Final Report

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Food Requirements for Artificial Conditioning of the Bay Scallop *Argopecten irradians* Lamarck to Maturity

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Abstract

A system was designed and tested to determine the food requirements for conditioning bay scallops to reproductive maturation under controlled conditions.

The algae Tetraselmis suecica was cultured in large volumes and delivered to the scallops on a continuous basis at densities optimal for highest feeding rates throughout the experimental period of 100 days.

Scallops feeding on Tetraselmis showed a loss of weight and sporadic development of gametes under the experimental conditions.

Food supplies, feeding rates, assimilation efficiencies and maintenance energy expenditures indicate that Tetraselmis is consumed and partially assimilated, but it is nutritionally inadequate for the scallops to acquire and store the excess energy needed for reproductive development. The low assimilation of food by scallops suggests poor digestability and/or dietary inadequacy of Tetraselmis.

With a few modifications, the system is suitable for conducting quantitative, long-term studies on scallop food requirements. It can also be used to assess the nutritional adequacy of various diets for sustaining the long-term processes of growth and reproduction of scallops and other bivalves.

Further studies with diets of different quality are necessary to determine an adequate food source for this species.

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I. Introduction

In the natural environment, the bay scallop Argopecten irradians reaches maturity and spawns once a year between mid-June and mid-August. Thus larval culture for seed scallop production with gametes procured from naturally maturing animals is only possible for a short period in the year. To produce seed scallops over a longer period for aquaculture purposes, the induction of gamete production by scallops out of season under artificial conditions and/or the advancement of gamete maturation is an attractive alternative (Loosanoff and Davis, 1963). There have been continuing efforts to artificially condition scallops to maturity out of season by exposing animals with developing gametes to elevated temperatures (Turner and Hanks, 1960; Sastry, 1963; 1966; Castagna and Duggan, 1971). These methods have not always been successful since all individuals may not respond similarly to the same treatment conditions or because the gametes produced are reduced in quantity and/or quality and may result in nonviable larvae (Turner and Hanks, 1976; Bayne, 1976; Sastry, 1968; 1979).

The production of gametes is a complex process involving a sequence of events, namely, a multiplication of gonial cells by juvenile or reproductively neutral individuals, activation of gametogenesis, and growth and differentiation of gametes to maturity. Activation of gametogenesis and the subsequent growth and differentiation of gametes is dependent upon the ability of an individual to divert ingested nutrients to gamete production in the face of competing demands by the interrelated processes of maintenance and somatic growth. The ability of an animal to divert nutrients to gamete production is also dependent

upon the age of an individual and is extremely sensitive to the interactive effects of temperature and food availability (both quality and quantity). Thus the maintenance of scallops under favorable temperature conditions and the provision of an adequate amount of suitable quality food to provide an energy excess is a necessary prerequisite for successful production of gametes under artificial conditions. It is also known that scallops are differentially sensitive to temperature and food conditions in the environment in different phases of the sequence of gamete development (e.g. multiplicative phase of primary germ cells and gonial cells, oocytes). These sequential events may be disrupted when the required environmental conditions are not met either by an increase or decrease of temperature or due to inadequacy of available food. Therefore, a definition of the food requirements (quality and quantity) of scallops at temperatures favorable for gamete production is necessary for successful conditioning on a reliable basis for aquaculture.

The objectives of this investigation were to determine the food concentrations for highest feeding rates and the amount of food consumed by scallops during the period of gonad development. A further objective has been to determine the viability of larvae produced by the artificially conditioned scallops.

In addition to monitoring the amount of food supplied to and consumed by scallops, more detailed observations were made throughout this experiment in order to determine how the animals respond to high food concentrations and how they partition their food into maintenance, somatic growth and gamete production.

II. Materials and Methods

This experiment was designed to provide known amounts of food to scallops at different temperatures on a long term basis to determine the food and temperture requirements for reproductive maturation of young scallops out of season. First year scallops were collected from Charlestown pond, R.I. in December, 1980 and January, 1981 (ambient temperture 0-1 C) when they were still small (total weight 13-23 g, shell depth 4.4-5.3 cm) and well before gamete development normally takes place. The system used for conditioning these animals permitted simultaneous conditioning of animals at 2 food levels and 2 tempertures, using a single food and seawater source. Figure 1 is a schematic diagram of the system used to culture and deliver food to scallops under four different experimental conditions. Details of its operation are provided in the following sections.

Algal Culture

The algal culture system was designed to continuously produce large volumes of monospecific food on a long term basis and conveniently deliver this food to the animals. Tetraselmis suecica (now identified as Platymonas suecica) was chosen as the food source because it can be cultured in large quantities and has been reported to be an "outstanding" food source for clam and oyster juveniles (Walne, 1970) and is well assimilated by mussels (Widdows and Bayne, 1971; Thompson and Bayne, 1972; 1974). T. suecica was obtained in culture from the Narragansett Laboratory of EPA and has been maintained in this

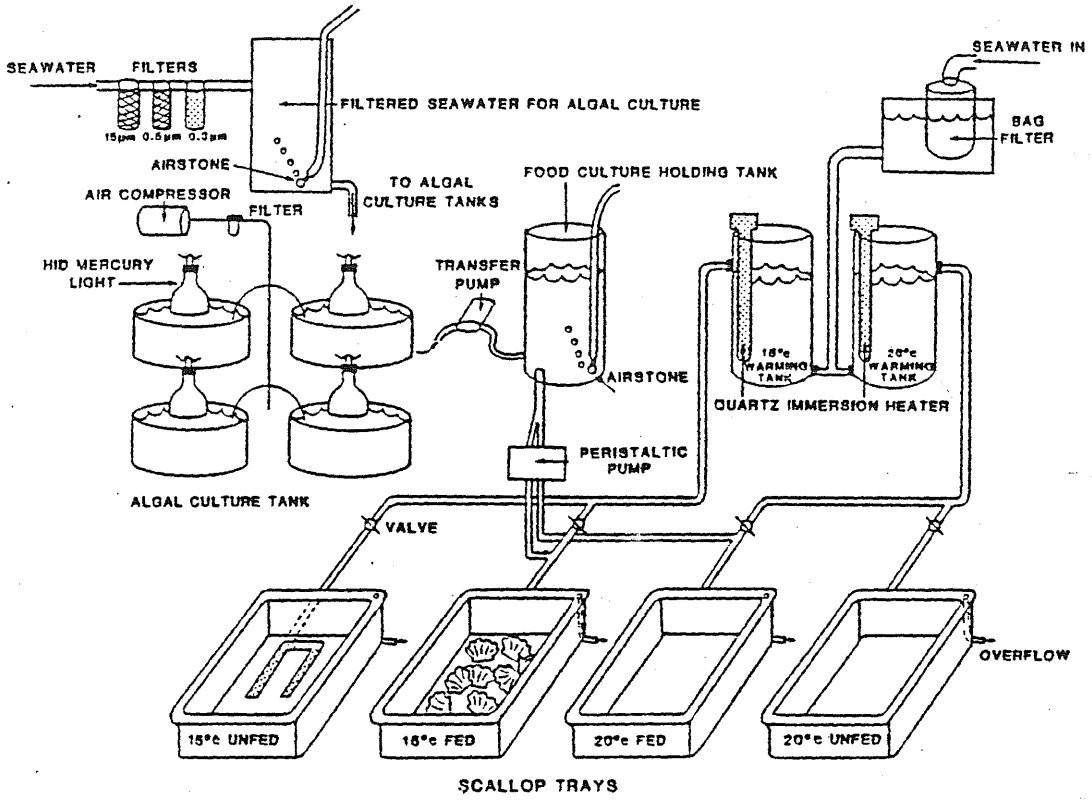

Figure 1. Schematic diagram of the large-scale algal culture system for production and continuous, quantitative delivery of the food to bay scallops to accelerate reproductive maturation under controlled conditions.

laboratory for several years in half-strength f medium (Guillard and Ryther, 1962). Quantities sufficient for mass culture are prepared by serial transfer and growth into 100 ml, 1.5 l and 40 l quantities. Food cultures were grown in 200 l quantities in 4 fiberglass tanks (92 cm diameter, 36 cm deep) on a semi-continuous basis. Water for the culture medium was prepared by filtration through a carbon-filled 10 um cartridge filter, a 0.5 um cartridge filter and a 0.3 um microfiber filter. Occassional contamination of cultures by protozoa suggests that some organisms were bypassing this system so the filtered water was further sterilized with the addition of 50 ml "Clorox"/200 l of seawater and mixed with an airstone overnight. Prior to use it was dechlorinated by the addition of 50 ml 0.3 M sodium thiosulfate and mixed for an hour. This procedure was used by Curtis (1979) to successfully culture oyster spat, animals which are probably more sensitive than the much larger young scallops used here. Each 200 l of water was fertilized by the addition of 15 g $NaNO_3$ (laboratory grade), 1 g $NaH_2PO_4 \cdot H_2O$ and 0.25 g ferric sequestrene. This is equivalent to half-strength f medium for nitrate and phosphate and 1/8 strength for ferric sequestrene. It is assumed some trace metals are introduced with the laboratory grade $NaNO_3$. Vitamins were not added to the 200 l cultures. This medium is the result of culture work done by Richard Steele at EPA, Narragansett and shown to result in excellent growth of Tetraselmis. Culture tanks were covered with 1/8 inch plexiglass lids and rapidly mixed with sterile-filtered air from a 0.75 HP air compressor (approximately 4 cubic feet per minute) divided among the four tanks and delivered through perforated silicone tubing. Lighting was continuous from a 175 watt HID mercury lamp over each tank about 0.5 m from the surface.

Culture temperature was maintained at about 20 C under these conditions. In each tank, 180 liters of water with nutrients was innoculated with 20 l of Tetraselmis culture and allowed to grow to a density of about 500 cells/ul, typically taking about 5 days, after which about 50 l/day were removed and replaced with fresh water and nutrients. The food culture was pumped over to a holding tank from which animals were fed under the control of a Harvard peristaltic pump. Food density, organic and inorganic content, and actual volume delivered to experimental animals was monitored on a daily basis to get an accurate assesssment of the amount of food available to and consumed by the animals.

Scallop Growth

The flow-through seawater system was designed and constructed to maintain at least 120 scallops on a long term basis with continuous food supply and accurate temperature control. The scallops were held on a perforated shelf in flow-through trays 59 cm square and 12 cm deep (41.8 liter volume). Four trays were established, each initially with 30 or 31 scallops. Water temperature in the trays was raised from the collection temperature (0-1 C) to experimental temperatures (15 and 20 C) at about 1 C per day, and the animals were held at experimental temperatures for a week prior to initial measurements. At each temperature there was one fed tray and one unfed tray. Water to the trays came by way of a 10 um bag filter eliminating most natural food, so that the experimental diet could be well characterized. The seawater was delivered via a small holding tank into larger warming tanks containing quartz immersion heaters, and was gravity fed through flow

meters to each tray. Water entered the bottom of the tray, with or without food, through a perforated pipe distributor and overflowed through a ring of drain holes at the top. Four airstones per tray were used to aid in suspension of the food in the trays. Flow rate was maintained at about 150 ml/min and was monitored daily on calibrated flow meters. Daily measurements (dry weight and ash-free dry weight) were made of the particulate content of the water from the warming tanks (input) as well as that of the water leaving each of the trays. Trays were cleaned weekly and settled material measured, thus allowing a measure of the food presumably used by the scallops.

Measurement of Feeding Rates

Preceding the long-term growth measurements a series of short-term feeding measurements were made to determine optimal food levels for delivery into the scallop holding system. Animals were held overnight in the experimental chamber at 15 C or 20 C as appropriate and fed at up to about 1 mg/scallop/hr Tetraselmis, less when experimental feeding levels were expected to be lower. Each scallop was then placed in a 4 l aquarium of filtered sea water and sufficient Tetraselmis culture added to bring its concentration to the determined level. A sample was taken for counting and fluoresence was measured with a Turner model 111-003 fluorometer with a flow-through door. At approximately 20 min intervals the water was resampled with the fluorometer and brought back to its original water and food culture levels. After 2 hours acclimation, measurements were recorded of initial and final times and fluorometer readings for each 20 minute period for about 8 periods, sometimes less

if problems were encountered. Food levels of approximately 1000, 2500, 7500, 10,000, and 15,000 cells/ml were used, and 6 to 15 animals were run at each level at 15 C and 20 C, though as few as 3 animals were used for two of the final data points due to procedural difficulties. Final readings were corrected for changes in a control tank containing an empty scallop shell. Feeding rate (l/hr) was calculated as;

$$F = \ln(Co/Ct) \times V/t$$

where Co is the initial fluorometer reading, Ct is the final fluorometer reading, V is the volume of the aquarium and t is the time over which the measurement was taken. Each scallop was used for two days measurements following which they were weighed and measured.

Utilization of Energy by Scallops

Under the four experimental conditions studied here, several different physiological processes can affect the growth rates of scallops. These include respiration and excretion which can account for much of the food assimilated by the animals. To get a better understanding of how these processes interact with food consumption to affect growth rates, they were measured at intervals throughout the experiment. Five scallops per tray were taken at about 30 day intervals and used for the physiological and growth measurements. Respiration and excretion were measured using 0.5 l plastic flow-through chambers. Source water and effluent from each chamber were sampled periodically and measured for dissolved O_2 by Winkler titration and ammonia by the

Solorzano technique (1969). It is also important to understand not only how much the scallops grow, but in what tissues that growth occurs under the experimental conditions provided here. Conceivably, the food supplied could be used for somatic growth rather than reproductive development. The animals used for physiological measurements were therefore weighed, dissected and the individual organs weighed, freeze-dried and reweighed to assess what proportion of food was being used for gonad development. Egestion rates can provide an indication of how much material animals were filtering out of the water and how much organic material they were assimilating from the food. To monitor the progress and experimental effect on this process, relative to growth rates, egestion was monitored weekly in the feeding trays. Five animals in each tray were placed in perforated plastic boxes and held for about one hour. The resulting egesta (feces plus pseudofeces) was collected quantitatively and filtered, rinsed with 0.9% ammonium formate, dried, weighed, ashed and reweighed.

Evaluation of Reproductive Condition

Evaluation of the progress and rate of reproductive development of the animals under four different conditions was done by periodically removing five animals from each condition and examining the gonads. At approximately 30 day intervals, portions of the freshly dissected ovary and testis from each of 20 animals were examined with light microscopy for the size, stage and abundance of developing gametes.

III. Results

Food Production

The system described here was capable of producing large quantities of algal culture on a continuous basis and delivering this food to scallops in known quantities. This is essential for long term growth experiments in bivalves. With four 200 liter culture tanks, we could produce about 10 grams dry weight of Tetraselmis per day (at about 500 cells/ul) allowing for one tank to be in the process of growing out or otherwise non-productive. On a sustained basis, 50-200 l of seawater were filtered, sterilized and warmed to room temperature each day, to renew the algal culture used for food.

Food Supply and Feeding Rates

Table I shows the amount of food delivered to each scallop over the course of this experiment. The total amount of food supplied was 9-10 grams dry weight per animal during the 100 days of the experiment, more than adequate even if consumption and assimilation rates are quite low. The rate of food delivery and turnover times are shown in Table II. An average turnover rate of 5-6 times per day should permit adequate input of fresh food and water while minimizing food wastage and cost of heating the input seawater from ambient temperatures (0-5 C). Some of this food was not consumed because it flowed out of the system while much of it either settled in the trays as individual cells or as feces and pseudofeces. The amount apparently utilized was 2.9 and 5.0 grams

Table I. Food budget (mg dry wt./animal/100 days). The apparent assimilation plus remineralization is calculated as: Food In minus Food Out minus Food Settled in the holding trays, and represents the food that "disappeared" based on tray input and output.

	15° Unfed	15° Fed	20° Fed	20° Unfed
Food In	353.3	9188	10400	207.0
Food Out	97.0	3739	2781	101.3
Food Settled	138.8	2502	2589	64.3
Assimilation + Remineralization	117.5	2947	5030	41.4

Table II. Rate of food delivery and turnover times for four experimental conditions. The values are averages of daily measurements in calibrated flow meters over 100 days.

Condition	Mean Flow Rate (ml/min) SW	Food	Total	Turnover Time (minutes)	Turnovers Per Day
15° Unfed	156.6	0	156.6	267	5.4
15° Fed	144.6	21.9	166.5	251	5.7
20° Fed	147.2	22.3	169.5	247	5.8
20° Unfed	154.7	0	154.7	270	5.3

dry weight per animals over the 100 days of the experiment at 15 and 20 C respectively (Table I).

The short-term measurements of feeding rate suggests that at low levels of food the scallops filter at a maximal rate of about 9 l/hr/g dry weight at 15 C and 17 l/hr/g dry weight at 20 C (Figure 2). This rate is maintained with increasing food density up to some critical level, lower at the higher temperature, above which an approximately uniform ingestion rate is maintained as the filtration rate decreases. This rate is similar at both 15 C and 20 C and corresponds to an ingestion rate of over 12 mg dry weight Tetraselmis per hour per g. dry weight of scallop. Thus, if food levels in the trays were maintained above the critical level, about 7.5 cells/ul at 15 C or 5.0 cells/ul at 20 C, the scallops should be getting all the food they can use. These food levels correspond to 1.3 and 0.85 grams dry weight of Tetraselmis per liter. The food supply was controlled to approximate this optimal food density, but the scallops routinely failed to clear the water of food due to very low filtration rates ralative to those expected from the short-term measurements. Food density therefore occasionally rose to higher levels, but could rapidly be reduced by temporarily increasing the seawater flow. The actual long-term filtration rates were estimated both from loss of material through the trays and from deposited egesta. The former estimate is likely to be an over-estimate due to remineralization of settled cells and egesta in the trays prior to cleaning. The latter estimate is likely to be an underestimate due to assimilation, but our calculation on the basis of ash in the food should minimize this error. The results are shown in Table III. The long-term measurement of filtration rate at high food levels is thus 10-20 times

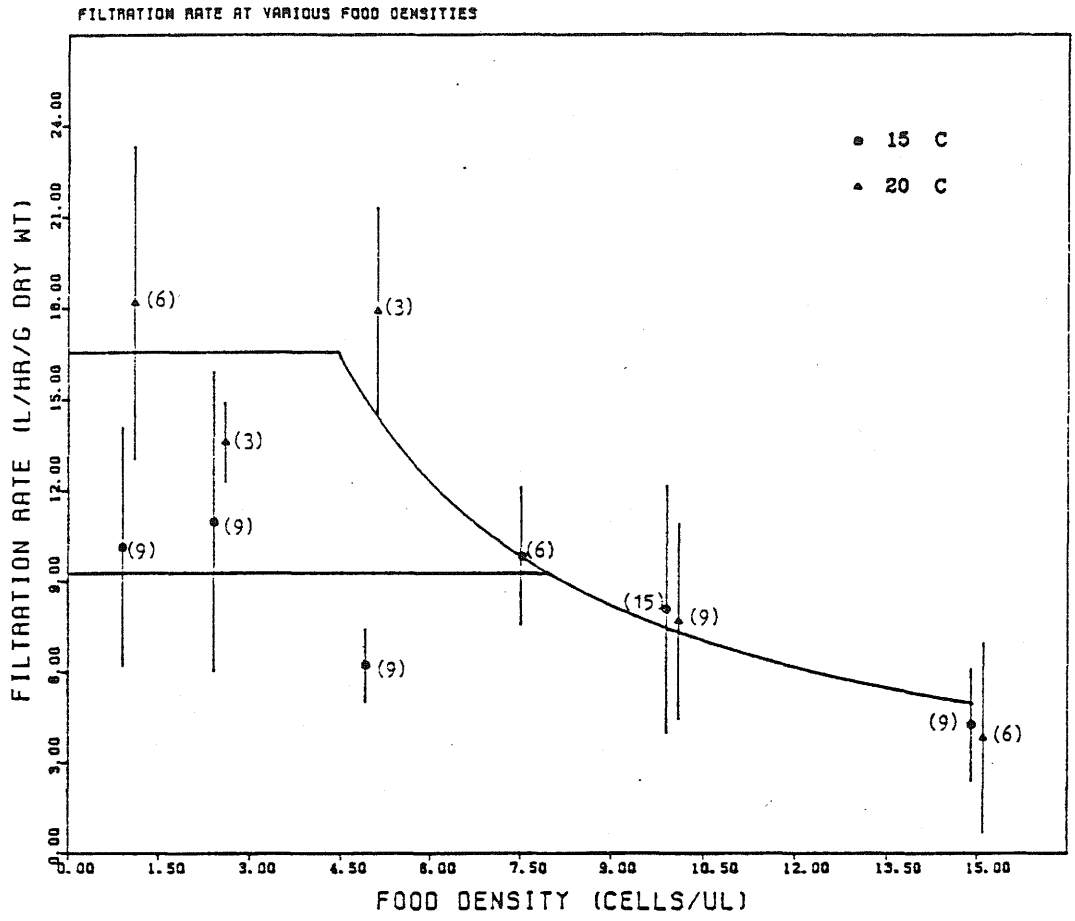

Figure 2. Filtration rates (liters/hr/g. dry wt.) of bay scallops in different concentrations of Tetraselmis suecica at 15 or 20 C in short-term laboratory experiments. The solid lines represent a model of filtration behavior: (a) horizontal lines represent maximum filtration rates at low food concentrations, (b) curved line at higher food concentrations represents the maximum ingestion rate (about 12 mg dry wt./hr/g dry wt. scallop), since ingestion can remain constnt as food density increases and filtration rate decreases. (Numbers in parentheses = number of animals, error bars are $\pm$ 1.0 standard deviation).

Table III. Estimates of filtration rates (liters/hr/animal) under the four experimental conditions calculated by (A) loss of material through the trays and (B) ash in egesta and incoming seawater. The values are averages over the 100 days of the experiment. The values for unfed animals are based on (A) natural particulates that were passed through the seawater filter and (B) small amounts of feces produced by the unfed animals.

	15° Unfed	15° Fed	20° Fed	20° Unfed
A	0.45	0.63	0.95	0.27
B	0.11	0.15	0.19	0.15

Table IV. Mortality and disposition of original experimental animals under the four experimental conditions.

Group	Initial # of Animals	# Removed for Measure	Mortality Measurement Interval 1 (29 days)	2 (40 days)	3 (31 days)	# Animals Remaining
15° Unfed	30	20	0	0	7	3
15° Fed	31	20	0	0	0	11
20° Fed	31	20	0	2	6	3
20° Unfed	30	14	0	16	-	0

lower than that expected from short-term measurements at the same density.

Survival of Scallops

Under all experimental conditions, the scallops survived for at least 30 days (Table IV). The long duration of the experiment did result in some mortality, which occurred in a predictable manner, based on food supplies and temperatures. The mortality rate after 30 days was higher for starved animals than fed animals and was higher at 20 C than at 15 C at both food levels. No animals died when fed at 15 C.

Reproductive Condition

The reproductive condition of scallops was evaluated at four periods throughout the experiment. The evaluation reflects only an average qualitative assessment of the state of gamete development based on a sample of five animals since there was no consistent trend for any group and substantial variation within samples. Spermatozoa and large oocytes were found in a few animals, but with no pattern with respect to temperature, food condition or time. Many animals in all experimental conditions showed no gamete development. The most mature gametes were observed after 70 days in the ovary and testis from an animal held at 15 C without food, but all other animals in this sample showed no development.

Energy Utilization

The total amount of food utilized under the four experimental conditions is shown in Table I. This is expressed as coloric equivalents in Table V along with components of energy expenditure. The metabolic energy expenditure and actual weight changes are also shown in this table and clearly show that the scallops did not utilize the food available to them. The apparent food utilization less the metabolic energy expenditure gives an expected growth rate of 83.6 and 161 cal per animal per day (approximately 18.5 and 36 mg dry weight per animal per day respectively). The actual growth rates however were -31.0 and -36.9 cal per animal per day indicating that the food apparently used in the trays was not converted into scallop biomass. It is likely that much of it may have been remineralized, despite adequate turnover in the trays and weekly cleaning.

The difference between the expected and actual growth represents the material remineralized in the trays, about 74% of total utilization under both feeding conditions. Weight loss per interval is shown in table VI. It is clear that fed animals lost less weight than starved animals for the first 70 days of the experiment indicating that food was being utilized, but to an inadequate extent.

The pattern of metabolic energy expenditure (Figure 3, respiration) in general decreased with time in unfed groups, compensating to some extent for apparent inadequate energy intake. It remained relatively constant throughout for fed animals. The metabolic cost was greater at 20 C and greater for fed animals. After 30 days, the metabolic energy expenditure was similar for starved animals, regardless of holding

Table V. Energy budget per animal (cal/day). Apparent utilization includes remineralization (Table I). Expected growth is based on apparent utilization minus respiration and excretion. Remineralization is the amount of apparent utilization that did not appear as actual growth of the scallops.

	15° Unfed	15° Fed	20° Fed	20° Unfed
Apparent Utilization	6.16	154.5	266.2	2.17
Respiration	31.4	61.9	90.0	29.4
Excretion	4.08	8.95	15.2	4.05
Weight Change	-42.5	-31.0	-36.9	-20.2
Expected Growth	-29.3	83.6	161.0	-31.3
Remineralization (Expected-Actual Growth)	13.2	114.6	197.9	-11.1
% of Apparent Utilization		74%	74%	

Table VI. Changes in scallop weight (mg dry weight per animal per day) under the four experimental conditions.

Condition	Measurement Interval		
	1 (29 days)	2 (40 days)	3 (31 days)
15° Unfed	-12.9	-9.8	-6.3
15° Fed	-9.6	-5.1	-7.0
20° Fed	-5.7	-4.1	-16.3
20° Unfed	-8.9	-4.9	-

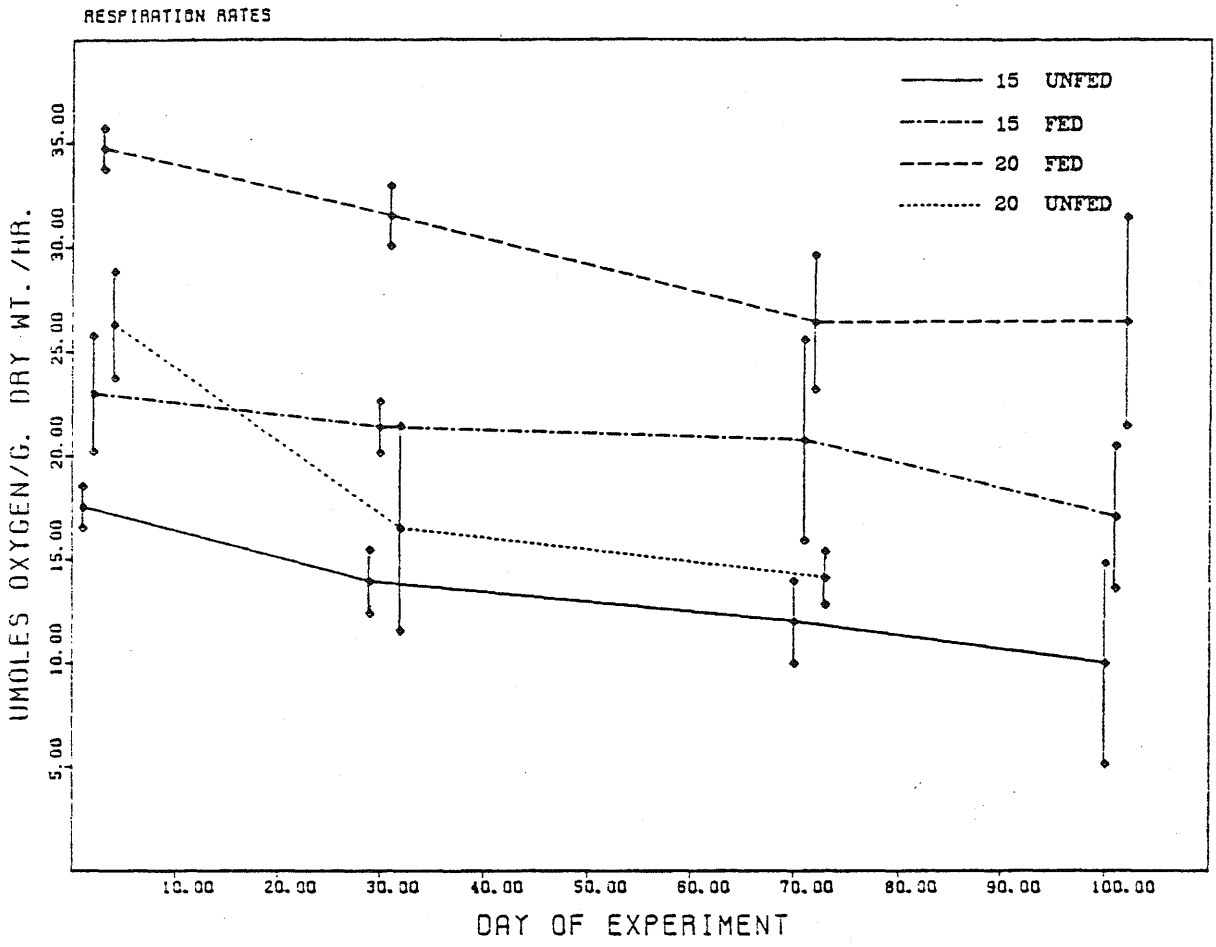

Figure 3. Energy utilized by fed and unfed scallops for maintenance, measured as respiration (1 ml O_2 = 4.78 cal), under the four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

temperature.

The energy expenditure due to excretion is shown in Figure 4. It was higher for fed animals and higher at 20 C than at 15 C. The excretion rates remained quite constant during the experiment.

The pattern of weight change is shown in Figure 5. The rate of weight loss is clearly greater in starved animals at the beginning of the experiment leading to consistently lower weights. The rate of weight loss is very consistent in both fed groups regardless of temperture difference. These weights have been standardized by a volume index (based on size of the shell) to account for possible size specific mortality (Figure 6). The pattern is similar showing the greater weight loss in unfed animals and greater weight loss at 20 C than at 15 C for unfed animals.

Of the major tissue components of scallops, the adductor muscle shows the greatest decrease in absolute and relative weight (Figures 7 and 8 respectively), roughly 60-80% over the duration of the experiment. The weight loss was greater for starved animals (Figure 7).

Weight loss in gonad tissue was also consistent and substantial (Figure 9). In general about 50-60% of the tissue weight was lost over the 100 day period. The relative weight change is shown as the gonad index in Figure 10.

Weight loss in the digestive gland was similar at both tempertures, but differed markedly between fed and unfed groups (Figure 11). Fed animals lost 25-35% of initial digestive gland weights while starved animals lost 50-60%. Relative weights actually increase slightly (Figure 12) due to the greater loss of weight in other tissues.

The O/N ratios (Figure 13) reflect the nature of fuel being

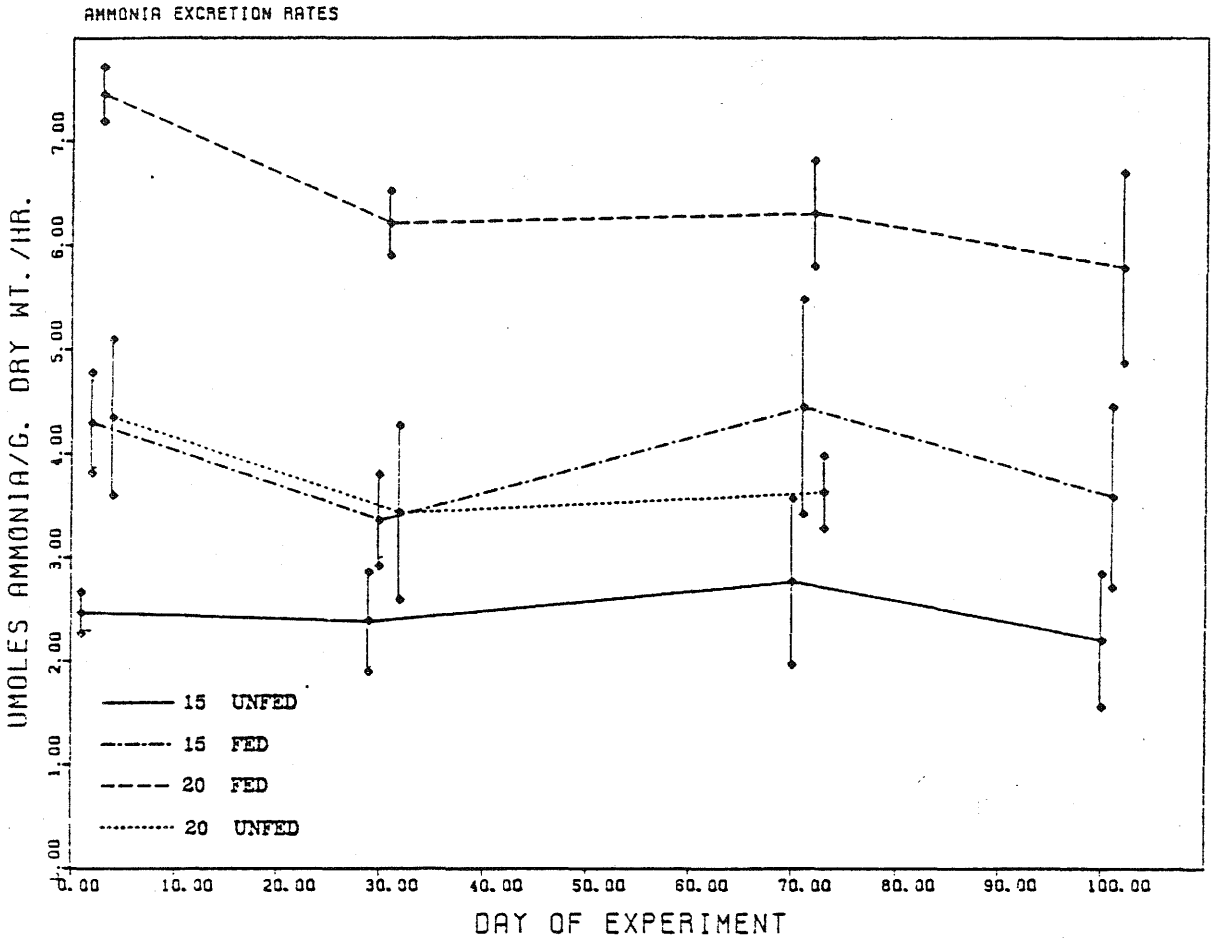

Figure 4. Energy loss from excretion (one gram ammonia = 4.7 kcal) in bay scallops under the four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

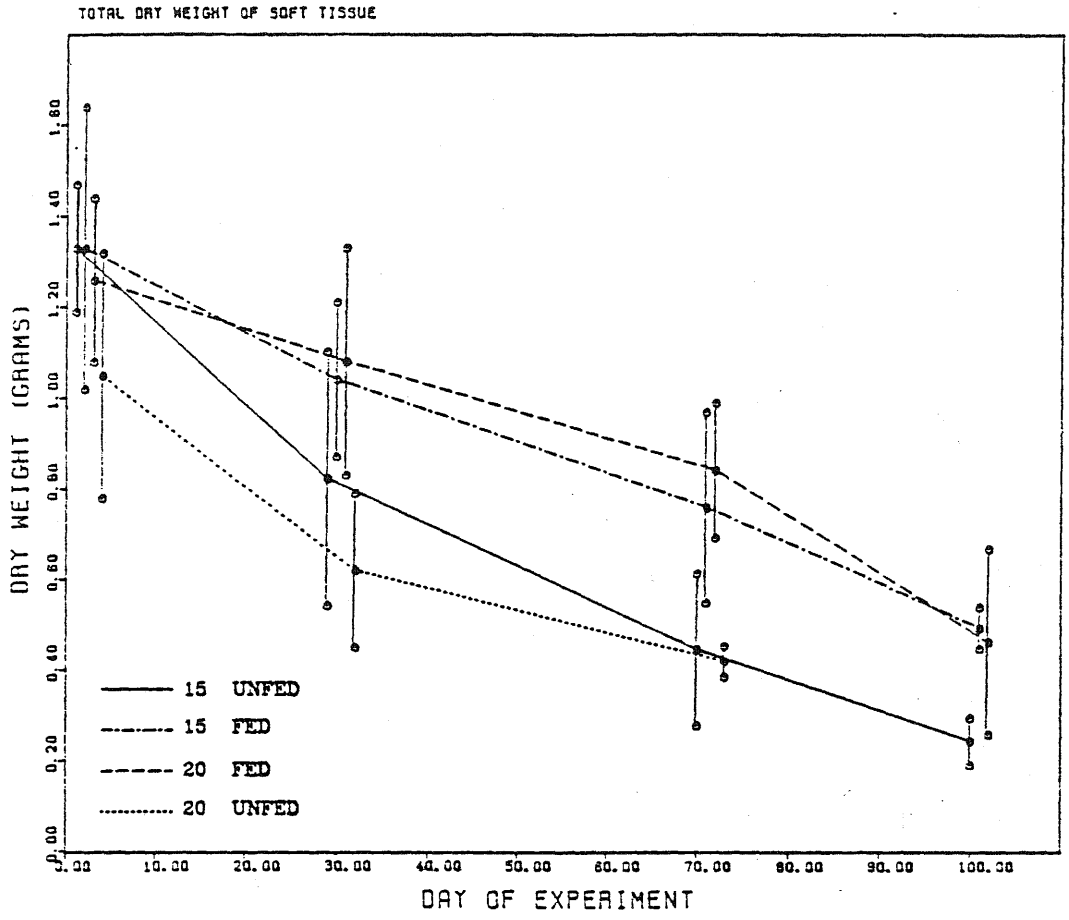

Figure 5. Total dry weight of soft tissue of scallops held under four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

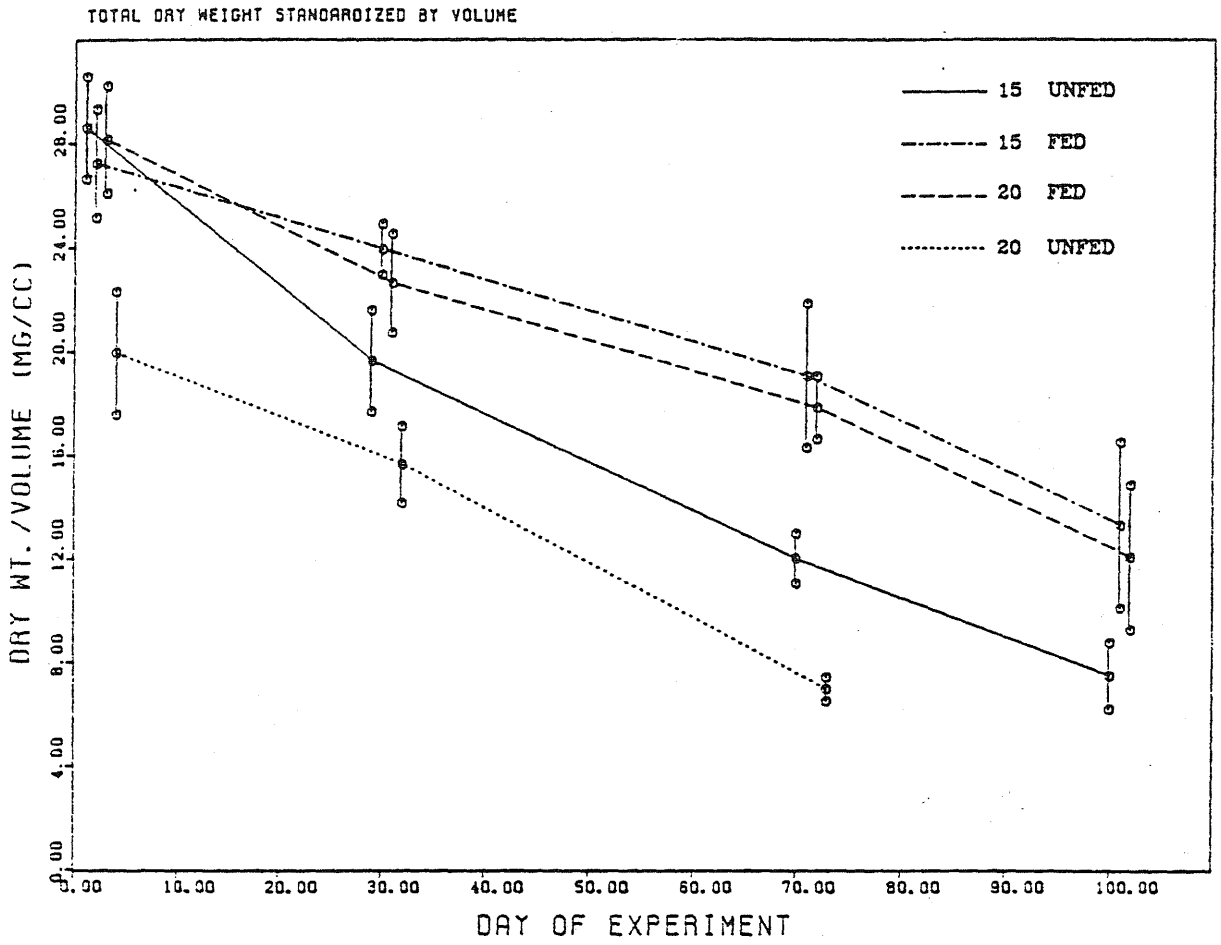

Figure 6. Changes in the total dry weight of soft tissues standardized by a volume index (volume index = shell depth squared x shell width x 2/3) to correct for possible size specific mortality. (Error bars = ± 1.0 standard deviations, n = 5).

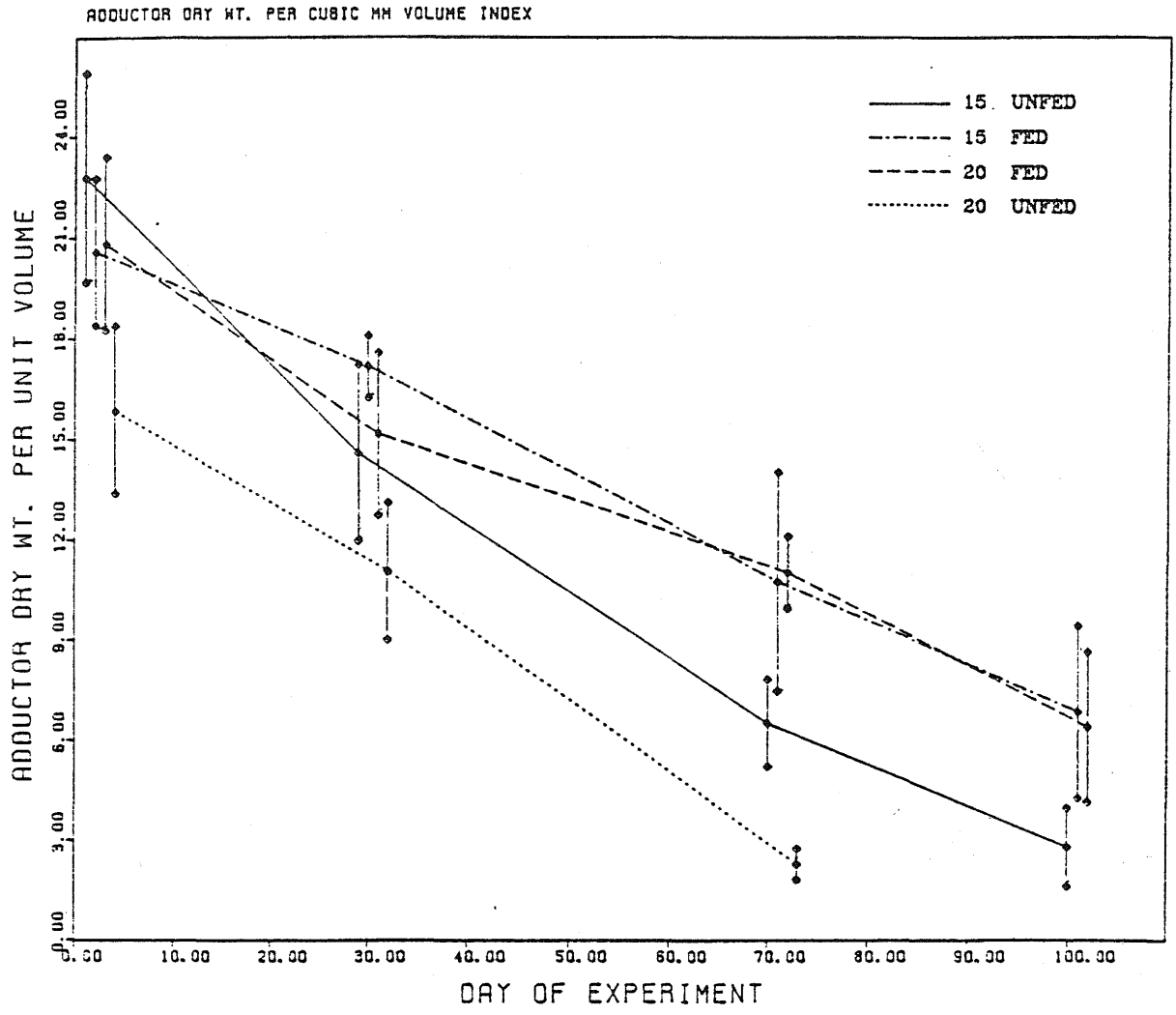

Figure 7. Adductor muscle weights standardized by a volume index (dry weight per cubic mm volume index) for scallops held under the four experimental treatment conditions. (Error bars = ± 1.0 standard deviations, n = 5).

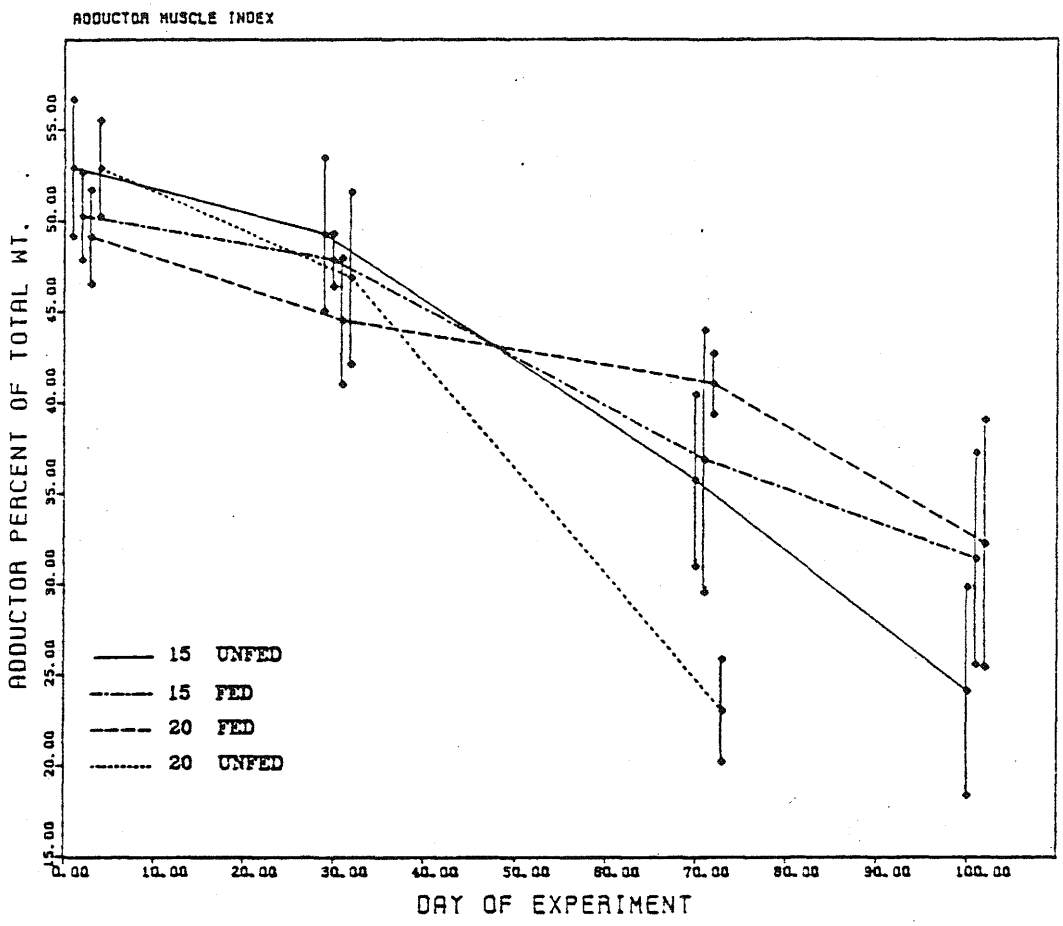

Figure 8. Adductor muscle weight expressed as a percentage of total body soft tissue dry weight for scallops held under the four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

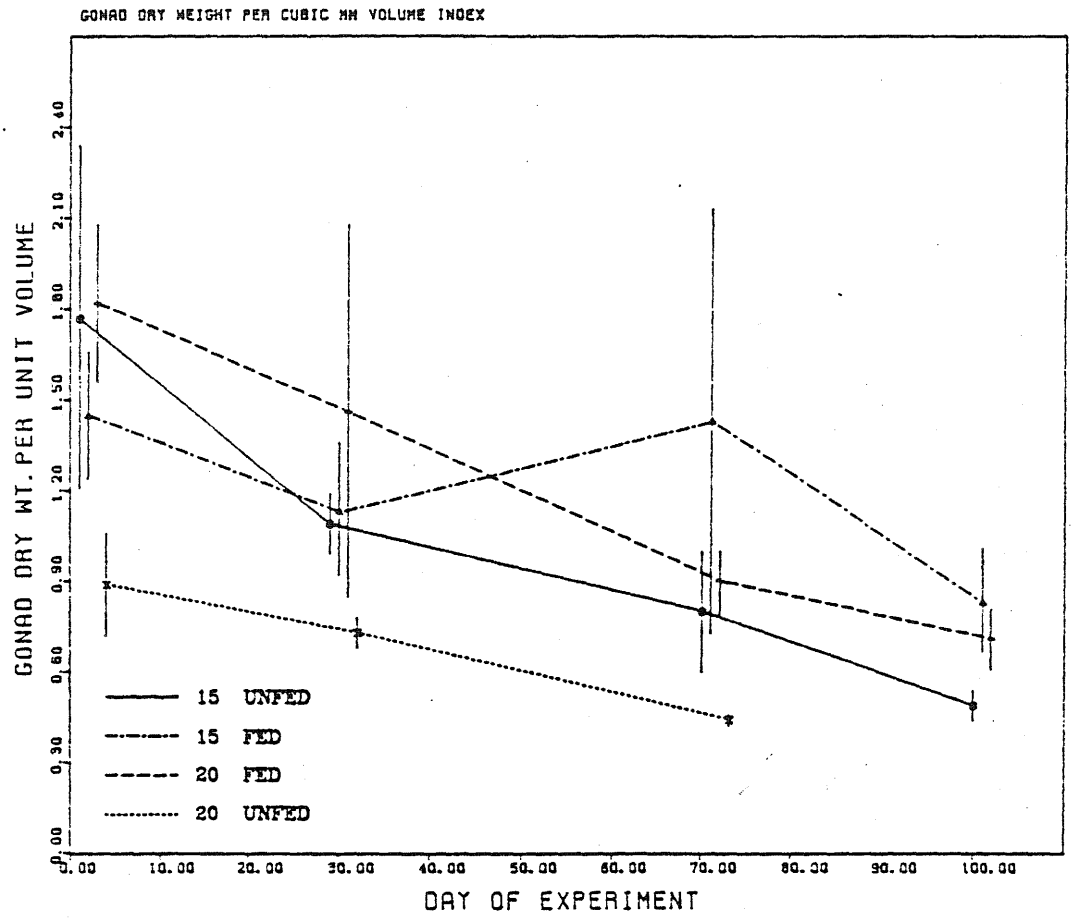

Figure 9. Gonad weights standardized by a volume index (dry weight per cubic mm volume index) for scallops held under the four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

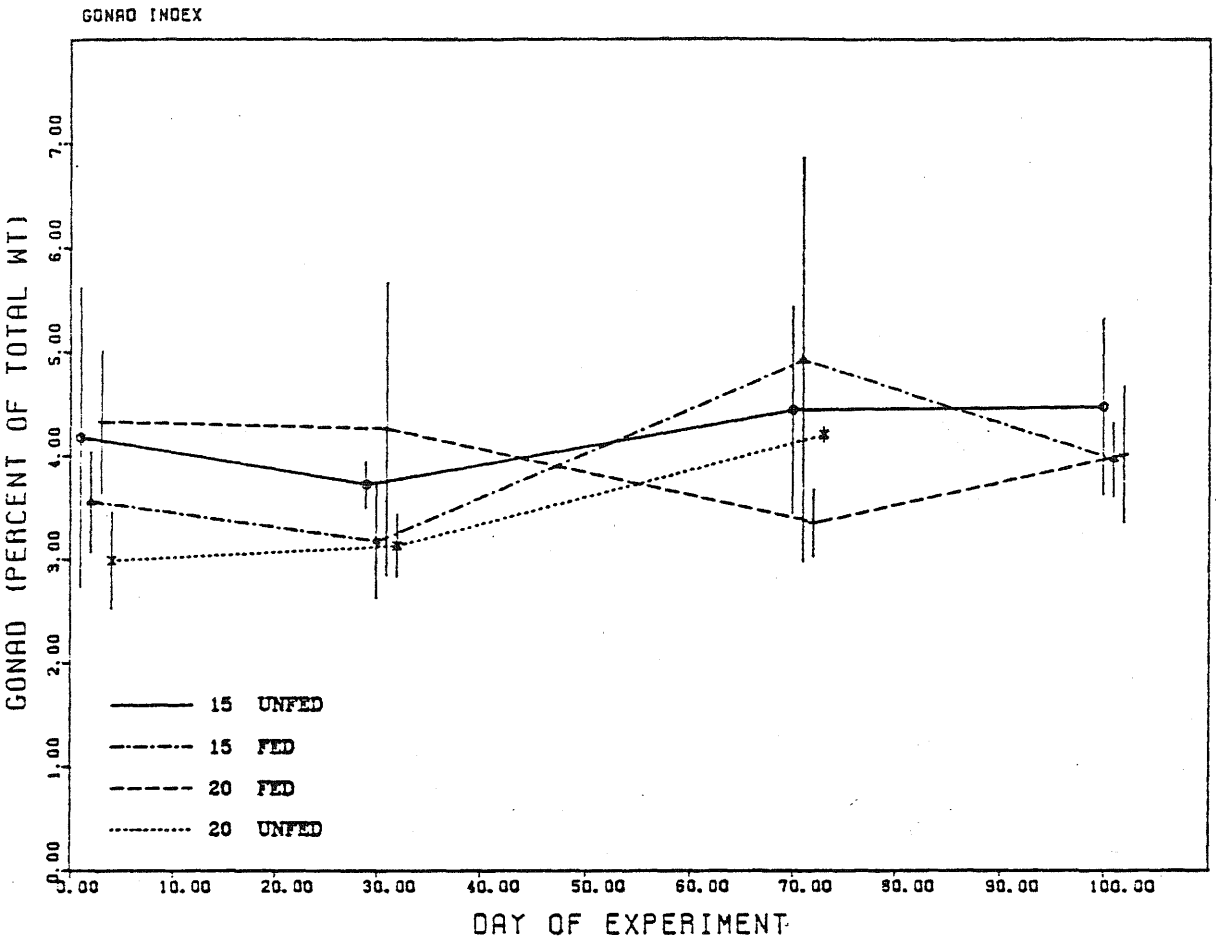

Figure 10. Gonad weights expressed as a percentage of total body soft tissue dry weight for scallops held under the four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

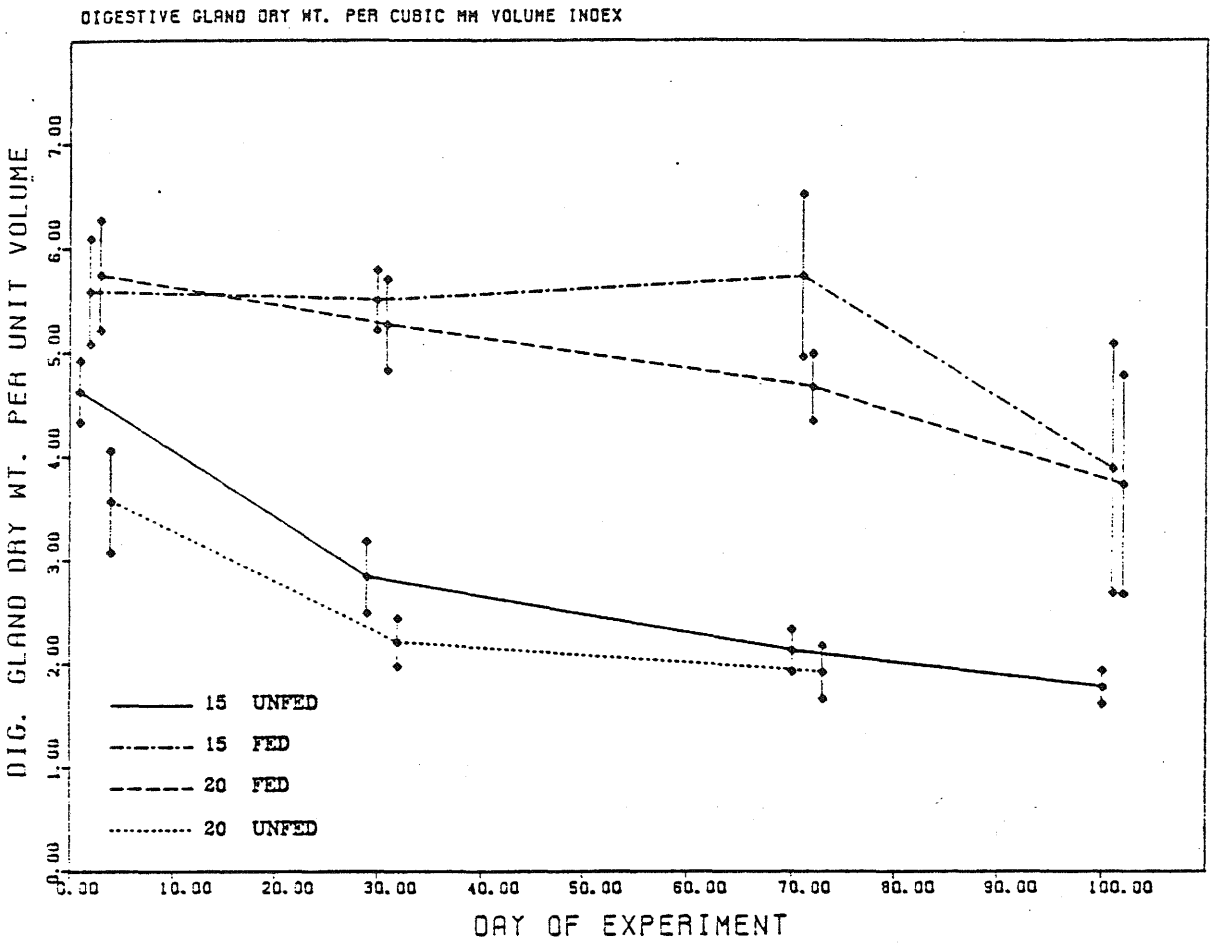

Figure 11. Digestive gland weights standardized by a volume index (dry weight per cubic mm volume index) for scallops held under the four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

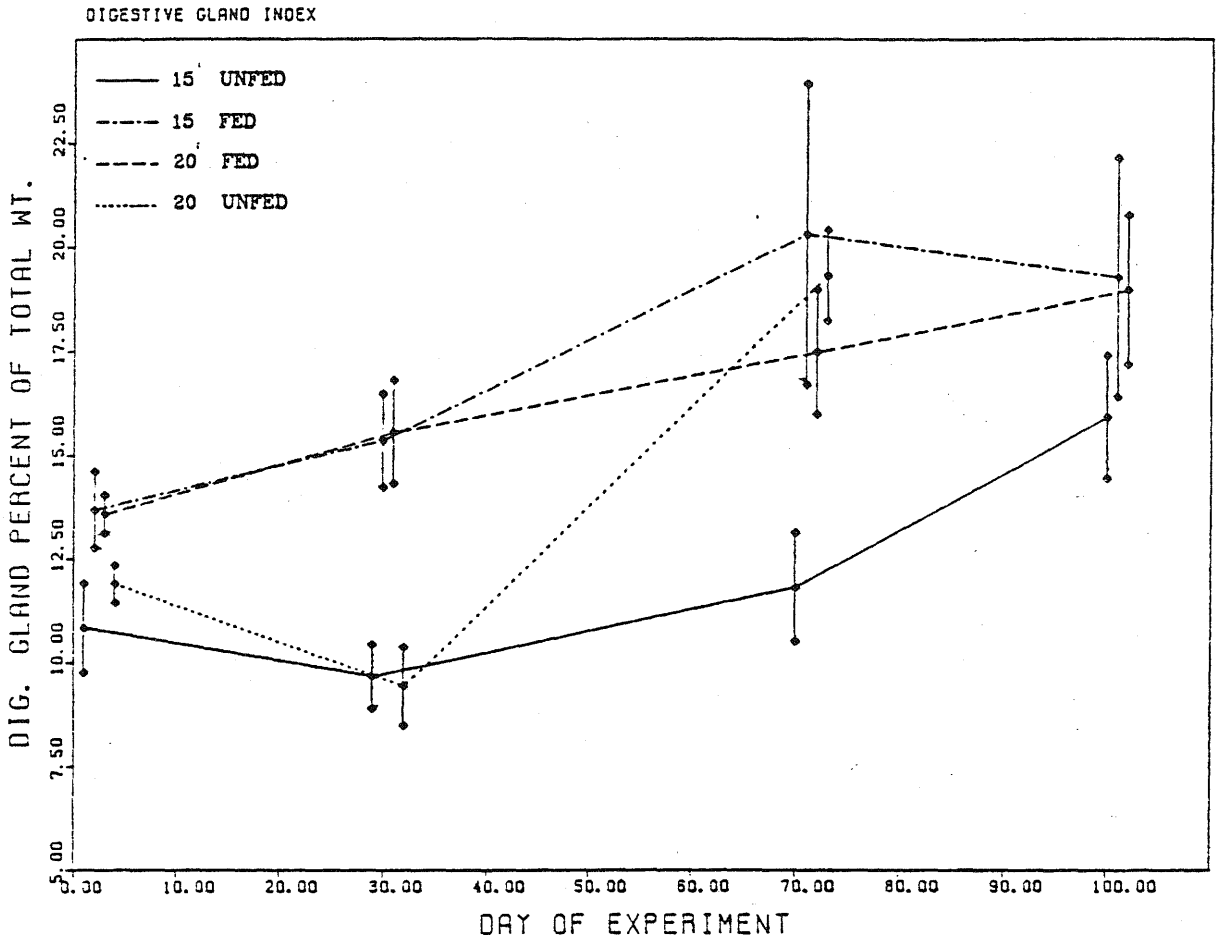

Figure 12. Digestive gland weights expressed as a percentage of total body soft tissue dry weight for scallops held under the four experimental conditions. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

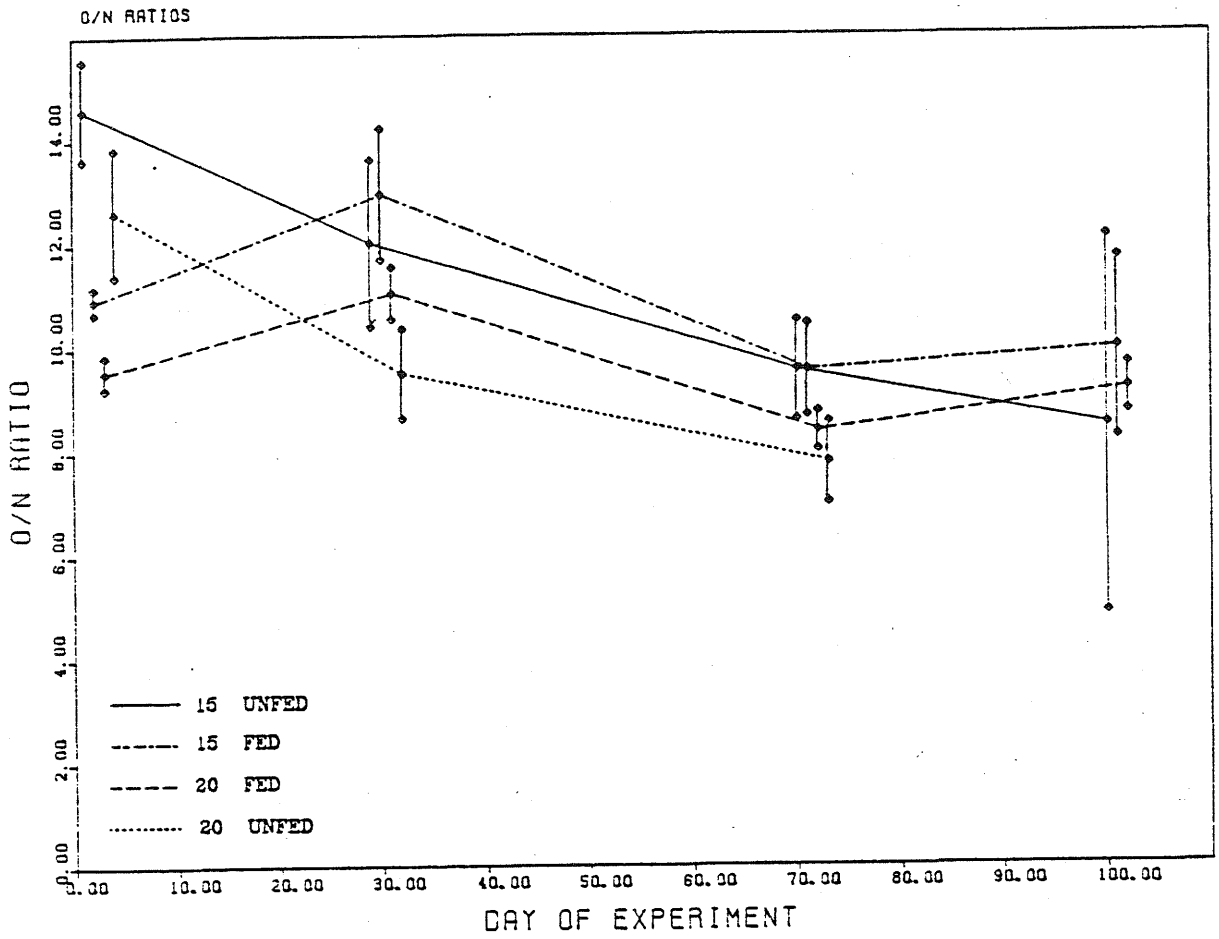

Figure 13. Oxygen:nitrogen ratios (O/N) indicating the types of substrates metabolized by the fed and unfed scallops held under the four experimental conditions. Lower ratios reflect a greater proportion of protein metabolized relative to carbohydrate and lipid. (Error bars = $\pm$ 1.0 standard deviations, n = 5).

metabolized by the animals. A low ratio reflects high protein metabolism while higher values reflect greater carbohydrate and lipid metabolism since these substrates contain no nitrogen. The pattern of fuel utilization suggests slightly less protein metabolism at the start of the experiment and a consistent, but slight increase, particularly for starved animals, as the experiment progressed. Over all, the O/N ratio suggests that all animals metabolized an appreciable proportion of protein throughout the experiment.

Quality of Gametes

Preliminary work was done on larval development from gametes procured from animals which matured naturally in local Rhode Island ponds. This provides baseline information to assess the quality of gametes produced by the artificially conditioned animals. Field collected animals were spawned in the laboratory a couple of months in advance of their natural breeding period. The resulting larvae developed to the veliger stage, but when maintained in high densities, abnormalities were very common. In low density cultures, larvae develop up to the metamorphic stage. In our experience with larvae from gametes procured from scallops in local ponds and cultured in filtered local pond water, the abnormalities in the veliger stage are particularly high. Although these abnormalities are suspected to have resulted from the pond water quality, comprehensive investigations are necessary to resolve this problem.

IV. Discussion and Conclusions

The artificial acceleration of growth and maturation of economically important bivalve molluscs is an important goal of fisheries biologists and aquaculturists. Towards this goal, our investigation has provided a practical system for culturing the food and maintaining animals, but has also pointed out some serious deficiencies (or uncertainties) in our knowledge of the nutritional aspects of bivalves.

Short-term measurements show that Argopecten has a high capacity for filtering seawater (9 to 17 l/hr/g dry weight scallop between 15 and 20 C), suggesting that it may be able to obtain adequate food supplies even at relatively low food concentrations. A maximum ingestion rate, first reached at 5000-7500 cells/ml, is about 12 mg dry weight of food per hour per gram dry weight scallop, which could permit high growth rates. However, our data also suggest that short term measurements may not be adequate for predicting the long-term response of scallops to high food supply and maximum ingestion rates.

Food was provided to the animals at or above these short-term optimal concentrations, but the animals failed to filter much of this food over the long duration of the conditioning experiment. In addition, food concentrations occassionally increased at the beginning of the experiment due to clogging of the seawater valves. Primarily because of the reduced filtration rates, compounded by reduction in seawater flow, food occassionally accumulated in the trays and the actual target food concentrations had to be reduced relative to initially predicted levels.

The long-term filtration rate estimates were considerably lower than the short-term rates which suggests that the animals cannot continue to feed at maximal rates on a long term basis.

The lack of growth despite abundant food supply suggests that the animals may not be assimilating the food adequately. A number of workers have measured assimilation efficiency using the ash-ratio method (Conover, 1966). Although this procedure has been used to measure the assimilation efficiencies of bivalves (Thompson and Bayne, 1972; 1974; Vahl, 1973; Widdows and Bayne, 1971), when assimilation efficiency is low or when the organic content of the food is high (as in our study) small errors in the ratios can result in very large errors in the calculated assimilation efficiency. An alternative method of estimating the assimilation efficiency using the conventional energy budget equation (Crisp, 1971; assimilation efficiency = A/C, where A = P + R + U and C = A + F) gave approximate assimilation efficiencies of 17% at 15 C and 26% at 20 C averaged over the entire experiment. These values are lower than might be expected but not too low to support growth given adequate food supplies. Moore (1966) reported an assimilation efficiency of 34% using natural particulates. Scallops may not be highly efficient assimilators even under natural conditions. A low feeding rate (and thus low assimilation) may have been a limiting factor in the scallop's energy acquisition rather than poor assimilation efficiency _per se_. A low feeding rate may be a physiological adjustment to (or consequence of) low digestibility of _Tetraselmis_ as suggested by Epifanio (1979). If this is the case, had the feeding rate been higher, the assimilation efficiency would have been lower. The scallops used for short-term feeding measurements might not have had sufficient time

feeding on Tetraselmis for the undigested material to back up in the gut, or for responding to conditions of not digesting its food.

A number of factors may have affected the scallops to produce the response observed in this study. Although Walne (1970) found that Tetraselmis was a good food for clam and oyster juveniles, and several studies with mussels (Widdows and Bayne, 1971; Thompson and Bayne, 1972; 1974) have shown it to be well assimilated, a more recent study (Epifanio, 1979) gave different results. Epifanio (1979) found that Tetraselmis was a rather poor food for oysters, and suggested low digestability as a cause. The conflicting results of these studies might be due to food concentration (Epifanio, 1979; Romberger and Epifanio, 1981). Perhaps Walne (1970) in giving less food to his animals allowed them to digest what they consumed. In this regard it should be noted that densities in the present study were rarely, if ever, as high as in Epifanio's experiment and normally were within the optimal range found by Walne. They were generally appreciably higher, however, than the densities Thompson and Bayne (1972) found optimal for mussels. Although all these studies used Tetraselmis suecica, the size and weight of the organisms used by different workers varied (Table VII). The variation in weight per cell indicates the possibility that there may be several clones or species and may account for the differences in feeding results reported by different workers. Whether or not such differences between clones or species account for the differences in feeding results can not be answered here, and further study is warranted.

It is also possible that the conditions of algal culture (Ukeles, 1976), the composition of growth medium, and the growth phase of the

Table VII. Volumes and weights of Tetraselmis suecica reported by workers feeding this alga to bivalves. These volumes and weights indicate differences in the nutritional content of cells grown under specific culture conditions and may be due to different clones of this species.

	Volume/Cell	Dry wt./Cell
Walne (1970)	335 um^3	-
Thompson & Bayne (1974)	-	66 pg
Epifanio (1979)	515 um^3	292 pg
This Study	430 um^3	135 pg

cells used for feeding (Parsons et al., 1961; Walne, 1970; 1973; Scott, 1980), could affect the nutritional quality of the diet. The results of this study indicate that although Tetraselmis could be grown in sufficiently large quantities, it may not constitute a complete diet to support growth and reproduction of scallops. Walne (1970) reported improved growth of his animals when the Tetraselmis was mixed with coarsely filtered sea water, thus adding wild plankton to the diet. The improved dietary value of mixed diets over uni-algal cultures is a widely observed phenomenon (Walne, 1970; 1973; Epifanio, 1979; Romberger and Epifanio, 1981). Epifanio (1979) suggests an incomplete diet as a possible cause for his problem, but later work (Romberger and Epifanio, 1981) suggests that the indigestibility may be the cause. The observed response may also be caused by excessive food levels and inability to use such large amounts of food.

The survival of all fed animals at 15 C, their steady energy expenditure for maintenance, and their consistent weight loss over the entire experimental period, suggests that energy derived from the ingested food is inadequate to maintain all the energy demanding processes. The low assimilation by scallops receiving food supplies that could theoretically sustain these long-term processes suggests: (1) that the animals are unable to filter the avilable food at maximal rates on a sustained basis, (2) the food ingested is not digested and assimilated at the levels required, or (3) the algal diet is nutritionally inadequate for this species. Further studies to determine the qualitative food requirements, using the system and procedures developed here, are suggested for artificially conditioning bay scallops under controlled conditions.

V. Summary and Recommendations

A system has been designed, fabricated and tested for quantitative determinations of the optimal food concentrations for maximal feeding rate and to estimate the food requirements for artificial conditioning bay scallops over long periods.

When Tetraselmis was delivered to scallops at densities optimal for maximum ingestion rates, the long-term filtration rates were much lower than expected from determinations made from short-term laboratory experiments.

The low assimilation, resulting from either lower feeding rate, poor digestibility of Tetraselmis, or the nutritional quality of the diet, appears insufficient to allow the scallops to acquire and store excess energy for growth and reproduction.

This study suggests that in addition to supplying food at concentrations optimal for the highest feeding rate, the nutritional quality of the diet is an important consideration for sustaining the processes of growth and reproduction.

Our results suggest several lines of investigation for further study. With a few modifications, the system designed and tested is suitable for determining the qualitative and quantitative food requirements for growth and reproduction of scallops and other bivalves held under experimental conditions over long periods.

A diet that can be readily digested and assimilated by scallops fed at optimal daily ration levels must be established before animals can be conditioned to reproduce under controlled conditions. In addition the optimal daily ration must be determined with relatively long-term

experiments.

Testing the effects of different quality diets on scallops' ability to acquire and store energy and to produce viable larvae will permit optimization of aquacultural procedures for this species.

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