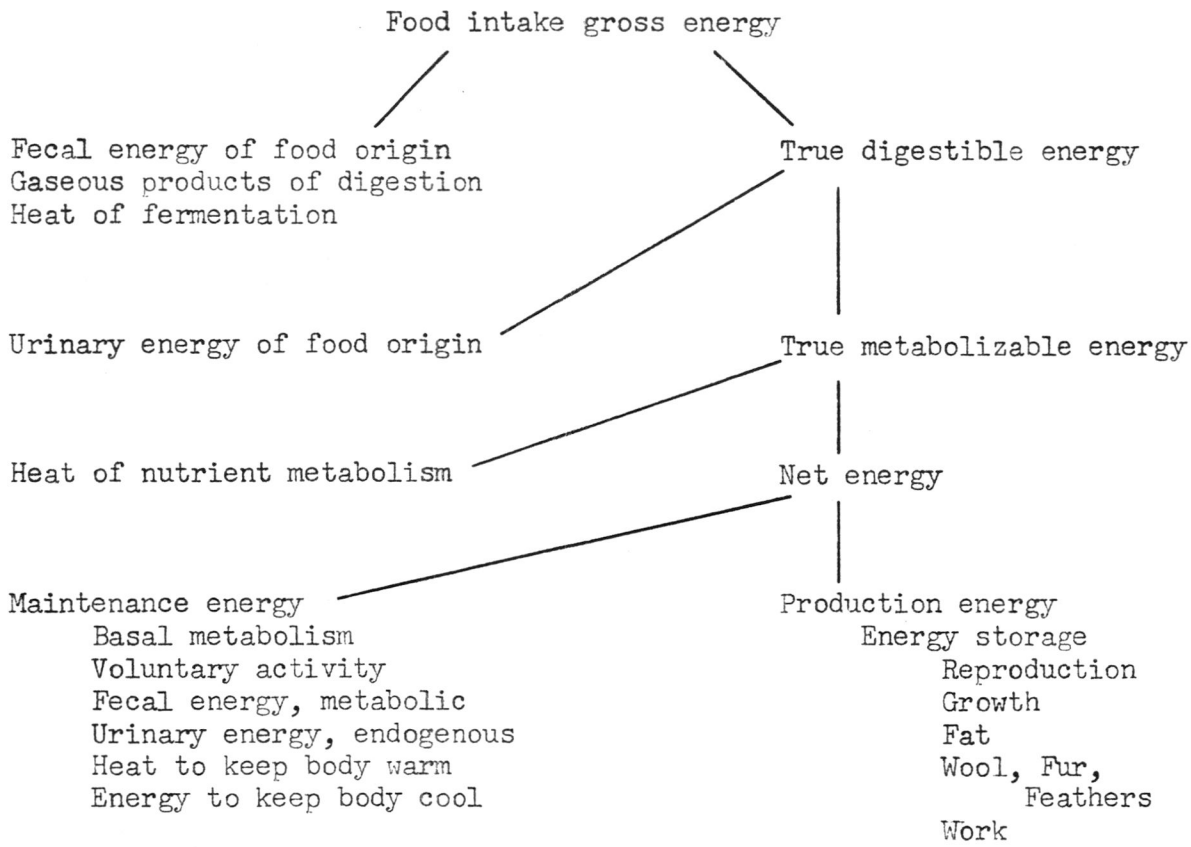
$$C = F + U + \Delta B + R$$

$$R = Rs + Rd + Ra$$



The energy equation and diagram of Davis and Warren (1968).

APPENDIX 2: ENERGY FLOW



Adapted from Harris' true energy distribution scheme.

APPENDIX 3: ENERGY EQUATION

$$E_o + E_f = H_{basal} + H_{SDA} + H_{activity} + E_{excretion} + E_t$$

E_o = energy content of the organism at time zero.

E_f = energy content of the food consumed during time interval "t".

H_{basal} = energy required for the metabolic cost of existence.

H_{SDA} = energy needed for food handling or specific dynamic action.

$H_{activity}$ = energy used for exercise.

Eexcretion = energy lost as compounds in the urine and feces.

E_t = remaining energy content of the organism at time "t".

Krueger's et. al. (1968) energy balance equation and adapted definitions.

LITERATURE CITED

Bagnel, T. B. and Erich Baum. 1968. Eggs and early life history, p. 159-181. In W. E. Ricker (ed.), Methods for assessment of fish production. Blackwell Scientific Publications, Oxford, 313 p.

Beamish, F. W. H. and L. M. Dickie. 1969. Metabolism and biological production in fish, p. 215-242. In S. D. Gerking (ed.), The biological basis of freshwater fish production. Blackwell Scientific Publications, Oxford, 495 p.

Blaxter, J. H. S. 1969. Development: eggs and larva, p. 178-252. In W. S. Hoar and D. J. Randall (ed.), Fish physiology, Vol. III. Academic Press, New York, 485 p.

Brody, Samuel. 1945. Bioenergetics and growth. Hafner Publishing Company Incorporated, New York, 1023 p.

Cummins, Kenneth W. and John C. Wuycheck. 1971. Caloric equivalents for investigations in ecological energetics. Mitt. int. Ver. Limnol., No. 18, 158 p.

Davis, G. E. and C. E. Warren. 1968. Estimation of food consumption rate, p. 204-225. In W. E. Ricker (ed.), Methods for assessment of fish production. Blackwell Scientific Publications, Oxford, 313 p.

Forester, Roy P. and Leon Goldstein. 1969. Formation of excretory products, p. 313-350. In W. S. Hoar and D. J. Randall (ed.), Fish physiology, Vol. I. Academic Press, New York, 465 p.

Fry, F. E. J. 1969. Effects of environmental factors on the physiology of fish, p. 1-99. In W. S. Hoar and D. J. Randall (ed.), Fish physiology, Vol. VI. Academic Press, New York, 559 p.

Golterman, H. L. 1969. Methods for chemical analysis of fresh waters. Blackwell Scientific Publications, Oxford, 166 p.

Harris, Lorin E. 1966. Biological energy interrelationships and glossary of energy terms. National Academy of Sciences and National Research Council, Washington, D. C., 35 p.

Hollett, A. and F. R. Hayes. 1946. Protein and fat of the salmon egg as sources of energy for the developing embryo. Can. J. Res. 24(D): 39-50.

Krueger, Hugo M., James B. Saddler, Gray A. Chapman, Ian J. Tinsley, and Robert R. Lowry. 1968. Bioenergetics, exercise, and fatty acids of fish. Amer. Zool. 8: 119-129.

Laurence, Geoffrey C. 1969. The energy expenditure of largemouth bass

larvae, Micropterus salmoides, during yolk absorption. Trans. Amer. Fish. Soc. 98: 398-405.

Mansueti, Romeo. 1958. Eggs, larvae, and young of the striped bass Roccus saxatilis. Chesapeake Biological Laboratory, Contribution No. 112. Solomons, Maryland, 33 p.

Phillips, A. M. 1969. Nutrition, digestion, and energy utilization, p. 391-432. In W. S. Hoar and D. J. Randall (ed.), Fish physiology, Vol. I. Academic Press, New York, 465 p.

Smith, S. 1947. Studies in the development of the rainbow trout Salmo irideus. I. the heat production and nitrogenous excretion. J. Exp. Biol. 23: 357-378.

Smith, S. 1952. Studies in the development of the rainbow trout Salmo irideus. II. the metabolism of carbohydrates and fats. J. Exp. Biol. 29: 650-660.

Warren, D. E. and G. E. Davis. 1967. Laboratory studies on the feeding, bioenergetics, and growth of freshwater fish, p. 151-174. In S. D. Gerking (ed.), The biological basis of freshwater fish production. Blackwell Scientific Publications, Oxford, 495 p.