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THE EFFECT OF DIFFERENT ALGAL PROTEIN CONCENTRATIONS ON THE GROWTH, PROTEIN CONVERSION, AND NITROGEN BALANCE OF JUVENILE MERCENARIA CAMPECHIENSIS, THE SOUTHERN HARD CLAM

City University of New York PH.D. 1980

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THE EFFECT OF DIFFERENT ALGAL PROTEIN CONCENTRATIONS ON THE GROWTH, PROTEIN CONVERSION, AND NITROGEN BALANCE OF JUVENILE *MERCENARIA CAMPECHIENSIS*, THE SOUTHERN HARD CLAM

BY

BARRY GOLDSTEIN

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1980

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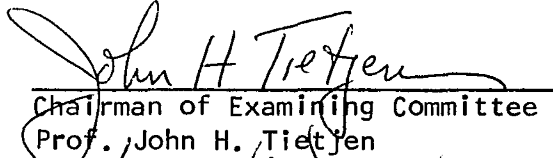

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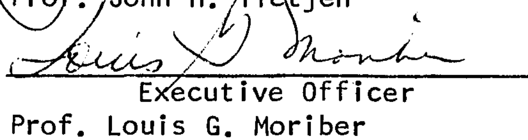

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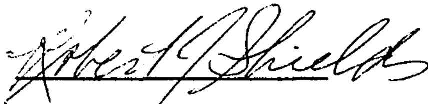

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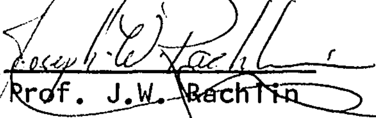

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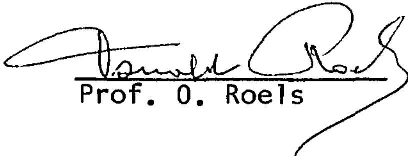

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An ABSTRACT of

THE EFFECT OF DIFFERENT ALGAL PROTEIN CONCENTRATIONS
ON THE GROWTH, PROTEIN CONVERSION, AND
NITROGEN BALANCE OF JUVENILE
MERCENARIA CAMPECHIENSIS,
THE SOUTHERN HARD CLAM

BY

BARRY GOLDSTEIN

Dr.O.A.Roels, Advisor

A Tahitian strain of *Isochrysis* sp. was grown in outdoor continuous culture and fed at four different cell densities to juveniles of the Southern hard clam, *Mercenaria campechiensis*. These cell densities were: $1X10^4$, $5X10^4$, $1X10^5$, and $5X10^5$ cells/ml. Controls consisted of trays without animals receiving an inflow cell density of $5X10^4$ cells/mland trays with animals, but receiving only filtered seawater. Each treatment went to duplicate populations of 100 animals; each population had a whole wet weight of 10 grams. The total flow rate to each population was 120 ml per minute.

The incoming filtered seawater, incoming algal culture, and effluent from each shellfish population was collected daily to be analyzed for nitrite, nitrate, ammonia, urea, dissolved free amino acids (DFAA), soluble protein (SP), total dissolved nitrogen (TDN), and particulate protein nitrogen (PPN).

A nitrogen balance for juvenile *Mercenaria campechiensis* in a continuous flow system was calculated; 85 to 95 percent of all total incoming nitrogen was accounted for in the different treatments.

The pattern of change in concentration of the various nitrogen containing compounds as a result of the passage through the shellfish culture containers is described. There was a significant excretion of ammonia only by those populations receiving an inflow algal protein concentration (APC) of 5.75 μgat. PPN/l. If there was any excretion of DFAA or urea, there was an equal uptake of these compounds by microorganisms present in the shellfish culture containers. There was a significant uptake of both nitrite and nitrate by the algae present in the copious biodeposits of those shellfish populations receiving an inflow APC of 56.01 μgat. PPN/l. There was a significant uptake of soluble protein by those shellfish populations receiving $\geq$ 5.75 μgat. PPN/l.

Protein Stripping Efficiency, Protein Retention Efficiency, Food Chain Efficiency, Ecological Efficiency, Protein Conversion Efficiency, and Gross Growth Efficiency were all maximal for those animals receiving an average weekly inflow APC of 5.75 μgat. PPN/l, which corresponds to an ambient APC of 2.93 μgat. PPN/l.

Ingested ration increased with increasing ambient APC; this does not conform to current models of food concentration and ingestion.

Weekly biomass production, regardless of how measured, was maximal for those animals receiving the intermediate inflow APC of 5.75 and 11.33 μgat. PPN/l. There was no significant difference at the 95 % confidence level in weekly biomass production between those clam populations receiving these intermediate inflow APC. There was less

growth exhibited by those clam populations receiving higher or lower inflow algal protein concentrations. These results do not conform to current models of bivalve mollusc feeding and growth.

An inflow algal protein concentaation of 5.75 μgat. PPN/l flowing at a rate of 120 ml/min. to 10 grams live weight of juvenile *Mercenaria campechiensis* contained in 250 ml of seawater results in an ambient algal protein concentration of 2.93 μgat. PPN/l. It is concluded that this ambient algal protein concentration is optimum for the growth and protein conversion efficiency of juvenile *Mercenaria campechiensis*.

ACKNOWLEDGEMENTS

There is no way that I could sufficiently express my thanks, love, and appreciation to my wife, Jacqueline Aboulafia Goldstein, for her constant emotional support, hard work, and unflagging love and encouragement through the many years required for the completion of this thesis. Quite simply, I could not and would not have finished, but for her.

I would like to thank Dr. Oswald Roels for his ungrudging financial support and guidance for so many years. Thanks are due to the other members of my Committee, particularly Dr. John Tietjen who has been a valuable advisor for my whole graduate education at CUNY.

I would like to thank the following fine people for their support, assistance, and advice: Robert Godbout, Paul McDonald, Phyllis McDonald, Ludo Van Hemelryck, Diane Spence, Marion Trout, and Bruce Sharfstein.

I would also like to thank my family and my wife's family for their support and encouragement, most especially my parents, Mr. and Mrs. GeorgeGoldstein, who helped in every way they could. Dad-I am a doctor.

Lastand not least, I want to thank my wonderful children, Joshua and Jessica, for bringing joy and distraction to me when the going got tough.

Thank you all.

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INTRODUCTION

World overpopulation has put a severe strain on the ability of agriculture to supply the world's nutritional needs, particularly of protein. It is often difficult for people to supply themselves with the right amounts and proportions of the essential amino acids from plant protein sources. Most animal proteins are more complete in their complement of essential amino acids and thus are a more desirable protein source.

The amount of arable land not presently under cultivation is limited and, at present rates of population increase, insufficient to supply projected needs.

In fact, Pimentel, et al., (1975) have shown that petroleum and land resource limitations make it impossible to feed even the present world population a diet comparable to that consumed in the U.S., in which 60% of the total protein intake is of animal origin.

This problem has motivated the search for alternative methods of food production, particularly of animal protein. Aquaculture, the farming and husbandry of freshwater and marine organisms, is one alternative that is being actively pursued. At its present level of development it cannot compete with natural fisheries in supplying low cost animal protein. However, with increasing pollution, reclamation of "wasted" estuarine areas for industrial development and overfishing, natural fisheries' yields are stabilizing or decreasing; aquaculture can supplement these natural fisheries. It may well be the only source of some foodstuffs, if present trends continue.

Aquaculture may be the best alternative or supplement to agriculture in areas of the world where agriculture cannot supply local needs, but where water is available for intensive aquaculture. Such intensive aquaculture may produce a less expensive product, pound for pound, than standard agriculture. This is because most aquatic animals are of the same density as the medium and thus do not need to expend energy to support themselves against gravity. Other aquatic animals, particularly bivalve molluscs, are sedentary and do not expend energy in searching out their food.

Other advantages of bivalve molluscs that would encourage their intensive culture is that they utilize phytoplankton, which are not normally nor directly utilized by man. Also, utilizing a herbivore such as bivalve molluscs as a source of animal protein is much more efficient than, for example, using fish meal protein to grow chickens for human consumption.

Some sources of animal protein that can be produced relatively cheaply, such as Fish Protein Concentrate from trash fish, are not readily accepted for human consumption. Bivalve molluscs, on the other hand, are often a traditional food source and/or can gain consumer acceptance readily.

In more developed areas of the world, pollution and overfishing have caused a great decrease in the annual yield of shellfish fisheries. In these areas, bivalves have become a luxury item fetching a high price in the market place. This high price has encouraged investigations into the intensive aquaculture of these shellfish for profit.

Thus, the profit motive and the search for suitable forms of inexpensive animal protein have motivated investigations into the intensive culture of several species of bivalve molluscs.

The successful cultivation of bivalves, as well as other organisms, requires among other things, control of the reproductive cycle of the organism, knowledge of its salinity and temperature requirements, and knowledge of its nutritional requirements. This latter criterion requires the investigation into the best type(s) and amounts of food. Criteria for deciding which is the best type and/or amount of food includes, obviously, amount of growth, but also feeding rate, food chain efficiency, ecological efficiency, protein conversion efficiency, and condition index.

These criteria help in deciding which is the best feeding regime for the organism, but may not indicate which is the best feeding regime insofar as the total culture system is concerned. An organism cannot be cultured without regard to what its role in the culture system is. If a particular food type is difficult and/or expensive to grow, it may not be the best food organism to use in the culture system, even though it may be very nutritional for the bivalve. A particular feed density that is iptimal for the greatest growth of the bivalve may result in such a great rate of

ammonia excretion that the animals may poison themselves.

For reasons like these, a complete study into the nutritional requirements of a bivalve for possible intensive aquaculture must take into account the bivalves role in the complete managed food chain. How does the culture system as a whole or particular parts thereof affect the bivalve and how does the bivalve affect the system?

An excellent way to gauge these effects is by constructing a nitrogen balance of the entire managed food chain. A nitrogen balance is constructed because:
1) nitrogen is often the limiting nutrient to the growth of the primary trophic level (Ryther and Dunstan, 1971);
2) nitrogenous waste products of the bivalve can be toxic to the bivalves themselves or other organisms downstream; 3) these nitrogenous waste products may be used for the growth of macrophytes; and 4) because animal protein production is often the primary goal of such managed food chains.

An important by product of studying the nitrogen dynamics of the bivalve in continuous flow, managed food chain is that the results give a fairly good picture of the role of that bivalve in the nitrogen cycle of its natural environment. Such a type of study may not be as realistic as a field study, but it is more controllable and subject to intensive investigation, including studying the effect of varying different elements of the animal's biotic and abiotic environment. Those studies in which a small number of animals are unfed for 24 hours prior to the experiment, placed into a small bowl of static, synthetic seawater and the change in concentration of different nitrogen compounds measured in the medium may be even more controlled and precise than studies involving a continuous flow managed food chain. However, they are so removed from "real" life as to render the results interesting but almost irrelevant.

I believe that studying the physiological responses of an organism to the biotic and abiotic factors of its environment is best accomplished in a continuous flow, managed food chain.

Field studies can point to the questions to be asked and validate the results of studies in such a managed food chain.

In my study, I constructed a nitrogen balance for juvenile *Mercenaria campechiensis*, the southern hard clam fed *Isochrysis* sp. at different densities. Juveniles were used because relatively little is known of the bioenergetics and nitrogen cycling of juvenile shellfish. Also, the greater growth rate of juveniles results in more measurable growth of the organism in a shorter period of time. The generally greater metabolism of juveniles results in more measurable changes in various physiological responses, such as ammonia excretion, in a shorter period of time. I used *Mercenaria campechiensis* because there is very little information in the literature on its growth and physiology, although it is abundant in great quantities throughout the Gulf Coast. Its growth is generally greater than *Mercenaria mercenaria* or their reciprocal hybrids and it is much more tolerant to high temperatures than *Mercenaria mercenaria*. Although there is no great commercial fishery for *Mercenaria campechiensis* at present, pollution and/or habitat destruction may lead to its demise before a commercial fishery can be developed. The information on the growth and physiology of *Mercenaria campechiensis* reported here can lay the groundwork for the cultivation of this clam, whether for experimental purposes, spat production for restocking natural hibitats or for intensive aquaculture of the organism for food and/or profit. The high cost of energy for controlling water temperatures to permit year-round growth of shellfish and other animals has made intensive aquaculture more difficult and expensive in colder climates; limited sunshine throughout the year limits the amount (and type) of phytoplankton that can economically be grown for animal food. Thus, aquaculture in general (except for specific cold water crops) will tend to operate as close to the equator as possible; legal, political and economic considerations will determine exactly where.

Thus, Mercenaria campechiensis appears to be a good candidate for warm water, intensive shellfish aquaculture and the information reported here lays the groundwork for such cultivation. It also gives a good picture of the nitrogen dynamics of the organism. This information can give an indication of the possible role of juvenile Mercenaria campechiensis in the nitrogen cycle of its natural environment and this can be very important because an understanding of nitrogen transfer in ecosystems can help clarify the inter-relationships of the organisms of that ecosystem. This is especially true in marine and estuarine ecosystems where nitrogen is the major limiting nutrient (Ryther and Dunstan,1971)

My work has indicated that the possible conversion of ingested protein to shellfish protein is approximately 33%. Thus, some 2/3 of all ingested protein is transformed and recycled back to the ecosystem. The effect of this recycling on the nitrogen cycle of the ecosystem could be very important. For example, bivalves can be an important source of regenerated nitrogen. The regenerated nitrogen may play a role in maintaining standing crops of phytoplankton, especially during warm weather when the thermocline prevents the entry of new nitrogen into the photic zone.

The regenerated nitrogen comes from the urine of the bivalves and from diffusional losses. The various uses of amino acids in bivalves (protein anabolism, catabolism, osmoregulation, nucleic acid synthesis, etc.) often require transamination and deamination resulting in nitrogenous waste products excreted in the urine. Other sources of these nitrogenous end products in the urine are the breakdown of nucleic acids and proteins as these materials age and are normally replaced by the organism.

The qualitative and quantitative nature of the urine of lamellibranchs is not well known and yet this information is important in understanding the impact of such urine on the primary producers of the ecosystem.

It is well-known that coastal phytoplankton can use ammonia

and urea as a source of nitrogen.

Wheeler, et al. (1974) found that 9 of the 25 species of algae that were studied could use several amino acids as a nitrogen source for growth, although none could use the amino acids for heterotrophic growth in the dark.

Although not ideal, experiments in which shellfish are placed in static bowls of seawater have shown that bivalves excrete nitrogenous end products other than ammonia. For example, the composition of the urine of Mya arenaria was: Amino acid-N (18%), Ammonia-N (21.5%), Purine-N (5%), Urea-N (4.5%), Uric Acid-N (trace%) and Unidentified-N (51%), (Albritton, 1954).

Lum and Hammen (1964), found that ammonia accounts for 75% of the excreted catabolites in Modiolus demissus, although total nonprotein nitrogen (NPN) was not measured. Amino acid nitrogen accounted for the other 25%. He did not find evidence for urea excretion by Modiolus demissus.

This was not the case with Crassostrea virginica which was found to release 0.32-0.86μM. urea/g tissue/day in a similar static bowl experiment. This difference may be due to the presence of measurable urease activity in the digestive gland of Modiolus demissus not found in Crassostrea. Ammonia-N constituted 65% of the total excreted nitrogen, urea-N 13.2%, amino acid-N 5.2%, and unidentified-N 16.6%, (Hammen, et al., 1966).

Little work has been done on the relationship of such variables as feeding regime, temperature, salinity, and size of the animal with the type and amount of urinary excretions in lamellibranchs. Such information is important to fully understand the impact of bivalves on their ecosystem. Therefore, a detailed nitrogen balance of Mercenaria campechiensis under different feeding regimes was constructed to clarify the role of this clam in the nitrogen cycle of its ecosystem as well as lay the groundwork for its intensive cultivation.

MATERIALS AND METHODS

Seawater Filtration System

Raw seawater from the Aransas Pass on the Texas Gulf Coast was pumped through two Sethco filter holders. The first held six 10 μm Sethco polypropylene disposable depth filters. The effluent from these filters was passed through a filter holder with eighteen 1 μm polypropylene disposable depth filters. This filtered seawater was stored in a 1900 l covered black cylindrical polyethylene tank.

The water level in this tank was controlled by a mechanical float valve. Filtered seawater was pumped to a constant head device providing a constant head, gravity flow system. Filtered seawater from this system was distributed to indoor and outdoor mass algal cultures and to the shellfish culture containers. All parts of the filtered seawater system were PVC or polyethylene. (Figure 1)

The disposable filters were changed as necessary to maintain an adequate supply of properly filtered seawater.

The filtered seawater reservoir was large enough so that minor fluctuations in salinity and temperatures were moderated;also because the reservoir was in a temperature controlled room.

Algal Culturing System

Throughout this study, a Tahitian strain of Isochrysis,a golden-brown naked flagellate, obtained from Dr. K.C. Haines of the St. Croix Artificial Upwelling Project,Rust-Op-Twist, USVI, was used.

It was maintained in test tubes containing Guillard's F/2 medium. The test tubes were illuminated by Cool White fluorescents. When needed for mass culture, a test tube with cell density of $\geq 1X10^6$ cells/ml was added to a 50 ml erlenmeyer flask containing Guillard's F/2 medium for an initial cell density of approximately $2X10^4$ cells/ml. When a cell density of $\geq 1X10^6$ cells/ml had been reached, the contents of the flask were put into a 10 l glass carboy, also containing Guillard's F/2 medium. All cultures were axenic as proven by routine sterility testing. These carboy cultures were maintained under artificial illumination and constant aeration--Cool White fluorescents providing 100 μeinsteins/m^2/sec.

Figure 1   Experimental Design.

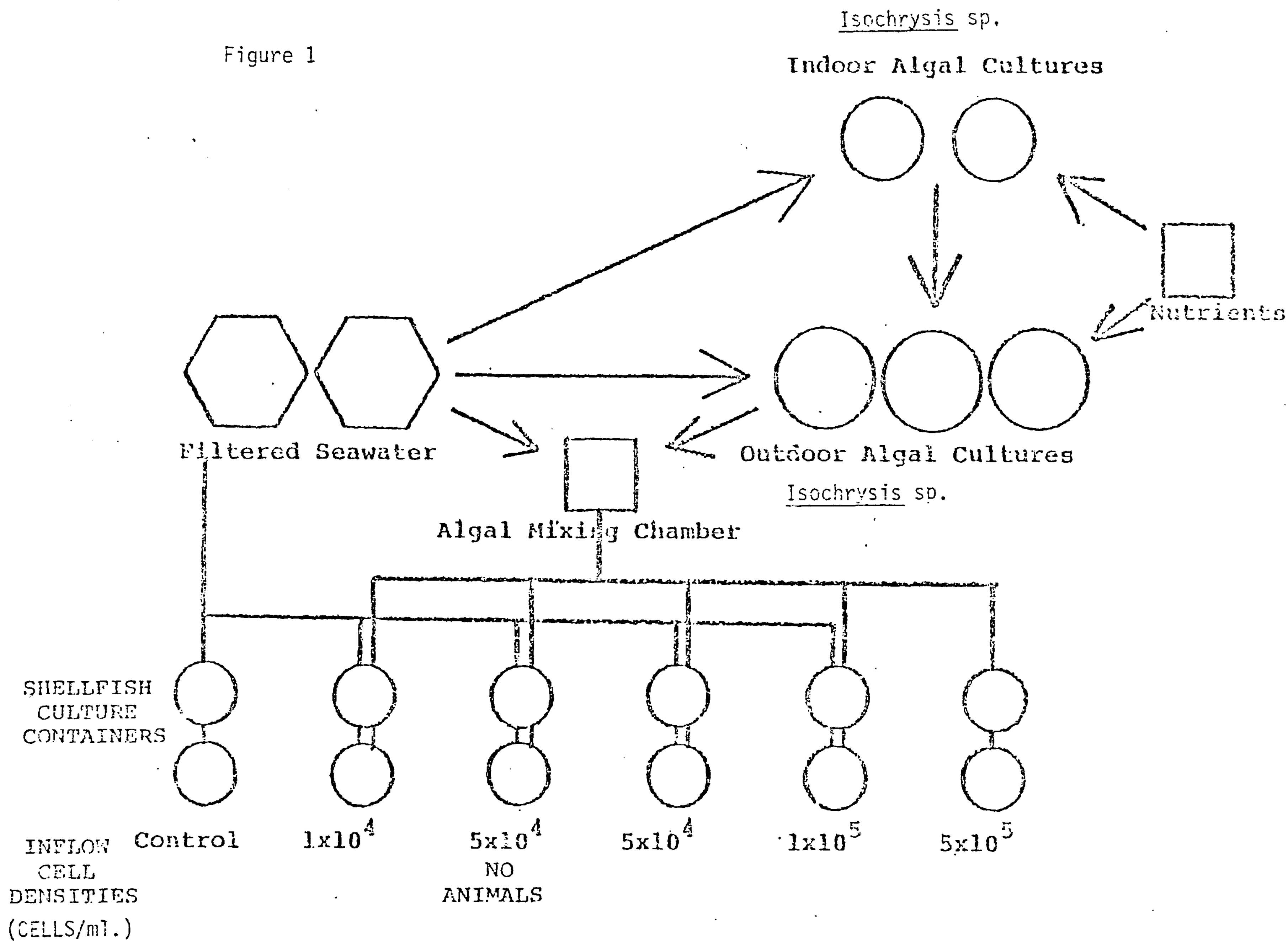


Figure 1
Isochrysis sp.
Indoor Algal Cultures
Nutrients
Filtered Seawater
Outdoor Algal Cultures
Isochrysis sp.
Algal Mixing Chamber
SHELLFISH
CULTURE
CONTAINERS
INFLOW
CELL
DENSITIES
(CELLS/ml.)
Control
1x10^4
5x10^4
NO
ANIMALS
5x10^4
1x10^5
5x10^5

When the cell density of the 10 l glass carboy reached $\geq 1X10^6$ cells/ml, its contents were introduced into one of three 1000 liter fiberglass tanks of 1.8 meters diameter by 0.6 meters high. Initially only 1 cm. of water was present in the tanks, representing a volume of approximately 120 l when the carboy was added. This water was enriched by the addition of a highly concentrated (at Guillard's 100F level) nutrient solution to the filtered seawater resulting in enrichment at the 150 μgat. NO_3-N/l level. Illumination came from cool white fluorescents suspended above the tank. Continuous aeration ensured a source of CO_2 and even exposure of the algae to the light source and to the added nutrients. The algae were grown in batch culture in these indoor tanks.

When cell densities reached $\geq 1X10^6$ cells/ml, the volume of the tank was doubled with enriched, filtered seawater. This procedure was followed until the water depth in the tank was 40 cm. representing a volume of approximately 1000 l. When the cell density reached $\geq 1X10^6$ cells/ml, half the contents of the tank was pumped outdoors into one of three 750 l cylindrical polyethylene tanks; these had a height of 1.2 m. Sufficient enriched seawater was added to bring the tanks to full volume. Under constant, vigorous aeration, the culture was allowed to acclimate to growing outdoors in batch until a cell density of $\geq 1X10^6$ cells/ml was achieved. The algae were then started on continuous culture, usually at a turnover rate of 0.4 times/day. Incoming filtered seawater was controlled by varying the length and diameter of capillary tubing at the end of the inflow pipe. Nutrient media (at Guillard's 100F level) was precisely metered into the tanks using a peristaltic Sage pump (model 375A). The result was the continuous entry of filtered seawater enriched to the level of 150 ugat. NO_3-N/l. All nutrients known to be necessary for the growth of most marine phytoplankton were present in this enrichment, equivalent to Guillard's F/12 nutrient levels (Guillard and Ryther, 1962)

The outflow from these cultures was pumped to shellfish tanks via a constant head system. The continuous algal cultures were maintained until (1) the cell density fell below $5X10^5$ cells/ml or(2)became contaminated with more than 5% (by number) of other algal species.

The inoculation sequence assured that at least one outdoor continuous culture was always on line to feed the shellfish.

DISTRIBUTION SYSTEMS

Filtered Seawater

A modified plastic carboy, with a tube on the side serving as an overflow, maintained a constant level of filtered seawater to the shellfish. The diameter of the overflow tube was sufficient to handle any inflow surges and still maintain a constant water level. Inflow to the carboy was always greater than needed for the shellfish to assure a constant overflow and thus a constant head. The filtered seawater traveled from this constant head device by gravity flow to a horizontal 3/4" schedule 80 PVC pipe manifold. Nipples on the manifold at regular intervals carried the filtered seawater to the shellfish culture containers. This system maintained constant flow rates of filtered seawater to the experimental shellfish.

Algal Culture

A modified plastic carboy, with a side tube serving as an overflow, was used for the algal culture mixing chamber as well.

Filtered seawater and outdoor algal culture, both from separate constant head pans, entered the mixing chamber spearately. Twice daily, the relative flows of each were adjusted to ensure that a cell density of $5X10^5$ cells/ml was maintained in the mixing chamber. The rapid turnover prevented growth in this mixing chamber and vigorous aeration ensured good mixing. The outflow of the mixing chamber went to a horizontal 3/4" PVC manifold from which tubes ran to the shellfish culture containers.

The algal mixing chamber ensured a constant cell density and maintained a constant flow rate of algal culture to the shellfish culture containers.

SHELLFISH

Experimental Animals

Adult *Mercenaria campechiensis* were collected in late February 1978 in an intertidal area of Redfish Bay, an estuarine area between the mainland and the barrier islands of the Texas Gulf Coast near Corpus

Christi, Texas, and acclimatized in the laboratory for ripening of the gonads. They were fed Isochrysis sp. from the outdoor continuous cultures described.

Spawning was induced by thermal shock (brought to 32°C from 22°C in one hour), and by the addition of gonad suspension stripped from male and female Mercenaria campechiensis. The experimental animals used in this study were the spawn of one female and two males. Therefore, their genetic makeup is presumed to be less variable than it would have been in the case of a wild set.

After spat settlement (some 800,000 animals) there were no mortalities. The lack of mortality indicates that water quality was good and that Isochrysis sp. is a good food for juvenile Mercenaria campechiensis from spat settlement on.

A subsample of eleven hundred animals were chosen from the population for this study on the basis of similar shell length 7.62 ± 0.40 mm. They were divided into eleven (11) populations of 100 animals each. The average wet weight of these eleven populations was 10.22 g ± 0.01. The eleventh population was used for determining the initial values of shell length, whole wet weight, protein content, etc. for the remaining ten (10) experimental populations. The average individual shell length of the juveniles was 7.62 ± 0.40 mm.

Shellfish Culturing System

The shellfish culture containers were plastic round bowls with tapered sides. The diameter across the top was 14.5 cm. and the diameter across the bottom was 10 cm. There was a plastic standpipe in the middle of the bowl to maintain a constant water volume of 250 ml. Inputs of algal culture and/or filtered seawater were arranged to create a vortex motion in the containers to ensure thorough mixing. The clams were evenly spaced on the bottom of the bowl and occupied approximately 56% of the bottom area.

Each shellfish culture container had two inflows, one from the algal mixing chamber and one from the filtered seawater constant head device. Different proportions of these were sent to each culture container to provide the different experimental cell densities shown in Table I .

Table 1 Flow Rates and Cell Densities of Experimental Treatments.

Table 1

TREATMENT	ALGAL CULTURE FLOW (ml/min.)	FILTERED SEA WATER FLOW (ml/min.)	CELL DENSITY (cells/ml.)
1	120	0	5×10^5
2	24	96	1×10^5
3	12	108	5×10^4
4	2.4	117.6	1×10^4
5	0	120	0

Duplicate populations were used for each treatment. Treatment V was a control of filtered seawater alone. Another control consisted of an identical experimental setup receiving $5X10^4$ cells/ml, but lacking animals.

Each experimental population had 100 animals with initial mean shell length of 7.62 mm and a mean whole wet weight of 0.10 g. Each experimental population, regardless of feed density, received the same total flow rate, 120 ml/min.

The entire experimental set up was in a temperature controlled room and was covered by heavy black plastic to exclude light and to prevent the animals from reacting to shadows.

During the experimental period, the salinities ranged from 24 to 29ppt. and the water temperatures varied from 23°C to 28°C.

Sampling Shellfish Effluents

At 10 a.m. each morning, the shellfish culturing area was quietly approached so as not to cause the clams to close their shells and cease normal activities. The curtains covering the shellfish area did not have to be opened because the effluent lines were easily accessible outside the curtained area. The last 15 to 30 cm of the effluent tube was wiped with a clean paper towel. The end of the tube was then put into the sample container (3.79 l capacity) and the container filled. The effluent from each individual shellfish culture was collected at the same time as were samples of the filtered seawater and algal culture.

Measuring Influent Flow Rates

After the shellfish effluents had been collected, the side curtains were drawn up to expose the influent lines.

Starting with the treatment receiving only algal culture, the flow rate of the algal mixture and/or the filtered seawater was individually measured. This was done by inserting the end of each line into the graduated cylinder and timing the flow for one minute. The end of the tube was held at exactly the same level as it was during normal operations in order to accurately measure the flow rate actually being presented to the shellfish. In addition, the end of the tube was never immersed in the liquid accumulating in the graduated cylinder as this too

would give a false lower reading. Each measurement was done twice.

Culling

At the end of each week, after the effluents and other samples had been taken and flow rates checked, the animals were removed from their containers. They were patted dry and whole population wet weight was measured. Each population was then randomly culled back to initial whole population wet weight (10.0g). The culled animals were frozen for later analyses of weight and protein content. The animals were then returned to the experimental culture containers.

After the animals had been removed from their experimental containers, their biodeposits (tank deposits) were removed and stored for later analysis of protein content.

While the animals were out of the experimental set up, the system was inspected, any minor adjustments made and the lines rinsed out with fresh water under high pressure. The lines were rinsed with filtered seawater before the animals were put back on line.

ANALYTICAL METHODS

Cell Densities

Cell densities were measured with a Speirs-Levy Eosinophil counter.

Particulate Protein Nitrogen (PPN)

The method of Dorsey *et al*., (1977) was modified for use of the AAII. The Auto Analyzer dispensed the 1N phenol reagent and the absorbance was read on the AA colorimeter.

Soluble Protein Nitrogen (SP)

This method was developed by the author. The sample is filtered through a 47 mm Gelman glass fiber filter, 0.45 μ pore size. The procedure is run on this filtrate and any particulate matter present is considered in solution or suspension.

The protein present is precipitated by the addition of 5.0 ml of concentrated perchloric acid per 100 ml of sample. The sample is carefully mixed by swirling and cooled in a circulating water bath for

two hours at $5^{o}C$. The sample is then filtered through double 25 mm Gelman glass fiber filters (0.45 μ pore size). The sample container, filter holder and filters are washed with glass-distilled water. The precipitated protein remaining on the filters is then determined by the particulate protein nitrogen method of Dorsey, et al., (1977).

The lower limit of sensitivity of the method is determined by the reagent blank. At least 0.10 μgat. protein Nitrogen must be present on the filters. Thus a 100 ml sample with a concentration as low as 1.0 ugat. protein-N/l is sufficient for an assay.

The method is linear over the range of 1.0 to 80 μgat. protein N/l.

Standard methodologies were used to analyze Nitrate plus Nitrite (Technicon Industrial Method 43-69 w), Ammonia (Berg and Abdullah, 1977), Urea (DeManche et al, 1973), Dissolved Free Amino Acids (Coughanower and Curl, 1975) and Total Dissolved Nitrogen (D'Elia et al, 1977)

Shellfish Wet Weight, Dry Weight and Protein Content

Animals were dried by patting with a paper towel and weighed on a Mettler analytical balance H54AR (precision: $\pm$ 0.01 mg). They were then frozen for later analysis. This gave the whole wet weight value.

When needed for further analyses, the frozen animals were placed in pre-tared aluminum weighing dishes and allowed to remain at room temperature for at least two hours to ensure that all the animals were gaping. They were then dried in an oven at $70^{o}C$ for 24 hours. This gave the whole dry weight value.

No more than 5.0 g whole dry weight of clams were put into 100 ml of 1N NaOH in a 125 ml glass Erlenmeyer flask. The flask was covered and boiled at $100^{o}C$ for 100 minutes along with another flask containing a standard of Bovine Serum Albumin and another with a blank of just 1N NaOH. The flasks were swirled and allowed to come to room temperature.

Duplicate 0.5 ml aliquots were taken from each flask and put into acid-washed, glass distilled water rinsed test tubes. Protein was determined on this alkaline digest by the method of Dorsey, et al., (1977).

The supernatant from the flasks was decanted. The remaining shells were rinsed repeatedly with Glass Distilled Water (GDW) to remove all traces of NaOH. They were then dried to constant weight at $70^{o}C$

for 24 hours in a pre-tared aluminum weighing dish to obtain dry shell weight. Dry Meat weight was considered to be the difference between whole dry weight and dry shell weight.

Tank Deposits

The tank deposits accumulated over one week in each shellfish container were collected at the end of the week in a 1l polyethylene screw cap bottle and the volume was brought up to 1l with filtered seawater.

The bottle's contents were filtered onto 47 mm Gelman glass fiber filters (0.45u pore size). Different numbers of filters were necessary for different samples depending on the amount of particulates present. The filters were stored frozen for later analysis of protein content. When needed, the filters were put into a pre-tared aluminum weighing dish and dried in an oven to constant weight at 70°C for 24 hours.

The filters were digested as described for the clams and protein was determined using the method of Dorsey, et al.,(1977).

Statistical Tests

The factorial analysis of variance (ANOVA) for both independent groups and repeated measures of Edwards, (1972) was used. The Scheffe' test for pairwise differences and the one sample T-Test described by Edwards (1972) was used. The 95% confidence level was the minimum acceptable confidence level.

RESULTS

Experimental Design

Flow Rate Control

The daily variation of flow rate to any replicate shellfish population over the week never varied more than 5.4% from the weekly mean and was much less for most replicates. The variation of the actual flow rate from the intended flow rate for any replicate population never exceeded 5.4% and was frequently less.

This very small variation indicates that flow rate to any population can be considered a constant in the experiment rather than a variable.

Constancy of Incoming Algal Protein Concentrations (APC)

The algae were grown in mass outdoor continuous cultures with no control over environmental conditions. These cultures had to be serially diluted with filtered seawater to produce five different, quite distinct incoming algal protein concentrations. The mean inflow APC and standard deviation over four weeks versus the corresponding treatment number indicatesnooverlap in inflow APC among treatments (Table 2). The densest treatment had an inflow concentration of particulate protein more than 300 times greater than that of the least dense treatment. The experimental design required that the particulate protein was presented to the shellfish populations in precise steps of concentration. The inflow APC of treatment 1 was almost exactly 5 times that of treatment 2, as intended. The inflow APC of treatment 3 was 4.48 times that of treatment 4 whereas, a factor of 5 was planned. As designed, treatment 2 was almost exactly twice (1.97 times) that of treatment 3.

Controls

There were two kinds of controls in the experiment. One was a control shellfish population that received filtered seawater only. The other was an experimental setup receiving 5.75 μgat. PPN/liter, but having no animals.

The concentration of particulate protein presented to the control animals receiving only filtered seawater was an order of magnitude

Table 2 Inflow Algal Protein Concentrations of Experimental Treatments (μgat. N/l)

Table 2

TREATMENT I	TREATMENT II	TREATMENT III	TREATMENT IV	TREATMENT V	WEEK
49.633	10.056	5.109	1.152	0.162	1
54.997	11.119	5.635	1.247	0.150	2
65.499	13.216	6.680	1.452	0.1445	3
53.914	10,947	5.577	1.280	0.206	4
56.011 ± 6.74	11.335 ± 1.338	5.750 ± 0.66	1.283 ± 0.125	0.166 ± 0.028	AVERAGE ± STANDARD DEVIATION

less than the lowest feed density receiving Isochrysis sp.. There was an increase of only 0.03 g whole dry weight for the entire experimental period of 28 days by these control animals.

The purpose of the second control was to determine if there were any changes in concentration of any nitrogen-containing compound due to the experimental set-up alone.

There was an average weekly inflow of 141.0 mg of algal protein to this control treatment. Only 2.1% of this settled out to be recovered as tank deposit PPN. Thus, settling out of algal cells due to the experimental set up was not significant.

A T-test was used to determine if the change in concentration (difference between inflow and outflow concentrations) of a compound was statistically significant at the 95% confidence level. The change in concentration of all the nitrogen containing compounds were so small that they were not significant at the 95% confidence level due to the experimental set-up.

Experimental Alga

The choice of the Tahitian strain of Isochrysis sp. was a good one. It alone supported the growth of the juvenile clams of this study from spatfall on with no mortalities. Feeding rates and growth were very good using this algae alone. This was to be expected since recent studies have shown Isochrysis to be an excellent food organism for bivalves (Epifanio, 1979a, 1979b)

Nitrogen Balance

Overall Nitrogen Balance

A nitrogen balance is an accounting of all the nitrogen containing compounds entering and leaving a system. In the present study, the concentration of a number of nitrogenous compounds flowing into and from the experimental groups of shellfish was determined. The total amount of nitrogen going to each group of shellfish was determined by summing the inflow amounts (in mg nitrogen) of particulate protein, nitrite ion, nitrate ion, ammonium ion/ammonia, dissolved free amino

acids, urea and soluble protein. The total amount of nitrogen leaving each group of shellfish was determined in a similar manner except that the protein of the biodeposits (tank deposits) and the protein gain of the shellfish themselves were added to this total.

The fraction of the total inflow nitrogen accounted for was determined by the calculation: $100 - \frac{(N\ in - N\ out) \times 100}{N\ in}$

A summary of these calculations for each of the experimental treatments are shown in table 3 .

Total Dissolved Nitrogen was determined in all influents and effluents. This analysis measures all dissolved nitrogen regardless of its form. Strong oxidizing agents and high temperatures and pressures (via autoclaving) oxidize all N-containing compounds to nitrate ion which is then assayed directly.

Thus, a different nitrogen balance can be constructed using PPN and TDN only. The percent of inflow nitrogen accounted for when using PPN and TDN only is very constant (see table 4).

That fraction of the total nitrogen accounted for by each individual chemistry is in table 5 . PPN of the outflow includes the PPN of the Tank Deposits (biodeposits) and the PPN of the gain in protein of the shellfish.

Individual N-Containing Compounds

Ammonia

The general pattern of ammonia excretion was an increase in ammonia-N excretion with increasing inflow Algal Protein Concentration (APC). Maximum excretion of ammonia-N was noted for those shellfish receiving an inflow APC of 5.75 μgat. PPN/l. Further increases in inflow APC decreased ammonia excretion (table 6).

A T-Test was used to determine if the change in concentration (difference between inflow and outflow concentrations) of ammonia-N was significant at the 95% confidence level. Only the change in ammonia-N concentrations of treatment 3 was significant at the 95% confidence level. This treatment resulted in the fastest growing animals.

All experimental treatments had the same size, weight, and number

Table 3 Nitrogen Balance of Juvenile *Mercenaria campechiensis*.

Table 3

Treatment	ΣN in (mg.)/week	ΣN out (mg.)/week	Percent Accounted For
1	1761.41	1502.32	85.29%
2	485.39	425.06	87.57%
3	325.88	298.18	91.49%
4	198.28	180.63	91.10%
5	166.39	159.59	95.91%

Table 4 Nitrogen Balance Using PPN and TDN only.

Table 4

Treatment	Average Weekly Nitrogen In (mg)	Average Weekly Nitrogen Out (mg)	Accounted For(%)
1	2202.002	1983.078	90.06
2	755.488	680.079	90.02
3	574.676	521.757	90.79
4	430.024	382.847	89.26
5	393.864	351.261	89.18

Table 5 Percent of Total Nitrogen Accounted For By Individual Nitrogen Compounds.

Table 5

TRT	PPN In	PPN Out	NO_2^- In	NO_2^- Out	NO_3^- In	NO_3^- Out	$NH_4^+ + NH_3$ In	$NH_4^+ + NH_3$ Out	DFAA In	DFAA Out	UREA In	UREA Out	SP In	SP Out
1	49.9	48.9	1.6	1.8	40.1	42.2	1.1	1.1	1.8	2.2	1.7	1.4	3.8	2.6
2	36.6	30.3	1.8	2.0	46.4	50.6	2.4	4.3	3.1	4.0	5.0	5.0	4.7	3.9
3	27.7	21.1	1.9	2.1	50.6	51.3	3.3	7.6	4.1	6.0	7.2	7.3	5.2	4.6
4	10.2	7.9	2.2	3.1	58.8	58.3	5.1	7.4	5.9	8.3	11.5	8.6	6.4	6.5
5	1.6	1.9	2.3	2.5	62.9	63.8	5.9	6.5	6.8	7.7	13.7	9.9	6.9	7.6

Table 6 Average Weekly Inflow and Effluent Concentrations (μgat. N/l). (Each value represents the mean of 56 replicate samples)

Table 6

Treatment		Particulate Protein	Nitrite	Nitrate	Ammonia	DFAA	Urea	Soluble Protein	TDN
1	In	56.01	1.77	45.76	1.28	2.05	1.88	4.49	85.31
	Out	45.40	1.67	41.06	1.05	2.06	1.34	2.62	80.73
2	In	11.33	0.55	14.49	0.76	0.97	1.54	1.51	37.15
	Out	7.03	0.53	13.81	1.17	1.10	1.36	1.06	35.40
3	In	5.75	0.39	10.58	0.69	0.84	1.50	1.14	31.14
	Out	2.93	0.39	9.84	1.44	1.14	1.42	0.90	29.45
4	In	1.28	0.27	7.45	0.64	0.73	1.47	0.84	26.32
	Out	0.69	0.36	6.71	0.85	0.95	0.98	0.76	23.54
5	In	0.16	0.24	6.67	0.63	0.70	1.46	0.76	25.12
	Out	0.16	0.26	5.49	0.66	0.78	1.01	0.80	22.27

of animals at the start of the experiment. Thus, any difference in ammonia excretion rates during the first week must be due primarily to differences in feeding regime. The excretion of ammonia-N per gram of dry meat weight for the first week is maximum for those shellfish receiving an inflow APC of 5.57 µgat. PPN/l. Those shellfish receiving more or less APC had lower excretion rates (figure2).

Urea

An analysis of variance was done on urea-N concentrations of the outflows and on changes in concentrations (difference between inflow and outflow concentrations). There was no significant difference among treatments for either outflows or net concentrations. Average weekly inflow concentrations for each treatment were nearly identical (table 6).

A T-test showed that changes in urea-N concentrations were not significant at the 95% confidence level for any treatment. Thus, urea was neither excreted nor taken up in any treatment or excretion was balanced by uptake by microorganisms.

Dissolved Free Amino Acids

There was an increase in DFAA excretion with increasing inflow APC until a maximum "excretion" rate was recorded by those clams receiving an intermediate inflow APC of 5.75 µgat. PPN/l. Further increases in inflow APC resulted in a decreased excretion rate (table6).

A T-test could not detect a significant difference between the average weekly mean value of DFAA-N of inflow and effluent concentrations. In other words, there was no significant uptake or excretion of DFAA in any of the experimental treatments at the 95% confidence level.

Soluble Protein

An ANOVA showed a significant difference (at the 95% confidence level) among treatments in the difference between inflow and effluent soluble protein concentrations. There is a net uptake of solubleprotein that is greatest at the densest feed treatment and decreases with decreases in inflow APC until there is a net excretion of soluble protein by those animals receiving just filtered seawater. However, a T-test showed that the difference between the average weekly inflow and effluent concentrations

Figure 2 Weight Specific Ammonia Excretion Rates as a function of Inflow and Outflow Algal Protein Concentration.

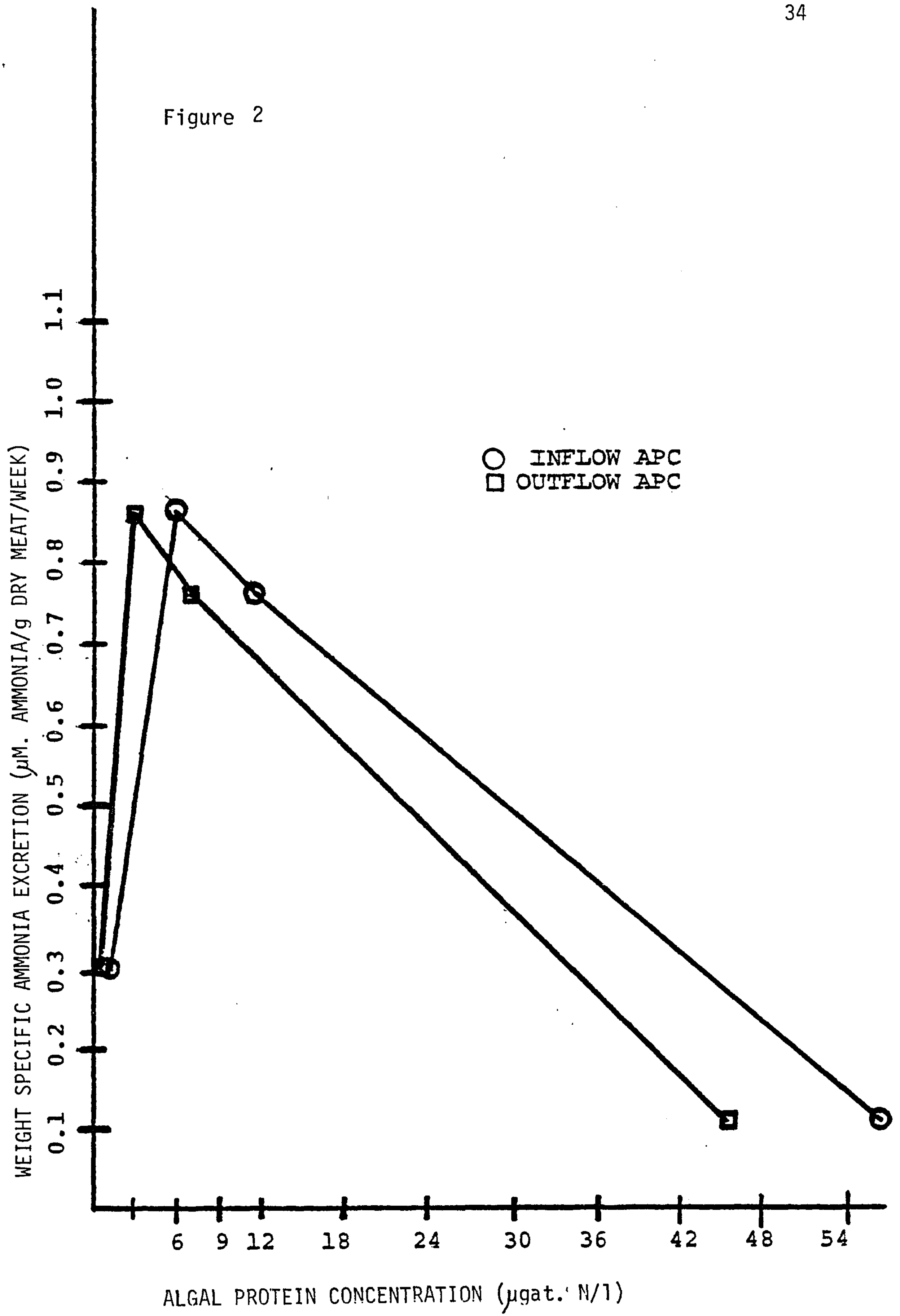


Figure 2
WEIGHT SPECIFIC AMMONIA EXCRETION (µM. AMMONIA/g DRY MEAT/WEEK)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
INFLOW APC
OUTFLOW APC
6
9
12
18
24
30
36
42
48
54
ALGAL PROTEIN CONCENTRATION (µgat. N/l)

of soluble protein for the two lowest feed densities was not significant at the 95% confidence level. In other words, there is a net uptake of soluble protein by those shellfish populations receiving an inflow APC greater than or equal to 5.75 µgat. PPN/l.

A multiple regression of the difference between inflow and effluent concentrations of soluble protein on ingested protein showed a strong (R^2=0.87) positive 2nd order relationship of the two that was significant at the 99% confidence level; $Y = 0.15 + 0.18X + 0.027X^2$

Nitrate

Most of the nitrate flowing to the shellfish came from the mass algal cultures as excess nitrate supplied to the algae. This is shown by the decrease in nitrate concentration of the inflows as the algal cultures were diluted more and more with filtered seawater to make up the different feed densities. Filtered seawater had an average of 6.7 µgat. NO_3^- - N/l.

An ANOVA indicated a significant difference at the 95% confidence level among treatments in the difference between nitrate concentrations of the inflow and effluent. However, Scheffe' tests for pairwise differences indicated that only the densest treatment showed a net change in concentration of NO_3^- which was significantly different at the 95% confidence level from the other treatments. There was a net uptake of nitrate in this treatment most likely by living algae present in the copious biodeposits of these shellfish populations (table 6).

Nitrite

The pattern of inflow nitrite concentrations indicate that most of the nitrite was coming from the algal cultures. The average weekly nitrite concentration of the filtered seawater was 0.24 µgat. N/l.

An ANOVA indicated a significant difference at the 95% confidence level among treatments in changes in nitrite concentration between inflow and effluent. However, a Scheffe' test for pairwise differences indicates that only the treatment with the greatest inflow APC was significantly different (at the 95% confidence level) from the rest in changes of nitrite concentration between inflow and effluent. There was a net uptake of nitrite in this treatment, also, most likely by living algae in the biodeposits of this treatment (table 6).

Diurnal Variations in the Nitrogen Balance

Algal Cultures

Algal protein concentrations were generally highest at the 6p.m. sampling time and lowest at 2a.m.. Concentrations of protein and nitrate were intermediate at the 10a.m. sampling time. The nitrite present in the algal cultures may be due to leakage from the algal cells as a consequence of nitrate uptake when nitrate is not limiting (S.M. Anderson, personal communication). This is supported by the presence of slightly higher nitrite concentrations at 6p.m. when more nitrate is being processed by the algal cells to produce protein (Table 7)

Ammonia concentrations in the algal cultures were very low and not very different from those of the filtered seawater entering the cultures. It would seem that ammonia is not used to any significant degree by the algae because so much nitrate was present.

The urea concentrations in the algal cultures are very low and even lower than in the filtered seawater entering the cultures which might indicate some uptake of urea by the algae.

Dissolved free amino acid (DFAA) nitrogen concentrations in the algal cultures were generally higher than those of nitrite-N, ammonia-N or urea-N. The FAA concentrations of the algal cultures at different times of the day forms no discernible pattern. There is also no discernible pattern of differences in DFAA concentrations of the filtered seawater and the algal cultures at different times of the day.

Concentrations of soluble protein tend to be higher in the algal cultures than in the filtered seawater. It would seem that the soluble protein is produced in the algal cultures. Time of day differences in soluble protein concentrations in the algal cultures are not pronounced, but concentrations tend to be highest at 2a.m. Total Dissolved Nitrogen (TDN) values are so strongly influenced by nitrate concentrations that Total Dissolved Nitrogen (TDN) concentrations follow the same time of day pattern as do nitrate concentrations.

Shellfish

There was no significant difference between inflow and outflow

Table 7

DIURNAL VARIATIONS IN CONCENTRATIONS (10^{-3} µgat. N/l)

Time	Hour
1	1000
2	1800
3	0200

FILTERED SEAWATER

DAY OF MONTH	TIME	PPN	NO_2^-	NO_3^-	NH_4^+	DFAA	UREA	SP	TDN
02	1	129	210	7193	480	0	3271	1172	31102
02	2	123	200	7352	550	0	3901	1873	34658
02	3	548	210	7756	510	640	1382	2614	34246
09	1	164	150	8524	360	583	896	0	19978
09	2	259	230	9111	290	1329	992	842	20459
09	3	230	260	9157	400	1997	1289	1606	21306
16	1	310	171	4498	751	1870	2791	130	17738
16	2	102	135	6171	720	1854	821	189	20962
16	3	63	204	6746	912	1854	572	501	28326
23	1	274	73	5741	409	1117	739	44	14511
23	2	476	677	3199	325	2720	851	165	25990
23	3	119	621	4095	1265	1587	814	344	24117

ALGAL MIXING CHAMBER

DAY OF MONTH	TIME	PPN	NO_2^-	NO_3^-	NH_4^+	DFAA	UREA	SP	TDN
02	1	49838	1800	37287	500	4163	843	1557	73174
02	2	58171	1850	29450	400	3287	1694	4470	72523
02	3	34238	1602	37509	1149	2410	1050	6530	79257
09	1	55669	1580	31187	380	946	834	1002	56029
09	2	62669	1970	22675	270	1008	631	304	52598
09	3	53458	1720	34249	270	928	984	3728	62344
16	1	48802	1041	23271	1752	8847	316	1801	49450
16	2	21723	1492	19970	1425	1391	790	1971	48561
16	3	24019	1246	39050	976	1043	765	2073	84864
23	1	61061	1632	14971	1061	1425	697	872	43263
23	2	73827	332	8316	869	626	2328	1264	22157
23	3	45552	480	1661	863	2540	923	688	29995

PPN concentrations at different times of day at the 95% confidence level. PPN net concentrations are an indicator of the amount of algae consumed. Thus, the shellfish consumed approximately the same amount of algae throughout the day. Therefore, sampling at 10a.m. gave a balanced picture of daily feeding.

The changes in concentrations between inflow and outflow of ammonia, urea, DFAA, soluble protein, nitrite, and nitrate of any treatment over the course of the day were not statistically different at the 95% confidence level.

Protein Dynamics

For case of discussion, various rates and efficiencies have been discussed as a function of inflow algal protein concentration. However, in a well-mixed, homogeneous system the animals experience an algal cell density that is the same as the effluent cell density. It is this ambient algal protein concentration that the animals respond to. In this study, all the efficiencies follow the same pattern whether as a function of inflow or effluent APC. The corresponding values for inflow and outflow APC are shown in Table 8 for ready comparison.

It should be noted, however, that these ambient APC are the result of a number of factors other than inflow APC, including the number and biomass of animals, the flow rate and the volume of the shellfish culture containers. A variation in any of these might well result in a completely different ambient APC and, consequently, different growth and feeding.

Therefore, it should be kept in mind that all these factors have been kept constant and only the inflow APC has been varied.

The following rates and efficiencies were calculated to be used as criteria in deciding which ambient APC was optimal for maximum feeding (feeding rate), maximum conversion of available food into shellfish biomass (food chain efficiency) and maximum conversion of ingested food into shellfish.

Feeding Rates

The difference between inflow and outflow concentration of particulate protein nitrogen is a measure of the feeding rate of the shellfish

Table 8 Inflow and Outflow Algal Protein Concentrations.

Table 8

Treatment	Inflow APC (μgat. PPN/l)	Outflow APC (μgat. PPN/l)
1	56.01	45.40
2	11.33	7.03
3	5.75	2.93
4	1.28	0.69
5	0.17	0.17

(Tenore, et al., 1973). The average weekly feeding rate measured as the percentage of inflow PPN stripped from the water by the clams, will be called Stripping Efficiency. As the inflow concentration of Protein-N increases from 1.3 µgat. N/l to 5.75 µgat. N/l there is an increase in the Stripping Efficiency. The maximum average weekly Stripping Efficiency of 48.6% is reached by those clams receiving an inflow protein concentration of 5.75 µgat. PPN/l. There is then a sharp decline in Stripping Efficiency with further increases in the concentration of protein available (figure 3).

The average weekly feeding rate, measured as the percentage of inflow PPN removed from the water by ingestion and/or biodeposition will be called Retention Efficiency. Retention Efficiency will necessarily be lower than Stripping Efficiency since there will be a greater difference in inflow and outflow concentrations when the protein of the tank deposits (biodeposits) are considered.

The relationship of Protein Retention Efficiency to inflow APC is non-linear. There is an initial slight increase in Protein Retention Efficiency with increasing inflow APC and then a precipitous decline with further increases in inflow APC (figure 4).

Ingested Ration

The absolute amount of algae ingested was measured as the difference between inflow and outflow particulate protein nitrogen (PPN) concentrations, corrected for the PPN of the tank deposits (biodeposits).

Ingested ration is positively correlated with both inflow and outflow PPN concentrations (figures 5,6). There is little indication that a plateau will be reached where ingestion is constant and independent of algal cell concentration.

Food Chain Efficiency

Food chain efficiency is a measure of the efficiency with which the food supply is exploited and is the ratio of net production (growth) to available food, whether or not the food is ingested (Tenore, et al., 1973). For this work, food chain efficiency, in terms of protein is:

$$\frac{\Delta \text{ Clam Protein/Week}}{\text{Inflow Particulate Protein/Week}} \times 100\%$$

Figure 3 Protein Stripping Efficiency as a function of inflow and outflow algal protein concentration.

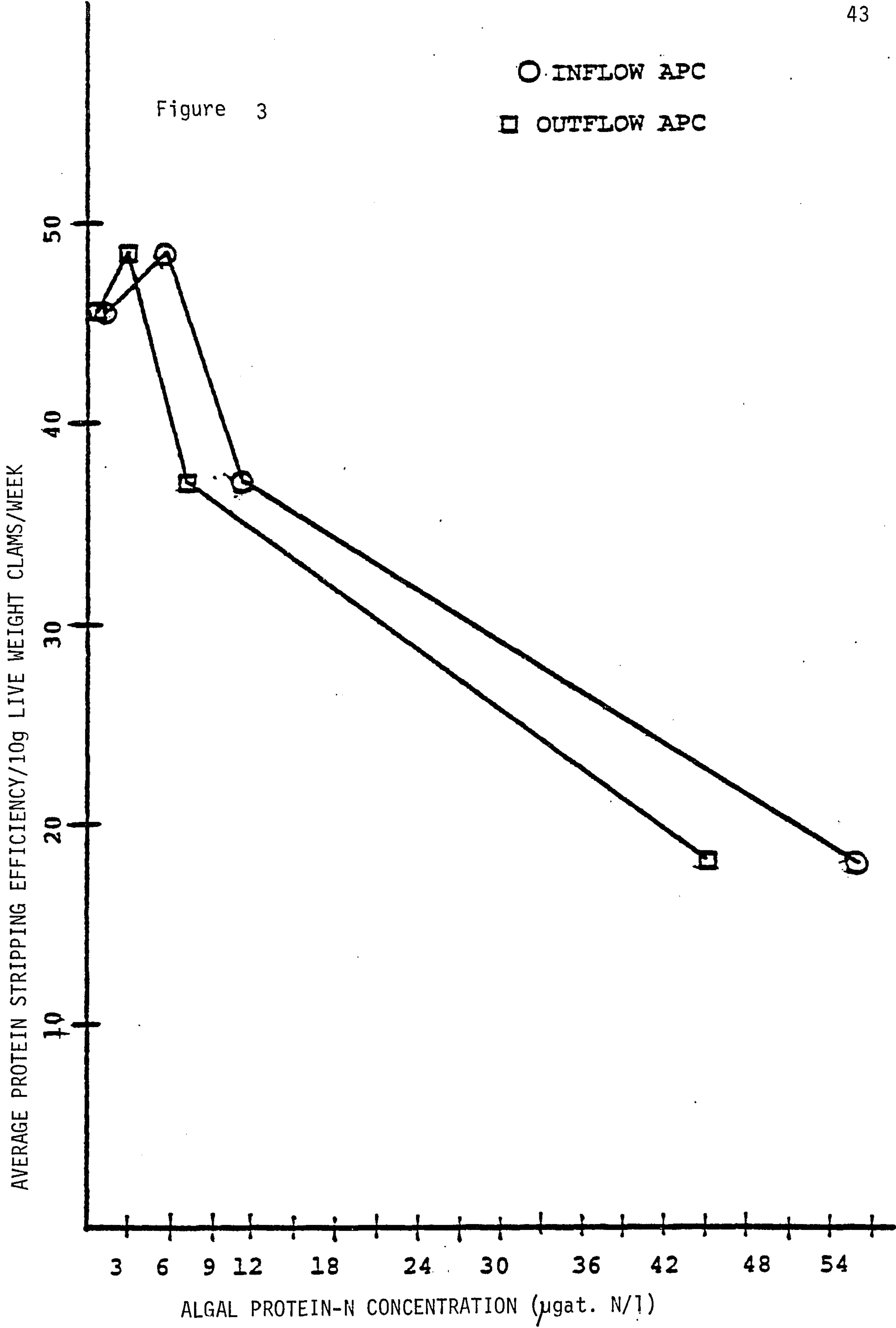


Figure 3
O INFLOW APC
□ OUTFLOW APC
AVERAGE PROTEIN STRIPPING EFFICIENCY/10g LIVE WEIGHT CLAMS/WEEK
10
20
30
40
50
3 6 9 12 18 24 30 36 42 48 54
ALGAL PROTEIN-N CONCENTRATION (μgat. N/l)

Figure 4 Protein Retention Efficiency as a function of inflow and outflow algal protein concentration.

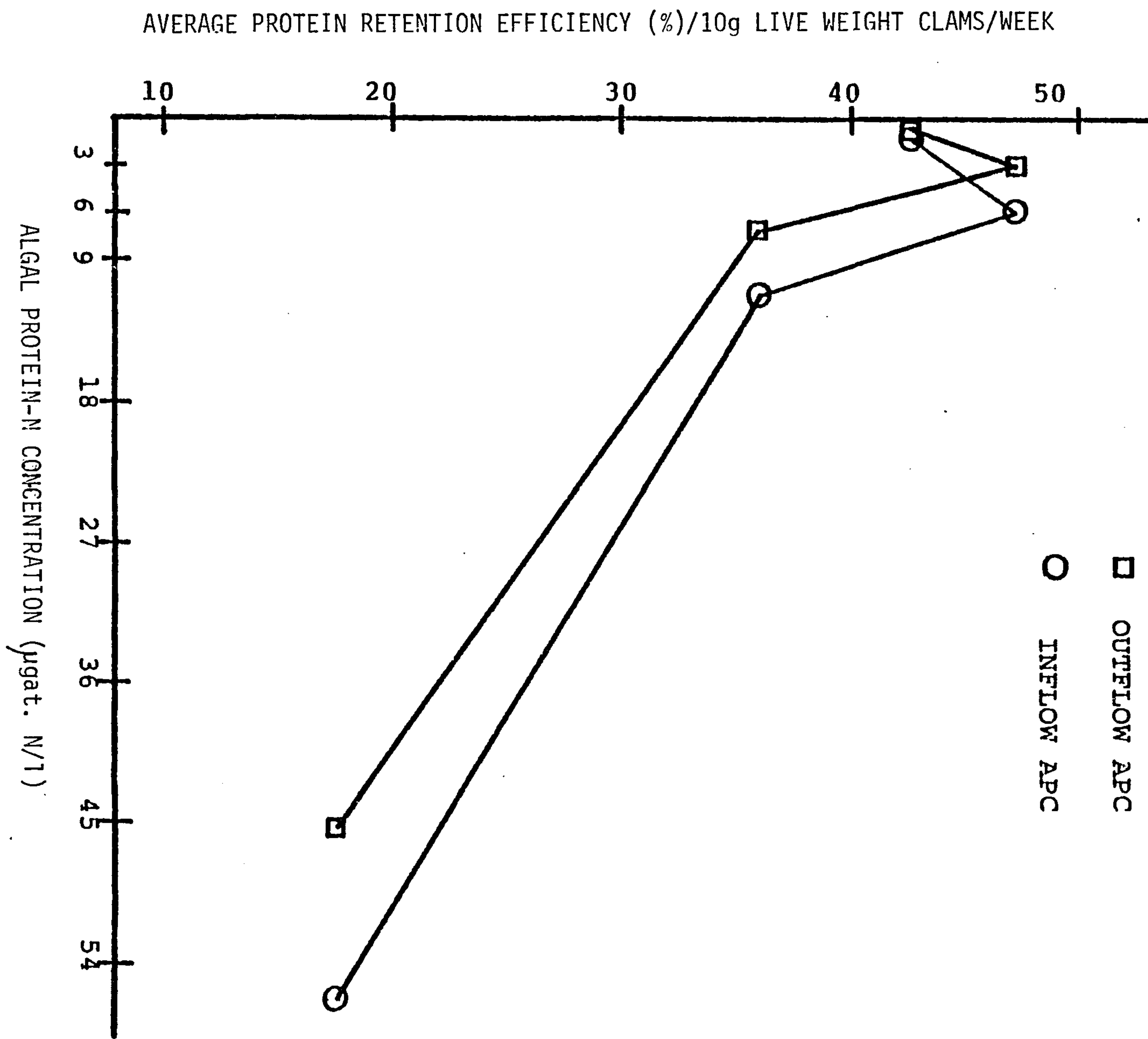


Figure 4

Figure 5 Ingested Algal Protein as a Function of Inflow Algal Protein Concentration.

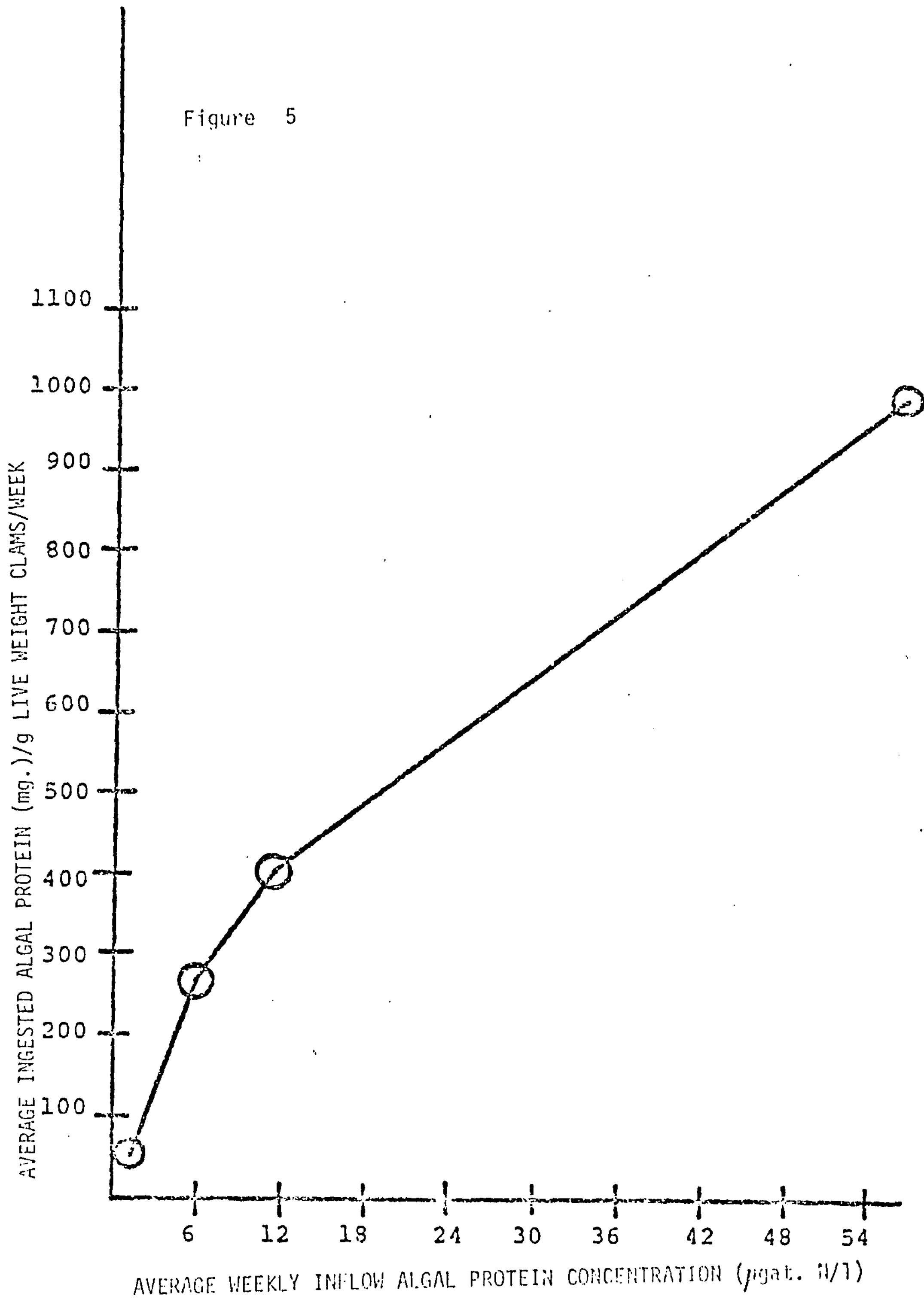
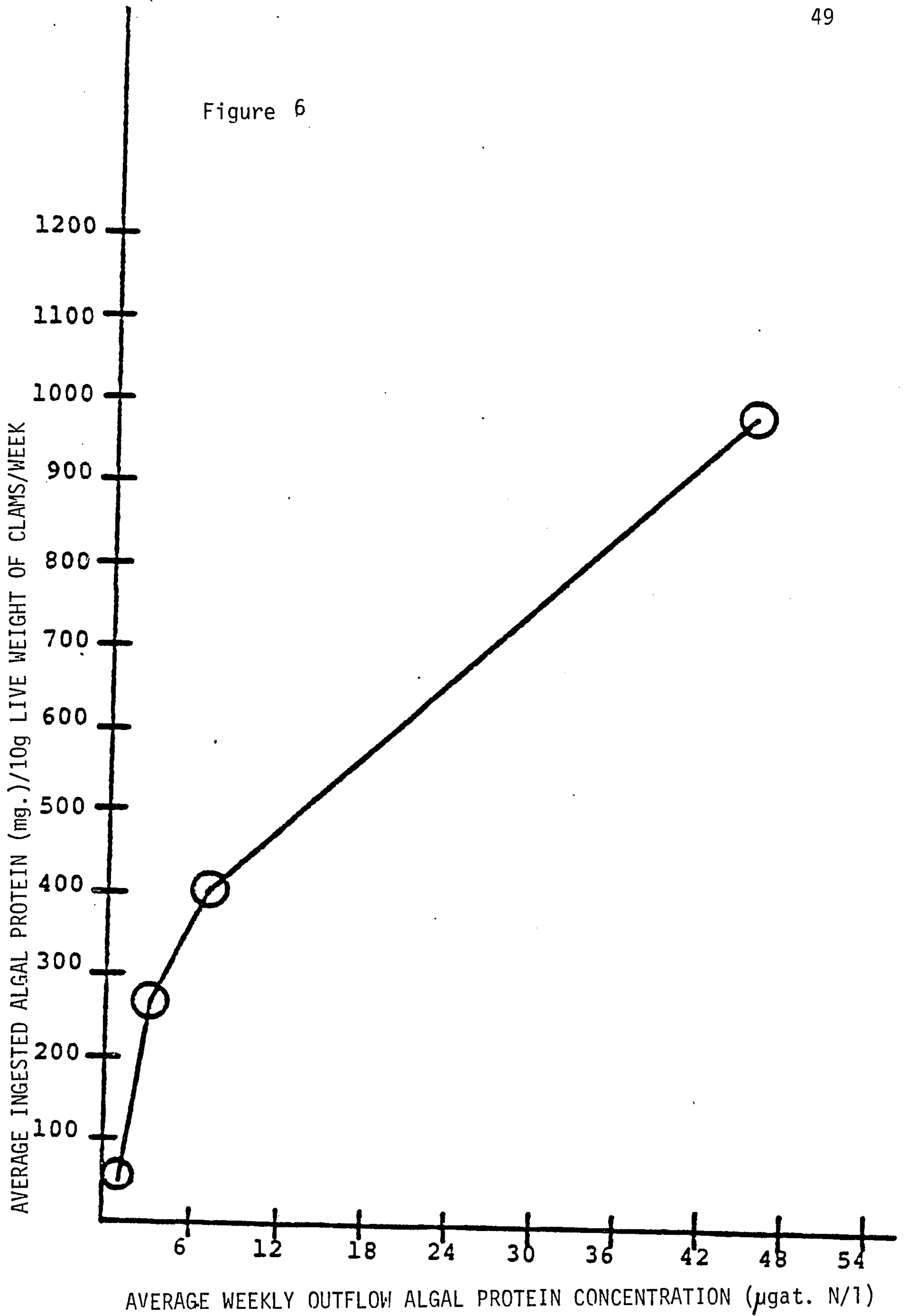


Figure 5
1100
1000
900
800
700
600
500
400
300
200
100
AVERAGE INGESTED ALGAL PROTEIN (mg.)/g LIVE WEIGHT CLAMS/WEEK
6
12
18
24
30
36
42
48
54
AVERAGE WEEKLY INFLOW ALGAL PROTEIN CONCENTRATION (μgat. N/l)

Figure 6 Ingested Algal Protein as a Function of Outflow Algal Protein Concentration.

Figure 6



There is an increase in food chain efficiency with increasing inflow APC until a maximum weekly average food chain efficiency of 16.89% is achieved by those clams receiving an inflow APC of 5.75 μgat. PPN/l. Thereafter, there is a sharp decline in food chain efficiency with increasing APC (figure 7).

These results clearly indicate that those animals receiving an inflow APC of 5.75 μgat. PPN/l convert available protein most efficiently into shellfish protein. A better measure of the amount of available food may be the amount of algal protein surrounding the animal and the outflow APC is the best measure of this surrounding algal protein concentration. Therefore, a better measure of food chain efficiency may be calculated as:

$$\frac{\Delta \text{ Clam Protein/Week}}{\text{Outflow APC/Week}}$$

The pattern of food chain efficiencies when calculated this way is the same as when calculated in the above manner, but the values are appreciably higher (table 9) with the maximum food chain efficiency being 33.68% or about twice the maximum food chain efficiency of 16.89% calculated in the above manner.

Table 9 Food Chain Efficiency Based on Outflow APC

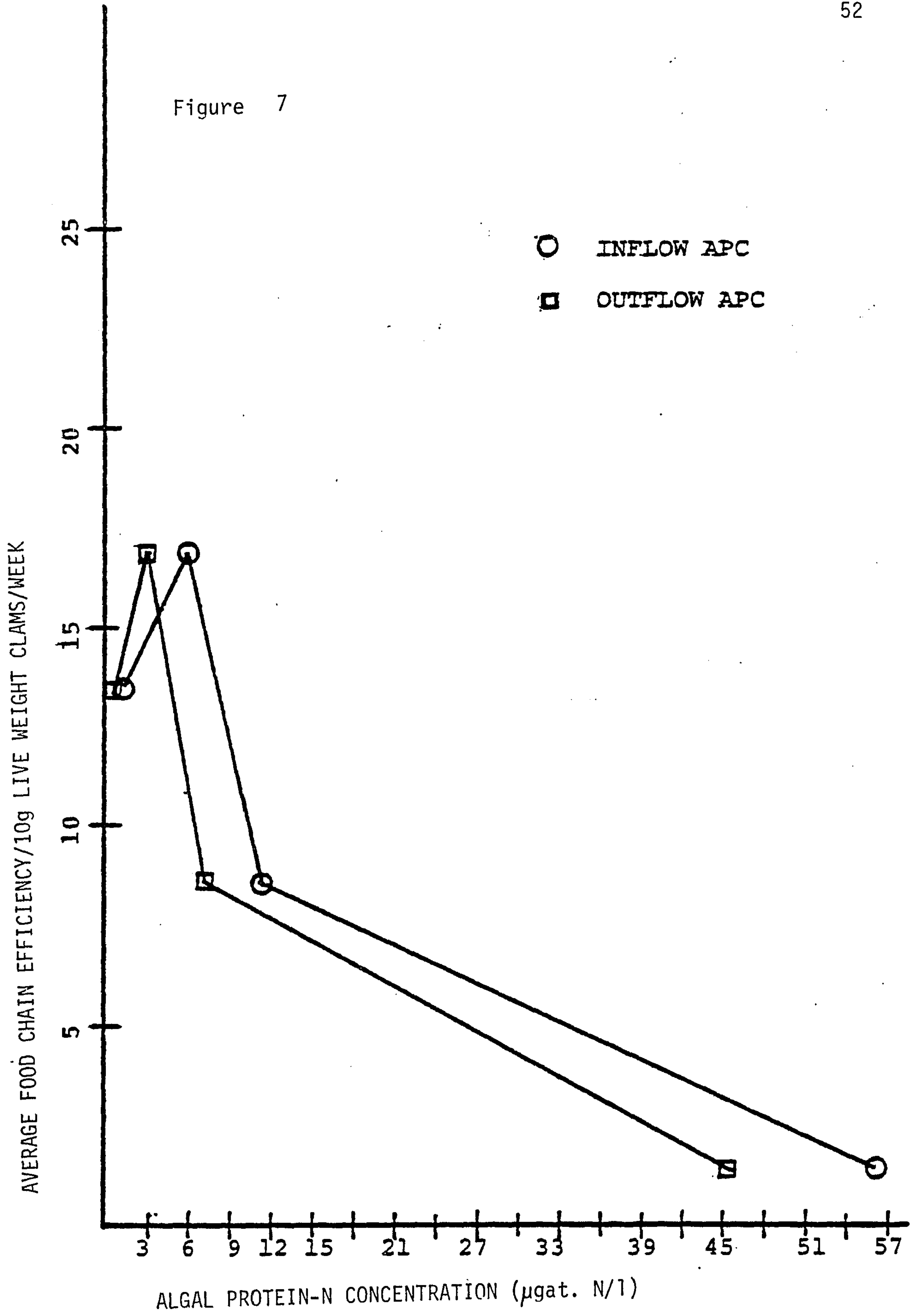
Ambient APC (μgat. PPN/l)	Food Chain Efficiency (%)
0.69	25.46
2.93	33.68
7.03	14.53
45.40	1.78

Ecological Efficiency

Ecological efficiency, as defined by Tenore, et al., (1973), is the ratio of net production (growth) to ingested food by the population. This ratio is equivalent to the Coefficient of Growth Efficiency of the IBP

Figure 7 Food Chain Efficiency as a function of inflow and outflow algal protein concentration.

Figure 7



(Holme and McIntyre, 1971) or, most properly, protein conversion rate, since the protein of the prey and predator are used in its calculation. In my study, food ingested, in terms of protein, is defined as the difference between ingoing and outgoing protein levels. A gross ecological efficiency is defined as:

$$\frac{\text{Average}\,\Delta\,\text{Clam Protein}}{\text{Average Stripped PPN}} \times 100\%$$

A net ecological efficiency is defined as:

$$\frac{\text{Average}\,\Delta\,\text{Clam Protein}}{\text{Average Retained PPN}} \times 100\%$$

My net ecological efficiency corresponds most closely to K_1, gross growth efficiency. The same pattern as before is apparent (figure 8). With an increase in inflow APC there is a concomitant increased gross ecological efficiency. A maximum weekly average gross ecological efficiency of 35.10% is achieved by those clams receiving an inflow APC of 5.75 μgat. PPN/l. Thereafter, there is a rapid decrease in gross ecological efficiency with increasing inflow APC.

When net ecological efficiency is plotted versus inflow APC (figure 9), it can be seen that those animals receiving an inflow APC of 5.75 μgat. PPN/l are most efficient in converting ingested or retained algal protein into clam protein. A similar pattern is observed with outflow concentrations. The decline in efficiency with increasing inflow APC is not as great for net ecological efficiency. This would seem to be due to the great amount of tank deposits (biodeposits) associated with those animals receiving the highest inflow levels of algal protein. The amount of protein stripped is very close to the amount of protein retained for all but the densest treatment where there is proportionally less retained than stripped (figure 10). This is due to greater biodeposition at this feed density. Thus, the net ecological efficiency will be proportionally less for this densest treatment as compared to gross ecological efficiency.

Protein conversion rate is equivalent to the net ecological efficiency (protein) of my study and follows the same pattern as net ecological efficiency as a function of inflow or outflow APC (figure 11) or of ingested algal protein (figure 12).

Gross growth efficiency (K_1) as a function of ingested algal protein also follows a similar pattern (figure 13).

Figure 8 Gross Ecological Efficiency as a function of inflow and outflow algal protein concentration.

Figure 8

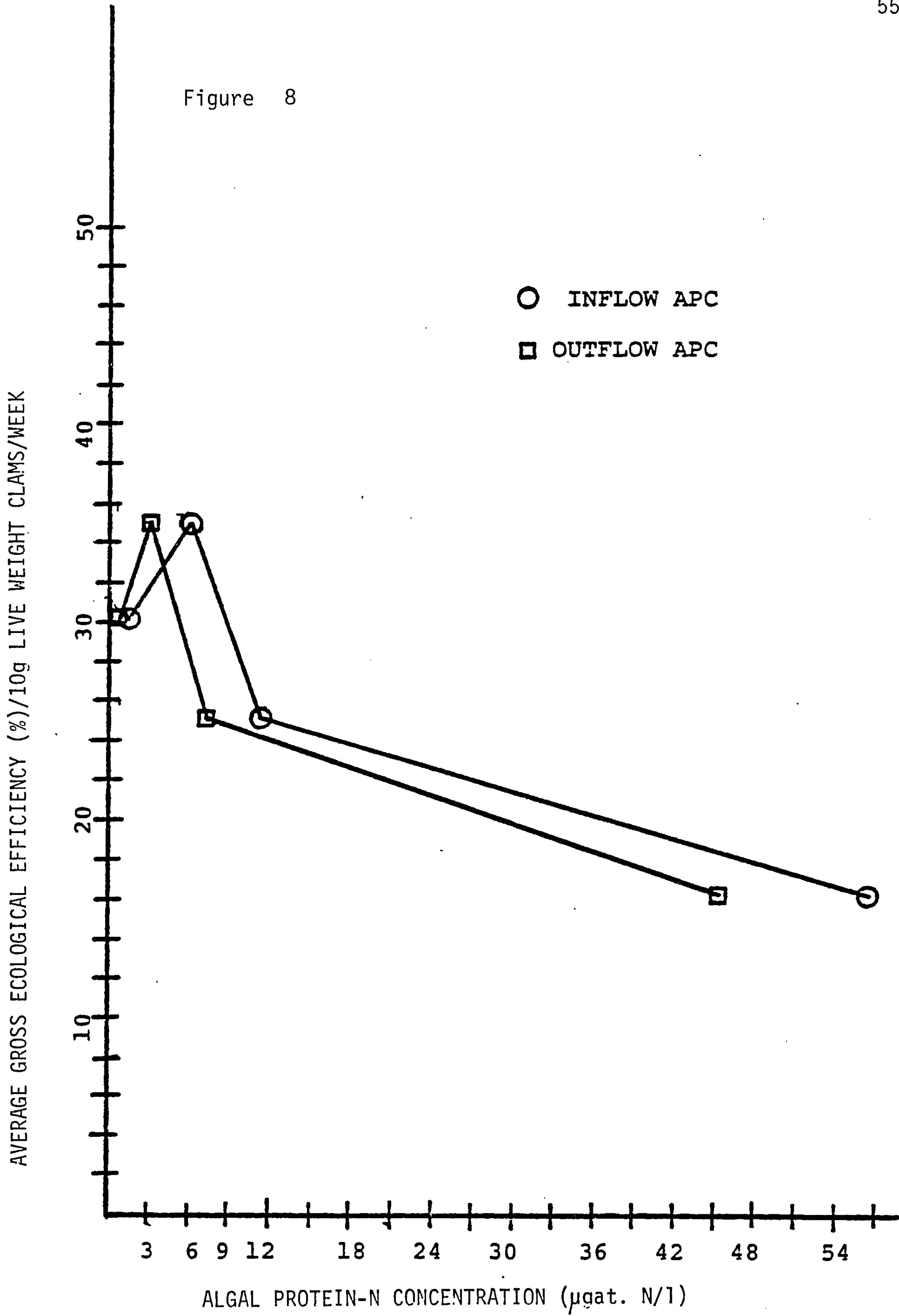


Figure 9 Net Ecological Efficiency as a function of inflow and outflow algal protein concentration.

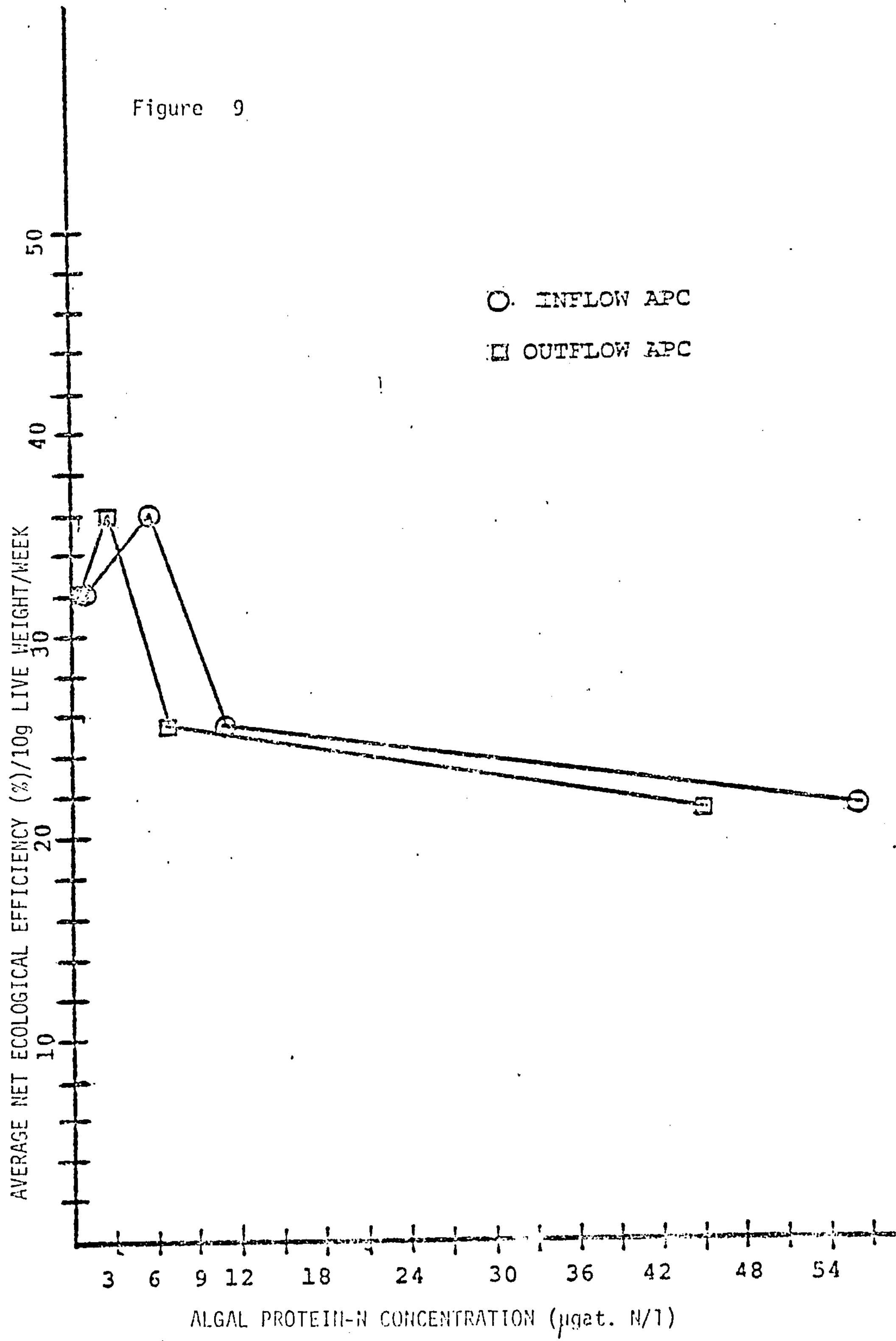


Figure 9
INFLOW APC
OUTFLOW APC
AVERAGE NET ECOLOGICAL EFFICIENCY (%)/10g LIVE WEIGHT/WEEK
50
40
30
20
10
3
6
9
12
18
24
30
36
42
48
54
ALGAL PROTEIN-N CONCENTRATION (μgat. N/l)

Figure Stripped Protein and Retained Protein of the Experimental Treatments for Week 1

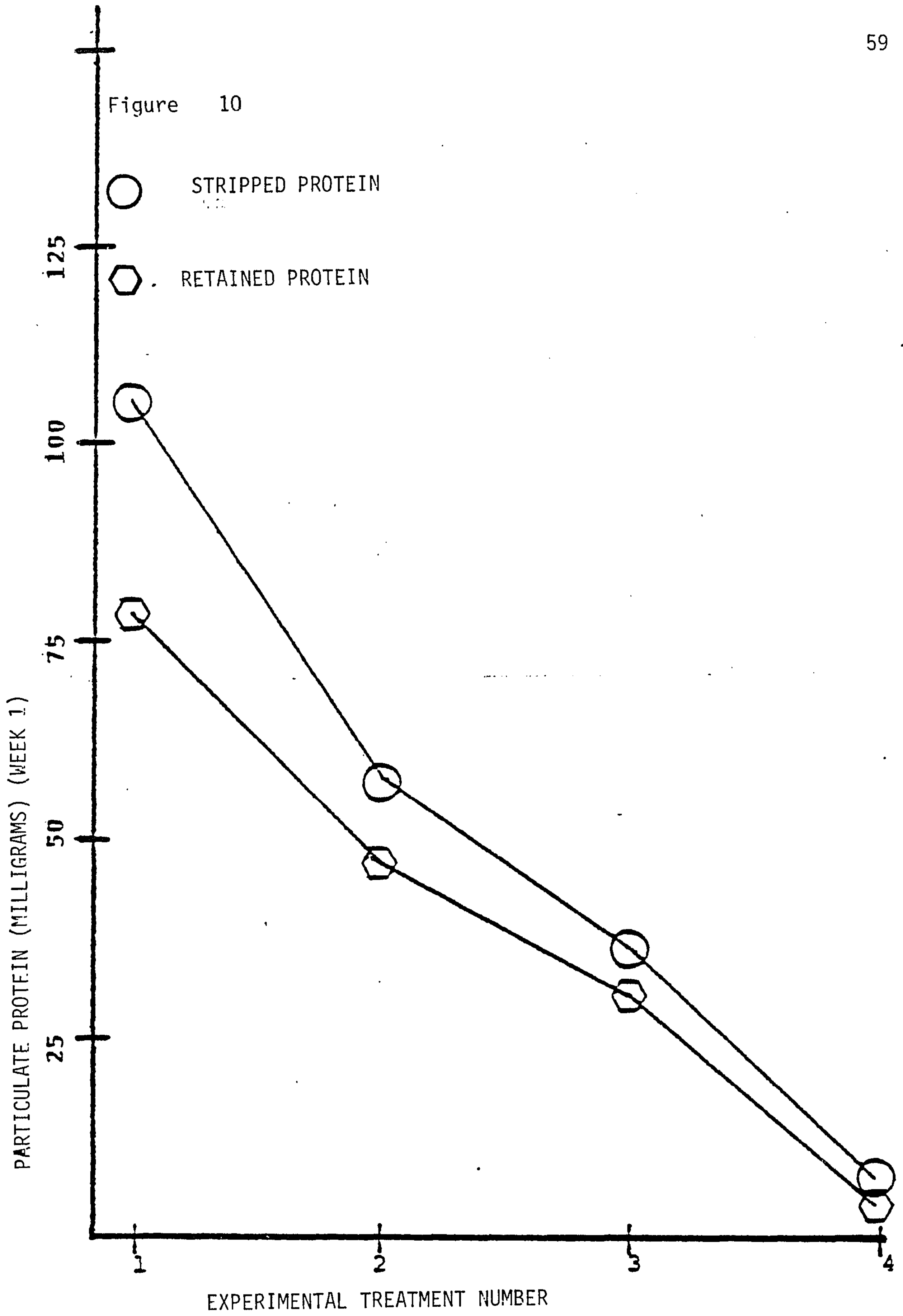


Figure 10
STRIPPED PROTEIN
RETAINED PROTEIN
PARTICULATE PROTEIN (MILLIGRAMS) (WEEK 1)
25
50
75
100
125
1
2
3
4
EXPERIMENTAL TREATMENT NUMBER

Figure 11 Protein Conversion Efficiency as a function of outflow algal protein concentration.

Figure 11

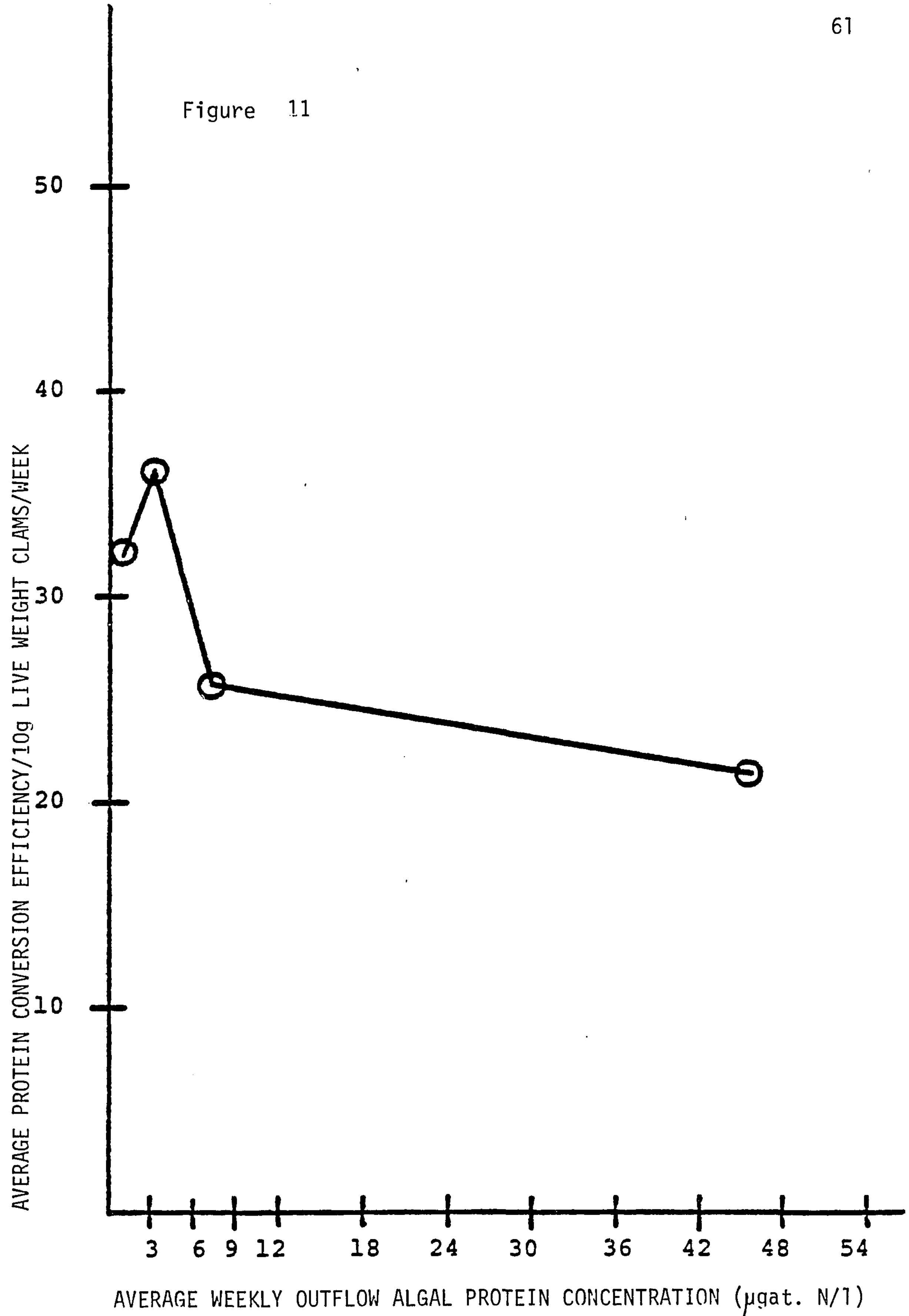


Figure 12 Protein Conversion Efficiency as a function of ingested algal protein.

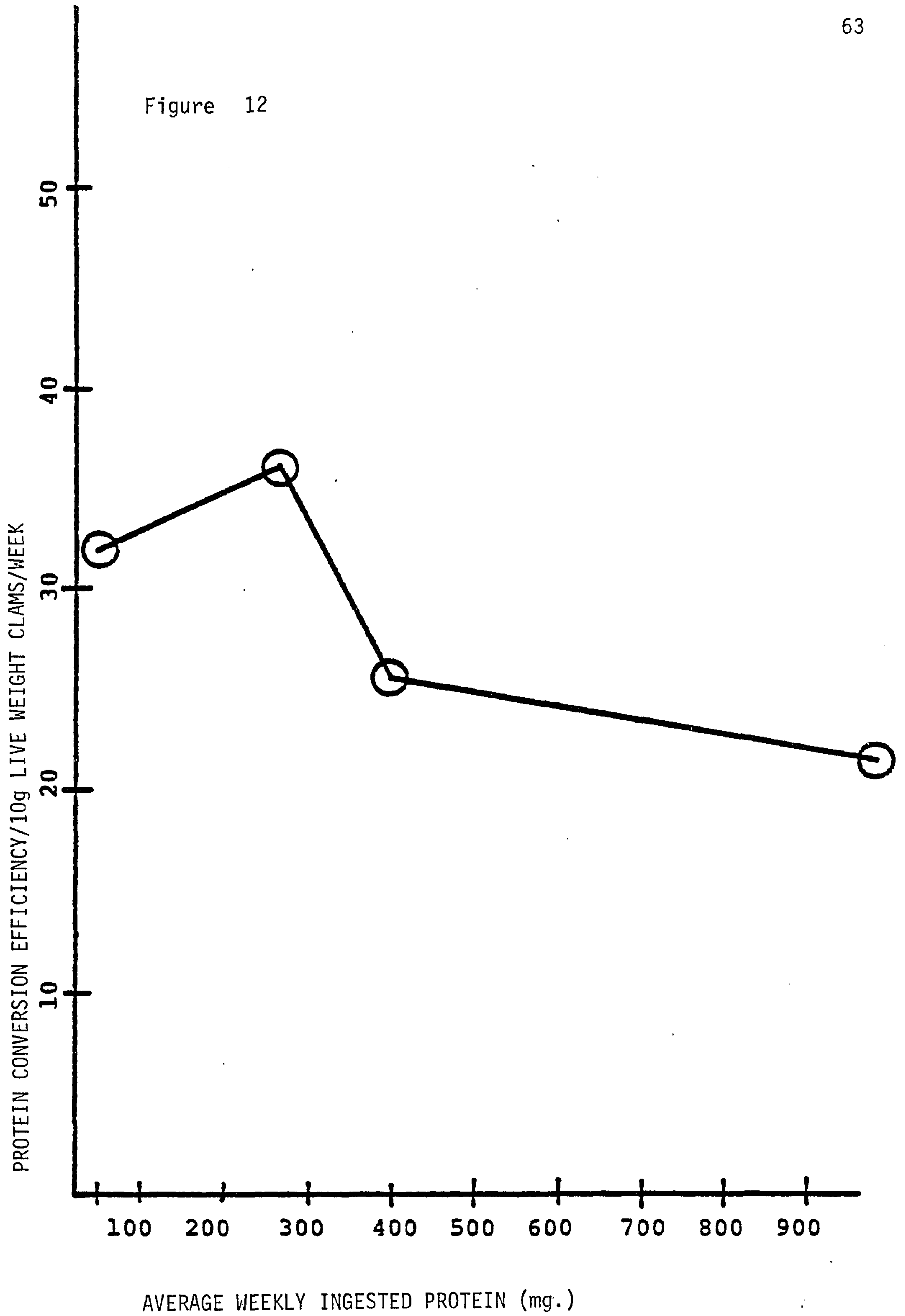


Figure 12
PROTEIN CONVERSION EFFICIENCY/10g LIVE WEIGHT CLAMS/WEEK
10
20
30
40
50
100
200
300
400
500
600
700
800
900
AVERAGE WEEKLY INGESTED PROTEIN (mg.)

Figure 13 Gross Growth Efficiency as a function of ingested protein.

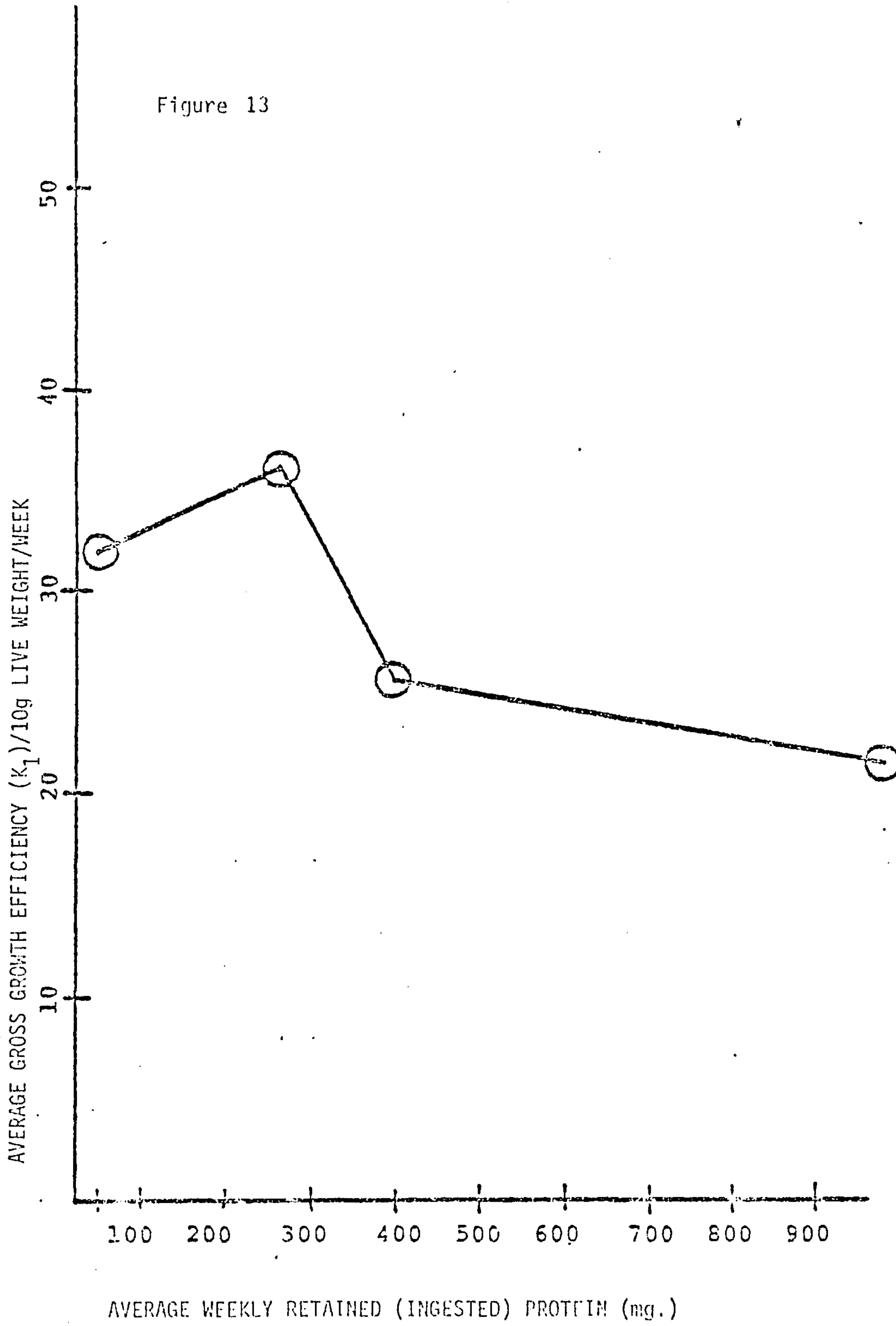


Figure 13
50
40
30
20
10
AVERAGE GROSS GROWTH EFFICIENCY (K_1)/10g LIVE WEIGHT/WEEK
100
200
300
400
500
600
700
800
900
AVERAGE WEEKLY RETAINED (INGESTED) PROTEIN (mg.)

Average Weekly Biomass Production

The pattern of weekly biomass production as a function of treatment or inflow APC is the same whether biomass is measured as whole wet weight, whole dry weight, dry meat weight, or protein (figures 14 to 17). The pattern of biomass production as a function of ingested ration is similar (figures 18 to 20). There is a very sharp increase in weekly biomass production with increasing inflow APC until maximum production is reached at an inflow APC of 5.75 μgat. PPN/l. There is then a less steep decline in weekly biomass production with increasing inflow APC. Statistically, there is a significant difference among treatments in weekly biomass production at the 99% confidence level. However, a Scheffe' test for pairwise differences showed no significant difference between the weekly biomass production of those clam populations receiving an inflow APC of 5.75 and 11.335 μgat. PPN/l. These algal protein concentrations correspond to approximately 5×10^4 cells/ml to 1×10^5 cells/ml and to an ambient APC of 2.93 μgat. PPN/l. and 7.03 μgat. PPN/l in the environment experienced by the clams. The corresponding ambient algal carbon values are 272 to 652 μg.C/l.

Individual Animal Weights

Individual animal weight is calculated by dividing the culled biomass by the number of animals culled. The general pattern of biomass per animal is that 1) the animals in every treatment are getting larger over time except for the control treatment receiving no algae, and 2) the largest animals for every week are always those receiving an inflow APC of 5.75 to 11.33 μgat.PPN/l (figure 21). This pattern was true whether biomass was measured as whole wet weight, whole dry weight, dry meat weight, or protein.

Differences in individual animal weights among treatments was significant at the 99.9% confidence level. However, a Scheffe' test for pairwise differences revealed no significant difference in individual animal weight between those populations receiving 5.75 and 11.33 μgat. PPN/l or experiencing 2.93 μgat. PPN/l and 7.03 μgat. PPN/l in their environment. Thus, there was the same biomass increase by those individuals receiving these different inflow APC.

Figure 14 Weekly Whole Wet Weight Production as a function of inflow and outflow algal protein concentration.

Figure 14

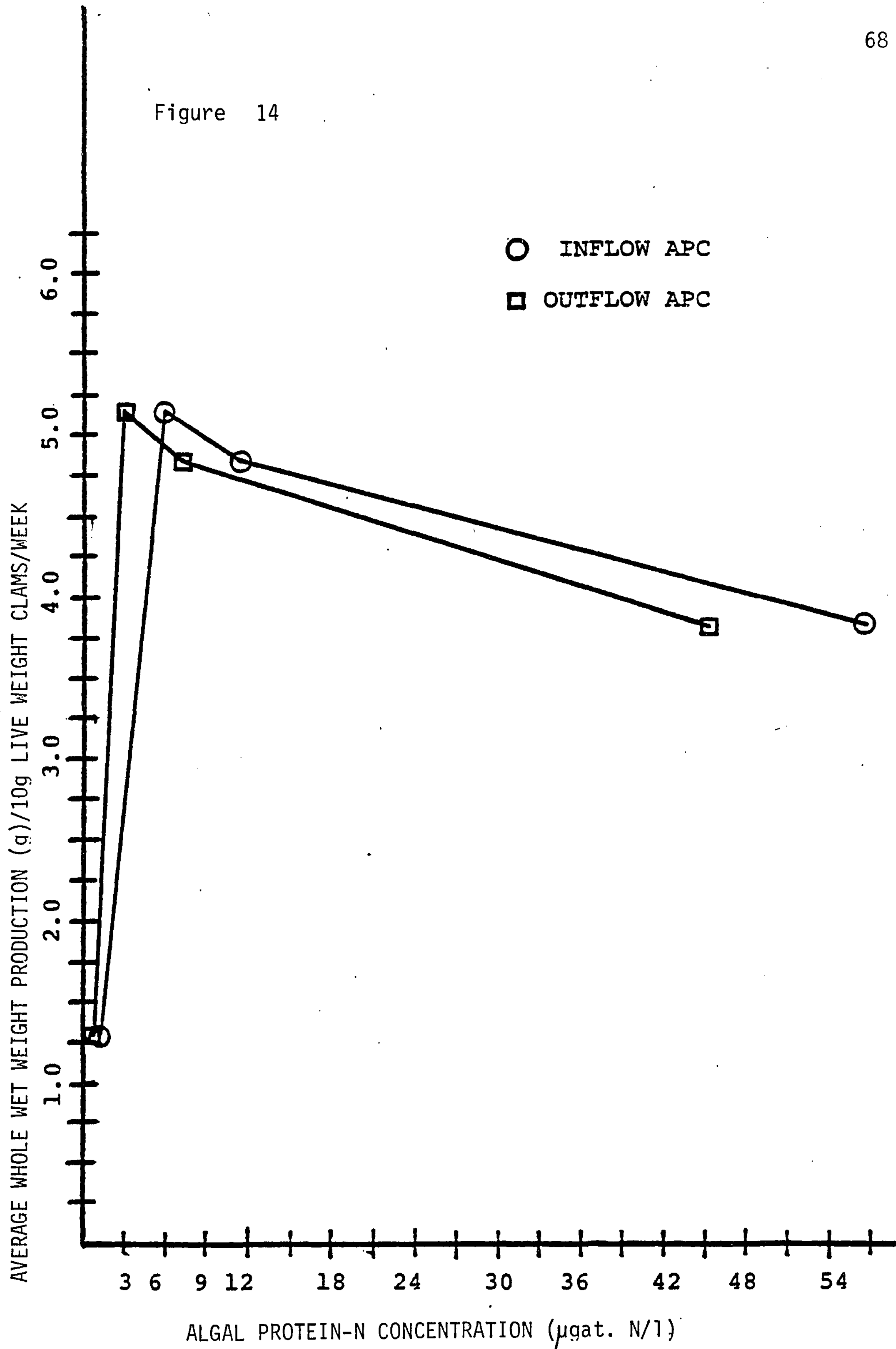


Figure 15 Weekly Whole Dry Weight Production as a function of inflow and outflow algal protein concentration.

Figure 15

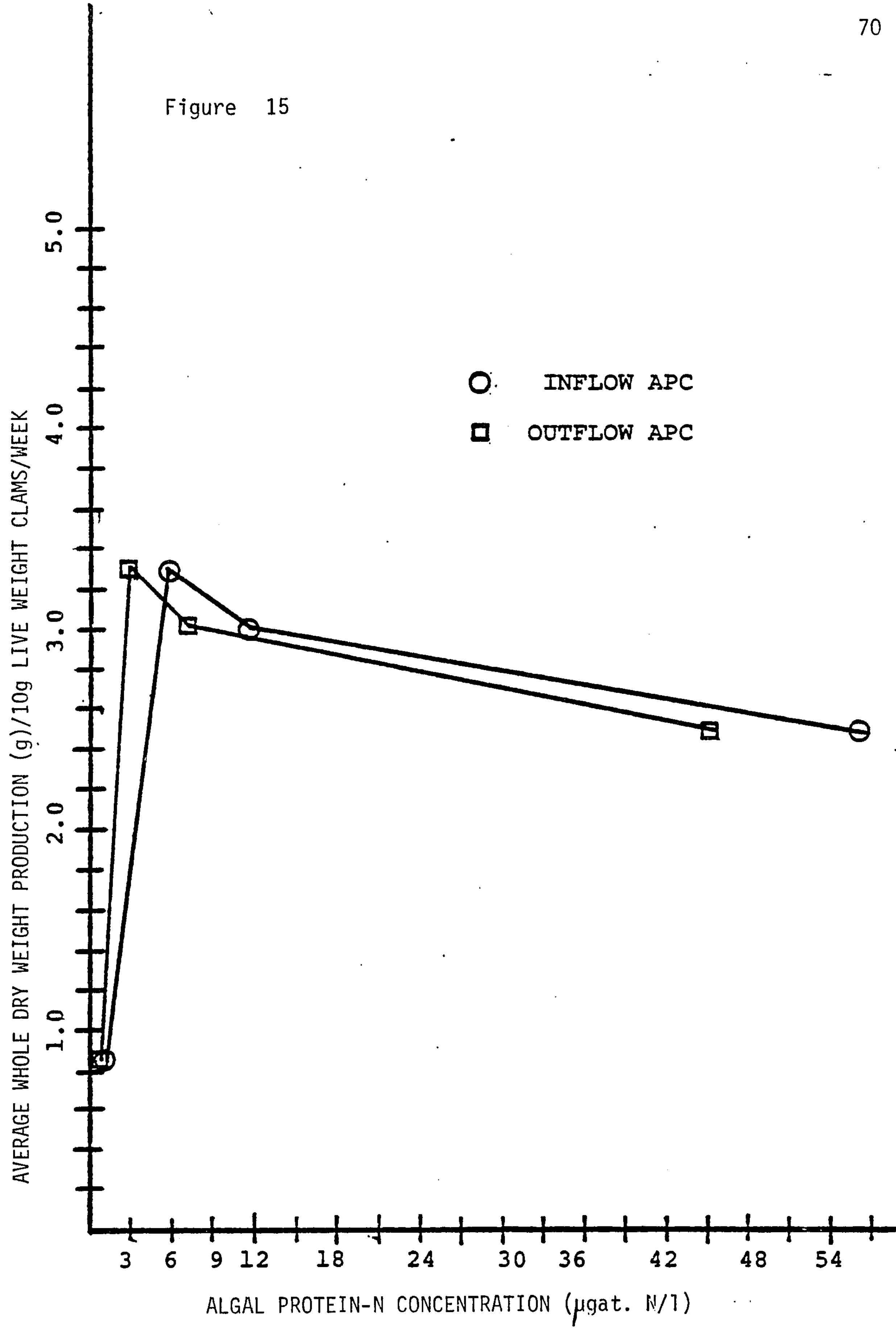


Figure 16 Weekly Dry Meat Production as a function of inflow and outflow algal protein concentration.

Figure 16

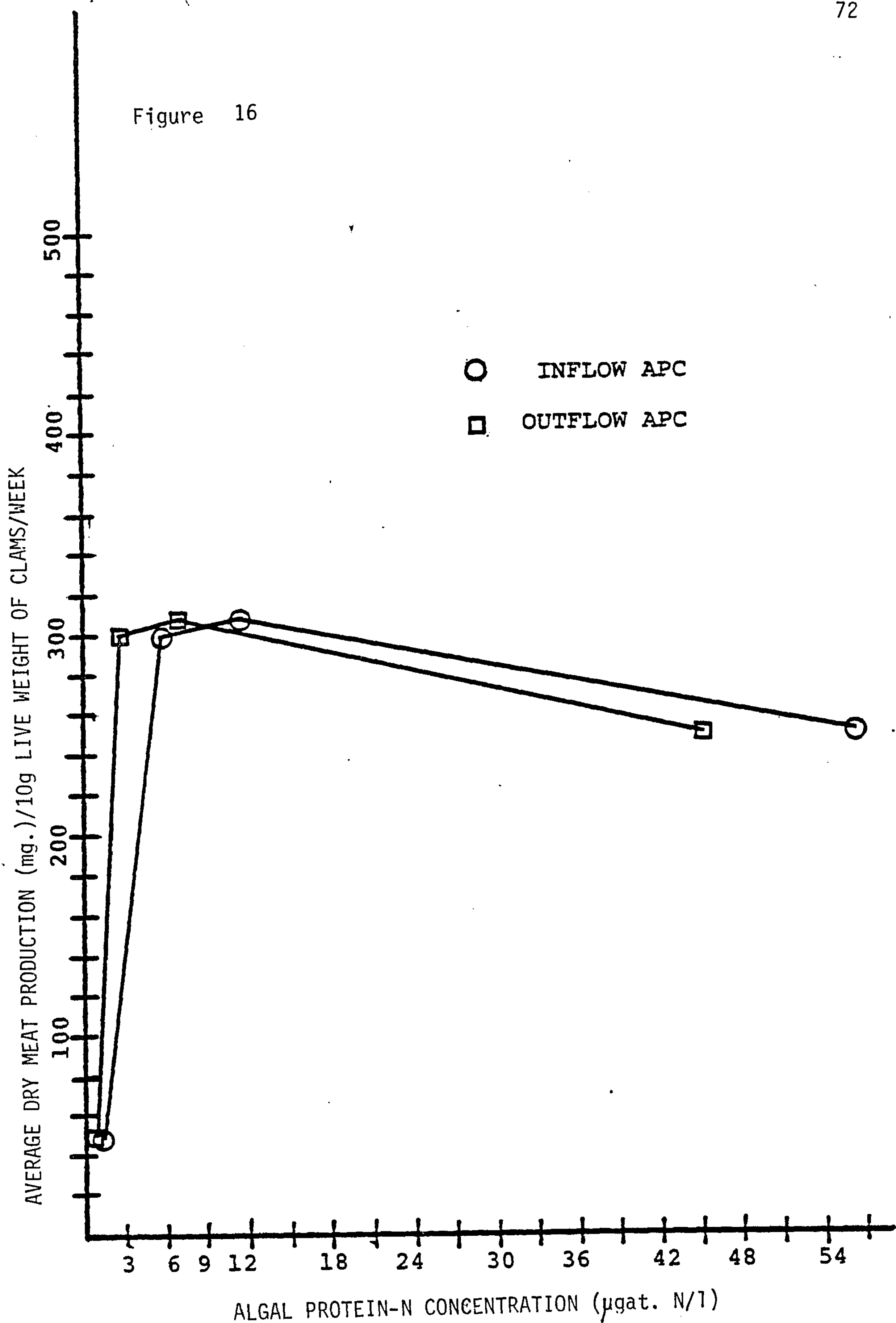


Figure 17 Weekly Protein Production as a function of inflow and outflow algal protein concentration.

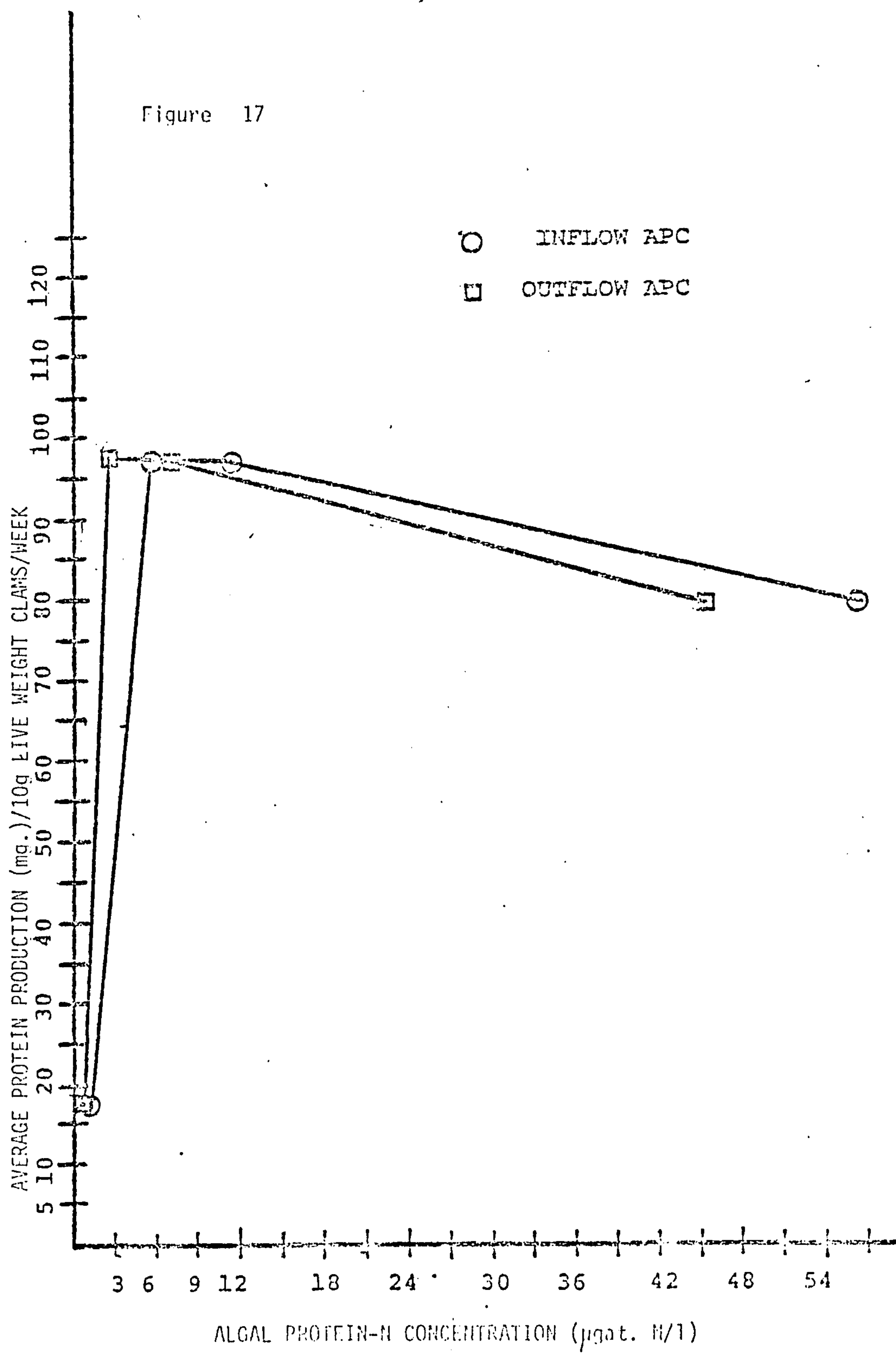


Figure 17
INFLOW APC
OUTFLOW APC
AVERAGE PROTEIN PRODUCTION (mg.)/10g LIVE WEIGHT CLAMS/WEEK
5 10 20 30 40 50 60 70 80 90 100 110 120
3 6 9 12 18 24 30 36 42 48 54
ALGAL PROTEIN-N CONCENTRATION (μgat. N/1)

Figure 18 Whole Wet Weight Production as a function of ingested algal protein.

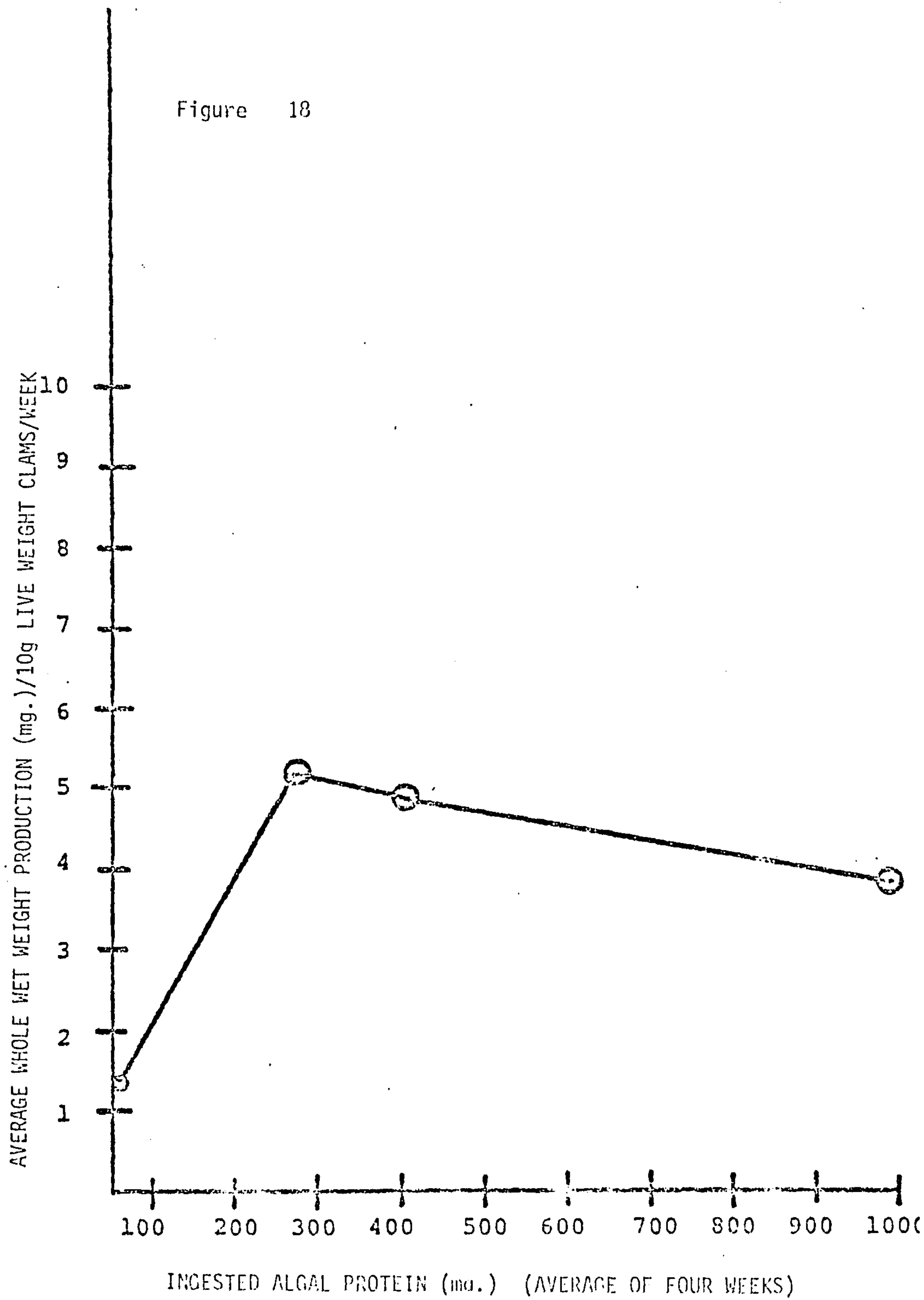


Figure 18
AVERAGE WHOLE WET WEIGHT PRODUCTION (mg.)/10g LIVE WEIGHT CLAMS/WEEK
1
2
3
4
5
6
7
8
9
10
100
200
300
400
500
600
700
800
900
1000
INGESTED ALGAL PROTEIN (mg.) (AVERAGE OF FOUR WEEKS)

Figure 19 Dry Meat Production as a function of Ingested Algal Protein.

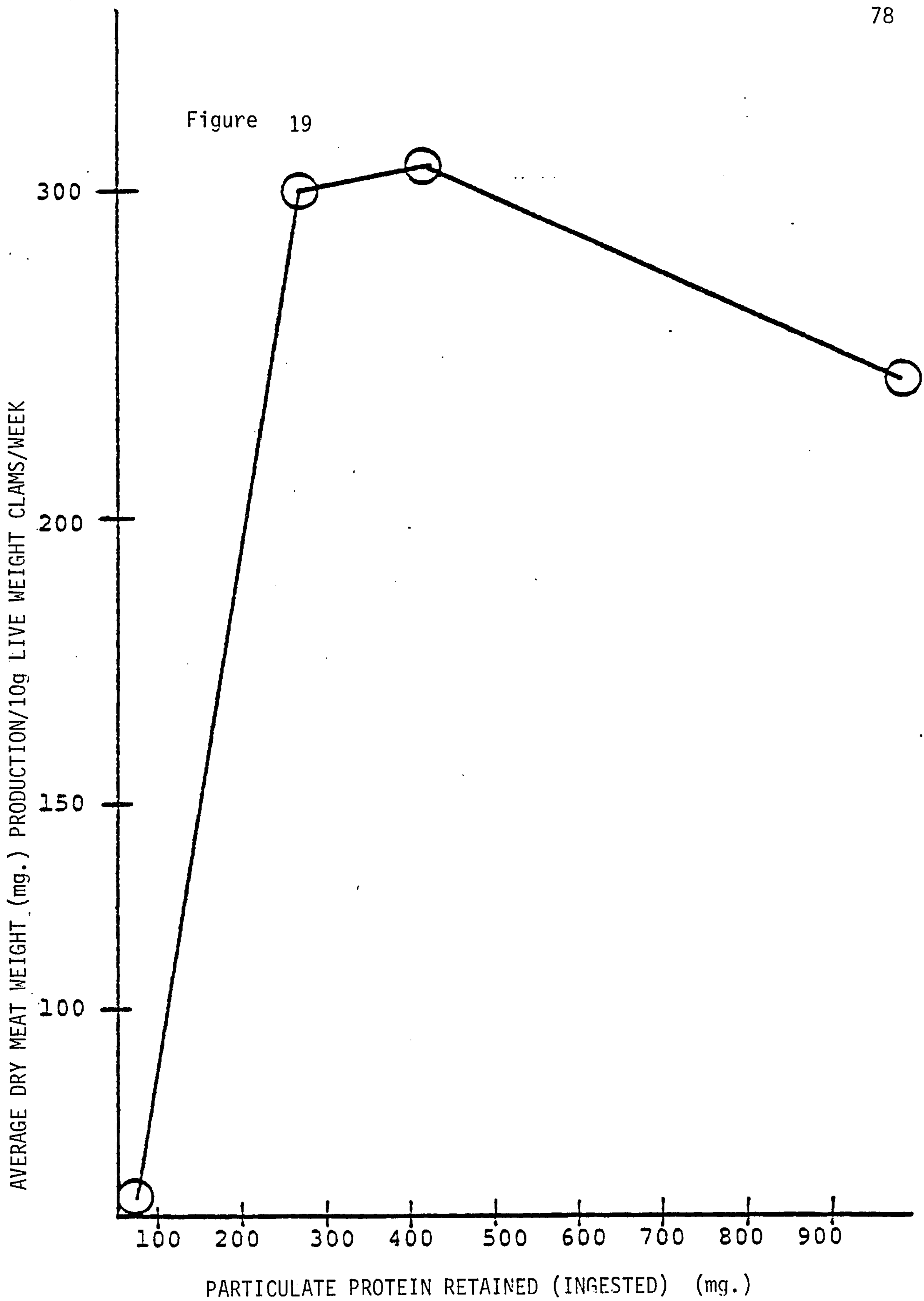


Figure 19
300
200
150
100
AVERAGE DRY MEAT WEIGHT (mg.) PRODUCTION/10g LIVE WEIGHT CLAMS/WEEK
100
200
300
400
500
600
700
800
900
PARTICULATE PROTEIN RETAINED (INGESTED) (mg.)

Figure 20 Protein Production as a function of ingested algal protein.

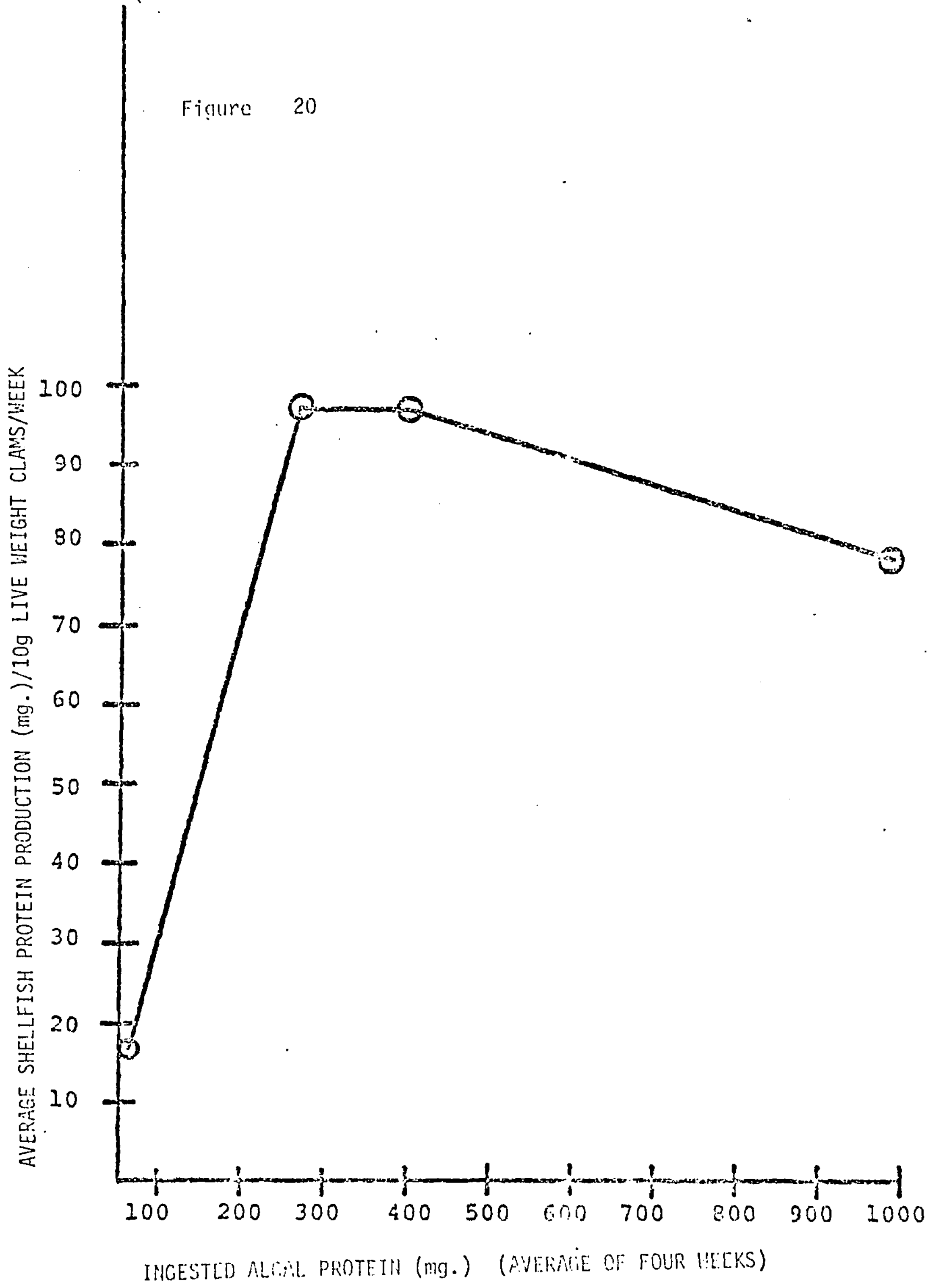


Figure 20
AVERAGE SHELLFISH PROTEIN PRODUCTION (mg.)/10g LIVE WEIGHT CLAMS/WEEK
10
20
30
40
50
60
70
80
90
100
100
200
300
400
500
600
700
800
900
1000
INGESTED ALGAL PROTEIN (mg.) (AVERAGE OF FOUR WEEKS)

Figure 21 Whole Dry Weight Increases Over Time.

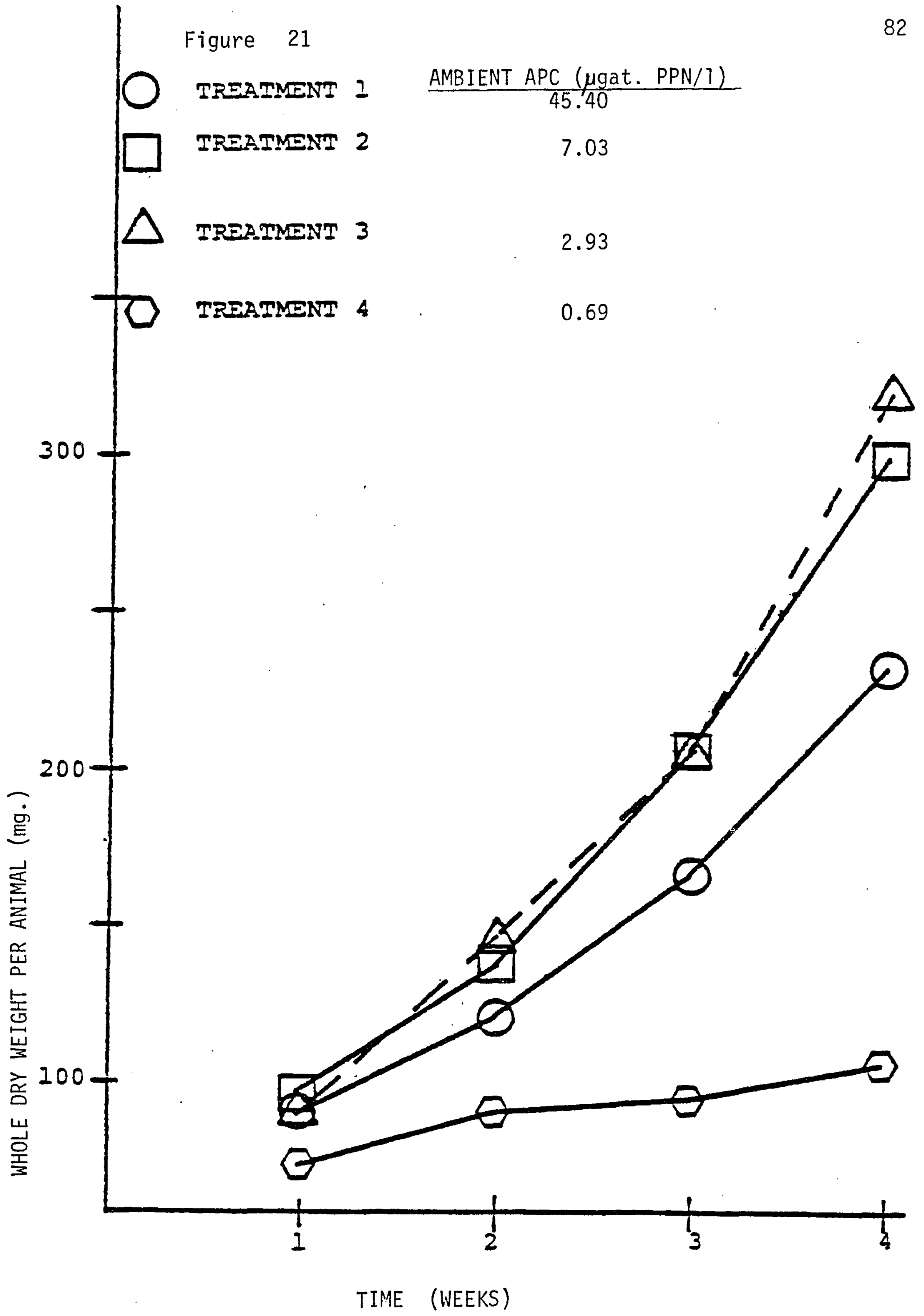


Figure 21
TREATMENT 1
TREATMENT 2
TREATMENT 3
TREATMENT 4
AMBIENT APC (μgat. PPN/l)
45.40
7.03
2.93
0.69
300
200
100
WHOLE DRY WEIGHT PER ANIMAL (mg.)
1
2
3
4
TIME (WEEKS)

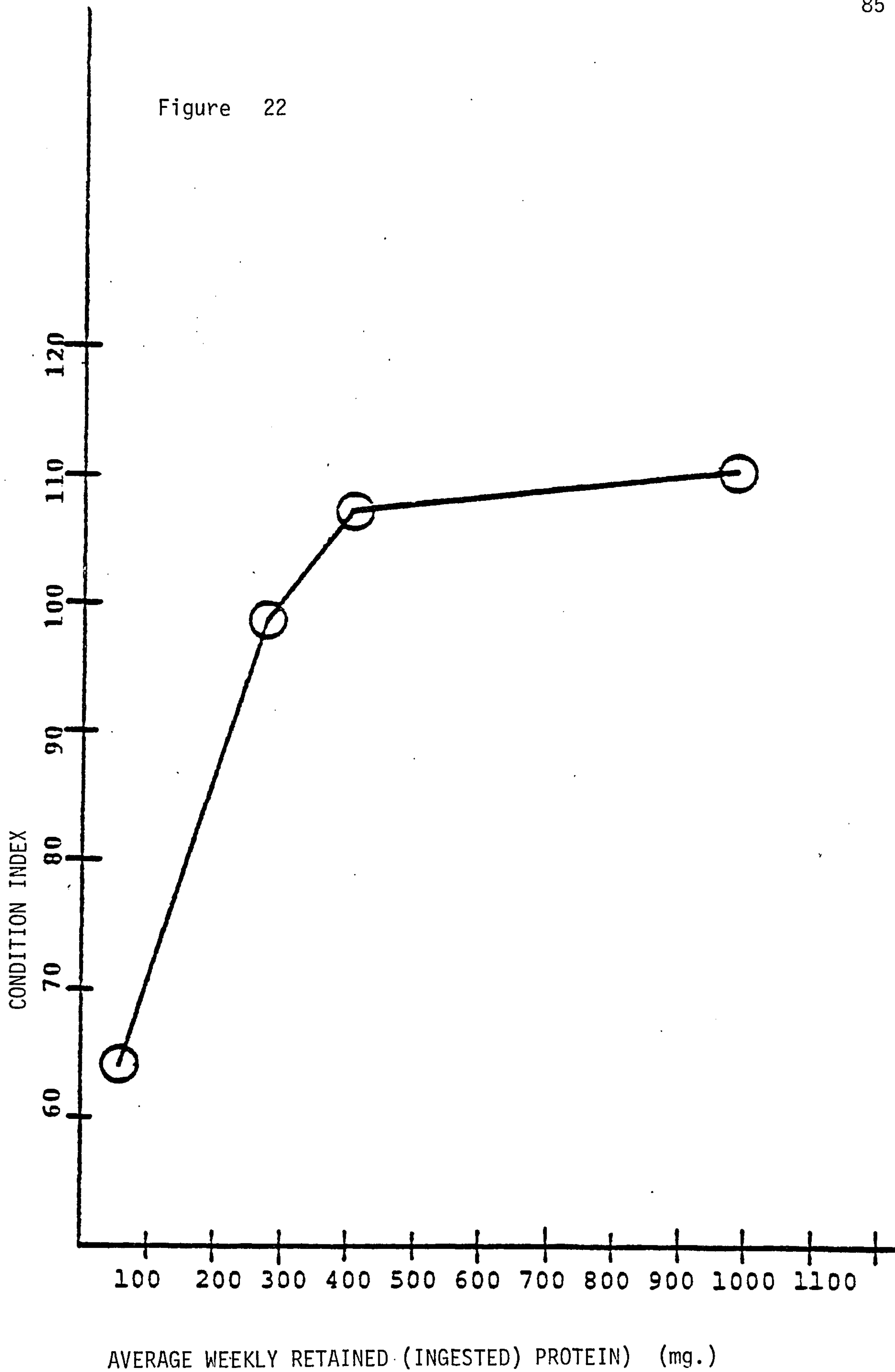
Condition Index

Condition Index is defined as the ratio of dry meat weight to dry shell weight X 1000 and is a commonly accepted measure of the general health or condition of a bivalve mollusc.

There were significant differences in the average weekly condition index among treatments at the 99.9% level. There is an increase in the condition index with an increase in inflow APC or ingested ration. The relationship is nonlinear (figure 22). There is a sharp increase in condition index with an increase in ingested ration (in terms of protein) and then a leveling off with a further increase in ingested ration. A Scheffe' test for pairwise differences showed no significant difference in condition index for the shellfish receiving an inflow APC of 5.75, 11.33 and 56.01 μgat. PPN/l. Only those shellfish receiving an inflow of 1.28 μgat. PPN/l had a condition index significantly lower than the others. It would seem that once a certain amount of protein is ingested, there is little improvement in the condition of the animal with further feeding.

Figure 22 Condition Index as a function of ingested algal protein.

Figure 22



DISCUSSION

Biomass

Winter (197), has suggested that bivalves consume phytoplankton in proportion to the algal cell concentration up to a plateau where consumption is constant and independent of the particle concentration. Finally, there is a decrease in the quantity of phytoplankton consumed with any further increase in algal cell density.

In my work, the absolute amount of phytoplankton consumed (measured as PPN) increased with increasing algal concentrations. There was little indication of reaching a plateau where the amount of phytoplankton consumed is constant and independent of algal concentration. Langton *et al.*, (1977) found a similar relationship of consumption and feed density for *Tapes*, as did Thompson and Bayne (1974), for *Mytilus edulis*.

However, in terms of *percentage* of inflow APC that is ingested, Winter's model seems to hold up. This percentage, here called Protein Stripping Efficiency, increases with increasing inflow APC, reaches a maximum and then decreases with further increases in inflow APC.

This response of *Mercenaria campechiensis* to increasing algal protein concentrations closely matches the response of *Mercenaria mercenaria* to increasing algal concentrations (Tenore and Dunstan, 1973). Tenore and Dunstan (*loc. cit.*) measured feeding rates as the difference between incoming and outgoing particulate carbon concentrations. In my study, feeding rate is called Protein Stripping Efficiency and is measured as the difference in incoming and outgoing particulate protein concentrations.

Tenore and Dunstan (*loc. cit.*), discuss an "absolute" and a "relative" feeding rate. Absolute feeding rate is defined as the *amount* of food removed whereas relative feeding rate is defined as the percentage of food available that is removed, all on a dry meat basis.

Their "relative feeding rate" is equivalent to Protein Stripping Efficiency in this study since at the beginning of each experimental week all populations had the same dry meat weight.

The relative feeding rate of *Mercenaria mercenaria* appears to respond to increasing algal concentrations in the same way that the Protein Stripping Efficiency of *Mercenaria campechiensis* responds to increasing

algal concentrations.

The greatest feeding rate reported by Tenore and Dunstan (loc. cit.) for Mercenaria mercenaria was at 650 ug C/l or a cell density of approximately 4 X 10^5 cells/ml. Mixed species of diatoms were used. In this study, the maximum Protein Stripping Efficiency occurred at an inflow APC of 5.75 μgat. particulate protein N/l or approximately 5 X 10^4 cells/ml of Isochrysis sp. This is equivalent to approximately 534 μg C/l--close to the optimum level (650 μg C/l) reported by Tenore and Dunstan, (1973).

Discrepancies in results can be attributed to differences in size of experimental animals, differences in food organisms, and species differences. However, it is striking that the pattern is similar for these two species of clams, since the mussel and oyster (Tenore and Dunstan, loc. cit.) show distinctly different patterns.

Furthermore, Tenore and Dunstan, (loc. cit.) found that the absolute feeding rate of the clam continued to increase with increasing food concentration. This finding is in agreement with the results of my work and of that of Thompson and Bayne (1974) in their work with Mytilus edulis.

Food chain efficiency is a measure of the efficiency with which the food supply is exploited. In my study the maximum food chain efficiency was achieved by those animals receiving an inflow APC of 5.75 μgat PPN/l or a cell density of approximately 5 X 10^4 cells/ml, corresponding to a PPN concentration in the animals environment of 2.93 μgat PPN/l. Not surprisingly, this was the inflow APC at which the greatest feeding rate was demonstrated.

Tenore, et al. (1973), calculated a gross food chain efficiency for their experimental Mercenaria mercenaria (at an optimum feed density of 650 μg C/l) of 15.12%. The maximum food chain efficiency demonstrated in my study was 16.9% by those animals receiving an "optimum" feed density of 534 μg C/l. The results are very comparable and may indicate that these two species respond similarly to food concentration in their environment.

Parsons, et al., (1961)reported a protein to carbon ratio of 0.94 for Monochrysis lutherii in exponential growth; this alga is closely related to the Isochrysis used in my study. Tenore, et al., (1973) reported that the carbon content of Mercenaria mercenaria was 42.55 percent of the dry meat weight. Food chain efficiency may be calculated with carbon values such as these that have been

derived from these conversion factors. It must be emphasized that they are only approximations to be used for comparative purposes.

The maximum food chain efficiency in terms of carbon was achieved by these shellfish populations receiving an inflow algal protein concentration of 5.75 μgat. PPN/l. Those populations of shellfish receiving higher or lower inflow APC achieved lower food chain efficiencies (table10).

Table 10 Carbon Food Chain Efficiencies

Inflow APC (μgat. PPN/l)	Food Chain Efficiency (%)
56.01	1.82%
11.33	10.98%
5.75	20.99%
1.28	16.15%

When a carbon food chain efficiency is calculated using the outflow algal density instead of inflow algal density as a measure of available food, the highest of all efficiencies are achieved (table 11).

Table 11 Carbon Food Chain Efficiencies Based on Outflow APC

Inflow APC (μgat. PPN/l)	Food Chain Efficiency (%)
56.01	2.3
11.33	18.5
5.75	41.72
1.28	30.45

Thus, for every unit of algal carbon surrounding those shellfish populations receiving an inflow APC of 5.75 μgat. PPN/l, 0.42 units of shellfish carbon are produced (exclusive of the shell).

Tenore, et al., found that gross ecological efficiency, a measure of the efficiency with which an animal converts ingested food into growth or biomass increase, was highest for the clam at 23.69 percent when compared to the mussel or oyster. Their ecological efficiency was calculated on a carbon basis. In order to compare my results with those of Tenore and coworkers, I have calculated my results on a carbon basis using conversion factors obtained from the literature.

Gross ecological efficiencies thus calculated show the same pattern of response to increasing ambientalgal protein concentrations as do those calculated directly from measured protein values, but are higher (Table 12)

Net ecological efficies may also be calculated on a carbon basis. Net ecological efficiencies thus calculated show the same pattern of response to increasing ambient algal protein concentrations as do those calculated directly from measured protein values, but also are higher (Table 13)

Whereas the highest gross ecological efficiecy of Mercenaria mercenaria as determined by Tenore, et al., (1973) was 23.69 percent calculated on a carbon basis, the juvenile Mercenaria campechiensis of my study achieved a net ecological efficiency of 47.71 percent.

TABLE 12

GROSS ECOLOGICAL EFFICIENCY BASED ON CARBON

AMBIENT APC (μgat. PPN/1)	GROSS ECOLOGICAL EFFICIENCY (%)
0.69	36.16
2.93	43.71
7.03	32.00
45.40	21.22

TABLE 13

NET ECOLOGICAL EFFICIENCY BASED ON CARBON

AMBIENT APC (μgat. PPN/1)	NET ECOLOGICAL EFFICIENTY (%)
0.69	40.85
2.93	47.71
7.03	34.92
45.40	29.23

It is obvious from comparing food chain and ecological efficiencies that the clams were not fully exploiting available food. Although this may be due only to the large amount of food presented to the animals relative to their biomass, a more complex interaction may be a better explanation of events. At optimum inflow concentrations of algae, there may be too much food (considering the flow rate and biomass of animals) for the animals to remove more of it. At lower inflow concentrations, resulting in even lower ambient APC, feeding rates may be supressed so that even though less total amounts of food are given, the animals cannot efficiently process it. At higher food concentrations, feeding rates may also be supressed and there is a sizeable amount of biodeposition because there was too much food available so that food is less efficiently processed.

Gross growth efficiency is the efficiency with which ingested food is converted into body tissue. Thompson and Bayne (1974), have described the general relationship of gross growth efficiency (K1) and ingested ration (R). At a very low ration K1 is negative. Small increases in ingested ration (R) result in greatly improved growth efficiencies. When K1 is zero, R is a measure of the maintenance ration. At higher values of R, K1 increases sharply towards a maximum value, before declining.

This was the pattern demonstrated in my study. However, the gross growth efficiencies reported in this study are higher than those reported in the literature in general and those given for bivalves by Tenore and Dunstan, 1973. This may be due to several factors; the fact that juvenile animals were used, the calculation of K1 on a protein basis and the maintenance of constant weight of animals throughout the experimental period. However, a K1 calculated on a carbon basis results in even higher values than when calculated on a protein basis in my study. This may indicate a generally higher conversion rate of juvenile Mercenaria campechiensis than the larger Mercenaria mercenaria individuals used in Tenore and Dunstan's (1973) study.

The K1 in this study was comparable, however, to those reported by

Langton, et al. (1977) in which K1 was also calculated on a protein basis. In fact, they reported even higher values.

Traditional models of feeding and growth predict that once maximum growth is attained at an optimum ingested ration an increase in ingested ration will not result in a decrease or increase in growth; saturation has occurred. This was not the case in my study. Increased ingested ration after optimum ration resulted in a decrease in growth. This was probably due to a greatly reduced Protein Conversion Rate at highest feed densities. Thus, even though more ration was ingested at these high feed densities, it was converted less efficiently into tissue production.

Nitrogen Balance

A nitrogen balance was constructed to gauge the effects of the shellfish populations on the flow of nitrogen through the culture system and the effects of the culture system on the shellfish populations. That is, for example, how does ammonia excretion vary with inflow APC; what are the other excretory products if any, of the shellfish; and are any dissolved nitrogen-containing substances taken up by the shellfish? These questions and others could best be answered by the construction of a nitrogen balance.

A nitrogen balance is an accounting of all the "nitrogen" (nitrogen containing compounds) entering and leaving a system. In this study, the inflow and effluent concentrations of eight different nitrogen-containing compounds were determined. The change in concentration of a particular compound between inflow and effluent is a measure of how the compound was affected by the system. If the net concentration is positive, then some of that compound was removed by the system. If the net concentration is negative, then some of that compound was generated by the system.

An overall determination of the fate of incoming nitrogen was accomplished by summing the individual concentrations or amounts of the different compounds. The difference between the total nitrogen of the inflow and of the effluent divided by the total nitrogen of the inflow is the fraction of total nitrogen not accounted for. In this study, the percent of inflow nitrogen accounted for varied between treatments from 85 to 95%.

The unaccounted for nitrogen may in part be attributed to loss of free ammonia to the atmosphere from the system and from the sample bottles during analyses. Additionally, only particulate protein nitrogen (PPN) was

assayed for in the algal cells and in the shellfish. Nitrogen present as nucleic acids, amino sugars or in other forms were not assayed for and are thus not accounted for. Also, some organic nitrogen compound (s) may have been formed as the result of chemical transformation and not detected in the effluents from the shellfish by the analytical techniques used in this study. Finally, some of the nitrogen unaccounted for is actually the accumulation of sampling, measurement and calculation error.

Only rarely does a single nitrogen containing compound other than PPN or nitrate account for more than 10% of the total nitrogen accounted for. Thus, quantitatively, PPN and nitrate are the most important components of the nitrogen balance, but some of the other nitrogen containing compounds have a qualitative importance . However, in many instances, the weekly mean change in concentration between inflow and effluent of a particular compound (s) was not statistically significant at the 95% confidence level. For example a T-test could not detect any significant differences between the mean change in concentration of urea of any treatment and zero or no change. Any urea excreted by the animals may have been taken up by microorganisms. Hammen (1968) could find no urea excreted by Mercenaria mercenaria, a closely related species.

No significant change (at the 95% confidence level) in nitrate concentration as a result of passage through the shellfish culture containers was observed. This was true for all treatments except treatment 1, which had the highest levels of inflow APC. There was a small net uptake of nitrate and nitrite in this treatment, probably by living algae in the copious tank deposits (biodeposits) of this treatment.

Soluble protein is numerically more important in the overall quantitative nitrogen balance than nitrite. There are relatively high levels of soluble protein associated with high levels of particulate protein. However, net changes in concentrations of soluble protein are more strongly correlated with the amount of protein ingested than with the amount of protein offered to the clams (PPN in).

It would seem that soluble protein is taken in along with or as a result of the ingestion of particulate protein. There is a definite, significant (at the 95% confidence level) change in concentration of soluble protein in passage through the shellfish culture containers.

Although mean net changes in DFAA concentration were not statistically significant between inflow and effluent, the pattern of concentration change is quite interesting. Hammen (loc. cit.), has proposed that the cell membranes of bivalves are permeable in both directions to DFAA so that uptake or loss for purposes of excretion, osmotic adjustment or nutrition is easily accomplished.

Thus, it is difficult to explain the DFAA excretion rate of the animals receiving different inflow APC, especially since the changes in concentrations involved are so low.

One might explain the greatest excretion of DFAA by those animals growing fastest because they could bioenergetically afford to let excess DFAA leak out, whereas, the animals receiving less food could not, although those animals receiving only filtered seawater were under an obvious nutrient stress and might also be expected to "excrete" large amounts of DFAA (Gabbot and Bayne, 1973). Lower excretion rates in animals receiving more food than the fastest growing animals might actually be due to the concurrent uptake of DFAA by microorganisms present in the biodeposits of these heavily-fed animals while DFAA-N was being "excreted" by the animals.

There was an increase in the ammonia excretion rate with increasing inflow APC until a maximum excretion rate was achieved by those clams receiving an intermediate inflow APC of 5.75 μgat. PPN/l. Further increases in inflow APC resulted in decreasing ammonia excretion rates.

The clams with the greatest excretion rate of ammonia were the fastest growing animals. Ammonia is an end product of algal protein catabolism and shellfish protein anabolism. Thus, those animals growing faster were processing protein at a faster rate and generating more ammonia per unit time.

The concentrations of ammonia in the system were low, in general, and the mean net changes in concentration (a measure of excretion) of ammonia were not statistically significant at the 95% confidence level except for the fastest growing clams. It is of interest, nevertheless, to compare these excretion rates with those in the literature on *Mercenaria mercenaria*, a closely related species. The values reported by Hammen (1968) and Srna and Baggaley (1976) have been converted into the same units used throughout this study.

Hammen (1968) reported that Mercenaria mercenaria with a mean wet weight of 51.0 g excreted ammonia at a rate of 0.463 ugat. (NH_3 + NH_4^+) N/l per gram of tissue per day. Srna and Baggaley (1976) reported an excretion rate of 0.55 μgat. (NH_3 + NH_4^+) N/l/g/day for a hypothetical average clam (Mercenaria mercenaria) of 51.0 g whole wet weight. Thus, similar values of ammonia excretion are reported by these investigators using similar techniques.

Srna and Baggaley (1976), found ammonia excretion rates to increase linearly with increase in the dry meat weight of the clams. Small animals with an average dry meat weight of 0.78 g excreted an average of 20.6 μgat. (NH_3 + NH_4^+) N/l/g/day.

In my study, clams with a range of 5.5 to 18.2 mg dry meat weight excreted 0.065 to 0.134 μgat NH_3 - N/g/day. According to the regression equation of Srna and Baggaley (1976), Mercenaria mercenaria of these weights would excrete 0.16 to 0.49 μgat. (NH_3 + NH_4^+) N/l/g/day, or somewhat higher than Mercenaria campechiensis.

It appears that the juvenile Mercenaria campechiensis of my study excreted ammonia at lower rates than Mercenaria mercenaria. The excretion rates of this study, however, were measured in a fundamentally different type of experimental system than that used by Hammen (loc. cit.) or Srna and Baggaley (1976). My study employed a continuous flow system whereas the work of Hammen (1968) and Srna and Baggaley (1976) involved measurements of ammonia excretion on nonfeeding animals in a static system. Although direct comparisons, therefore are not possible, some idea of the differences in excretion rates of Mercenaria mercenaria and Mercenaria campechiensis might be gotten from such comparisons.

Tenore, et al. (1973) measured inflow and effluent ammonia nitrogen concentrations in a continuous flow system. They reported that Mercenaria mercenaria (average individual dry meat weight of 0.74 g) regenerated an average ammonia nitrogen of 3.72 X 10^{-3} μgat. N/l per g dry meat weight for an eleven week period or 3.38 X 10^{-4} μgat. N/l/g/dry meat weight/week. In my study, the average weekly dry meat weight of the animals differed significantly between treatments and ranges from 5.5 mg to 18.2 mg. The average weekly ammonia excreted ranged from 434 X 10^{-3} to 853 X 10^{-3} μgat. NH_4- N/l per g dry meat weight. It appears that my experimental animals excreted much more ammonia per gram of dry meat weight.

It is generally acknowledged that the weight specific ammonia excretion of a bivalve increases with decreasing weight. This may explain the greater excretion rate of the smaller animals in this study. However, direct comparisons with previous studies of Tenore et al., (1973) are difficult to make since the experimental systems, although similar in concept, were different in design. Food type, density, and flow rate and animal size, density and species were all different between systems.

CONCLUSIONS

The small changes in concentration of nitrogen containing compounds between influent and effluent noted in my study may be the result of measuring concentrations in a continuous flow system in which the volume of seawater flowing past the animals is very great compared to the biomass of the animals. Thus, very large amounts of a compound have to be taken up or generated by the shellfish for there to be a significant change in concentration between influent and effluent.

However, I believe that measuring concentration changes of a nitrogen containing compound in such a system gives more realistic results than other types of studies. A study in which the clam is not fed for 24 hours prior to the experiment and then placed in a bowl of standing synthetic seawater for 24 hours does give larger, more measurable changes in concentration of a particular compound. However, the results can hardly be used to describe the normal metabolic activity of the animals. My methods more closely approximate the normal metabolic activity of a feeding animal.

An improvement of my method may be to increase the biomass to volume ratio. This will result in greater concentration changes of a nitrogen containing compound as it passes through the shellfish culture containers in a continuous flow system. This may result in the better resolution of concentration changes associated with static methods of excretion measurements, but maintain the realism of a continuous flow system.

A mariculture system must determine the environmental or ambient algal density that will result in the maximum growth and then ensure this APC by suitably adjusting inflow APC, inflow rates, clam density, and container size. The maximum growth achieved in my study was by those shellfish populations receiving an average inflow APC of 5.75 µgat. PPN/l, resulting in an average outflow APC of 2.93 µgat. PPN/l; this was the average algal protein concentration surrounding the animals. If one ensured that this (2.93 µgat. PPN/l) APC surrounded populations of juvenile Mercenaria campechiensis, maximum growth would be attained. For more than 10 g live weight of clams, an inflow APC greater than 5.75 µgat. PPN/l

at 120 ml/min or a flow rate greater than 120 ml/min at this APC may be required for a given container size. This optimum feeding regime of 5.75 μgat PPN/l at 120 ml/min flowing to 10 g live weight of clams in 250 ml of seawater resulted in accelerated growth as compared to growth in the natural environment. Menzel (1962) reported an increase in the average size of juvenile Mercenaria campechiensis from 8mm to 11.4mm for a one month growing period. In my study, the clams grew from 7.5mm to 13mm in 4 weeks -- an increase of 5.5mm or 38% better growth than in the natural environment.

Further, this feeding regime produced animals with a condition index that was not significantly different at the 95% confidence level from the maximum condition index demonstrated by those animals receiving ten times the amount of protein.

Since feeding rates and food chain efficiencies were relatively low with the experimental feeding regimes, a decreased flow rate, but with the same optimum ambient algal concentration ensured, might well result in increased efficiencies for a managed food chain in terms of increased shellfish growth per unit of available algae.

However, more experimental data are needed to develop a model describing the interactive effects of such variables as inflow rate, inflow algal protein concentration, and animal biomass, etc., on the growth and conversion efficiency of juvenile Mercenaria campechiensis.

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