MATURATION OF PENAEID SHRIMP: DIETARY FATTY ACIDS

A Dissertation

Presented to

the Faculty of the Department of Biophysical Sciences

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

of Doctor of Philosophy

By

Stephen Randall Missler

December, 1979

ACKNOWLEDGEMENTS

This dissertation is dedicated to my wife Betty, for her patience, understanding, and assistance during its preparation; and to my parents for their direction and guidance in the achievement of this goal. A special thanks to Betty's mother, Minerva Jackson, for her kind assistance during the research.

I would particularly like to thank Dr. Addison L. Lawrence for his assistance in the biological aspects of the dissertation, and for his collaboration during the progress of this study. I would also like to acknowledge Dr. Jim McVey and the National Marine Fisheries Service in Galveston, Texas for providing many of the samples used during research.

I am deeply indebted to Dr. Brian S. Middleditch for his instruction and supervision during my graduate years, and without whose constructive criticisms would have made this presentation impossible.

ABSTRACT

The objective of this study was to obtain for the first time ovarian maturation and spawning of penaeid shrimp in captivity. This objective was achieved. Past efforts to obtain this result centered on the adjustment of parameters such as photoperiod, temperature, and water quality. The approach used in this study was to manipulate the chemistry of cultured shrimp through dietary modifications, in order to mimick the chemistry of wild shrimp. Fatty acids were the compounds selected for study. Metabolic profiling using gas chromatography and combined gas chromatography-mass spectrometry was used in comparing fatty acid distributions in wild and cultured shrimp.

Examination of the fatty acid profiles from several species of penaeid shrimp during maturation showed that these animals have high relative concentrations of C_{20} and C_{22} polyunsaturated fatty acids, particularly 20:4, 20:5, and 22:6. This suggested a requirement for polyunsaturated fatty acids in the diet. Examination of the fatty acid content in a traditional feedstock revealed low relative concentrations of polyunsaturated fatty acids. Several marine invertebrates were subsequently analysized, in order to find an economical source of these acids for use as a feedstock supplement. The addition of marine invertebrates containing high relative concentrations of polyunsaturated fatty acids resulted in immediate ovarian maturation and spawning in P. setiferus and P. stylirostris. This represents the first attainment of reproducible ovarian maturation and spawning of penaeid shrimp grown in

mariculture.

Fatty acid profiles from the eggs and larvae of P. styliros-tris grown in culture were compared to those obtained from the eggs and larvae of wild type shrimp. The results demonstrated that eggs from cultured shrimp had higher relative concentrations of polyunsaturated fatty acids. However, cultured postlarvae contained lower relative concentrations of 18:2 and 18:3 compared to postlarvae from wild shrimp. It was concluded that eggs of cultured shrimp had sufficient relative concentrations of polyunsaturated fatty acids; furthermore, it was suggested that requirements for these acids in developing larvae and postlarvae could be met by modifying the diet.

Experiments designed to alter the fatty acid content in shrimp larval diets were conducted with rotifers. Rotifers were fed on algae containing high relative concentrations of polyunsaturated fatty acids. Fatty acid profiles in rotifers were found to change significantly, and it was concluded that this change reflected the algal diet. It was suggested that rotifers fed on algae containing the appropriate fatty acids could serve as a live feedstock for developing shrimp.

A mathematical model was constructed to simulate the fatty acid distributions in the food web of a shrimp pond. Initial data were obtained by fitting the model to fatty acid distributions found in typical food web constituents. The model was tested by studying the effect that an input of fertilizer would have on fatty acid distributions in the pond. It was concluded that the model can be used to indicate the experiments or observations which need

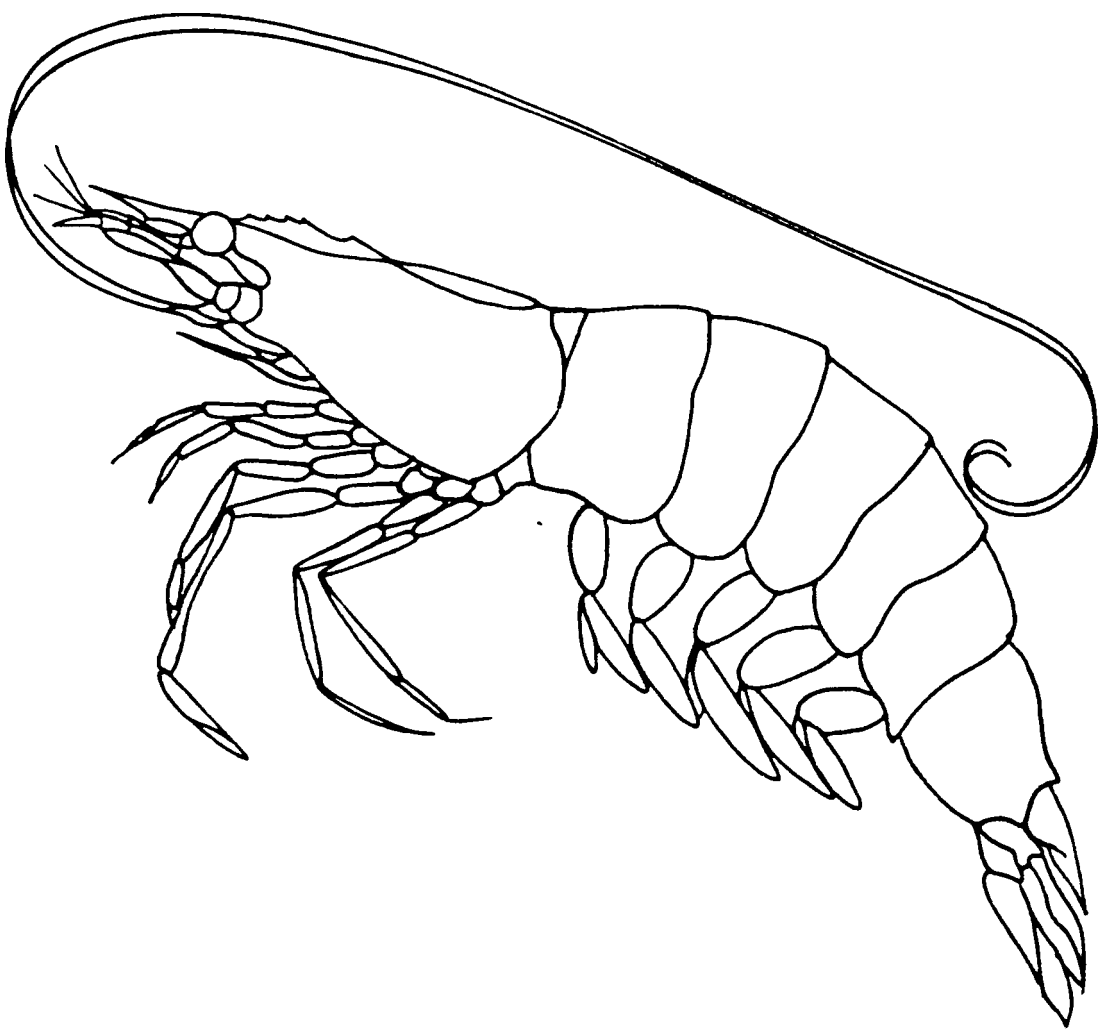
to be conducted in order to obtain a more complete understanding of the dynamics of shrimp ponds.

Experiments were initiated using simplex optimization on ingredients in dry feedstocks in order to optimize the food conversion ratio and cost of the feed. Although only a few iterations had been conducted, the technique had already attained a food conversion ratio of 2.3. This is equal to the best results obtained by trial and error experiments in the laboratory; and it was suggested that further iterations should provide food conversion ratios having lower values. Additionally, a theoretical optimization procedure was discussed in order to obtain acceptable response values in a short period of time. This technique was designed to allow the mariculture operator to vary ingredients in shrimp feedstocks in order to take advantage of fluctuations in costs of these ingredients.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES



1.

INTRODUCTION

1. INTRODUCTION

While the demand for shrimp has steadily increased, pollution, inflation, and rising fuel costs have had an adverse effect on the shrimping industry in the United States. As a result, increased interest has been expressed in recent years for the achievement of large-scale commercial shrimp farming. The attainment of such a goal would provide an answer to demand but, perhaps more importantly, would open the door to selective shrimp breeding for the purpose of producing superior varieties of shrimp. By controlling shrimp genetics it would be possible to produce shrimp which reach marketable size in less time, have improved disease resistance, and have better taste.

1.1. Statement of the problem.

Reviews of the history (Hanson and Goodwin, 1977) and techniques (Wickins, 1976; Kirk, 1979) of mariculture have been presented. Currently, the large-scale production of shrimp is attained by the "grow out" of shrimp larvae spawned from gravid females obtained in the wild (Hudinaga and Kittaka, 1975; O'Connor, 1979). Previous attempts to achieve gonadal maturation and spawning of shrimp in culture have met with little success, except in isolated instances (Johnston and Fielding, 1956; Conte et al., 1978). This failure is probably due to a lack of knowledge concerning the biochemistry of shrimp during growth and maturation. In this respect, nutritional insufficiences in the diet could cause biochemical imbalances, thereby reducing the chances for

spawning. Before reproduction can be achieved on the scale necessary for commercial investment, there is a need to define the relationships between biochemical changes in shrimp during the maturation process, nutritional requirements of the shrimp, and the chemical components in shrimp diets. The class of compounds chosen for study were fatty acids. Fatty acids are an important energy source and are major components of cell membranes, occuring in a large number of polar and nonpolar lipids (Gurr and James, 1975; Harrison and Lunt, 1975). Furthermore, certain polyunsaturated fatty acids are precursors of prostaglandins which are known to be important in human reproduction (von Euler and Eliasson, 1967).

1.2. Literature review.

Reviews on crustacean metabolism have been presented (Huggins and Munday, 1968; Yamaoka and Scheer, 1970). A review of lipids in crustaceans has been presented by Morris and Culkin (1976). O'Connor and Gilbert (1968) have reported on the role of lipids in the metabolism of crustaceans.

It is interesting that crustaceans reflect the lipid composition of their diet (Pathak *et al.*, 1952; Lovern, 1953; Jezyk and Penicnak, 1966). Of particular interest is the fact that the coconut crab has a lipid content very similar to that of coconuts (Andre and Richert, 1960). These data suggest that crustaceans have a strong dietary requirement for lipids. Sedgwick (1979) notes that lipids act as an energy source in the shrimp *Penaeus merguiensis*, sparing proteins for growth. In addition, lipids

have been shown to be essential in the diet of various penaeid shrimp (Deshimaru and Kuroki, 1974; Colvin, 1976; Guary et al., 1976; Kanazawa et al., 1977a).

As mentioned previously, fatty acids are important constituents of lipids. A complete discussion of fatty acid biosynthesis and degradation has been presented by Lehninger (1975). In vertebrates and other animals, fatty acids are formed in the cytosol by a series of reactions mediated by the fatty acid synthetase complex. In the reactions, one molecule of acetyl coenzyme A (Ac CoA) and seven molecules of malonyl CoA are combined through a series of successive condensations to form one molecule of palmitic acid. The net reaction is:

$$\text{Acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 14\ H^+ \longrightarrow$$
$$CH_3(CH_2)_{14}COOH + 7CO_2 + 8CoA + 14NADP^+ + 6H_2O$$

Palmitic acid (16:0) is the end product of saturated fatty acid synthesis in most animals. Odd-chain fatty acids, which occur in many marine organisms, are formed by using propionyl CoA rather than acetyl CoA as the starting molecule. Stearic acid (18:0) and other long-chain saturated fatty acids may be formed by enlongation of palmitic acid using a different set of enzymes, and requiring Ac CoA rather than malonyl CoA. Monoenoic acids such as palmitoleic acid (16:1) and oleic acid (18:1) may be formed from their saturated precursors using a specific monooxygenase system which places a cis double bond in the Δ^9-position. Polyenoic long-chain fatty acids are formed by successive desaturation

and enlongation reactions. Double bonds are usually separated by methylene units. Polyunsaturated fatty acids can be grouped into categories according to the precursor fatty acids from which they are derived: palmitoleic, oleic, linoleic, and linolenic acids (Table 1.1). Figures 1.1 and 1.2 show the pathways for the formation of fatty acids from these precursors. Linoleic and linolenic acids are not synthesized in many animals due to the lack of enzymes which can desaturate in positions more than nine carbon atoms from the carboxylic acid moiety. Such fatty acids, which are required for growth but cannot be synthesized by the organism, are called "essential" and must be derived from dietary sources. The ultimate sources of these polyunsaturated acids are plants.

Polyunsaturated fatty acids, particularly linoleic acid (18:2), linolenic acid (18:3), arachidonic acid (20:4), 5,8,11,14,17-eicosapentaenoic acid (20:5), and 4,7,10,13,16,19-docosahexaenoic acid (22:6), have been shown to be required in the diets of fish (Watanabe et al., 1974, 1975; Takeuchi and Watanabe, 1976, 1977a,b; Yu and Sinnhuber, 1979). These fatty acids are found to comprise a relatively large portion of the fatty acid composition in many crustaceans (Wolfe et al., 1965; Gallagher and Brown, 1975; Takahashi and Yamada, 1976). Furthermore, linoleic acid (18:2) and linolenic acid (18:3) have been shown to be essential in the diets of the crayfish Astacus astacus (Zandee, 1966) and the prawn Penaeus japonicus (Kanazawa et al., 1977b). Although there have been publications concerning the effects of fatty acid composition of diets on the growth and molting cycle of penaeid shrimp (Teshima and Kanazawa, 1976; Ando et al., 1977), there is

Table 1.1. Classification of fatty acids according to their parent compound.[a]

Parent fatty acid	Chain structure	Nomenclature
Palmitoleic acid	$CH_3-(CH_2)_5-CH=CH-$	n-7 (ω-7)
Oleic acid	$CH_3(CH_2)_7-CH=CH-$	n-9 (ω-9)
Linoleic acid	$CH_3-(CH_2)_4-CH=CH-$	n-6 (ω-6)
Linolenic acid	$CH_3-CH_2-CH=CH-$	n-3 (ω-3)

[a]Polyenoic acids derived from these compounds will have the same terminal chain structure as the parent fatty acid.

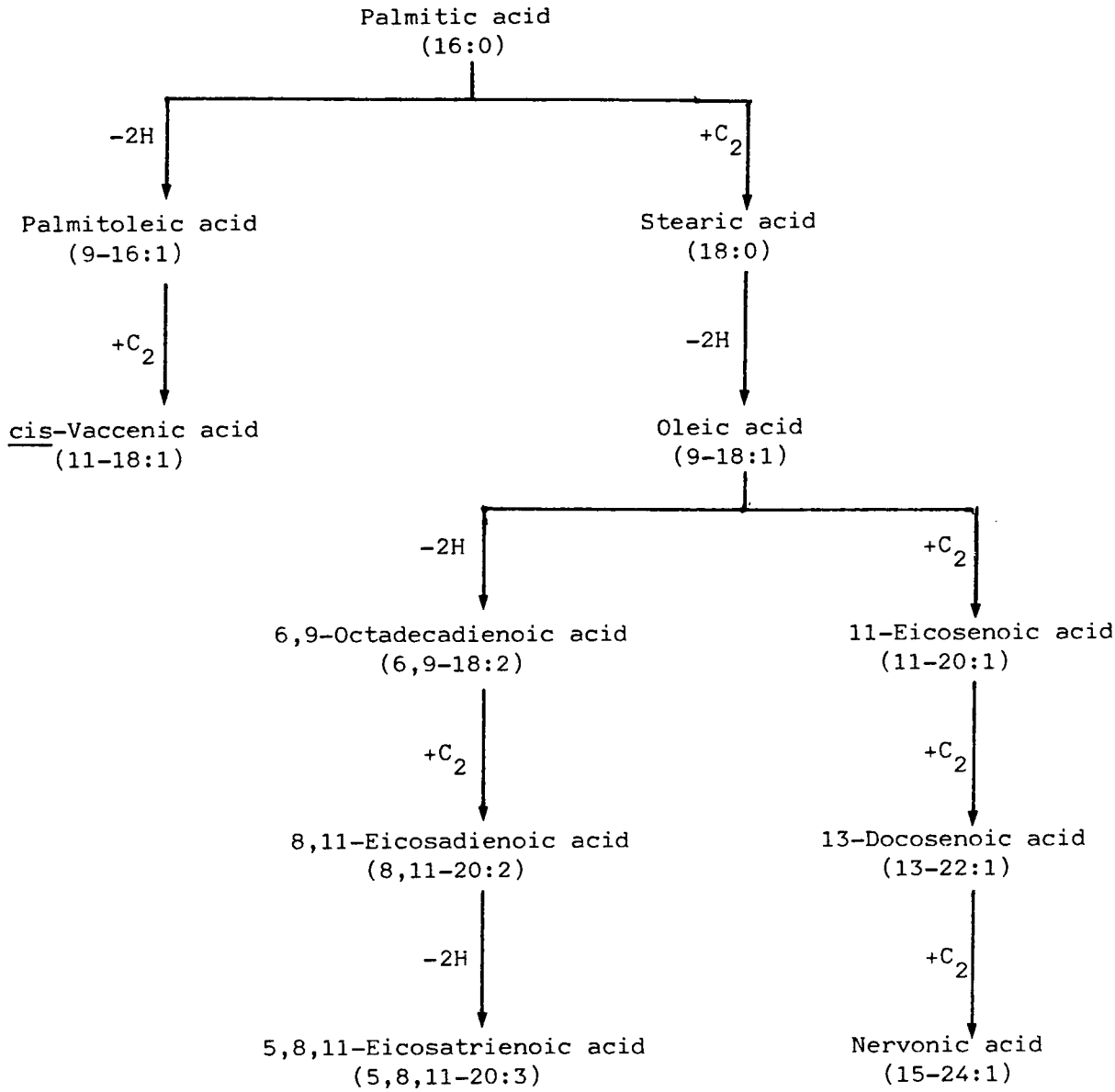

Palmitic acid
(16:0)
-2H
+C2
Palmitoleic acid
(9-16:1)
Stearic acid
(18:0)
+C2
-2H
cis-Vaccenic acid
(11-18:1)
Oleic acid
(9-18:1)
-2H
+C2
6,9-Octadecadienoic acid
(6,9-18:2)
11-Eicosenoic acid
(11-20:1)
+C2
+C2
8,11-Eicosadienoic acid
(8,11-20:2)
13-Docosenoic acid
(13-22:1)
-2H
+C2
5,8,11-Eicosatrienoic acid
(5,8,11-20:3)
Nervonic acid
(15-24:1)

Figure 1.1. Synthesis of some saturated and unsaturated fatty acids from palmitic acid.

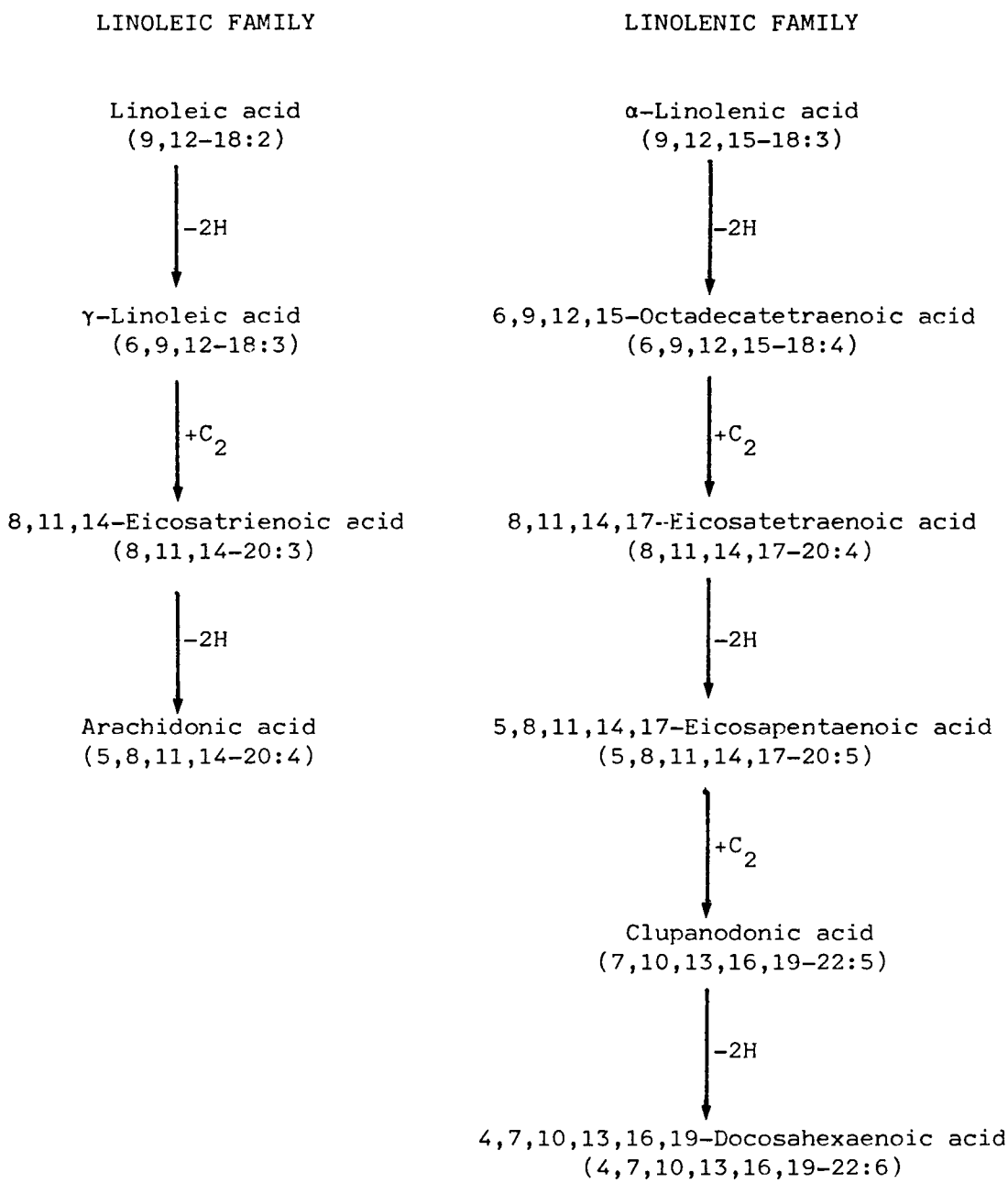

LINOLEIC FAMILY
Linoleic acid
(9,12-18:2)
-2H
γ-Linoleic acid
(6,9,12-18:3)
+C2
8,11,14-Eicosatrienoic acid
(8,11,14-20:3)
-2H
Arachidonic acid
(5,8,11,14-20:4)
LINOLENIC FAMILY
α-Linolenic acid
(9,12,15-18:3)
-2H
6,9,12,15-Octadecatetraenoic acid
(6,9,12,15-18:4)
+C2
8,11,14,17-Eicosatetraenoic acid
(8,11,14,17-20:4)
-2H
5,8,11,14,17-Eicosapentaenoic acid
(5,8,11,14,17-20:5)
+C2
Clupanodonic acid
(7,10,13,16,19-22:5)
-2H
4,7,10,13,16,19-Docosahexaenoic acid
(4,7,10,13,16,19-22:6)

Figure 1.2. Synthesis of polyunsaturated fatty acids from linoleic and linolenic acids.

relatively little material concerning this subject during the maturation process (Morris, 1973).

1.3 Rationale for study.

As noted previously, relatively few studies have been conducted concerning the biochemical processes involved in the maturation of shrimp. It was indicated that fatty acids are essential for growth in shrimp, and lack of ovarian maturation and spawning in captivity. In addition, the polyunsaturated fatty acids found in shrimp are precursors of the prostaglandins, which in humans are known to be involved in reproduction. Although endogenous prostaglandins have not been found in crustaceans, experiments conducted *in vitro* using lobster stomach and gill homogenates have resulted in the conversion of the 20:3 acid to prostaglandin E_1 in low yield (Christ and van Dorp, 1973). If prostaglandins are important in the reproductive processes of shrimp, the lack of polyunsaturated fatty acid precursors in the diet might inhibit spawning.

The technique of metabolic profiling has been useful in elucidating biochemical disorders in humans (Mamer *et al.*, 1974). Notable among these are the discovery of new inborn errors in metabolism (Tanaka *et al.*, 1966; Jellum *et al.*, 1972, 1973) and the diagnosis of diabetes mellitus by the analysis of volatiles in urine (Zlatkis *et al.*, 1973). The versatility, sensitivity, and simplicity of this technique make it ideally suited to the study of biochemical changes that occur in shrimp during reproductive maturation. Additionally, it will provide information which would

allow the design of suitable diets for shrimp grown in culture.

2.

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1. Sample collection.

Wild specimens of *Penaeus stylirostris* and *Penaeus vannamei* were obtained from the Pacific Ocean off the southern coast of Panama. Wild specimens of *Penaeus setiferus* were collected in the Gulf of Mexico off the coast of Texas. All specimens were collected during the spawning season which runs from May to September. Females were classified according to gonadal maturation as determined by gonadal indices (Lawrence *et al.*, 1979). The gonads, hepatopancreas, and tail muscle were removed as soon as possible after collection and kept frozen until analyzed.

2.2. Materials.

All solvents used were Mallinckrodt Nanograde except for anhydrous diethyl ether (U.S.P.), and hydrochloric acid and sodium hydroxide (reagent grade). Samples were exposed only to glass, stainless steel, aluminum, and Teflon. All glassware was rinsed with chloroform prior to use.

2.3. Extraction and liquid chromatography.

2.3.1. Extraction.

The extraction technique used is based on that presented by Christie (1973). Frozen samples were placed in test tubes containing 10 ml of tap water and homogenized 15-30 seconds with a Polytron PT 10/35. The homogenates were transfered to 50 ml centrifuge tubes equipped with Teflon-lined screw caps. 4ml of 4M

sodium hydroxide were added and the samples were heated for 2 hours at 110°. Samples were cooled to room temperature and extracted with 2 x 15 ml of diethyl ether (extract A). The samples were then acidified with 2 ml of concentrated hydrochloric acid and extracted with 2 x 15 ml of diethyl ether (extract B). In each extraction the samples were shaken 5 minutes and centrifuged at 2000 rpm to separate the phases (occasionally it was necessary to cool the emulsion for 20 minutes in a freezer in order to separate the phases). Extract A was either saved for concurrent steroid analysis (Middleditch et al., unpublished)) or discarded. The solvent was reduced on extract B using a Buchi-Brinkman Rota-vapor R rotary evaporator. The sample was then taken up in chloroform and stored in air tight vials in the freezer until liquid chromatography was performed.

2.3.2. Liquid chromatography.

Following extraction, fatty acids were further purified using silica gel chromatography. Silica gel (J. T. Baker Chemical Co., 60-200 mesh) was activated by drying in an oven at 110° overnight. The silica gel was filled to a height of 20cm in a 40 x 1cm glass column equipped with a 100 ml reservoir at the upper end and a glass frit and Teflon stopcock at the lower end. The column was then rinsed with 100 ml of benzene and air bubbles removed by placing the system under 10 psi pressure using nitrogen. The sample was carefully added to the top of the silica gel and pushed into the column using nitrogen. The column was eluted initially with 30 ml of benzene which was discarded. Fatty

acids were collected in a round bottom flask following elution with 30 ml of ethyl acetate. The volume was reduced using a rotary evaporator, and the samples taken up in chloroform and stored as previously mentioned, prior to gas chromatography.

2.4 Derivatization.

Fatty acids were converted to their methyl esters using dimethylformamide dimethylacetal ("Methyl-8") from Pierce Chemical Co. (Dabre, 1978). Derivatization was performed on a microscale in the following manner: 10-20 mg of concentrated fatty acids were placed in a 3.5 ml glass vial equipped with a Teflon-lined screw cap. 100 µl of "Methyl-8" were added, the vial capped tightly, and the solution heated for 5 minutes at 60°. The solution was then brought to an approximate concentration of 1 mg/ml of individual fatty acids. This mixture was then injected into the gas chromatograph. No interfering peaks resulting from the derivatization procedure were detected in the range of fatty acids under examination (C_{12} to C_{24}).

2.5 Gas chromatography.

Gas chromatography was performed using a Perkin-Elmer 3920B instrument equipped with heated injection ports and flame ionization detectors. Fatty acid profiles were obtained using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh) programmed from 100 to 270° at 4°/min. Additional analyses were performed using a 2m x 2mm (i.d.) glass column packed with 10% SP-2330 on Chromosorb W AW (100-120 mesh)

programmed from 130 to 230° at 4°/min., or using a 20m or 30m x 0.25mm (i.d.) WCOT glass capillary coated with Silar-10C and programmed from 90 to 210° at 4°/min. In all cases nitrogen was used as the carrier gas. The injection port and interface temperatures were 250° and 280°, respectively. Identification of fatty acids was made based on their retention index (I) values as compared to those of known standards, and by GC-MS.

2.6 Gas Chromatography-mass spectrometry.

Combined gas chromatography-mass spectrometry was performed using a Hewlett-Packard 5992A instrument equipped with a jet separator and using an electron energy of 70 eV. Chromatography was performed using either a 2m x 2mm (i.d.) glass column packed with 5% Silar-10C on Chromosorb W AW (100-120 mesh) or a 30m x 0.25mm (i.d.) WCOT capillary column coated with Silar-10C, and using the appropriate temperature programs.

2.7 Quantitation.

2.7.1 Introduction. Accurate quantification is necessary in order to derive conclusions concerning the levels of fatty acids in shrimp and their diets. It is commonly accepted that the use of internal standards is the best technique for this purpose.

An internal standard must have chemical properties similar to those of the sample, such that losses incurred during purification are equally reflected. Normally, a fatty acid that does not appear in the fatty acid profiles of the sample would be used as a standard (e.g., an odd chain or a branched chain acid).

However, the diversity of fatty acids from marine sources greatly limits the selection of fatty acids that can be used as a standard. Grant (1971) suggests that in such instances it is possible to use a standard which is already an intrinsic component of the sample mixture. A correction is then necessary which is made by analyzing the sample twice, once with and once without the standard. Since internal standards are added prior to extraction for the most accurate results, this method would require two simultaneous extractions. In many instances this is impossible due to the lack of sufficient amounts of the sample, or to sample uniqueness.

Blomstrand et al. (1978) devised a method for the quantification of erucic acid in rapeseed oils using [1-^{14}C]erucic acid as an internal standard. Resolution of the standard and sample was made possible by the use of GC-MS, and the technique proved to be very accurate.

The analysis of animal tissue often requires the homogenization of the total organ. Where this is impractical, large sample sizes must be used in order to obtain results which are representative of the whole organ. As a result, it is necessary to use a large quantity of standard in order to achieve the best possible accuracy (Grant, 1971). In the analysis of marine animals this would typically require 1 mg of standard for every 1 to 5 g of tissue analyzed. The use of [1-^{14}C]erucic acid as suggested by Blomstrand would require using 2.84 mCi of radioactively labelled standard for each analysis. Although radioactive samples have been used in mass spectrometry on a limited basis (Burstein et al., 1975), it is not acceptable as a routine procedure (Brooks

and Middleditch, 1977). Additionally, the cost of using this amount of labelled erucic acid would be prohibitive. Based on the use of 1 mg of [1-^{14}C]stearic acid, this would amount to $420 per analysis for the internal standard alone. An alternative would be to add smaller amounts of labelled fatty acid to an aliquot of homogenized tissue. However, this defeats the purpose of the internal standard, which provides the best accuracy when added to the entire sample prior to analysis.

The use of deuterium labelled fatty acids overcomes the problems associated with radioactivity in Blomstrand's technique, and provides an economical solution as well. I now report on the use of [2,2-^{2}H]stearic acid as an internal standard for the routine quantification of marine fatty acids.

2.7.2. Materials and methods. [2,2-^{2}H]Stearic acid (98 atom%, Merck and Co.) was made to a concentration of 10 mg/ml in methanol. Prior to extraction, 1 mg (100 µl) of the standard was added to each sample. The samples were then purified in the manner mentioned previously. A diagram of the analytical procedure is shown in Figure 2.1.

2.7.3. Results and discussion. The mass spectra of stearic acid (18:0) and [2,2-^{2}H]stearic acid methyl esters are shown in Figure 2.2. The mass chromtograms obtained by selective ion monitoring (SIM) of ions at m/e 298 and m/e 300 (representing the molecular ions of the methyl esters of stearic acid and deuterium labelled stearic acid, respectively) are presented in Figures 2.3 and 2.4.

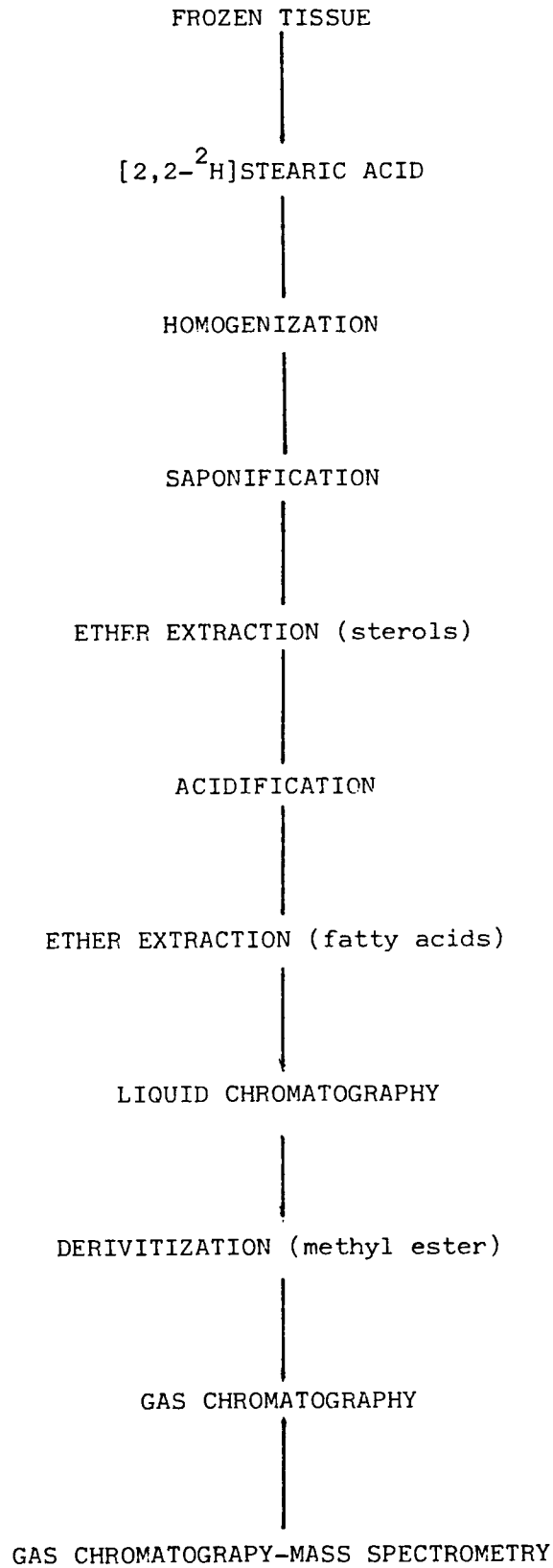

FROZEN TISSUE
[2,2-^{2}H]STEARIC ACID
HOMOGENIZATION
SAPONIFICATION
ETHER EXTRACTION (sterols)
ACIDIFICATION
ETHER EXTRACTION (fatty acids)
LIQUID CHROMATOGRAPHY
DERIVITIZATION (methyl ester)
GAS CHROMATOGRAPHY
GAS CHROMATOGRAPY-MASS SPECTROMETRY

Figure 2.1. Flow chart indicating the steps in the analysis of fatty acid composition.

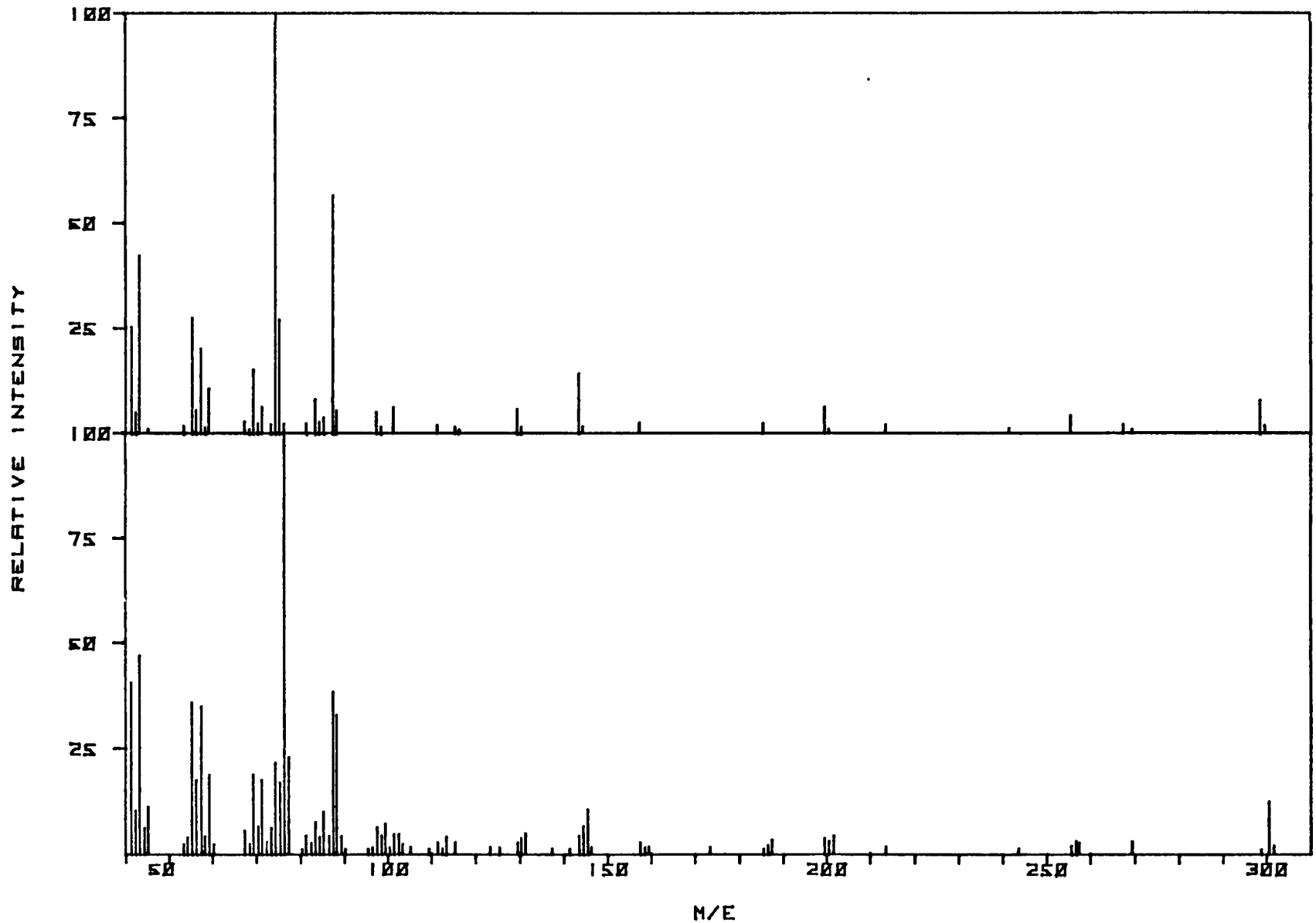

100
75
50
25
100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 2.2. Mass spectra of methyl stearate (top) and methyl [2,2-^{2}H]stearate (bottom). Note the increase in molecular weight of the deuterated compound. The molecular ion in this case occurs at m/e 298 for the unlabeled compound.

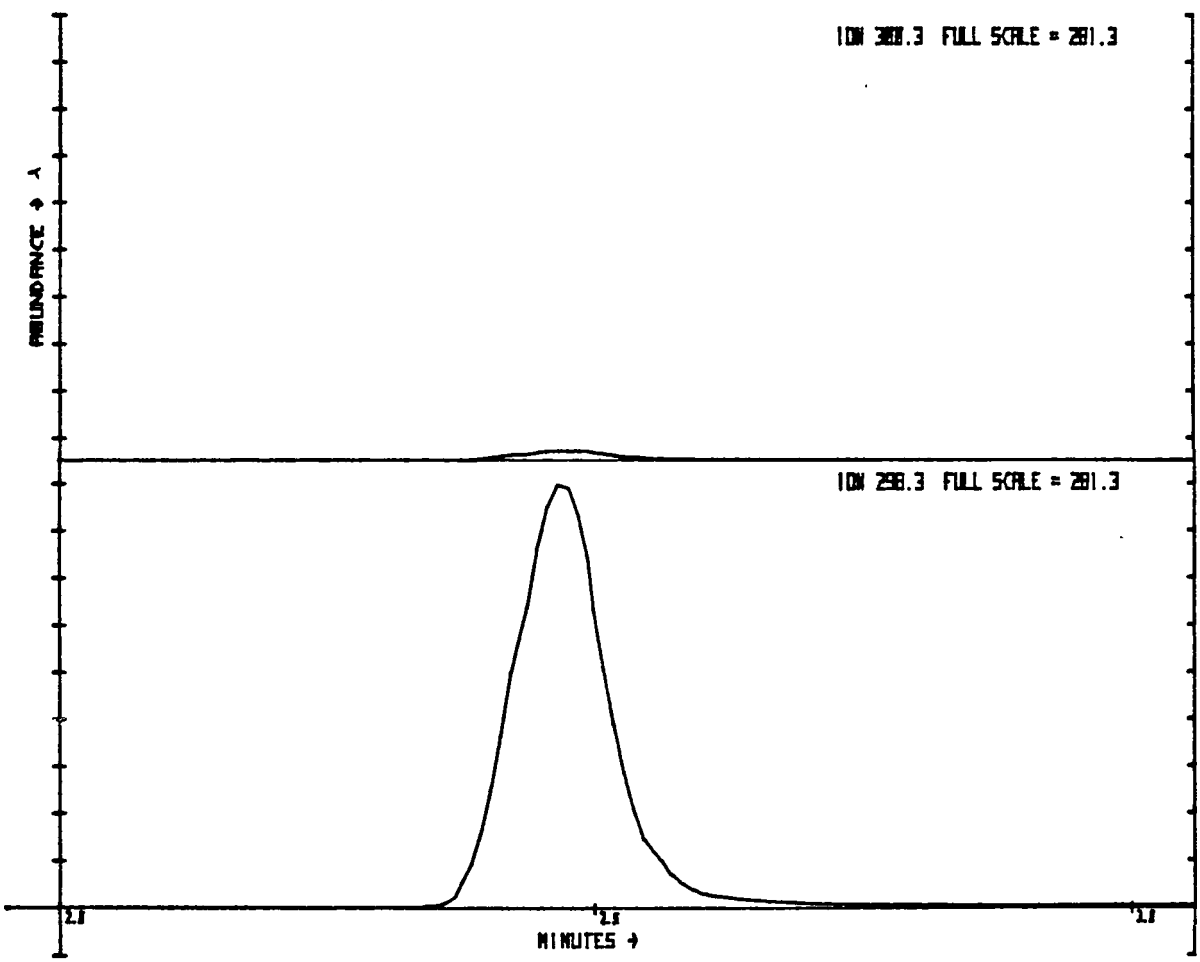

ION 300.3 FULL SCALE = 201.3
ABUNDANCE →
ION 298.3 FULL SCALE = 201.3
2.0
2.5
3.0
MINUTES →

Figure 2.3. Selective ion monitoring of ions at m/e 298 and m/e 300 for methyl stearate.

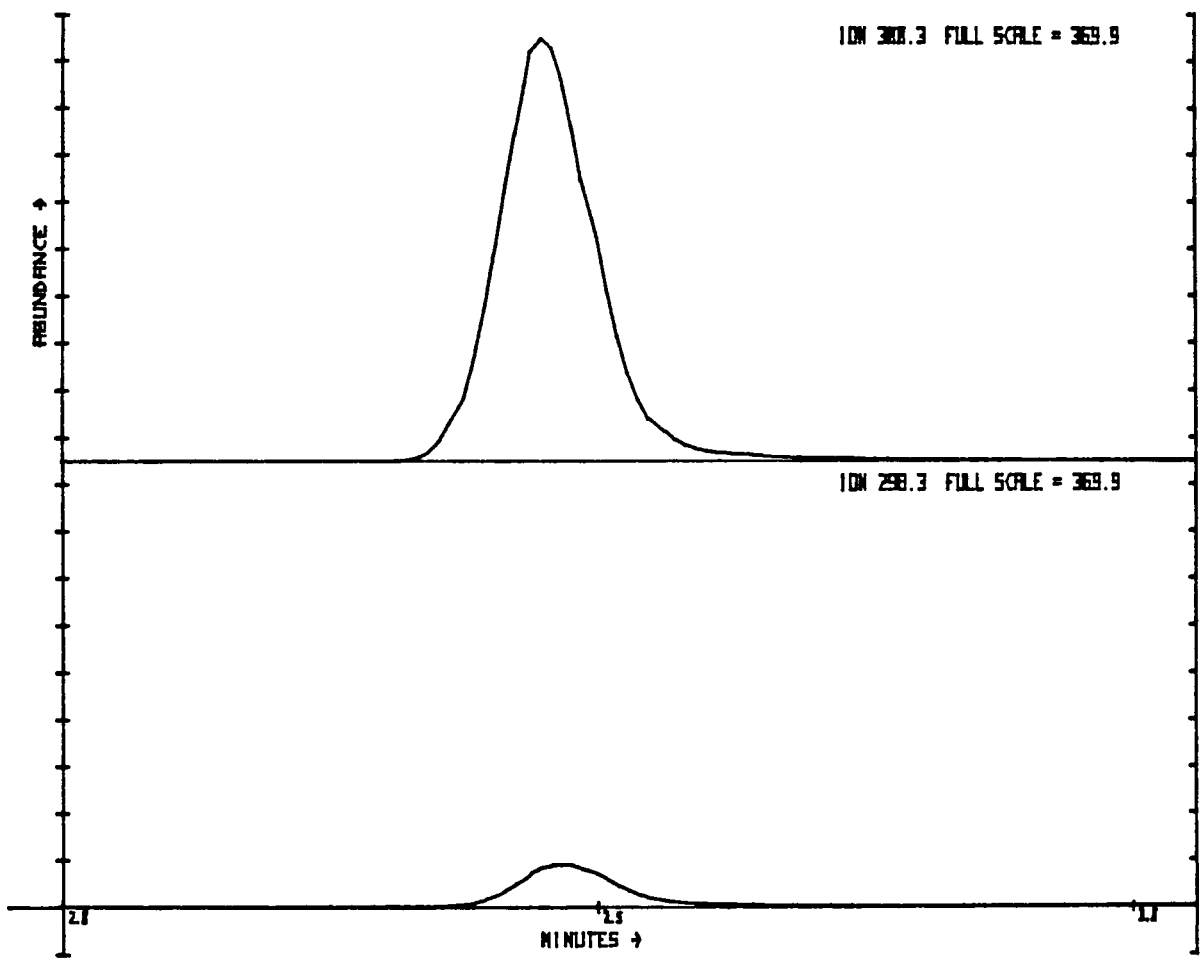

ION 300.3 FULL SCALE = 369.9
ABUNDANCE →
ION 298.3 FULL SCALE = 369.9
2.0
2.5
3.0
MINUTES →

Figure 2.4. Selective ion monitoring of ions at m/e 298 and m/e 300 for methyl [2,2-^{2}H]stearate.

The calibration curve in Figure 2.5 was obtained using 100 ng of [2,2-^{2}H]stearic acid. The curved line is a result of contributions that unlabelled methyl stearate adds to the response at m/e 300 and that labelled stearic acid methyl ester adds to the response at m/e 298. Correcting for these contributions results in the straight line in Figure 2.5. Corrections were made according to the equation given by Blomstrand:

$$A = (B - C)/(1 - B/D),$$

where A = the corrected ratio between the peak heights at m/e 298 and m/e 300, B = the uncorrected ratio between the peak heights at m/e 298 and m/e 300, C = the ratio between the peak heights at m/e 298 and m/e 300 from [2,2-^{2}H]stearic acid methyl ester (see Figure 2.4), D = the ratio between the peak heights at m/e 298 and m/e 300 from unlabelled methyl stearate (see Figure 2.3).

The determination of the amount of stearic acid in a sample was calculated using the corrected calibration curve. Calculations of the quantities of other peaks in the chromatogram were made using the equation:

$$Q_I = A_I(Q_S + Q_D)/A_{S+D},$$

where Q_I = the quantity of the peak of interest, Q_S = the quantity of unlabelled stearic acid, Q_D = the quantity of labelled stearic acid, A_{S+D} = the abundance of the GC peak comprised of labelled and unlabelled methyl stearate, A_I = the abundance of the GC

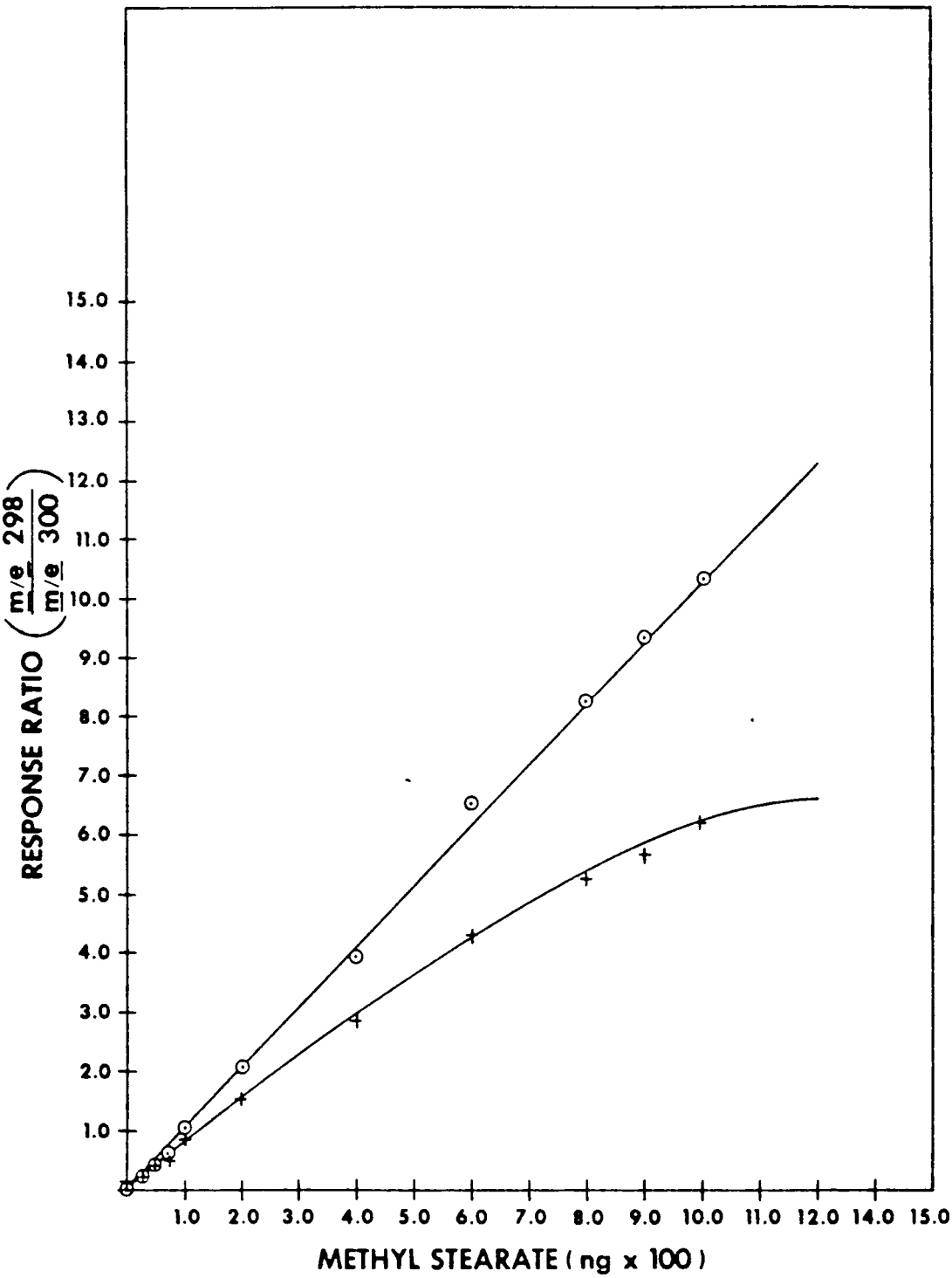

RESPONSE RATIO (m/e 298 / m/e 300)
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 14.0 15.0
METHYL STEARATE (ng x 100)

Figure 2.5. Calibration curve obtained by comparing the response ratios at m/e 298 to m/e 300, using variable amounts of methyl stearate and a constant quantity (100 ng) of methyl[2,2-^{2}H]stearate. The curved line is the plot of uncorrected response ratios. The straight line is the plot of response ratios corrected using the formula given in the text.

peak of interest. An example of quantitative results obtained with this technique is presented in Table 2.1. The chromatogram for this sample is shown in Figure 2.6 and the GC-MS data obtained by SIM are shown in Figure 2.7.

The precision of this technique is based on the fact that no ions at m/e 298 and m/e 300 are produced in substantial quantities by any fatty acid methyl ester except methyl stearate and methyl [2,2-^{2}H]stearate. Methyl oleate produces some ions at m/e 298; however, the retention times of methyl esters of oleic and stearic acid are significantly different, thus avoiding any confusion.

Although the use of internal standards with GC-MS has some inherent problems (Millard, 1978), it is the best technique for quantitative analysis. The accuracy of the technique is comparable to that obtained by the use of internal standards with GC (Blomstrand et al., 1978). The data presented here show that the use of internal standards labelled with deuterium provide the accuracy and economy needed to run routine analysis of fatty acids from marine invertebrates (at this writing the cost of the internal standard was $0.15 per analysis). With minor modifications, the technique could easily be applied to the quantification of sterols and other lipids from animal sources.

2.8. Mass spectra of standards.

The mass spectra of methyl esters of authentic samples of all of the fatty acids encountered during this study are presented in Appendix 1. Table 2.2 lists the various standards, their molecular formulae, molecular weights, retention index values on OV-1 and

Table 2.1. Fatty acid content in the tail muscle of Penaeus setiferus.

Peak number[a]	Identity	Relative %	mg/g[b]
1	14:0	0.9	0.22
2	15:0	0.6	0.13
3	16:0	18.9	4.31
4	16:1	5.9	1.34
5	17:0	2.1	0.47
6	17:1	1.3	0.30
7	18:0	19.5	2.40
8	18:1	11.7	2.67
9	18:2	0.8	0.17
10	20:1	2.1	0.47
11	20:3	0.6	0.13
12	20:4	4.5	1.03
13	20:5	17.6	4.01
14	22:4	0.8	0.17
15	22:5	1.7	0.39
16	22:6	18.3	4.18

[a]For peak numbers refer to Figure 2.6.

[b]Values in milligrams of fatty acid per gram dry weight.

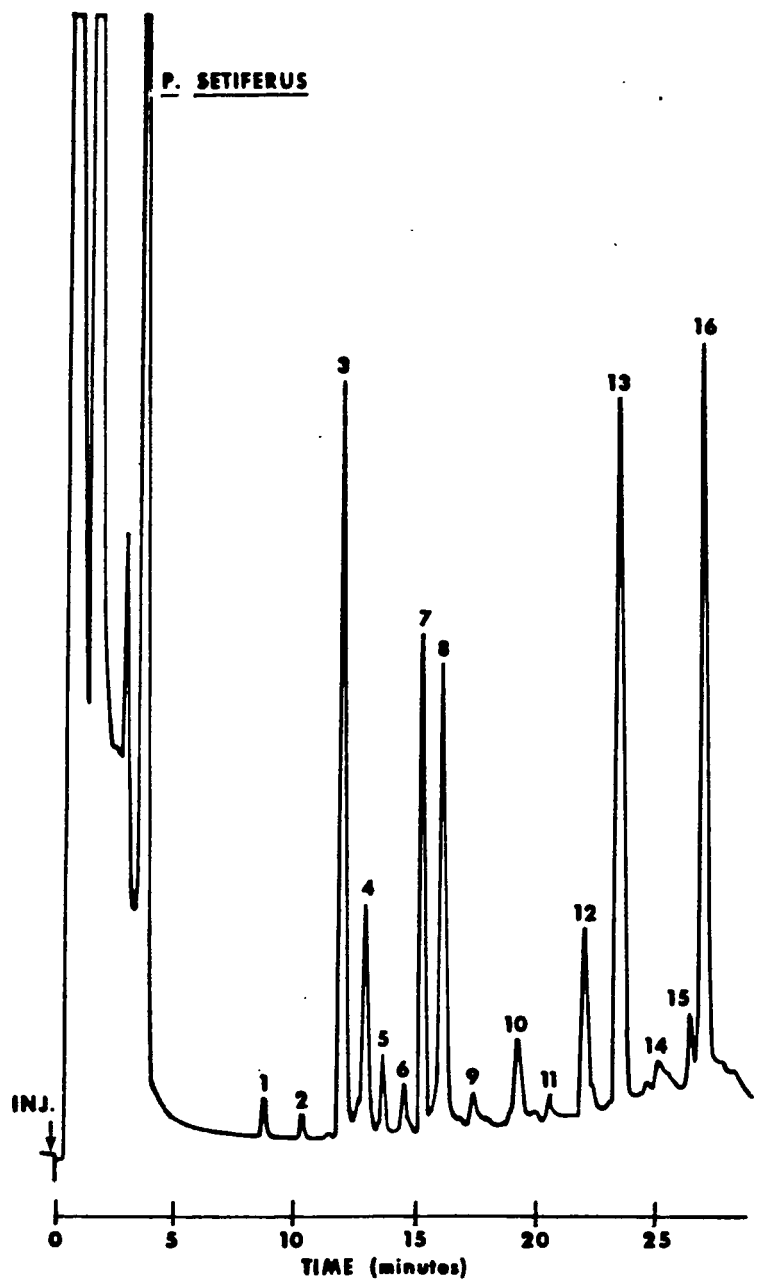

P. SETIFERUS
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
0
5
10
15
20
25
TIME (minutes)

Figure 2.6. A chromatogram of fatty acid methyl esters obtained from P. setiferus and quantitatively analyzed using [2,2-^{2}H]stearic acid as an internal standard. Chromatogram was obtained using a 2m x 2mm (i.d.) glass column packed with 5% SP-2330 on Chromosorb W AW (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecanoate	15:0
3.	palmitate	16:0
4.	palmitoleate	16:1
5.	heptadecanoate	17:0
6.	heptadecenoate	17:1
7.	stearate + [2,2-^{2}H]stearate	18:0
8.	oleate	18:1
9.	linoleate	18:2
10.	eicosenoate	20:1
11.	eicosatrienoate	20:3
12.	arachidonate	20:4
13.	eicosapentaenoate	20:5
14.	docosatetraenoate	22:4
15.	docosapentaenoate	22:5
16.	docosahexaenoate	22:6

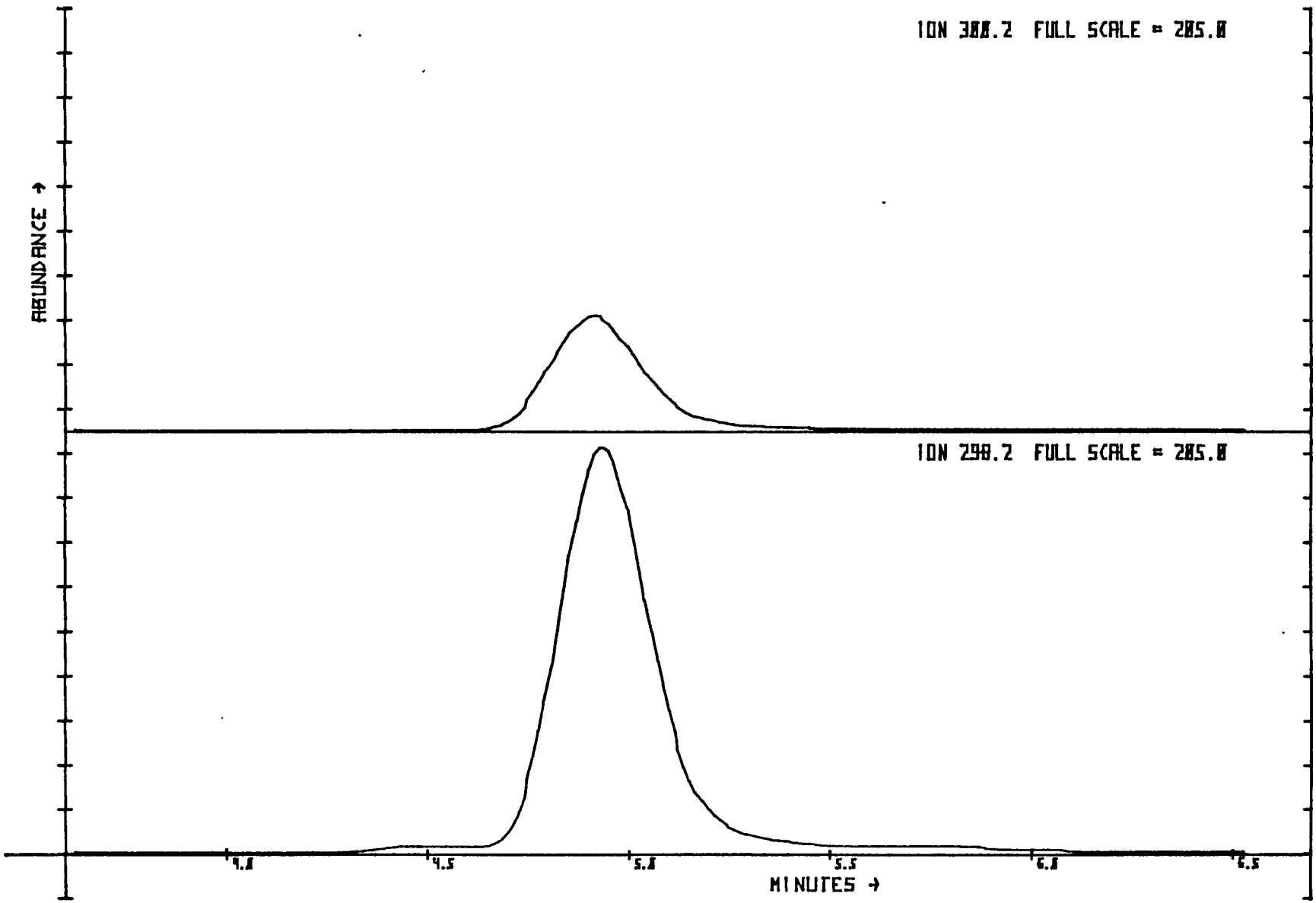

ION 388.2 FULL SCALE = 285.0
ABUNDANCE →
ION 298.2 FULL SCALE = 285.0
4.0
4.5
5.0
5.5
6.0
6.5
MINUTES →

Figure 2.7. Selective ion monitoring of m/e 298 and m/e300 for the sample shown in Figure 2.6. The peaks in this figure correspond to peak 7 in Figure 2.6. Note that the gas chromatogram does not resolve methyl stearate from the labelled compound. However, by monitoring the individual molecular ions by GC-MS, resolution of the two compounds is easily achieved.

Table 2.2 Reference data for standards of fatty acid methyl esters.

Fatty acid methyl ester	Formula	M	I (OV-1)	I (Silar-10C)	significant ions
SATURATED ESTERS					
Methyl laurate	12:0	214	1510	1875	43, 55, 74, 87, 214
Methyl myristate	14:0	242	1710	2080	43, 55, 74, 87, 242
Methyl palmitate	16:0	270	1910	2280	43, 55, 74, 87, 270
Methyl stearate	18:0	298	2110	2480	43, 55, 74, 87, 298
Methyl arachidate	20:0	326	2310	2675	43, 55, 74, 87, 326
Methyl behenate	22:0	354	2515	2875	43, 55, 74, 87, 354
Methyl lignocerate	24:0	382	2715	3080	43, 55, 74, 87, 382
MONOUNSATURATED ESTERS					
Methyl palmitoleate	16:1	268	1880	2345	41, 55, 69, 74, 268
Methyl oleate	18:1	296	2080	2540	41, 55, 69, 74, 296
Methyl eicosenoate	20:1	324	2280	2740	41, 55, 69, 74, 324
POLYUNSATURATED ESTERS					
Methyl linoleate	18:2	294	2070	2620	41, 55, 67, 81, 294
Methyl linolenate	18:3	292	2070	2720	41, 55, 67, 79, 292
Methyl homo-γ-linolenate	20:3	320	2240	2845	41, 55, 67, 79, 320
Methyl arachidonate	20:4	218	2220	2920	41, 55, 67, 79, 91
Methyl docosahexaenoate	22:6	342	2415	3290	41, 55, 67, 79, 91

Silar-10C columns (see Section 2.4), and abbreviated mass spectral data.

2.8.1. Saturated methyl esters. The mass spectra of the C_{14} to C_{24} even-carbon straight-chain saturated fatty acid methyl esters are presented in Figures 1-7 of Appendix 1. The normal saturated methyl esters tend to have similar fragmentation patterns and can easily be distinguished from the unsaturated species. The example of methyl laurate (Appendix 1, Figure 1) will be used in the following discussion.

The most characteristic feature of the mass spectrum of a saturated methyl ester is the base peak at m/e 74. The major fragment ion of this peak is formed by a McLafferty rearrangement involving β-cleavage at the carboxyl end of the molecule (Figure 2.8). The stability of this fragment ion as compared to others in the spectrum is responsible for its high relative abundance.

The ions appearing at m/e 87 and 143 are fragments having the general formula $^{+}CH_2(CH_2)_nCOOCH_3$ in which n = 1 and 5, respectively (Budzikiewicz et al., 1967). These ions are thought to be formed as a result of reciprocal hydrogen transfers during fragmentation, as shown in Figure 2.9.

The ions appearing at m/e 43, 57, 71, and 95 are fragments having the general formula $C_nH_{2n+1}^{+}$ in which n = 3, 4, 5, and 6, respectively. These probably represent fragments formed by cleavage at the terminal end of the molecule. Ions having the general formula $C_nH_{2n}^{+\bullet}$ and $C_nH_{2n-1}^{+}$ where n = 3, 4, 5, and 6 as before, are thought to be formed by subsequent degradation of the

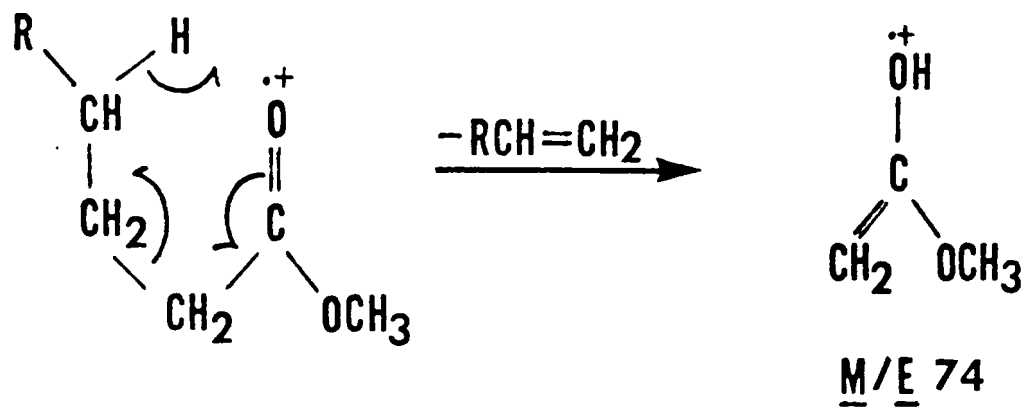

R
H
CH
O
CH2
C
CH2
OCH3
-RCH=CH2
OH
C
CH2
OCH3
M/E 74

Figure 2.8. Mc Lafferty rearrangement of the molecular ion of methyl laurate (12:0).

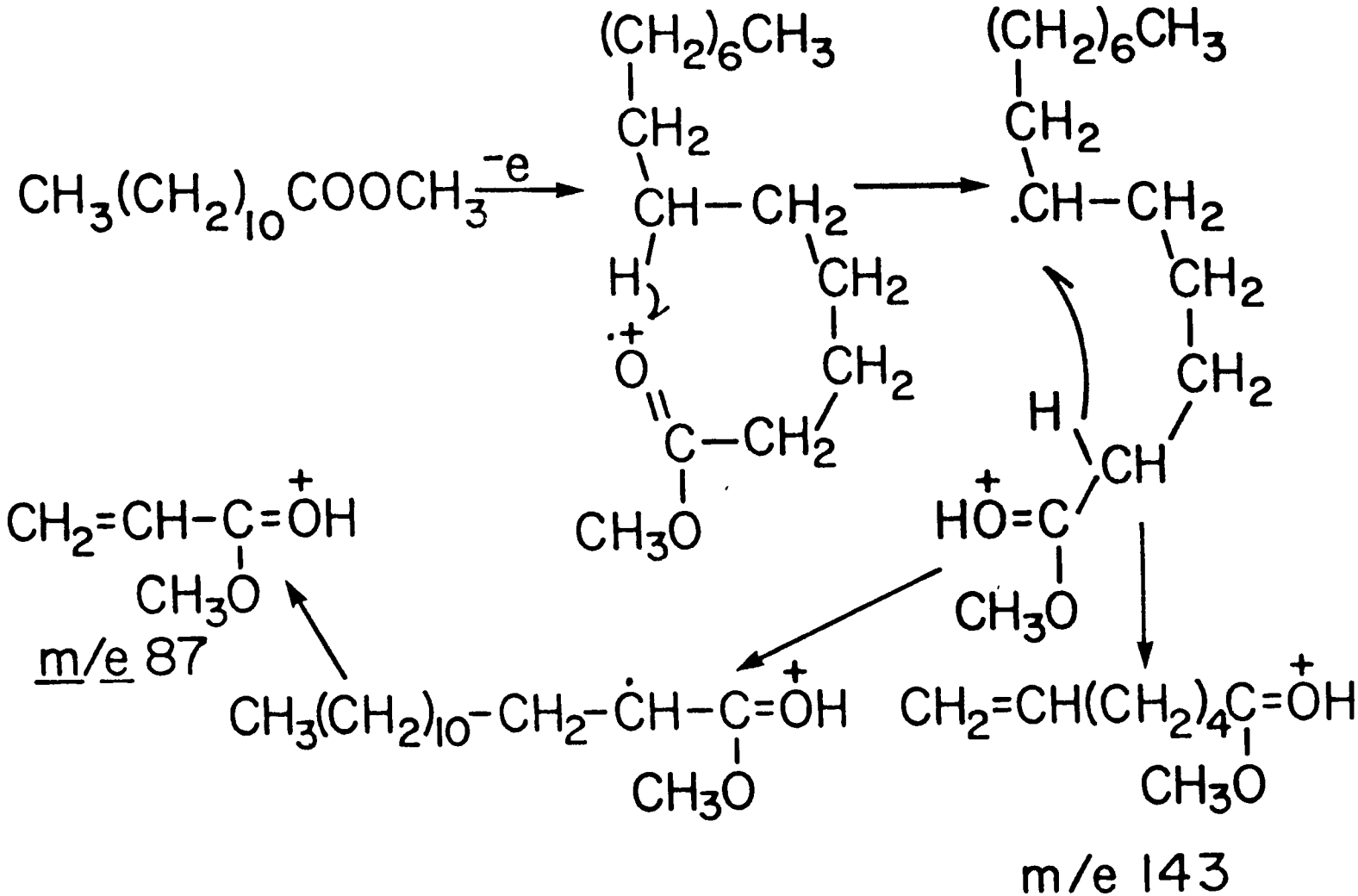

CH3(CH2)10COOCH3
-e
(CH2)6CH3
CH2
CH
CH2
H
CH2
O
CH2
C
CH2
CH3O
(CH2)6CH3
CH2
CH
CH2
CH2
CH2
H
CH
HO=C
CH3O
CH2=CH-C=OH
CH3O
m/e 87
CH3(CH2)10-CH2-CH-C=OH
CH3O
CH2=CH(CH2)4C=OH
CH3O
m/e 143

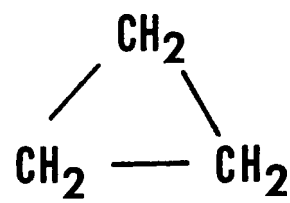
Figure 2.9. Example of reciprocal hydrogen transfers which could explain the formation of ions at m/e 87 and m/e 143.

$C_nH_{2n+1}^+$ fragments (Budzikiewicz et al., 1967). Budzikiewicz notes that the relatively large abundance at m/e 43 may be ascribed in part to a protonated cyclopropane fragment (Figure 2.10).

Perhaps the most informative peak appears at m/e 214. This is the molecular ion, giving the molecular weight of methyl laurate. Other saturated methyl esters also produce molecular ions, which appear at m/e values corresponding to their molecular weights.

2.8.2. Monounsaturated fatty acid methyl esters. The mass spectra of three of the monounsaturated fatty acid methyl esters are presented in Figures 8-10 of Appendix 1. As was true with the saturated esters, monounsaturated fatty acid methyl esters have similar fragmentation patterns. Some of the fragmentations that occur in these spectra are the same as those in the saturated methyl ester spectra, and will not be discussed again. The methyl ester of palmitoleic acid (16:1) will be used as an example in the following discussion.

In comparing the mass spectrum of 16:1 (Appendix 1, Figure 8) with that of its saturated counterpart 16:0 (Appendix 1, Figure 3), a substantial increase in the relative abundances of the ions at m/e 41, 55, and 69 is observed in the former. As mentioned previously, contributions to these peaks are thought to be due to degradations of the $C_nH_{2n+1}^+$ ions. However, this would not account for the higher relative abundances of these peaks in the unsaturated compound. Several authors have proposed that hydrogen rearrangements in the molecular ions of unsaturated alkanes and



H+

M/E 43

Figure 2.10. Structure of protonated cyclopropane. The stability of this ion is believed to account for its relatively large abundance at m/e 43 (Budzikiewicz et al., 1967).

fatty acids result in a shift of the double bond along the molecule prior to fragmentation (Budzikiewicz et al., 1967; Boon et al., 1977). If the radical moves toward the terminal end of the molecule, it is possible that an allylic cleavage could occur (Figure 2.11). Different fragmentation sites along the chain could therefore produce increases in the peak abundances at m/e 41, 55, and 69 with respect to those of the saturated compound. Relative peak abundances would be based on the stabilities of the fragment ions. Similar cleavages along the molecule could also be responsible for other ions in the spectrum (e.g., m/e 83, 97, and 111).

The ion at m/e 236, $[M-32]^{+\bullet}$, represents a loss of methanol from the molecular ion, probably resulting from hydrogen rearrangements and subsequent fragmentation as shown in Figure 2.12. As mentioned previously, the ability of unsaturated fatty acids to undergo bond shifts could result in the fragmentation process shown in the figure. The stability of the fragment ion supports this hypothesis. This type of fragmentation would not be expected to occur in a saturated fatty acid methyl ester, due to the absence of the double bond. Both saturated and unsaturated fatty acid methyl esters do produce $[M-31]^{+}$ fragment ions as a result of α-cleavage and loss of $CH_3O^{\bullet}$.

The most significant peak in the spectrum is again that of the molecular ion, which occurs at m/e 268. Other monounsaturated methyl esters will also produce molecular ions which correspond to their respective molecular weights.

2.8.3. Polyunsaturated methyl esters. The mass spectra of five polyunsaturated esters are presented in Figures 11-15 of Appendix

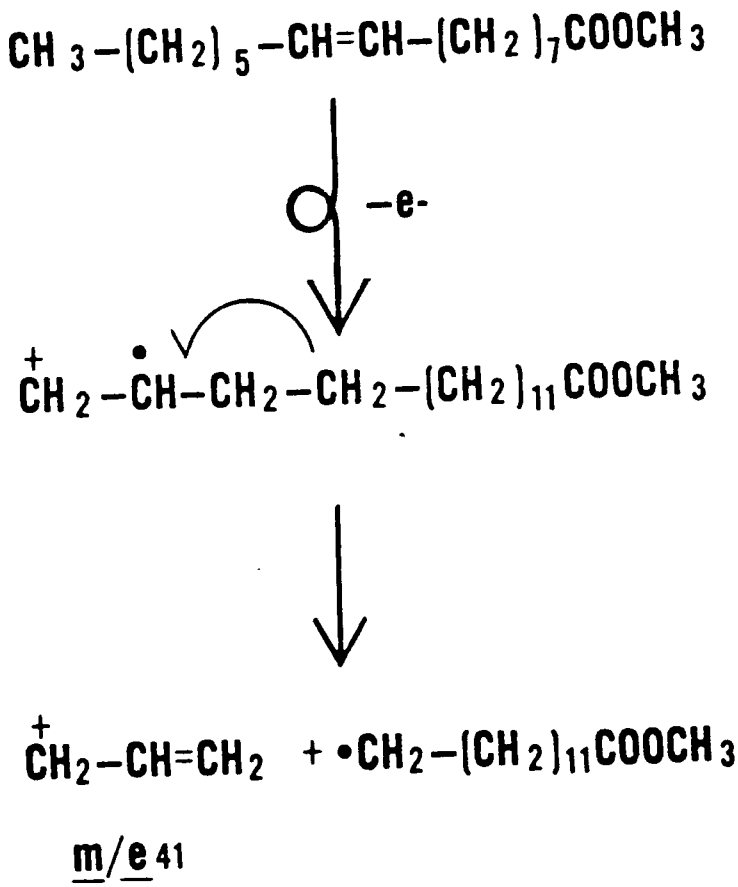
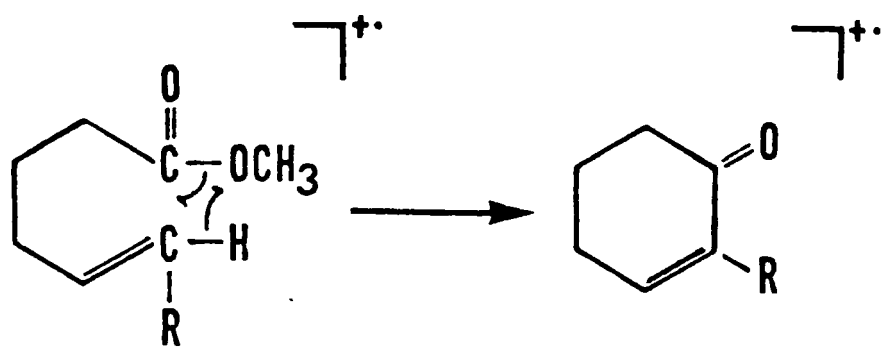

CH3–(CH2)5–CH=CH–(CH2)7COOCH3
–e-
+
CH2–CH–CH2–CH2–(CH2)11COOCH3
+
CH2–CH=CH2
+ •CH2–(CH2)11COOCH3
m/e 41

Figure 2.11. Rationalization of formation of the ion at m/e 41. Double bond migration or a shift in the radical site is believed to initiate an allylic cleavage at the terminal end of the molecule, resulting in the formation of a fragment ion which appears at m/e 41.



$M^{+\cdot}$ $[M-32]^{+\cdot}$

Figure 2.12. Rationalization of the formation of the $[M-32]^{+\cdot}$ ion. Double bond migration, loss of methanol and resultant formation of the stable ring structure could account for the appearance of this ion.

1. Difficulties in identifying polyunsaturated fatty acids result from the absence of molecular ions and the similarity of their fragmentation patterns.

The migration of the double bond in a monounsaturated methyl ester may be used as an explanation for the formation of fragment ions in the more desaturated species. For example, the mass spectrum of methyl linoleate (18:2), shown in Figure 10, Appendix 1, has a base peak at m/e 67 which may be explained as shown in Figure 2.13. Should both double bonds migrate to the terminal end of the chain, an allylic cleavage could result which would produce this fragment ion. The relative abundance of this peak could be explained by the stability afforded the fragment ion due to the conjugation of double bonds. Further experimentation using deuterium labelling might elucidate this fragmentation mechanism. Similar fragment ions having the general formula $C_nH_{2n-3}^+$ would rationalize the peaks occuring at other masses in the spectrum (e.g., m/e 81, 95, 109, and 123). The molecular weight of methyl linoleate is given by the appearance of the molecular ion at m/e 294.

The mass spectra of the methyl esters of linolenic acid (18:3), arachidonic acid (20:4), 5,8,11,14,17-eicosapentaenoic acid (20:5), and 4,7,10,13,16,19-docosahexaenoic acid (22:6) are all very similar. The base peak in all cases occurs at m/e 79. This would correspond to the fragment ion $C_6H_7^+$ and an allylic cleavage can be conceived to rationalize this (Figure 2.14). Contributions to this ion might also result through degradation of fragments with slightly higher m/e values (e.g. 81). Other frag-

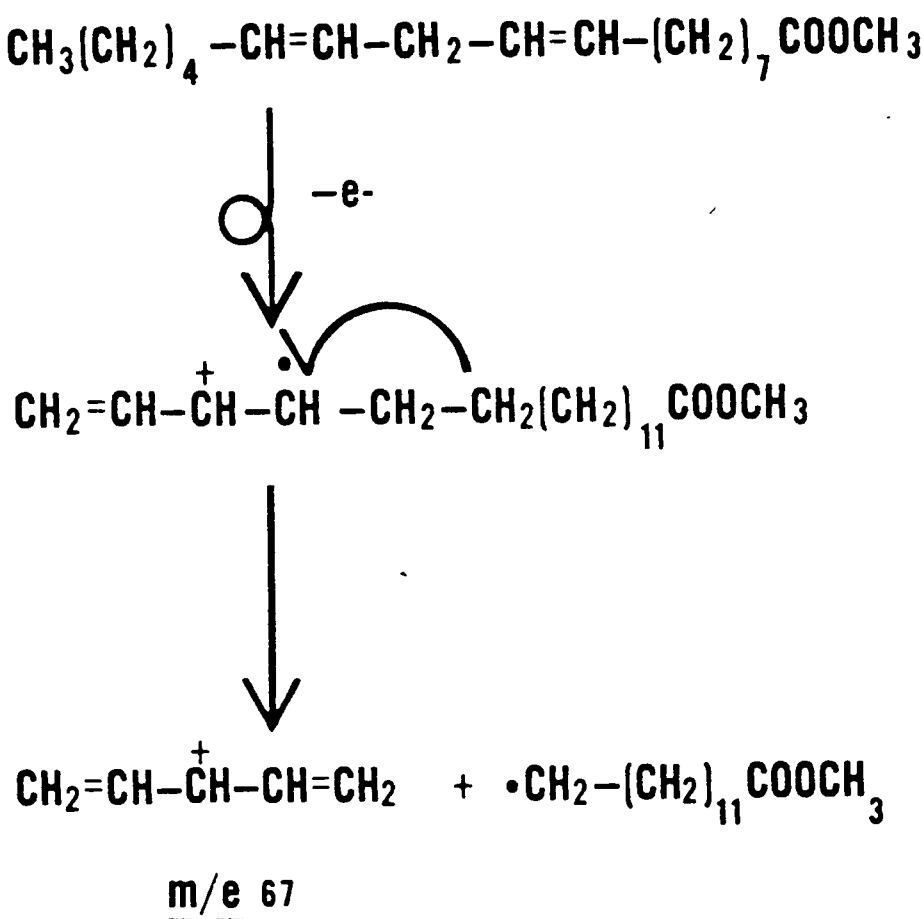

CH3(CH2)4-CH=CH-CH2-CH=CH-(CH2)7COOCH3
-e-
CH2=CH-CH-CH-CH2-CH2(CH2)11COOCH3
CH2=CH-CH-CH=CH2 + •CH2-(CH2)11COOCH3
m/e 67

Figure 2.13. Allylic cleavage resulting after the migration of double bonds to the terminal end of the molecule could rationalize the formation of the ion at m/e 67.

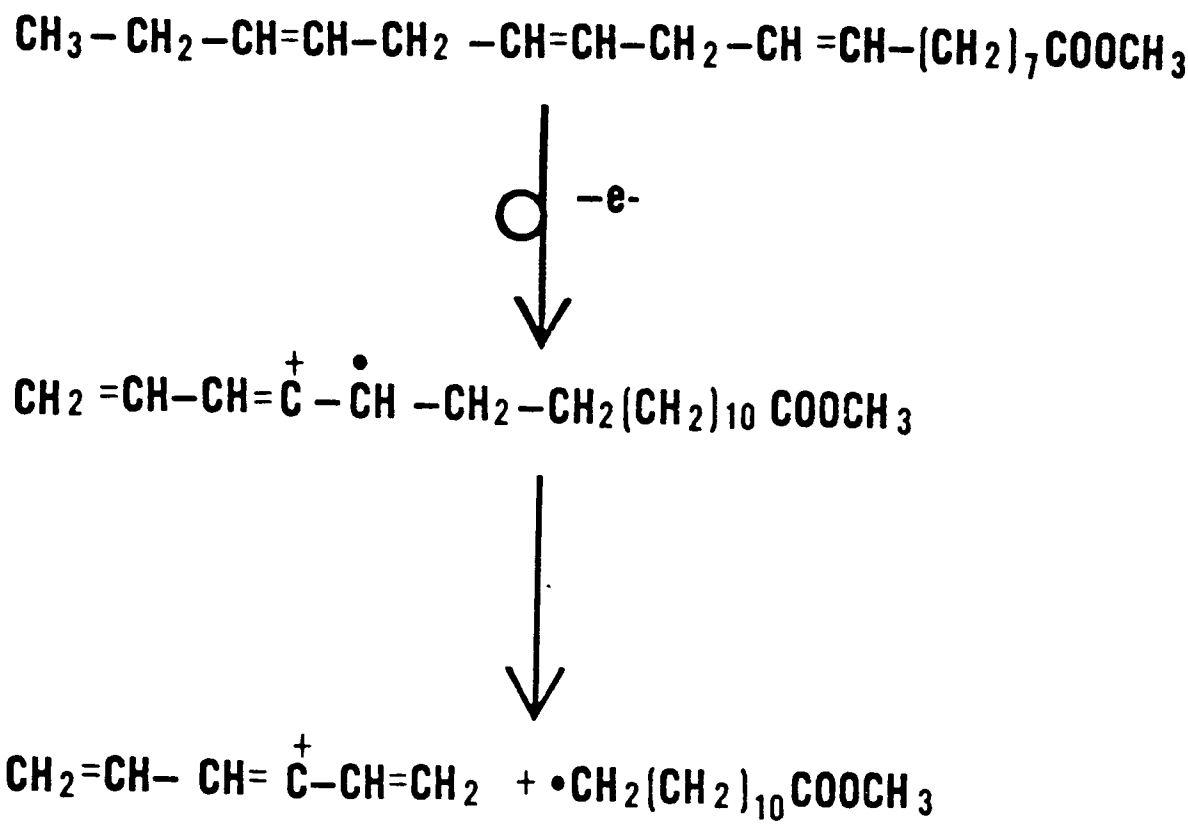

CH3 – CH2 –CH=CH–CH2 –CH=CH–CH2–CH =CH–(CH2)7COOCH3
–e-
CH2 =CH–CH=C – CH –CH2–CH2(CH2)10 COOCH3
CH2=CH– CH= C–CH=CH2 + •CH2(CH2)10COOCH3
m/e 79

Figure 2.14. Allylic cleavage resulting after the migration of double bonds to the terminal end of the molecule could rationalize the formation of the ion at m/e 79. Formation of this fragment would be enhanced by the stable conjugated system shown in this figure.

ment ions in the spectra have already been discussed. The molecular ion of methyl linoleate, although small, gives the molecular weight for the compound, thus simplifying its identification. The spectra of 20:4, 20:5, and 22:6, however, have no molecular ions. In these cases one must either depend on retention index values, or other techniques in order to verify the identification.

3.

RESULTS AND DISCUSSION

3. RESULTS AND DISUSSION

The purpose of this study, as stated in the introduction, was to obtain an understanding of the biochemical interrelationships between shrimp nutrition and maturation. The approach taken to the elucidation of the problem was to use metabolic profiling to evaluate the fatty acid composition of shrimp as compared to that of their diet. When differences among the fatty acid distributions of these profiles were determined to be significantly different ($p<0.05$), a Student's t-test was performed in order to determine which of the major fatty acids in the profiles were significantly different ($p<0.05$) in relative concentraction.

3.1. Fatty acid profiles of wild shrimp.

In the initial experiments, fatty acid profiles of wild shrimp were determined. These results were later used as a guide in the evaluation of different diets. Wild Penaeus stylirostris and Penaeus vannamei were simultaneously collected from the Gulf of Panama on May 11, 1977.

3.1.1. Penaeus stylirostris. Table 3.1 presents the analytical data obtained for Penaeus stylirostris. A typical fatty acid profile obtained from the gonad of a mature female (stage 5) is presented in Figure 3.1. Profiles were obtained using a 2m x 2mm glass column packed with OV-1 on Supelcoport (100-120 mesh). The majority of samples contained a full range of saturated and unsaturated fatty acids from C_{14} to C_{22}. A tabulation of the data

Table 3.1. Relative concentrations of major fatty acids in tissues of P. stylirostris from the Gulf of Panama.[a]

Specimen[b]	Tissue[c]	n	16:0	16:1	18:0	18:1	20:1	20:4+5[d]	22:5+6[e]
Male	G	2	7.4±1.0[f]	2.5±0.2	7.7±0.2	12.6±0.2	8.0±0.5	27.1±0.6	18.0±0.6
	H	2	18.0±0.3	4.1±0.1	9.3±1.1	10.8±0.4	6.1±0.1	12.9±0.1	11.4±0.0
	T	2	17.2±0.1	4.8±0.1	14.6±0.1	13.2±0.1	2.0±0.1	20.4±1.2	16.1±0.4
Female (stage 1)	G	2	9.4±0.1	4.1±0.1	7.6±0.7	19.5±0.4	10.5±1.6	14.0±0.5	7.8±1.5
	H	2	18.2±2.1	5.3±0.1	12.3±1.8	13.2±0.1	10.1±0.4	8.5±0.1	5.6±1.9
	T	2	17.2±0.1	5.4±1.1	14.4±0.1	12.9±0.8	2.1±0.8	21.7±0.5	14.8±0.7
Female (stage 2)	G	2	15.6±1.2	6.5±0.0	9.3±0.9	15.7±1.8	6.6±1.0	12.0±2.1	10.3±2.3
	H	2	22.1±2.9	5.7±0.1	12.0±1.9	13.9±1.1	8.8±1.5	8.3±2.1	6.7±2.3
	T	2	18.2±0.6	5.9±0.4	14.1±0.4	13.1±0.1	2.1±0.4	20.7±0.1	15.5±0.6
Female (stage 3)	G	2	21.7±1.7	9.1±0.1	9.6±0.0	14.8±1.5	2.8±0.4	13.9±0.2	10.4±0.6
	H	2	25.3±2.2	6.9±0.1	10.7±0.7	14.0±1.1	6.2±0.4	8.5±2.1	5.4±1.5
	T	2	18.8±0.0	7.6±0.6	15.1±0.4	14.5±0.3	1.9±0.1	17.5±0.6	11.2±0.4
Female (stage 5)	G	2	21.7±2.3	8.5±0.4	9.8±0.7	14.5±0.6	3.1±0.7	12.6±0.1	9.5±0.6
	H	2	24.5±1.0	6.8±0.4	10.9±0.4	13.1±0.3	6.4±0.6	8.6±1.3	5.2±0.1
	T	2	19.0±0.7	6.8±0.0	15.7±1.0	14.4±0.2	2.1±0.2	16.7±1.3	12.3±0.8

Footnote to Table 3.1.

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]For females, stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 4 animals were collected.

[c]G = gonad, H = hepatopancreas, T = tail muscle.

[d]20:4 and 20:5 not resolved on OV-1.

[e]22:5 and 22:6 not resolved on OV-1.

[f]Mean ± standard deviation.

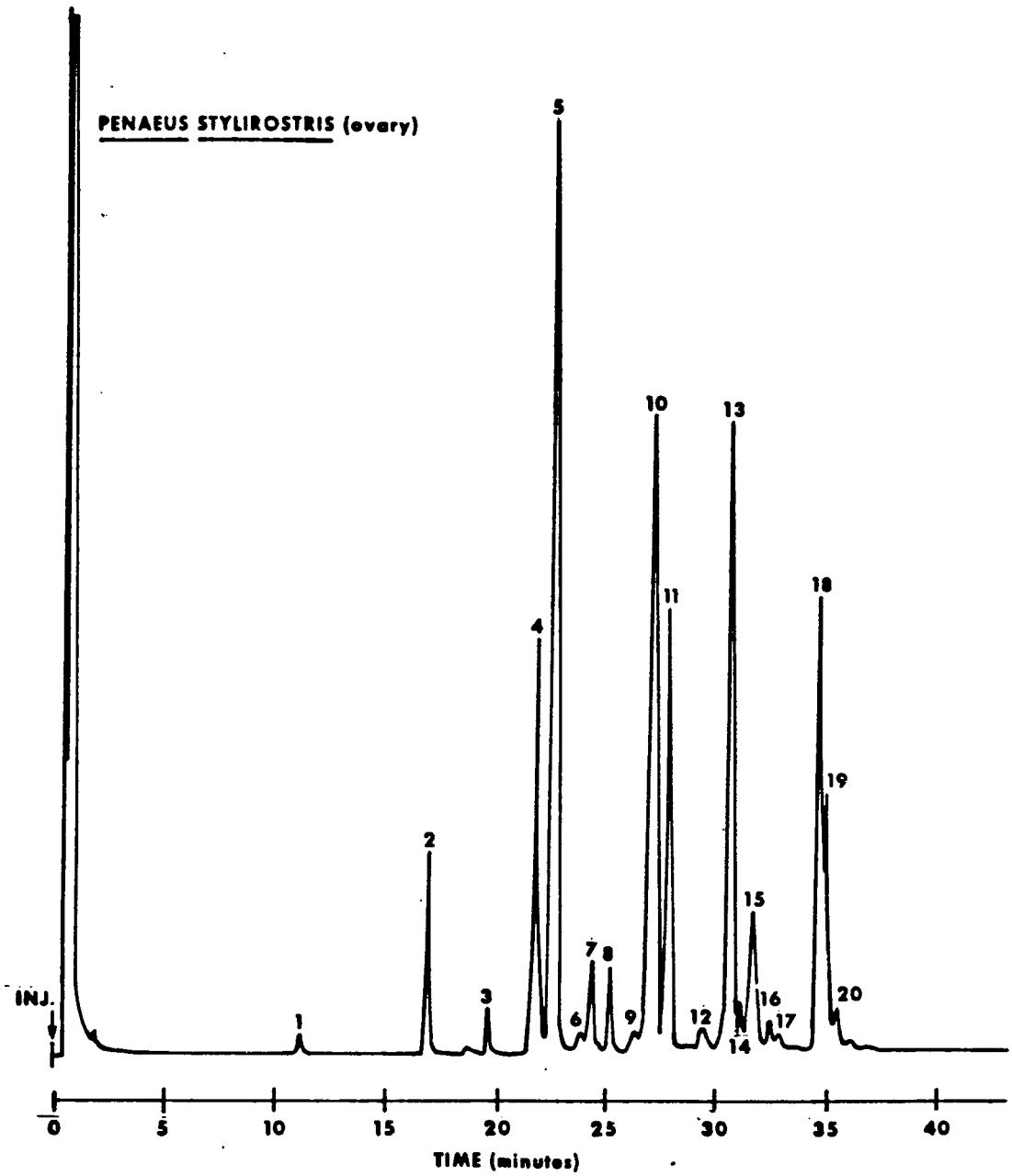

PENAEUS STYLIROSTRIS (ovary)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 3.1.. Fatty acid profile from the ovary of a mature (stage 5) female P. stylirostris. Chromatography was performed using a 2m x 2mm (i. d.) glass column packed with 3% OV-1 on Supelcoport (100--120 mesh.).

Identification of methyl esters

1.	laurate	12:0
2.	myristate	14:0
3.	pentadecaneate	15:0
4.	palmitoleate	16:1
5.	palmitate	16:0
6.	heptadecanoate (br)	br17:0 ?
7.	heptadecenoate	17:1
8.	heptadecanoate	17:0
9.	linoleate	18:2
10.	oleate	18:1
11.	stearate	18:0
12.	nonadecenoate	19:1
13.	arachidonate + eicosapentaenoate	20:4+5
14.	eicosatrienoate	20:3
15.	eicosenoate	20:1
16.	heneicosenoate (br) ?	br21:1 ?
17.	eicosanoate	20:0
18.	docosapentaenoate + docosahexaenoate	22:5+6
19.	docosatetraenoate	22:4
20.	docosatrienoate	22:3

from Figure 3.1, and respective mass spectra (when available) are presented in Appendix 2. Comparisons were made between the spectra of fatty acid methyl esters from shrimp tissues and those of standards whenever possible. Otherwise, the sample spectra were identified by mass spectral interpretation (see Section 2.8). Compounds that were not scanned by the mass spectrometer or which gave only weak spectra were identified by using retention index values.

In most of the samples examined, palmitic acid (16:0) was the major individual constituent. Saturated fatty acids typically comprised about 40% of the total fatty acid content, while monounsaturated compounds accounted for about 30% of the total content. Interestingly, approximately 25% of the total content consisted of polyunsaturated fatty acids. The remaining 5% included compounds such as branched chain fatty acids, or components which were unidentified.

The data in Table 3.1 include the values obtained for the major fatty acids: palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), eicosenoic (20:1), arachidonic (20:4), 5,8,11,14,17-eicosapentaenoic (20:5), 7,10,13,16,19-docosapentaenoic (22:5), and 4,7,10,13,16,19-docosahexaenoic (22:6) acids. These acids comprise approximately 75-80% of the total fatty acid content. Although 20:4 and 20:5 as well as 22:5 and 22:6 were not separated with the stationary phase (OV-1) used in this early experiment, subsequent analysis using a polar stationary phase (SP-2330) revealed that they were both present. In the majority of cases, 20:5 constituted approximately 70-75% of the 20:4 + 20:5

peak, while 22:6 comprised 75-80% of the 22:5 + 22:6 peak.

The distribution of fatty acids in the hepatopancreas does not change with ovarian maturation. Furthermore, no significant changes are detected when comparing the distributions in the hepatopancreas of males to those of females at stage 1 or stage 5.

The chi-square test shows that no significant changes occur in fatty acid distribution in the ovaries when comparing successive stages of maturation. A significant difference can be shown, however, when a comparison is made between the profiles from ovaries at stage 1 versus stage 5. In this case, stage 5 animals have increased relative percent concentrations of 16:0 and 16:1, while there is a subsequent decrease in the relative percent concentrations of 18:1 and 20:1 ($p<0.05$). Additionaly, there are significant differences ($p<0.05$) in the fatty acid distributions between male gonads and ovaries at stage 1. In this respect, males have lower relative percent concentrations of 16:1 and 18:1, but higher relative percent concentrations of the polyunsaturated fatty acids 20:4, 20:5, and 22:6. Distributional differences can also be found between the fatty acid contents in males and stage 5 females. In this respect, males have lower relative percent concentrations of 16:0 and 16:1, but higher relative percent concentrations of 20:1, 20:4, 20:5, and 22:6. This suggests that the testis may require more polyunsaturated acids than the ovary.

If a comparison is made between fatty acid profiles of the gonads and hepatopancreas, only two differences are significant. The ovaries of stage 1 females have lower relative percent concentrations of 16:0 and 16:1, and higher relative percent

concentrations of 18:1, 20:4, and 20:5, than the hepatopancreas of these animals. Additionally, the male gonads also show lower relative percent concentrations of 16:0 and 16:1, while 18:1, 20:4, 20:5, 22:5, and 22:6 are more abundant than corresponding relative percent concentrations in the hepatopancreas.

As was true in the hepatopancreas, no change in fatty acid profiles is observed in the tails during ovarian maturation. Furthermore, a comparison of the distribution of fatty acids in the tail muscle of males to those of females at stage 1 and stage 5 reveals no differences. However, when one compares the tail muscle of males and females (at all stages) to the hepatopancreas, quite noticeable differences can be shown. In all cases, 20:1 occurs at lower relative percent concentrations in the tail muscle, while those of the polyunsaturated fatty acids 20:4, 20:5, 22:5, and 22:6 are higher. This would suggest that the tail muscle has a requirement for polyunsaturated fatty acids and accumulates these at the expense of 20:1. Comparisons made between the gonads and tails show this same pattern when observing males and females at stages 1 and 2 of ovarian maturation.

3.1.2. Penaeus vannamei. The data obtained for Penaeus vannamei are presented in Table 3.2. Due to small sample sizes no fatty acids were recovered from the ovaries of stage 1 females. A typical chromatogram is given in Figure 3.2. Profiles were obtained using a 2m x 2mm (i.d.) glass column with OV-1 as the stationary phase. The identities of individual components were the same as those for P. stylirostris.

Table 3.2. Relative concentrations of major fatty acids in tissues of P. vannamei from the Gulf of Panama.[a]

Specimen[b]	Tissue[c]	n	16:0	16:1	18:0	18:1	20:1	20:4+5[d]	22:5+6[e]
Male	G	1	41.4	3.5	26.3	15.7	0.0	2.7	0.0
	H	2	25.9±1.8[f]	6.0±0.4	13.1±0.7	13.0±1.0	7.5±0.8	5.4±0.1	3.5±0.4
	T	2	14.9±0.4	4.7±0.4	15.2±0.8	12.0±0.5	2.3±0.2	18.7±0.6	14.1±0.3
Female	G	0	-	-	-	-	-	-	-
(stage 1)	H	1	20.1	5.8	8.4	10.0	6.3	12.8	8.8
	T	1	13.9	4.5	12.5	10.7	2.6	19.8	15.8
Female	G	2	26.7±6.3	10.1±0.8	12.0±4.0	18.4±2.6	2.4±0.5	8.0±8.6	4.9±6.7
(stage 2)	H	2	23.2±1.7	6.5±1.1	12.4±0.8	14.1±0.8	7.6±0.8	10.1±3.0	6.0±1.9
	T	2	20.4±3.0	7.2±1.0	17.2±2.2	15.5±2.0	3.6±0.0	11.7±4.2	7.2±3.9
Female	G	2	20.0±0.3	8.7±1.1	9.0±1.7	15.1±2.3	3.0±0.6	14.1±2.0	10.9±3.3
(stage 3)	H	2	19.6±1.5	6.1±0.6	10.4±0.2	14.2±2.3	9.0±0.7	8.7±1.8	6.9±1.6
	T	2	20.0±0.0	5.9±0.7	15.3±0.2	13.3±0.1	6.0±1.2	13.9±1.8	11.1±1.3
Female	G	2	20.6±2.4	8.6±1.1	9.5±0.8	14.1±1.0	2.9±0.2	13.8±0.9	10.4±1.2
(stage 5)	H	2	19.1±2.1	6.8±0.1	10.7±1.1	14.6±0.6	8.0±0.8	10.2±0.4	6.3±0.7
	T	2	21.5±0.4	6.6±0.3	16.1±0.3	13.6±0.1	6.9±0.1	11.1±0.3	8.7±0.6

Footnote to Table 3.2

[a]Totals do not equal 100% because minor fatty acids are not listed

[b]For females, stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 4 animals were collected.

[c]G = gonad, H = hepatopancreas, T = tail muscle.

[d]20:4 and 20:5 not resolved on OV-1.

[e]22:5 and 22:6 not resolved on OV-1.

[f]Mean ± standard deviation.

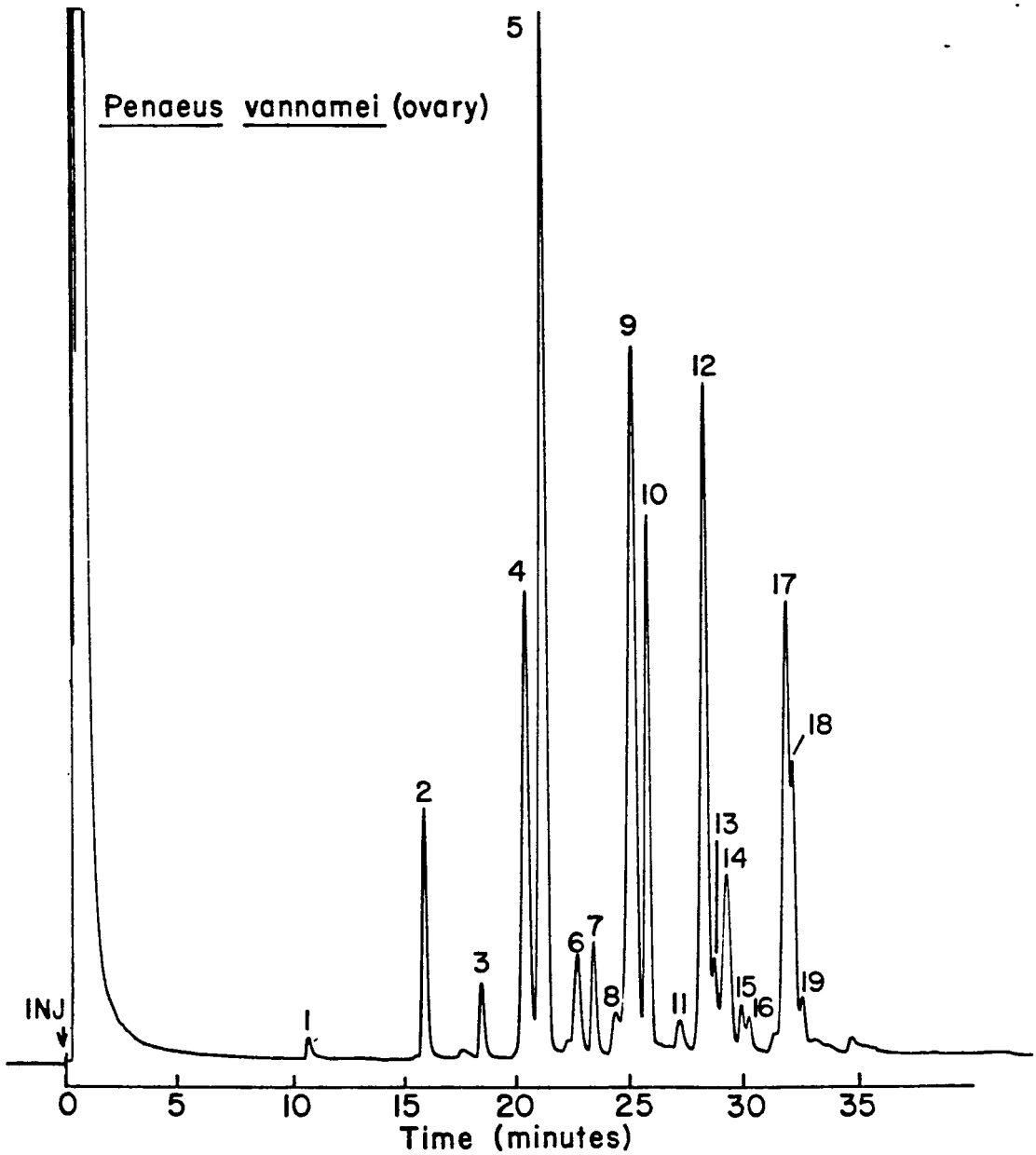

Penaeus vannamei (ovary)
INJ
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
0
5
10
15
20
25
30
35
Time (minutes)

Figure 3.2. Fatty acid profile from the ovary of a mature (stage 5) female P. vannamei. Chromatography was performed using a 2m x 2 mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	laurate	12:0
2.	myristate	14:0
3.	pentadecanoate	15:0
4.	palmitoleate	16:1
5.	palmitate	16:0
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	linoleate	18:2
9.	oleate	18:1
10.	stearate	18:0
11.	nonadecenoate	19:1
12.	arachidonate + eicosapentaenoate	20:4+5
13.	eicosatrienoate	20:3
14.	eicosenoate	20:1
15.	heneicosenoate (br) ?	br 21:1 ?
16.	eicosanoate	20:0
17.	docosapentaenoate + docosahexaenoate	22:5+6
18.	docosatetraenoate	22:4
19.	docosatrienoate	22:3

The major individual fatty acid in most cases was palmitic acid. The distribution of saturated, monounsaturated, and polyunsaturated fatty acids was similar to that obtained for P. stylirostris.

The fatty acid distribution in the hepatopancreas of this species did not vary with ovarian maturation. A difference was noted when comparing the profiles of males to those of stage 1 females. In this respect, the male's hepatopancreas had lower relative percent concentrations of polyunsaturated fatty acids 20:4, 20:5, 22:5, and 22:6, than the stage 1 female.

The chi-square test did not reveal any significant changes in metabolic profiles of the ovaries during the maturation process. A comparison of profiles in gonads of males versus stage 5 females revealed significant differences: 18:0 was at higher relative percent concentrations in males, while 20:1 occured at lower relative concentrations than in the females. Although no differences were found between the ovaries and hepatopancreas, the male gonad had higher relative concentrations of 16:0 and 18:0 than its respective hepatopancreas. It must be pointed out, however, that the metabolic profile obtained from the one male gonad analyzed appears to be atypical judging by values obtained for male gonads in P. stylirostris. Before conclusions can be drawn, other specimens should be analyzed. Unfortunately, difficulty in obtaining these animals has prevented this.

The chi-square test revealed that there were no significant difference in the distribution of fatty acids in ovaries of females during the maturation process. Furthermore, no differences in

distribution were found between the ovaries and their respective hepatopancreas; however, male gonads were found to have higher relative concentrations of 16:0 and 18:0 than in the hepatopancreas. Again, this gonad may be atypical in nature.

Variations in the distribution of fatty acids in metabolic profiles of the tail muscle between stage 1 and stage 2 females were determined by the chi-square test. However, due to the small sample size analyzed for the stage 1 animal, the t-test did not find significant differences among the major fatty acids. Differences were found between stage 1 and stage 5 animals. In this respect stage 1 animals had higher relative concentrations of 20:4 and 20:5, while having lower relative concentrations of 16:0, 18:1, and 20:1. Although no variations were found between the tail muscle and hepatopancreas of females, there were lower relative concentrations of 16:0 and 20:1, and higher relative concentrations of polyunsaturated fatty acids 20:4, 20:5, 22:5, and 22:6 in the tail muscles of males as opposed to the hepatopancreas. This same difference was also encountered when comparing male gonads to the tail muscles, although 18:0 replaces 20:1 as being lower in concentration in the tail.

A comparison of data obtained for P. stylirostris and P. vannamei is appropriate at this point. Both species were collected simultaneously from the same general area, thus comparisons of the animals should give an indication of the importance that diet has on fatty acid composition. Based on the premise that diet is the principle factor in determining fatty acid content in shrimp, one would expect metabolic fatty acid profiles to be quite similar

between closely related species raised in the same environment. Indeed, statistical comparisons between the species reveal considerable similarity. Only in the cases of males and stage 2 females were any significant differences in fatty acid distribution found.

3.1.3. Penaeus setiferus. Wild specimens of P. setiferus were collected on July 22, 1977 off the Texas coast in the Gulf of Mexico. Data from fatty acid profiles in the white shrimp Penaeus setiferus are presented in Table 3.3. A typical chromatogram is shown in Figure 3.3. Profiles were obtained using a 2m x 2mm (i.d.) glass column packed with OV-1.

P. setiferus contains a full range of fatty acids from C_{14} to C_{22} as was true with the other species. The concentrations of the acids are nearly evenly distributed between monounsaturated (30%) and polyunsaturated compounds (30%) while there are slightly more saturated fatty acids (40%).

There is no variation of the relative percent concentrations of fatty acids in the hepatopancreas during ovarian maturation. Comparisons between the fatty acid distribution in the hepatopancreas of males and stage 1 females reveal significantly lower relative concentrations of 16:1 in the males. No such difference was found when comparing males to stage 5 females.

The fatty acid distribution in the ovaries did not change significantly during maturation. A comparison of male gonads and ovaries of stage 1 females showed lower relative percent concentrations of 16:0 and 18:1 in the males. Furthermore, comparisons made between the gonads of males and stage 5 females revealed

Table 3.3. Relative concentrations of major fatty acids in tissues of P. setiferus from the Gulf of Mexico.[a]

Specimen[b]	Tissue[c]	n	16:0	16:1	18:0	18:1	20:1	20:4+5[d]	22:5+6[e]
Male	G	2	9.6±1.8[f]	2.7±0.4	8.2±0.5	10.8±0.0	4.0±0.6	31.2±1.6	20.4±3.0
	H	2	14.9±2.3	3.9±0.5	9.9±0.7	8.3±0.1	8.2±0.4	16.2±2.1	7.9±0.4
	T	1	18.3	5.6	18.3	14.4	2.1	19.5	10.3
Female	G	2	13.7±7.4	8.9±0.6	7.7±1.5	16.5±0.1	3.6±1.1	23.5±7.5	10.0±2.3
(stage 1)	H	2	26.5±4.2	8.5±0.3	10.3±0.9	13.3±2.8	3.0±2.5	7.7±8.8	5.2±2.8
	T	2	21.1±0.0	8.7±1.5	13.8±1.2	14.5±0.2	3.5±0.1	17.5±0.1	11.4±2.5
Female	G	2	19.0±2.8	10.2±2.8	10.2±2.6	19.8±2.5	5.7±2.7	13.4±11.7	5.1±4.8
(stage 2)	H	2	16.2±1.6	11.0±1.6	11.2±0.6	12.3±2.1	7.6±4.6	15.3±12.2	5.7±4.0
	T	1	19.2	8.2	16.5	15.9	1.8	22.7	8.9
Female	G	2	21.5±0.7	9.7±0.9	10.3±0.7	15.6±0.8	6.6±1.3	11.7±2.5	6.8±2.4
(stage 3)	H	2	19.7±0.9	9.0±3.5	11.7±0.4	10.8±1.2	9.0±0.2	10.5±0.2	6.0±0.4
	T	2	17.7±1.4	9.4±1.9	15.0±2.5	15.7±3.1	2.4±0.8	15.9±6.6	8.9±5.6
Female	G	2	20.0±1.4	9.8±0.1	9.2±0.6	13.7±0.9	4.1±1.1	14.8±0.3	8.2±1.0
(stage 5)	H	2	18.6±4.3	8.2±0.1	10.2±0.3	11.1±2.3	6.0±2.5	17.4±6.9	8.4±2.5
	T	2	21.0±1.2	8.8±0.2	14.3±0.2	15.3±2.2	2.8±1.1	16.8±2.5	10.9±0.3

Footnote to Table 3.3

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]For females, stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 4 animals were collected.

[c]G = gonad, H = hepatopancreas, T = tail muscle.

[d]20:4 and 20:5 not resolved on OV-1.

[e]22:5 and 22:6 not resolved on OV-1.

[f]Mean ± standard deviation.

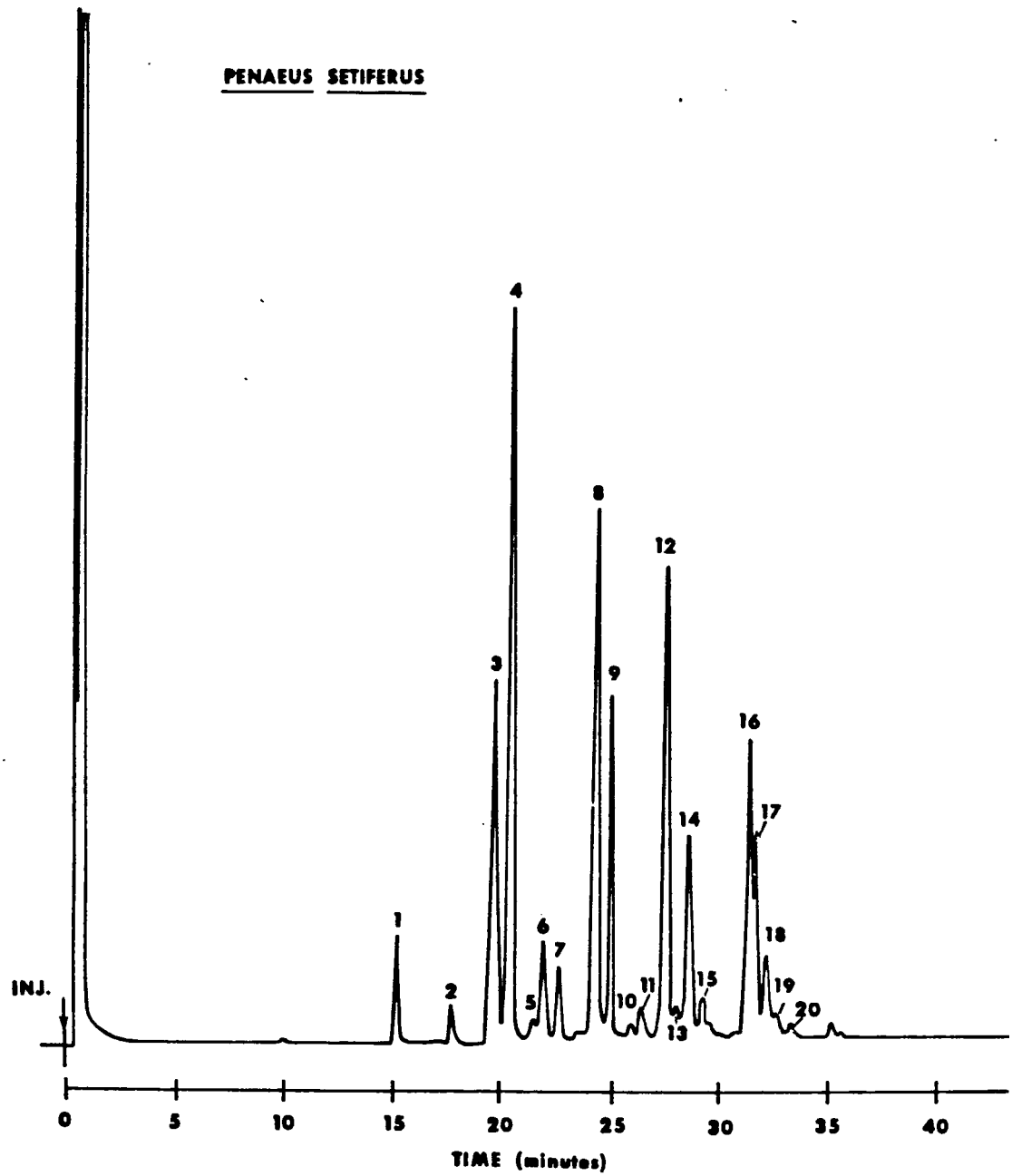

PENAEUS SETIFERUS
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 3.3. Fatty acid profile from the ovary of a mature (stage 5) female P. setiferus. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecanoate	15:0
3.	palmitoleate	16:1
4.	palmitate	16:0
5.	heptadecanoate (br)	br 17:0
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	oleate	18:1
9.	stearate	18:0
10.	nonadecanoate (br)	br 19:0
11.	nonodecenoate	19:1
12.	arachidonate + eicosapentaenoate	20:4+5
13.	eicosatrienoate	20:3
14.	eicosenoate	20:1
15.	heneicosenoate ?	21:1 ?
16.	docosapentaenoate + docosahexaenoate	22:5+6
17.	docosatetraenoate	22:4
18.	docosatrienoate	22:3
19.	docosadienoate ?	22:2 ?
20.	docosenoate ?	22:1 ?

significantly lower relative concentrations of 16:0, 16:1, and 18:1, plus higher relative percent concentrations of the polyunsaturated fatty acids 20:4, 20:5, 22:5, and 22:6. In a comparison made between the gonads and hepatopancreas of P. setiferus, no significant differences could be found in the females. However, the male gonad had higher relative percent concentrations of 18:1, 20:4, 20:5, 22:5, and 22:6 and lower relative percent concentrations of 20:1 than the hepatopancreas.

The chi-square test revealed no significant changes in fatty acid distributions in the tail muscles with respect to sex or ovarian maturation. Differences were found between the hepatopancreas and tail muscles of males and stage 3 females. In both cases relative percent concentrations of 20:1 were reduced in the tail muscle. Additionally, the relative percent concentrations of 18:0 were found to be higher in the tail muscle of males when compared to the hepatopancreas. No other significant differences could be found.

Comparisons of the fatty acid distributions in P. setiferus are similar to those in the other species. The chi-square test revealed no significant differences except when comparing to the ovaries and hepatopancreas of stage 1 females in P. stylirostris, and to the hepatopancreas of stage 1 females and the gonads and hepatopancreas of males in P. vannamei.

In summary, the ovaries of most animals did not vary in fatty acid distribution when comparing sequential stages of maturation. Additionally, no significant changes were found to occur in the hepatopancreas during ovarian maturation. Furthermore, the

fatty acid distribution in the tail muscle was found to be unaffected by ovarian maturation or sex. Comparisons between the gonad and hepatopancreas show that no changes in distribution occur except in stage 1 females and in males. However, there are significant differences when comparing the hepatopancreas to the tail muscle in males and females. It is interesting that in all species, the gonad, hepatopancreas, and tail muscle of males are significantly different from one another. When differences in fatty acid distribution occur between tissues (especially in P. stylirostris males), the relative percent concentrations of poly-unsaturated fatty acids are generally greatest in the tail muscle, and greater in the gonads than in the hepatopancreas.

3.1.4. Discussion. It is relevant at this point to make some comments concerning the nature of the various tissues under study. The hepatopancreas was chosen for study primarily because it has been shown to be a storage device for lipids in crustaceans (Van Weel, 1970). Guary et al. (1974) have verified this fact in the shrimp Penaeus japonicus, showing much higher concentrations of lipids, primarily triglycerides (a storage lipid), in the hepato-pancreas. The function of the hepatopancreas is in digestion, and this organ has often been refered to as the digestive gland. It is also the main tissue involved in absorption of lipids from the diet. Crustaceans absorb lipids virtually unchanged from the diet (Huggins and Munday, 1968); consequently, one would expect to find fatty acids in this tissue which will to a large degree reflect the nature of fatty acids in the diet. Furthermore, one might

expect that little change should be found when comparing fatty acid profiles from the hepatopancreas of different shrimp of the same species raised in the same environment, regardless of sex or degree of ovarian maturation. This hypothesis is supported by the data presented here.

The gonads were examined due to their obvious importance in reproduction. The ovaries in shrimp are known to increase in total lipid content as maturation progresses (Lawrence et al., 1979). Lipids are most likely stored in the yolk portion of eggs prior to spawning, to be used by larvae during development. Guary et al. (1974) and Lawrence et al. (1979) have provided data which suggest that, during maturation, a mobilization of lipids occurs from the hepatopancreas to the ovary. This is supported by the fact that a large majority of the lipids in mature females is comprised of triglycerides. Furthermore, Teshima and Kanazawa (1978) have shown that the hepatopancreas is capable of releasing triglycerides and phospholipids into the hemolymph. This would suggest, then, that similarities should be found between fatty acid profiles in the ripe ovaries and the hepatopancreas. This same similarity would not be expected in immature ovaries, or in male gonads. Indeed, the results presented here show that this hypothesis is true for fatty acid distributions.

The tail muscle was selected for study because it comprises much of the body mass of the shrimp, and because of its commercial importance. This tissue is quite different from either the hepatopancreas or gonads in that it is primarily composed of protein. The principle lipids in the tail are cholesterol esters and

phospholipids, which are major constituents of cell membranes. A study of this tissue would be expected to reveal fatty acid profiles much different in distribution from those of the hepatopancreas or ovaries. Indeed, the fatty acid content of the tail muscle should be more indicative of the shrimp's requirements for fatty acids than that of other tissues examined. The results obtained with Penaeus stylirostris in particular verify that the tail muscle is significantly different in fatty acid distribution from the other tissues. Additionally, increased relative concentrations of polyunsaturated fatty acids in the tail muscle suggest that these particular fatty acids are required for growth. Insufficient amounts of these acids would result in poor health and could account for the lack of ovarian maturation in past experiments.

As mentioned previously, similarities in the tail muscle fatty acid distributions between P. stylirostris and P. vannamei were attributable to their common diet. What is more remarkable is that similarities were found between the fatty acid profiles of these species and those in P. setiferus, even though P. setiferus was obtained from a completely different environment. This suggests that if fatty acids are important for maturation, a dietary regimen which works for one species should be satisfactory for all three.

The data obtained for the three species of penaeid shrimp under examination suggest that similarities should exist with other penaeid species. Indeed, the work presented by Guary et al. (1974) on Penaeus japonicus collected from the Sea of Japan agrees favorable with material presented in the previous sections. Considering the ovaries of females in Guary's paper to represent

stage 5 females, values for 16:0 (17.4%), 16:1 (10.1%), 18:0 (5.7%), 18:1 (11.9%), 20:1 (4.4%), 20:4 (4.8%), 20:5 (12.6%), and 22:6 (9.4%) are equivalent to those results obtained for stage 5 ovaries in the penaeid species examined here (see Tables 3.1-3.3).

3.2. Fatty acids in cultured shrimp diets.

The previous section showed that high relative percent concentrations of polyunsaturated fatty acids are found in shrimp. As mentioned in the introduction, higher animals do not have the capabilitity of synthesizing polyunsaturated fatty acids, and must depend on dietary sources. Deficient levels of these compounds in prepared diets fed to cultured shrimp could be responsible for poor survival rates and the inability to obtain ovarian maturation. Therefore, close scrutiny of the fatty acid content in diets must be made.

This section presents the results obtained by determining the fatty acid content of various types of shrimp diets. Initially, a traditional diet was examined to determine whether a deficiency of polyunsaturated acids existed, since this diet was incapable of promoting ovarian maturation in shrimp.

3.2.1. Traditional diet. The tabulation of fatty acids obtained from a commercially available marine chow (Marine Ration #40, Ralston--Purina Co.) is presented in Table 3.4. The fatty acid profile, obtained by performing chromatography using a 2m x 2mm (i.d.) glass column packed with OV-1 is shown in Figure 3.4. Comparisons with fatty acid profiles from wild shrimp reveal that there

Table 3.4. Relative concentrations of fatty acids obtained from MR-40.

Fatty acid	Relative %
14:0	4.2
16:0	29.7
16:1	4.5
18:0	10.5
18:1	43.1
20:4+20:5[a]	4.8
22:4	0.9
22:5+22:6[b]	2.4

[a] 20:4 and 20:5 not resolved on OV-1.

[b] 22:5 and 22:6 not resolved on OV-1.

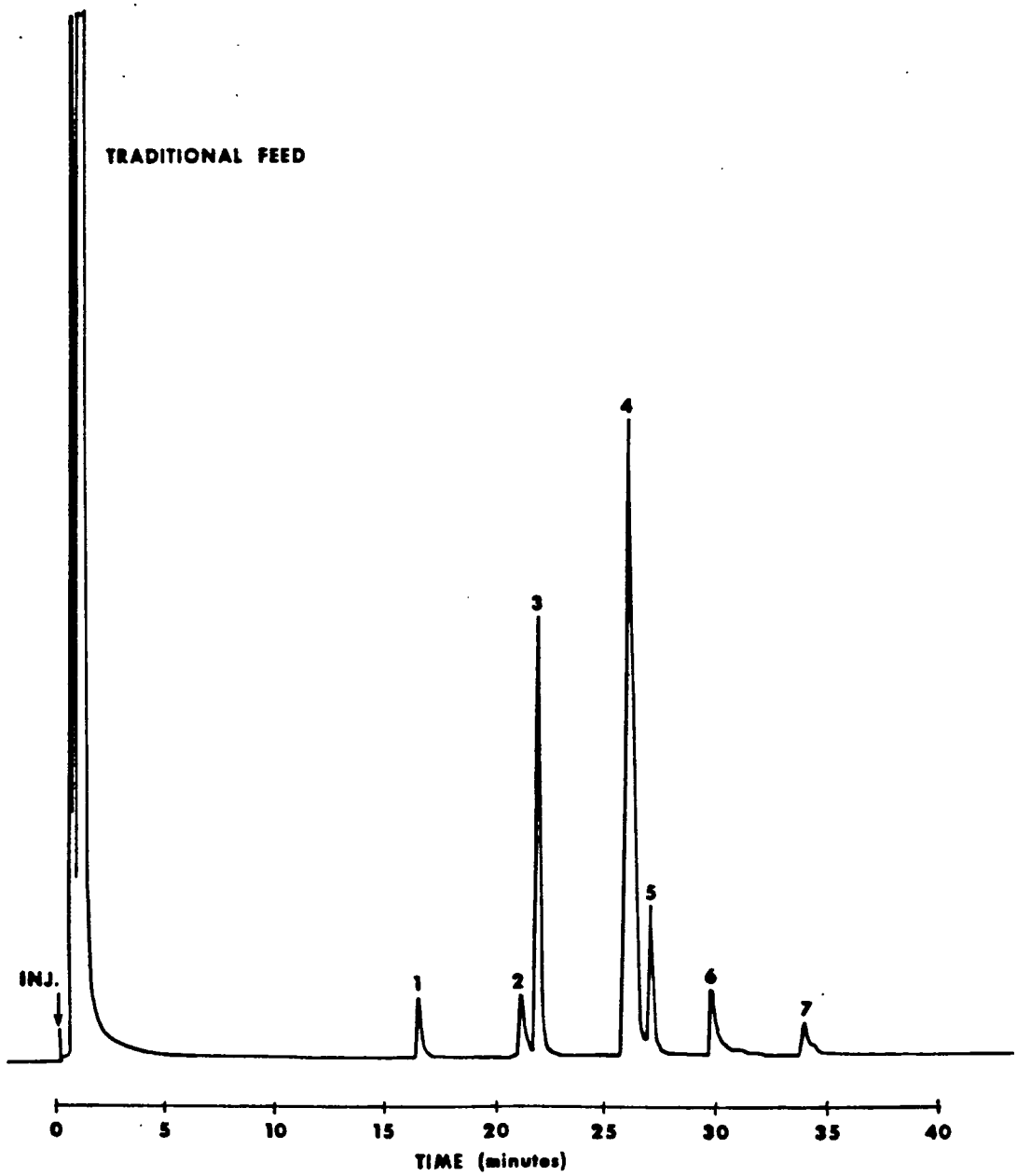

TRADITIONAL FEED
INJ.
1
2
3
4
5
6
7
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 3.4. Fatty acid profile from a traditional feedstock (MR-40). Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1. muristate	14:0
2. palmitoleate	16:1
3. palmitate	16:0
4. oleate	18:1
5. stearate	18:0
6. arachidonate + eicosapentaenoate	20:4+5
7. docosapentaenoate + docosahexaenoate	22:5+6

Table 3.5. Relative concentrations of major fatty acids from some marine invertebrates collected from the Gulf of Mexico.[a]

Specimen	16:0	16:1	18:0	18:1	20:1	20:4+5[b]	22:5+6[c]
Annelids							
Glycera dibranchiata	12.9	4.5	6.6	10.7	15.3	21.5	10.0
Glycera sp.	11.2	3.1	8.7	6.4	12.2	14.0	12.9
Marphysa sanguina	11.2	3.6	7.6	7.0	9.1	21.1	18.0
Nereis succinea	16.9	20.9	4.3	9.4	4.2	14.8	2.7
Nereis viridens	18.7	4.2	4.9	15.8	10.2	20.7	7.2
Pista palmata	18.5	26.5	2.7	8.2	3.6	18.3	5.0
Spiochaetopterus oculatus	19.0	8.6	7.1	6.5	7.7	19.8	5.1
Bivalves							
Brachidontes recurvus	23.7	11.5	4.1	8.0	8.6	10.0	10.7
Crassostrea virginica	23.6	5.5	3.7	8.8	6.8	18.1	12.7
Cyrtopleura costata	22.2	16.8	6.8	6.2	4.0	20.6	9.2
Tagelus plebeius	25.6	15.9	9.1	10.3	10.7	3.0	3.7
Cephalopod							
Loligo opalescens	23.3	0.4	7.0	2.7	5.4	17.9	38.9
Crustaceans							
Balanus sp.	21.2	23.3	4.3	11.0	7.8	7.3	4.2
Uca minax	14.9	13.4	6.7	17.9	10.5	16.5	6.4
Gastropod							
Nassarius vibex	13.9	7.6	9.2	9.7	12.0	15.7	8.5

Footnote to Table 3.5.

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]20:4 and 20:5 not resolved on OV-1.

[c]22:5 and 22:6 not resolved on OV-1.

is a substantial lack of polyunsaturated fatty acids in the dry feed, with a preponderance of saturated and monounsaturated C_{16} and C_{18} fatty acids. If this feedstock is to be used for the purposes of maturation, the recommendation (based on the hypothesis that ovarian maturation requires a diet containing polyenoic fatty acids) would be to supplement the diet with a source of polyunsaturated fatty acids. It should be noted that the ingredients used in MR-40 may have contained polyunsatured fatty acids, but that these could have been lost due to autoxidation during processing or storage.

3.2.2. Food supplements. A number of marine invertebrates were screened in order to find an economical food supplement having substantial amounts of polyunsaturated fatty acids. Samples indigenous to the Gulf of Mexico were collected from West Bay, Galveston, Texas. Additionally, the blood worm Glycera dibranchiata and the sand worm Nereis viridens (obtained from F. H. Hammond, Wholesale Blood Worms and Sand Worms, Wisscasset, Maine) were examined. The relative concentrations of major fatty acids are listed in Table 3.5. All organisms were found to contain a full range of saturated and unsaturated fatty acids from C_{14} to C_{24}. High relative percent concentrations of polyunsaturated fatty acids in these organisms should meet the requirements that shrimp may have for these compounds. The commercial blood worm and sand worm, as well as an indigenous blood worm (Glycera sp.), had fatty acid profiles most similar to those found in the ovaries of wild shrimp. Chromatograms from these animals are shown in

Figures 3.5-3.7. Additionally, the squid Loligo opalescens (obtained commercially from Southeast Packing Co., Galveston, Texas), had notably high relative concentrations of polyunsaturated fatty acids (Figure 3.8). The commercial availability of the blood worm, sand worm, and squid makes these invertebrates an economically acceptable source of polyunsaturated fatty acids for use as food supplements.

Wild specimens of P. setiferus were obtained off of the Texas coast in the Gulf of Mexico on June 12, 1978. None of the animals had maturing ovaries. Shrimp were stocked at a density of $6.5/m^2$ into four raceways (8.5 x 1.2 m) and two circular tanks (3.0 m diameter), and maintained on a diet supplemented with blood worms, sand worms, and squid. One-half of the animals had their eyestalks unilaterally ablated. Eyestalk ablation has been reported to enhance ovarian maturation in shrimp (Caillouet, 1972; Aquacop, 1975). Ovarian development occured within 3 to 4 weeks after the experiment was initiated: 63 egg collections yielded a total of 4.3 million eggs (Brown et al., 1979).. None of the eggs produced in this experiment were fertilized because the spermatophores of males had a bacterial infection (Vibrio sp.).

A second experiment was conducted in a similar manner starting on November 28, 1978, and resulted in spawning on January 15, 1979. This experiment demonstrates that reproducible ovarian maturation can be obtained even at a time of the year when natural populations are not breeding.

Although spermatophore transfer was not accomplished in these two experiments, eggs were found to undergo cortical reaction

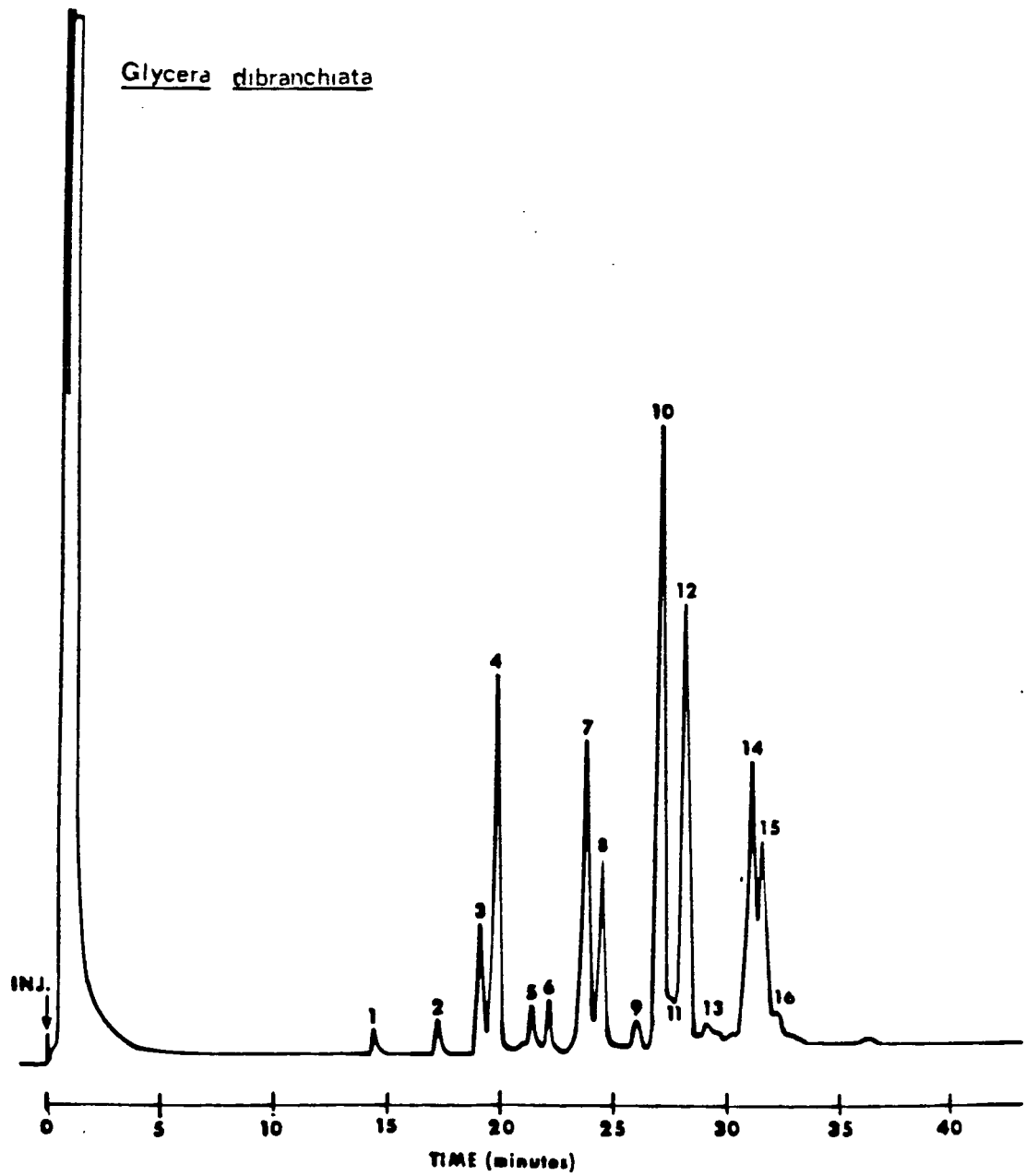

Glycera dibranchiata
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 3.5. Fatty acid profile from the blood worm Glycera dibranchiata. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecanoate	15:0
3.	palmitoleate	16:1
4.	palmitate	16:0
5.	heptadecenoate	17:1
6.	heptadecanoate	17:0
7.	oleate	18:1
8.	stearate	18:0
9.	nonadecenoate	19:1
10.	arachidonate + eicosapentaenoate	20:4+5
11.	eicosatrienoate	20:3
12.	eicosenoate	20:1
13.	heneicosenoate (br) ?	br 21:1 ?
14.	docosapentaenoate + docosahexaenoate	22:5+6
15.	docosatetraenoate	22:4
16.	docosatrienoate	22:3

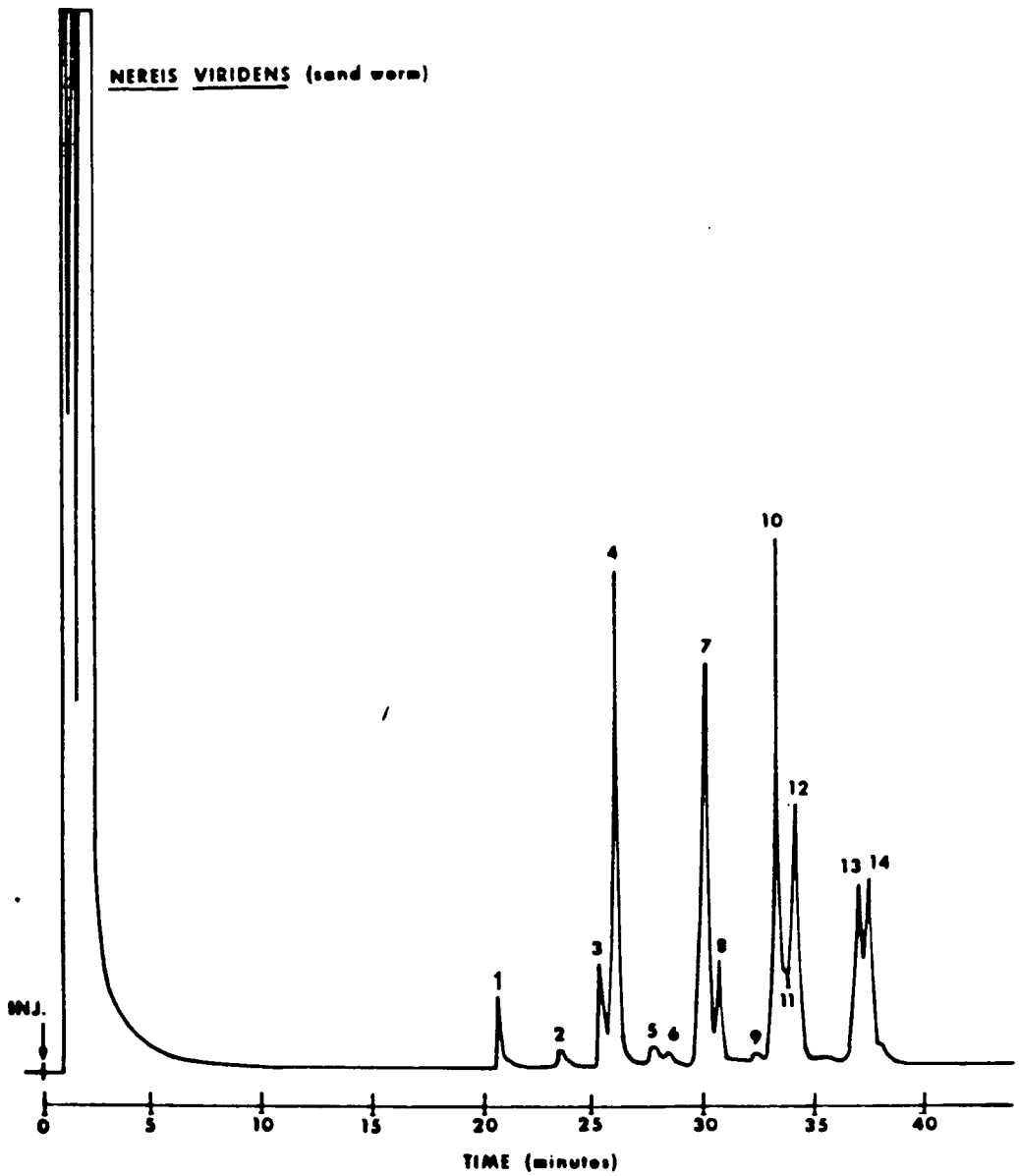

NEREIS VIRIDENS (sand worm)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 3.6. Fatty acid profile from the sand worm Nereis viridens. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecanoate	15:0
3.	palmitoleate	16:1
4.	palmitate	16:0
5.	heptadecenoate	17:1
6.	heptadecanoate	17:0
7.	oleate	18:1
8.	stearate	18:0
9.	nonadecenoate	19:1
10.	arachidonate + eicosapentaenoate	20:4+5
11.	eicosatrienoate	20:3
12.	eicosenoate	20:1
13.	docosapentaenoate + docosahexaenoate	22:5+6
14.	docosatetraenoate	22:4

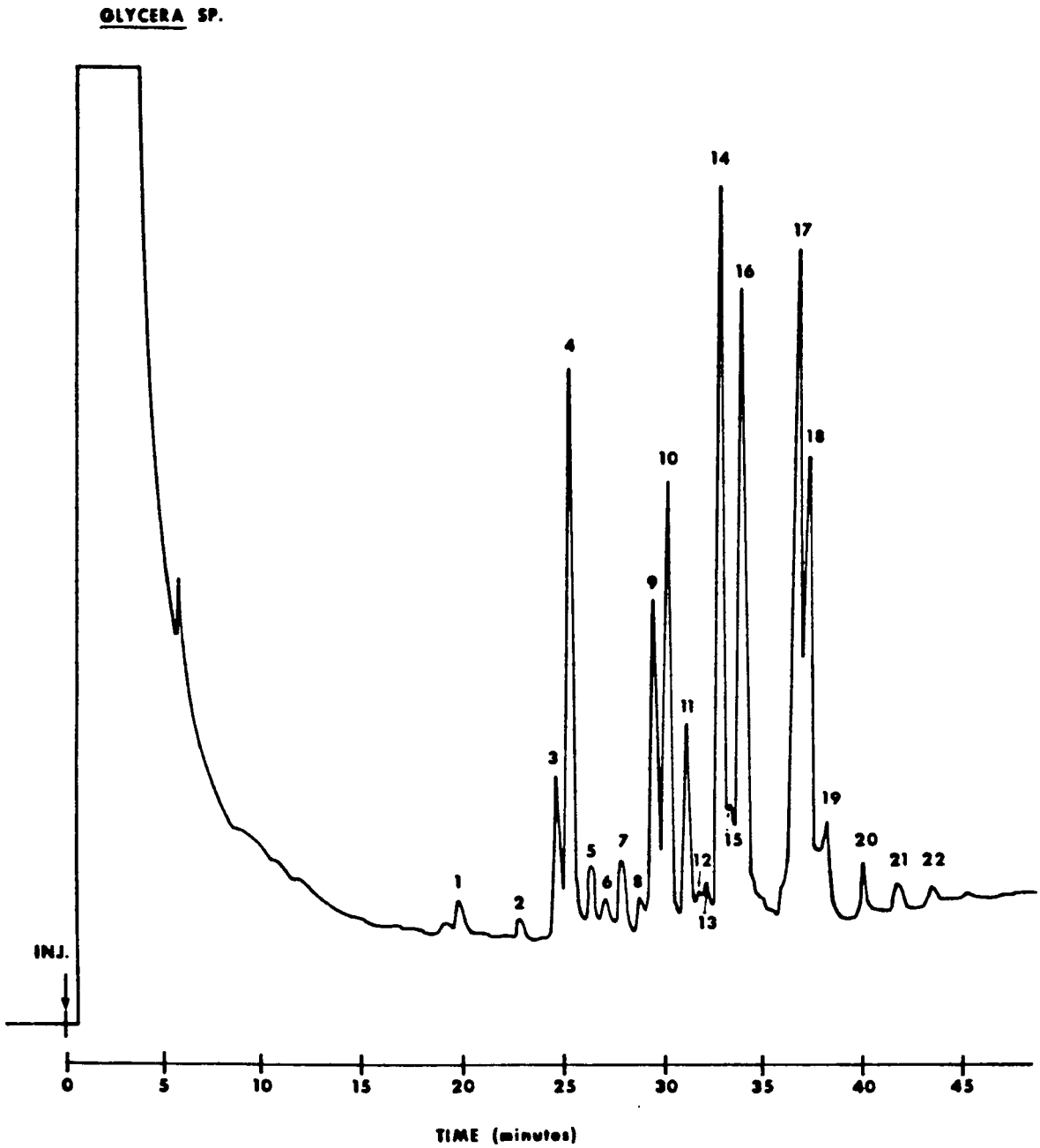

GLYCERA SP.
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
0
5
10
15
20
25
30
35
40
45
TIME (minutes)

Figure 3.7. Fatty acid profile from the blood worm Glycera sp., indigenous to West Bay. Chromatography was performed using a 2m x 2 mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecenoate	15:0
3.	palmitoleate	16:1
4.	palmitate	16:0
5.	heptadecanoate (br)	br 17:0
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	linoleate	18:2
9.	oleate	18:1
10.	stearate	18:0
11.	unknown	
12.	nonadecanoate (br)	br 19:0
13.	nonadecenoate	19:1
14.	arachidonate + eicosapentaenoate	20:4+5
15.	eicosatrienoate	20:3
16.	eicosenoate	20:1
17.	docosapentaenoate + docosahexaenoate	22:5+6
18.	docosatetraenoate	22:4
19.	docosadienoate ?	22:2 ?
20.	unknown	
21.	unknown	
22.	unknown	

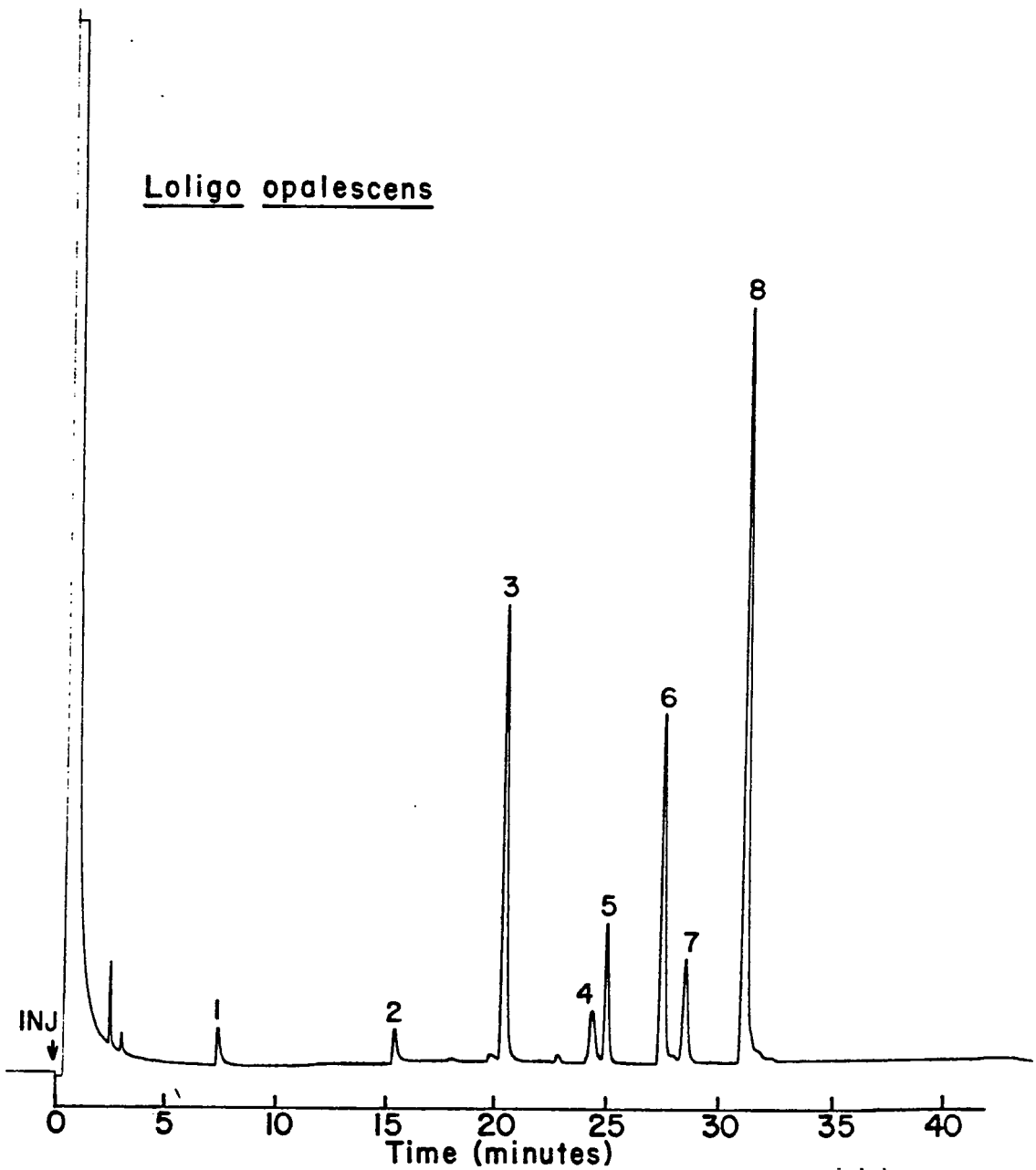

Loligo opalescens
INJ
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35
40
Time (minutes)

Figure 3.8. Fatty acid profile from the squid Loligo opalescens. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1. undecananoate ?	11:0 ?
2. myristate	14:0
3. palmitate	16:0
4. oleate	18:1
5. stearate	18:0
6. arachidonate + eicospentaenoate	20:4+5
7. eicosenoate	20:1
8. docosapentaenoate + docosahexaenoate	22:5+6

and formation of the hatching membrane by the method described by Clark et al. (1975). This indicates that eggs could be fertilized if the spermatophore were implanted. Later experiments using P. stylirostris did result in spermatophore transfer and production of viable eggs and larvae. Further studies on these eggs and larvae are described in Section 3.4.2.

3.2.3. Discussion. The encougaging results obtained by using marine invertebrates containing high relative concentrations of polyunsaturated fatty acids supports the hypothesis that these compounds are required for improved growth and ovarian maturation in penaeid shrimp.

Success in using the polychaetes and squid as feedstock supplements is most likely related to the diet of these individuals. The essential fatty acids probably derive ultimately from phytoplankton, and other routes through the food web to shrimp could be envisioned.

It must be noted at this point that it is not impossible for a factor other than dietary fatty acid content to be responsible for the promotion of ovarian development. α-Tocopherol (vitamin E), for example, is a naturally occuring antioxidant often found associated with polyunsaturated fatty acids in organisms. It has been demonstrated that α-tocopherol deficiencies in carp result in symptoms not unlike those encountered in diets lacking essential fatty acids (Watanabe et al., 1977; Watanabe and Takashima, 1977). Indeed, lack of α-tocopherol in the diet of carp resulted in a decrease in the concentration of polyunsaturated fatty acids and

caused a regression of ovarian maturation. However, now that spawning is possible in captivity on a reproducible basis, experiments can be performed to resolve some of these issues.

3.3 Metabolic profiles of cultured shrimp.

Once maturation was achieved successfully in captivity, studies could be conducted to optimize the feeding regimen. Initially, it was necessary to ascertain what qualitative and quantitative differences existed between shrimp grown in culture and those retrieved from the wild. In this study a new set of data from wild animals was obtained using a different GC column to separate the polyunsaturated fatty acids (see Section 2.5). Additionally, fatty acids were quantitated in both wild and captive animals (see Section 2.7). The results presented in this section were obtained using Penaeus setiferus.

3.3.1. Wild shrimp. Wild specimens of P. setiferus were obtained in the Gulf of Mexico on June 12, 1978. Table 3.6 lists the major fatty acids and relative per cent concentrations for wild shrimp obtained using a 2m x 2mm (i.d.) glass column packed with SP-2340. Additionally, quantitative data are presented in Table 3.7. A typical metabolic pattern obtained for these samples using the polar column is presented in Figure 3.9. Note that in this chromatogram 20:4 and 20:5 as well as 22:5 and 22:6, are completely separated. A tabulation of the various components of this sample is given in Table 3.8. The qualitative data obtained here agree with those presented in Section 3.1.3.

Table 3.6. Relative concentrations of selected fatty acids in tissues of P. setiferus from the Gulf of Mexico.[a]

Specimen[b]	Tissue[c]	n	16:0	16:1	18:0	18:1
Male	G	4	13.5±4.6[d]	4.1±0.6	10.1±3.7	9.9±1.3
	H	5	20.7±3.2	6.3±0.7	9.9±2.1	8.0±1.0
	T	5	15.7±1.8	5.9±0.8	12.7±3.7	11.2±1.8
Female	G	1	19.1	7.6	8.3	19.8
(stage 1)	H	2	24.8±1.1	7.5±1.3	8.5±0.1	9.6±0.8
	T	2	17.6±1.4	8.3±1.8	11.0±0.8	12.8±0.2
Female	G	2	21.8±3.3	11.0±1.1	8.0±1.1	16.1±0.4
(stage 2)	H	2	21.8±2.1	10.9±0.2	8.4±0.3	10.9±1.6
	T	2	17.7±1.9	8.5±0.6	11.2±1.2	12.0±0.1
Female	G	2	20.2±2.8	10.9±0.6	7.8±0.4	15.2±0.8
(stage 3)	H	3	22.1±2.3	10.7±0.8	8.3±0.3	9.4±1.5
	T	3	18.5±1.1	8.7±0.3	11.9±0.9	11.8±0.3
Female	G	6	21.2±2.8	10.5±1.4	8.8±2.3	15.2±0.9
(stage 5)	H	6	21.4±1.2	9.6±2.1	7.9±0.6	9.8±1.0
	T	6	17.8±1.4	7.9±0.7	10.6±1.9	12.3±1.5

Table 3.6. continued.

Specimen	Tissue	n	20:1	20:4	20:5	22:6
Male	G	4	0.7±0.2	9.5±2.9	17.3±2.5	17.5±0.9
	H	5	4.3±0.9	4.7±1.5	7.3±1.4	5.1±0.8
	T	5	0.8±0.2	6.9±1.6	13.9±5.4	12.8±3.4
Female	G	1	1.6	8.3	10.1	6.9
(stage 1)	H	2	4.2±1.1	4.6±0.6	8.0±2.3	5.6±0.6
	T	2	1.1±0.2	6.6±0.9	14.6±3.5	11.5±1.2
Female	G	2	2.1±0.1	4.3±0.6	9.4±2.1	6.8±2.1
(stage 2)	H	2	3.8±0.6	5.0±0.1	7.7±1.1	5.0±0.6
	T	2	0.9±0.1	6.2±1.8	13.1±2.7	10.7±2.5
Female	G	2	1.7±0.1	4.3±0.7	12.8±2.1	8.7±1.6
(stage 3)	H	3	3.1±0.7	4.4±0.4	8.6±2.2	4.8±0.1
	T	3	0.9±0.3	5.1±1.2	16.2±4.5	11.4±3.3
Female	G	6	1.8±0.3	4.1±1.0	9.9±2.5	7.0±2.0
(stage 5)	H	6	3.5±1.3	4.4±0.5	8.8±2.2	5.4±1.1
	T	6	1.1±0.5	6.1±1.4	14.1±2.2	11.7±1.7

Footnote to Table 3.6.

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]For females, stage of developement of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 4 animals were collected.

[c]G = gonad, H = hepatopancreas, T = tail muscle.

[d]Mean ± standard deviation.

Table 3.7. Quantitative analysis of major fatty acids in tissues of P. setiferus from the Gulf of Mexico.

Specimen[a]	Tissue[b]	n	16:0	16:1	18:0	18:1	20:1
Male	G	2	6.7±1.7[c,d]	3.7±2.5	5.0±2.2	3.5±0.1	0.2±0.0
	H	2	39.1±7.8	11.6±3.1	15.9±5.8	14.4±1.8	7.2±3.1
	T	2	2.7±0.1	1.0±0.1	2.3±0.1	1.8±0.4	0.2±0.1
Female (stage 3)	G	1	14.2	8.8	5.9	11.4	1.3
	H	1	28.3	13.9	9.8	9.3	3.5
	T	1	2.9	1.4	1.9	1.9	0.1
Female (stage 5)	G	2	13.8±1.5	10.9±3.4	5.8±0.3	10.6±0.5	1.1±0.1
	H	2	8.3±3.8	4.9±2.8	3.3±1.5	4.4±2.4	0.9±0.1
	T	2	3.8±1.6	1.7±0.8	2.3±0.7	2.4±0.9	0.2±0.1

Table 3.7. continued.

Specimen[a]	Tissue[b]	n	20:4	20:5	22:6	Total mg/g	Total mg/organ
Male	G	2	2.7±0.1	5.9±1.1	6.4±1.0	37.9± 7.4[e]	9.7± 3.4
	H	2	7.3±3.6	13.8±8.3	10.1±5.5	177.8±51.5	59.3± 2.1
	T	2	1.2±0.1	2.9±0.1	2.7±0.1	17.7± 1.7	81.2±24.0
Female (stage 3)	G	1	3.8	11.2	7.4	78.1	63.2
	H	1	5.7	13.6	5.7	120.9	67.7
	T	1	1.0	3.4	2.4	17.9	92.6
Female (stage 5)	G	2	3.1±0.1	8.4±0.1	5.6±0.1	69.1± 3.4	110.2± 2.9
	H	2	1.8±0.9	4.2±2.5	2.2±1.4	40.2±20.0	30.8± 7.9
	T	2	1.1±0.2	3.3±1.6	2.7±1.2	21.0± 8.8	125.7±46.0

Footnotes to Table 3.7.

[a]For females, stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 1, stage 2, or stage 4 animals were quantitatively analyzed.

[b]G = gonad, H = hepatopancreas, T = tail muscle.

[c]Values are in milligrams of fatty acid per organ dry weight, unless otherwise indicated.

[d]Mean ± standard deviation.

[e]Value is the sum of major fatty acids plus those not listed.

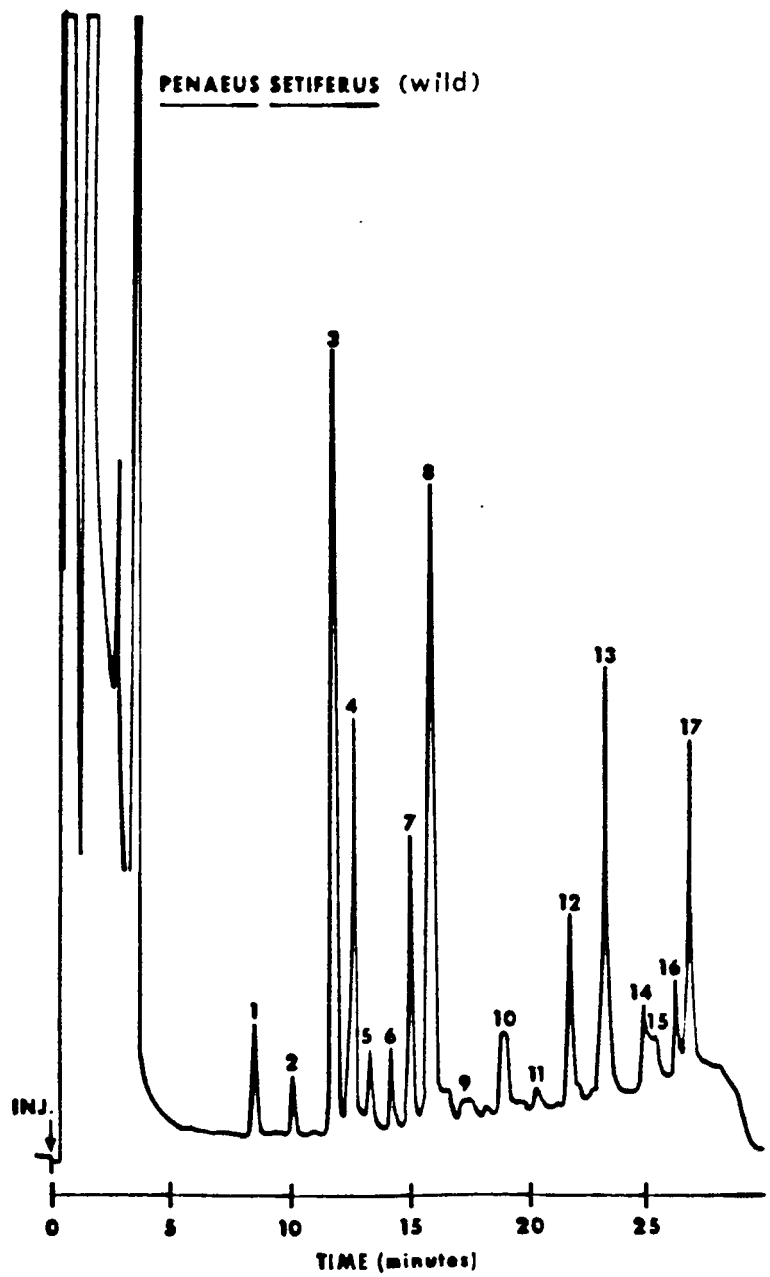

PENAEUS SETIFERUS (wild)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
0
5
10
15
20
25
TIME (minutes)

Figure 3.9. Fatty acid profile from wild type P. setiferus ovary (stage 3). Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 10% SP-2330 on Chromosorb W AW (100-120 mesh). For tabulation see Table 3.8.

Table 3.8. Tabulation of methyl esters from Figure 3.9.

Identification of methyl esters	
1. myristate	14:0
2. pentadecanoate	15:0
3. palmitate	16:0
4. palmitoleate	16:1
5. heptadecanoate	17:0
6. heptadecenoate	17:1
7. stearate + [2,2-^{2}H]stearate	18:0
8. oleate	18:1
9. nonadecanoate + linoleate	19:0+18:2
10. eicosenoate	20:1
11. eicosatrienoate	20:3
12. arachidonate	20:4
13. eicosapentaenoate	20:5
14. docosatrienoate	22:3
15. docosatetraenoate	22:4
16. docosapentaenoate	22:5
17. docosahexaenoate	22:6

In the quantitative analyses for wild shrimp, some interesting observations can be made. As expected, the hepatopancreas and ovaries contain relatively large concentrations (mg/g) of fatty acids, particularly when compared to the tail muscle. However, the tail muscle contains a larger quantity (mg/organ) of fatty acid by virtue of its size. Note that the hepatopancreas is extremely high in lipid content, being composed of up to 18% fatty acid in the case of male samples as compared to 3.8% in the testis and 1.8% in tail muscle. This supports the idea that the hepatopancreas functions as a storage tissue for lipids.

3.3.2. Cultured shrimp. P. setiferus were maintained in captivity on a diet supplemented with blood worms, sandworms, and squid (see Section 3.2.2). The qualitative (Table 3.9) and quantitative (Table 3.10) fatty acid data obtained from animals maintained on this diet for three months are presented. A typical chromatogram is shown in Figure 3.10. The peak height of methyl stearate in this figure is elevated due to the addition of the internal standard. The true peak height is determined as discussed in Section 2.7. There were no significant differences in fatty acid profiles of the tail muscles in wild and cultured animals. The ovaries of stage 2 females grown in the laboratory were lower in relative concentrations of 16:1 and higher in 20:5 and 22:6 than wild animals, although these differences were not found to be significant in the more matured ovaries. Only in the hepatopancreas were consistantly significant differences found between wild and cultured shrimp: the relative concentrations of 16:0 and

Table 3.9. Relative concentrations of selected fatty acids in tissues of P. setiferus grown in the laboratory.[a]

Specimen[b]	Tissue[c]	n	16:0	16:1	18:0	18:1
Female	G	0	-	-	-	-
(stage 1)	H	1	21.0	4.4	5.5	7.4
	T	1	14.7	3.9	10.1	10.0
Female	G	3	19.6±1.0[d]	5.1±0.9	6.5±0.0	15.9±1.5
(stage 2)	H	3	25.6±2.5	5.8±1.4	5.1±0.7	9.5±0.6
	T	3	19.0±1.0	4.9±0.3	11.0±0.5	13.1±1.1
Female	G	2	20.4±0.5	6.8±0.0	5.8±0.2	14.8±0.9
(stage 3)	H	2	29.4±1.8	8.1±0.1	3.7±0.4	8.3±0.2
	T	2	19.9±1.4	6.2±0.4	10.2±0.4	12.6±1.2
Female	G	2	21.0±1.0	6.9±1.3	5.7±0.0	16.1±1.6
(stage 4)	H	2	26.5±2.3	8.5±1.2	2.9±0.1	9.3±0.8
	T	2	17.9±1.1	5.6±0.2	9.7±0.1	13.6±0.3
Female	G	2	23.0±0.8	7.1±1.8	7.0±0.1	14.7±0.4
(stage 5)	H	1	26.0	4.7	5.7	8.9
	T	2	18.5±2.1	4.8±1.1	10.8±0.8	12.7±0.2

Table 3.9. continued.

Specimen[b]	Tissue[c]	n	20:1	20:4	20:5	22:6
Female	G	0	-	-	-	-
(stage 1)	H	1	8.1	2.1	12.5	17.2
	T	1	0.6	10.2	17.9	18.2
Female	G	3	3.2±0.2	3.8±0.8	16.8±0.7	18.8±0.8
(stage 2)	H	3	7.2±0.4	2.0±0.6	10.4±0.9	13.2±2.2
	T	3	1.7±0.1	4.8±0.3	16.5±0.7	17.6±0.9
Female	G	2	3.3±0.4	3.0±0.1	15.5±0.2	19.6±0.1
(stage 3)	H	2	7.2±0.6	1.6±0.1	8.9±0.8	10.8±2.3
	T	2	2.0±0.1	4.4±0.1	16.7±1.3	17.8±0.8
Female	G	2	2.9±0.1	2.8±0.3	13.5±0.7	18.6±1.8
(stage 4)	H	2	6.1±0.6	1.7±0.0	11.2±1.3	14.0±0.4
	T	2	1.8±0.2	4.3±0.6	15.0±0.9	17.0±1.8
Female	G	2	3.8±0.8	2.5±0.1	12.6±0.4	18.5±1.4
(stage 5)	H	1	8.3	2.1	9.1	13.0
	T	2	2.1±0.1	4.5±0.4	18.1±1.3	19.6±2.0

Footnote to Table 3.9.

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]Stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn.

[c]G = gonad, H = hepatopancreas, T = tail muscle.

[d]Mean ± standard deviation.

Table 3.10. Quantitative analysis of major fatty acids in tissues of P. setiferus grown in the labratory.

Specimen[a]	Tissue[b]	n	16:0	16:1	18:0	18:1	20:1
Female	G	0	-	-	-	-	-
(stage 1)	H	1	69.8[c]	14.7	18.3	24.4	26.9
	T	1	3.7	1.0	2.5	2.5	0.3
Female	G	3	22.5± 6.5[e]	5.8± 1.9	7.4±1.8	18.0± 3.6	3.6± 1.0
(stage 2)	H	3	90.8±56.3	21.1±13.8	17.0±6.9	32.1±14.9	24.4±11.7
	T	3	4.1± 1.0	1.1± 0.3	2.4±0.6	2.8 ± 0.8	0.4± 0.1
Female	G	2	24.8± 3.3	8.3± 1.3	7.0±0.8	18.1± 3.9	4.0± 0.1
(stage 3)	H	2	94.2±46.5	26.2±14.1	11.7±5.2	27.1±15.6	23.9±14.7
	T	2	4.6± 0.4	1.4± 0.1	2.4±0.1	2.9± 0.3	0.5± 0.1
Female	G	2	31.2± 1.5	10.1± 1.1	8.5±0.8	23.8± 0.0	4.4± 0.6
(stage 4)	H	2	116.5± 5.4	37.4± 6.9	12.6±0.1	40.9± 5.1	26.7± 3.7
	T	2	4.8± 0.4	1.5± 0.0	2.6±0.1	3.6± 0.0	0.5± 0.1
Female	G	2	34.6± 7.6	10.8± 4.5	10.4±1.8	21.9± 3.4	5.8± 2.3
(stage 5)	H	1	51.4	9.3	11.3	17.5	16.5
	T	2	4.2± 0.1	1.1± 0.1	2.5±0.1	2.9± 0.4	0.5± 0.1

Table 3.10. continued.

Specimen[a]	Tissue[b]	n	20:4	20:5	22:6	Total mg/g	Total mg/organ
Female	G	0	-	-	-	-	-
(stage 1)	H	1	7.0	41.5	56.9	331.5[d]	161.2
	T	1	2.6	4.5	4.6	25.1	93.1
Female	G	3	4.2±0.5	19.1± 3.8	21.2± 4.2	114.1± 26.8	42.6± 15.4
(stage 2)	H	3	6.5±2.4	35.0±15.0	43.1±15.1	345.0±180.2	166.4±125.2
	T	3	1.0±0.2	3.5± 1.0	3.7± 0.7	21.4± 5.1	106.1± 7.3
Female	G	2	3.7±0.7	18.9± 3.2	24.0± 3.9	122.0± 19.1	61.8± 5.7
(stage 3)	H	2	5.4±3.2	29.9±18.8	37.2±26.5	326.0±178.6	225.1±103.3
	T	2	1.0±0.0	3.8± 0.3	4.1± 0.1	23.0± 0.2	120.5± 12.7
Female	G	2	4.2±0.8	20.1± 2.9	27.8± 5.4	148.7± 14.4	170.8± 32.2
(stage 4)	H	2	7.6±0.5	49.3± 3.6	61.7± 0.4	440.3± 16.9	457.5±103.2
	T	2	1.2±0.2	4.0± 0.3	4.5± 0.6	26.5± 0.4	169.4± 5.4
Female	G	2	3.6±0.6	18.7± 3.0	27.5± 2.9	149.7± 27.6	176.8± 37.8
stage 5)	H	1	4.1	18.0	25.7	197.4	106.1
	T	2	1.1±0.7	4.1± 0.7	4.5± 0.9	22.6± 2.3	105.5± 1.1

[a]Stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn.

[b]G = gonad, H = hepatopancreas, T = tail muscle.

[c]Values are in milligrams of fatty acid per gram dry weight, unless otherwise indicated.

[d]Value is the sum of major fatty acids plus those that are not listed.

[e]Mean ± standard deviation.

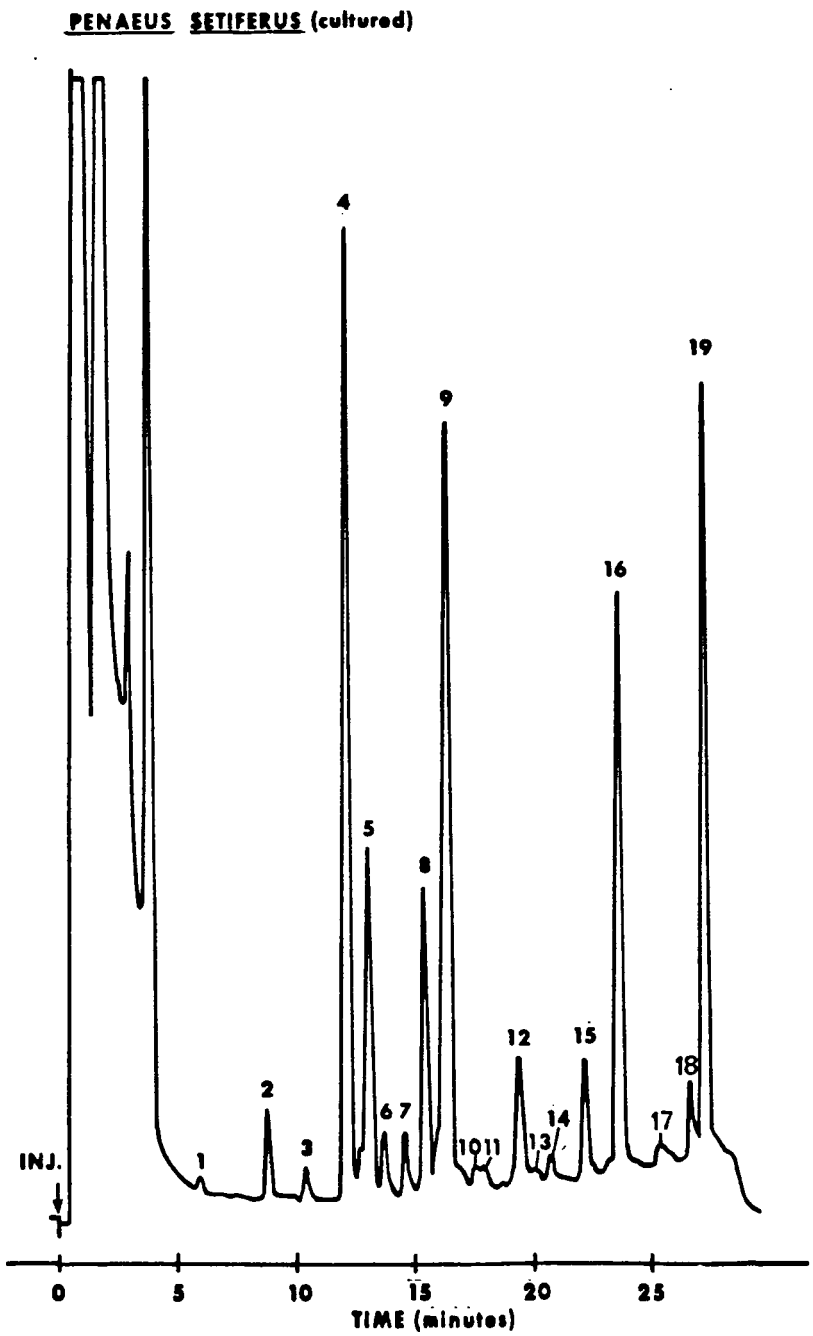

PENAEUS SETIFERUS (cultured)
INJ.
1
2
3
4
5
6 7
8
9
10 11
12
13
14
15
16
17
18
19
0
5
10
15
20
25
TIME (minutes)

Figure 3.10. Fatty acid profile from cultured P. setiferus ovary (stage 5). Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 10% SP-2330 on Chromosorb W AW (100-120 mesh).

Identification of methyl esters

1.	laurate	12:0
2.	myristate	14:0
3.	pentadecanoate	15:0
4.	palmitate	16:0
5.	palmitoleate	16:1
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	stearate + [2,2-^{2}H]stearate	18:0
9.	oleate	18:1
10.	nonadecanoate	19:0
11.	linoleate	18:2
12.	eicosenoate	20:1
13.	eicosadienoate ?	20:2 ?
14.	eicosatrienoate	20:3
15.	arachidonate	20:4
16.	eicosapentaenoate	20:5
17.	docosatrienoate + docosatetraenoate	22:3+4
18.	docosapentaenoate	22:5
19.	docosahexaenoate	22:6

Table 3.11. Relative concentrations of major fatty acids in tissues from unilaterally ablated P. setiferus grown in the laboratory.[a]

Specimen[b]	Tissue[c]	n	16:0	16:1	18:0	18:1
Female	G	0	-	-	-	-
(stage 2)	H	1	12.8	3.5	11.0	9.0
	T	1	14.5	2.8	12.5	13.3
Female	G	3	20.9±1.8[d]	7.2±1.0	5.8±0.7	16.4±0.8
(stage 3)	H	3	27.6±0.7	9.0±2.5	2.8±1.1	8.9±1.1
	T	2	18.7±0.4	5.9±0.1	10.2±0.4	13.7±0.1
Female	G	1	22.7	7.3	6.0	15.4
(stage 4)	H	1	23.4	4.9	5.3	9.0
	T	1	18.5	5.2	9.9	13.5
Female	G	1	26.3	8.8	6.3	14.2
(stage 5)	H	1	24.1	4.8	6.5	8.2
	T	1	20.2	5.4	10.5	12.3

Table 3.11. continued.

Specimen[b]	Tissue[c]	n	20:1	20:4	20:5	22:6
Female	G	0	-	-	-	-
(stage 2)	T	1	3.2	6.7	26.1	14.5
	T	1	1.5	8.4	23.3	14.0
Female	G	3	3.5±0.2	3.2±0.4	14.2±1.5	17.9±1.2
(stage 3)	H	3	7.0±0.2	1.6±0.3	9.4±1.7	11.8±1.1
	T	2	2.1±0.0	4.7±0.2	16.6±0.1	17.4±0.8
Female	G	1	4.1	2.4	13.6	15.2
(stage 4)	H	1	10.1	1.6	10.1	10.3
	T	1	1.9	4.5	18.7	17.2
Female	G	1	3.2	1.6	10.8	12.1
(stage 5)	H	1	8.7	1.7	11.2	9.2
	T	1	2.0	4.4	19.5	16.7

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]Stage of development is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 1 animals were collected.

[c]G = gonad, H = hepatopancreas, T = tail muscle.

[d]Mean ± standard deviation.

Table 3.12. Quantitative analysis of major fatty acids in tissues of unilaterally ablated P. Setiferus grown in the laboratory.

Specimen[a]	Tissue[b]	n	16:0	16:1	18:0	18:1	20:1
Female	G	0	-	-	-	-	-
(stage 2)	H	1	4.7[c]	1.3	4.0	3.3	0.2
	T	1	2.3	0.4	2.0	2.1	0.5
Female	G	3	25.9± 6.1[e]	9.0± 2.4	7.0±0.8	20.2± 3.8	1.1±0.3
(stage 3)	H	3	134.4±48.3	46.3±27.3	12.6±0.8	44.6±19.9	15.5±5.2
	T	2	5.0± 0.4	1.6± 0.1	2.7±0.3	3.7± 0.2	1.3±0.2
Female	G	1	29.3	9.4	7.8	20.0	2.7
(stage 4)	H	1	58.1	12.2	13.2	22.4	5.1
	T	1	4.2	1.2	2.2	3.0	1.0
Female	G	1	37.6	12.6	9.1	20.3	4.5
(stage 5)	H	1	49.0	9.8	13.2	16.7	17.6
	T	1	4.1	1.1	2.2	2.5	0.4

Table 3.12. continued.

Specimen	Tissue	n	20:4	20:5	22:6	total mg/g	total mg/organ
Female	G	0	-	-	-	-	-
(stage 2)	H	1	2.4	9.6	5.3	36.5[d]	8.2
	T	1	1.3	3.7	2.2	15.8	54.5
Female	G	3	3.9±0.3	17.3±1.7	22.2± 5.1	123.1± 20.8	74.2± 17.4
(stage 3)	H	3	7.9±2.3	44.0±9.8	58.1±24.4	458.1±165.1	417.9±139.6
	T	2	1.3±0.1	4.4±0.3	4.6± 0.1	26.5± 1.8	132.6± 20.9
Female	G	1	3.1	17.6	19.7	129.2	124.6
(stage 4)	H	1	4.1	25.0	25.6	248.1	98.2
	T	1	1.0	4.2	3.8	22.4	109.3
Female	G	1	2.3	15.4	17.3	142.9	296.3
(stage 5)	H	1	3.4	22.8	18.6	203.0	89.5
	T	1	0.9	4.0	3.4	20.4	115.9

Footnotes to Table 3.12.

[a]Stage of development of ovaries is indicated: stage 1 is immature, stage 5 is about to spawn. No stage 1 animals were collected.

[b]G = gonad, H = hepatopancreas, T = tail muscle.

[c]Values are in milligrams of fatty acid per gram dry weight, unless otherwise indicated.

[d]Values are the sum of major fatty acids plus those not listed.

[e]Mean ± standard deviation.

22:6 were generally higher, while those of 16:1, 18:0, and 20:4 were generally lower in laboratory animals as compared to wild animals.

3.3.3. Ablated shrimp. Qualitative (Table 3.11) and quantitative (Table 3.12) data on fatty acids obtained from unilaterally ablated P. setiferus, grown under the same laboratory conditions as unablated animals (see Section 3.2.2), are presented. As mentioned previously, eyestalk ablation has been reported to enhance ovarian maturation in shrimp. However, no significant differences between the relative concentrations or absolute amounts of fatty acids could be found when comparing ablated and unablated animals.

3.3.4. Discussion. As mentioned previously, dietary lipids are absorbed virtually unchanged by the hepatopancreas of crustaceans. Therefore, fatty acid profiles obtained from the hepatopancreas may be used as an indicator in evaluating the nutritional quality of fatty acids from the diet. In these early experiments it is best to mimick the chemical composition of the natural diet, based on the assumption that poor nutrition is responsible for the absence of ovarian maturation in cultured shrimp. The fact that significant differences were found between fatty acid profiles from the hepatopancreas of wild and cultured shrimp suggests that the laboratory diet does not completely mimick the fatty acid content obtained from a natural diet. However, the fact that the tail muscles of both wild and cultured animals were found not to be statistically different in fatty acid distribution suggests that the

nutritional requirements for fatty acids in cultured shrimp have been obtained using the laboratory diet. Indeed, laboratory matured animals may have been overfed, as indicated by the higher amounts of fatty acids (mg/g) in stage 5 animals compared to those matured in the wild. This represents a significant advance in shrimp mariculture, as demonstrated by the ability to obtain reproducible ovarian maturation and spawning (see Secton 3.2.2). Before more conclusions can be drawn, however, it is necessary to study the eggs and larvae spawned from cultured shrimp. Any deficiencies in the diet of parent shrimp will likely be reflected in the eggs.

3.4. Fatty acid profiles of shrimp eggs and larvae.

The viability of eggs and larvae obtained from cultured shrimp is crucial if a commercially feasible production system is to be attained. Nutritional deficiencies in the diet of parent shrimp will be reflected in the eggs. As a result, reduced viability or an overall reduction in the amount of shrimp obtained per spawn would cause economic losses. In a controlled system, however, one should expect to produce more viable larvae per spawn than are obtained from wild shrimp. Therefore, it is important to optimize nutritional requirements not only for the purpose of obtaining ovarian maturation, but to produce a maximum number of viable eggs.

3.4.1. Wild P. stylirostris eggs and larvae. Fatty acid changes during the development of eggs of wild Penaeus stylirostris

spawned in the laboratory have been presented by Ward (1979). The results presented in Ward's thesis were obtained in this laboratory. Relative concentrations of major fatty acids are given in Table 3.13. Chromatograms of fatty acids obtained from the eggs and postlarvae are presented in Figures 3.11 and 3.12, respectively. Palmitic acid (16:0) has the highest relative concentration in eggs (23.1%), while oleic acid (18:1) is highest in postlarvae (25.4%).

The relative percent concentrations of certain fatty acids decreased during development. In this respect the greatest decreases were found in 16:1 (47%), 22:4 (36%), 22:5 (48%), and 22:6 (76%). It is interesting to note that the greatest increases in relative percent concentration during development from egg to postlarvae were found in 18:2 (478%) and 18:3 (300%). The large increases in these last two fatty acids can be attributed to dietary contributions, since developing larvae and postlarvae begin to feed on aquatic microorganisms such as algae. Several algae have been shown to have linoleic and linolenic acids as major constituents of their total fatty acid content (Schneider et al., 1970).

No statistical differences were detected when comparing the relative percent concentrations of major fatty acids obtained from eggs to the same fatty acids from the ovaries of mature (stage 5) P. stylirostris females (see Table 3.1).

3.4.2. Cultured P. stylirostris eggs and larvae. Penaeus stylirostris obtained from the spawn of wild shrimp matured in

Table 3.13. Relative concentrations of major fatty acids in eggs and post-larvae from wild P. stylirostris.[a]

Fatty acid	Eggs	Postlarvae	% change
16:0	23.1±2.2[b]	19.2±0.5	- 17
16:1	10.2±0.7	5.4±0.5	- 47
18:0	7.7±0.7	10.5±0.5	+ 36
18:1	16.6±0.4	25.4±0.8	+ 53
18:2	0.9±0.1	5.2±0.4	+478
18:3	1.8±0.1	7.2±1.4	+300
20:4	3.4±0.4	5.4±0.1	+ 47
20:5	8.3±1.4	9.7±0.8	+ 17
22:6	6.4±1.0	1.5±0.4[c]	- 76

[a]Obtained from Table 2, Ward (1979). Percentages do not total 100% because minor fatty acids are not listed.

[b]Mean ± standard deviation. n = 6 Unless otherwise stated.

[c]n = 3.

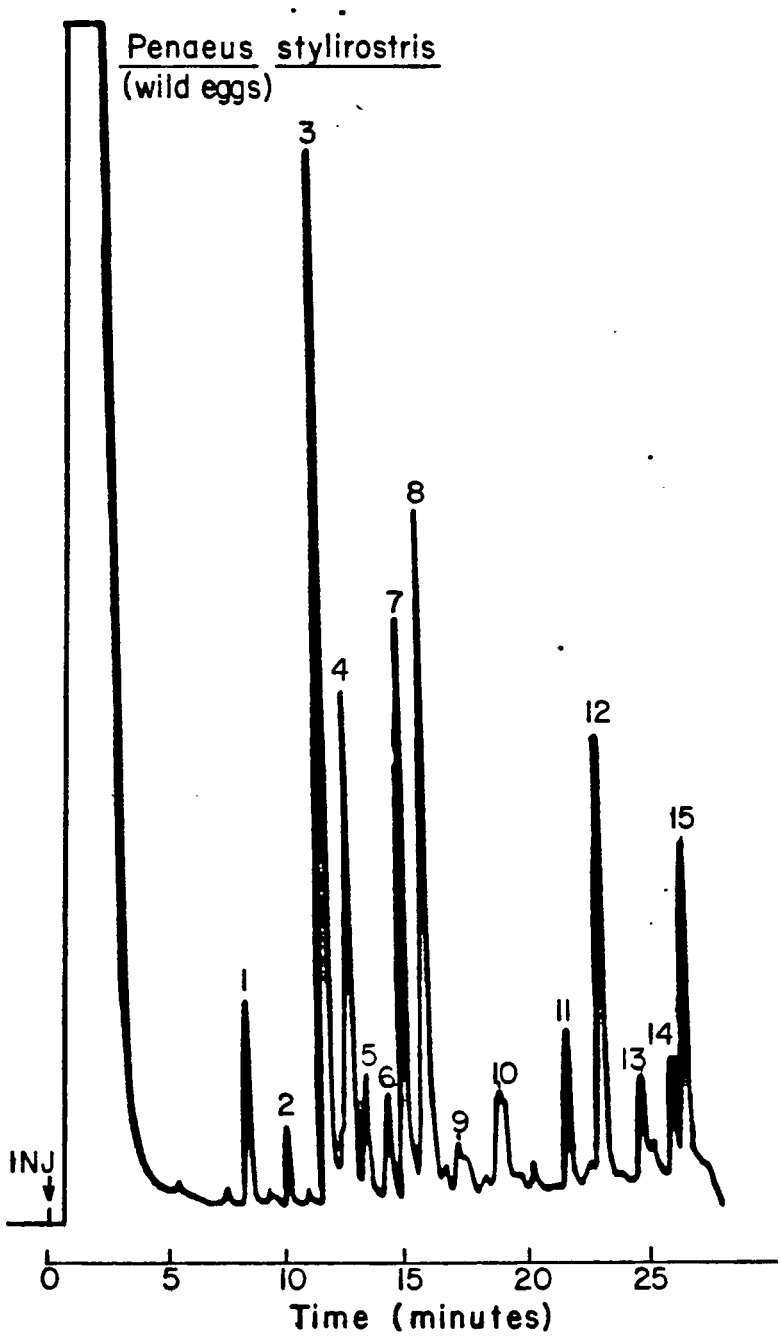

Penaeus stylirostris
(wild eggs)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
INJ
0
5
10
15
20
25
Time (minutes)

Figure 3.11. Fatty acid profile from wild type eggs of P. stylirostris. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 10% SP-2330 on Chromosorb W AW (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecanoate	15:0
3.	palmitate	16:0
4.	palmitoleate	16:1
5.	heptadecenoate	17:1
6.	heptadecanoate	17:0
7.	stearate + [2,2-^{2}H]stearate	18:0
8.	oleate	18:1
9.	linoleate	18:2
10.	linolenate	18:3
11.	arachidonate	20:4
12.	eicosapentaenote	20:5
13.	docosatrienoate + docosatetraenoate	22:3+4
14.	docosapentaenoate	22:5
15.	docosahexaenoate	22:6

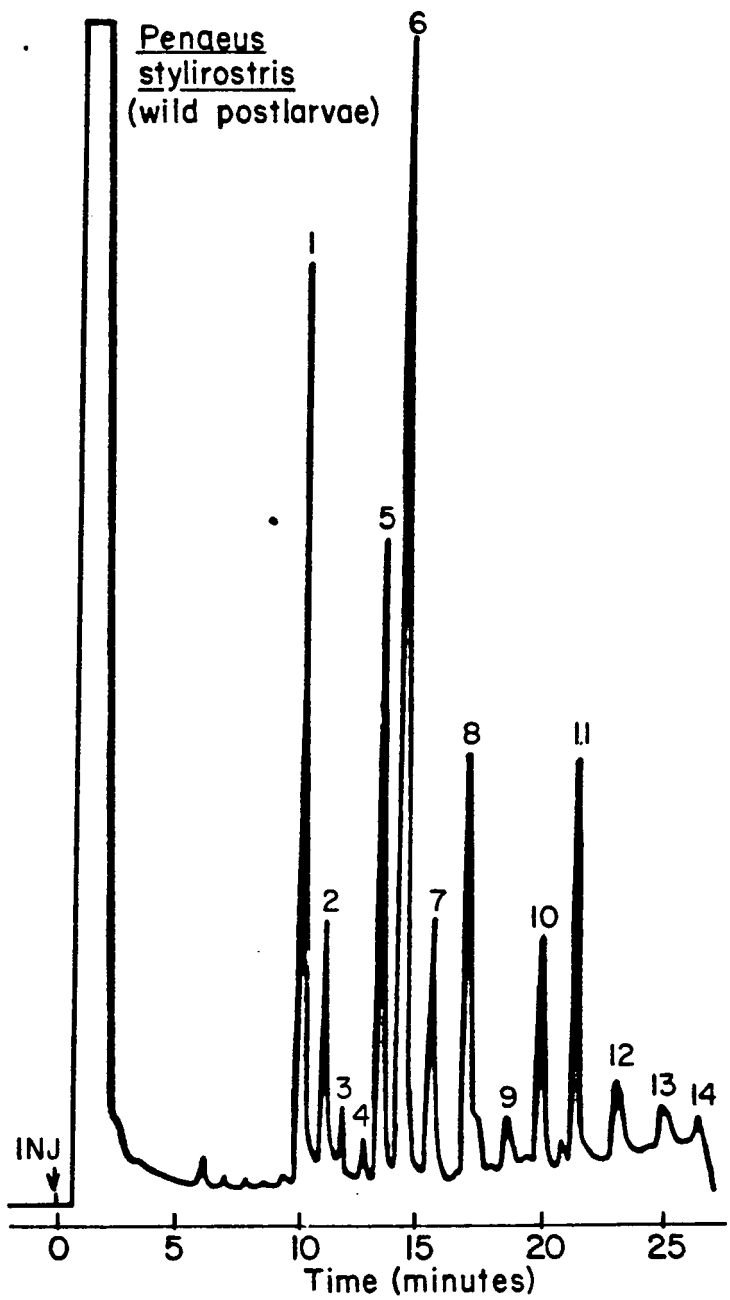

Penaeus
stylirostris
(wild postlarvae)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
INJ
0
5
10
15
20
25
Time (minutes)

Figure 3.12. Fatty acid profile from wild type postlarvae of P. styliro-stris. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 10% SP-2330 on Chromosorb W AW (100-120 mesh).

Identification of methyl esters

1.	palmitate	16:0
2.	palmitoleate	16:1
3.	heptdecenoate	17:1
4.	heptadecanoate	17:0
5.	stearate + [2,2-^{2}H]stearate	18:0
6.	oleate	18:1
7.	linoleate	18:2
8.	linolenate	18:3
9.	eicosatrienoate	20:3
10.	arachidonate	20:4
11.	eicosapentaenoate	20:5
12.	docosatrienoate + docosatetraenoate	22:3+4
13.	docosapentaenoate	22:5
14.	docosahexaenoate	22:6

captivity, were maintained on a maturation diet supplemented with bloodworms, sandworms, and squid. Spawning in the cultured animals occurred during the period from August 30, 1979 to September 20, 1979. Eggs were collected and hatched in separate tanks from the adults. Developing larvae were maintained on a diet of bakers' yeast and the algae Tetraselmis chuii. Table 3.14 presents the relative concentrations of major fatty acids obtained from eggs, larvae, and feeds. Chromatograms of fatty acids from eggs (Figure 3.13), postlarvae (Figure 3.14), yeast (Figure 3.15), and Tetraselmis chuii (Figure 3.16) are shown. Fatty acid profiles were obtained using a 30 m Silar-10C capillary column.

Due to the small sample sizes, no statistical differences were found when comparing the various stages of development in these samples. However, comparisons with values obtained for eggs and postlarvae obtained from wild P. stylirostris (Table 3.13) show some interesting differences. In this respect, there are significantly higher relative concentrations of 20:5 and 22:6 in the eggs from cultured shrimp as compared to those from wild shrimp. Furthermore, postlarvae of cultured shrimp have significiantly lower relative concentrations of 16:0 and 18:0 compared to postlarvae of wild shrimp. Postlarvae of cultured animals also have considerably lower relative concentrations of 18:2 and 18:3 when compared to postlarvae from wild P. stylirostris.

Although no statistical differences were found between the postlarvae and eggs from cultured shrimp due to the small sample sizes, cursory observation of the data would indicate a decrease in the relative concentrations of palmitic acid and 4,7,10,13,16,19-

Table 3.14. Relative concentrations of major fatty acids in eggs and larvae from P. Stylirostris grown in the laboratory, and their feeds.[a]

Specimen	n	16:0	16:1	18:0	18:1	18:2
Eggs	2	27.0±6.9[b]	7.4±2.4	5.3±2.3	13.6±0.2	0.0±0.0
Nauplii	2	23.0±0.2	4.9±2.3	6.8±1.6	11.6±1.6	0.0±0.0
Protozoea	2	24.2±3.3	4.2±3.4	7.2±3.2	10.2±6.1	0.0±0.0
Postlarvae	1	12.3	10.1	4.0	23.0	2.8
Baker's yeast	1	14.0	43.8	3.6	35.9	2.2
Tetraselmis chuii	1	19.8	3.8	0.1	15.9	17.6

Table 3.14. continued.

Specimen	n	18:3	20:4	20:5	22:6
Eggs	2	0.0±0.0	2.0±0.1	13.6±0.2	14.2±1.1
Nauplii	2	0.0±0.0	2.5±0.3	17.9±0.8	15.4±0.4
Protozoea	2	0.0±0.0	2.4±1.6	11.7±9.5	10.8±1.8
Postlarvae	1	0.0	2.2	11.8	7.9
Baker's yeast	1	0.0	0.0	0.0	0.0
Tetraselmis chuii	1	16.1	1.4	3.0	0.0

[a]Percentages do not total 100% because minor fatty acids are not listed.

[b]Mean ± standard deviation.

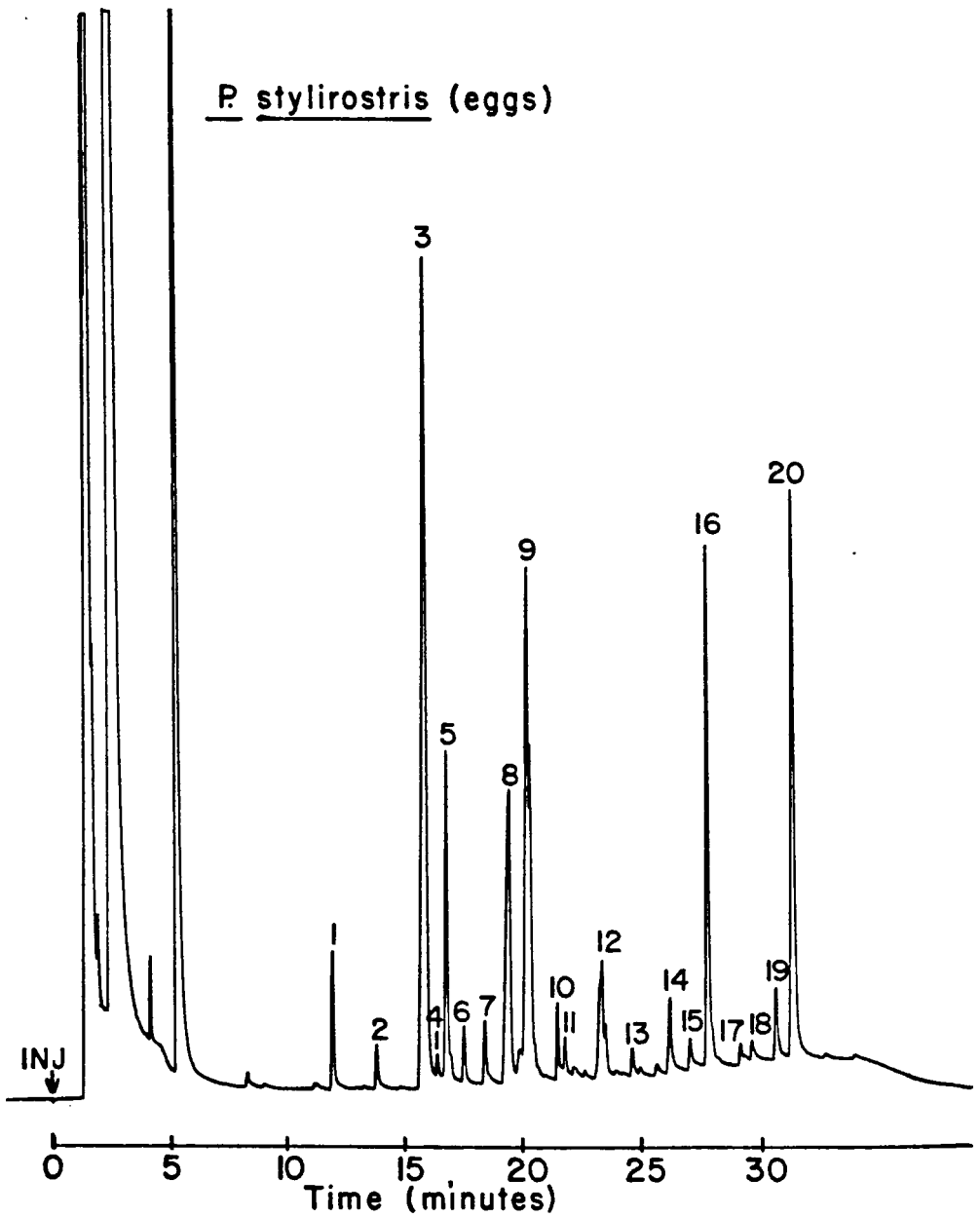

P. stylirostris (eggs)
INJ
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
0
5
10
15
20
25
30
Time (minutes)

Figure 3.13. Fatty acid profile from eggs of cultured P. stylirostris. Chromatography was performed using a 30m x 0.2mm (i.d.) glass capillary coated with Silar 10C.

Identification of methyl esters

1.	myristate	14:0
2.	pentadecanoate	15:0
3.	palmitate	16:0
4.	heptadecenoate (br)	br 17:1
5.	palmitoleate	16:1
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	stearate + [2,2-^{2}H]stearate	18:0
9.	oleate	18:1
10.	linoleate	18:2
11.	nonadecanoate	19:0
12.	eicosenoate	20:1
13.	eicosatrienoate	20:3
14.	arachidonate	20:4
15.	docosadienoate ?	22:2 ?
16.	eicosapentaenoate	20:5
17.	docosatrienoate	22:3
18.	docosatetraenoate	22:4
19.	docosapentaenoate	22:5
20.	docosahexaenoate	22:6

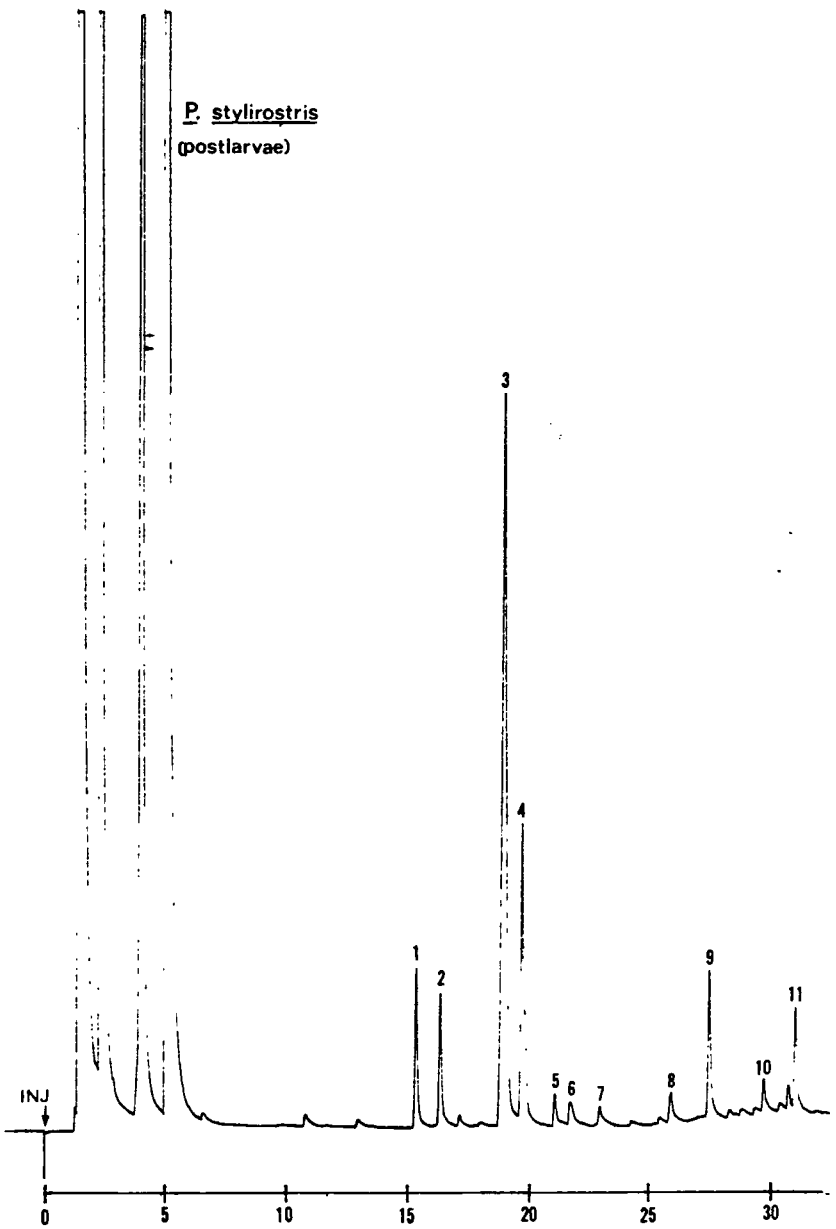

P. stylirostris
(postlarvae)
INJ
1
2
3
4
5
6
7
8
9
10
11
0
5
10
15
20
25
30

Figure 3.14. Fatty acid profile from postlarve of cultured P. stylirostris. Chromatogram was obtained using a 30m x 0.2mm glass capillary coated with Silar-10C.

Identification of methyl esters

1.	palmitate	16:0
2.	palmitoleate	16:1
3.	stearate + [2,2-^{2}H]stearate	18:0
4.	oleate	18:1
5.	linoleate	18:2
6.	nonadecanoate	19:0
7.	eicosenoate	20:1
8.	arachidonate	20:4
9.	eicosapentaenoate	20:5
10.	docosatetraenoate	22:4
11.	docosahexaenoate	22:6

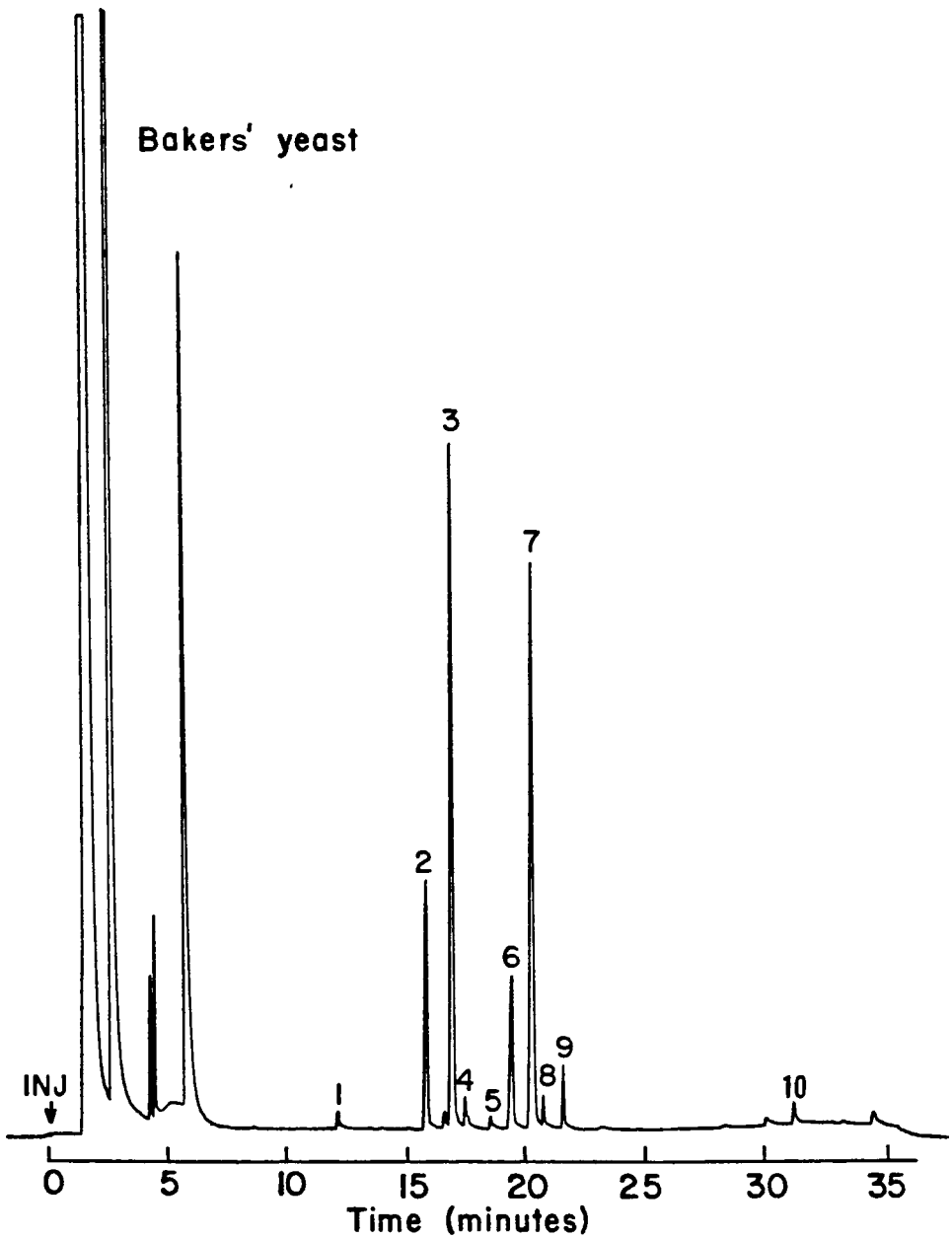

Bakers' yeast
INJ
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
35
Time (minutes)

Figure 3.15. Fatty acid profile from bakers' yeast. Chromatography was performed using a 30m x 0.2mm glass capillary coated with Silar - 10C.

Identification of methyl esters

1.	myristate	14:0
2.	palmitate	16:0
3.	palmitoleate	16:1
4.	heptadecanoate	17:0
5.	heptadecenoate	17:1
6.	stearate + [2,2-^{2}H]stearate	18:0
7.	oleate	18:1
8.	nonadecanoate	19:0
9.	linoleate	18:2
10.	docosapentaenoate ?	22:5 ?

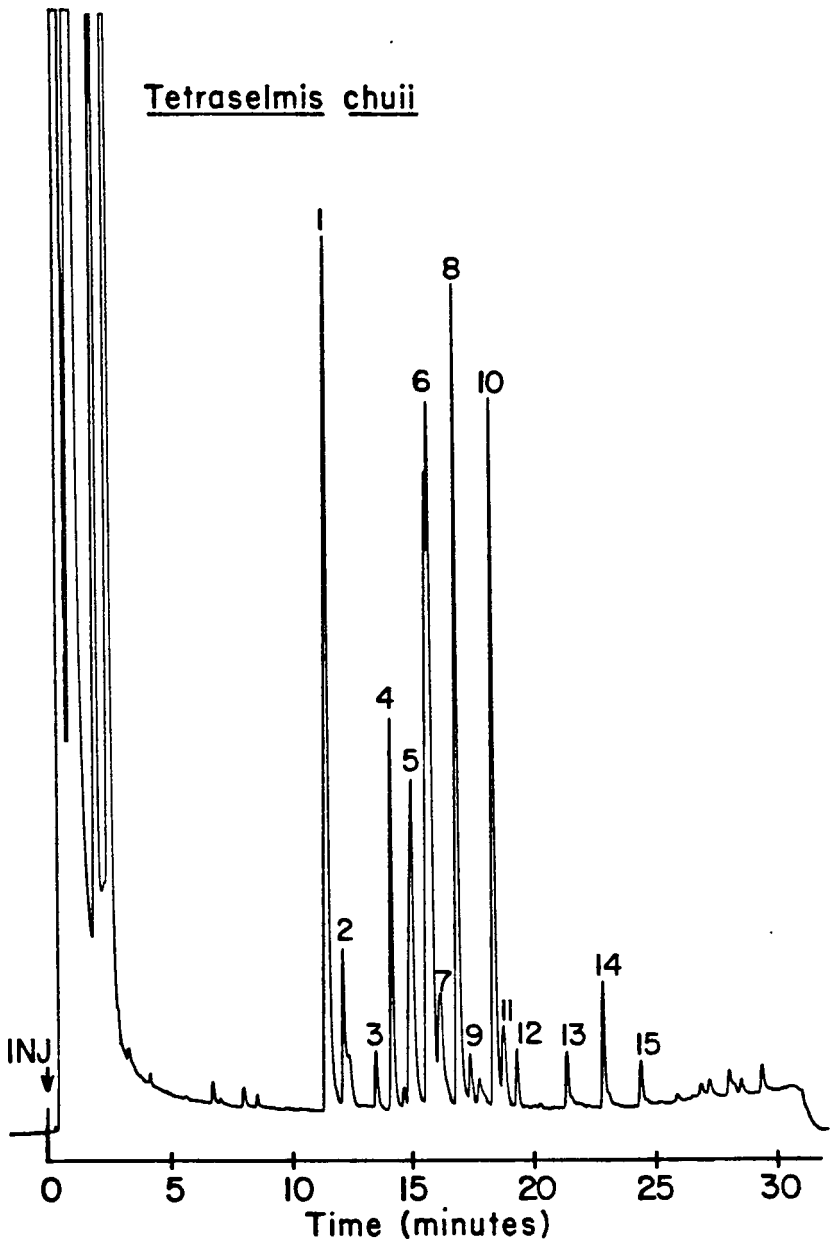

Tetraselmis chuii
INJ
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0
5
10
15
20
25
30
Time (minutes)

Figure 3.16. Fatty acid profile from Tetraselmis chuii. Chromatography was performed using a 30m x 0.2mm (i.d.) glass capillary coated with Silar-10C.

Identification of methyl esters

1.	palmitate	16:0
2.	palmitoleate	16:1
3.	hexadecadienoate	16:2
4.	hexadecatrienoate	16:3
5.	stearate + [2,2-^{2}H]stearate	18:0
6.	oleate	18:1
7.	heptadecenoate (br) ?	br17:1 ?
8.	linoleate	18:2
9.	nonadecenoate	19:1
10.	linolenate	18:3
11.	arachidate	20:0
12.	eicosenoate	20:1
13.	arachidonate	20:4
14.	eicosapentaenoate	20:5
15.	docosatrienoate ?	22:3 ?

docosahexaenoic acid during development, while the relative concentration of 18:1 increased. This would indicate that the postlarvae are tending to adopt fatty acid profiles more similar to those of their diet (i.e. bakers' yeast and Tetraselmis chuii). The low relative concentrations of polyunsaturated C_{20} and C_{22} compounds in both feeds and high relative concentrations of 18:1 in bakers' yeast support this hypothesis.

3.4.3. Discussion. Differences between the fatty acid distributions found in the eggs and postlarvae of wild and cultured P. styliros-tris were primarily from the saturated and polyunsaturated fatty acid classes. Ovarian maturation was accomplished using similar diets; however, the fact that wild animals were maintained on this diet for a relatively short period (compared with cultured animals) may not have allowed complete lipid turnover in the shrimp. As a result, lipids mobilized to the ovaries during the maturation process may in part reflect the natural diet of parent shrimp. The fact that eggs from cultured shrimp were higher in 20:4 and 20:5 than those from wild shrimp (a difference also noted in the fatty acid distribution between the maturing ovaries of wild and cultured P. setiferus, Section 3.3.2), could also be explained as differences in fatty acid distributions in the diet of parent shrimp.

3.5. Fatty acids in the food web.

A complete mariculture system can be envisioned which would incorporate separate growing facilities for shrimp at various stages of development. Such a production system would require three

different types of diet. Shrimp selected as breeding stock would be maintained on a diet which has been shown to facilitate ovarian maturation and produces the maximum number of viable, fertilized eggs. Eggs, developing larvae, and postlarvae would be fed a diet which provided the essential nutrients and maximized growth. The final grow out system would use an economical diet which produced the maximum gain in biomass at the lowest food conversion ratio (ratio of total food used to the total gain in biomass) in juvenile animals. A maturation diet which has been shown to give good success has been discussed in Section 3.2.2. The following sections will discuss the development of economical feedstocks designed to meet the nutritional requirements of larvae and juveniles.

3.5.1. Algae and rotifers. Several authors have reported on the use of rotifers as feeds for the culture of fish larvae and planktonic crustaceans such as Artemia salina (Watanabe et al., 1978; Hirayama et al., 1979). In these studies, rotifers have been shown to reflect the fatty acid profiles of their diet. It may be possible to feed rotifers on algae which are high in relative concentrations of polyunsaturated fatty acids, in order to incorporate these compounds in the rotifers' lipids. Rotifers high in polyunsaturated fatty acids could then be fed to shrimp larvae. In this manner, the necessary fatty acids required for growth in shrimp could be selectively introduced into the shrimp's diet using rotifers as a live feed.

The rotifers Brachionus plicatilis and Lepadella ovalis (95:5) were maintained in culture on a diet of yeast (Torula sp.). A

Table 3.15. Relative concentrations of major fatty acids in rotifers grown in the laboratory, and their diets.[a]

Specimen	n	16:0	16:1	18:0	18:1	20:1	18:2+3[b]	20:4+5[c]	22:5+6[d]
Starved 24 hrs.	2	16.3±0.9	10.5±0.8	8.8±0.5	21.5±0.4	5.5±0.6	17.6±0.1	3.1±0.4	0.6±0.3
Rotifers fed *Torula* sp.	1	15.6	14.1	5.1	19.0	4.3	21.6	5.0	1.5
Rotifers fed *Tetraselmis chuii*	2	15.1±1.9	9.5±1.4	8.3±1.3	20.6±1.0	7.0±3.2	21.3±1.7	13.2±0.5	1.1±0.5
Rotifers fed *Chlorella* sp.	2	16.4±0.1	15.0±0.3	7.2±0.2	16.0±0.3	5.6±0.2	16.6±0.7	7.7±0.1	1.2±0.1
Torula sp.	1	15.7	8.2	3.6	32.1	0.0	35.7	0.0	0.0
Tetraselmis chuii	1	25.2	2.3	12.8	32.8	0.0	f	4.3	0.0
Chlorella sp.	1	30.2	28.1	0.5	3.0	1.2	2.7	17.5	0.5

Footnotes to Table 3.15.

[a]Values do not total 100% because minor fatty acids are not listed.

[b]18:2 and 18:3 are not resolved on OV-1.

[c]20:4 and 20:5 are not resolved on OV-1.

[d]22:5 and 22:6 are not resolved on OV-1.

[e]Mean ± standard deviation.

[f]18:2 insufficiently resolved from 18:1.

portion of these rotifers was rinsed for 24 hours in fresh sea water and fed either on Tetraselmis chuii or Chlorella sp. for a period of 2 hours. Rotifers were then frozen and stored until analyzed. This techique was designed to allow rotifers to become satiated with algae, without fully digesting them. In this manner, algal fatty acids would be passed more directly to shrimp larvae without being altered by the rotifers. Table 3.15 presents the relative concentrations of major fatty acids obtained from rotifers and their diets. Chromatograms of fatty acids were obtained using a 2m x 2mm (i.d.) OV-1 column and are presented in Appendix 3. Statistical differences ($p<0.05$) were found when comparing the relative fatty acid concentration of rotifers fed Tetraselmis chuii (Figure 4, Appendix 3) to those fed Chlorella sp. (Figure 6, Appendix 3). In this respect, rotifers that were fed Tetraselmis chuii had lower relative concentrations of 16:0, 20:4, and 20:5, and higher relative concentrations of 18:1. These results reflect the differences in fatty acid distribution between Tetraselmis chuii and Chlorella sp.

This experiment demonstrates that it is possible to selectively adjust the relative concentrations of fatty acids in rotifers to meet the nutritional requirements of shrimp larvae. Live feeds such as rotifers overcome the problem of autoxidation of polyunsaturated fatty acids during processing and storage of dry feeds. A production system could be envisioned in which inorganic fertilizer is added to the pond in order to stimulate growth of the algae. Rotifers could then be maintained on the algae. Once algal fatty acids were incorporated into the rotifers, eggs and larvae of

shrimp would be introduced into the system. In this manner an economical and nutritional shrimp feedstock could be obtained, which overcomes the problems associated with storage.

3.5.2. Food web of a shrimp pond: Computer modeling. The idea of using inorganic fertilizer to stimulate growth of algae has interesting possibilities. Algae are primary producers of fatty acids in the food web. In a closed system it would be possible to select specific species of algae which synthesized the fatty acids required by shrimp. These fatty acids would then be transferred through the food web by lower order invertebrates such as polychaete worms. Specific invertebrates could be selected which produce fatty acid profiles most similar to those found in wild shrimp, and which are not competitors with shrimp for food. Cultured shrimp would then receive part or all of their nutritional requirements by consuming these invertebrates.

A computer model was designed to test the feasibility of such a system. Data were obtained on various constituents in the food web of a quarter acre shrimp pond at the Barney M. Davis power plant at Corpus Christi, Texas. Shrimp ponds were filled with water from Laguna Madre to a depth of 4 feet. Tanks were stocked with shrimp and harvested every 3 to 4 months. The particular pond examined was stocked with P. stylirostris maintained on a commercial shrimp chow (Marine Ration #20, Ralston-Purina Co.). Specimens representative of the food web were collected on September 30, 1979, just prior to harvesting the shrimp. Average shrimp weight was approximately 25 grams, and the total weight

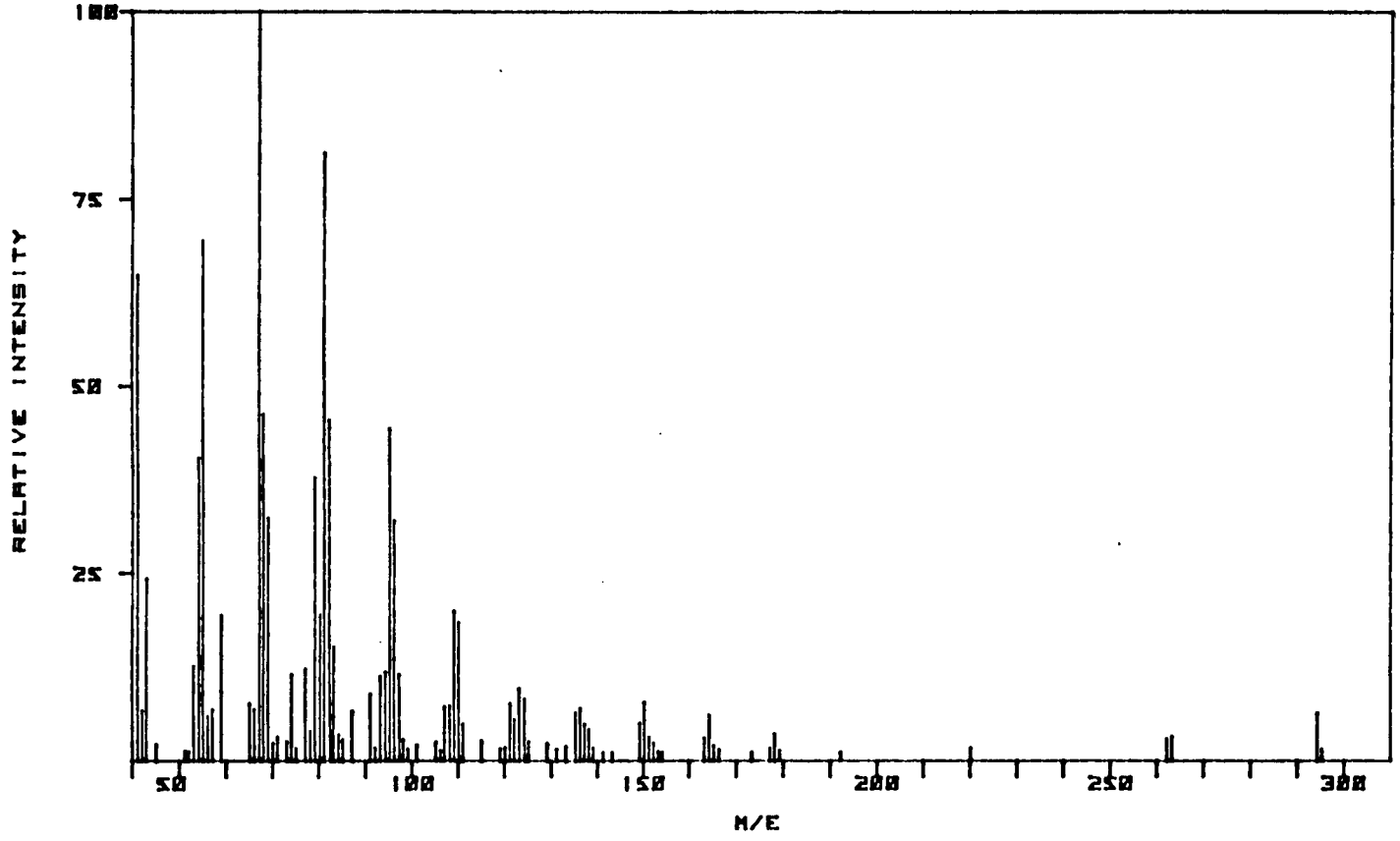
harvested was approximately 115 kg.

The relative concentrations of major fatty acids are presented in Table 3.16. Quantitative results are given in Table 3.17. The majority of samples contained a full range of fatty acids from C_{14} to C_{24}, palmitic acid being the most abundant individual constituent. All specimens were found to contain polyunsaturated fatty acids. Sediment was found to contain rather high relative concentrations of 22:6 (23.6%); this may be ascribed to small organisms such as polychaetes which inhabit the sediment. Fatty acids found in water samples collected from the pond are probably contributed by algae, bacteria, small planktonic organisms, and detritus suspended in the water column.

A schematic of the computer model used to predict fatty acid distributions within the shrimp pond is presented in Figure 3.17. The pond was considered as a closed system, inputs occuring with the addition of fertilizer and shrimp feed. Fatty acid transfers are indicated by arrows. Units of pool size are in kilograms; rates (R_1-R_3) are kg $week^{-1}$; and rate constants (k_1-k_5) are $week^{-1}$. The initial pool sizes in the primary producers, intermediary consumers, and shrimp are a, b, and c, respectively. The pool sizes after time t are expressed as C_y^x, where the superscript denotes the origin of the fatty acid and the subscript the pool referred to (A = primary producers, B = intermediary consumers, C = shrimp, D = input to primary producers, E = output from primary producers, F = output from intermediary consumers, G = output from shrimp, H = input to shrimp, and I = input to intermediary producers).

Table 3.16. Relative concentrations of major fatty acids from constituents of the food web in a shrimp pond.[a]

Specimen	16:0	16:1	18:0	18:1	18:2	18:3	20.1	20:4	20:5	22:6
Algal mat 1	17.5	4.3	1.9	6.7	8.4	7.8	0.0	1.7	3.0	1.3
Algal mat 2	39.3	9.0	1.3	7.9	3.5	0.8	6.7	1.2	5.5	1.2
Arenicola sp.	23.1	3.5	3.8	10.9	10.6	0.0	4.6	10.2	6.0	1.8
Gammarus mucronatus	39.4	4.8	5.9	15.3	5.6	1.2	0.8	4.0	8.0	4.8
Hydroides dianthus	19.1	15.7	5.6	12.3	5.6	0.0	7.7	3.6	4.2	4.8
Menidia beryllina	25.2	2.0	7.3	12.1	2.5	0.0	0.0	4.9	2.9	32.7
Mysidopsis bahia	33.8	1.2	1.9	17.8	12.3	0.9	0.6	1.5	3.1	4.6
Nassarius vibex	26.5	1.4	7.9	18.1	8.4	0.0	2.8	5.6	2.8	2.8
Penaeus stylirostris	22.4	1.9	7.7	17.1	21.6	0.8	1.6	4.5	7.8	5.2
Pond water	28.5	4.9	0.0	28.5	13.0	0.0	0.0	0.8	3.3	1.6
Sediment	19.4	4.2	2.9	12.0	4.9	1.9	2.1	3.9	15.9	23.6
Uca minax	24.6	16.3	4.2	9.9	5.4	0.0	2.8	3.9	10.1	2.7


100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Footnotes to Table 3.16.

[a]Totals do not equal 100% because minor fatty acids are not listed.

[b]Unidentified green algae matted to drainage structures in the pond.

[c]Unidentified green algae matted to the surface of sediments.

Table 3.17. Quantitative analysis of major fatty acids from constituents of the food web of a shrimp pond.[a]

Specimen	16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:4	20:5	22:6	total
Algal mat 1	0.28	0.07	0.03	0.11	0.13	0.12	0.00	0.03	0.05	0.02	1.57
Algal mat 2[c]	0.21	0.05	0.01	0.04	0.02	0.00	0.04	0.01	0.03	0.01	0.54
Arenicola sp.	1.89	0.28	0.31	0.89	0.87	0.00	0.38	0.83	0.49	0.15	8.16
Gammarus mucronatus	1.20	0.15	0.18	0.47	0.17	0.04	0.02	0.12	0.25	0.15	3.05
Hydroides dianthus	0.27	0.22	0.08	0.18	0.08	0.00	0.11	0.05	0.06	0.07	1.43
Menidia beryllina	1.05	0.08	0.30	0.51	0.10	0.00	0.00	0.20	0.20	1.36	4.17
Mysidopsis bahia	1.84	0.07	0.10	0.97	0.67	0.05	0.03	0.08	0.17	0.25	5.45
Nassarius vibex	1.45	0.08	0.43	0.99	0.46	0.00	0.15	0.30	0.15	0.15	5.46
Penaeus stylirostris	1.87	0.16	0.64	1.43	1.80	0.07	0.13	0.37	0.65	0.43	11.04
Pond water	220[d]	37.80	0.00	220	101	0.00	0.00	6.30	25.20	12.60	745
Sediments	0.05	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.04	0.07	0.28
Uca minax	3.99	2.64	0.68	1.60	0.88	0.00	0.46	0.64	1.64	0.44	16.21

Footnotes to Table 3.17.

[a]Values are in milligrams of fatty acid per gram wet weight, unless otherwise specified. Values do not equal 100% because minor fatty acids are not listed.

[b]Unidentified green algae matted to drainage structures in the pond.

[c]Unidentified green algae matted to the surface of sediments.

[d]Values for pond water are in nanograms per milliliter.

FATTY ACID MODEL

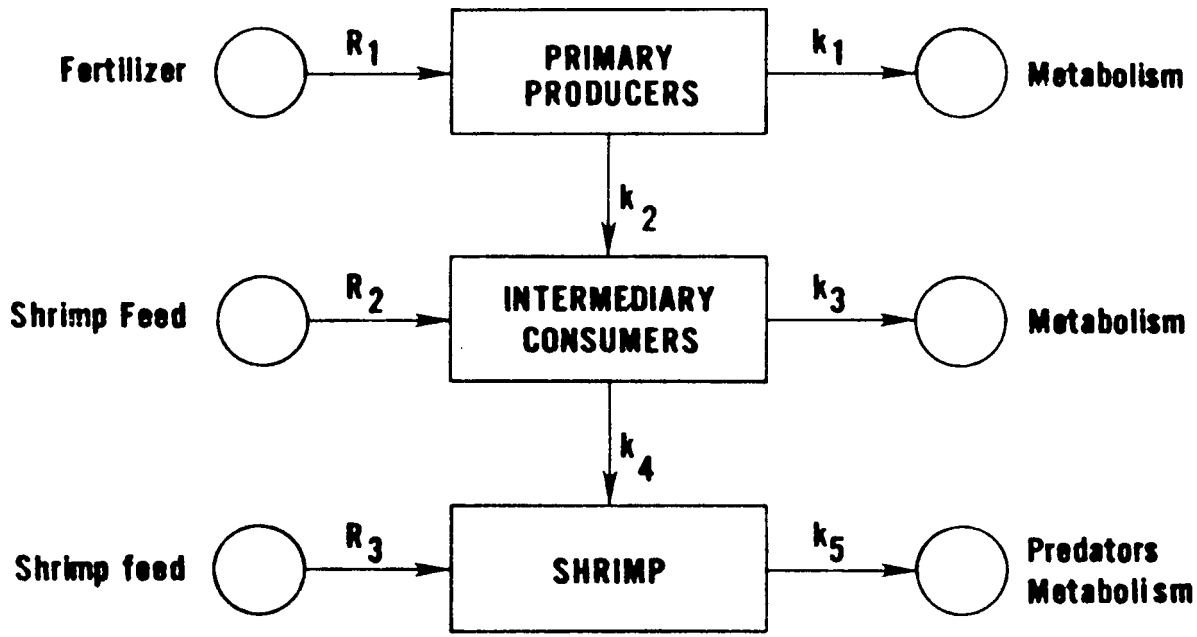


Figure 3.17. Schematic of fatty acid computer model. Inputs into the pond are designated by rates R_1-R_3; and rate constants are designated as k_1-k_5. Primary producers are algae, while intermediary consumers include polychaete worms.

The initial pool in shrimp will be depleted by factors such as metabolism and predation, so

$$C_C^C = ce^{-k_5 t}$$

Direct input to shrimp by shrimp feed will also contribute to this pool, so

$$C_C^H = R_3/k_5(1-e^{-k_5 t})$$

The initial pool in intermediary producers will be depleted by transfer to competitors and shrimp, so

$$C_B^B = be^{-(k_3+k_4)t}$$

and the contribution from this pool to shrimp will be given by

$$C_C^B = [bk_4/(k_5-k_3-k_4)][e^{-(k_3+k_4)t}-e^{-k_5 t}]$$

Direct input to intermediary producers from shrimp feed will also contribute to this pool, so

$$C_B^I = [R_2/(k_3+k_4)][1-e^{(k_3+k_4)t}]$$

and this input will also supplement the shrimp pool in the following manner

$$C_C^I = R_2 k_4 [1/(k_5(k_3+k_4)) + e^{-(k_3+k_4)t}/((k_3+k_4)(k_3+k_4-k_5)) + e^{-k_5 t}/(k_5(k_5-k_3-k_4))]$$

The initial pool size in the primary producers will be depleted, so

$$C_A^A = ae^{-(k_1+k_2)t}$$

and fatty acids initially in this pool will contribute to the pools in intermediary consumers

$$C_B^A = [ak_2/(k_3+k_4-k_1-k_2)][e^{-(k_1+k_2)t} - e^{-(k_3+k_4)t}]$$

and shrimp

$$C_C^A = ak_2k_4[e^{-(k_1+k_2)t}/((k_1+k_2-k_3-k_4)(k_1+k_2-k_5)) + e^{-(k_3+k_4)t}/((k_1+k_2-k_3-k_4)(k_5-k_3-k_4)) + e^{-k_5 t}/((k_1+k_2-k_5)(k_3+k_4-k_5))]$$

Finally, direct input to primary producers will contribute to the pools in primary producers

$$C_A^D = R_1/(k_1+k_2)[1-e^{-(k_1+k_2)t}]$$

intermediary consumers

$$C_B^D = R_1 k_2 [1/((k_1+k_2)(k_3+k_4)) + e^{-(k_1+k_2)t}/((k_1+k_2)(k_1+k_2-k_3-k_4)) + e^{-(k_3+k_4)t}/((k_3+k_4)(k_3+k_4-k_1-k_2))]$$

and the shrimp

$$C_C^D = R_1 k_2 k_4 [1/((k_1+k_2)(k_3+k_4)k_5) + e^{-(k_1+k_2)t}/((k_1+k_2)(k_1+k_2-k_3-k_4)(k_5-k_2-k_1)) + e^{-(k_3+k_4)t}/((k_3+k_4)(k_1+k_2-k_3-k_4)(k_3+k_4-k_5)) + e^{-k_5 t}/(k_5(k_1+k_2-k_5)(k_5-k_3-k_4))]$$

The total quantity of material in each pool is given by the expressions

$$C_A = C_A^D + C_A^A$$

$$C_B = C_B^D + C_B^A + C_B^I + C_B^B$$

$$C_C = C_C^D + C_C^A + C_C^I + C_C^B + C_C^H + C_C^C$$

Programs were written on a Hewlett-Packard 9825A programmable calculator. The language used was HPL, which is very similar to BASIC. One program was used to calculate pool sizes and concentrations for output on the line printer (Figure 3.18) and the other for plotting concentration profiles (examples are given in

Appendix 4).

The H.-P. 9825A has a dynamic range of 10^{99} to 10^{-99}, 0, and -10^{-99} to -10^{99}. Thus, it cannot work with numbers lower than e^{-228}. This is a slight problem in some calculations since, when rate constants are high, there is a limitation on the time period encompassed by a simulation. This problem can be circumvented by running serial simulations.

Considering the total system, fertilizer and shrimp feeds would encompass the major input devices of fatty acids into the system. Shrimp feeds would introduce fatty acids directly into the system, while fertilizer does so indirectly by stimulating the growth of algae which then produce fatty acids. Rate constants were determined initially by fitting the model to data presented in Table 3.17.

Due to the lack of information on total quantities of algae and other organisms in the pond, certain assumptions had to be made. In this respect, primary producers were considered to exist primarily in the water column. Therefore, values obtained for the fatty acid content in pond water (from Table 3.17) were used in the calculation of rate constants in the initial model (algae were assumed to contribute the majority of fatty acids in this specimen). A reasonably accurate estimation of total water capacity (and thus total fatty acid content) in the pond could then be made. Intermediary consumers such as lower invertebrates and bacteria exist primarily in the sediment; therefore, values for fatty acid content in sediment were used in the calculation of rate constants for the initial model. At this particular pond a layer of

sediment approximately 5 cm thick covered a hard layer of clay. It was assumed that the majority of organisms (and thus fatty acids) are present in the first centimeter of sediment. Finally, the production system of greatest interest is one which can make maximum use of the food web in meeting dietary requirements for shrimp. Therefore, rates R_2 and R_3 in the initial model were considered to be zero in order to study the impact of fertilizer alone on the fatty acid distribution in the pond. As mentioned previously, fertilizer introduces fatty acids into the pond indirectly, by stimulating their production in algae. Since a variety of factors such as water quality, photoperiod, and temperature affect the growth of algae (and thus the production of fatty acids), it is difficult to estimate a conversion ratio for the quantity of fatty acids derived from a certain amount of fertilizer. Therefore, for the purposes of the model at this point, the rate R_1 will be considered in terms of kilograms of fatty acid per week rather than kilograms of fertilizer per week. Once experimental data are available on conversion ratios, then kilograms of fertilizer per week can be considered.

The approach used in testing the model was to select empirical values which appear to adequately account for the analytical data obtained in Table 3.17, and the determine the affects of varying the input rate R_1 on the pool sizes.

Simplex optimization (Deming and King, 1974) was used to arrive at reasonable values for various parameters, based on the following data:

1) the time frame considered is a 12 week period period in which P. stylirostris weighing 2 grams each have

been grown to 25 grams;

2) the concentration (mg/g) of total fatty acids in shrimp was considered constant during this interval (10 mg/g);

3) the initial quantity of fatty acids in the shrimp pool based on 10,000 animals per pond was 0.25 kg, and the quantity considering 100% survival was 3.15 kg;

4) accumulation of fatty acids in shrimp was considered to be linear over the time interval;

5) the total amount of fatty acids in the intermediary consumer pool was considered to be 16.5 kg, based on fatty acid concentrations in the sediment and considering a 1 cm thickness and 6.5×10^3 kg/m^3 wet weight of sediment;

6) the quantity of total fatty acids in the primary producer pool was considered to be 0.094 kg based on the fatty acid content in pond water and considering a 4 ft depth over the quarter acre pond; and

7) the total quantity of fatty acids in the primary producer and intermediary consumer pool was considered to be essentially constant over the time interval (i.e. near equilibrium).

The response value for simplex optimization was selected such that the conditions listed above were optimized. The initial rate constants k_1-k_5 and rate R_1 are given in Figure 3.18. A plot of the

UNIVERSITY OF HOUSTON

DEPT. OF BIOPHYSICAL SCIENCES

FATTY ACID MODEL

TIME INTERVAL: 12.00 weeks

RATE:	(kg/wk)
R(1)	0.30
R(2)	0.00
R(3)	0.00

CONC.:	initial		final	
	(mg/g)	(kg)	(mg/g)	(kg)
PRIMARY	0.001	0.093	0.001	0.100
INTERMEDIARY	0.250	16.450	0.251	16.485
SHRIMP	10.000	0.250	10.000	3.152

RATE CONST.:	(1/wk)
K(1)	0.0010
K(2)	3.0000
K(3)	0.0030
K(4)	0.0150
K(5)	0.0030

SIMULATION NUMBER 1

Figure 3.18. Tabulation obtained from the H.-P. 9825A for initial rates R_1-R_3 and rate constants k_1-k_5, based on the input of fatty acids due to addition of fertilizer only.

quantity of fatty acids in the shrimp pool using these parameters is shown in Figure 1, Appendix 4.

The rate R_1 necessary to meet the initial criteria was found to be 0.3 kg/week. This corresponds to the production of 191 kg/week of algae (based on 1.57 mg/g wet weight for algal mat 1). Rate constants k_1, k_3, and k_5 each representing loss of fatty acids due to autoxidation and metabolism, are relatively small in comparison to k_2 and k_4, which represent transfer of fatty acids from one pool to the next. Therefore, only a small portion of fatty acids absorbed by each pool are lost to metabolic processes.

The effect of a 10-fold increase or reduction of rate R_1 on the quantity of fatty acids in each pool is given in Figures 2-7, Appendix 4. An increase in R_1 has a marked effect upon the quantity of total fatty acids in the intermediary consumer pool at the end of the time interval (2.8 times the quantity of that in Figure 3.18). The increase affects the pool size in shrimp to a lesser extent, and does not appreciably affect the pool size in algae. This indicates that the majority of the fatty acids produced in the pond are absorbed very rapidly by organisms living in the sediment.

A 10-fold decrease in R_1 does not have an appreciable effect on the fatty acid content in intermediary consumers or in shrimp. However, there is a 10-fold reduction in the amount of fatty acids present in the primary producer pool. This information indicates that the use of fertilizer in the production of shrimp may be feasible. A pond may be fertilized prior to addition of shrimp such that fatty acid concentrations in the intermediary consumers reach

those levels shown in the initial data (Figure 3.18). Fertilization can then be halted and the pond stocked by shrimp, which will then utilize the fatty acids stored by intermediary consumers.

3.5.3. Discussion. Perhaps the best use of the model at present is to indicate what experiments or observations need to be conducted in order to obtain a more complete understanding of the dynamic processes involved in the shrimp pond. For example, the model at this point does not distiguish between fatty acid distributions in bacteria and those in lower invertebrates (both are combined in the intermediary consumer pool). More than likely the majority of fatty acids obtained from sediment are derived from bacteria. Population counts would be recommended to determine approximate amounts of bacteria and lower invertebrates in the pond of equilibrium.

Once more information is available concerning physical, chemical, and biological processes occuring in the pond, the model can be expanded. Modeling can then allow the mariculture operator to predict the effects that such parameters as stocking capacity, feeding regimens and competitors have on the production of shrimp.

3.6. Simplex optimization of shrimp feedstocks.

The previous sections have demonstrated that enough information is now abailable concerning shrimp biochemistry and nutrition, to enable commercial mariculture systems to raise shrimp in captivity and provide their own seedstock. However, the ultimate goal in mariculture is to produce these results in the most

economical manner. The use of fresh marine invertebrates as food supplements for the purposes of maturation would not be feasible on a large scale for a number of reasons:

1) Problems of storage are involved when trying to maintain large stocks of invertebrates.
2) Availability of the desired invertebrates cannot be guaranteed, particularly during winter months.
3) The expense of obtaining suitable quantities of marine invertebrates for use as food supplements would be prohibitive.

For these reasons the use of dry feedstocks is desirable.

Shrimp feeds are normally composed of a variety of constituents including various meals, vitamins, minerals, and binders. Table 3.18 lists the components of a typical dry feed used for shrimp mariculture. The selection of components and relative compositions are arrived at most frequently by trial and error experimentation, coupled with the limited knowledge available concerning shrimp biochemistry. Often, nutrients in one component of the feedstock will duplicate those of another. Obviously, a feed that incorporates as few ingredients as possible will be the least expensive to manufacture. Of course one cannot totally sacrifice the quality of a feed (in terms of shrimp survivability or promotion of maturation) while trying to produce a cheaper feedstock. What has to be accomplished is the optimization of all parameters (i.e. quality of the feed and expense of production) in order to obtain the most economically beneficial system.

Table 3.18. Constituents of an experimental maturation feedstock (1023-77-1 HP from the National Marine Fisheries Service, Galveston, Texas).

Constituent	Relative Percent
Shrimp meal	14%
Fish meal	13%
Fish protein concentrate	14%
Soy protein	14%
Yeast protein	13%
Wheat flour	9%
Rice gel	5%
Whey	2%
Menhaden oil	4%
Herring oil	2%
Soy oil	2%
Vitamin mix	2%
Fish solubles	5%
Lecithin	1%

3.6.1. Simplex optimization. The typical approach to optimizing a feedstock has been to conduct a large number of experiments using different concentrations of various ingredients in the feedstock. This "hit-and-miss" technique then allows the observer to select that composition which has produced the best reponse (i.e. shrimp survival rate or increase in biomass and/or cost of manufacturing the feed). Obviously, with many variables to consider, this technique would require a large number of experiments before arriving at an optimum response value. In the event that there is factor dependence (i.e. response can only be improved by varying two or more factors simultaneously), the number of experiments required to reach an acceptable response value would dramatically increase. Furthermore, there is no guarantee that this value represents the optimum.

An alternate approach to this problem is the use of an optimization algorithm. Because of its effectiveness and simplicity of design, the technique of simplex optimization is most acceptable (Nelder and Mead, 1965). The modified simplex optimization procedure discussed by Deming and Morgan (1973) is applicable to the optimization of constituents in feedstocks.

Various meals to be used in experimental feeds designed by simplex optimization were examined initially to determine their fatty acid content. Qualitative and quantitative results are presented in Table 3.19 and Table 3.20, respectively. It is interesting that shrimp meal contains relatively low concentrations of 20:4, 20:5, and 22:6 compared with squid and menhaden meals. Fatty

Table 3.19. Relative concentrations of major fatty acids in selected meals.

Meal	16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5	22:6
Artemia meal	21.1	14.1	3.4	18.7	6.3	13.9	2.3	2.3	0.0
Menhaden meal	24.4	5.4	7.1	12.0	1.7	0.0	4.2	8.1	22.5
Rice bran	21.2	0.7	1.5	35.4	30.8	0.7	0.0	0.0	0.0
Shrimp meal	29.9	10.8	12.1	14.3	2.4	0.9	1.8	1.2	0.6
α-Soy protein	46.1	6.9	13.1	5.5	15.7	1.8	1.4	0.5	0.0
Squid meal	28.4	2.6	9.1	5.1	0.0	0.0	5.7	14.8	19.9

[a]Totals do not equal 100% because minor fatty acids are not listed.

Table 3.20. Quantitative analysis of major fatty acids from selected meals.[a]

Meal	16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5	22:6
Artemia meal	17.56	11.76	2.81	15.63	5.27	11.59	1.93	1.93	0.00
Menhaden meal	3.35	0.74	0.98	1.64	0.23	0.00	0.57	1.11	3.08
Rice bran	9.31	0.31	0.67	15.51	13.49	0.31	0.00	0.00	0.00
Shrimp meal	5.48	1.97	2.22	2.63	0.44	0.16	0.33	0.22	0.11
α-Soy protein	3.18	0.48	0.91	0.38	1.08	0.13	0.10	0.03	0.00
Squid meal	8.81	0.79	2.82	1.59	0.00	0.00	1.79	4.58	6.17

[a]Values are in milligrams of fatty acid per gram dry weight.

acid concentrations in shrimp meal are also lower than values obtained for wild Penaeus setiferus (Table 3.7). Joseph and Myers (1975) have previously shown that significant differences in fatty acid composition exist between sun-dried meals manufactured from penaeid shrimp wastes as compared to raw wastes. This can be attributed in part to autoxidation of polyunsaturated fatty acids during processing and storage. Autoxidation would explain low concentrations of fatty acids found in shrimp meal to be used in simplex optimization experiments. Squid meal probably contained high relative concentrations of these acids because it was prepared fresh in the laboratory.

The variable constituents (ingredients) and their relative percent compositions of feedstock weight used in the first iteration are given in the Table 3.21. Separate optimization routines were conducted to maximize the response for the food conversion ratio, C/R (amount of food consumed/change in weight of surviving shrimp), and the response for the price per pound of shrimp, $/lb [$\Sigma$(cost of constituent x relative concentration) x food conversion ratio]. Response functions were maximized by feedstocks which produced the lowest food conversion ratios or which had the lowest cost per pound of shrimp produced. Prices of variable constituents as of September, 1979 are given in Table 3.22. Juveniles (5g) of P. stylirostris maintained on the experimental feedstocks for a period of 2 weeks were used in evaluating responses. Interestingly, both optimization experiments found the feedstock for vertex 9 to produce the best response.

The reflection based on the 10 best responses was calculated, and the resulting feedstock constructed. To save time, the contrac-

Table 3.21. Relative compositions of variable ingredients used in the initial simplex.

	VERTEX										
INGREDIENT	1	2	3	4	5	6	7	8	9	10	11
Menhaden meal	25.0	22.5	20.0	17.5	15.0	12.5	10.0	7.5	5.0	2.5	0.0
Menhaden oil	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0.0
Menhaden solubles	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0.0
Mineral mix	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0.0
Rice bran	19.0	21.7	24.5	27.3	30.0	32.8	35.5	38.2	41.0	43.8	46.5
Sand	10.0	9.0	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0
Shrimp meal	0.0	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0
α-Soy protein	25.0	22.5	20.0	17.5	15.0	12.5	10.0	7.5	5.0	2.5	0.0
Squid meal	0.0	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0
Vitamin mix	2.5	2.3	2.0	1.7	1.5	1.2	1.0	0.8	0.5	0.2	0.0
Response (C/R)[a]	15.6	3.5	3.7	5.4	75.2	2.8	8.1	5.5	2.3	-[b]	-
Response ($/lb)[c]	14.09	2.97	2.90	3.88	50.01	1.69	4.42	2.71	0.98	-	-

Footnotes to Table 3.21.

[a]Response determined by the food conversion ratio (amount of food consumed / change in biomass).

[b]Response values for vertices 10 and 11 were negative due to a decrease in the weight of surviving biomass. In simplex calculations these responses were assigned very large values such that they would be discarded in future iterations.

[c]Response determined by Σ(price of constituent x percent composition of feed) x food conversion ratio.

Table 3.22. Price list of variable ingredients used in the preparation of shrimp feedstocks for simplex optimization experiments. Prices are as of September, 1979.

Constituent	Price
Menhaden meal	$ 0.16/lb
Menhaden oil	$ 0.25/lb
Menhaden solubles	$ 0.20/lb
Mineral mix	$ 1.61/lb
Rice bran	$ 0.07/lb
Sand	$ 0.02/lb
Shrimp meal	$ 0.16/lb
α-Soy protein	$ 1.67/lb
Squid meal	$ 0.90/lb
Vitamin mix	$11.82/lb

tions C_R, C_W, and the expansion E were also calculated. Table 3.23 lists the relative compositions of ingredients obtained from these calculations. The expansion calculation produced negative values for relative compositions of some constituents, and was immediatly assigned a bad response. Feedstocks designed as a result of the reflection R, and the contractions C_R and C_W were used in experiments to determine their response functions. The responses determined by reflection for both simplex optimization routines were accepted and used in performing the second iteration.

The reflection R, contractions C_R and C_W, and the expansion E were calculated for the second iteration using retained vertices (those feedstocks which gave the best responses). The relative compositons of ingredients based on these calculations are given in Table 3.24. The reflection and expansion were assigned bad responses because of negative values calculated for some ingredients. Therefore, only the responses obtained for C_R and C_W contractions will be considered for the next iteration of both simplex optimization routines. These values have not been fully evaluated at this point.

Although there have not been a sufficient number of interations conducted to draw any conclusions concerning the optimum concentrations of ingredients, some interesting observations can be made. The reflections generated by both iterations have tried to increase the amount of sand in the feedstock. Analyses performed on total lipid extracts (Lawrence *et al.*, unpublished) have indicated that cultured shrimp contain lipid amounts greatly exceeding those found in wild shrimp. This would indicate that

Table 3.23 Relative percent compositions of ingredients from the reflection R, contractions C_R and C_W, and the expansion E, calculated from the initial simplex.

	METHOD OF CALCULATION			
INGREDIENT	R	C_R	C_W	E
Menhaden meal	24.5	19.0	8.0	35.5
Menhaden oil	4.9	3.8	1.6	7.1
Menhaden solubles	4.9	3.8	1.6	7.1
Mineral mix	4.9	3.8	1.6	7.1
Rice bran	19.5	25.5	37.7	7.3
Sand	9.8	7.6	3.2	14.2
Shrimp meal	0.5	6.0	17.0	-10.5
α-Soy protein	24.5	19.0	8.0	35.5
Squid meal	0.5	6.0	17.0	-10.5
Vitamin mix	2.5	1.9	0.8	14.2
Response (C/R)[a]	4.74	20.64	3.07	-[b]
Response ($/lb)[c]	4.25	15.27	1.47	-

[a]Response values obtained by the food conversion ratio. For calculation, see text.

[b]Responses for expansion were considered bad due to negative percent composition for some ingredients.

[c]Response value obtained by the price per pound of shrimp. For calculation, see text.

Table 3.24. Relative percent compositions of ingredients used in the reflection R, contractions C_R and C_W, and expansion E calculated from the second iteration.

	METHOD OF CALCULATION			
INGREDIENT	R	C_R	C_W	E
Menhaden meal	32.0	24.0	8.0	48.0
Menhaden oil	6.4	4.8	1.6	9.6
Menhaden solubles	6.4	4.8	1.6	9.6
Mineral mix	6.4	4.8	1.6	9.6
Rice bran	11.4	20.2	37.8	-6.2
Sand	12.8	9.6	3.2	19.6
Shrimp meal	-6.9	1.1	17.1	-22.9
α-Soy protein	32.0	24.0	8.0	48.0
Squid meal	-6.9	1.1	17.1	-22.9
Vitamin mix	3.2	2.4	2.4	4.8
Response (C/R)	-[a]	N/A[b]	N/A	-
Response ($/lb)	-	N/A	N/A	-

[a]Response values for the reflection and expansion were automatically assigned bad values due to negative percent concentrations calculated for some ingredients.

[b]Response values for the contractions C_R and C_W have not been fully evaluated at this time.

cultured shrimp are being overfed. The attempt by simplex optimization to increase the amount of sand in the feedstock seems to indicate this as well. Both reflections have also tried to reduce the amount of squid meal and shrimp meal, another indication that shrimp may be overfed.

It must be pointed out that at this time, simplex optimization has achieved a food conversion ratio of 2.3. This value is equal to that obtained by trial and error experimentation in our laboratories (Lawrence et al., unpublished). Further simplex experiments should be able to obtain lower values.

3.6.2. Discussion. As indicated by the results, the simplex optimization technique has already moved in the direction of the optimum response. Unfortunately, it was not discovered until the second iteration that certain ingredients could not be varied with respect to one another. This fault lies in the design of the initial simplex; therefore, simplex optimization has been started anew, using initial values which will allow variation of all ingredients with respect to each other. Such a design is imperative since it will allow the isolation of those ingredients which have the greatest effect on response.

Once an optimum has been attained by the simplex optimization routine, biochemical information can be derived from the resultant feedstock. Fatty acid data on meals used in construction of the feedstock (Tables 3.19 and 3.20) can then be evaluated. Final concentrations of fatty acids in a feedstock may be much smaller (or larger) than originally anticipated. Therefore, simplex

optimization can give an indication as to nutritional requirements of the shrimp for specific fatty acids. This concept can also be extended to other nutrients such as protein, carbohydrate, and cholesterol. By determining the biochemical nature of the optimal feedstock, and by examining the progress of the simplex optimization routine, it will most likely be possible to reduce the number of ingredients used in preparation of the feed.

Although simplex optimization offers the best solution to reaching a universal optimum (considering the ingredients used for preparation of the feedstock) it is still a time-consuming process. From an economical standpoint, the prices of meals such as soy protein and menhaden may change considerably in only a few months time. Large price fluctuations might dictate a change in feedstock preparation. Therefore, the commercial mariculture operator will need a method which can obtain a reasonably good optimum in a short period of time. One solution to this problem would utilize simplex optimization as a "compass" to indicate the direction of the optimum on the response surface. Several small simplex experiments could be performed simultaneously, using initial values for ingredients which gave responses covering a wide range of the response surface. In this manner small simplex optimization routines would be initiated at different points on the response surface. The direction taken by each simplex would accomplish two things:

1) Each simplex would tend to move in the direction of the optimum, thus indicating at which point on the response surface the optimum value exists.

2) Small simplex optimization routines would "silhouette" the optimal region, thereby reducing the range of values to be considered for each ingredient of the feedstock.

By examining each simplex routine, the operator would have an indication of the concentrations of ingredients to be used in the feedstock in order to obtain responses within the region of the optimum. Several simultaneous trial and error experiments could then be performed in order to obtain the most acceptable response from this region. Figure 3.19 illustrates this concept on a two dimensional basis.

One of the disadvantages of this technique is that bimodal or multimodal response surfaces (i.e. surfaces which have several local optima whose responses may be less desirable than that of the universal optimum) could result in false interpretation of data from each simplex. Therefore, it will be necessary to obtain more information concerning response surfaces when dealing with optimal values for ingredients in feedstock preparations, in order to test the validity of this concept.

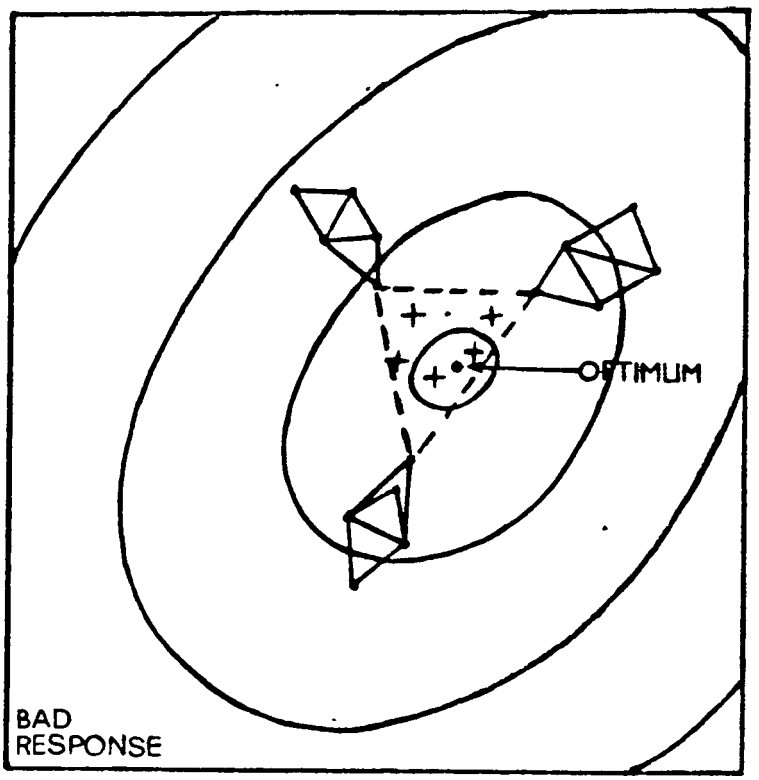

OPTIMUM
BAD
RESPONSE

Figure 3.19. Two dimensional illustration of the concept involving the use of simultaneous simplex optimizations as a method of rapidly localizing the response optimum. Response surface is displayed as a contour map with the optimum response at its center. Triangles indicate the progress of each simplex. Once a few iterations have been performed, the optimum can be isolated in the area of the response surface indicated by dotted lines. Several trial and error experiments ("+" marks) can then be performed based on values derived from evaluating the direction that the simplex routines have taken. The concentrations of ingredients obtained from the experiment which produced the best results would then be used in the manufacure of feedstocks.

4.

SUMMARY

4. SUMMARY

The object of this study was to obtain for the first time reproducible ovarian maturation of penaeid shrimp grown in mariculture. This objective was achieved. Past efforts to obtain this result centered on the adjustment of parameters such as photoperiod, temperature, and water quality, as mentioned in the introduction. The approach used in this study was to manipulate the chemistry of cultured shrimp through dietary modifications, in order to mimick the chemistry of wild shrimp. Since lipids were known to be the principle source of energy in crustaceans, this class of compounds was of primary interest. Fatty acids are major lipid components, and were the compounds selected for study. Metabolic profiling using gas chromatography and combined gas chromatography-mass spectrometry was used in comparing fatty acid distributions of wild and cultured shrimp.

The fatty acid profiles obtained from the gonads, hepatopancreas, and tail muscle of the penaeid shrimp Penaeus stylirostris, P. vannamei and P. setiferus were examined. Each contained a full range of fatty acids from C_{14} to C_{22}, with palmitic acid generally being the main individual constituent. All tissues were found to contain high relative concentrations of polyunsaturated fatty acids, particularly arachidonic acid, 5,8,11,14,17-eicosapentaenoic acid, and 4,7,10,13,16,19-docosahexaenoic acid. This is interesting, since precursors of these acids are known to be required in the diet of higher animals. Additionally, 20:4 and 20:5 are known to be precursors of prostaglandins, which have been

shown to play a role in mammalian reproduction. Although little is known about the role of 22:6 in reproduction, the polyolefin 3,6,9,12,15,18-heneicosahexaene has been shown to accumulate exclusively in the reproductive tissue in phytoplankton, suggesting a possible reproductive role for this hydrocarbon (Youngblood and Blumer, 1973). Although the metabolic pathway for production of this hydrocarbon is uncertain, decarboxylation of 22:6 can easily be envisioned.

Based on the assumption that dietary insufficiencies were responsible for the absence of ovarian maturation in cultured shrimp, the fatty acid distribution of a traditional diet was examined. The results of this analysis revealed that the marine chow used in mariculture at that time was relatively low in relative concentrations of polyunsaturated fatty accids when compared to wild penaeid shrimp. Therefore, several varieties of marine invertebrates were screened as possible food supplements. The polychaete worms Nereis viridens, and Glycera dibranchiata, and the squid Loligo opalescens were found to have high relative concentrations of polyunsaturated fatty acids. Because of their commercial availability, these animals were selected for use as food supplements in shrimp diets.

The addition of marine invertebrates containing high relative concentrations of polyunsaturated fatty acids resulted in immediate ovarian maturation and spawning of P. setiferus. Eggs were not fertilized in this experiment due to a bacterial infection in the males. Application of the diet to P. stylirostris in later experiments resulted in the production of viable shrimp larvae. This

represents the first attainment of reproducible ovarian maturation and spawning of penaeid shrimp in the U.S. Furthermore, these results suggested that the absence of polyunsaturated fatty acids in the traditional diet was responsible for the inability to obtain ovarian maturation of penaeid shrimp grown in mariculture.

The attainment of reproducible ovarian maturation was a significant advance on shrimp mariculture. The next step was to determine whether shrimp larvae had a requirement for polyunsaturated fatty acids. Metabolic profiling of fatty acid fractions from wild P. stylirostris eggs reflected those found in adult (stage 5) females. During development these profiles were found to change dramatically, typified by increases in the relative concentrations of linoleic (18:2) and linolenic (18:3) acids, and a decrease in palmitic acid (16:0). These changes were attributed to the diet of postlarvae. Comparisons of the fatty acid profiles between wild and cultured eggs revealed that cultured eggs had substantially higher relative concentrations of polyunsatured fatty acid 20:5 and 22:6, but lower relative concentrations of 16:0 and 18:0. In addition, postlarvae of cultured animals had significantly lower relative concentrations of 18:2 and 18:3 compared to wild postlarvae. The conclusion was that cultured shrimp eggs had sufficient relative concentrations of fatty acids. It was suggested that nutritional requirements for polyunsaturated fatty acids in developing larvae and postlarvae could be met by dietary alterations.

Rotifers (which are readily consumed by shrimp larvae) were fed for a period of 2 hours on algae until satiated. This technique

was designed to incorporate algal fatty acids into shrimp, without being altered by the rotifers. Fatty acids distributions in rotifers fed on algae revealed that these rotifers had profiles which were beginning to reflect those of their algal diet. It was concluded that diets could be selectively altered to meet the nutritional requirement for fatty acids in developing shrimp larvae and postlarvae.

The results obtained with algae and rotifers suggested that it might be possible to meet all or part of the dietary requirements of shrimp by making maximum use of the food web in shrimp ponds. A computer model was designed to allow observation of fatty acid distributions within the food web. The initial model was constructed using data obtained from constituents of the food web in a typical shrimp pond. Rates and rate constants were obtained by fitting the initial model to the fatty acid distributions found in food web constituents. The model was tested by studying the effect that fertilizer had on fatty acid pool sizes. Due to the simplicity of the model at this point, it is best used as an indicator of what observations or experiments need to be conducted in order to obtain a more complete understanding dynamics of shrimp ponds. Once more data are available, the model can be made more comprehensive. It can then be used to make predictions concerning the productivity of shrimp ponds based on perturbations of the system. An example of this would be a prediction of the effect of competitors on the weight change in shrimp over a period of time. It could also be used to predict the influence of temperature, salinity, and many other physical parameters on shrimp production.

The fact that ovarian maturation and spawning was achieved through alteration of the diet suggested that the nutritional quality of shrimp diets had not been optimized. Therefore, experiments were initiated using simplex optimization to accomplish two things:

1) optimize dietary ingredients to obtain the best food conversion ratio (which is based in part on shrimp survival and weight gain), and
2) arrive at a diet which has the least cost per pound of shrimp produced.

Fatty acid compositions of various ingredients to be used in simplex experiments were analyzed prior to the initial iteration. High relative concentrations of polyunsaturated fatty acids were found in squid and menhaden meals, but were surprisingly low in shrimp meal. This was attributed to autoxidation of labile polyunsaturated fatty acids in shrimp during processing or storage; and it may be necessary to incorporate an antioxidant into meals to inhibit autoxidation. Once an optimum has been reached by simplex optimization, the fatty acid content of the optimal feedstock will be examined. Comparison of fatty acid profiles from this feedstock and those in constituent meals, combined with an evaluation of simplex data, may allow the elimination of a number of ingredients. Additionally, the content of fatty acids in the optimal feedstock will indicate the importance of dietary polyunsaturated fatty acids in meeting nutritional requirements of shrimp.

Although relatively few iterations had been performed at the time of this writing, simplex optimization had already achieved the

best food conversion ratio obtained through trial and error experiments (Lawrence et al., unpublished). Continuation of the experiments should provide a feedstock which has a very low food conversion ratio (i.e. around 1.0) and which is economical to produce.

Since the prices of various ingredients in feedstocks can fluctuate considerably within a small period of time, it would be economically advantageous to change the composition of feedstocks. This would require that a new optimal composition be derived; however, because simplex experiments take about one month each to complete, obtaining a new optimum every 3 to 4 months using simplex alone would not be possible. Therefore, a theoretical optimization procedure was discussed which used a number of small, simultaneous simplex optimization experiments initiated at different sectors of the response surface. This technique would indicate the concentrations for which ingredients in the feedstock are tending, in order to reach the optimum response. A number of trial and error experiments could then be performed using concentrations of ingredients which would give responses within the optimal region defined by the simplex experiments. The best of these experiments would then be used in the preparation of feedstocks.

This study has demonstrated that an understanding of biochemical processes in shrimp can be used in resolving problems associated with shrimp mariculture. However, the amount of literature published in this area indicates that far more information on shrimp biochemistry is necessary. It would be interesting to

determine whether the relationship between ovarian maturation and the requirement for polyunsaturated fatty acids in the diet is connected to prostaglandins. Furthermore, very little data are available concerning the endocrinology of shrimp. Several authors have indicated that hormones regulating molting in crustaceans are derived from organs in the eyestalks (Passano, 1953, 1960; Carlisle, 1957; Lockwood, 1967; Stewart and Green, 1969); and the attainment of ovarian maturation after eyestalk ablation (see Section 3.2.2) indicates that eyestalks may have a regulatory function in shrimp reproduction. Future biochemical studies may make it possible to produce larger shrimp in less time by such techniques as activating the production of growth stimulating hormones. Furthermore, breeding stock could be maintained in a continual reproductive state by administration of the appropriate reproductive hormones.

5. REFERENCES

1. Ando, T., A. Kanazawa, S. Teshima, J. Patrois, and H. J. Ceccaldi, Bull. Jap. Soc. Sci. Fish. 43, 1445 (1977).

2. Andre, E. and M. T. Richert, Olegineux 15, 479 (1960).

3. Aquacop, Proceedings of the 1975 World Mariculture Society Meeting, 123 (1975).

4. Blomstrand, R. and L. Svensson, Lipids 13, 283 (1978).

5. Boon, J. J., v. d. Graaf, P. J. W. Schuyl, F. d. Lange, J. W. d. Leeuw, Lipids 12, 717 (1977).

6. Brooks, C. J. W. and B. S. Middleditch, "Gas Chromatography-Mass Spectrometry", in Specialist Periodical Report: Mass Spectrometry, Vol. 4, R. A. W. Johnstone, Senior Reporter, Chemical Society, London (1977), p. 146.

7. Brown, A., Jr., J. B. McVey, B. S. Middleditch, and A. L. Lawrence, Proc. 1979 World Mariculture Society Meeting, in press.

8. Budzikiewicz, H, C. Djerassi, and D. H. Williams, Mass Spectrometry of Organic Compounds, Holden-Day, Inc., Cambridge (1967).

9. Burstein, S., B. S. Middleditch, and M. Gut, J. Biol. Chem. 250, 9028 (1975).

10. Caillouet, C. W. Proceedings of the 1972 World Mariculture Society Meeting, 205 (1972).

11. Carlisle, D. B., J. Marine Biol. Ass. U.K. 36, 291 (1957).

12. Christ, E. J. and D. A. van Dorp, Adv. Biosci. 9, 35 (1973).

13. Christie, W. W., Lipid Analysis, Pergamon Press, Oxford (1973).

14. Clark, W. H., A. Yudin, W. J. Persyn, and A. Persyn, Proceedings of the International Symposium on Physiology of Reproduction in the Invertebrates, (1975).

15. Colvin, P. M., Aquaculture 8, 81 (1976).

16. Conte, F. S., M. S. Duronslet, W. H. Clark and J. C. Parker, Proc. 1978 World Mariculture Society Meeting, in press.

17. Darbre, A., in Handbook of Derivatives for Chromatography, K. Blau and G. King, eds., Heyden and Son, Ltd., Bristol (1978), p. 39.

18. Deming, S. N. and S. L. Morgan, Anal. Chem. 45, 278A (1973).

19. Deming, S. N., and P. G. King, Research/Development 25, 22 (1974).

20. Deshimaru, O. and K. Kuroki, Bull. Jap. Soc. Sci. Fish. 40, 413 (1974).

21. Gallagher, M. and W. D. Brown, J. Agric. Food Chem. 23, 630 (1975).

22. Grant, D. W., Gas-Liquid Chromatography, Van Nostrand Reinhold Company, New York (1971).

23. Guary, J. C., M. Kayama, and Y. Murakami, Bull. Jap. Soc. Sci. Fish. 40, 1027 (1974).

24. Guary, J. C., M. Kayama, Y. Murakami, and H. Ceccaldi, Aquaculture 7, 245 (1976).

25. Gurr, M. I. and A. T. James, Lipid Biochemistry: An Introduction, John Wiley and Sons, Inc., New York (1975).

26. Hanson, J. A. and H. L. Goodwin, Shrimp and Prawn Farming in the Western Hemisphere. Dowden, Hutchinson and Ross, Inc., New York (1977).

27. Harrison, R. and G. G. Lunt, Biological Membranes Their Structure and Function, John Wiley and Sons, New York (1975).

28. Hirayama, K., K. Takagi, and H. Kimura, Bull. Jap. Soc. Sci. Fish. 45, 11 (1979).

29. Hudinaga, M. and J. Kittaka, Bull. Jap. Soc. Sci. Fish. 41, 843 (1975).

30. Huggins, A. K. and K. A. Munday, in Advances in Comparative Physiology and Biochemistry, Vol. 4, O. Lowenstein, ed., Academic Press, New York (1968), p. 271.

31. Jellum, E., O. Stokke, and L. Eldjarn, Clin. Chem. 18, 800 (1972).

32. Jellum, E., O. Stokke, and L. Eldjarn, Anal. Chem. 45, 1099 (1973).

33. Jezyk, F. and A. J. Penicnak, Lipids 1, 427 (1966).

34. Johnston, M. C. and J. R. Fielding, Tulane Stud. Zool. 4, 175 (1956).

35. Joseph, J. D., and S. P. Meyers, Feedstuffs 47, 35 (1975).

36. Kanazawa, A., S. Teshima, and S. Tokiwa, Bull. Jap. Soc. Sci. Fish. 43, 849 (1977a).

37. Kanazawa, A., S. Tokiwa, M. Kayama, and M. Hirata, Bull. Jap. Soc. Sc. Fish. 43, 1111 (1977b).

38. Kirk, R. G., Aquaculture 16, 95 (1979).

39. Lawrence, A. L., D. Ward, S. Missler, A. Brown, J. P. McVey, and B. S. Middleditch, Proc. 1979 World Mariculture Society Meeting, in press.

40. Lehninger, A. L., Biochemistry, Worth Publishers, Inc., New York, (1975).

41. Lockwood, A. P. M., in Aspects of the Physiology of Crustacea, W. H. Freeman, San Francisco (1967), pp. 8182.

42. Lovern, J. A., Biochem. J. 54, 126 (1953).

43. Mamer, O. A., W. J. Mitchell, and C. R. Scriver, ed., Application of Gas Chromatography-Mass Spectrometry to the Investigation of Human Disease, McGill University, Montreal (1974).

44. Millard, B. J., Quantitative Mass Spectrometry, Heyden and Son Ltd., London (1978).

45. Morris, R. J., J. Mar. Biol. Ass. U.K. 53, 27 (1973).

46. Morris, R. J. and F. Culkin, Oceanogr. Mar. Biol. Ann. Rev. 14, 391 (1976).

47. Nelder, J. A., and R. Mead, Computer J. 7, 308 (1965).

48. O'Connor, C., Aquaculture 16, 153 (1979).

49. O'Connor, J. D. and L. I. Gilbert, Am. Zoologist 8, 529 (1968).

50. Passano, L. M., Physiol. Comparat. Oecol. 3, 155 (1953).

51. Passano, L. M., in The Physiology of Crustacea, Vol. 4, T. H. Waterman, ed., Academic Press, New York (1960), p. 473.

52. Pathak, S. P., C. V. Agarwal, and S. S. Mathur, J. Am. Oil Chemists' Soc. 29, 593 (1952).

53. Schneider, H., E. Gelpi, E. O. Bennett, and J. Oro, Phytochemistry 9, 613 (1970).

54. Sedgwick, R. W., Aquaculture 16, 7 (1979).

55. Stewart, J. R. and J. P. Green, Am. Zool. 9, 579 (1969).

56. Takahashi, H. and M. Yamada, Bull. Jap. Soc. Sci. Fish. 42, 769 (1976).

57. Takeuchi, T. and T. Watanabe, Bull. Jap. Soc. Sci. Fish. 42, 907 (1976).

58. Takeuchi, T. and T. Watanabe, Bull. Jap. Soc. Sci. Fish. 43, 541 (1977a).

59. Takeuchi, T. and T. Watanabe, Bull. Jap. Soc. Sci. Fish. 43, 947 (1977b).

60. Tanaka, K., M. A. Budd, M. L. Efron, and K. J. Isselbacher, Proc. Natl. Acad. Sci. 56, 236 (1966).

61. Teshima, S. and A. Kanazawa, Bull. Jap. Soc. Sci. Fish. 42, 1129 (1976).

62. Teshima, S. and a. Kanazawa, Bull. Jap. Soc. Sci. Fish. 44, 1269 (1978).

63. Van Weel, P. B., Chemical Zoology 2, 97 (1970).

64. Von Euler, U. S. and R. Eliasson, Prostaglandins, Academic Press, New York (1967).

65. Ward, D. G., Biochemical Changes During Development of Eggs, Larvae, and Postlarvae of the Shrimp Panaeus stylirostris (Stimpson), Masters Thesis, presented to Departments of Biology and Biophysical Sciences, University of Houston, 1979.

66. Watanabe, T., C. Ogino, Y. Koshishi, and T. Matsunaga, Bull. Jap. Soc. Sci. Fish. 40, 493 (1974).

67. Watanabe, T., O. Utsue, I. Kobayashi, and C. Ogino, Bull. Jap. Soc. Sci. Fish. 41, 257 (1975).

68. Watanabe, T., M. Matsue, T. Kawabata, and C. Ogino, Bull. Jap. Soc. Sci. Fish. 43, 813 (1977).

69. Watanabe, T. and F. Takashima, Bull. Jap. Soc. Sci. Fish. 43, 819 (1977).

70. Watanabe, T., C. Kitajima, T. Arakawa, K. Fuckusho, and S. Fujita, Bull. Jap. Soc. Sci. Fish. 44, 1109 (1978).

71. Wickins, J. F., Oceanogr. Mar. Biol. Ann. Rev. 14, 435 (1976).

72. Wolfe, D. A., P. Venkata Rao, and D. G. Cornwell, J. Am. Oil Chemists' Soc. 42, 633 (1965).

73. Yamaoka, L. H., and B. T. Scheer, Chemical Zoology 2, 321 (1970).

74. Youngblood, W. W. and M. Blumer, Marine Biology 21, 163(1973).

75. Yu, T. C. and R. O. Sinnhuber, Aquaculture 16, 31 (1979).

76. Zandee, D. I., Arch. Intern. Physiol. Biochim. 74, 614 (1966).

77. Zlatkis, A., W. Bertsch, H. A. Lichtenstein, A. Tishbee, F. Shunbo, H. M. Liebich, A. M. Coscia, and N. Fleischer, Anal. Chem. 45, 763 (1973).

APPENDIX 1.

APPENDICES

5.

REFERENCES

Figure 1. Mass spectrum of methyl laurate (M = 214).

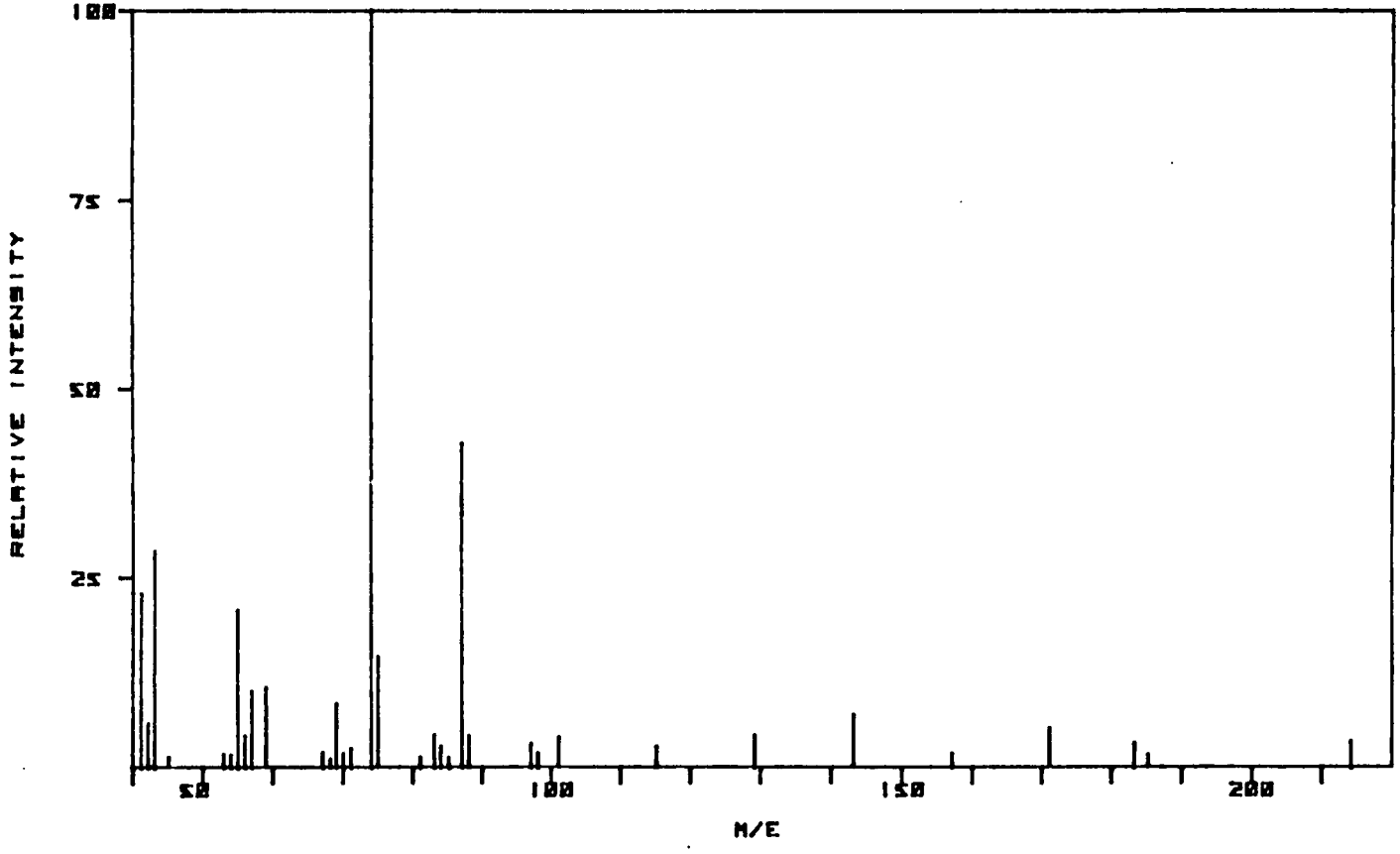

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
M/E

Figure 2. Mass spectrum of methyl myristate (M = 242).

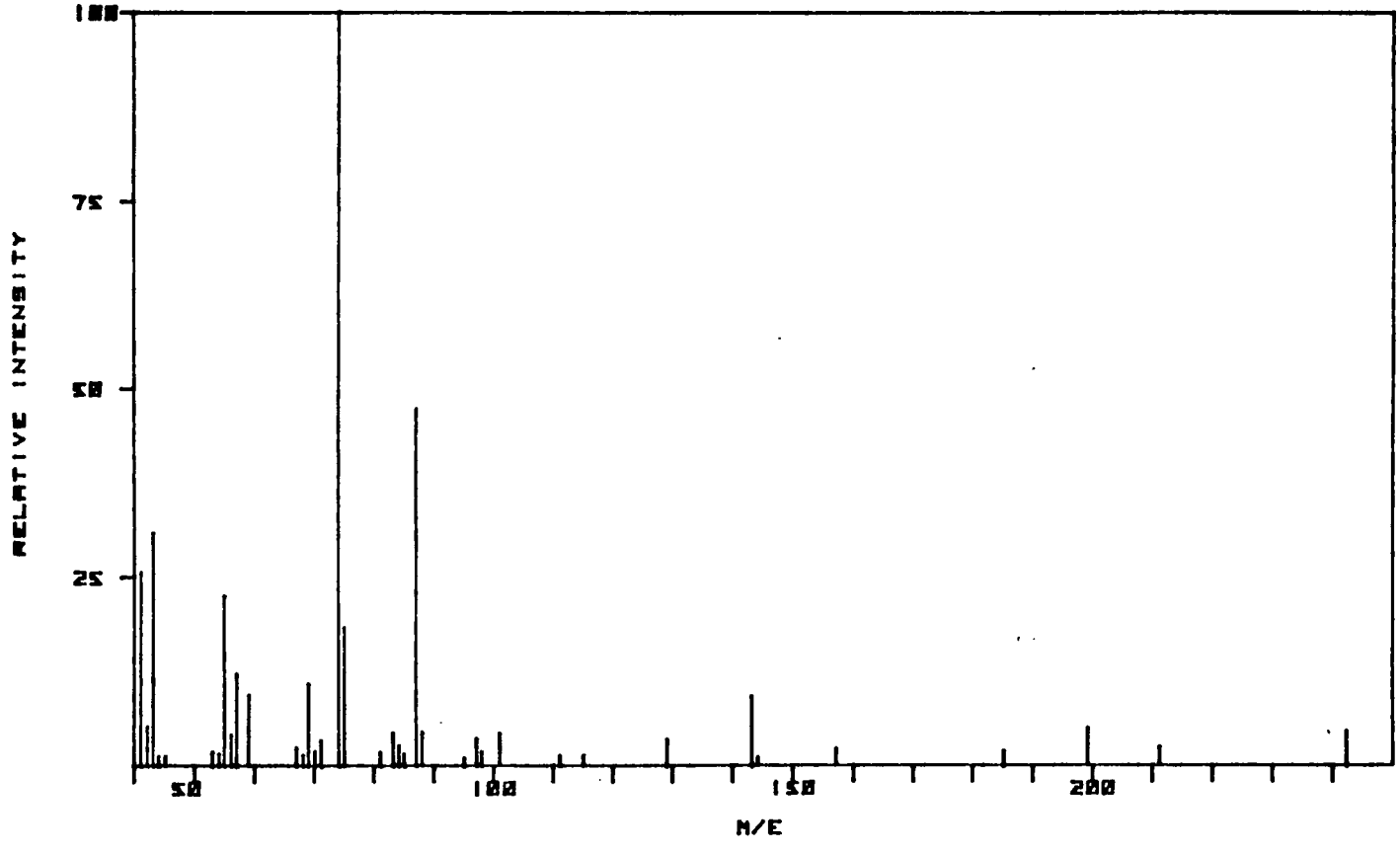

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
M/E

Figure 3. Mass spectrum of methyl palmitate (M = 270).

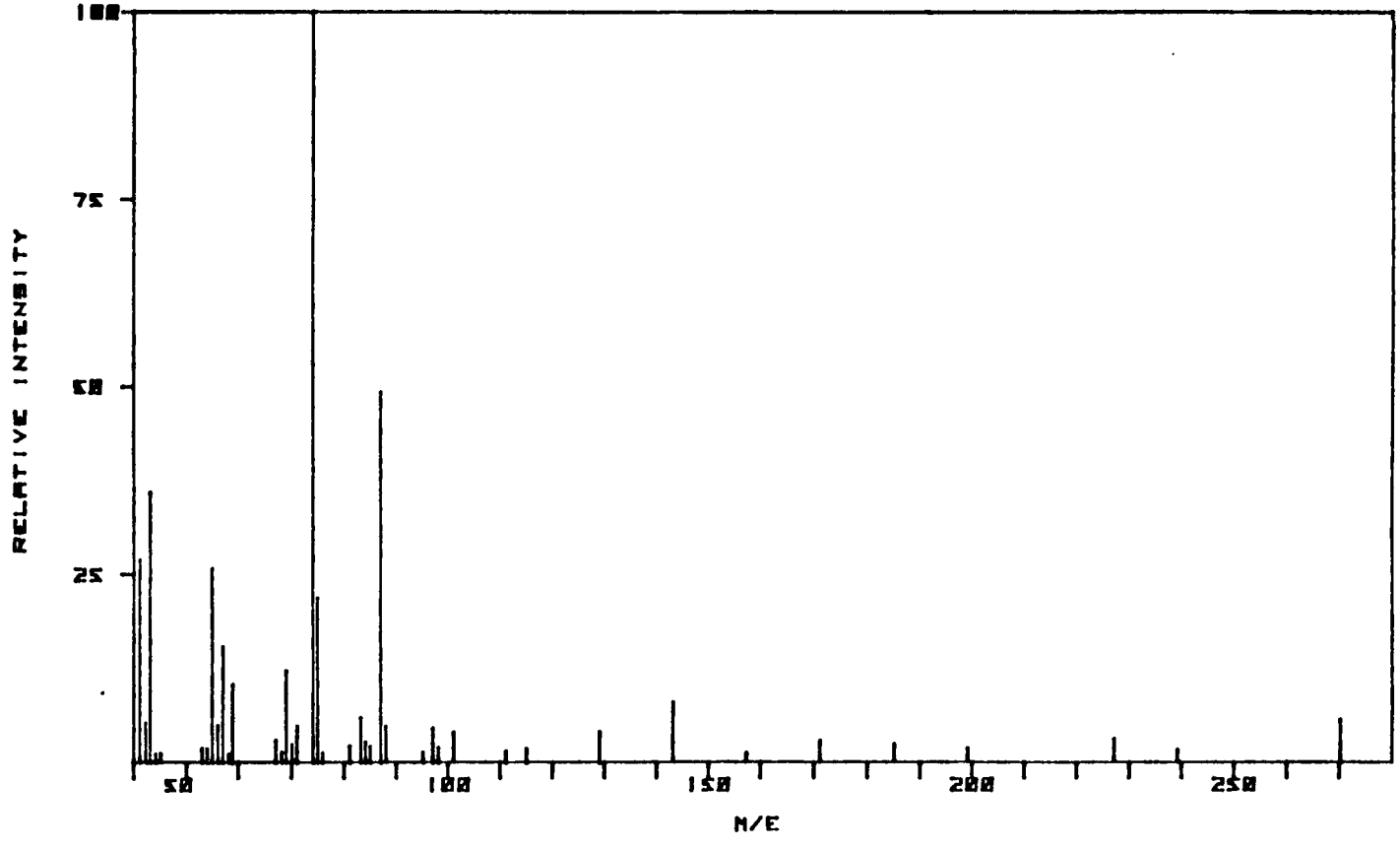

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
M/E

Figure 4. Mass spectrum of methyl stearate (M = 298).

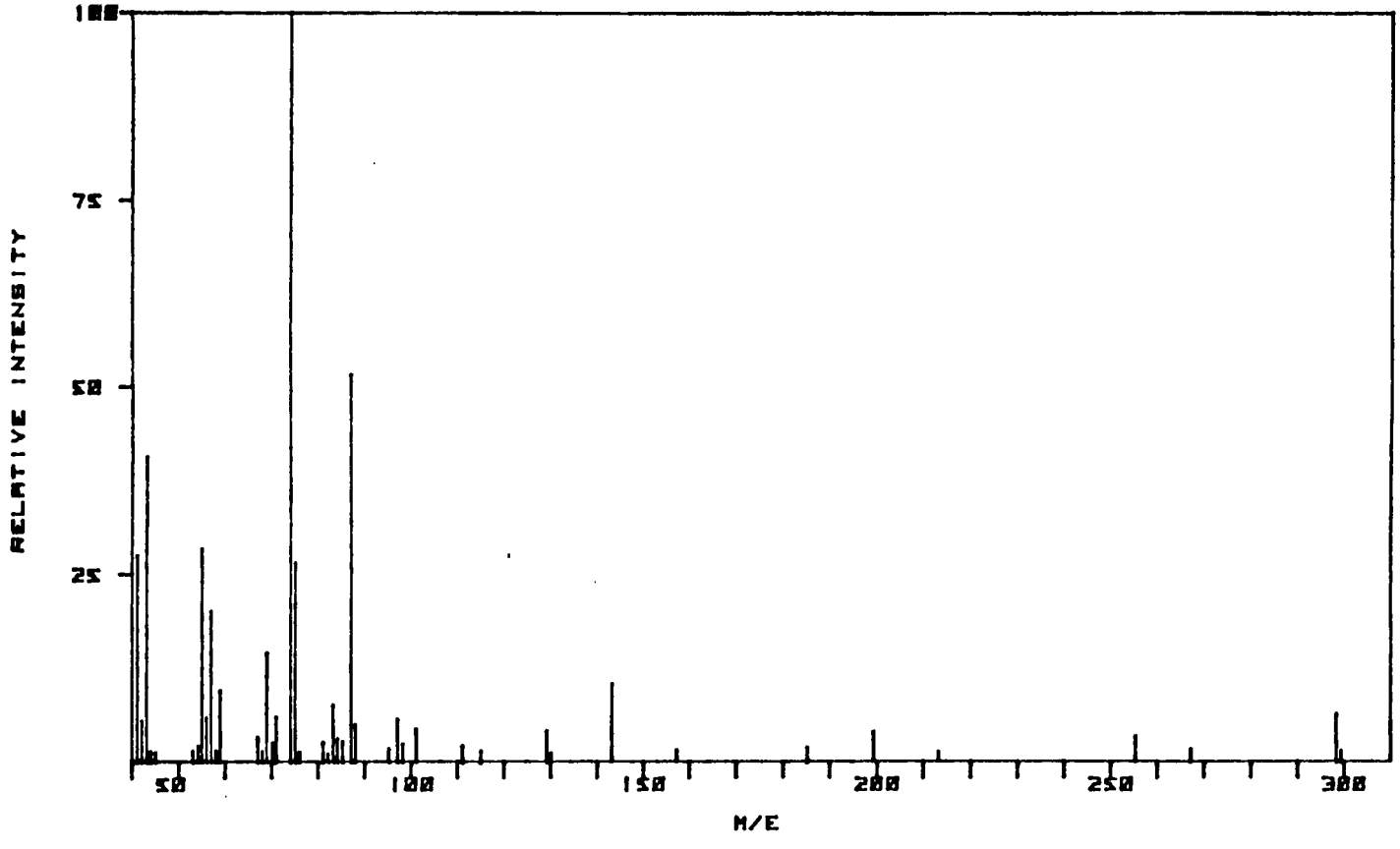

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 5. Mass spectrum of methyl arachidate (M = 326).

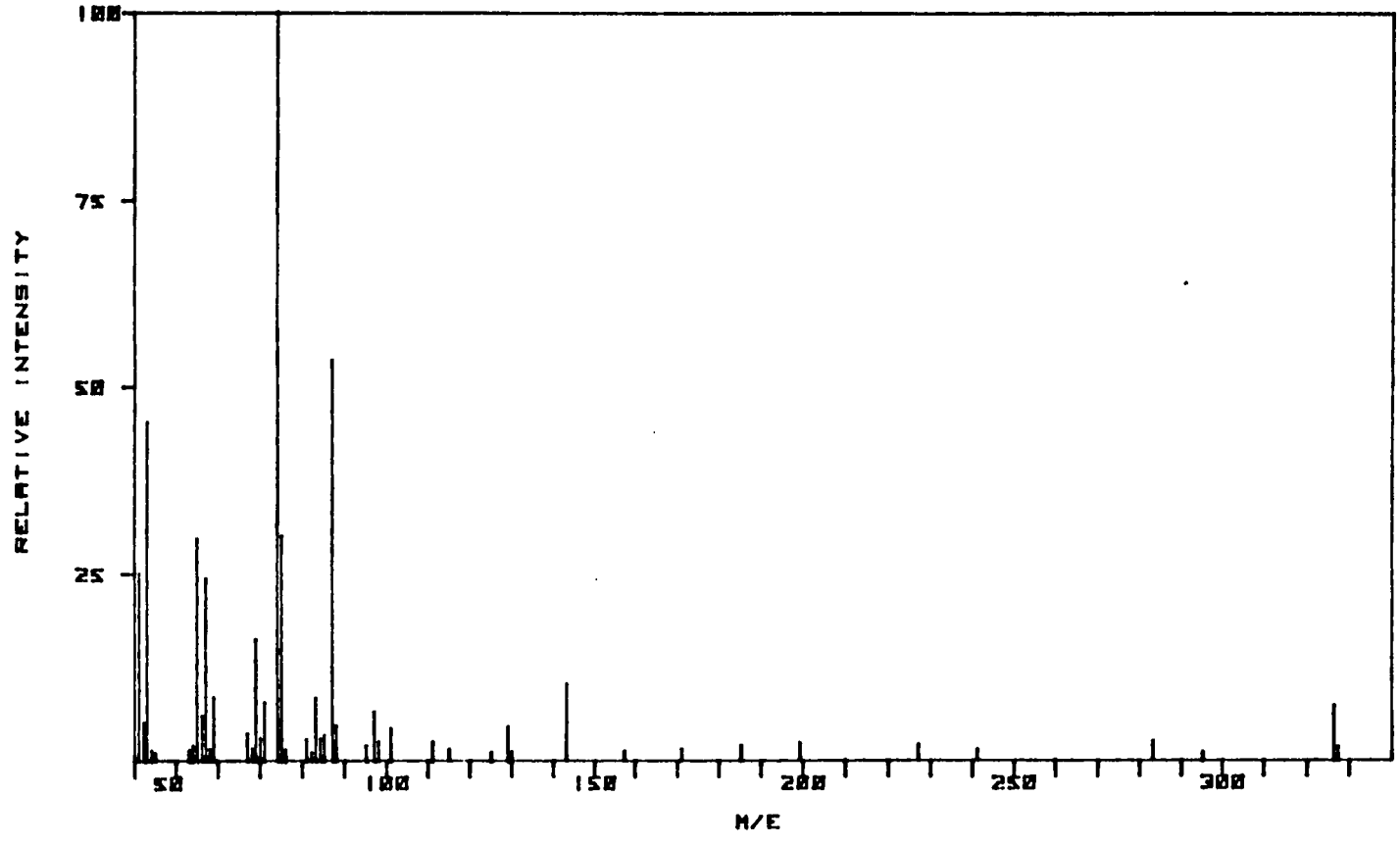

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 6. Mass spectrum of methyl behenate (M = 354).

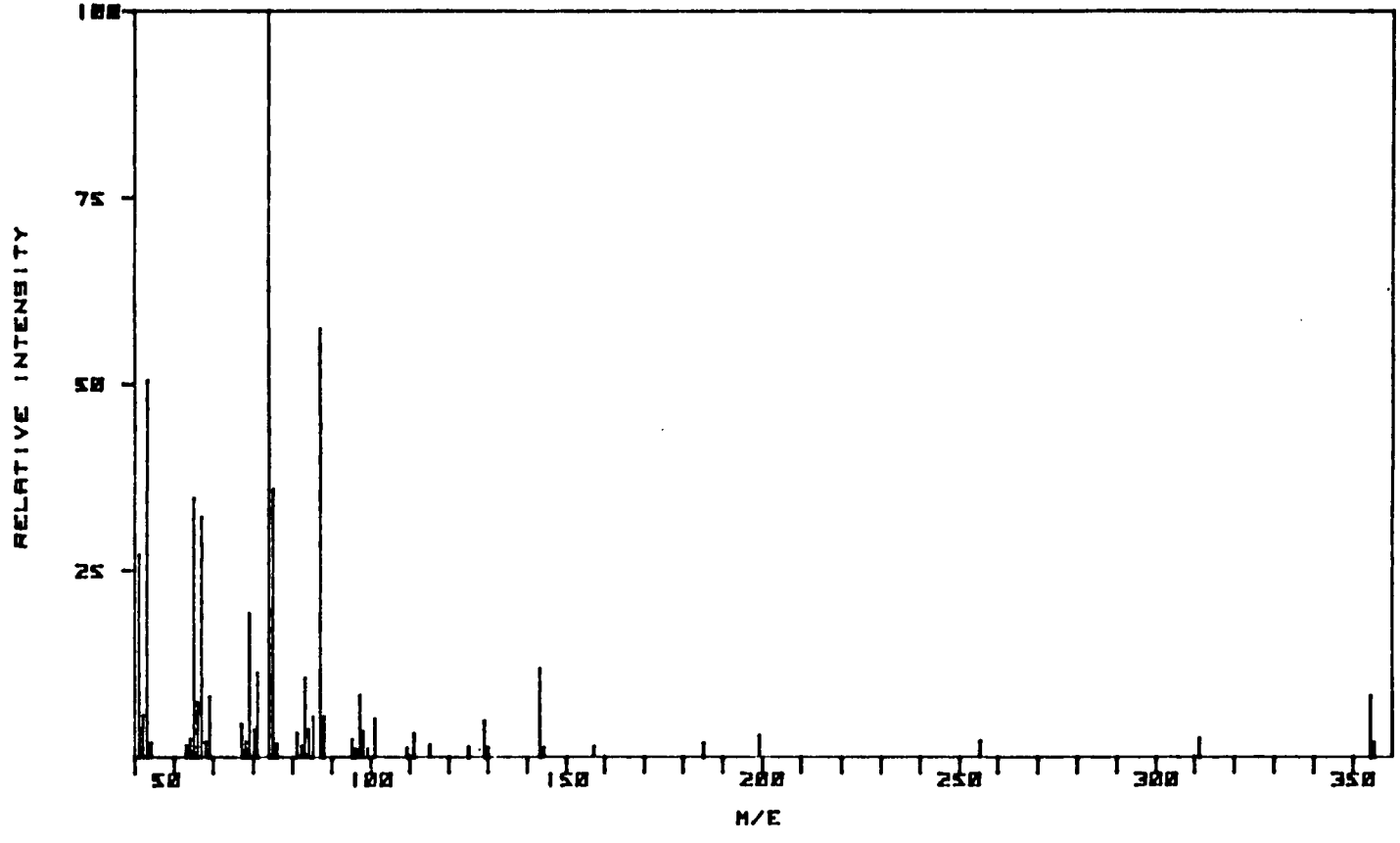

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
350
M/E

Figure 7. Mass spectrum of methyl lignocerate (M = 382).

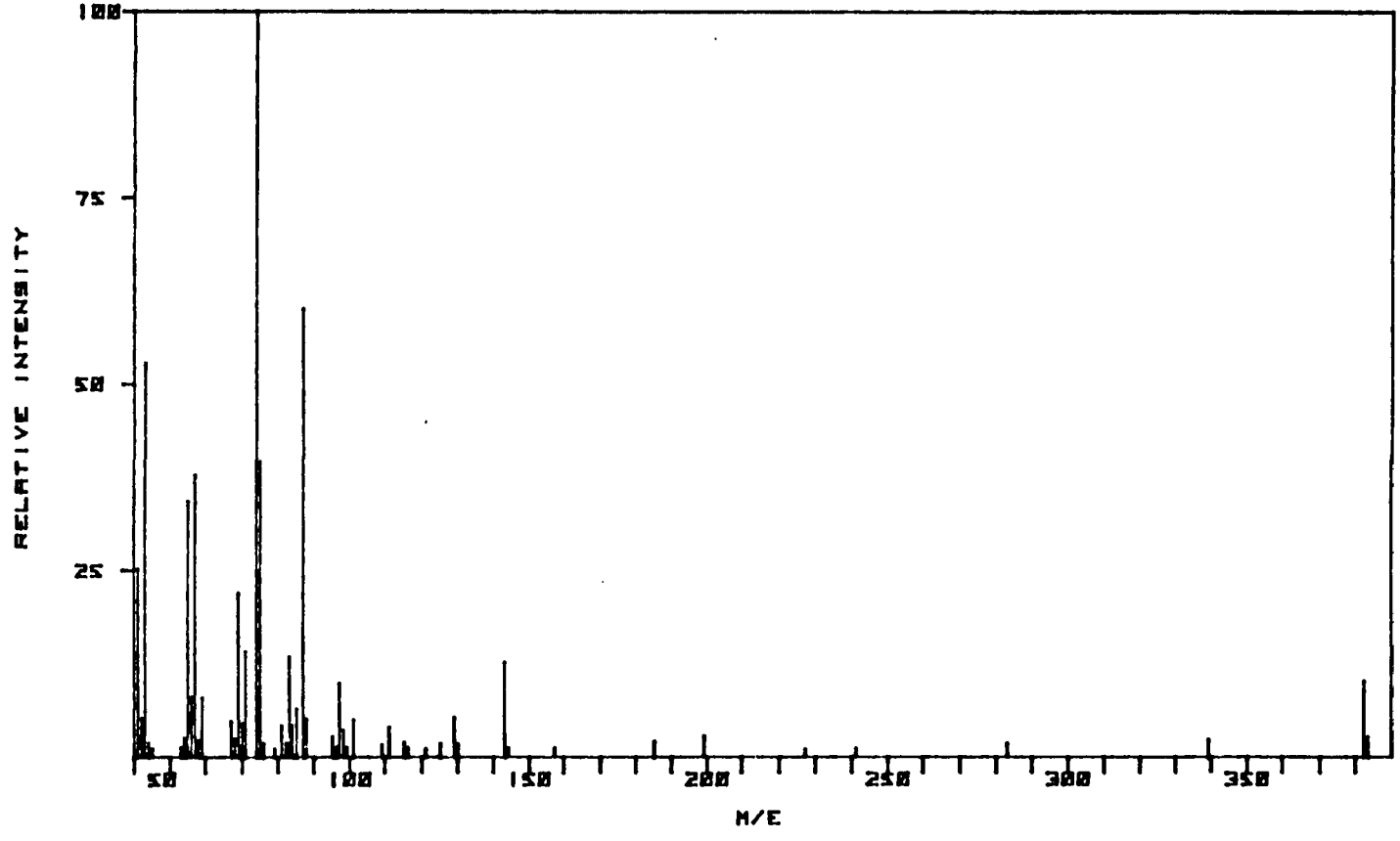

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
350
M/E

Figure 8. Mass spectrum of methyl palmitoleate (M = 268).

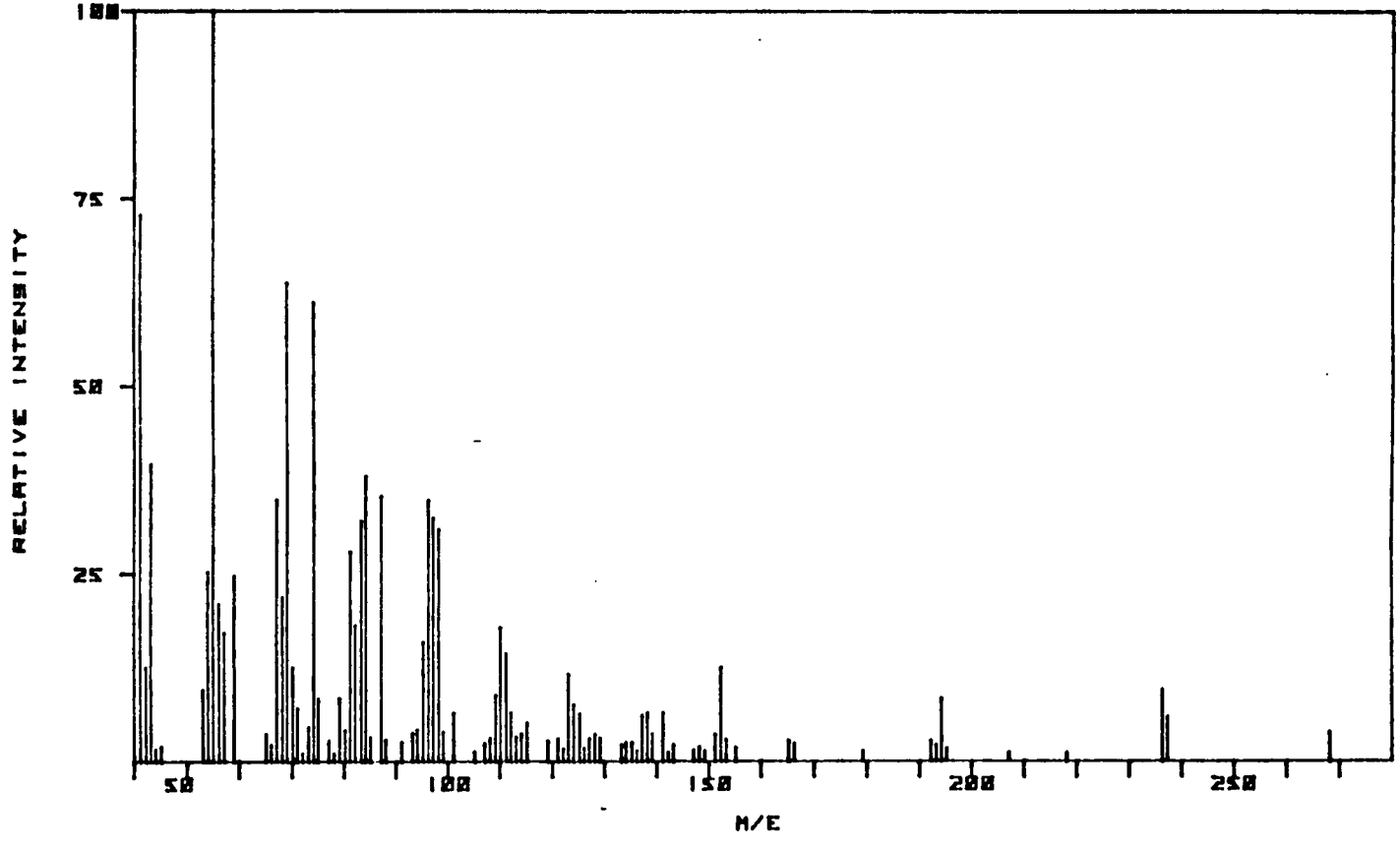

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
M/E

Figure 9. Mass spectrum of methyl oleate (M = 296).

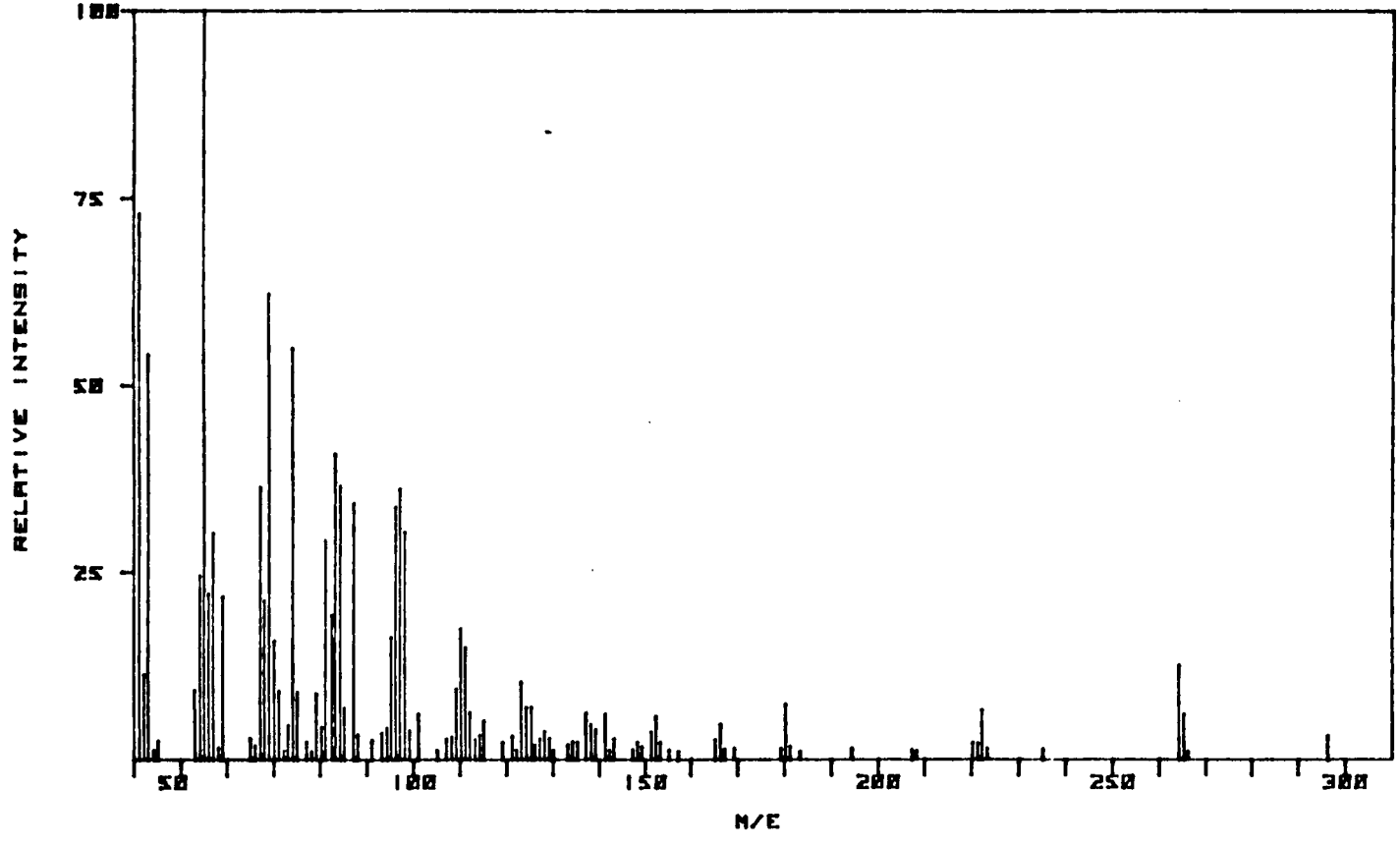

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 10. Mass spectrum of methyl eicosenoate (M = 324).

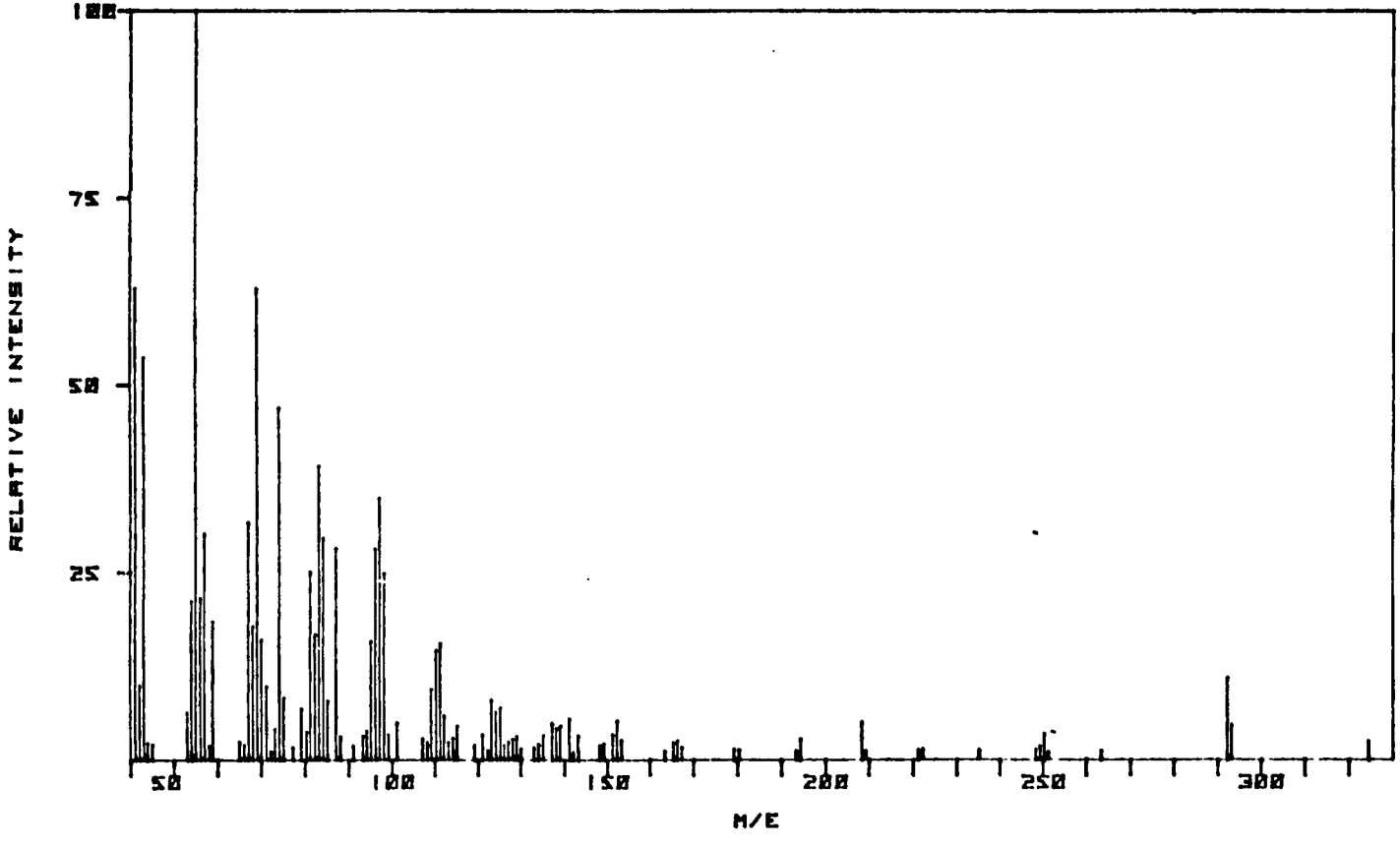

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 11. Mass spectrum of methyl linoleate (M = 294).

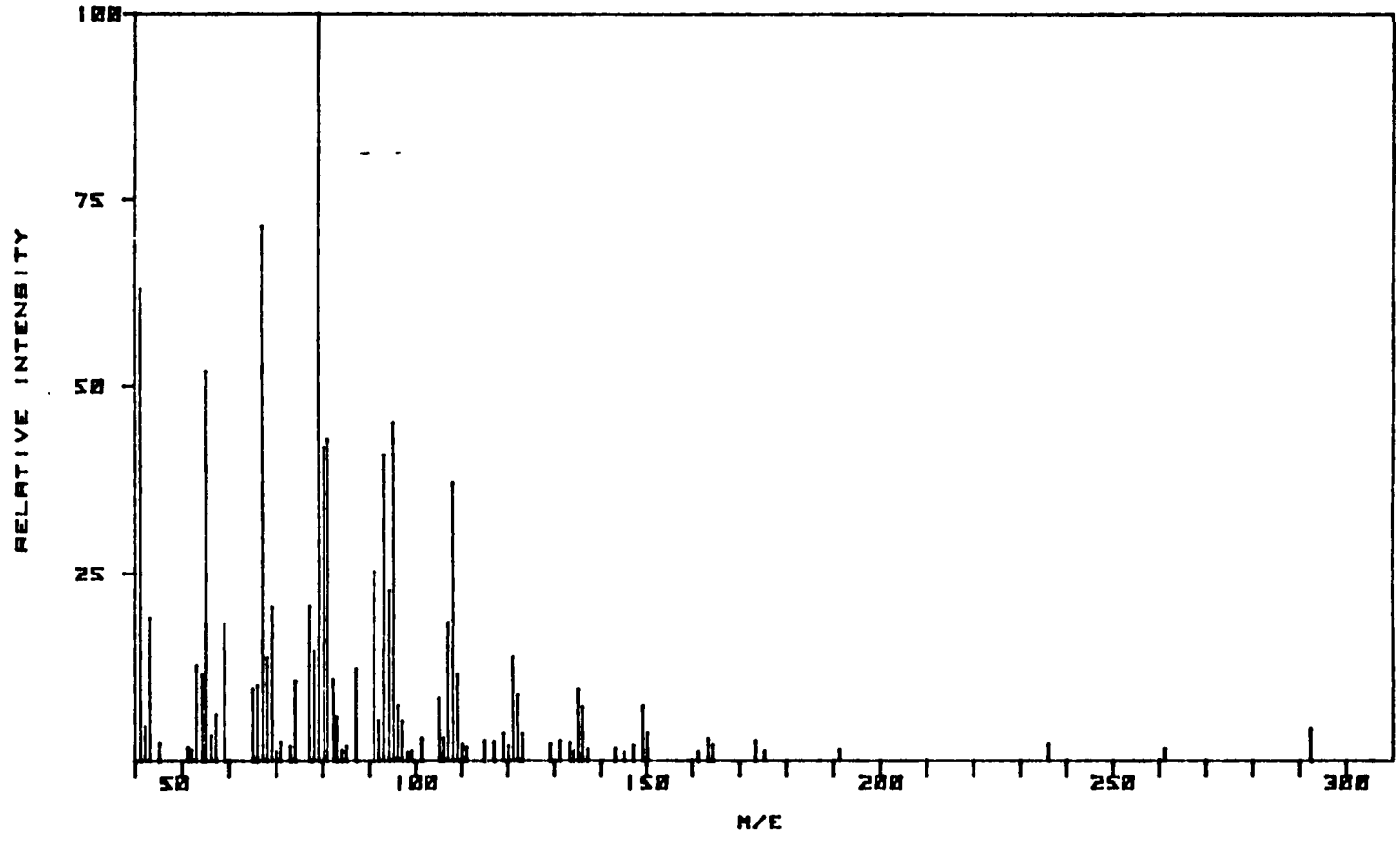

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 12. Mass spectrum of methyl linolenate (M = 292).

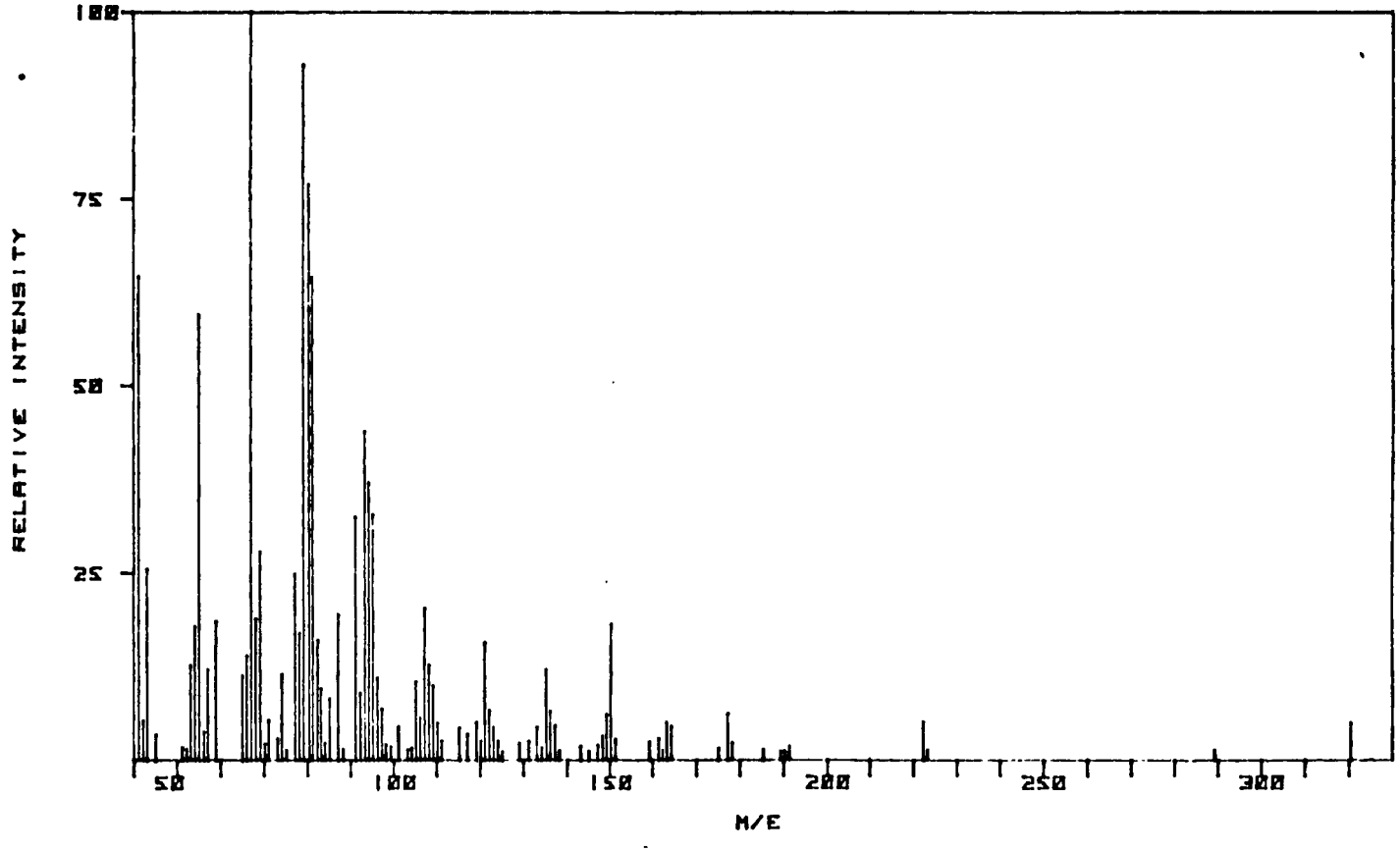

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 13. Mass spectrum of methyl eicosatrienoate (M = 320).

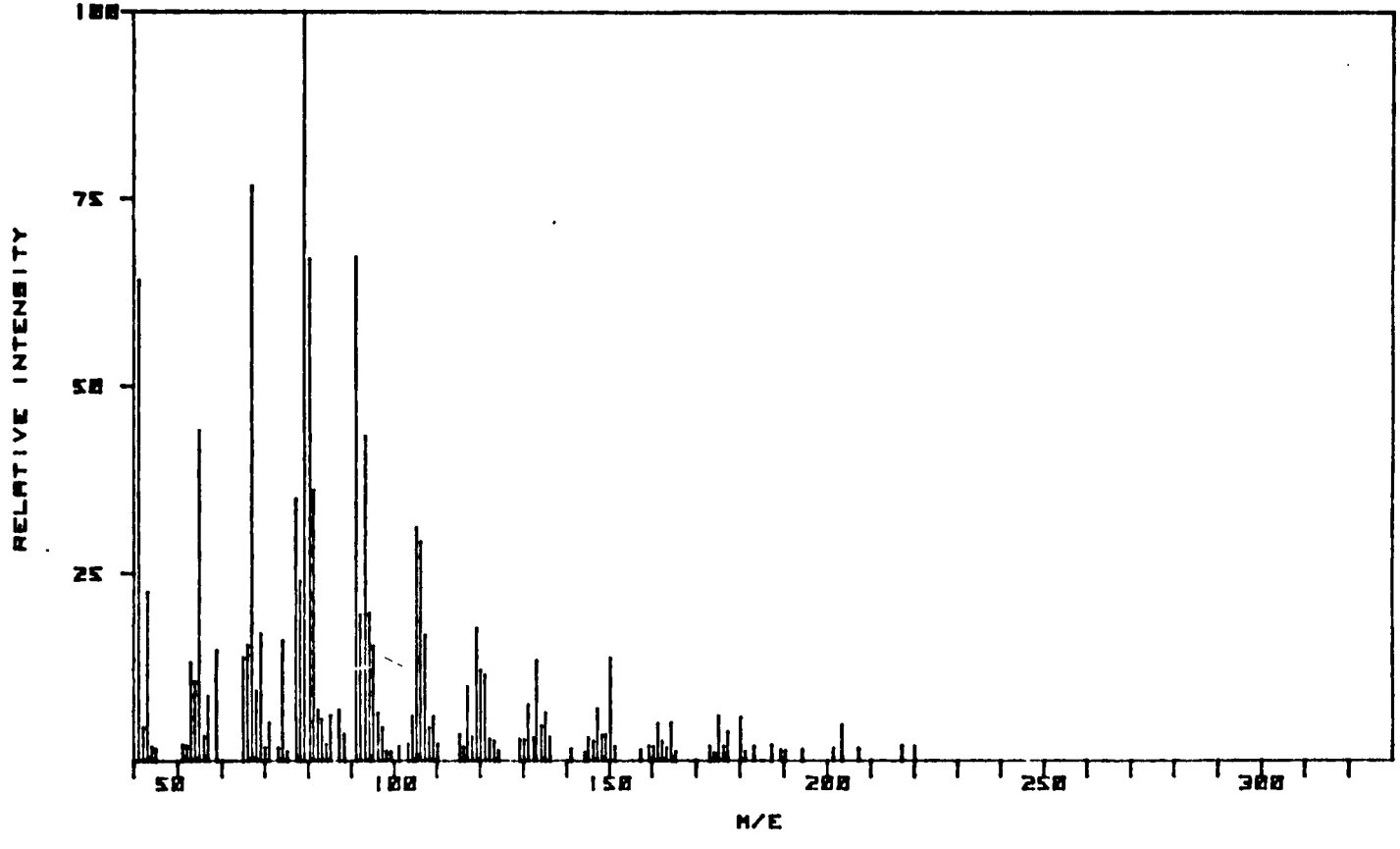

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 14. Mass spectrum of methyl arachidonate (M = 318).

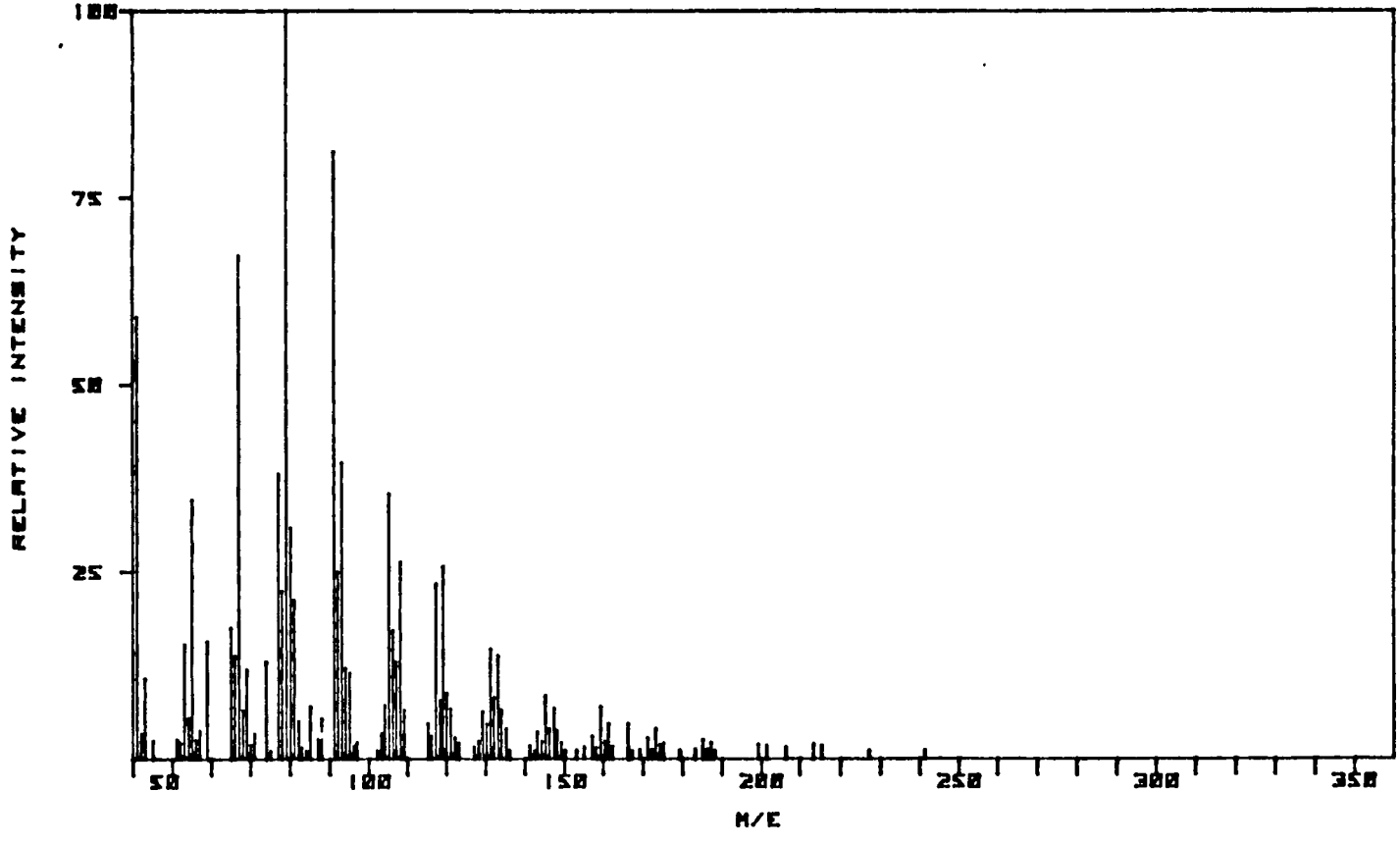

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
350
M/E

Figure 15. Mass spectrum of methyl docosahexaenoate (M = 342).

APPENDIX 2.

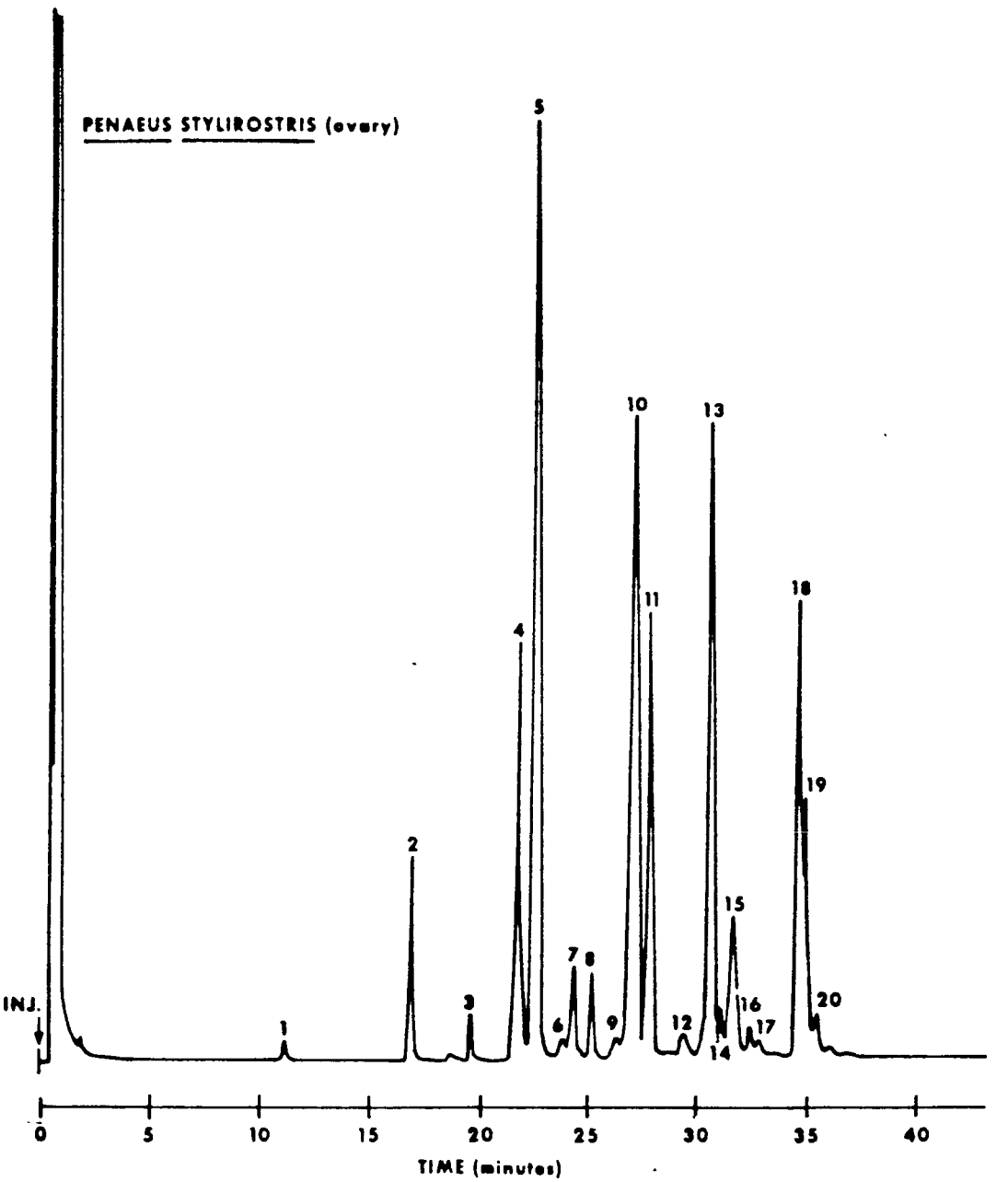

PENAEUS STYLIROSTRIS (ovary)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 1. Fatty acid profile from the ovary of a mature (stage 5) female P. stylirostris. Chromatography was performed using a 2m x 2mm (i. d.) glass column packed with 3% OV-1 on Supelcoport (100--120 mesh.).

Identification of methyl esters

1.	laurate	12:0
2.	myristate	14:0
3.	pentadecaneate	15:0
4.	palmitoleate	16:1
5.	palmitate	16:0
6.	heptadecanoate (br)	br17:0 ?
7.	heptadecenoate	17:1
8.	heptadecanoate	17:0
9.	linoleate	18:2
10.	oleate	18:1
11.	stearate	18:0
12.	nonadecenoate	19:1
13.	arachidonate + eicosapentaenoate	20:4+5
14.	eicosatrienoate	20:3
15.	eicosenoate	20:1
16.	heneicosenoate (br) ?	br21:1 ?
17.	eicosanoate	20:0
18.	docosapentaenoate + docosahexaenoate	22:5+6
19.	docosatetraenoate	22:4
20.	docosatrienoate	22:3

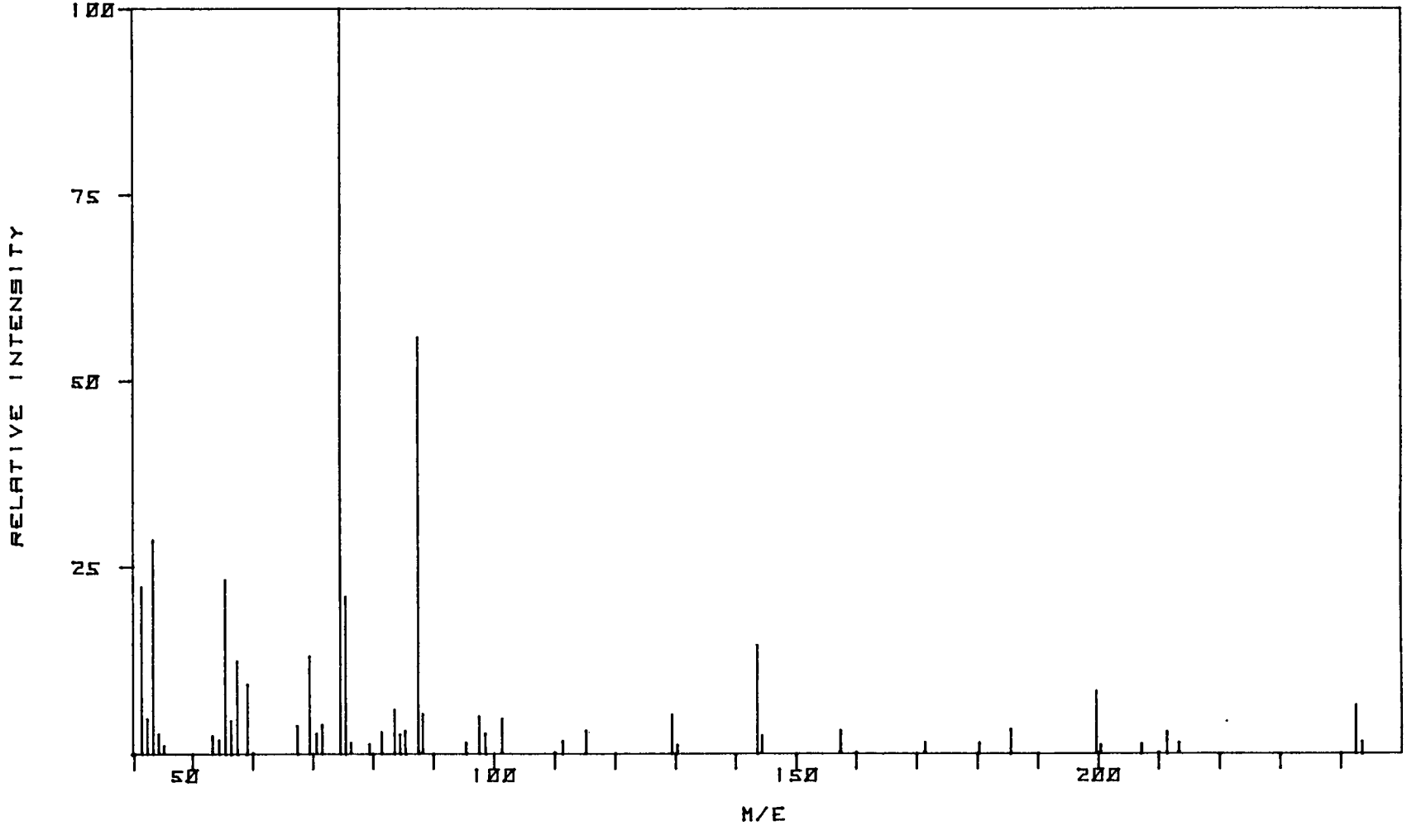

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
M/E

Figure 2. Mass spectrum of methyl myristate from P. stylirostris (M = 242).

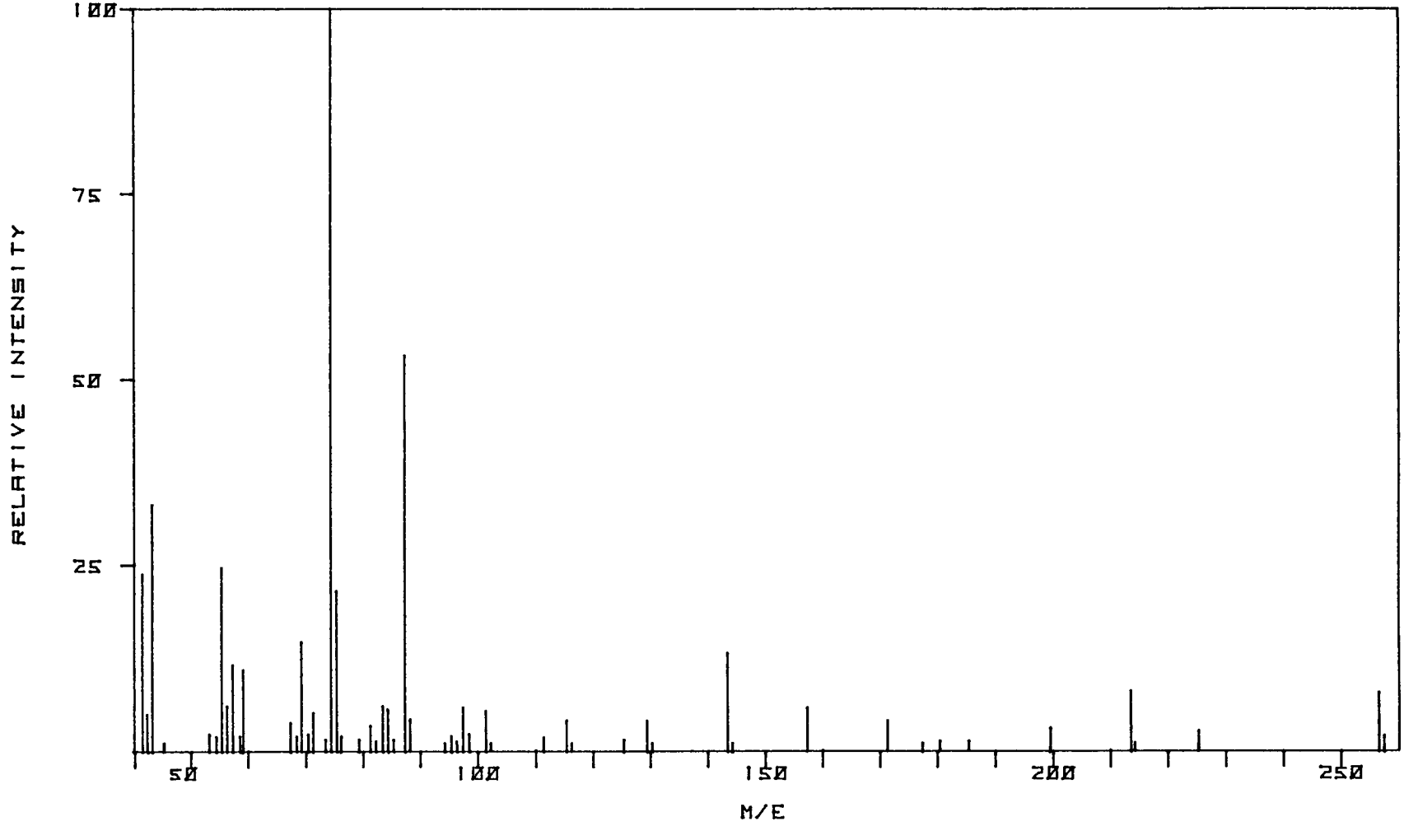

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
M/E

Figure 3. Mass spectrum of methyl pentadecanoate from P. stylirostris (M = 256).

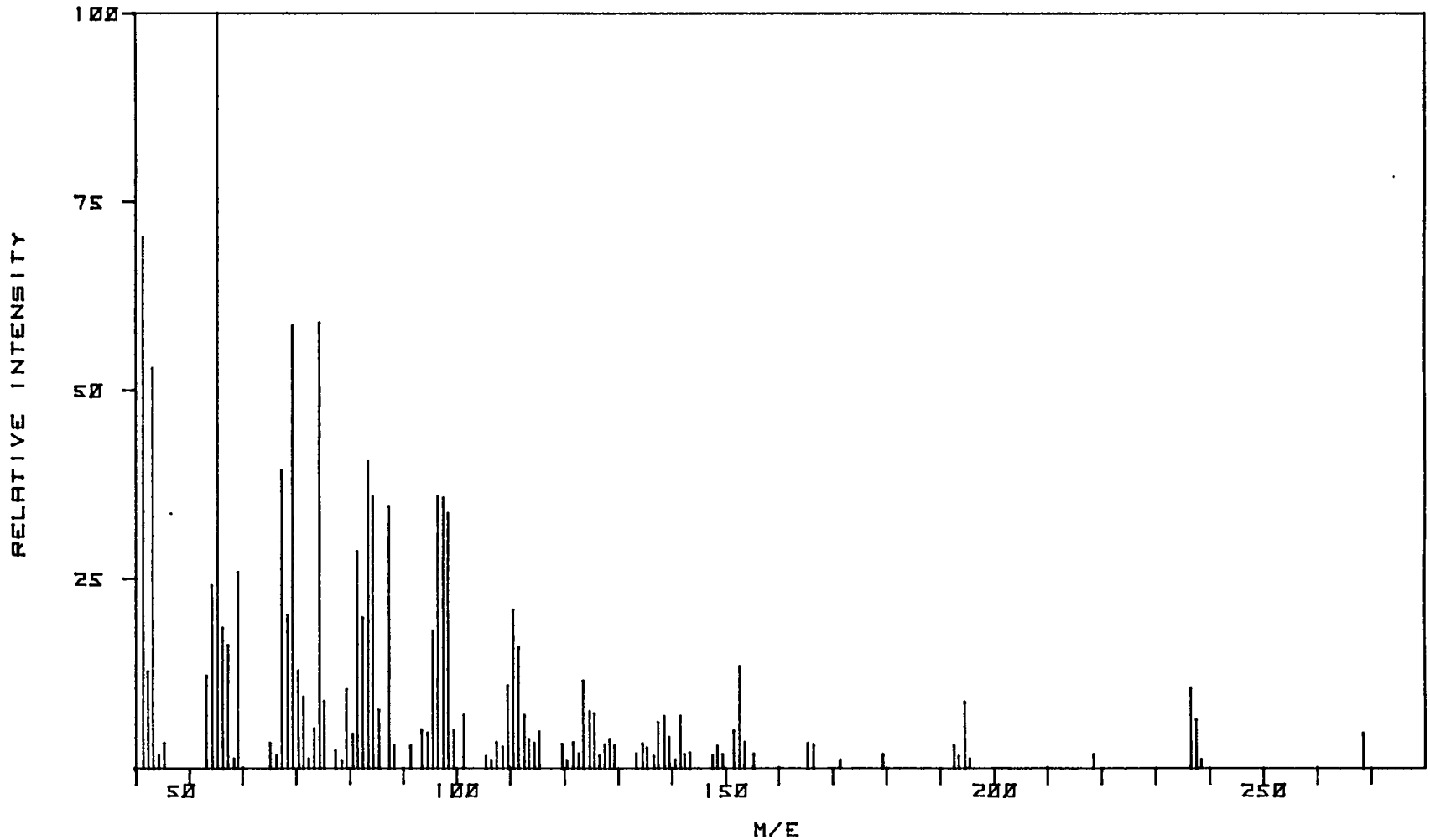

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
M/E

Figure 4. Mass spectrum of methyl palmitoleate from P. stylirostris (M = 268).

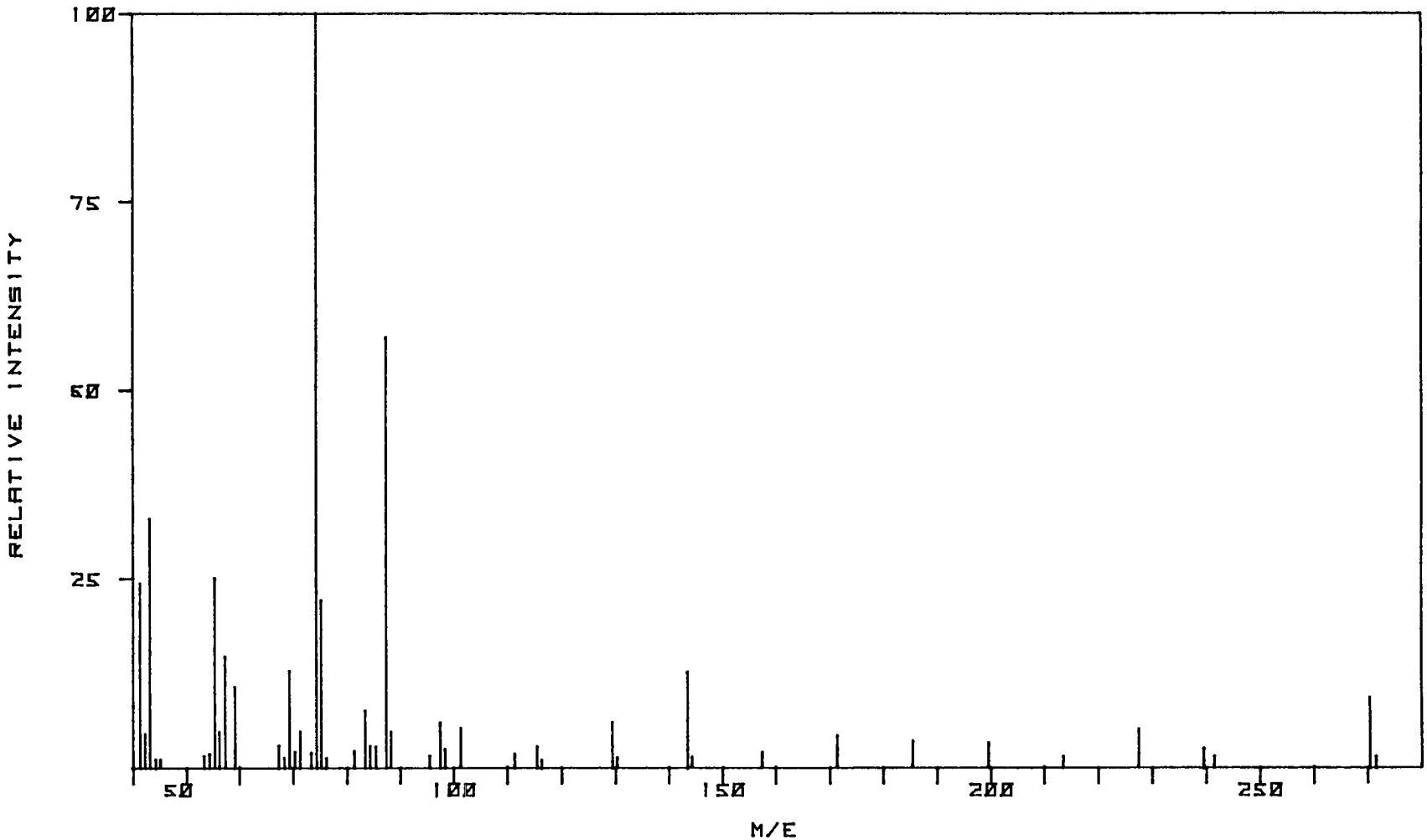

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
M/E

Figure 5. Mass spectrum of methyl palmitate from P. stylirostris (M = 270).

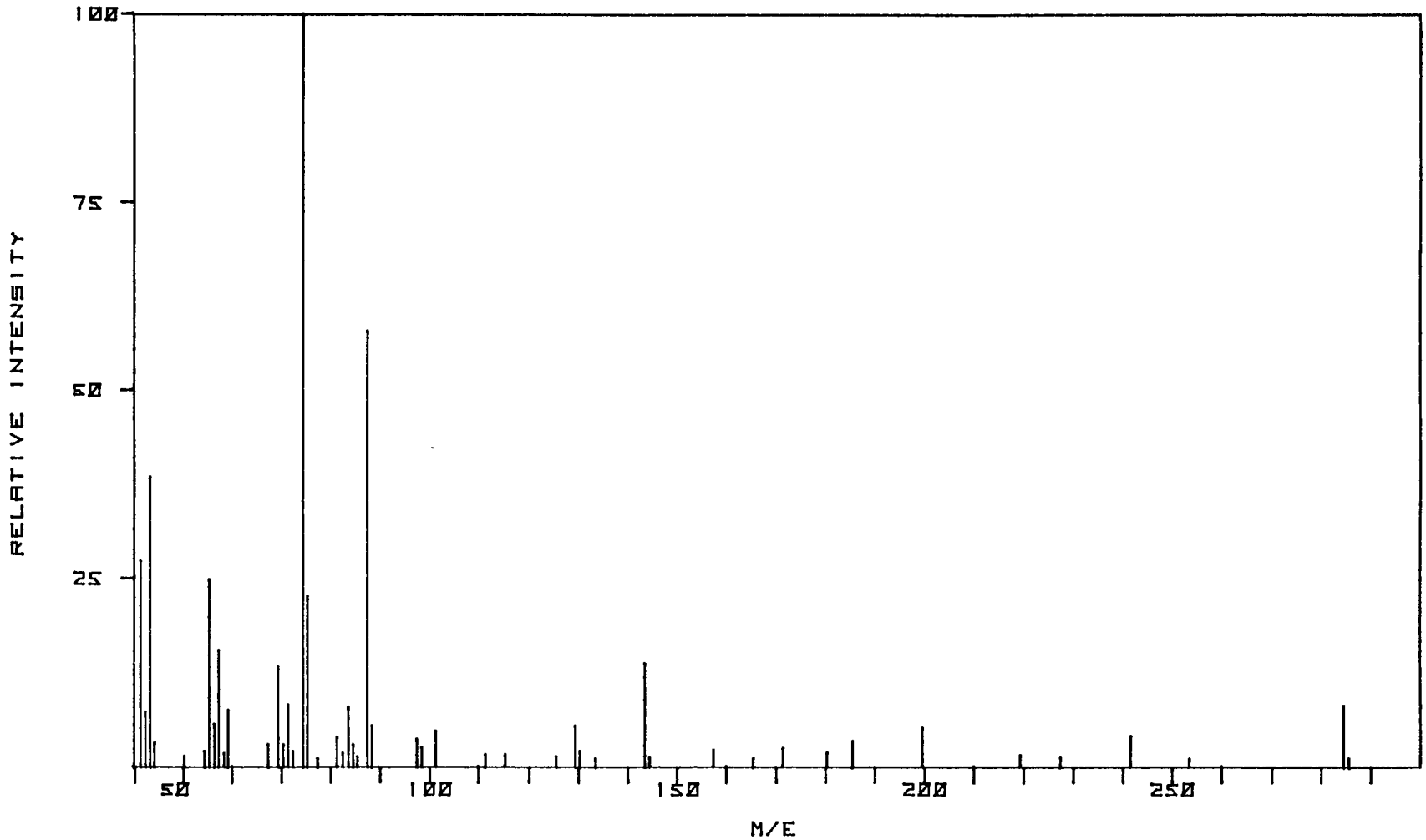

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
M/E

Figure 6. Mass spectrum of methyl heptadecanoate from P. stylirostris (M = 284).

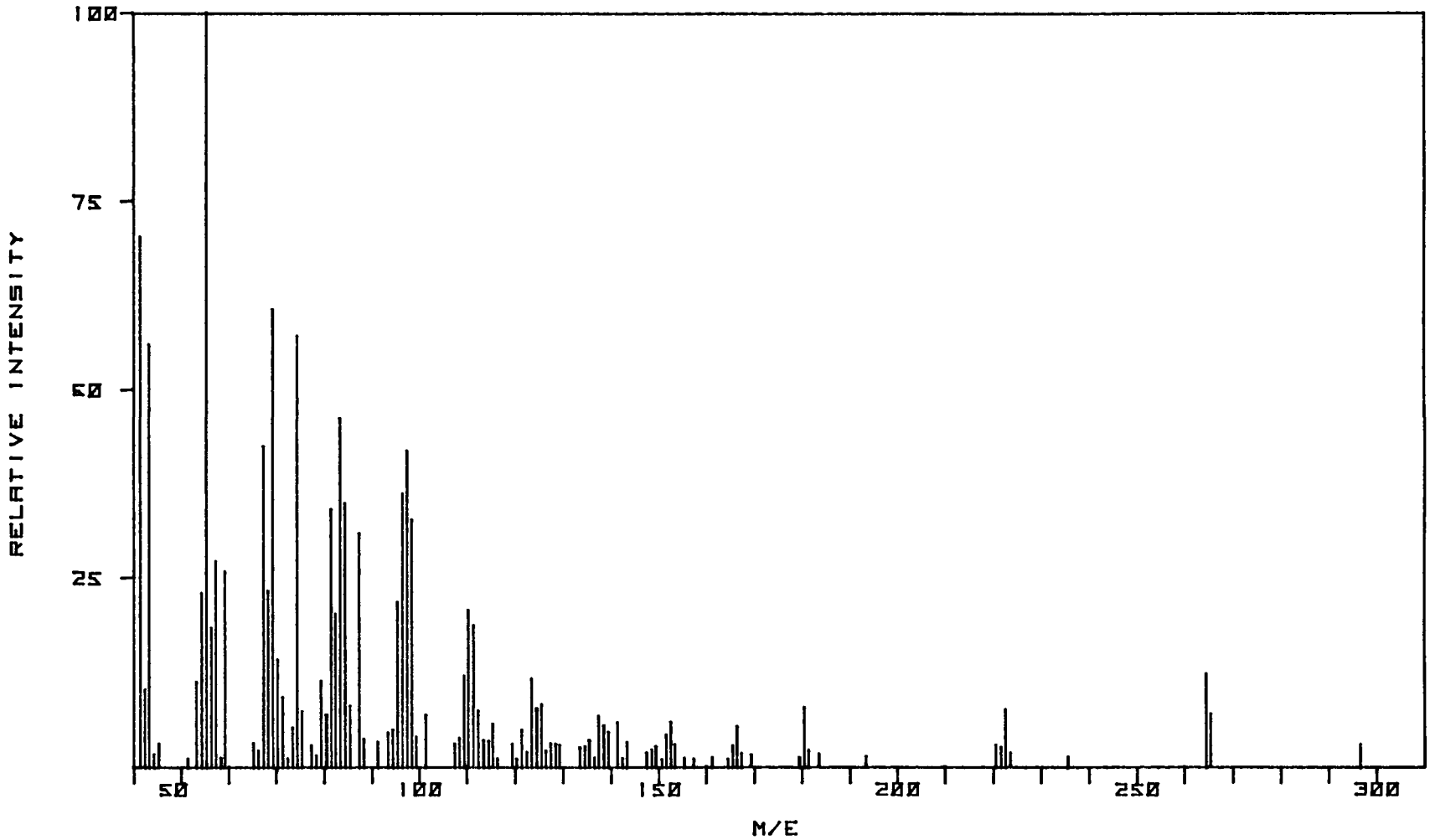

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 7. Mass spectrum of methyl oleate from P. stylirostris (M = 296).

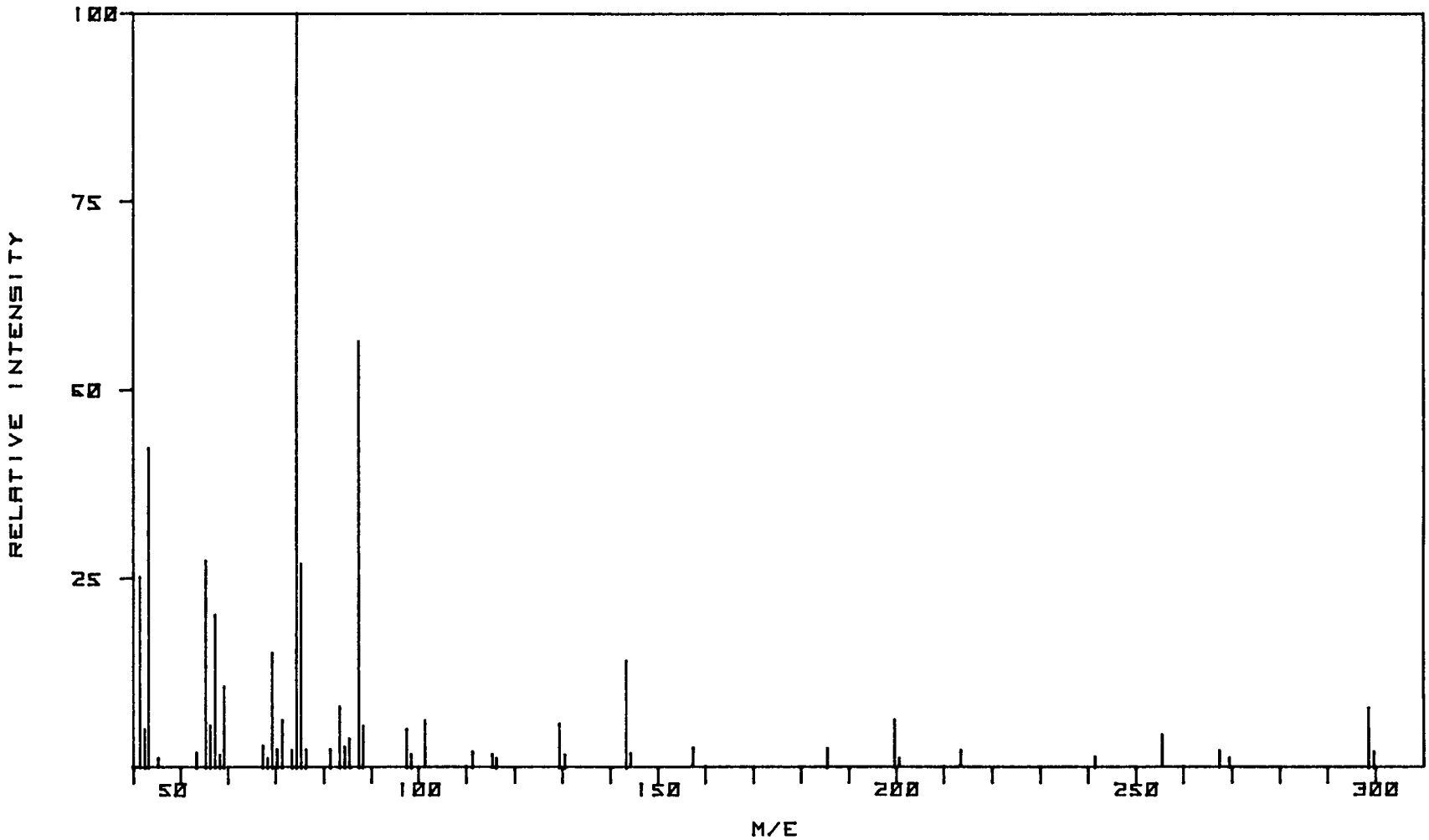

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 8. Mass spectrum of methyl stearate from P. stylirostris (M = 298).

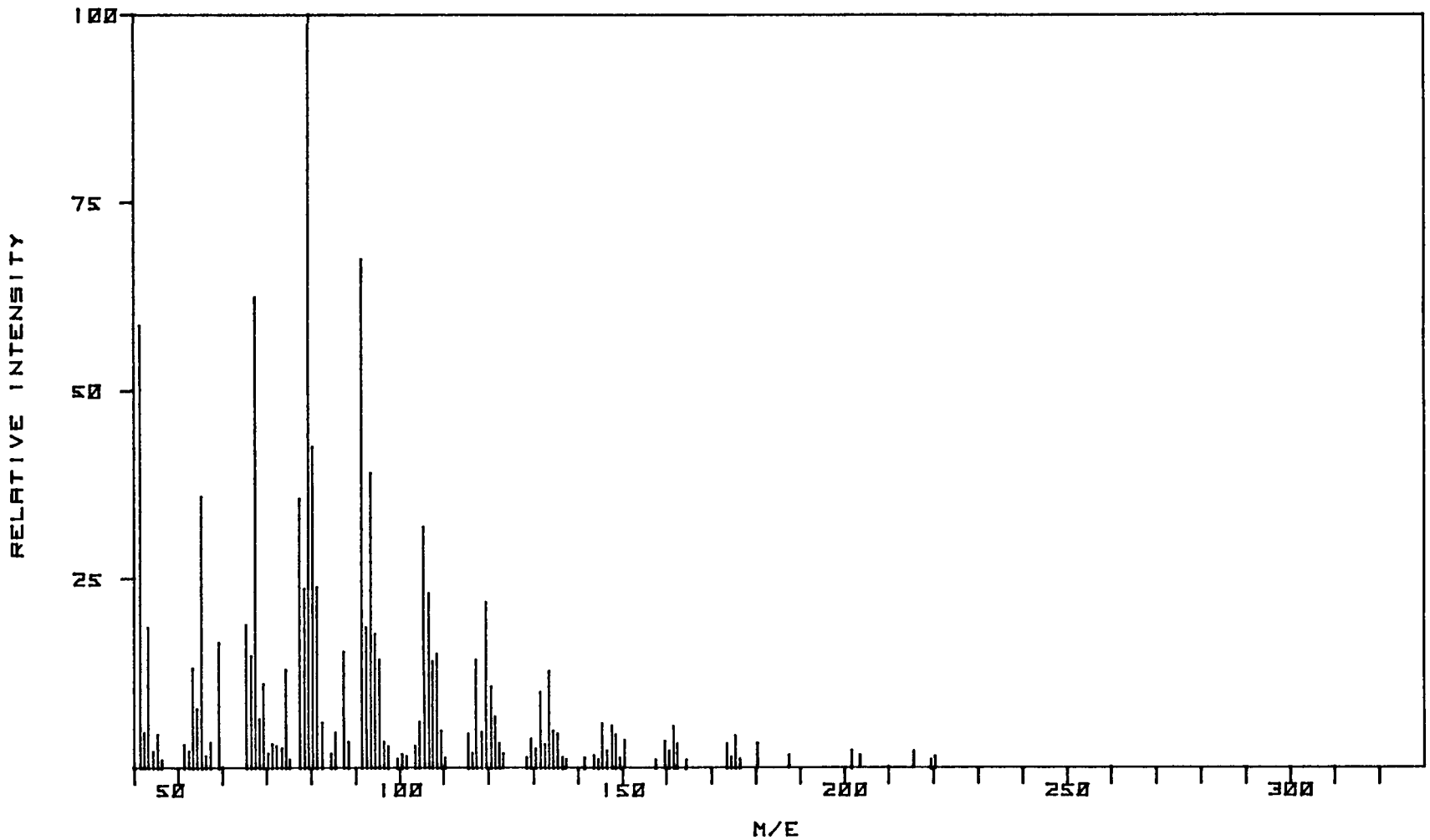

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 9. Mass spectrum of methyl arachidonate (M = 318) + methyl eicosapentaenoate (M = 316) from P. stylirostris.

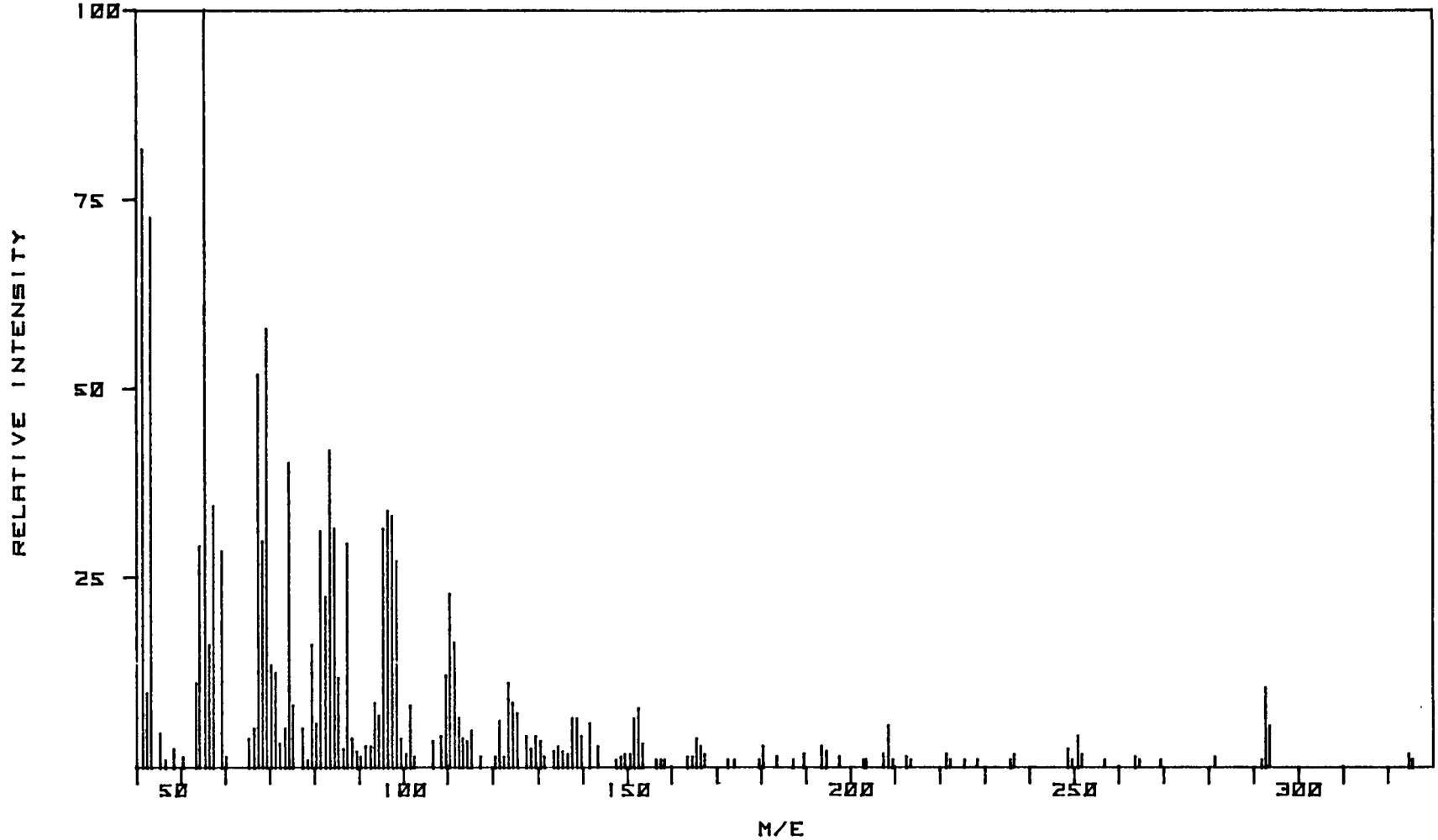

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
M/E

Figure 10. Mass spectrum of methyl eicosenoate from P. styliros-tris (M = 324).

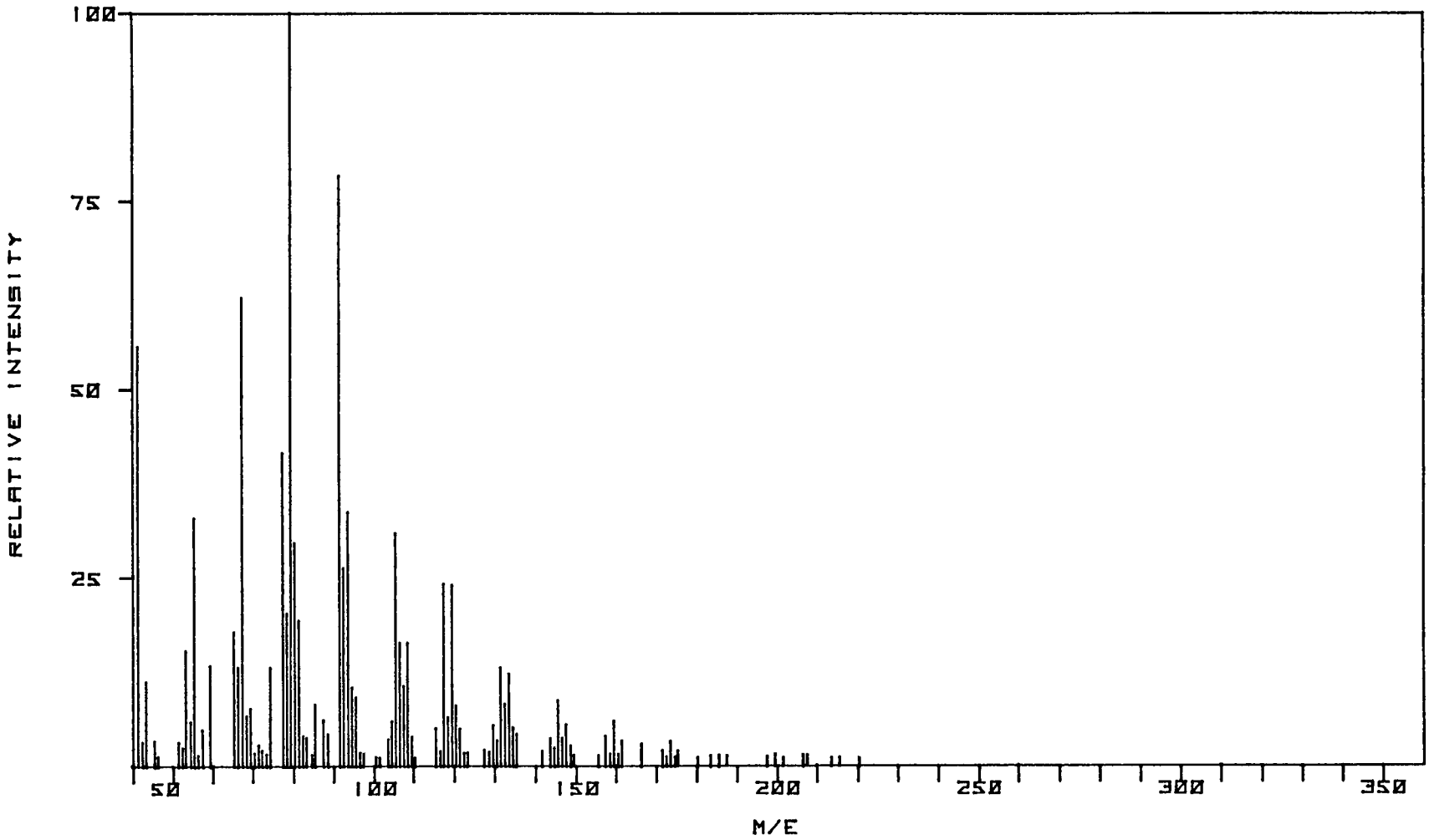

100
75
50
25
RELATIVE INTENSITY
50
100
150
200
250
300
350
M/E

Figure 11. Mass spectrum of methyl docosapentaenoate (M = 344) + methyl docosahexaenoate (M = 342) from P. stylirostris.

APPENDIX 3.

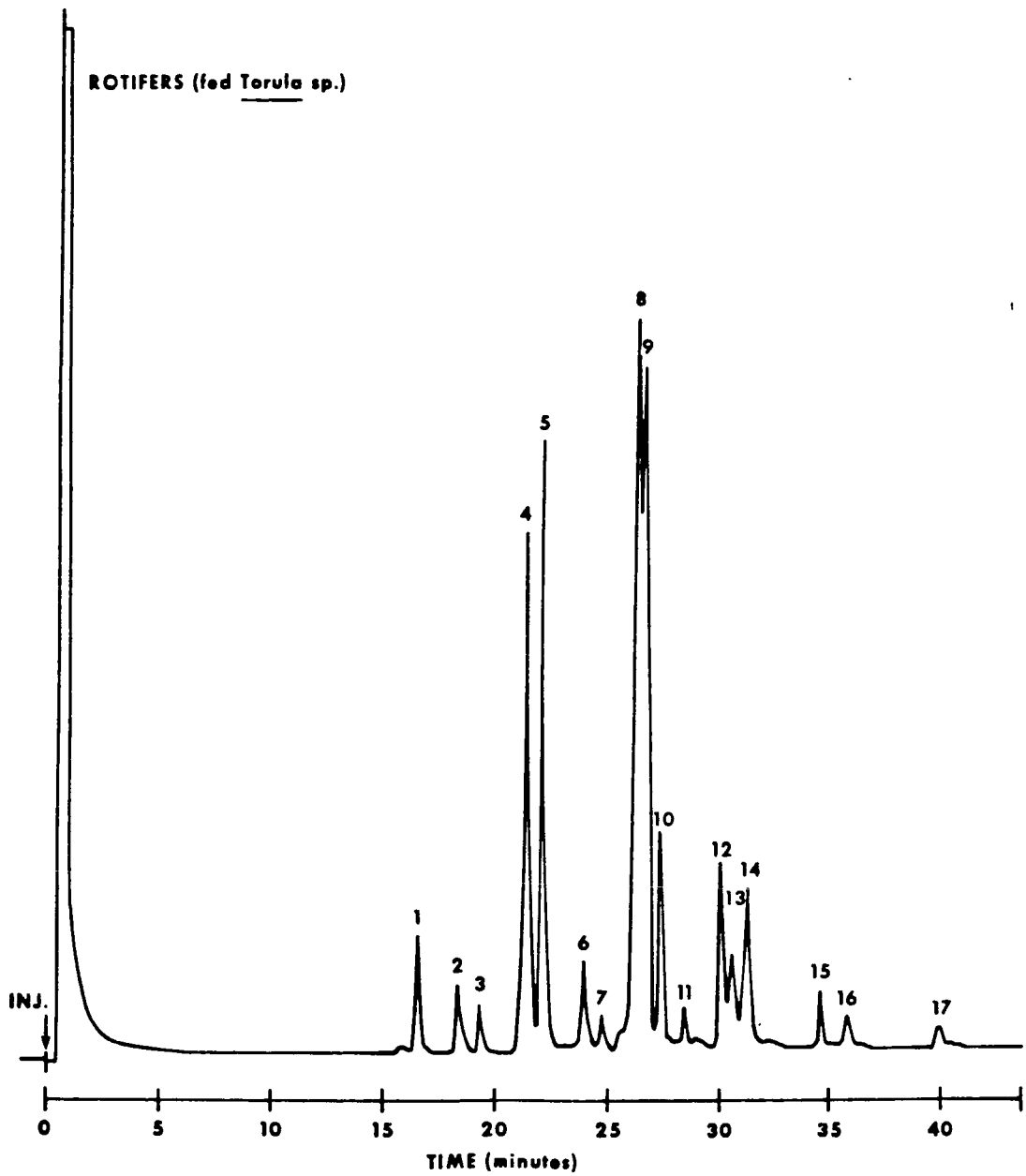

ROTIFERS (fed Torula sp.)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 1. Fatty acid profile from rotifers maintained on a diet of Torula sp. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecenoate	15:1
3.	pentadecanoate	15:0
4.	palmitoleate	16:1
5.	palmitate	16:0
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	linoleate + linolenate	18:2+3
9.	oleate	18:1
10.	stearate	18:0
11.	nonadecenoate	19:1
12.	arachidonate + eicosapentaenoate	20:4+5
13.	eicosatrienoate	20:3
14.	eicosenoate	20:1
15.	docosapentaenoate + docosahexaenoate	22:5+6
16.	docosatrienoate	22:3
17.	unknown	

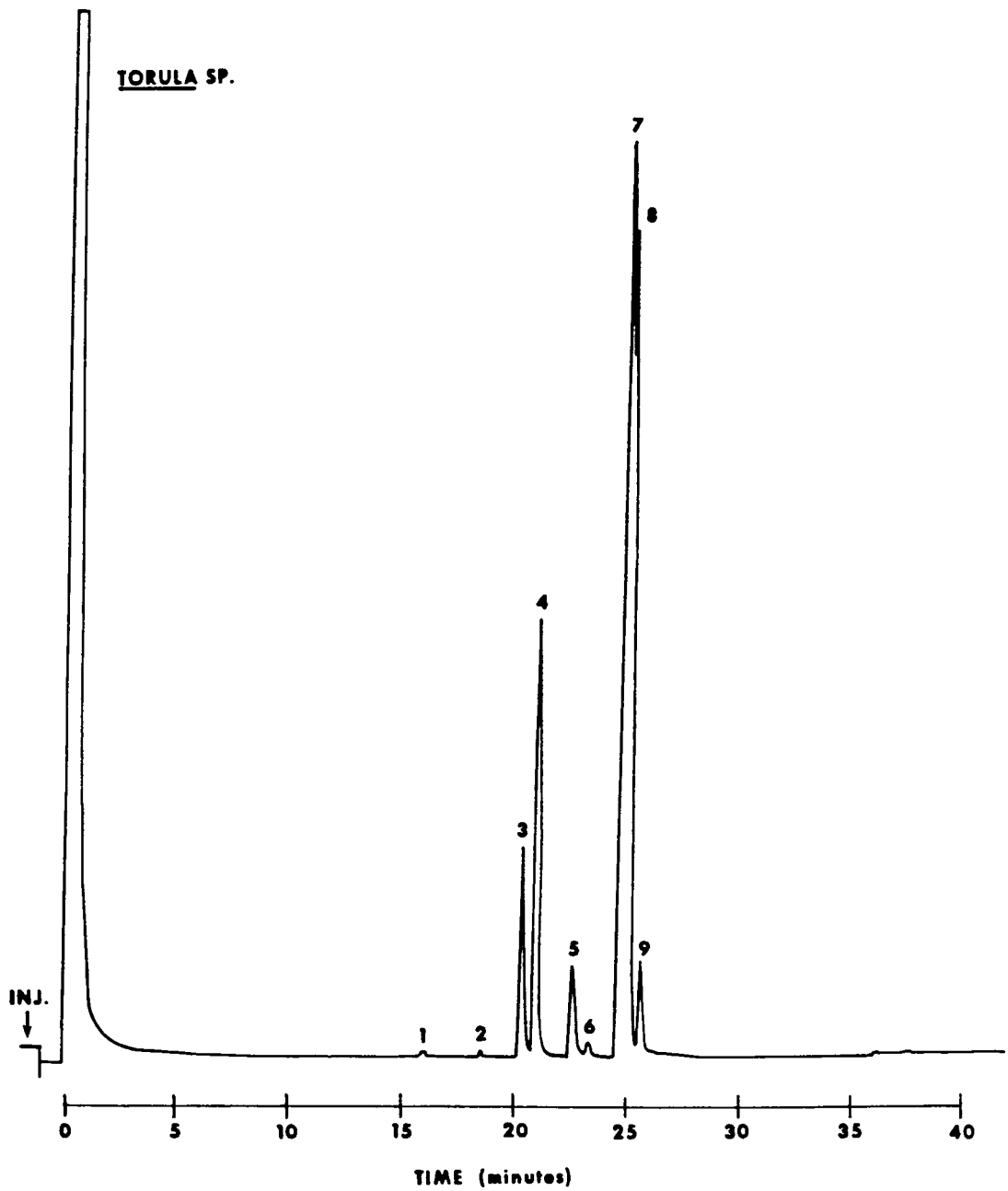

TORULA SP.
INJ.
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 2. Fatty acid profile from Torula sp. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1. myristate	14:0
2. pentadecanoate	15:0
3. palmitoleate	16:1
4. palmitate	16:0
5. heptadecenoate	17:1
6. heptadecanoate	17:0
7. linoleate + linolenate	18:2+3
8. oleate	18:1
9. stearate	18:0

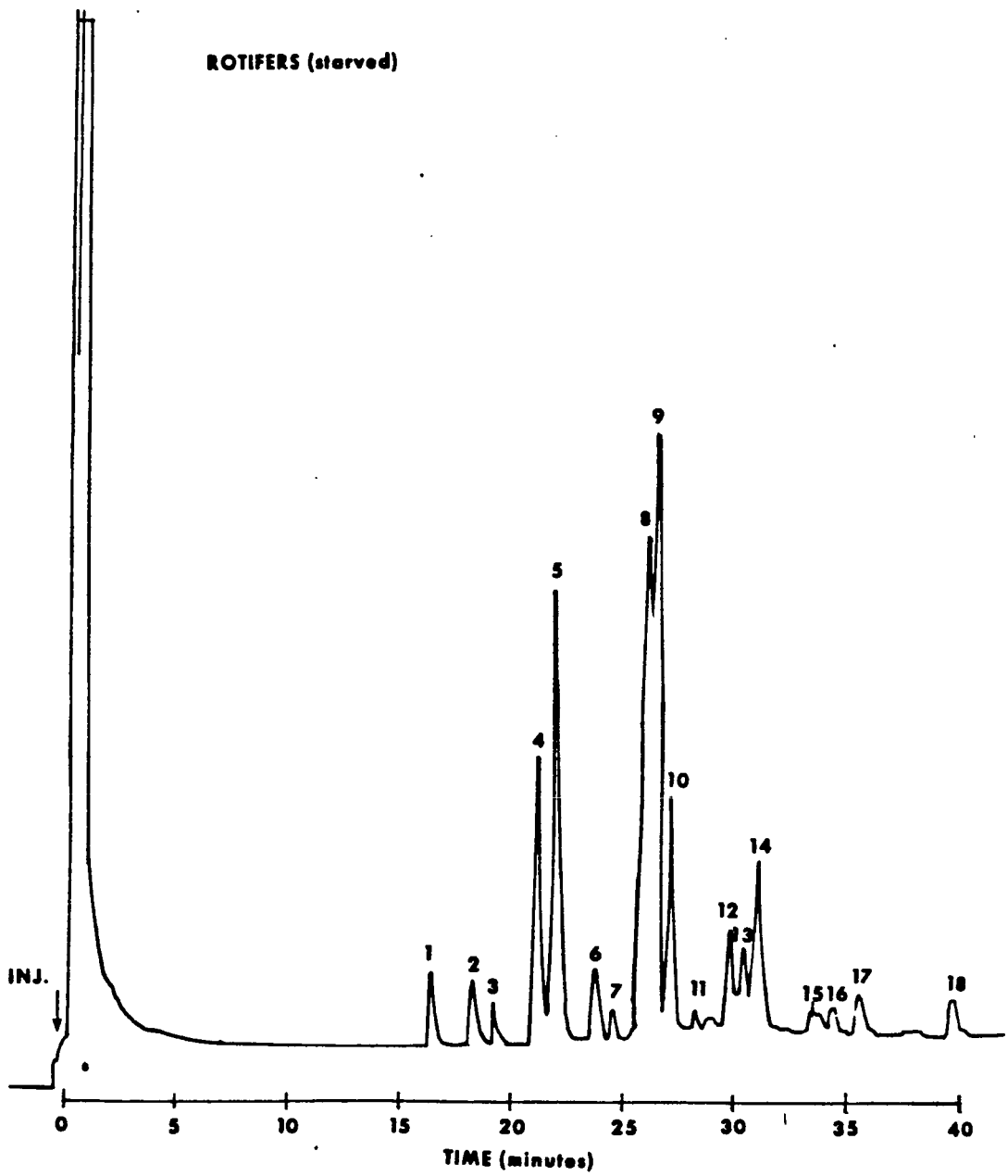

ROTIFERS (starved)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 3. Fatty acid profile from rotifers which were fed on Torula sp. and starved for a period of 24 hours prior to freezing. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecenoate	15:1
3.	pentadecanoate	15:0
4.	palmitoleate	16:1
5.	palmitate	16:0
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	linoleate + linolenate	18:2+3
9.	oleate	18:1
10.	stearate	18:0
12.	arachidonate + eicosapentaenoate	20:4+5
13.	eicosatrienoate	20:3
14.	eicosene	20:1
15.	docosapentaenoate + docosahexaenoate	22:5+6
16.	docosatetraenoate ?	22:4 ?
17.	docosenoate ?	22:1 ?
18.	unknown	

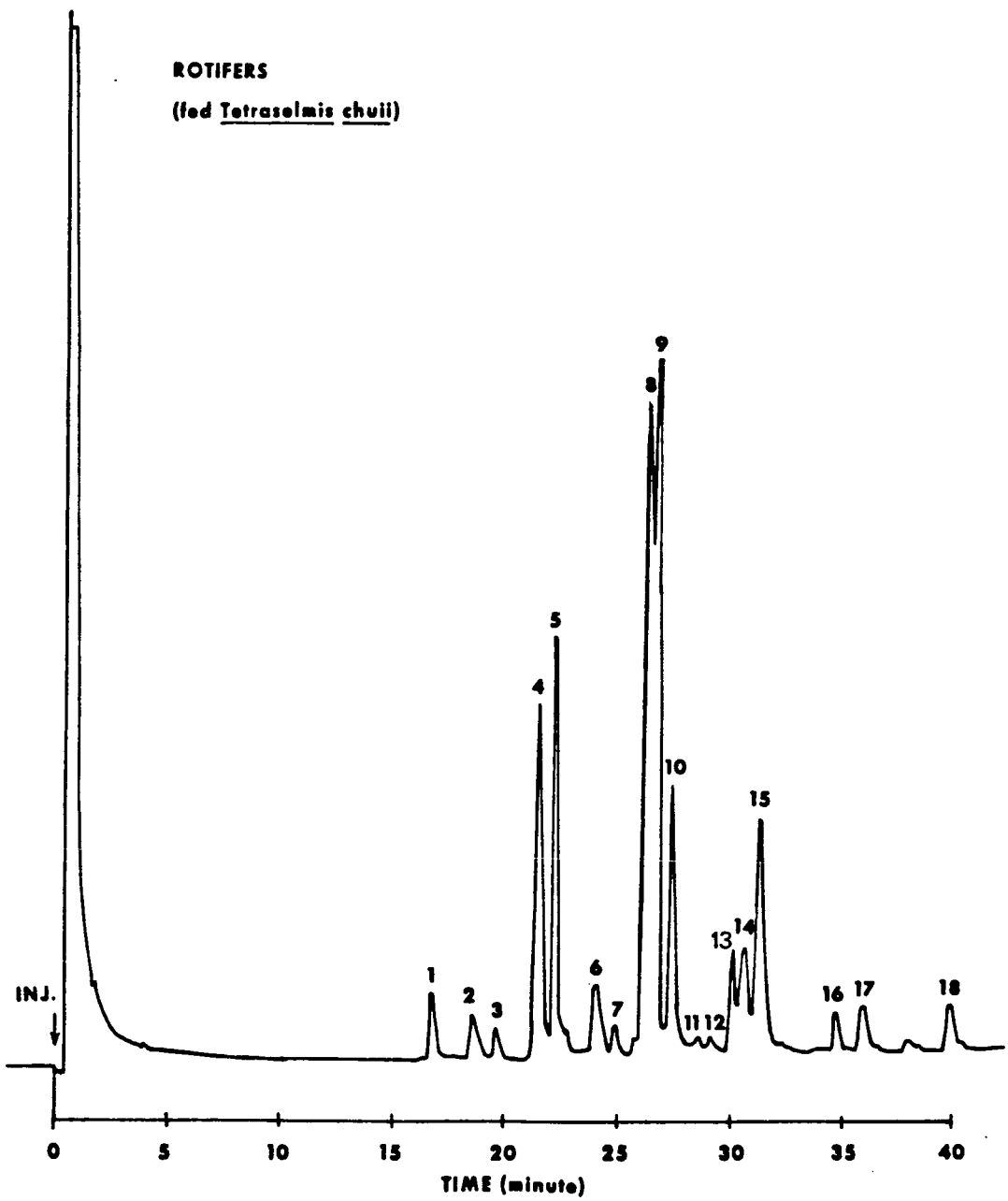

ROTIFERS
(fed Tetraselmis chuii)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
0
5
10
15
20
25
30
35
40
TIME (minute)

Figure 4. FAtty acid profile from rotifers maintained on a diet of Tetraselmis chuii. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecenoate	15:1
3.	pentadecanoate	15:0
4.	palmitoleate	16:1
5.	palmitate	16:0
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	linoleate + linolenate	18:2+3
9.	oleate	18:1
10.	stearate	18:0
11.	nonadecanoate (br) ?	br 19:0 ?
12.	nonadecenoate	19:1
13.	arachidonate + eicosapentaenoate	20:4+5
14.	eicosatrienoate	20:3
15.	eicosenoate	20:1
16.	docosapentaenoate + docosahexaenoate	22:5+6
17.	docosatrienoate	22:3
18.	unknown	

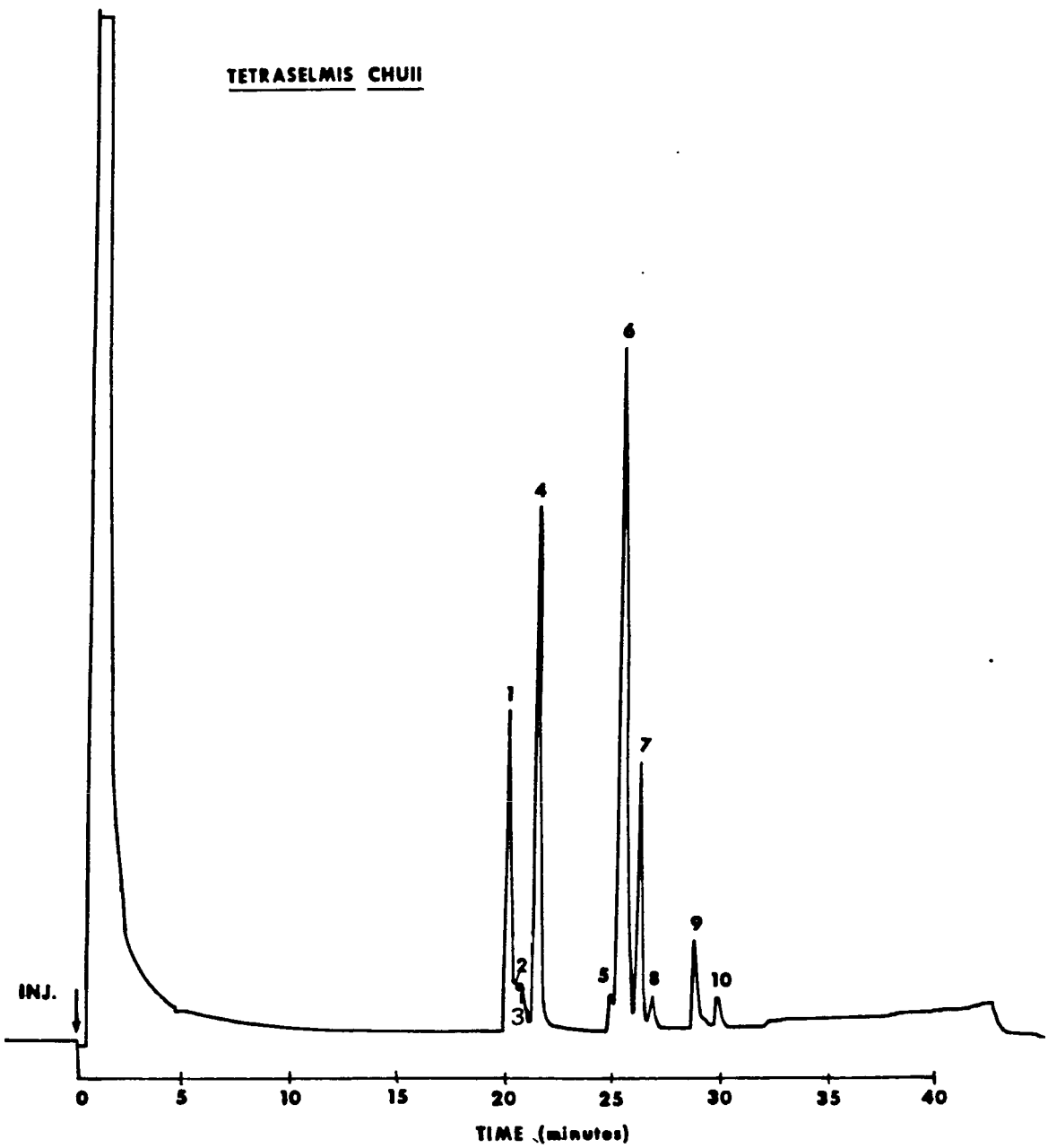

TETRASELMIS CHUII
INJ.
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 5. Fatty acid profile from Tetraselmis chuii. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	hexadecatrienoate	16:3
2.	hexadecadienoate	16:2
3.	palmitoleate	16:1
4.	palmitate	16:0
5.	unknown	
6.	oleate + linoleate + linolenate	18:1+2+3
7.	stearate	18:0
8.	nonadecanoate (br) ?	br 19:0 ?
9.	arachidonate + eicosapentaenoate	20:4+5
10.	eicosenoate	20:1

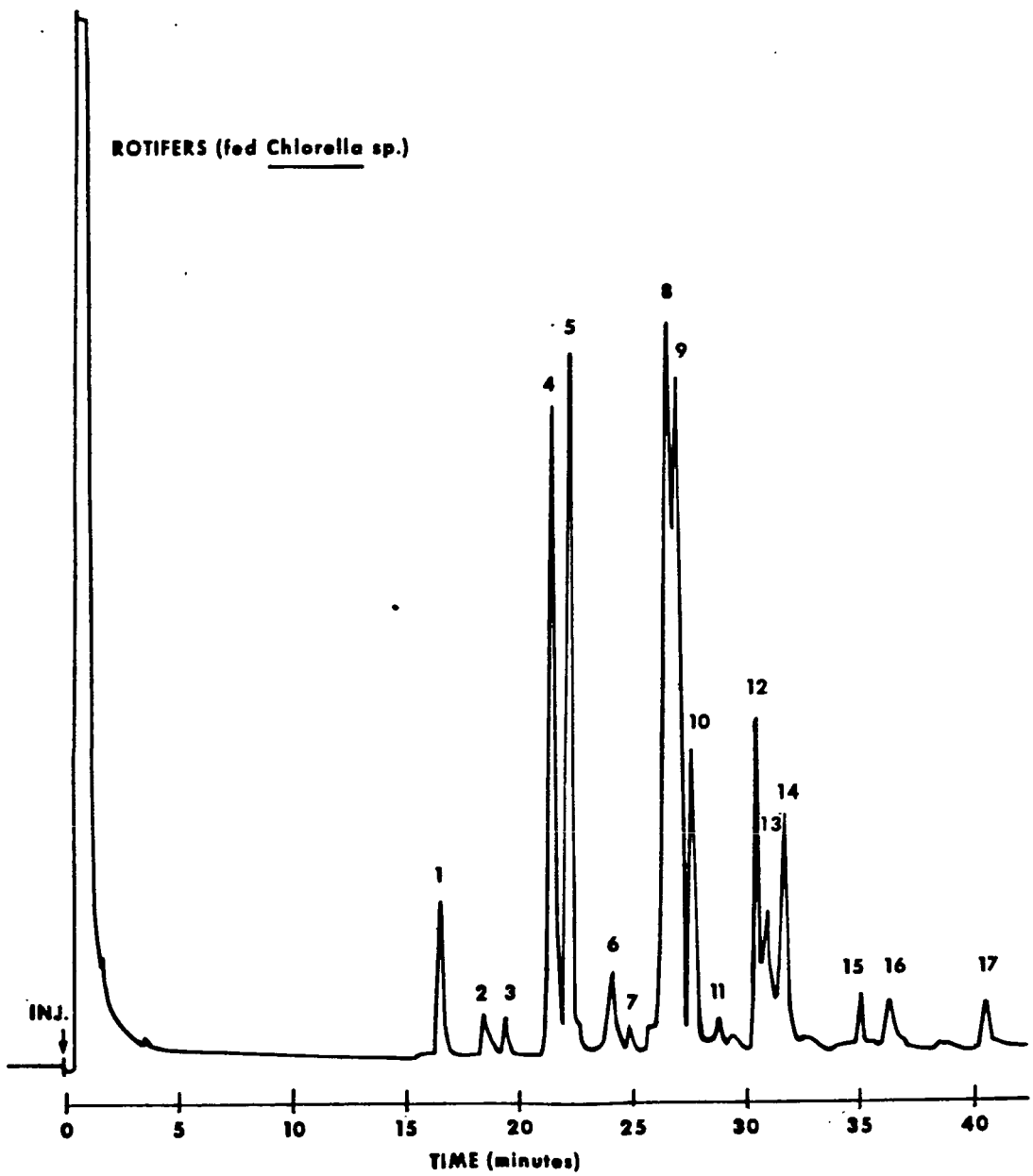

ROTIFERS (fed Chlorella sp.)
INJ.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
0
5
10
15
20
25
30
35
40
TIME (minutes)

Figure 6. Fatty acid profile from rotifers maintained on a diet of Chlorella sp. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecenoate	15:1
3.	pentadecanoate	15:0
4.	palmitoleate	16:1
5.	palmitate	16:0
6.	heptadecenoate	17:1
7.	heptadecanoate	17:0
8.	linoleate + linolenate	18:2+3
9.	oleate	18:1
10.	stearate	18:0
11.	nonadecanoate (br) ?	br 19:0 ?
12.	arachidonate + eicosapentaenoate	20:4+5
13.	eicosatrienoate	20:3
14.	eicosenoate	20:1
15.	docosaheptaenoate + docosahexaenoate	22:5+6
16.	docosatrienoate	22:3
17.	unknown	

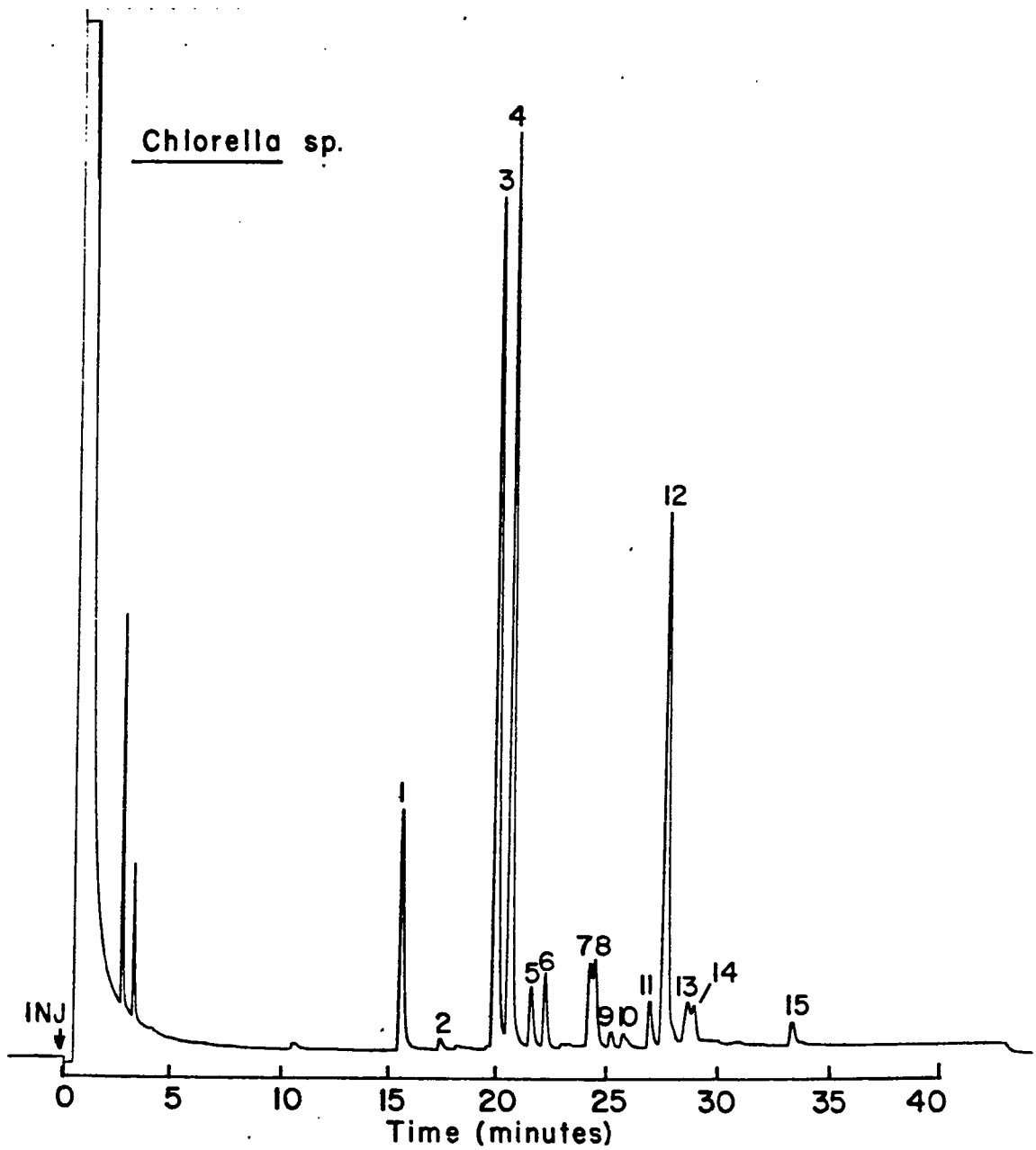

Chlorella sp.
INJ
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0
5
10
15
20
25
30
35
40
Time (minutes)

Figure 7. Fatty acid profile from Chlorella sp. Chromatography was performed using a 2m x 2mm (i.d.) glass column packed with 3% OV-1 on Supelcoport (100-120 mesh).

Identification of methyl esters

1.	myristate	14:0
2.	pentadecenoate	15:1
3.	palmitoleate	16:1
4.	palmitate	16:0
5.	heptadecanoate (br)	br 17:0
6.	heptadecenoate	17:1
7.	linoleate + linolenate	18:2+3
8.	oleate	18:1
9.	stearate	18:0
10.	unknown	
11.	nonadecenoate	19:1
12.	arachidonate + eicosapentaenoate	20:4+5
13.	eicosadienoate ?	20:2 ?
14.	eicosene	20:1
15.	docosapentaenoate + docosahexaenoate	22:5+6

APPENDIX 4.

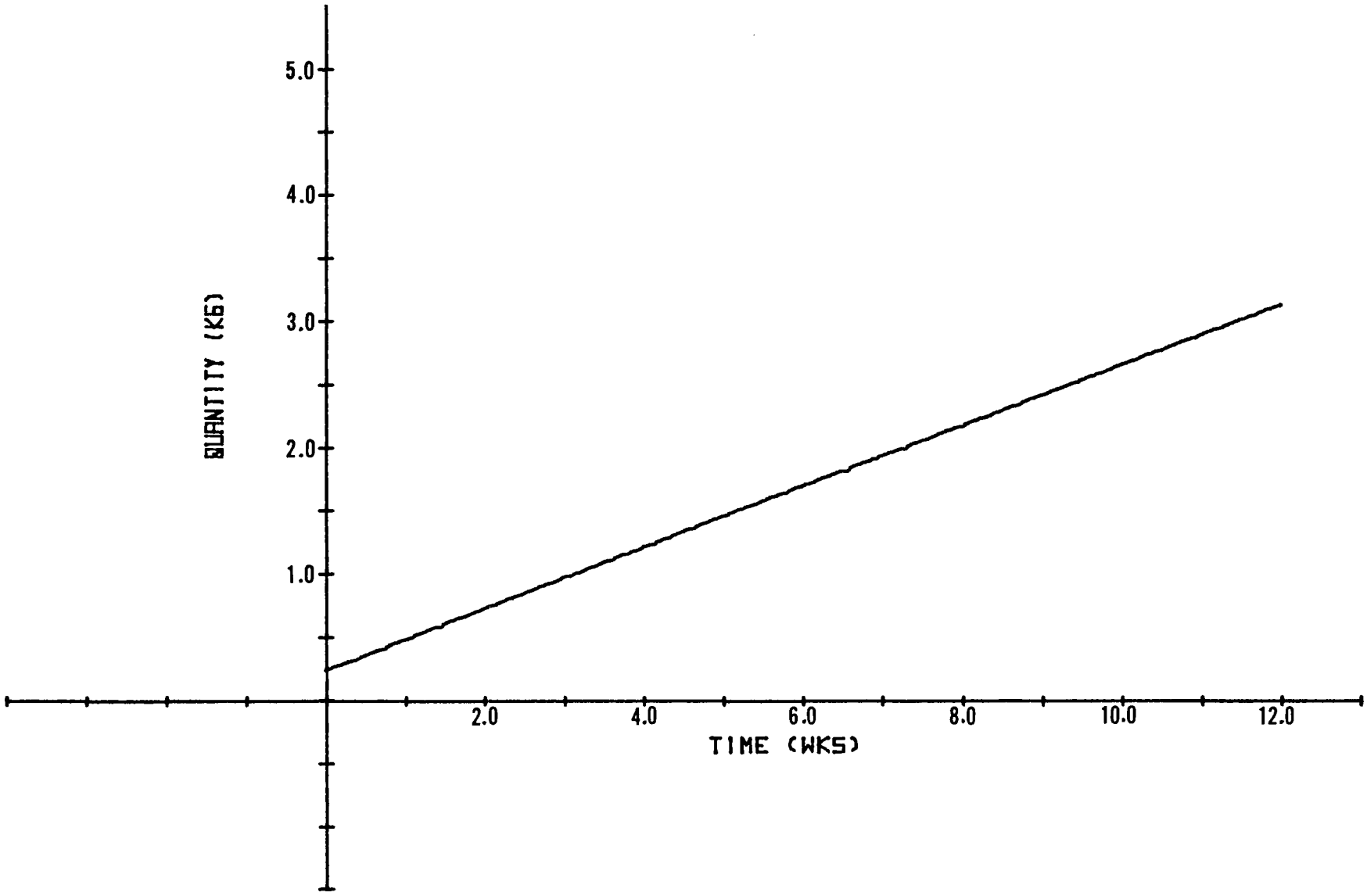

5.0
4.0
3.0
2.0
1.0
QUANTITY (KG)
2.0
4.0
6.0
8.0
10.0
12.0
TIME (WKS)

Figure 1. Plot of the amount of fatty acids in the shrimp pool using initial values given in Figure 3.18.

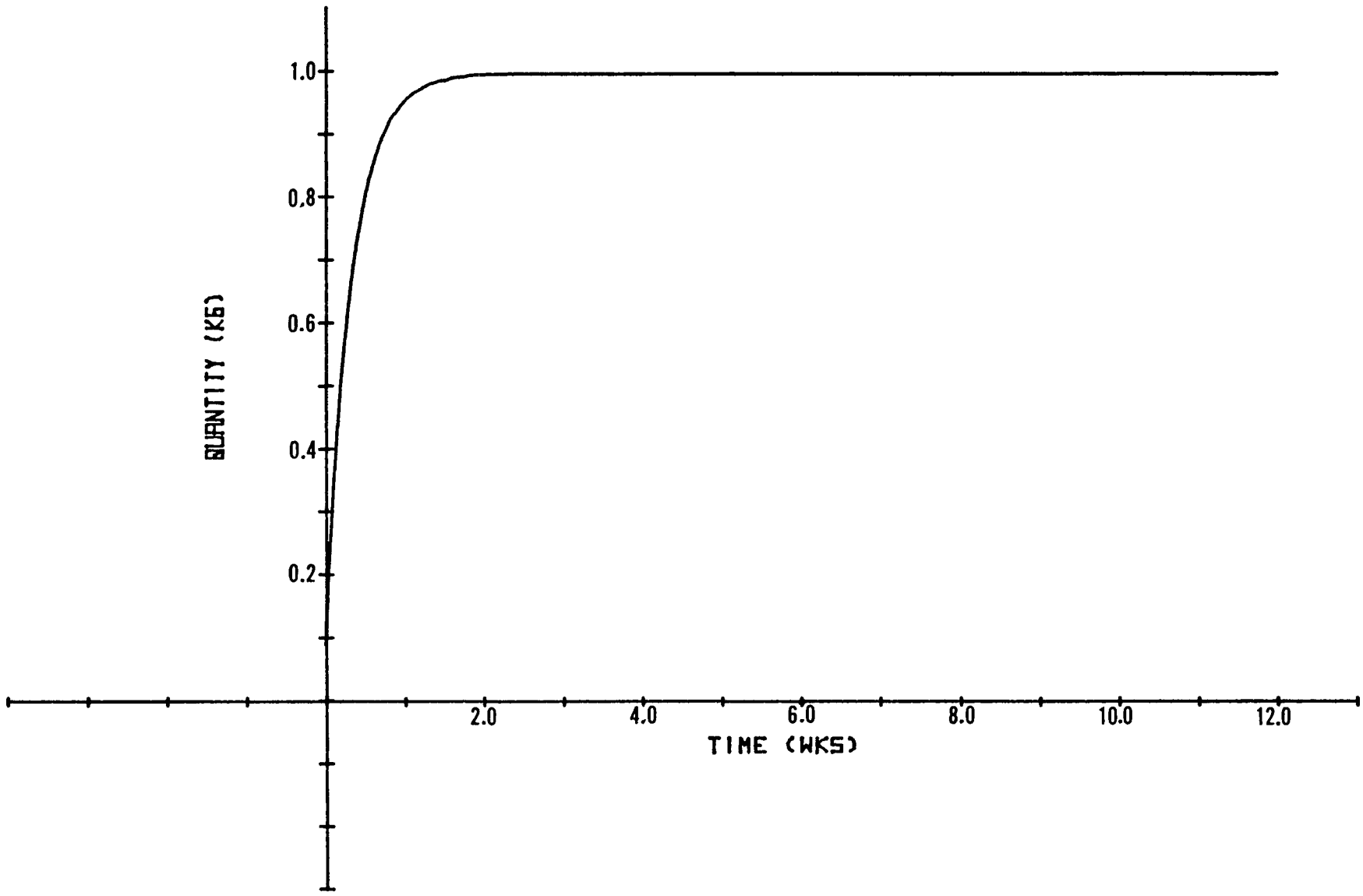

1.0
0.8
0.6
0.4
0.2
QUANTITY (K5)
2.0
4.0
6.0
8.0
10.0
12.0
TIME (WKS)

Figure 2. The effect of a 10-fold increase in rate R_1 on the quantity of fatty acids in the primary producer pool.

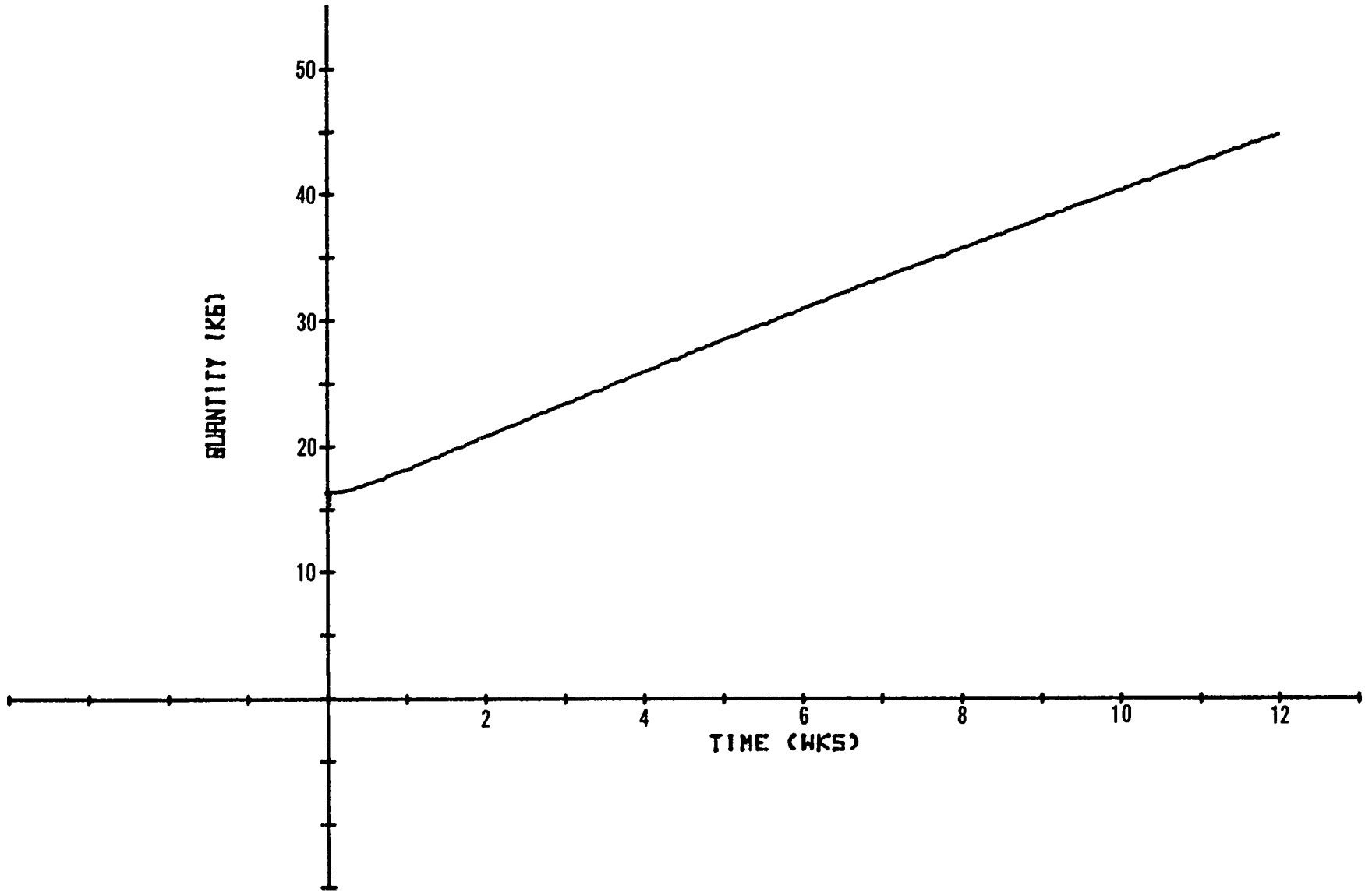

50
40
30
20
10
QUANTITY (KG)
2
4
6
8
10
12
TIME (WKS)

Figure 3. The effect of a 10-fold increase in the rate R_1 on the quantity of fatty acids in intermediary consumer pool.

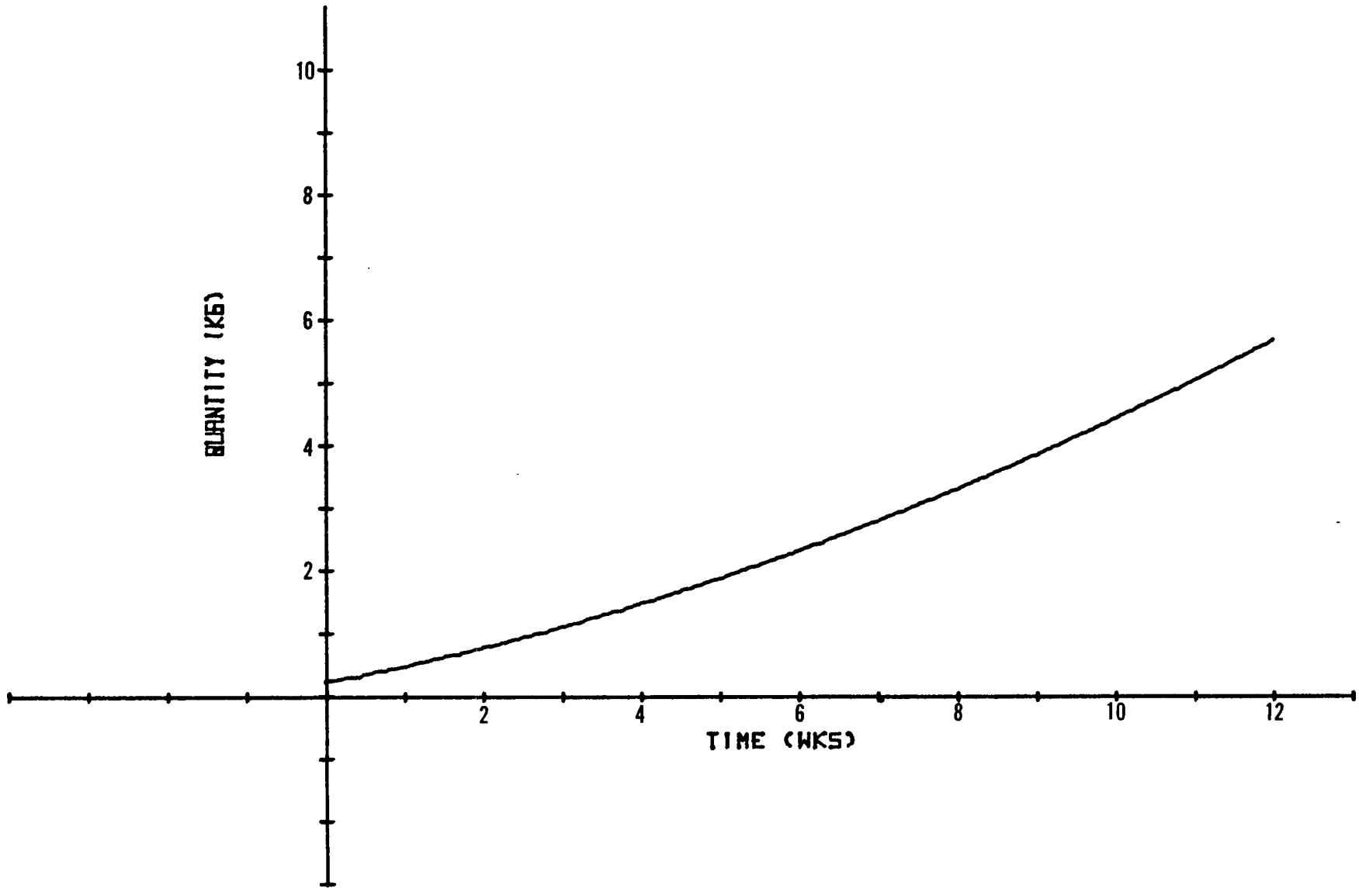

QUANTITY (KG)
10
8
6
4
2
2
4
6
8
10
12
TIME (WKS)

Figure 4. The effect of a 10-fold increase in rate R_1 on the quantity of fatty acids in the shrimp pool.

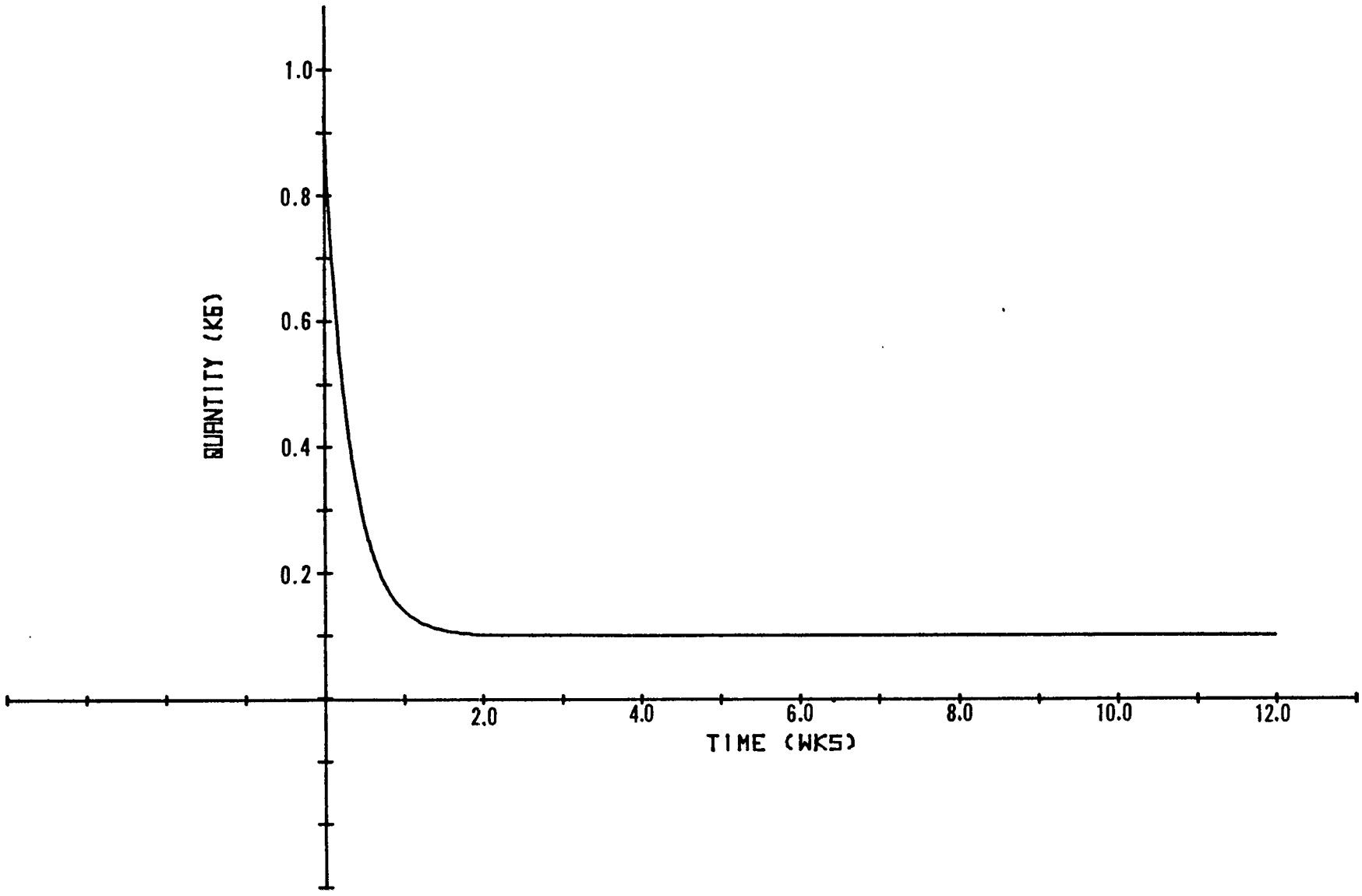

1.0
0.8
0.6
0.4
0.2
QUANTITY (KG)
2.0
4.0
6.0
8.0
10.0
12.0
TIME (WKS)

Figure 5. The effect of a 10-fold reduction in rate R_1 on the quantity of fatty acids in the primary producer pool.

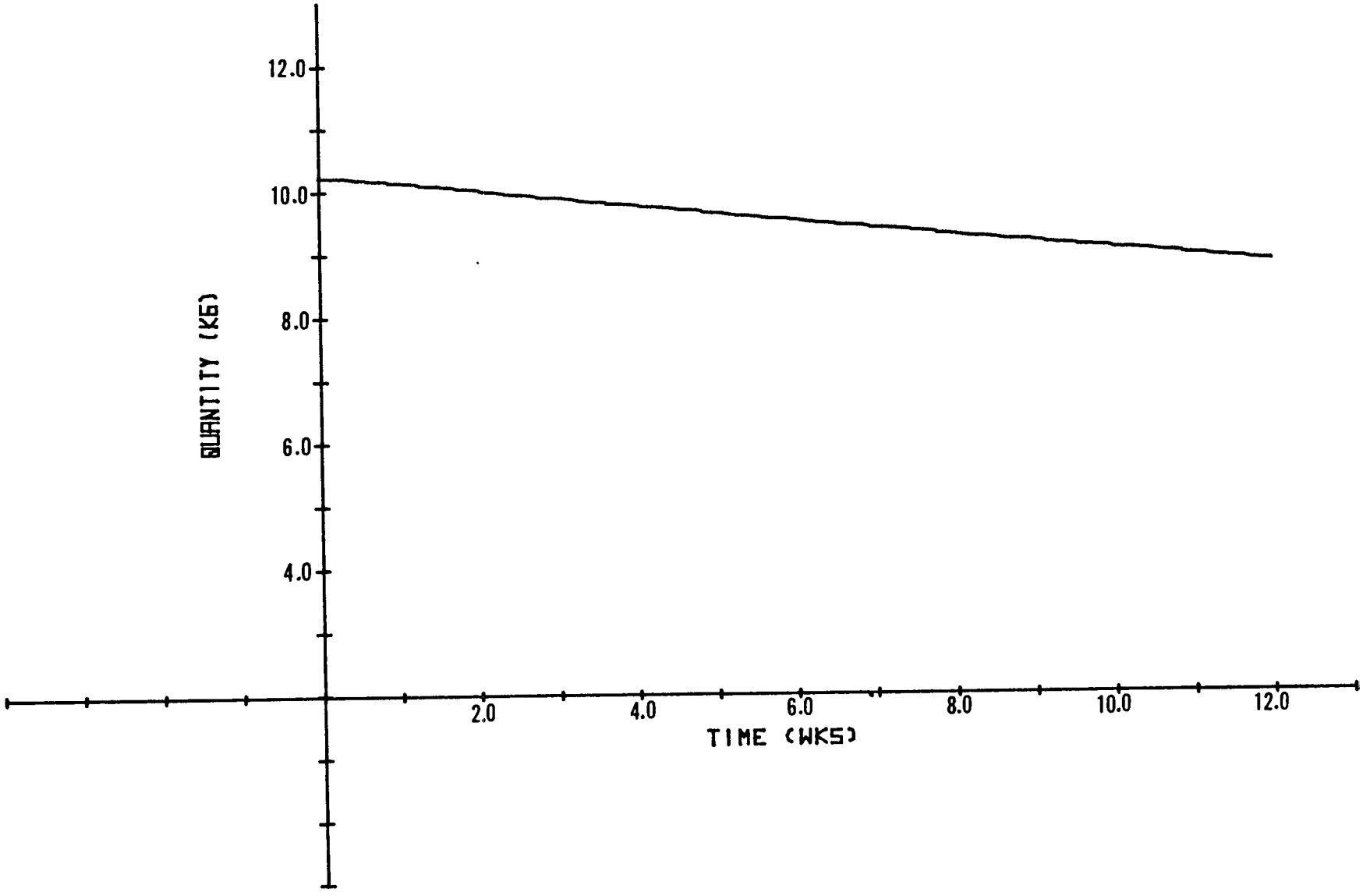

12.0
10.0
8.0
6.0
4.0
QUANTITY (KG)
2.0
4.0
6.0
8.0
10.0
12.0
TIME (WKS)

Figure 6. The effect of a 10-fold reduction in rate R_1 on the quantity of fatty acids in the intermediary consumer pool.[1]

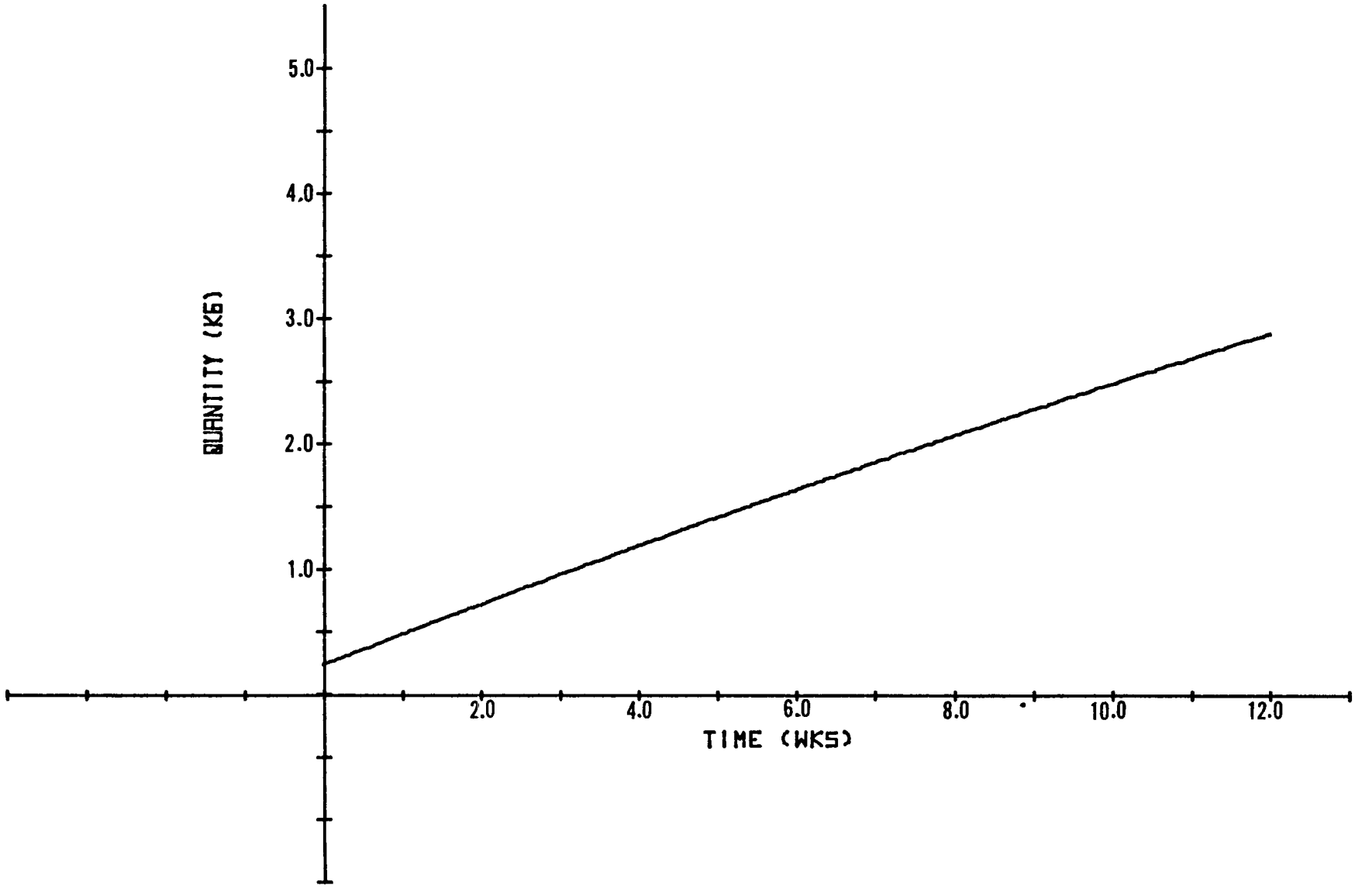

5.0
4.0
3.0
2.0
1.0
QUANTITY (KG)
2.0
4.0
6.0
8.0
10.0
12.0
TIME (WKS)

Figure 7. The effect of a 10-fold reduction in rate R_1 on the quantity of fatty acids in the shrimp pool.