

ENERGETICS OF A HOST-PARASITE RELATIONSHIP
AS ILLUSTRATED BY THE LEECH MALMIANA NUDA,
RICHARDSON AND THE SHORTHORN SCULPIN
MYOXOCEPHALUS SCORPIUS (L.)

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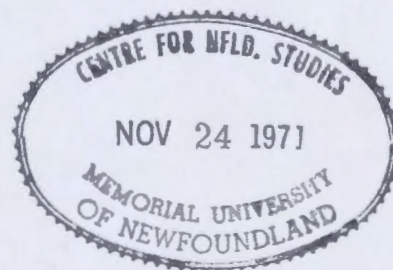
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AS ILLUSTRATED BY THE LEECH *MALMIANA NUDA*,
RICHARDSON AND THE SHORTHORN SCULPIN
MYOXOCEPHALUS SCORPIUS (L.)

by



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A thesis presented to the Memorial University
of Newfoundland in partial fulfilment of the
requirements for the degree of Master of
Science in Biology.

June, 1971.

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ACKNOWLEDGEMENTS

The author wishes to express his gratitude to all those who aided in this study, particularly to the faculty and staff whose ideas and equipment he begged or borrowed for a period of two years. Special thanks are given to Dr. C. C. Davis. His supervision and friendship are a reminder that there is more to scientific study than pure research.

ABSTRACT

The weekly energy budget was balanced for two groups of the sculpin, *Myoxocephalus scorpius* at 10°C. An accurate estimate of weekly energy expenditure could be made by calculating the metabolic energy, measuring the waste production and measuring the energy incorporated into new tissue. The metabolic energy component was measured as the difference between weekly energy input and the sum of calories of growth and calories of waste production. The logarithm of the calculated respiration rate was proportional to the logarithm of body weight, and equal to approximately twice the resting respiration rate as measured in a flowing water-type respirometer. There was no significant difference between the summed components of energy expenditure and weekly energy input.

There was a significant difference between the expected energy expenditure and the observed energy input of fish parasitized with a known weight of the leech *Malmiana nuda*. This difference was proportional to the biomass of parasites and equal to 2238 calories/gram of leech.

The resting energy consumption of the leech was estimated from measurements of resting oxygen consumption. Resting oxygen consumption was proportional to weight, and environmental temperature. It was calculated that the resting leech would consume 63 m./gram/week at 10°C. (equivalent to approximately 285 calories). At this rate of energy expenditure, there would be an additional 75 calories of waste produced. Growth would account for 1240 calories/gram of new tissue.

The energy input also was estimated by measuring the amount

of blood in the gut of recently fed leeches. It was observed that a leech would contain about 2 ml. of blood/gram. One ml. of blood was equal to 575 calories, resulting in an estimated energy input of approximately 1100 calories. This suggests that a leech continuously fed would absorb about 1600 calories of energy per week, or the equivalent of 2 3/4 ml. of blood.

It was suggested that the ratio of energy loss from the host to energy expenditures of the parasite would be an indication of the degree of adaptation of the system. A well adapted host parasite relationship would exert a metabolic demand which is little more than the energy requirements of the parasite.

INTRODUCTION

The two criteria commonly used to distinguish associations are: the relative loss or gain from the association, and the degree of inter-dependence of the components (Croll, 1966). Often, an association is classified according to the latter criterion, with the ecologist assuming the energy relations. The measurement of the flow of energy between the host and symbiont may provide quantitative evidence which, combined with the observed dependences, provides a more meaningful distinction of symbiotic relationships.

Ecological systems can be defined in terms of energy flow. A primary consumer is an organism that utilizes some of the energy stored by producers; a secondary consumer is one that uses some of the energy stored by primary consumers. Energy flow is equally important when discussing relations within trophic levels. One of the major factors affecting the size of an individual or population is the supply of energy (Slobodkin, 1962). If we accept the principle that a parasite removes energy from the host, then we must accept that the absorbed energy will be resolved as a deficit to the host's energy budget.

For any animal (or population) per unit time, the following holds true:

$$Q_c - Q_w = Q_g + Q_s + Q_d \quad (\text{Winberg, 1956}) \quad (1)$$

Where:

Q_c = energy of food consumed

Q_w = energy of waste materials

Q_s = energy released for metabolism

Q_d = energy released in digestion, assimilation and storage of materials consumed

Q_g = total change in energy value of body material.

This relationship is normally expressed with:

$$Q_s + Q_d = Q_r \quad (2)$$

Where: Q_r = total energy metabolically utilized (i.e. total heat production). This factor can be expressed in terms of oxygen consumption using the appropriate oxy-caloric conversion factor (Walkey and Meakins, 1970).

For a parasitized animal the following will exist:

$$Q_c - Q_w = Q_g + Q_r + Q_p \quad (\text{Walkey and Meakins, 1970}) \quad (3)$$

Q_p is the additional load of the parasite. To calculate Q_p it is necessary to measure the other four parameters.

At present we are concerned with systems in which the immediate benefit is to only one component (i.e. predator, micro-predator and parasite). The distinction between a micro-predator and a parasite is difficult to make, and concerns the life history and mode of feeding of the organism. A parasite and a micro-predator can be considered the same system if energy flow pattern alone is considered. Both types involve a transferal of energy from one component to the other.

It has been known for some time that parasite infections can have an effect on the nutrition of the host. Lower than normal weight classes were obtained from fish samples collected by Hubbs (1927) and Cross (1935). Both authors found a direct correlation between weight difference and intensity of infection. The species infecting the fish

varied from individual to individual, and level of parasitemia was arbitrarily decided. Krull (1934) described large mortality of fish fry due to infections of *Cercaria bessiae*. The dead fish superficially appeared normal. Recently, Castro and Olson (1967) observed host weight losses associated with infections of *Trichinella spiralis*. There are, of course, many other examples. (Hunter and Huncer, 1938; Moose, 1963).

Parasites also have been proven to aid host's growth (Varley and Butler, 1933; Salt, 1931; Lincicome and Shepperson, 1963; Mueller, 1964). Examples of growth enhancement are observed between guinea pigs and *Spirometra mansonoides*, and mice and *Trypanosoma lewisi*. In both cases, increased growth rate was due to the presence of the parasite. Lincicome and Shepperson (1963) described weight gains in mice, but found no correlation between increased growth rate and numbers of trypanosomes. They theorized that this extra growth was due to some unidentified metabolic byproducts (Lincicome, 1959; Lincicome *et al*, 1960).

Mueller (1965a) observed that mice infected with plerocercoids of the tapeworm *Spirometra mansonoides* gained weight more rapidly than did controls. This was due to an increase in appetite with a slight increase in assimilation efficiency (Harlow *et al*, 1967). The enhanced growth rate was a result of leakage of a hormone-like substance from the parasite. (Mueller, pers. comm.). Hypophysectomized hosts gained weight twice as rapidly as hypophysectomized controls on the same diet (Mueller, 1968; Mueller and Reed, 1968). Infections also compensated the growth of thyroidectomized and diabetic rats. This

benefit to the host was an isolated incident. The Asian form of *Spirometra* had little effect on rats or mice (Mueller, pers comm.). There is no evidence of enhanced growth in the natural host (tadpoles, frogs and water snakes). It is probable that the substance(s) responsible for the increased growth rate are metabolic products or tapeworm constituents which are similar to the rat's hormones, but have no effect on the natural host (Mueller, pers comm.).

Leakage also causes amplified parasite effects in other systems. Arme (1968) suggests that an anti-gonadal substance is produced by *Ligula intestinalis* which causes suppressed gonad development. O'Kelly and Seifert (1970) suggest that the presence of an antagonistic substance causes the pronounced weight loss in tick infested cattle. It has been theorized that some of the instances of increased growth rates of stylopterized insect pupae are due to hormones produced by the parasite (Von Brand, 1952, 1966). In all of these cases, host and/or parasite nutritional parameters have not been measured, and the presence and nutritional effects of parasite hormones cannot be evaluated.

Recently, Walkey and Meakins (1970) attempted to calculate the parasite energy drain using the relationship between the three-spined stickleback, *Gasterosteus aculeatus* and plerocercoids of the tapeworm, *Schistocephalus solidus*. Unfortunately, it was impossible to compare changes in the host's weight, respiratory rate, etc., without having to consider the parasite's bulk as an important factor. The plerocercoids grew within the host's tissues, and excreted great quantities of wastes directly into the host's system. Nevertheless,

the authors did observe metabolic differences between control and infected fish. These consisted of an increased gross food conversion efficiency in parasitized animals, a slightly higher respiratory rate and a faster mortality during starvation. These were attributed to the presence of the parasite burden. The authors were unable to compare the changes with the metabolic requirements of the parasite.

The present study was initiated in order to calculate the energy cost of a parasite. The relationship between the shorthorn sculpin, *Myoxocephalus scorpius* (L.) (Huntsman, 1921) and the leech *Malmiana nuda* (Richardson, 1970) was chosen as a model relationship. The physiology and metabolism of fish have been well documented (Winberg, 1956; Davis and Warren, 1965; Brockson et al, 1968). The sculpin is present in adequate numbers in this locale and is comparatively easy to rear in the laboratory. *Malmiana nuda* is an ectoparasite, and can be added or removed from the system at will. Thus it is possible to accurately quantify the parasite burden and to establish the basic energy requirements of the leech when not on the host. The leech produces no wastes directly affecting the host tissue, while removing a noticeable amount of the host's body fluids. The anti-coagulant substances produced only affect local tissues, and have no direct effect on host metabolism (Mann, 1962). There appears to be no scar tissue or local bleeding on the host following feeding. The energetic consequences of the leech burden would be restricted to the energy requirements of the parasite and any increased physiological strain on the host.

MATERIALS AND METHODS

Fish of approximately the same age and size group were used exclusively for the growth experiments. It was estimated that these were group I fish since their individual weight was between 3.5 and 5.0 gm. (Ennis, 1970). These animals were small, easily worked with, and the parasite's effects would be noticeable. There has been no observed difference in growth rate between animals of different sexes (Ennis, 1970).

The fish were captured by divers using a suction device, and were transferred to holding tanks in the laboratory. The fish were from a number of localities, and an average of about 10 m. depth.

The energy requirements of the parasite burden were calculated indirectly and compared with direct estimates. Energy values were assigned to all components of the energy equation, and the total of these values compared with energy input. Following the six week controlled growth period, the difference between observed and expected growth rates of parasitized fish was assumed to be due to the presence of the parasite.

1. Calorimetry

The caloric value of all samples was measured by use of a Parr micro-bomb calorimeter, type 1411. The apparatus was standardized by burning ten replications of a standard preparation of benzoic acid at a pressure of 35 atm. O_2 . The standardization procedure was repeated periodically during the experimental period.

All samples were dried to constant weight at 65°C. and ground

with a mortar and pestle. The resultant powder was weighed in a platinum dish and placed in the bomb capsule. Five replications of each sample were burned and the results averaged. Following each burning, the platinum dish was reweighed, and the ash content of the sample calculated. Caloric contents were expressed as cal/gm. or cal/ash-free gm.

2. Food Analysis

The fish were fed exclusively on mantle muscle of squid (*Illex illecebrosus illecebrosus*). The food was analyzed for fat, protein and water content. The corresponding dry/wet weight ratio was calculated every two weeks.

Thawed muscle was homogenized in a Virtis No. 45 homogenizer and diluted with distilled water to about 40 mg. tissue per ml. Five ml. of sample were dried and weighed. The remaining fluid was analyzed for protein content using the biuret method (Layne, 1957). An aqueous solution of bovine serum albumin was serially diluted and used as a standard. The optical densities of the sample and of the standards were measured with a Bausch and Lomb Spectronic 20 spectrophotometer at a wavelength of 540 mμ. Protein concentration was expressed as wt. protein/gm. of dried tissue. At least three replications were performed per squid sample.

Dried tissue was analyzed for lipid concentration by a modification of the technique of Bligh and Dyer (1959). At least 2 gm. of dried tissue were mixed with 8 ml. of distilled water, 20 ml. of chloroform and 20 ml. of absolute methanol. The dissolved lipid phase was dried at 40°C. and the resulting solid weighed. Lipid concentration

was expressed as mg. lipid/gm. dried tissue.

Carbohydrate composition was calculated indirectly as the difference between the weight of dried tissue and the sum of the weights of protein, lipid and ash.

3. Measurement of Leech Basal Metabolism

The rate of oxygen consumption of the leech was measured within a closed system consisting of a 250 ml. flask containing 200 ml. of filtered seawater, stirring magnet and a 2 cm. thick layer of mineral oil (Fig. 1). Oxygen concentration was measured by the micro-Winkler technique (Allee and Oesting, 1934). Fifty ml. water samples were removed and fixed at the beginning and end of the test period. Each 50 ml. was divided into two samples and each sample measured for oxygen concentration. A piston burette was used for titration with 0.0025 N sodium thiosulfate. It was calculated that the minimum resolution of the system was approximately .005 ml. O_2 /L. The length of the test period was adjusted until about 10% of the available oxygen was used.

Two series of tests were conducted; rate of oxygen consumption vs. total animal weight and rate of oxygen consumption vs. temperature. All experimental animals had been kept in holding tanks at 5°C. Respiration vs. weight experiments were conducted at $5 \pm 1^\circ\text{C}$. Temperature regulation was accomplished by placing the test apparatus in a modified refrigerator. Individual animals were blotted dry and placed in wet weight classes of 0.005 gm. At least five animals were used per test, and each group was allowed 48 hours prior to the experiment to adapt to the respiration flask. The oxygen consumption

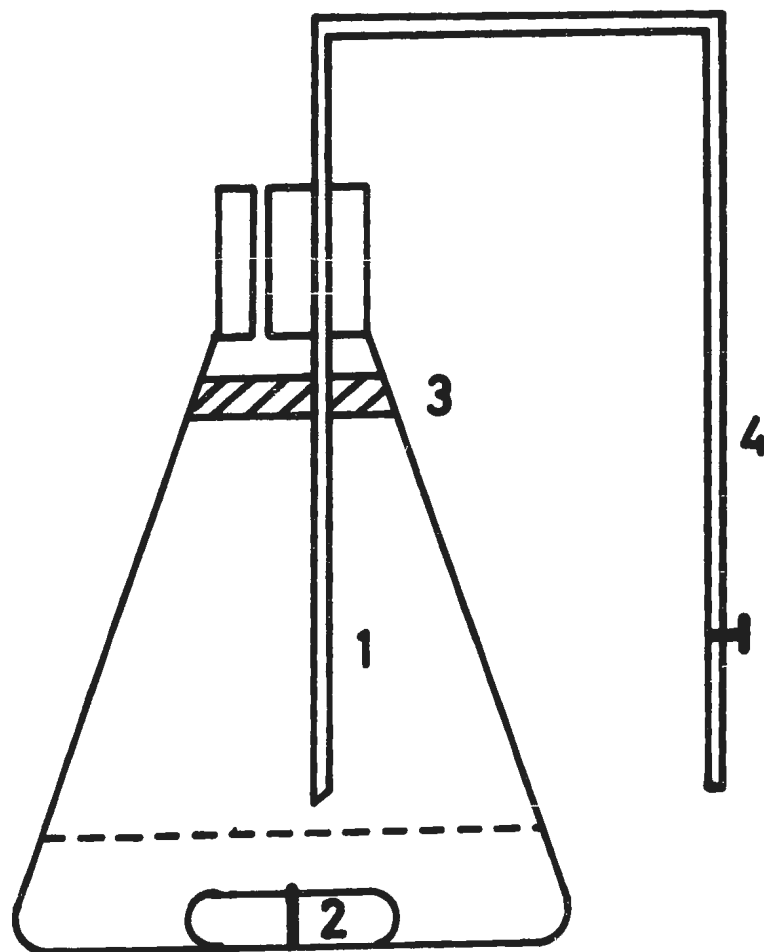


FIG. 1. APPARATUS USED TO MEASURE LEECH RESPIRATION RATE. 1 - RESPIRATION CHAMBER. 2 - STIRRING MAGNET. 3 - MINERAL OIL BOUNDARY. 4 - WATER SAMPLING TUBE.

rate for each group of animals was measured twice and averaged. At the end of each experiment, the animals were removed, dried at 65°C. and weighed.

Temperature experiments were conducted at 10°C., 7°C., 4°C., and 1°C., (all $\pm 1^\circ\text{C.}$). Groups of five or six animals were adapted to the experimental temperature for one week and adapted to the respiration flask for 2 days prior to measurement of oxygen consumption. Two replications were conducted with each group, and five groups were utilized per temperature. Oxygen consumption was measured by the technique described above.

4. Measurement of Fish Basal Metabolism

Oxygen consumption of fish was measured in a continuous flow respirometer (after Stroganov, 1964) (Fig. 2). Samples of seawater from taps placed before and after the chamber were analyzed for oxygen content by the normal Winkler method. The absolute oxygen decrease was controlled by regulating the rate of water flow. The rate was adjusted to allow 10% oxygen consumption. For most experiments a flow rate of 5 ml./min. was sufficient. The flow meters (Cole-Parmer, No.2) and system were standardized for flow rate prior to the experiment period. Rate of flow through both sampling tubes was regulated to within 1 ml./min. Prior to the experimental period, an inert dye was injected into the system to ascertain that water flow was uniform within the respiration chamber.

Fish were adapted for 4 hours in the respiration chamber. The length of the test period was usually 5 hours, and was sufficient to ensure at least 4-250 ml. water samples. After filling, the

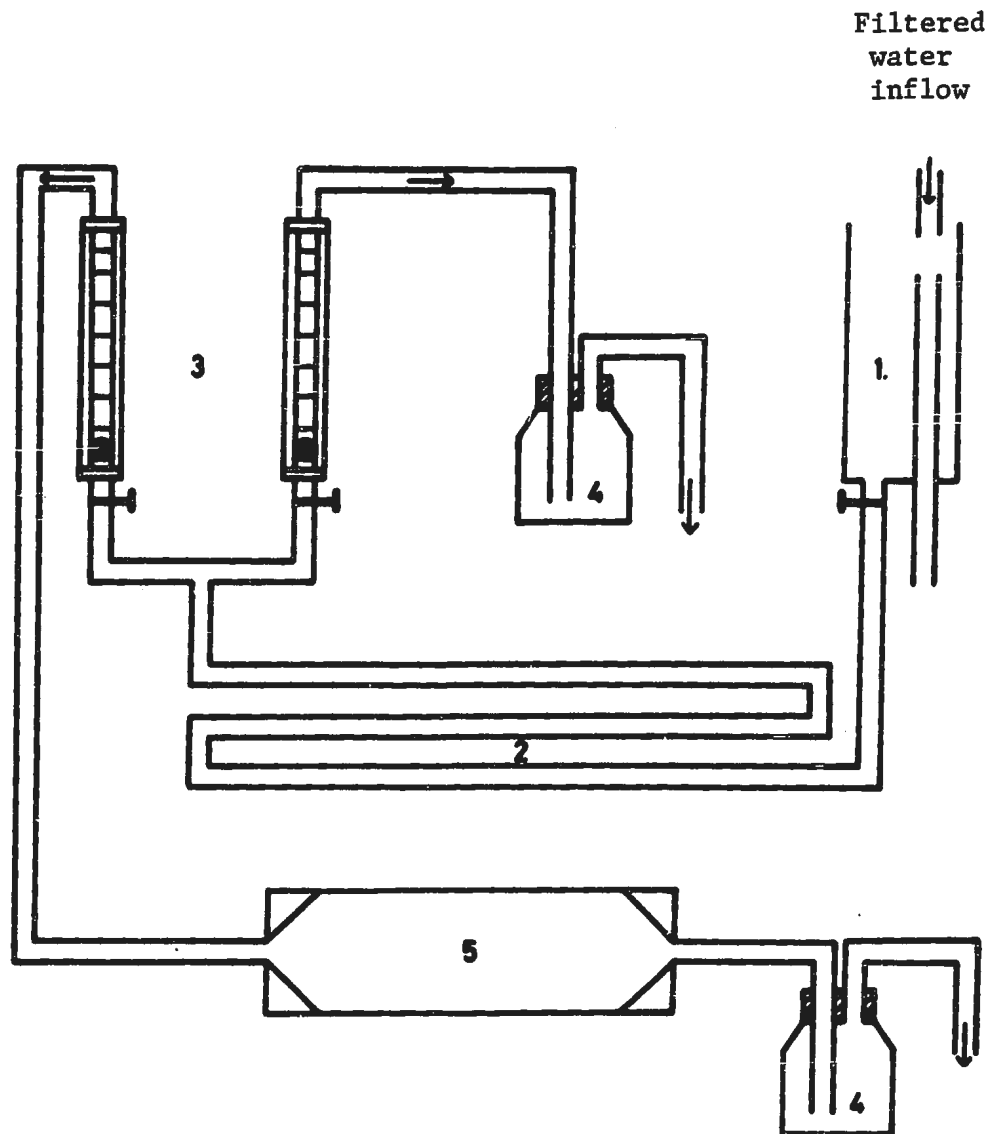


FIG. 2. DIAGRAM OF APPARATUS USED TO MEASURE FISH RESPIRATION RATE. 1 - CONSTANT PRESSURE APPARATUS. 2 - COOLING COILS. 3 - COLE-PARMER FLOW-RATE METERS. 4 - WATER SAMPLE BOTTLES. 5 - RESPIRATION CHAMBER. THE COOLING COILS, RESPIRATION CHAMBER AND SAMPLE BOTTLES WERE IN A WATER BATH AT 10° C.

sampling bottles were left connected to the system for 10 minutes to allow partial flushing.

One experiment was conducted at 10°C., to measure the diurnal changes in oxygen consumption rate. The fish were fed immediately prior to the start of the diurnal rate experiments.

Estimated weekly metabolic requirements were calculated by deriving the energy assumed to be due to respiration and dividing by the oxy-caloric equivalent. The resulting value was compared with the average of the 16 weeks and corrected for animal wet weight.

5. Growth Experiments

Two groups of fish were used; a control group and a test group. Both groups were controls for a period of five weeks prior to the tests. Each group consisted of five fish. Both groups were kept in the same size tanks (75 cm. by 300 cm. and 10 cm. deep), at 10°C. and at the same lighting. The incoming seawater was filtered through a "fulflo" filter (Commercial Filters Corp.) with an effective pore size of 10 microns. Once every five weeks the tanks were drained and thoroughly cleaned. After five weeks the test group was parasitized with a known wet weight of leeches.

Each morning, for a period of three hours both groups were placed in two-liter flasks supplied with running seawater. The test group flask contained a known weight of leeches. For two weeks prior to and following the experimental period, both fish groups were placed for three hours daily in the same flasks devoid of leeches.

It was assumed that both groups of fish were parasite-free. Subsequent autopsies revealed no noticeable metazoan infections.

Protozoan infections were not investigated.

Each day the fish were fed a weighed amount of chopped, thawed squid muscle. The feces were collected approximately every 12 hours, dried at 65°C. and the total weight recorded weekly. Fecal material was collected with an eye dropper. The feces calorie content was determined for the total group sample every 4 weeks. Each week the fish were blotted dry and weighed in a tared flask of water. Both groups were starved for 24 hours prior to weighing.

6. Leech Blood Meal Analysis

Blood meal size was estimated from the hemoglobin intake. Fish blood and homogenized starved leeches were diluted with Drabkin's solution (Dacie and Lewis, 1968). The percent absorbance of each sample was measured at wavelength intervals of 5 mμ from 325 mμ to 800 mμ. At a wavelength of 540 mμ the blood samples absorbed maximum light, while the leech extract exhibited no reaction. Samples of homogenized, fed leeches were compared with diluted whole blood samples.

RESULTS

A. Leech Biology

Under laboratory conditions, very high leech burdens were observed on larger sculpins. The leeches aggregated about the fin rays, opercular region and belly. Starved leeches placed on a clean fish immediately moved to the axial region of the pectoral fins. Leeches were observed on the head and caudal fins of the host, but only when the host was heavily parasitized.

Leeches in aquaria remained quiescent, but became active when a suitable host was placed in the immediate vicinity. The leeches were attached to the substrate by the posterior sucker, and waved the extended body in a random, searching movement (the "Suchbewegung" of Becker and Katz, 1965). When feeding, the leeches had both suckers attached to the host. Occasionally, leeches were seen moving about the surface of the fish, presumably searching for a vulnerable surface. No wound was evident following removal of the leech. The feeding generally lasted from two to three hours. Feeding leeches were occasionally observed on the winter flounder, *Pseudopleuronectes americanus* and the longhorn sculpin, *Myoxocephalus octodecemspinosus*. Presumably, any sedentary fish may act as a host when there is a large density of parasites.

Reproductive cocoons were observed between May and July, concurrent with a slight increase in water temperature. Copulating pairs were observed, but there was no indication of the "breeding clusters" previously observed in piscicolid leeches (Becker and Katz, 1965). The egg cocoons were oval, flattened capsules attached firmly

to rocks, shells or other hard, smooth objects. They were an average size of .94 mm. by 1.05 mm. and contained a prominent plug at one end (Fig. 3). Each cocoon contained only one embryo. At 4°C. the cocoons took about 6 weeks to fully develop. Development appeared to follow that described by Mann (1962) for the leech, *Piscicola* sp.

A skewed distribution was observed when numbers of individuals were plotted against weight classes. (Fig. 4). The random sample was from a population of animals in an aquarium containing a number of sculpins. Sculpins were observed feeding on leeches and it is possible that such predation accounts for mortality within the restricted system. If the sculpins ate relatively more large leeches, the normal weight distribution would be skewed as observed.

B. Leech Metabolic Demands

The relationships of oxygen consumption to temperature, and oxygen consumption to body weight were derived in order to evaluate the effects of these variables on parasite energy cost.

The results of 18 determinations of oxygen consumption of leeches ranging from a size of 8 mg. to 55 mg. are shown in Figure 5. From these data the following relationship was found by the method of least squares:

$$y = 0.156 x^{1.05} \quad \text{at } 5^{\circ}\text{C.} \quad (4)$$

Oxygen consumption at four different temperatures is shown in Figure 6. At a temperature of 10°C., the mean oxygen consumption was estimated to be 63 ml. O₂/gm./week.

LEECH PLUG

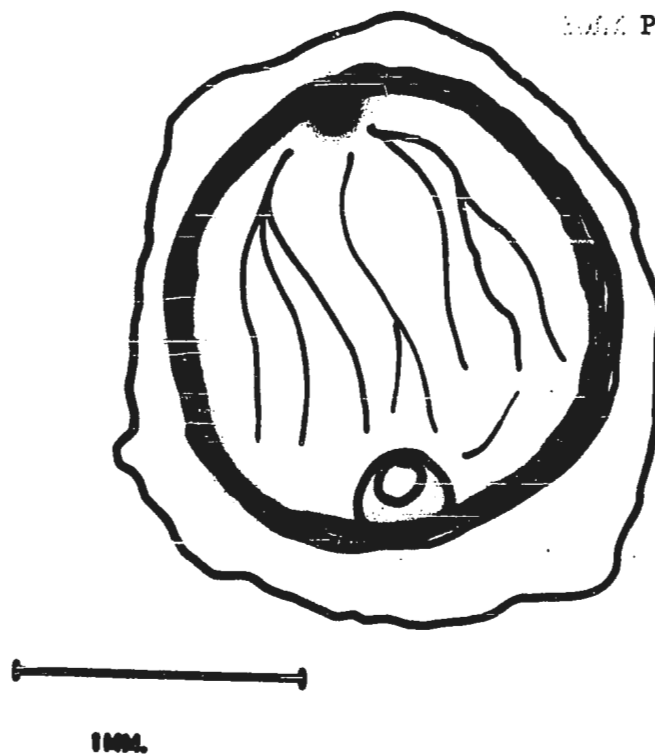


FIG. 3. LEECH EGG COCOON.

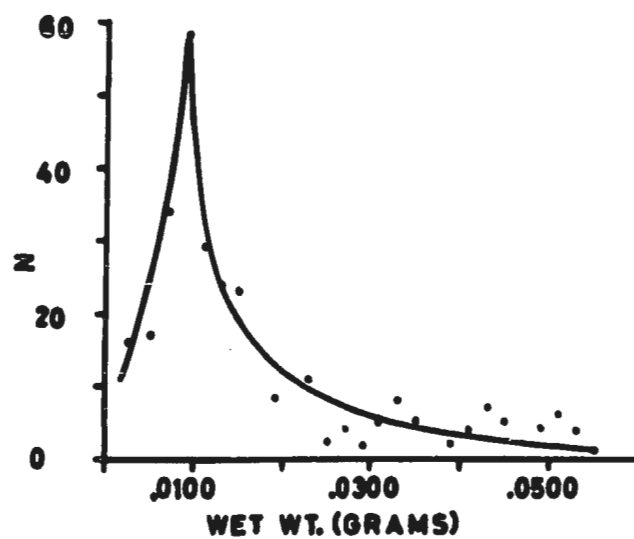


FIG. 4. NUMBER OF LEECHES IN 25 MG. WEIGHT CLASSES.

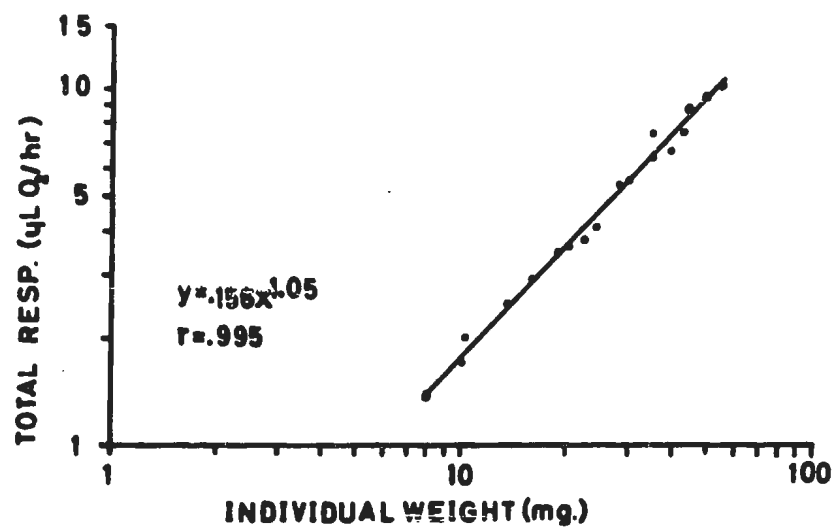


FIG. 5. TOTAL RESPIRATION OF LEECHES PLOTTED AGAINST INDIVIDUAL WEIGHT.

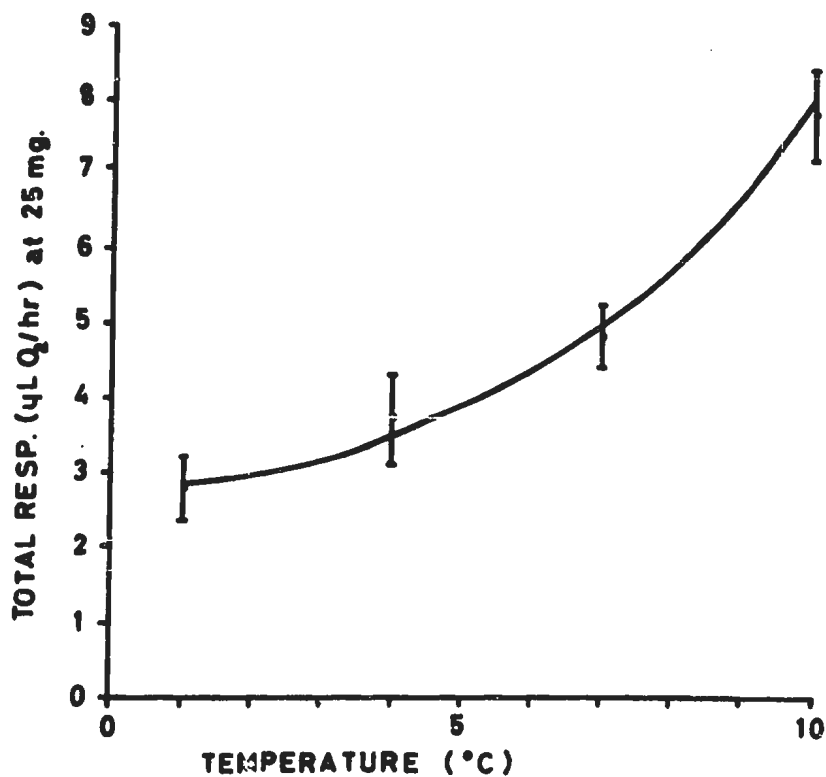


FIG. 6. TOTAL RESPIRATION OF LEECHES PLOTTED AGAINST TEMPERATURE. RESPIRATION RATE ADJUSTED FOR AN ANIMAL WEIGHING 25 MG. THE SOLID LINES INDICATE THE 95% CONFIDENCE INTERVAL.

The oxy-caloric equivalent was based on previous analyses of marine fish blood (Maclead *et al.*, 1960; Kamrask, 1966). It was calculated that a blood meal would consist of 90% protein and 5% carbohydrate, 5% lipid. An equivalent of 4.5 was derived using these data (see Appendix 4). The following theoretical energy equivalent was derived:

$$Q_c = Q_w + 290 \text{ cal/gm.} + 1240 \text{ dw} \quad (5)$$

It is difficult to calculate Q_w (energy content of wastes produced). It was estimated that approximately 20% of the diet will be waste energy, if the assumption is continued that the diet would be 90% protein. The corrected energy estimation would be:

$$Q_c = 65 \text{ cal.} + 290 \text{ cal./gm.} + (1240 \times \text{dw}) \quad (6)$$

The total energy input was also estimated from the average blood meal size. No significant difference ($p < 1\%$) was observed between the blood meal content of feeding individuals and blood meal size of starved animals fed just prior to measurement (Appendix 3, Table 1). The animals had been fed for a period of three hours every three days, and it was concluded that the blood meal size was equivalent to the blood meal size of the test parasites in the previous experiment.

The blood samples analyzed were from fish of sizes ranging from 4.5 gm. to 7.0 gm. One ml. of blood yielded an average of 0.10 gm. of dried tissue. Calorimetry data equaled an energy content of 5730 cal/gm. (Appendix 3, Table 2). Thus, 1 ml. of whole blood was equal to 573 cal. This would mean that the weekly input was 1150 cal/gm. of leech.

C. Normal Fish Metabolism

The results of the analyses of dried squid mantle are shown in Table 3, Appendix 3. The oxy-caloric equivalent of metabolism of squid mantle was calculated as 4.7 (Albanese, 1965). This is calculated from the average composition of the mantle (78% protein, 9.5% lipid and 12.5% carbohydrate) and assumes that all of the food is equally digested and metabolized (see Appendix 4).

The average calorie content per gram dry weight of each squid sample is shown in Table 4, Appendix 3). Although an analysis of variance indicates no significant difference between samples ($p < 1\%$) individual averages were used in computing weekly food ration.

The weekly values for feces production are shown in Table 5, Appendix 3. The average value of weight of dried feces/gram of dried food is 3.0% for group 1 and 3.5% for group 2 (control periods before and after parasitism). An analysis of variance indicates no significant difference between the two groups ($p < 1\%$). No significant difference was observed for test fish between the control and test periods ($p = 1\%$). The average calorie content was 1990 cal/gm. It was not possible to measure urine production. An approximation was determined from the total food protein and was considered as a constant 5% of the calorie input (Winburg, 1962).

An analysis of variance indicates a significant difference ($p < 1\%$) among the calorie contents of different fish (Table 12, Appendix 3). The tissue calorie/gram value is probably proportional to the total weight over a large range of values. Within the fish weight range employed for metabolic determinations, there was no

significant difference ($p < 1\%$). The range of dry/wet weight ratios are shown in Table 12, Appendix 3. Using the average value, 1 gram of fish (wet weight) contained 1185 calories (4995 cal/gm.(wet)).

The number of calories due to weekly respiration are shown in Tables 7 and 8, Appendix 3. Using these values O_2 consumption was calculated as ml. O_2 consumed/gm./day. The weight used was initial weight plus $\frac{1}{2}$ weekly growth.

Calculated oxygen consumption varied with animal weight (Fig. 7). This relationship was linear when plotted on logarithmic scale by the method of least squares and yielded the equation:

$$y = .83 X^{.88} \text{ at } 10^\circ\text{C.} \quad (7)$$

Normal resting oxygen consumption was also used to estimate metabolic rate. At 10°C. , a group of animals with a mean biomass of 13.55 gm. had a calculated oxygen consumption rate of 2.0 ml./gm./day. The diurnal fluctuations in normal metabolic rate are shown in Fig. 8. Animals fed at 10:00 hours showed a marked peak in respiratory rate at 11:00. These diurnal fluctuations occurred during a period of full artificial lighting from 09:00 to 17:00 hours.

A t-test was employed to test the difference between energy input (food) and calculated energy expenditure. The hypothesis:

$$Q_f - (Q_g + Q_r + Q_w) = 0 \quad (8)$$

was accepted for the weekly observations of the control group (group 1) and the control phase of group 2 experiments ($p < 1\%$, see Table 10, Appendix 3). The expected energy expenditure was the sum of calories of weighed dried feces, calories of growth (dw (gm.) \times 1185 cal/gm. wet), estimated calories of urine, and estimated calories of metabolism

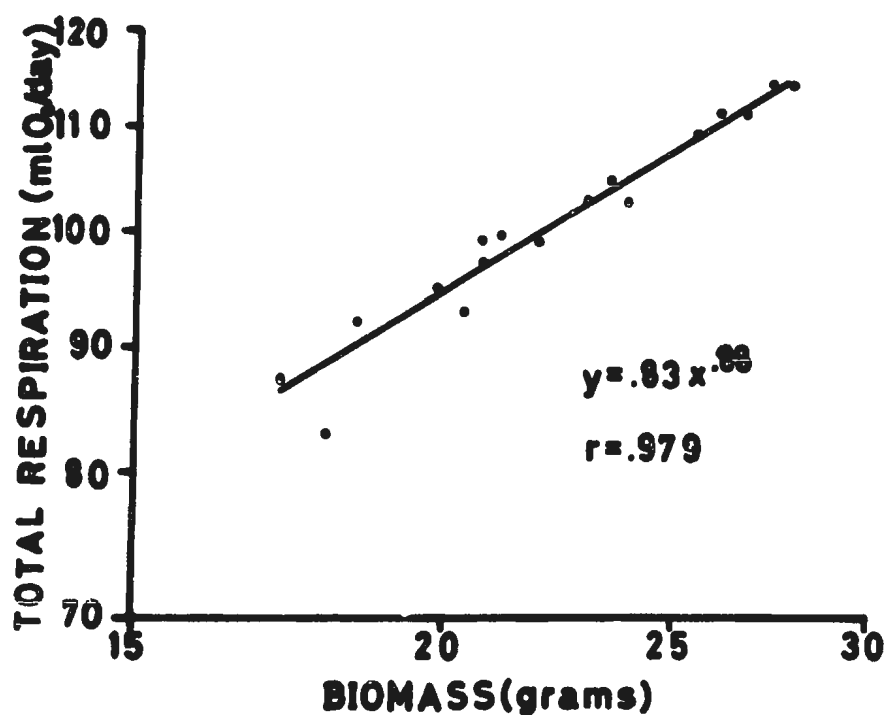


FIG. 7. CALCULATED TOTAL FISH RESPIRATION AT 10°C. PLOTTED AGAINST BIOMASS. (5 FISH/GROUP)

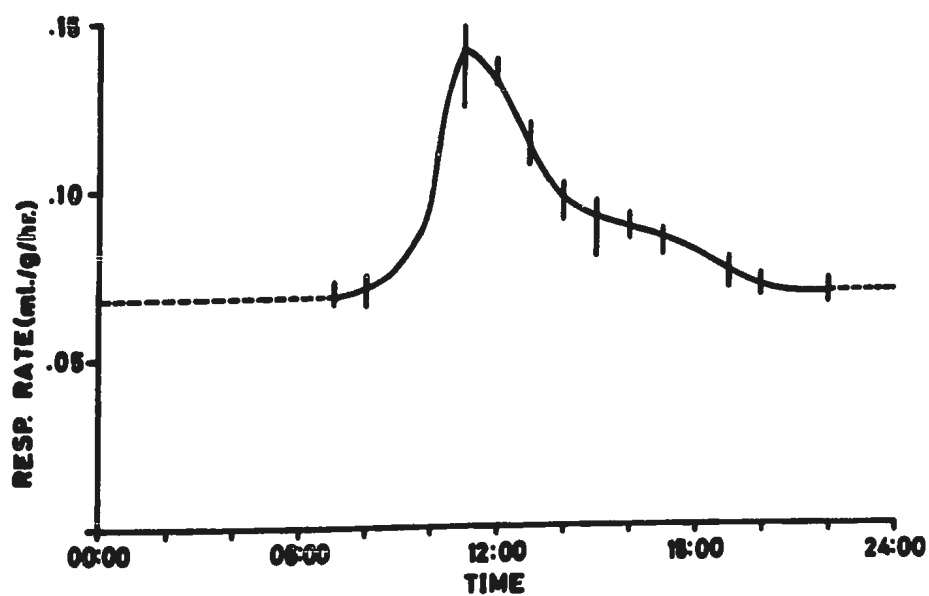


FIG. 8. OBSERVED DIURNAL CHANGES IN FISH RESPIRATION RATE. (AT 10°C.) THE SOLID LINES INDICATE THE 95% CONFIDENCE INTERVAL.

(estimated oxygen consumption based on the average oxygen consumption modified by the relationships to temperature and body size).

D. Metabolism of Parasitized Fish

The rate of growth of group 2 fish differed noticeably when these animals were parasitized (Fig. 9). A significant difference existed between the observed and the expected energy expenditure during this period ($p < 1\%$). It was observed that the calculated respiratory rate did not increase during the two weeks immediately prior to and after the parasitism (see Table 8, Appendix 3). During these periods the test fish had been placed in a 2 liter flask devoid of leeches for three hours a day. Therefore, it was assumed that this environment did not affect the fish's respiration rate, and the energy differences were due to the addition of the parasite burden.

Parasite burden was directly proportional to the observed energy anomalies (see Table 11, Appendix 3), and was expressed as:

$$Q_p = 2240 \text{ cal/gm. (wet weight) parasite/week.}$$

The energy required for leech growth was estimated from the calorie content of the leeches, and from the dry/wet weight ratios. This equalled:

$$Q_g = 1240 \text{ cal/gm. (wet weight).}$$

During four test weeks, the leeches produced egg cocoons. It was calculated that one egg cocoon resulted from an energy expenditure of 82 cal. This was calculated by subtracting the estimations of energy expenditure of metabolism, growth and waste production from the estimated input. These estimates were based on calorimeter data of leech tissues, and the observed energy requirements of the leeches

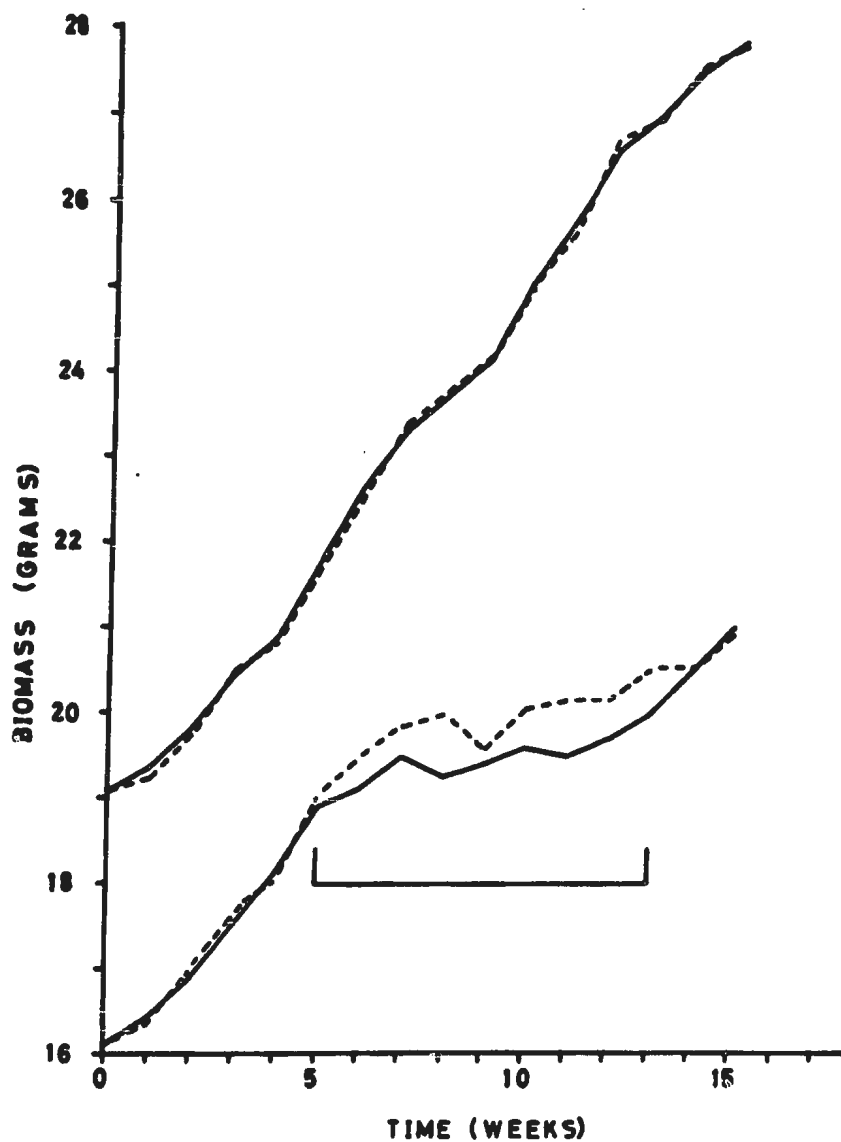


FIG. 9. OBSERVED AND EXPECTED WEEKLY GROWTH OF SCULPIN GROUPS. EXPECTED WEEKLY VALUES. — OBSERVED WEEKLY VALUES. THE SOLID BAR INDICATES TIME THAT THE TEST GROUP WAS PARASITIZED.

during the experimental periods in which they did not produce cocoons.

A total energy budget was derived by combining the above relationships.

$$Q_p = Q_w + Q_{gl} + Q_{rl} + Q_e + \text{stored energy} \quad (9)$$

$$Q_{gl} = Q_{wt} + Q_{rep}. \quad (10)$$

Where:

Q_p = energy attributable to parasite burden

Q_w = energy of leech waste burden

Q_{gl} = energy consumed in leech growth

Q_{rl} = energy consumed in leech respiration

Q_e = additional energy consumption of host due to the parasite burden

Q_{rep} = energy consumed by leech reproduction

Q_{wt} = energy involved in leech weight gain.

From the study of fish metabolic anomalies it was calculated that Q_p would equal 2240 cal/gm. of leech (wet weight). Where:

$$2240 \text{ cal/gm.} = Q_w + Q_{rl} + Q_{gl} + Q_e + \text{stored energy.} \quad (11)$$

The stored energy component must be included because of the blood held within the leech's gut.

DISCUSSION

A. Fish Metabolic Studies

Keys (1930) compared various methods of measuring the resting metabolism of *Fundulus parvipinnis* and concluded that the flowing water method was most accurate. Reproducibility of results was independent of light intensity and chamber size, but a minimum acclimation period of five hours was necessary before the start of each experiment. Using a continuous flow system, Spoor (1946) established the diurnal changes in respiratory rate of the goldfish, *Carassius auratus*. The shape of the derived curve agrees with the present work. Spoor observed an additional increase in respiratory rate following feeding. In the present work the sculpins showed a marked peak in respiration rate following morning feeding.

The area under the diurnal curve equals the total oxygen consumed per day (Fig. 8). These data indicate an average resting respiratory rate of 2 ml./gm./day, a value which is 45% of the calculated normal oxygen consumption. Walkey and Meakins (1970) estimated that the normal metabolic rate of stickleback, *Gasterosteus aculeatus* was about twice the average resting metabolism. They established this value by measuring resting and active metabolism and assuming that normal metabolism was the average of the two.

It is possible that the flowing water technique used in the present experiments precludes measurement of the lowest rate of oxygen consumption. In this case the values that were assigned to resting respiratory rate may be too high.

Normal metabolic rate was calculated by balancing the energy equation. The oxygen consumption rate was computed by calculating the calories per week due to respiration and dividing by a suitable oxy-caloric equivalent. This technique has also been used by Davis and Warren (1965), and involves subtracting the calorie values of waste and growth from the food calories. The oxy-caloric equivalent is based on food composition. The value used in this experiment was similar to the values assigned by Brett (1962) and Winberg (1960). Walkey and Meakins (1970) in their study of fish energetics fed the animals *Tubifex* sp. and used an oxy-caloric equivalent of 4.89 cal/ml., giving no reason for using this particular value. The oxy-caloric equivalent is a constant assuming that the composition of the diet and the metabolic processes are constant. In the present experiments, the fish were fed a constant diet. It was assumed that the relationship between calculated metabolic rate and actual oxygen consumption would remain constant throughout the experiments.

Plotting of calculated (normal) oxygen consumption against total weight leads to the equation:

$$y = 0.83 X^{.88} \text{ at } 10^{\circ}\text{C.} \quad (12)$$

Brett (1962) states that the value "a" in the equation $y = ax^b$ may vary from .2 to .5 at 20°C. This is due to inherent species, seasonal and hormonal differentiation. His review was based on measurements of resting metabolism. Winberg (1960) states that the value has been observed between 6.7 and 15.3. This is concluded from the observation of thirty different species of fish, and implies that the respiration rate of fish of the same weight but different species is not constant.

Beamish and Mookherjee (1964) discovered that the exponent (b) of the power equation $y = ax^b$ was greater with measurements of normal metabolism than with measurements of resting metabolism. Winberg (1960) was unable to conclude whether fish conform to the "surface law" (i.e. $B = 2/3$). He states that fish respiration studies are incomplete, and that it is impossible to reach any conclusion. The slope of the line varies from .6 to .8 depending on the species studied and the size range of experimental animals (Winberg, 1960; Beamish, 1964a).

The observed parameters in my experiments are within the ranges previously observed. The normal metabolism of sculpins does not appear to follow the surface law. It is possible that respiratory differences may exist between age classes, sex and physiological condition of the sculpins. All of the animals were within the same size group and physical environment, and were not sexually mature (Ennis, 1970). It was assumed that the normal respiration rate was constant between the two groups of experimental fish.

Seasonal variations in fish respiratory rate have been observed (Beamish, 1964b). Beamish kept the fish in a constant environment and proved these variations were inherent in the physiological and hormonal variations within the sexual cycle. The studies were conducted on mature fish, while the animals used in my experiments were not sexually mature. In the present work, the water temperature was kept constant, and there was no indication of seasonal variation in calculated respiratory rate during the experimental period from

September to January.

The values and observed relationships of metabolic energy consumption agree with those derived from values of resting oxygen consumption. Metabolic energy consumption can be calculated over long periods of time without disturbing the experimental animals. Values calculated from these data are indicative of true "normal" metabolism because they incorporate diurnal and behavioral differences in respiratory rate to measure the average metabolic rate in conditions approximating normal.

Winberg (1960) estimated that total feces production is normally 15% of the food dry weight. The values obtained in my experiments range from 3 to 3.5% and are much lower than normally recorded. The high protein and low ash content of squid mantle muscle would result in lower levels of feces production. Every precaution was taken to ensure that all feces were collected daily.

The high food protein level would also result in high levels of nitrogenous wastes, a factor which must be considered in the calculations of energy loss through waste production. Winberg (1960) describes a method of estimation of nitrogenous waste energy based on total nitrogen intake and the nitrogen utilized for growth. A value of 5% of the food energy was obtained assuming that the nitrogen used for growth was a fixed percentage. This is the same value used by Walkey and Meakins (1970). Gerking (1955a, 1955b) observed that protein absorption efficiency was constant, but that the amount of nitrogen used for growth varied from 5% to 33% depending on the age of the fish. His measurements were conducted on fish ranging in size

from 2 gm. to 32 gm. It was assumed that the ages of fish used in my experiments were the same and that the level of urinary nitrogen would be constant for all animals. The increase in size and age of individual fish during the experimental period was an average of 1.4 gm., and would only account for a theoretical change of 1% in protein absorption efficiency. This would cause a change of less than 1% urinary nitrogen.

The calorie and water content of *M. scorpius* agrees with the values previously recorded for fish (Davis and Warren, 1965; Cummins, 1967). The maximum deviation observed among replications of one fish sample was about $\pm 3\%$. Golley (1961) stated that he could attain a minimum deviation of $\pm 3\%$ due to the variation inherent within biological samples.

B. Leech Metabolic Studies

The respiratory rate of *Malmiana nuda* is higher than any observed by Mann (1956, 1962), who examined the oxygen consumption of five other species of leeches. *Piscicola geometra* shows the highest respiratory rate of those that Mann observed. He states that this may be due to three factors: the ecology of the animal, the high level of activity and the high level of respiratory activity. It is probable that the high respiratory rates of *P. geometra* and *M. nuda* are due to ecological and behavioral similarities. Both species are from oxygen rich environments and both species show high levels of activity during feeding and while searching for a host. This behavior is different from the other species examined, and can account for a higher normal metabolic rate (Mann, 1956).

It is possible that the observed respiratory rate is not a true measure of resting metabolism. In this case, it would not be valid to compare the present data with those of Mann (1956) who used a different technique. Wherever possible, all factors affecting the activity rate were minimized.

The slope of the line of respiration rate vs. weight indicates that respiratory rate is directly proportional to weight. Zeuthen (1953) observes that the slope of such lines may be a constant between 1 and 0. Exceptions have been observed where the slope is greater than 1 (Zeuthen, 1953; Brody, 1964). Nevertheless, these instances are only during brief phases of growth in the life of individual groups. The leeches sampled in these experiments are from one size group and represent a range of growth stages. The slope of the line observed for *Malmiana nuda* is 1.05 and closely agrees with the values recorded for *Erpobdella octoculata* (Mann, 1956). The other species of leeches examined have respiration curves with the slopes ranging from .69 to .82. In these species, the respiration rate seems to obey the "surface law" (Brody, 1945; Klieber, 1947) (i.e. $y = ax^{2/3}$). A slope of 1 indicates that oxygen is easily accessible to all cells, and that cells respire at a constant rate regardless of the animal's size. It is possible that in the other leech species the cellular metabolic rate is limited by the rate of oxygen diffusion, and therefore metabolic rate is proportional to surface area.

Mann (1956) observed no correlation between the slope of the respiration vs. weight curve and the taxonomy of the leech. However, there is a relationship between the three families and the respiratory

systems. Glossiphoniidae have no hemoglobin in the blood, and no accessory respiratory organs. Erpobdellidae have hemoglobin but no respiratory organs, and Ichthyobdellidae frequently have pulsatile vacuoles filled with a colourless coelomic fluid aiding respiration (Mann, 1962). The two Erpobdellid leeches studied have different responses. *E. testacea* has a slope of .81 when respiration is plotted against weight; *E. octoculata* has a slope of 1.06. The weight range of these two animals is different. *E. testacea* ranged from 6 - 37 mg., *E. octoculata* from 12 - 104 mg. If the respiration rate was limited by surface area, then the slope of the respiration curve would be .66. However, Erpobdellid leeches have hemoglobin in the blood. It is possible that the oxygen carrying capacity of *E. testacea* is not as well developed as that of *E. octoculata*, which, being a larger animal, would require a better developed system. Similarly, the fact that *Piscicola geometra* is smaller than *Malmiana nuda*, and has a respiratory line slope of .70 as opposed to 1.05 implies that the respiratory system of *M. nuda* (the larger animal) is more efficient. No accessory respiratory structures have been observed in *Malmiana nuda* (Richardson, 1970). It is possible that the oxygen carrying capacity of the coelomic fluid may be modified.

The respiratory rate varies with temperature according to Krogh's normal curve (Krogh, 1941). Lindeman (1935) investigated the respiratory rate of *Hirudo medicinalis*. Although he was primarily interested in the effects of oxygen concentration on respiratory rate, his data suggest that the respiratory rate plotted against temperature follows a curve similar to *Malmiana nuda*. Krogh (1941) states that

the curve obtained when plotting resting oxygen consumption against temperature is the same for all animals. This appears to be true for the species of leeches examined.

Energy consumption can be calculated from resting oxygen consumption providing that a suitable oxy-caloric equivalent is employed. Little work has been done on the digestion of rhyncobdellid leeches. Mann (1962) implies that purely sanguivorous leeches partially digest haemoglobin, surviving almost entirely on a diet of protein. The animals may also absorb fats and sugars present in the blood (Bradbury, 1956). The oxy-caloric equivalent is based on the assimilated diet. In this experiment, it was assumed that the diet was predominantly protein (90%) with the remaining diet an equal portion of fat and carbohydrate. Therefore, an oxy-caloric equivalent of 4.5 was used (Albanese, 1965). The energy consumption was then computed as 290 cal/gm./week. This figure may be inaccurate because of the assumption of the diet composition. Until more evidence is available on the digestion and food storage of leeches, it will be necessary to use this estimation.

Becker and Katz (1965) compared the wet weight of salmonid leeches, *Piscicola salmositica* before and after feeding. The starved leeches had been starved for 40 days, the repleted leeches had been allowed to feed for 24 hours. They observed that the average difference in weight was .75 gm. per 1 gm. of starved animal. This figure may be low. The starved leeches may have been digesting food reserves, and not blood stored in the gut. The fed animals may have digested some of the blood as they were feeding, and the blood fluids may have

been excreted as the leeches were feeding. In this case the difference in wet weight would not be due to the weight of whole blood, but to the weight of partially dehydrated blood. In contrast, my experiments estimated the amount of whole blood taken into the animal during feeding. These estimates were based on the total amount of undigested blood tissue, regardless of the water excreted by the leech during feeding. The leeches were sacrificed immediately after feeding and it was assumed that none of the hemoglobin would be digested during the feeding process. I observed that the blood meal size was an average of 2 ml. of whole blood per 1 gram of starved leech. The difference in wet weight between starved and fed leeches was about 2 grams per gram of starved animal. This indicates that some dehydration of blood meal may occur during feeding, as previously observed with the medicinal leech, *Hirudo medicinalis* (Mann, 1962). Since the leech weekly weight gain was negligible, it must be assumed that the imbibed blood is significantly dehydrated.

Energy input was estimated by measuring the average blood meal size. The values obtained measured the hemoglobin in the leech's gut. Accuracy of measuring the total blood volume depends upon the hemoglobin content of a standard volume of blood. Blood samples were taken from the tip of the pectoral fin and it was assumed that the hemoglobin content at this point was the same as that imbibed by the leeches. The data suggest that leeches consume 2 ml./gm./week of blood. This results in an additional energy input of 1150 cal/gm./week. It is improbable that leeches completely digest the blood. The energy value of the heme must be subtracted from the estimates of energy input, as it is probably excreted as additional waste.

There was no indication of the normal monthly rate of feeding. It was my observation that the leeches would feed whenever placed on or near a suitable host. In this respect, the weekly energy requirements would be dependent to a certain extent on the weekly rate of feeding. The survival time of starved leeches in my experiments appeared to be a great deal shorter than previously recorded. Becker and Katz (1965) observed that the salmonid leech could be starved for 40 to 50 days at 10°C. The medicinal leech can be starved for over 6 months (Mann, 1962). At 10°C., *Malmiana nuda* would live from 20 to 30 days following feeding. It is possible that the gut storage capacity or food reserves of these animals are not so great as other species.

C. Host-Parasite Relationships

The energy loss of the sculpin is not entirely due to the energy requirements of the leech. The excess energy loss could be explained in part by an increase in the host's metabolic rate. This is probably due to two factors: the increased physiological strain, and shock. In endoparasitic systems, leakage of metabolites and hormones into the host can result in an increase in metabolic rate (Von Brand, 1952). In the leech-sculpin system, it is unlikely that there is a direct host stimulation by leech waste products. However, the leech's saliva and anti-coagulant production may directly affect the host tissues. Becker and Katz (1965) observed some tissue damage and inflammation around the areas where leeches had previously fed. This reaction was only local and did not extend beyond the area of feeding. I observed no such reaction, although it is possible that

some local inflammation was present. The respiratory increase that I observed was about 5% of the host's metabolic rate. It is probable that this respiratory increase is due to the increased rate of blood and tissue formation and to stress caused by the mechanical irritation of the feeding leeches. These increases may not be observed with smaller burdens of the parasite under natural conditions.

Walkey and Meakins (1970) were unable to account for the observed host energy losses. This was due to the variation in control and parasitized fish's respiratory rate, and to the unknown energy demands of the parasite. They were able to conclude that the parasites utilize some of the host's assimilated energy, and that the assimilation conversion efficiency was greater for parasitized fish. In my experiments it was not possible to observe any difference in this conversion efficiency of parasitized fish. The amount of feces produced was very small. It is possible that any increase in conversion efficiency would not be discernable in feces production, but that there would be a decrease in nitrogenous wastes excreted. It is also possible that the efficiency of food absorption was at its maximum due to the low levels of food. Winberg (1960) observed that starved fish have a greater conversion efficiency than do animals that are regularly fed, and that the efficiency of conversion is inversely proportional to diet. The fish in my experiments were on a fixed ration. No measurements of conversion efficiency were conducted at different levels of nutrition. Therefore it is not possible to state which possibility is correct.

The energy removed from the host's system is proportional to the metabolic needs of the parasite:

$$Q_p = K \times Q_{cp} \quad (13)$$

K can never be less than 1. The value of K is proportional to the degree of adaption to the host's system. A well adapted system will cause little additional metabolic increase in the host. Harmful metabolites, tissue reactions or hormone leakage will cause increased energy loss and an increased value of K.

Parasites do not affect only the assimilated energy of the host, since increased appetite, behavioral and physiological changes are all associated with some parasite burdens. Thus, in order to calculate the effects of the parasite we must add another, more arbitrary factor (M). If we consider that any additional parasite effect is proportional to the metabolic rate of the parasite, then:

$$Q_p = K \times Q_{cp} + M \times Q_{cp} \quad (14)$$

$$Q_p = Q_{cp} (K + M) \quad (15)$$

M may be any value. If the parasite causes additional harm, M will be greater than 0. If the parasite benefits the host, M will be less than 0. Consequently, (K + M) may be > 0 or < 0 . A well adapted system is one in which $(K + M) < 0$. In this case, the benefits of the parasite offset the energy losses to the host, and the system will be "mutualistic" as opposed to "parasitic".

When calculating (K + M), we must consider the infested host in relation to the normal animal. In this case:

$$\frac{Q_I - Q_C}{Q_{cp}} = (K + M) \quad (17)$$

Where Q_I is a metabolic level of infected animal, Q_C is the metabolic level of the normal. The parameters measured are now energy flow, but any arithmetic parameter may be studied (i.e. behavioral actions, activity).

It is impossible to accurately evaluate M . We can calculate $(K + M)$ if we know the energy budget of host and parasite. In the present experiments, $K + M = 1.5$. Unfortunately, there are few data to compare with this value. Mueller (1965b) observed parasite-induced weight gains in guinea pigs as a result of increased appetite and absorption efficiency. $(K + M)$ is estimated to be negative. In appearance, this is a well adapted parasitic system. Unfortunately, the guinea pig is not the parasite's normal host. The "advantages" of the parasite are a result of a metabolic abnormality (Mueller, pers. comm.). Furthermore it is not possible to state that the increase in weight is beneficial to the host. Mueller (1966) observed no abnormal behavior or morphology due to the parasite's presence. In the absence of further evidence, it is possible to assume that the parasite in this particular instance is a benefit to the host.

It remains to be seen whether in specific instances $(K + M)$ varies with the level of parasitemia or the nutritional level of the host. It would be interesting to observe whether there are any consistencies between the value of $(K + M)$ and the type of relationship. Energetic investigations of the relationships between symbionts and/or mutualists should provide data which would indicate whether taxonomic or ecological relationships exist.

Rabaud (1928) states that the host's internal environment is so stable that it is difficult to conceive of speciation occurring. He concludes that only adverse changes will affect the parasite population, causing the new genotype to die immediately. Changes which improve the survival of the parasite will soon be absorbed by

the population. Single changes which vastly improve the parasite or population will be reflected in population changes. The most efficient parasite is one which least affects the host and ensures maximum survival. It may be justified to state that some mutualistic relationships are highly evolved parasitic relationships. The most primitive host-parasite systems are those in which energy is removed from the host resulting in increased host energy loss due to concurrent increased physiological imbalance. The observed weight loss in tick infested cattle is an example (O'Kelly and Seifert, 1970). A more advanced system entails a similar loss of energy to the host, with less physiological imbalance between host and parasite tissues. The parasite may partially compensate for host loss. Hormone leakage of *Spirometra mansonioides* results in increased assimilation and growth efficiency of infected rats and mice.

Eventually some systems could evolve in which both organisms benefit each other. As an example, gut symbionts are internal micro-competitors which are essential for the nutrition of the host. It is possible that some mutualistic systems are evolved from host-parasite systems.

In my experiments, it is possible to conclude that the leech is a micro-predator and not a parasite. This conclusion cannot be based solely on the pattern of energy transfer, but on the life history, and nature of the association. The leech is not strictly host specific, nor is it permanently attached to the sculpin for all or part of its lifetime. A major portion of the energy lost to the host is not the energy requirements of the leech but host respiratory increases due to

physiological strain. The system is not well adapted, but indicates that the leeches are directly associated with the sculpin on a random basis, feeding on any fish encountered. This mode of nutrition more closely resembles predatory systems than parasitic.

SUMMARY

The metabolic rate of the sculpin can be measured by difference if an accurate assessment can be made of weekly intake, egestion and stored energy. The calculated oxygen consumption rate is proportional to weight by the equation:

$$y = 0.81 \times .84 \quad \text{at } 10^{\circ}\text{C.}$$

Animals parasitized with a known biomass of starved leeches show a significant loss of energy. During the same period, control animals lost no energy. The energy loss of parasitized animals is proportional to the biomass of leeches and equal to 2240 cal/week/gram of parasite. There are no observed changes in energy absorption efficiency in the host animals.

Leech oxygen consumption is proportional to biomass (at 5°C.) by the relationship:

$$y = 0.156 \times 1.05$$

Leech oxygen consumption is also proportional to temperature. It is calculated that at 10°C. , the leeches would consume 63 ml. O_2 /week/gram. Using an oxy-caloric equivalent of 4.5, this would be equivalent to the consumption of 290 calories. At this rate of energy expenditure, it is calculated that an additional 75 calories/week would be waste material.

At the end of one week of continuous feeding, previously starved leeches contain about 2 ml. of blood/gram. The energy value of the blood is 570 cal./ml. This suggests that the leeches absorb an additional 1140 calories of blood per week (above from normal energy requirements). This equals an overall energy expenditure of 1500 cal/gram/week (1080 + 75 + 290).

It is suggested that the remaining energy loss is a result of increased host energy expenditure. It is possible that the relationship between energy loss from the host and estimated parasite energy intake is a measure of the degree of adaption of the system.

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APPENDIX I.

LEECH RESPIRATION
AND METABOLIC DATA.

TABLE 1.

TOTAL LEECH RESPIRATION AT DIFFERENT ANIMAL WEIGHTS.
RESPIRATION RATE VALUES ARE THE MEAN OF TWO MEASURE-
MENTS. EACH ANIMAL EXPERIMENTAL GROUP CONSISTED OF
5 ANIMALS PLACED IN 5 MG. WEIGHT CLASSES.

| Mean Weight (mg.) | Mean Respiration Rate (μ L/Hr) |
|----------------------|--|
| 35 | 7.5 |
| 17 | 2.8 |
| 10.5 | 2.0 |
| 8 | 1.4 |
| 39 | 6.7 |
| 24 | 4.1 |
| 35 | 6.5 |
| 30 | 5.6 |
| 55 | 10.8 |
| 50 | 9.6 |
| 14 | 2.5 |
| 23 | 3.8 |
| 45 | 8.8 |
| 10 | 1.7 |
| 19 | 3.5 |
| 43 | 7.6 |
| 28 | 5.4 |
| 20 | 3.9 |

TABLE 2.

LEECH RESPIRATION RATE AT DIFFERENT TEMPERATURES. VALUES ADJUSTED FOR AN ANIMAL WEIGHT OF 25 MG. THIS ADJUSTMENT WAS BASED ON THE ASSUMPTION THAT RESPIRATION RATE WAS IN DIRECT PROPORTION TO INDIVIDUAL WEIGHT. (TABLE 1. AND FIG. 5.).

| Temperature | 1 | 4 | 7 | 10 |
|------------------|-----|-----|-----|-----|
| Respiration Rate | 2.5 | 3.4 | 4.2 | 7.8 |
| (μ L/Hr) | 3.3 | 4.0 | 5.1 | 8.3 |
| | 2.7 | 3.0 | 4.9 | 7.8 |
| | 2.8 | 4.1 | 5.0 | 7.0 |
| | 2.6 | 3.9 | 5.0 | 7.9 |
| Mean | 2.8 | 3.7 | 4.2 | 7.8 |

TABLE 3.

CALORIE CONTENT OF LEECH.

| Dry/wet | Calorie Determinations (Cal/gr.) | | |
|-----------|----------------------------------|------|-----------|
| | 1 | 2 | Ind. mean |
| .210 | 6173 | 6206 | 6190 |
| .200 | | 6207 | 6207 |
| .198 | 5873 | 6100 | 5956 |
| .206 | 6107 | 6005 | 6061 |
| Mean .204 | | | 6067 |

APPENDIX II.

MEASUREMENT OF FISH DIURNAL
CHANGES IN RESPIRATION RATE.

APPENDIX III

DATA FROM FISH METABOLIC STUDIES.

TABLE 1.

LEECH BLOOD MEAL ANALYSIS. THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN THE VALUES OF ML. BLOOD/GM. (FULL) FOR THE STARVED GROUP AND THE RANDOM SAMPLE OF FEEDING LEECHES. (P=1%, T=2.94). *UNABLE TO CALCULATE VALUES.

| Random sample starved wt. * (gm.) | | Fed wt. (gm.) | ml. Blood | increase in weight * | ml. Blood/ gm. (fed wt.) |
|---|------------------|------------------|-------------|---------------------------|--------------------------------|
| | | .0136 | .0060 | | .44 |
| | | .0080 | .0051 | | .64 |
| | | .0037 | .0022 | | .60 |
| | | .0082 | .0059 | | .72 |
| | | .0131 | .0020 | | .15 |
| | | .0071 | .0049 | | .69 |
| | | .0038 | .0028 | | .74 |
| | | .0046 | .0030 | | .65 |
| | | .0182 | .0070 | | .38 |
| | | .0170 | .0047 | | .28 |
| | | .0040 | .0021 | | .53 |
| | | .0065 | .0042 | | .65 |
| | | .0043 | .0029 | | .67 |
| | | .0026 | .0018 | | .69 |
| | | .0030 | .0020 | | .66 |
| | | .0226 | .0098 | | .43 |
| | | | | | Mean: .56 |
| Starved Group | | | | | |
| Starved wt. | Fed wt. (gm.) | ml. Blood | increase in | ml. Blood/ gm(starved) | ml. Blood/ gm. (full) |
| .0015 | .0043 | .0032 | .0028 | 2.13 | .74 |
| .0029 | .0076 | .0053 | .0047 | 1.83 | .70 |
| .0008 | .0020 | .0015 | .0012 | 1.88 | .75 |
| .0014 | .0038 | .0027 | .0024 | 1.93 | .71 |
| .0018 | .0054 | .0043 | .0036 | 2.39 | .80 |
| .0026 | .0072 | .0047 | .0046 | 1.81 | .65 |
| .0034 | .0087 | .0055 | .0053 | 1.62 | .63 |
| .0028 | .0082 | .0055 | .0054 | 1.96 | .67 |
| .0012 | .0033 | .0020 | .0021 | 1.67 | .61 |
| .0009 | .0025 | .0017 | .0016 | 1.89 | .68 |
| | | | | Mean: 1.91 | Mean: .69 |

TABLE 2.

CALORIC ANALYSIS OF SCULPIN BLOOD SAMPLES.

| Volume Blood (ml.) | Dried wt. (gm.) | Dry/wet | Calorie content (cal/g.) |
|-----------------------|--------------------|-----------|--------------------------------|
| .40 | .042 | .11 | 5650 |
| .30 | .026 | .09 | 5780 |
| .20 | .020 | .10 | 5790 |
| .40 | .048 | .12 | 5680 |
| .30 | .020 | .07 | 5750 |
| | | Mean: .10 | Mean: 5730 |

TABLE 3.

DRIED SQUID ANALYSIS. GRAND MEAN PROTEIN
 % (DRY) = 78.0, GRAND MEAN LIPID % (DRY) = 9.5.
 ASH CONTENT IS LESS THAN 1% (FROM CALORIMETRY).
 THE CARBOHYDRATE CONTENT WOULD EQUAL 12.5 %.

| | 1 | 2 | 3 | 4 | 5 | GRAND MEAN % (DRY) |
|---------|------|------|------|------|------|--------------------------|
| Protein | 76.6 | 73.2 | 75.9 | 78.9 | 78.0 | 78.0 |
| % Dry | 75.8 | 80.1 | 79.6 | 80.4 | 72.4 | |
| | 81.5 | 82.9 | 80.1 | 76.6 | 79.0 | |
| | | 76.8 | 70.8 | 80.3 | 82.6 | |
| Mean | 78.0 | 78.3 | 76.6 | 79.1 | 78.0 | |
| Lipid | 8.4 | 8.6 | 11.3 | 10.4 | 10.0 | 9.5 |
| % Dry | 7.8 | 8.1 | 9.9 | 11.6 | 9.0 | |
| Mean | 8.1 | 8.4 | 10.6 | 11.0 | 9.5 | |

TABLE 4.

DRIED SQUID CALORIMETRY (CAL/GM.)

| Squid | Calorie Determinations (cal/gm.) | | | | | Mean (cal/gm.) |
|-------|----------------------------------|------|------|------|------|-------------------|
| | 1 | 2 | 3 | 4 | 5 | |
| 1 | 5720 | 5990 | 5720 | 5770 | 5730 | 5786 |
| 2 | 5870 | 5728 | 6119 | 5561 | 5720 | 5800 |
| 3 | 5970 | 5826 | 5752 | 5757 | 5690 | 5799 |
| 4 | 5805 | 5695 | 5804 | 5809 | 5745 | 5772 |
| 5 | 5346 | 5644 | 5988 | 5444 | 5672 | 5619 |

Between treatment M.S. = 29. d. of F. = 4

within treatment M.S. = 26. d. of F. = 20

$F_{(4,20)} = 1.12$ N.S.D. $P = 1\%$.

TABLE 5.

TOTAL WEEKLY FECES PRODUCTION AND % OF DRY FOOD. NO SIGNIFICANT DIFFERENCE ($P = 1\%$) IN % FECES PRODUCTION BETWEEN THE CONTROL AND TEST GROUP ($T = 1.0Z.$) NO SIGNIFICANT DIFFERENCE ($P = 1\%$) IN % FECES PRODUCTION BETWEEN THE CONTROL PERIOD AND THE TEST PERIOD OF THE TEST GROUP ($t = 1.51$).

| | Control Group | | | Test Group | | |
|------|------------------|-----------------|-----|------------------|-----------------|-----|
| Week | Dry wt. feces | Dry wt. food | % | Dry wt. feces | Dry wt. food | % |
| 1 | .0272 | .6151 | 4.4 | .0106 | .5493 | 1.9 |
| 2 | .0234 | .6763 | 3.5 | .0262 | .6420 | 4.1 |
| 3 | .0334 | .7028 | 4.8 | .0324 | .6495 | 5.0 |
| 4 | .0189 | .6672 | 2.8 | .0387 | .6142 | 6.3 |
| 5 | .0463 | .7865 | 5.9 | .0464 | .7140 | 6.5 |
| 6 * | .0163 | .7659 | 2.1 | .0193 | .6507 | 3.0 |
| 7 * | .0267 | .7635 | 3.4 | .0171 | .6864 | 2.6 |
| 8 * | .0201 | .7259 | 2.8 | .0168 | .6414 | 2.6 |
| 9 * | .0113 | .7177 | 1.6 | .0212 | .6099 | 3.5 |
| 10* | .0242 | .8309 | 2.9 | .0165 | .6558 | 3.6 |
| 11* | .0198 | .8291 | 2.4 | .0147 | .6604 | 2.5 |
| 12* | .0233 | .8958 | 1.2 | .0246 | .6909 | 2.2 |
| 13* | .0106 | .8142 | 2.6 | .0167 | .7481 | 3.6 |
| 14 | .0200 | .8660 | 1.8 | .0180 | .6855 | 2.6 |
| 15 | .0186 | .8092 | 2.3 | .0152 | .7063 | 2.2 |
| Mean | | | 3.0 | | | 3.5 |

* Time when Test Group parasitized.

TABLE 6.

CALORIE CONTENT OF FECES. GRAND MEAN IS 1990 CAL./GM.
NO SIGNIFICANT DIFFERENCE ($P = 1\%$) IN CALORIE CONTENT
BETWEEN FECES OF CONTROL AND THE TEST GROUP.

| Week | Caloric content (cal/gm.(dry)) | |
|---------|--------------------------------|------------|
| | Control Group | Test Group |
| 1 - 4 | 2125 | 1875 |
| 5 - 8 | 1815 | 2030 |
| 9 - 12 | 1984 | 1995 |
| 13 - 15 | 2140 | 1955 |
| Mean | 2016 | 1964 |

TABLE 7.
ENERGY BALANCE DATA FOR CONTROL ANIMALS.

| Date | Week No. | Fish wt. at end of wk. | Growth | | Food | | Est. wastes (cal.) | Calc. resp. rate (Ml O ₂ /gm./day) | Exp. resp. rate (Ml O ₂ /gm./day) | Exp. growth gm./wk. | Temp. | Diff. (exp. growth observed) |
|---------------------|----------|------------------------|--------|------|--------|------|--------------------|---|--|---------------------|-------|------------------------------|
| | | | gm. | cal. | gm. | cal. | | | | | | |
| Aug. 23 - Aug. 30 | 0 | 19.05 | | | | | | | | | 12 | |
| Aug. 31 - Sept. 6 | 1 | 19.32 | .27 | 320 | 2.3843 | 3559 | 179 | 4.91 | 5.007 | .17 | 12 | -119 |
| Sept. 7 - Sept. 13 | 2 | 19.80 | .48 | 569 | 2.6212 | 3913 | 184 | 4.97 | 4.993 | .42 | 12 | - 71 |
| Sept. 14 - Sept. 20 | 3 | 20.45 | .65 | 770 | 2.7242 | 4067 | 209 | 4.72 | 4.699 | .66 | 10 | + 12 |
| Sept. 21 - Sept. 27 | 4 | 20.90 | .45 | 533 | 2.5862 | 3861 | 173 | 4.69 | 4.695 | .45 | 10 | 0 |
| Sept. 28 - Oct. 4 | 5 | 21.80 | .90 | 1067 | 2.6217 | 4551 | 251 | 4.66 | 4.692 | .88 | 10 | - 24 |
| Oct. 5 - Oct. 11 | 6 | 22.75 | .95 | 1126 | 2.9802 | 4443 | 188 | 4.33 | 4.625 | .77 | 10 | -214 |
| Oct. 12 - Oct. 18 | 7 | 23.35 | .60 | 711 | 2.9710 | 4430 | 208 | 4.68 | 4.620 | .64 | 10 | + 47 |
| Oct. 19 - Oct. 25 | 8 | 23.75 | .40 | 474 | 3.0501 | 4209 | 187 | 4.64 | 4.620 | .41 | 10 | + 12 |
| Oct. 26 - Nov. 1 | 9 | 24.15 | .40 | 474 | 3.0155 | 4161 | 168 | 4.52 | 4.593 | .35 | 10 | - 59 |
| Nov. 2 - Nov. 8 | 10 | 25.05 | .90 | 1067 | 3.4912 | 4818 | 217 | 4.42 | | .90 | 9 | 0 |
| Nov. 9 - Nov. 15 | 11 | 25.80 | .75 | 889 | 3.4838 | 4808 | 188 | 4.70 | 4.843 | .52 | 10 | -273 |
| Nov. 16 - Nov. 22 | 12 | 26.65 | .85 | 1007 | 3.7480 | 5172 | 200 | 4.65 | 4.567 | .91 | 10 | + 71 |
| Nov. 23 - Nov. 29 | 13 | 27.05 | .40 | 474 | 3.4066 | 4581 | 160 | 4.52 | 4.544 | .38 | 10 | - 24 |
| Nov. 30 - Dec. 6 | 14 | 27.60 | .55 | 652 | 3.6233 | 4873 | 172 | 4.55 | 4.537 | .56 | 10 | + 12 |
| Dec. 7 - Dec. 13 | 15 | 27.90 | .30 | 356 | 3.3857 | 4553 | 140 | 4.49 | 4.505 | .29 | 10 | - 12 |

TABLE 8.
ENERGY BALANCE OF EXPERIMENTAL ANIMALS.

| Date | Week No. | Fish wt. at end of wk. | Growth | | Food | | Est. wastes (cal.) | Calc. resp. rate (Ml O ₂ /gm./day) | Exp. resp. rate (Ml O ₂ /gm./day) | Exp. growth gm./wk. | Temp. | Diff. (exp. growth observed) |
|---------------------|----------|------------------------|--------|------|--------|------|--------------------|---|--|---------------------|-------|------------------------------|
| | | | gm. | cal. | gm. | cal. | | | | | | |
| Aug. 23 - Aug. 30 | 0 | 16.10 | | | | | | | | | | |
| Aug. 31 - Sept. 6 | 1 | 16.40 | .30 | 356 | 2.1289 | 3178 | 132 | 5.10 | 5.286 | .22 | 12 | - 95 |
| Sept. 7 - Sept. 13 | 2 | 16.85 | .45 | 533 | 2.4884 | 3715 | 182 | 5.55 | 5.265 | .58 | 12 | +154 |
| Sept. 14 - Sept. 20 | 3 | 17.50 | .65 | 770 | 2.5175 | 3758 | 196 | 5.00 | 4.802 | .74 | 10 | +107 |
| Sept. 21 - Sept. 27 | 4 | 18.10 | .60 | 711 | 2.3808 | 3554 | 202 | 4.57 | 4.778 | .50 | 10 | -119 |
| Sept. 28 - Oct. 4 | 5* | 18.90 | .80 | 948 | 2.3801 | 4132 | 237 | 4.90 | 4.757 | .87 | 10 | + 83 |
| Oct. 5 - Oct. 11 | 6* | 19.10 | .20 | 237 | 2.5318 | 3775 | 171 | | 4.739 | .57 | 10 | +439 |
| Oct. 12 - Oct. 18 | 7* | 19.50 | .40 | 474 | 2.6710 | 3982 | 173 | | 4.731 | .71 | 10 | +368 |
| Oct. 19 - Oct. 25 | 8* | 19.25 | -.25 | -296 | 2.6950 | 3719 | 164 | | 4.727 | .49 | 10 | +878 |
| Oct. 26 - Nov. 1 | 9* | 19.40 | .15 | 178 | 2.5628 | 3537 | 166 | | 4.727 | .34 | 10 | +225 |
| Nov. 2 - Nov. 8 | 10* | 19.60 | .20 | 237 | 2.7556 | 3803 | 166 | | 4.469 | .68 | 9 | +569 |
| Nov. 9 - Nov. 15 | 11* | 19.50 | -.10 | -119 | 2.7746 | 3829 | 163 | | 4.724 | .56 | 10 | +783 |
| Nov. 16 - Nov. 22 | 12 | 19.70 | .20 | 237 | 2.8907 | 3989 | 189 | | 4.719 | .67 | 10 | +558 |
| Nov. 23 - Nov. 29 | 13 | 20.00 | .30 | 356 | 3.1303 | 4210 | 190 | | 4.713 | .82 | 10 | +617 |
| Nov. 30 - Dec. 6 | 14 | 20.50 | .50 | 593 | 2.8681 | 3857 | 200 | 4.66 | 4.701 | .47 | 10 | - 36 |
| Dec. 7 - Dec. 13 | 15 | 21.05 | .55 | 652 | 2.9552 | 3974 | 205 | 4.62 | 4.695 | .50 | 10 | - 59 |

* Period when parasitized (see Table 11).

TABLE 9.

SQUID DRY/WET RATIOS FOR FOOD CALCULATIONS. VALUES NOT INDICATED
WHEN DRY/WET RATIO IS THE SAME AS THE PREVIOUS MEASUREMENT.

| Squid No. | Date | Week No. | Dry/wet |
|-----------|--------------------|----------|---------|
| 1 | Aug. 30 - Sept. 27 | 1 - 4 | .258 |
| 1 | Sept. 27 - Oct. 4 | 5 | .300 |
| 2 | Oct. 4 - Oct. 18 | 6 - 7 | .257 |
| 3 | Oct. 18 - Nov. 22 | 8 - 12 | .238 |
| 4 | Nov. 22 - Dec. 13 | 13 - 15 | .233 |

TABLE 10.

T - TEST VALUES FOR GROWTH EXPERIMENTS TO TEST
THE HYPOTHESIS $Q_F - (Q_g + Q_r + Q_w) = 0$.

| | N | \bar{X} | S | T |
|---------------|----|-----------|-------|--------|
| Control | 15 | -42.8 | 91.1 | 1.754 |
| Test Group | 15 | 298.1 | 317.8 | 3.507* |
| Test Group | | | | |
| Control Phase | 7 | 5.0 | 100. | .121 |
| Parasitized | 8 | 554.6 | 199. | 7.394* |

* Significant difference at the 1% level of
significance.

TABLE 11.

FISH ENERGY ANOMALIES, CORRELATED WITH LEECH BURDEN.
 AVERAGE (NON-REPRODUCTIVE) ENERGY DEMAND IS 2238
 CAL./GM. LEECH/WEEK. AVERAGE ENERGY COST PER LEECH
 EGG COCOON IS 82 CAL. THIS WAS CALCULATED BY USING
 THE FORMULA $\text{ENERGY LOSS} = \frac{(\text{MEAN LEECH WT.} \times 2238)}{\text{No. OF EGG COCOONS.}}$

| Exp. week | Energy loss (cal) | Leech Biomass | | Energy loss * (cal/gm.) | No. eggs. | Cal/ cocoon |
|--------------|-------------------------|---------------|------|-------------------------------|--------------|----------------|
| | | Beg. | End | | | |
| 6 | 439 | .200 | .202 | 2173 | | |
| 7 | 368 | .069 | .069 | | 3 | 71 |
| 8 | 878 | .095 | .095 | | 6 | 111 |
| 9 | 225 | .092 | .102 | 2320 | | |
| 10 | 569 | .108 | .108 | | 5 | 48 |
| 11 | 783 | .087 | .100 | | 6 | 96 |
| 12 | 558 | .200 | .240 | 2309 | | |
| 13 | 617 | .280 | .295 | 2150 | | |

* Calculated only for periods when no cocoons were produced.

TABLE 12.

CALORIE CONTENT OF SCULPINS. THERE WAS A SIGNIFICANT DIFFERENCE AMONG ALL MEANS ($F = 18.3$), BUT NO DIFFERENCE AMONG VALUES FROM WEIGHT BETWEEN 1.0 AND 6.5 GM. ($F = 2.9$). $P = 5\%$.

| Wet wt. (gm.) | Dry wt. (gm.) | Dry/wet | Caloric Determinations (cal/gm.) | | | | | |
|------------------|------------------|---------|----------------------------------|------|------|------|------|------|
| | | | 1 | 2 | 3 | 4 | 5 | Mean |
| 16.0132 * | 3.4409 | .218 | 5450 | 5450 | 5250 | 5200 | 5400 | 5350 |
| 12.5642 * | 2.9275 | .233 | 5643 | 5447 | 5433 | 5099 | 5464 | 5417 |
| 14.7802 * | 3.2180 | .218 | 5352 | 5243 | 5515 | 5398 | 5542 | 5410 |
| 9.2769 * | 2.0847 | .225 | 5318 | 5505 | 5622 | 5382 | 5685 | 5502 |
| 4.3426 | 1.0335 | .238 | 5250 | 5100 | 5100 | 4950 | 4950 | 5070 |
| 1.4171 | .3342 | .236 | 4900 | 4930 | 4950 | 4930 | | 4928 |
| 5.0024 | 1.1494 | .238 | 5033 | 4901 | 4887 | 5000 | 4986 | 4961 |
| 3.6630 | .8205 | .239 | 4800 | 4978 | 4877 | 4788 | 5125 | 4914 |
| 6.0428 | 1.4503 | .240 | 5092 | 5066 | 5156 | 5010 | 5104 | 5086 |
| 4.9902 | 1.1727 | .235 | 5088 | 4909 | 4954 | 5012 | 5097 | 5012 |
| | Mean:- | .237 | | | | | | 4995 |

* Caloric content and dry/wet ratio not counted in calculation of means.

APPENDIX IV.

CALCULATION OF OXY-CALORIC EQUIVALENTS.

TABLE 1.

SUMMARY OF OXYGEN CONSUMED AND ENERGY RELEASED FROM CATABOLISM
OF VARIOUS FOODS. ¹ FROM KLEIBER, 1961. ² FROM ALBANESE.

| Material (1 gm.) | Required O ₂ | Release Energy |
|------------------------------------|-------------------------|----------------|
| Meat protein ¹ | 1.0L | 4.5 Kcal |
| Glucose ¹ | .7L | 3.5 Kcal |
| Glycerol Tripalmitate ¹ | 2.0L | 9.4 Kcal |
| Protein ² | 1.0L | 4.1 Kcal |
| Carbohydrate (starch) ² | .8L | 4.1 Kcal |
| Animal fat ² | 1.4L | 9.3 Kcal |

1 gram of squid:

| contains | which requires | and releases |
|-----------------------------|------------------------------------|--------------|
| .78 g, protein | .78 L O ₂ | 3.4 Kcal |
| .095 g. carbohydrate | .08 L O ₂ | .4 Kcal |
| .125 g. fat | .21 L O ₂ | 1.2 Kcal |
| Sum 1 g. (dry wt.) requires | 1.07 L O ₂ and releases | 5.0 Kcal. |

Therefore 1 L O₂ releases 4.67 Kcal of energy.

Similarly, 1 gram (dry wt.) of blood would

| contain | which requires | and releases |
|---------------------|------------------------------------|--------------|
| .90 g. protein | .90 L O ₂ | 3.9 Kcal |
| .05 g. fat | .04 L O ₂ | .2 Kcal |
| .05 g. carbohydrate | .09 L O ₂ | .5 Kcal |
| Sum 1 g. requires | 1.03 L O ₂ and releases | 4.6 Kcal. |

Therefore 1 L O₂ releases 4.47 Kcal of energy.

