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Essential Fatty Acid Metabolism and Requirements of the Lobster, Homarus americanus

by

Kim Elizabeth Harrison

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for the degree of Doctor of Philosophy

at

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Dedicated to my parents,
Commander James L. Harrison (USN, Ret.)
and Mrs. Franciszka (Galuski) Harrison,
for their never-failing love, encouragement,
personal sacrifice
and financial support.

It's been my Dad's influence, from the time of my very first memories of fishing and gardening and inspecting the marine life in rocky intertidal pools from Maine to California, that's instilled in me an appreciation of nature, a curiosity and desire to understand the adaptations of marine animals, and the drive to pursue an education and career in the marine sciences. Thanks, Dad.

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especially to Mrs. Amelia Gajewski and
Mr. and Mrs. Walter Galuski.

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ABSTRACT

In two long-term feeding trials, juvenile lobsters (Homarus americanus) were reared on semi-purified test diets supplemented with or deficient in selected (n-6) or (n-3) polyunsaturated fatty acids (PUFA) or the highly unsaturated fatty acid (HUFA), 22:6(n-3). An attempt was made to establish which fatty acids are essential for lobsters and to determine symptoms or disorders associated with EFA deficiency to thereby suggest functions of those fatty acids in lobsters. In the first feeding experiment 800 sibling juvenile lobsters were reared at 20°C and fed one of ten semi-purified test diets. Survival, growth, and whole body fatty acid composition were evaluated. In the second feeding trial, 100 sibling juveniles were reared at 10°C or 20°C and fed one of five semi-purified test diets. A multi-disciplinary approach was used to evaluate deficiency-related pathologies and to investigate the role of these nutrients in the lobster at the following levels of biological organization: (a) general: growth, molting, survivorship, (b) functional: metabolic rate, (c) structural: tissue and membrane histology, (d) compositional: tissue-specific fatty acid composition and (e) biochemical: fate of radio-labelled fatty acids.

Although juvenile lobsters could survive 10-12 weeks on diets deficient in both (n-3) and (n-6) PUFA, growth and molting rate were depressed, and rapid mortality occured after this critical time. A diet high in saturated fatty acids enhanced these deficiency effects. There was no discernable difference in the benefits of 18:3(n-3) vs. 18:2(n-6); each alone improved growth, molting rate and survival. There was an additional significant improvement in these factors by diets supplemented with 22:6(n-3) or with mixed HUFAs.

The oxygen consumption rate of lobsters fed the diet deficient in PUFA was significantly lower than that of lobsters fed diets with supplemental (n-3) or (n-6) PUFA. There was no affect of dietary fatty acids on ammonia excretion rates. The low O:N ratios indicated protein catabolism. The significantly lower O:N ratios of lobsters fed the PUFA-deficient diet indicated a starvation state and a possible degenerative condition or catabolism of body tissues for energy. These lobsters were notably lethargic prior to death.

Lobsters fed the PUFA-deficient diet had a significantly lowered hepatosomatic index (HSI). In addition, this is the first time evidence of degradative hepatopancreas associated with PUFA deficiency has been documented in crustaceans.

There were significant (and independent) effects of dietary fatty acids and temperature on lobster fatty acid composition. The major response to temperature was significantly lower proportions of 20:5(n-3) and significantly higher proportions of 16:0 in the polar lipids (PLs) of lobsters at 20°C as compared to those at 10°C. The response to diet and temperature varied with tissue type. The gill PLs were most responsive to changes in temperature, the muscle PLs were relatively unaffected by changes in diet or temperature, and the hepatopancreas PLs were primarily affected by diet but also by temperature. Although diet has a significant affect on the fatty acid composition of selected tissues of the lobster, the shifts in fatty acid composition in response to temperature appear to result from physiological acclimatization, and are independent of diet.

Incorporation of injected ^{14}C-18:3(n-3) into the phospholipids (predominantly PC, and also PE and PI) of the muscles and the hepatopancreas was demonstrated, indicating both uptake of this fatty acid from the hemolymph and short term turnover of membrane phospholipids. There was elongation of ^{14}C-18:3(n-3) to ^{14}C-20:3(n-3), negligible bioconversion of ^{14}C-18:3(n-3) to ^{14}C-20:5(n-3), and no evidence of bioconversion to ^{14}C-22:6(n-3). These data (and evidence from the effect of dietary fatty acids on the fatty acid composition of the tissues) indicate elongase activity and a lack of desaturase activity.

In conclusion, (n-3) and/or (n-6) fatty acids are essential for juvenile lobsters and HUFAs [e.g. 22:6(n-3)] have a higher EFA value than 18-C PUFAs. EFA deficiency in the lobster is manifested at each level of biological organization; symptoms include changes in fatty acid composition, degenerative tissues (hepatopancreas), depressed metabolic rate and tissue catabolism, lethargy, poor growth and depressed molting rates, and mortality.

ABBREVIATIONS

ANOVA	analysis of variance
H	hepatopancreas
H-CRD	Halifax Crustacean Reference Diet
% BW	percent of body weight
DWB	dry weight basis (see also WWB)
EFA	essential fatty acid
FA	fatty acid
FAME	fatty acid methyl ester
G	gills
GLC	gas-liquid chromatography
GL	glycolipid
HCO	hydrogenated coconut oil
HDL	high density lipoprotein
HSI	hepatosomatic index
HUFA	highly unsaturated fatty acid (having 20 or more carbons, and 3 or more methylene-interupted cis double bonds)
M	abdominal muscles
MFA	monounsaturated (monoenoic) fatty acid
NL	neutral lipid
PA	phosphatidic acid
PC	phosphatidylcholine
PE	phosphatidlyethanolamine
PI	phosphatidlyinositol
PL	polar lipid (phospholipid, phosphoglyceride)
PS	phosphatidylserine
PUFA	polyunsaturated fatty acid (having 18 or more carbons and 2 or more methylene-interupted double bonds)
SFA	saturated fatty acid
TAG	triacylglycerol
TL	total lipid
TLC	thin layer chromatography
VLDL	very low density lipoprotein
WWB	wet weight basis (see also DWB)

(Continued)

Nomenclature of Fatty Acids Frequently Cited in this Thesis[1]

Shorthand Notation[2]	Common Name	Systematic Name
SFAs		
14:0	myristic acid	
16:0	palmitic acid	hexadecanoic acid
18:0	stearic acid	octadecanoic acid
MFAs		
16:1(n-7)	palmitoleic acid	cis-9-hexadecenoic acid
18:1(n-9)	oleic acid	cis-9-octadecenoic acid
PUFAs		
18:2(n-6)	linoleic acid	9,12- octadecadienoic acid
18:3(n-3)	α-linolenic acid	9,12,15-octadecatrienoic acid
HUFAs:		
20:4(n-6)	arachidonic acid	5,8,11,14 - eicosatetraenoic acid
20:5(n-3)	eicosapentaenoic acid	5,8,11,14,17- eicosapentaenoic acid
22:6(n-3)	docosahexaenoic acid	4,7,10,13,16,19- docosahexaenoic acid

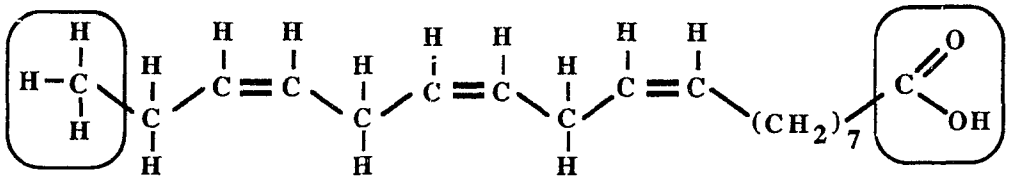

Figure 1. Structure of α-Linolenic Acid

1. For more complete listings, see Christie (1982)
2. Explanation of shorthand notation

   Convention adopted by IUPAC-IUB (1967, 1977, as cited by Christie (1982))

   X:Y(n-Z) X = the number of carbon atoms in the fatty acid chain;

   Y = the number of double bonds in fatty acid;

   (n-Z) = position of the last double bond

   n represents the number of carbon atoms in the chain

   Z = the number of carbon atoms from the terminal methyl group to the nearest double bond including the methyl C and the first carbon of the double bond

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PART I: GENERAL INTRODUCTION

THE FATTY ACID METABOLISM AND EFA REQUIREMENTS OF Homarus americanus AND OTHER DECAPOD CRUSTACEANS

1.0 BACKGROUND

Certain nutrients are termed "essential" for a given organism based on two criteria: 1) the requirement of that organism for the specific nutrient to fulfill roles in physiological, biochemical, physical-chemical and other processes in the body, and 2) the inability of the organism to produce the biochemical, or produce it from precursors at a rate sufficient to meet the body's requirements for it. Specific fatty acids (FAs) are known to be essential (EFAs) for animals as they meet the above criteria, and dietary deficiency can impair growth, development and reproduction. Aspects of EFA deficiency are discussed more fully in Sections 1.2.3 and 1.5.2.

For the lobster (Homarus spp.), evidence of EFA requirements has been indirect and non-conclusive, based on research on other crustaceans, and on lobster nutrition studies which have shown that dietary oils rich in polyunsaturated FAs (PUFA) and/or (n-3) highly unsaturated FAs (HUFA) have growth promoting effects (Castell & Covey, 1976; D'Abramo et al., 1980). The number and identity of FAs that are essential, their specific functions, and specific deficiency symptoms have not been determined for lobsters. In the wild, lobsters likely satisfy presumed EFA requirements through their natural diet of marine animals and plants, which includes polychaetes, molluscs, crustaceans, echinoderms, and fish (Weiss, 1971, referenced in Castell & Covey, 1976; Ennis, 1973; Scarratt,1980; Elner & Campbell, 1987); these organisms are high in PUFA and (n-3) HUFA. Hughes & Matthiessen (1962), Stewart et al. (1967a,b) and McLeese (1972a,b) reported early attempts to raise lobsters in captivity on natural diet ingredients. While marine organisms as diets or diet supplements may provide essential nutrients (including EFAs) for captive animals, these feeds vary in quality and availability, and they are more difficult to handle, store, and administer than synthetic feeds. In intensive culture there is a reliance on synthetic feeds to provide total nutrition and because lobster EFA are not yet determined, lobsters may be subjected to EFA-deficient diets. Since proper lipid

nutrition is required for survival, optimal growth and development, fecundity and viability of produced eggs, and general good health (disease and stress resistance), it is important to determine the EFA requirements for lobsters reared under the conditions of intensive culture.

Castell & Covey (1976) tested the effects of several dietary lipids (using natural lipid sources and semi-purified, casein based diets) on adult American lobster (Homarus americanus). Results suggested a requirement for (n-3) series FAs. Castell & Boghen (1979) compared the nutritional value for juvenile lobsters of 18:3(n-3)[1] and 22:6(n-3) with mixtures of 18:2(n-6) and 18:3(n-3). Unique effects of dietary lipids on FA metabolism were observed. However, interpretation of the findings is difficult since only the theoretical levels of dietary FAs were reported, and there was high mortality within four weeks on all treatments presumably from essential amino acid deficiency (of methionine and histidine) due to poor quality of the casein lot used in the basal diet. (Note: casein had been used effectively in lobster diets since 1973, pers. comm. J.D. Castell).

In 1980, Conklin et al. reported that juvenile lobsters could only be kept alive on a casein-based diet by supplementation with 6-7% soy lecithin[2]. In the absence of soy lecithin, lobsters would die in a partially molted condition or would have missing or deformed appendages upon completion of the molt. This condition was termed "molt death syndrome" (Conklin et al., 1980) and was described in detail by Bowser & Rosemark (1981). There is continued debate over whether there is an actual nutritional requirement for lecithin or whether it functions to maintain the physical integrity of the diet and also assists in emulsification and absorption of other nutrients (as well as spare the dietary requirement for specific FAs or choline, of which it is comprised).

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[1] Short-hand notation for fatty acids is explained in the section of abbreviations, page xviii.

[2] Commercial lecithin may be a mixture of phospholipids; the major component is typically phosphatidylcholine, which may constitute 25-97% of soybean lecithin (O'Leary and Matthews, 1990).

However, since lecithin is a phospholipid (PL), comprised of two fatty acyl chains (which may be EFAs), its use is inappropriate in diets testing for EFA deficiency; such studies require basal ingredients extracted of any trace lipids and controlled supplementation with purified FAs. Kean & co-workers (1985) reported that diets for H. americanus in which casein was replaced with protein purified from the rock crab (Cancer irroratus) supported good growth and survival without the need for supplemental soy lecithin .

Since "the imperative prerequisite" for studies investigating EFA deficiency is a well-defined, EFA-free experimental diet (Aaes-Jorgensen, 1961), the subsequent development of a crustacean reference diet and semi-purified test diets based on rock crab protein (Castell et al., 1989) has enabled testing (for the first time) for the EFA requirements of lobsters, determining their EFA deficiency symptoms, and broadening the current knowledge of lipid metabolism. This dissertation presents the rationale, methodology and findings from two feeding trials (using rock crab protein diets) and the associated experiments designed to investigate the EFA metabolism and requirements of lobsters (Homarus americanus).

The introduction which follows pertains to crustaceans, in general, and to Homarus americanus, in particular. It provides a review of: 1) FA synthesis and metabolism, 2) nutritional physiology pertaining to FAs, 3) factors affecting FA composition, and 4) previous research on dietary FA requirements. It concludes with an overview and rationale of this research program (Section 1.6), and in Section 1.7, states the overall objectives.

1.1 FATTY ACID SYNTHESIS AND METABOLISM IN Homarus americanus AND OTHER DECAPOD CRUSTACEANS

The limited information available on FA metabolism and nutrition of crustaceans was reviewed by Castell (1982). In the subsequent eight years there has been limited advancement in the understanding of specific biosynthetic pathways, enzyme activities, the factors affecting crustacean EFA metabolism or the roles and exact requirements of FAs for crustaceans. Advances in the field of crustacean phospholipid (PL) metabolism have been made and reviewed by Chapelle (1986) & co-workers, with emphasis on the biosynthesis of PLs and on adaptive mechanisms in PL metabolism in response to changes in environmental conditions such as temperature and salinity. This is discussed in Section 1.1.4 and in greater detail in Part III.

Vertebrate systems serve as a model for investigating and understanding crustacean lipid and FA metabolism, since the factors and mechanisms are far more thoroughly researched, and are understood in greater detail for vertebrate systems than for crustaceans. However, there are several general aspects of lipid and FA metabolism which are distinctly different in crustaceans. Firstly, the primary site of absorption, metabolism and synthesis of lipids in crustaceans is the hepatopancreas[3], whereas in vertebrates these functions are divided among the intestines, liver, and adipose tissue, respectively. Secondly, whereas in vertebrates, lipids are cleared from the bloodstream by the liver after absorption of dietary lipids into the blood via the intestines, in crustaceans, lipids are principally processed in the hepatopancreas prior to release into the blood stream. Thirdly, the circulating form of lipids released from the midgut and hepatopancreas of crustaceans is PLs in the form of high density lipoproteins (HDLs), unlike the chylomicrons of vertebrates which are released from the intestines and are high (~80%) in triacylglycerides (TAGs) (i.e. neutral

[3] The terms hepatopancreas, digestive gland and midgut gland are used interchangeably in the literature. Hepatopancreas will be used in this thesis, based on the arguments of Gibson and Barker (1979).

lipids, NLs) or the very low density lipoproteins (VLDL) released from the liver, also high in NLs. A fourth factor, which is unique to crustaceans, is that there is a cyclical change in lipid metabolism coincident with changes in the molt cycle, both under hormonal control.

1.1.1 De novo Fatty Acid Synthesis in Decapods

The ability of decapods, including the European lobster (Homarus gammarus), for de novo FA synthesis was first demonstrated by Zandee (1962, 1966, 1967), by examining the fate of injected [1-^{14}C]-labeled acetate. Label appeared in the saturated FAs (SFAs) and the monounsaturated FAs (MFAs), and did not appear in the PUFAs. Subsequent studies (also using [1-^{14}C]-acetate) on the crayfish Orconectes virilis and the crab Gecarcinus lateralis (O'Connor & Gilbert, 1968, 1969), the lobster Homarus americanus (Shieh, 1969, 1971), and the prawns Penaeus japonicus (Kanazawa & Teshima, 1977; Patrois et al., 1978) and P. monodon and P. merguiensis (Kanazawa et al., 1979c), support the earlier findings. Shieh examined incorporation of radioactive precursors into the glycerides (Shieh, 1971) and the PLs (Shieh, 1969) of the lobster. Farkas & co-workers (1981) found that incorporation of label was primarily in the PLs, and predominantly in the SFAs and MFAs (Farkas & Nevenzel, 1981). Unlike vertebrates, lobsters and other decapod crustaceans have little adipose tissue and de novo synthesis of lipids occurs primarily in the hepatopancreas. There is also subcutaneous "adipose"-like tissue.

The rate of de novo synthesis has been shown to vary with the molt cycle (Patrois et al., 1978), and is highest during the period of new tissue synthesis at early intermolt and early premolt. This was determined by measuring the percentage of incorporation of [1-^{14}C]-acetate into lipids of the prawn Penaeus japonicus, at each stage of the molt cycle. O'Connor & Gilbert (1969) have suggested that acceleration of lipid synthesis at premolt may be under hormonal control by glandular secretions from the base of the eyestalks.

1.1.2 The Bioformation of Monoenoic (Monounsaturated) Fatty Acids

In animals, monounsaturated FAs, (MFAs) are formed by an oxidative method in which a double bond is introduced directly into preformed SFAs, at carbon atoms C_9 and C_{10} from the carooxyl end (Figure 1, p. xviii). This conversion of SFAs to MFAs, is facilitated by a Δ^9-desaturase enzyme. The Δ^9-desaturase is a complex of three component proteins which are bound to membranes of the rough endoplasmic reticulum (which in vertebrates, occurs primarily in the liver). Studies indicate that there may be two desaturase complexes which differ in chain length specificity (Gurr & James, 1980). The more abundant Δ^9-desaturase has a maximum activity for C_{18} chain length FAs, and converts stearic acid (18:0) to oleic acid (18:1(n-9)). It also converts, in decreasing effectiveness, SFAs of C_{17}, C_{16} (converting palmitic acid, 16:0, to palmitoleic acid, 16:1(n-7)), C_{15} and C_{14} to their respective MFAs, and has very low activity for C_{19} and C_{20} FAs. A second Δ^9-desaturase introduces a double bond between C_9 and C_{10} of shorter chain FAs, with greatest activity for C_{14} FAs and with decreasing effectiveness on C_{13}, C_{12}, C_{11} and C_{10}.

Indirect evidence of Δ^9-desaturase activity has been reported for several crustaceans (Herrodek, 1970; Morris & Sargent, 1973), including the larvae and juveniles of the decapod Penaeus japonicus (Jones et al., 1979 & Kanazawa et al., 1979d, respectively). In these studies ^{14}C-labeled palmitic or stearic acids were administered and the subsequent occurrence of ^{14}C-labeled MFAs was measured.

The Δ^9-desaturase activity (in rat liver) is rapidly and dramatically affected by fasting and by the level of dietary PUFA (for overview, see Cook, 1985). In response to fasting and to low levels of dietary PUFA, there is a drop in Δ^9-desaturase activity which is restored to higher than normal levels upon refeeding. High levels of dietary PUFA (e.g. linoleic acid) may selectively inhibit Δ^9-desaturase activity and the formation of MFA.

Although there is some evidence of reduced monoene formation in the presence of dietary PUFA in crustaceans, further studies are required to verify this. Future studies should (1) control diet in addition to examining the rate of label incorporated into MFAs, and (2) report changes in the mass balance of tissue FAs and not just proportional changes.

1.1.3 Desaturation to Form Polyunsaturated Fatty Acids (PUFAs)

The enzyme systems of animals do not have the ability to introduce a double bond between an existing double bond and the methyl end of a FA (Figure 1). However, further desaturation of unsaturated FAs can occur towards the carboxyl end of the molecules through the action of Δ^4, Δ^5, and Δ^6-desaturases (see Gurr & James, 1980, for a review). This gives rise to "families" or "series" of FA metabolites with different chain lengths but similar terminal structures, arising from oleic acid, 18:1(n-9) ((n-9) series), linoleic acid, 18:2(n-6) ((n-6) series), and a-linolenic acid, 18:3(n-3) ((n-3) series) (Figure 2). While 18:1(n-9) can be synthesized de novo (or obtained from the diet), no animals tested thus far have been shown to have the ability to manufacture 18:2(n-6) or 18:3(n-3) de novo; they are supplied, preformed, in the diet and originate from plant sources. Terrestrial plants, in general, are high in 18:2(n-6), while marine plants and phytoplankton are generally high in C_{20} and C_{22} (n-3) FAs.

The first desaturation of each of these substrates (18:1(n-9), 18:2(n-6) or 18:3(n-3)) is by Δ^6-desaturase, which introduces a double bond between carbon atoms 6 and 7; this is the rate limiting step in the sequence of elongations and desaturations for each series (Figure 2). It is generally accepted that the (n-9), (n-6) and (n-3) series of reactions are metabolized by the same enzyme sequence, but this has not been proven unequivocally (see Horrobin, 1990 for review). The affinity of these FAs towards Δ^6-desaturase is in the order 18:3 > 18:2 >> 18:1, such that if supplied in equal amounts, there will be more end products of the 18:3(n-3) pathway. In mammals, deficiency of 18:3(n-3) and especially

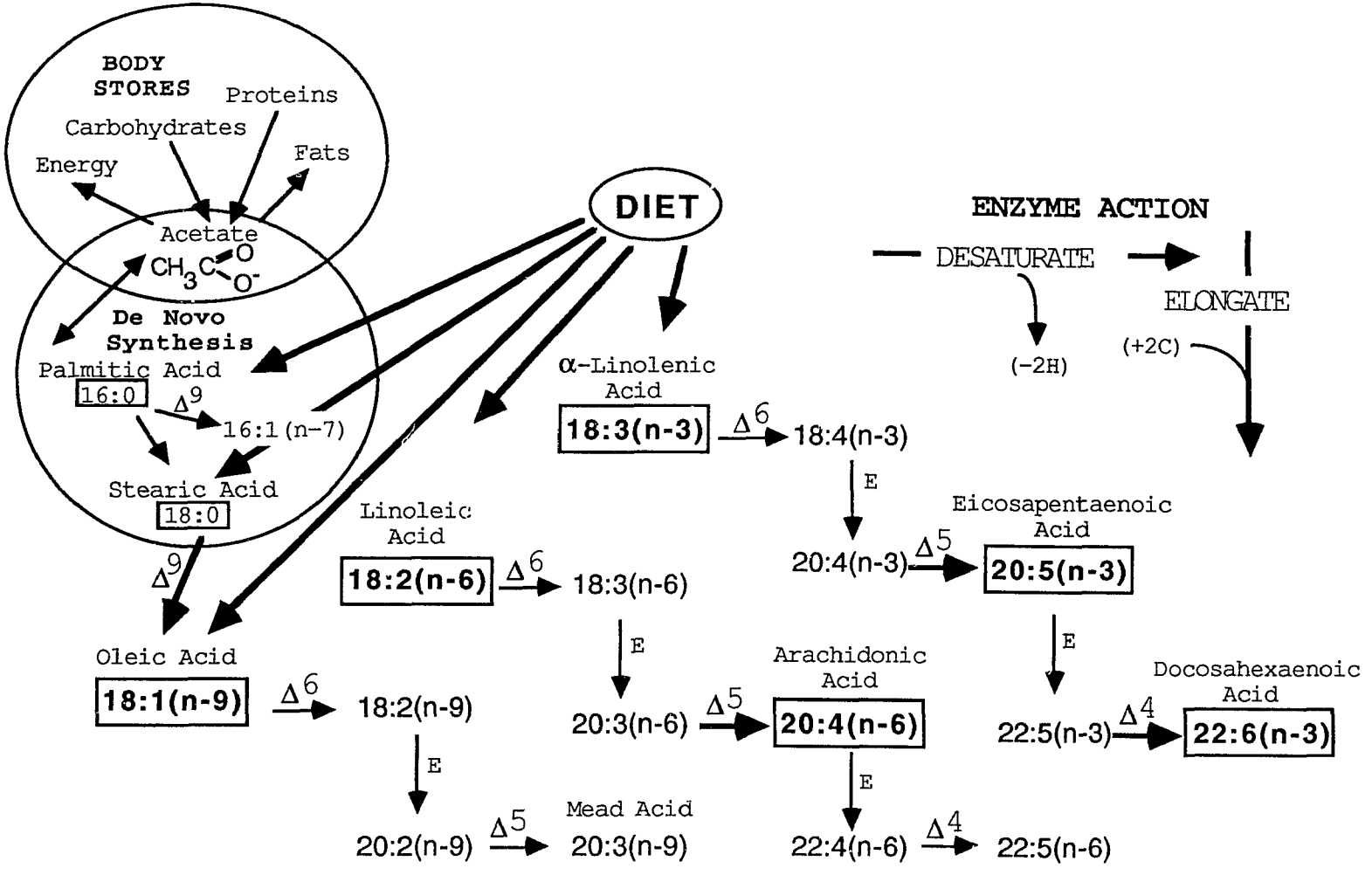

Figure 2. Pathways of Bioconversion of the Three Major Series of Unsaturated Fatty Acids.

Desaturase enzymes are shown as Δ9, Δ6, Δ5 and Δ4; affinity of each desaturase for substrates of the same carbon length: (n-3) > (n-6) > (n-9). Elongase enzymes shown as "E". Note that (n-9) series can be synthesized de novo whereas the (n-3) and (n-6) series fatty acids must be obtained from the diet. Fatty acids from the (n-9), (n-6), and (n-3) series pathways are not interconvertible. (Adapted from H. Cook, 1985, Fig. 6.6)

18:2(n-6) results in a relative increase in metabolites of 18:1(n-9). This has yet to be confirmed for crustaceans.

Among the first researchers to investigate lipid biosynthesis in arthropods, Zandee (1967) administered [1-^{14}C]-acetate to several crustaceans including the lobster Homarus gammarus. There was negligible label occurring in 18:2(n-6), 18:3(n-3) or in the C_{20}-C_{22} more highly unsaturated FAs.

The conversion of injected [1-^{14}C]-18:3(n-3) to 20:5(n-3) or 22:6(n-3) by the prawn Penaeus japonicus was examined by Kanazawa & co-workers (1979b). After 24 hours of incubation at 25°C, 20:5(n-3) constituted approximately 4% of labeled PL or NL, and 22:6(n-3) was approximately 2% of labeled PL. Results suggested that the prawn is capable of desaturation and elongation to form HUFAs. However, several feeding experiments have shown that inclusion of 20:5(n-3) and/or 22:6(n-3) in the diet has a greater growth enhancement effect than 18:3(n-3) (Kanazawa et al., 1977). This indicates that while the marine prawns exhibit a capacity for bioconversion of linolenic acid, the rate or efficiency of conversion is not sufficient to meet the demands for new tissue synthesis, and thus dietary 18:3, 20:5 and 22:6 (n-3) FAs are essential for growth.

1.1.4 Biosynthesis of Phospholipids

The synthesis of PLs has been demonstrated in several decapod crustaceans and the topic has been recently reviewed by Chapelle (1986). Bilinski (1962) first demonstrated the biosynthesis of phospholipids by the lobster Homarus americanus and the crab Cancer magister using 1-methyl-^{14}C-choline. The synthesis of phosphoglycerides from ^{32}P-disodium phosphate and from various ^{14}C-labeled precursors was demonstrated by Shieh (1969) in the lobster H. americanus. Later studies by Chapelle & co-workers (1977, 1979) using radioactive inorganic phosphate showed that synthesis of PLs in the muscles,

hepatopancreas and gills of the crab Carcinus maenas was by the same pathways as in vertebrates and that the activity of the reaction sequence $^{32}P_i$ -> ^{32}PA -> ^{32}PL was dependent on temperature of the environment.

1.2 FUNCTIONAL ASPECTS OF FATTY ACIDS IN THE PHYSIOLOGY AND BIOENERGETICS OF DECAPOD CRUSTACEANS

FAs occur in many forms in all living cells and cell membranes as constituents of NLs and PLs. The number of carbons, the number of double bonds and the position of the double bonds in a FA affect its functional properties. Within organisms, changes in the lipid constituents and FA composition can alter the physical properties of membranes (properties such as fluidity and permeability, which affect osmotic and ionic balance) and influence biochemical and physiological cell and membrane functions. This can be particularly important in adaptation of the organism to changes in the environment such as temperature or salinity. Some FAs function as metabolic regulators or serve as precursors of hormone-like eicosanoid compounds such as prostaglandins. Other FAs, esterified to a glycerol backbone as TAGs, provide an efficient form of energy storage in many animals, including decapod crustaceans.

1.2.1 Neutral Lipids (Principally Triacylglycerides)

NLs include mono-, di-, and tri- acylglycerols (IUPAC-IUB, 1967; 1977), (or formerly, mono-, di- and tri- "glycerides"), sterols and steryl esters. The acylglycerols, particularly TAGs, are a major dietary source of energy, and the predominant form of energy storage in crustacean adults, eggs, and pre-feeding larvae (Ward et al., 1979; Middleditch et al., 1979; Teshima & Kanazawa, 1983; Clarke, 1982). In post-embryonic stages of decapods, the hepatopancreas is the major organ for lipid absorption, processing and storage (Vogt et al., 1985). Thus, the hepatopancreas has the highest fat content (and

percentage composition as fat) of any tissue in the body, and NLs (principally TAGs) comprise the largest fraction of hepatopancreatic total lipids (Chapelle, 1977).

During sexual maturation the ovaries become an additional center for lipid metabolism (including lipogenesis by the smooth endoplasmic reticulum). There is increasing evidence, however, that hepatopancreatic lipids are the major source of lipid accumulation in maturing ovaries (Teshima & Kanazawa, 1988b). The NLs are accumulated in globules comprised primarily of non-EFAs (i.e. 16:0 and metabolites) (Teshima et al., 1988a). Ovarian lipids (and carbohydrates) provide fuel for the biosynthetic processes of oogenesis and vitellogenesis and are apparently taken up and accumulated by the developing oocytes.

1.2.2 Phospholipids (Principally Phosphoglycerides)

This review will restrict discussion to phosphoglycerides (or glycerophosphatides), a class of PLs which have a glycerol backbone to which two fatty acyl chains (which may constitute ~60% of the mass) are attached by ester linkages (positions 1 and 2) and a phosphate moiety is esterified at position 3, conferring a polar nature to the molecules. The phosphate moiety may be inorganic phosphate (as in phosphatidic acid), or phosphate bonded to either an organic base or polyhydroxy compound. Phosphoglycerides (hereafter termed PLs) are important components of cell membranes. They comprise 40 to 50% of the membrane and function to maintain structural and physiological properties of the membranes (see Gilbert & O'Connor (1970), for review). Chapelle (1986) reviewed evidence of the role of specific PLs in anisosmotic extracellular regulation in crustaceans. PLs are also important as the major transport form of lipids in the hemolymph, accounting for as much as 87 to 88% of the lipid composition of circulating high density lipoproteins (HDLs), generally in a protein to lipid ratio of 1:1 to 1.2:1 (Teshima & Kanazawa, 1978, 1979, 1980; Lee & Puppione, 1978).

Plasmalogens, a class of PLs in which an alkenyl group is attached to the glycerol backbone at position 1 by an ether bond, have been reported to occur in the tissue of marine animals. In particular, they occur in the gill tissues of crustaceans and other aquatic animals (Nevenzel et al., 1985; Chapelle & Benson, 1986; Chapelle et al., 1987). Plasmalogens will not be covered in further detail in this review.

1.2.3 Essential Fatty Acids

For the vertebrates, the EFAs are (n-3) and (n-6) PUFAs in which all double bonds are in the cis-configuration. While 18:2(n-6) and 18:3(n-3) have been shown to satisfy EFA requirements in the vertebrates, it is their metabolites that satisfy most known EFA functions. There are no specific functions attributed to 18:3(n-3) except as a primary EFA precursor. Linoleic acid (18:2(n-6)), however, in addition to its role as primary EFA precursor, is itself important in maintaining the structural integrity of cell membranes, and thus in regulating water permeability and the activity of membrane-bound enzymes.

For vertebrates, the (n-6) EFAs are "overwhelmingly more important" than the (n-3) EFAs (Horrobin, 1990). For crustaceans, the (n-3) FAs appear to have higher EFA value based on growth and survival studies and on the far greater abundance of (n-3) HUFA in the membrane PLs.

1.2.4 Prostaglandins

Prostaglandins are synthesized from specific PUFAs released from membrane-bound PLs. They are reported to have many important physiological functions in vertebrates and in arthropods other than crustaceans, and are particularly important in reproductive physiology. It is likely that part of the dietary requirement for EFAs is to provide precursors for prostaglandins. Middleditch et al. (1980b) reported that there were no published studies on the occurrence of endogenous prostaglandins in crustaceans, and

no studies have been published since their review. The metabolism and action of prostaglandins in decapod crustaceans is an important area for investigation and will likely provide information on the dietary requirements for (n-6) and/or (n-3) FAs.

1.3 FACTORS AFFECTING THE FATTY ACID COMPOSITION OF Homarus americanus AND OTHER DECAPOD CRUSTACEANS

The lipid composition of the membranes of an organism reflects the type of environment to which the organism has adapted over evolutionary time periods (i.e. genetically determined), as well as reflects endogenous factors and acclimatory responses to seasonal or shorter term fluctuations in environmental parameters (i.e. within the life span of an individual of the species) (Hazel & Prosser, 1974). For crustaceans, several studies conducted since the mid-1960's, have related their FA composition to their habitat (marine vs. fresh water), diet and temperature. However, details of the adaptive significance of these patterns of FA composition (i.e. the functions of the FA molecules at the membrane level), or of the de novo synthesis ability of crustaceans for these FAs under different conditions are not well known. Most studies to determine the FA composition of crustaceans have been performed over very short duration and with undefined nutritional history of the animals. While many factors affect the FA composition of PLs in crustaceans, the following trend is summarized by Chapelle (1986): 16:0 is the major SFA, 18:1 is the major monoene, and 20:5 is the major polyunsaturate. In addition, there is a general tendency for the PL composition of crustaceans to be comprised of "a wide range of HUFAs (primarily C_{20} and C_{22}) of the linolenic (n-3) series".

1.3.1 Endogenous Factors

1.3.1.1 Tissue-Specific Differences

In general, the tissues in decapod crustaceans in which the membrane properties or physiological properties are of primary importance (e.g. nervous tissue, gut epithelium, gills and muscle) are high in PLs. PLs are also the major lipid component of the hemolymph, principally associated with HDLs (high density lipoproteins). PLs comprise ~68% of hemolymph TL in Carcinus maenas (Brichon et al., 1980) and ~ 88% of the HDL_3 lipids of the hemolymph (Lee & Puppione, 1978). In crustaceans, the PLs are typically high in (n-3) PUFAs and HUFAs. In contrast, the hepatopancreas (Guary et al., 1974, Penaeus japonicus; D'Abramo et al., 1980, Homarus americanus) and ovaries (Gehring, 1974, P. duorarum; Jeckel et al., 1989a, Pleoticus muelleri) are high in TAGs, which are accumulated as energy stores and tend to be primarily SFAs or MFAs (Middleditch et al., 1980; Teshima et al., 1989).

Parnova (1982) examined the FA composition of the PLs of the nervous system of four species of decapod crustaceans (three marine crabs and one freshwater crayfish). The predominant FA was 20:5(n-3), compared to the nervous tissue of marine fish, in which 22:6(n-3) is the principal FA. However, Chapelle (1986) reports high levels of 22:6(n-3) in the nervous tissue of the crab Carcinus maenas and the lobster Homarus vulgaris (12.1 and 16.9% of PLs, respectively) as well as high levels of 20:5(n-3) (17.2 and 18.1%, respectively). The other major fatty acids (as percentage of PLs), in C. maenas and Homarus vulgaris, respectively, include 18:1(n-9) (12.2 & 15.8%), 18:0 (10.9 & 7.1%) and 16:0 (12.4 & 15.9%); 20:4(n-6) (6.8 & 5.7%), and 18:2(n-6) is only present at 0.4 & 1.7%.

Morris & Tentori (1985) examined the lipid composition of the epidermis and cuticle of the foregut (cardiac stomach) of the crab Carcinus maenas. The composition of the cuticle (dry weight basis), which acts as a barrier at the gut lumen, is predominantly protein (~50%) and chitin (~41%), and only ~3.5% lipid. Protein and free amino acids make up 51.5% and 5.9%, respectively, of the epidermis, and lipids comprise 12%. In both the cuticle and the epidermis, the total lipid fraction is 75-80% PLs and 11-13% sterols, however the FA composition of the PLs is significantly different between the two tissues. Cuticular PLs are predominantly short chain SFAs (~70% are 14:0 - 18:0) whereas the PLs of the epidermis are mainly long chain PUFAs (C_{18}-C_{20}), of which 20:5(n-3) and 22:6(n-3) comprise more than 40% of the total FAs.

1.3.1.2 Stage of Development

Teshima & Kanazawa (1982) examined the variations in whole body lipid composition during the larval development of the prawn (Penaeus japonicus) and in comparison to pre-spawning levels of ovarian FAs. They noted that the decrease in SFAs and MFAs from ovarian levels through early development may be attributed to utilization of these FAs for energy during embryogenesis and early growth of nauplii. During the same period of development, there was an increase in the proportion of 20:5(n-3) and 22:6(n-3) in the PLs. They postulated that these FAs may be preferentially incorporated into cell membranes and subcellular components during embryogenesis and metamorphosis. These results concur with an earlier study of changes in FA composition during larval development of P. setiferus (Ward et al., 1979).

1.3.1.3 Molt Cycle

O'Connor & Gilbert (1969) investigated alterations in lipid metabolism associated with premolt activity in two decapods: a land crab (Gecarcinus lateralis), and a

fresh-water crayfish (Orconectes virilis). During early premolt the animals exhibited active feeding and a concomitant accumulation of lipids in the hepatopancreas. The crayfish also accumulated lipids in the abdominal muscle. While they did not rule out an increased rate of absorption during premolt of preformed dietary lipids, or an enhanced capacity to incorporate exogenous FAs into tissue lipids, they attributed the increase to an increased capacity of de novo synthesis of both NLs and PLs from lipid precursors, arising from carbohydrate metabolism. There was no change in the FA composition of NL or PL fractions. Teshima et al. (1977), however, demonstrated that the FA composition as well as the ratio of NL to PL changes over the molting cycle in the marine shrimp Penaeus japonicus, with the greatest variation occurring at the intermolt. The variation in lipid synthesis (from [1-^{14}C]-acetate) during the molting cycle of P. japonicus was examined by Patrois & co-workers (1978). They, too, found that de novo lipid synthesis appeared to be greatest during early intermolt and at the beginning of premolt, which they attributed to times of new cuticle and tissue formation. Depletion of accumulated hepatopancreatic lipid reserves from mid-premolt to ecdysis[4] has also been shown for the marine shrimp Pleoticus muelleri (Jeckel et al., 1990).

Changes in the FA composition of gill PLs have been shown to vary with the molt cycle in the amphipod crustacean Gammarus duebeni (Morris et al., 1987). Within 12 hours following ecdysis, and lasting 3-4 days, the gill PLs have lower levels of PUFAs as compared to intermolt animals. They believe this change in the level of FA saturation is a mechanism to reduce water permeability and thus regulate water flux and minimize the potential osmotic stress of ecdysis.

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[4] Ecdysis is the process of molting or shedding of the exoskeleton.

1.3.1.4 Sexual Maturation

An increase in total ovarian lipids with sexual maturation in wild-caught crustaceans has been documented by Pillay & Nair (1973), Guary et al. (1974), Gehring (1974), Kulkarni & Nagabhushanam (1980), Read & Caulton (1980) and Galois (1984). Jeckel et al. (1989a) report that ovary weight increased from 0.63 to 3.47 g during maturation in wild-caught Pleoticus muelleri, with a 4-fold increase in the gonadosomatic index over approximately 10 weeks (rate of 0.10 units per day). Total ovarian lipids doubled from 10.2% dry weight to 19.3% of dry weight during that time.

Teshima et al. (1988a) investigated lipid metabolism in eyestalk ablated prawn Penaeus japonicus. They specifically related the induction of maturation to a ten-fold accumulation of lipid in the ovary.

Several of the above authors describe a decrease in total lipid of the hepatopancreas concomitant with the increase in ovarian lipids during maturation, and suggest mobilization of lipids to explain this. While there is further evidence for mobilization, depletion of the hepatopancreas total lipid could also be explained by the following: a decrease in either dietary lipid or total dietary calories, or a possible increase in energy consumption attributed either to increased biosynthetic activity during maturation, or to an increase in metabolic activity induced by eyestalk ablation, but independent of maturation.

Galois (1984) investigated changes in the lipid composition of the ovary, hepatopancreas, hemolymph and muscle of the shrimp, Penaeus indicus, during vitellogenesis. He concluded that the hepatopancreas lipid reserves contributed only partially to vitellogenesis. Depletion of hepatopancreas NL and PL fractions could not fully account for the increase in hemolymph PLs and profound rise in ovarian NLs and PLs, and

concluded that dietary lipids must be processed rapidly through the hepatopancreas and exported to the ovary.

Several studies which document the FA composition in the organs of different shrimp species have demonstrated that, throughout ovarian maturation, ovarian lipids contained higher proportions of 20:5(n-3) and 22:6(n-3) than the hepatopancreas (Teshima & Kanazawa, 1983; Jeckel et al., 1989a,b). The gonads and muscles of naturally maturing Pleoticus muelleri contained 20:5(n-3) and 22:6(n-3) at 9-14% and 7-12% of total FAs, respectively), and 20:4(n-6) ranged from 4 to 8% in female gonadal tissues, and 7 to 9.5% in the male reproductive tract. Changes in the FA profiles of crustaceans concomitant with induced maturation have been documented by Teshima et al. (1988b).

In most of the above maturation studies, however, nutrition has not been controlled. Thus changes in diet which may occur (in the wild) at the time of ovarian maturation likely contribute to the change in FA composition of the crustaceans. The affects of diet and maturation need to be investigated in a controlled study to determine the relative contribution of each to the changing FA profiles of the crustaceans.

1.3.2 Exogenous Factors

1.3.2.1 Temperature

As with other aquatic poikilotherms, there is a tendency in crustaceans for the concentration of total PLs and the proportion of unsaturated FAs to increase with a decrease in acclimation temperature (Chapelle et al., 1977; Chapelle, 1978a). This is particularly documented for the PL fraction of muscles (Martin & Ceccaldi, 1977) and gills (Morris et al., 1987). Brichon et al. (1980) demonstrated the effect of temperature on the PL classes of the hemolymph of Carcinus maenas acclimated to 13°C or 23°C for two weeks with no food. PC was significantly higher (77.3 vs. 74.8% of PLs) and PE was significantly

lower (12.3% vs. 14.7% of PL) at the higher temperature. These responses to temperature indicate the ability of crustaceans for homeoviscous adaptation in which the effects of temperature on the physical properties of membranes are counteracted by altering the degree of unsaturation of membrane lipids. This in turn affects the fluidity of the membranes, which is important in determining functional properties such as permeability or activity of membrane-bound enzymes.

Homeoviscous adaptation may be an important consideration in determining EFA requirements of Homarus americanus. The standard temperature for rearing lobsters in captivity is ~ 20°C, while in the wild, lobsters are commonly found at much lower temperatures (1-15°C). Part III of this dissertation concerns experiments conducted to investigate the affect of temperature and dietary FAs on H. americanus.

1.3.2.2 Salinity

In general, marine crustaceans contain higher proportions of long chain PUFAs (C_{20}-C_{22}) of the linolenic series while freshwater and terrestrial forms contain predominantly C_{16}-C_{18} SFAs and MFAs (O'Connor & Gilbert, 1968; Teshima et al., 1976; Chanmugam et al., 1983). These differences may be attributed to a response of membrane PL metabolism to external salinity, since regulation of membrane permeability may be achieved by altering the ratio of saturates/unsaturates and thus provide a mechanism for maintaining intracellular osmotic concentrations (Spaargaren & Mors, 1985).

In his review of lipids in marine organisms (1976), Sargent attributes the higher (n-3) PUFA levels characteristic of most marine organisms to the predominantly low and constant temperatures concomitant with life in the open oceans. The contribution of diet must also be considered since marine species tend to have diets higher in long chain PUFAs, which originate in the marine food web from the planktonic organisms (Sargent, 1976).

Studies by Morris & co-workers (1982) on an amphipod crustacean (Gammarus duebeni) found a correlation between gill PLs and long-term salinity acclimation. They attributed the lower proportion of unsaturated FAs in amphipods acclimated to 2% sea water (~ 0.7 ppt salinity) than in those acclimated to 100% sea water (~ 35 ppt) to an adaptive strategy of reducing the permeability across the gills in the very dilute media.

1.3.2.3 Season

Seasonal changes in the lipid levels and FA profiles of several decapods have been reported by Collatz (1972), Guary et al. (1975), Chapelle (1978) and Clarke (1979). In Penaeus setiferus, P. aztecus and P. duorarum there was an increase in the proportion of SFAs during periods of warm water temperatures and a concomitant decrease in monoenes and polyenoic FAs (Bottino et al., 1980). They attributed the response to seasonal changes in diet (and dietary FAs) rather than to a direct effect of temperature on lipid metabolism. This was based on the 2 month lag time in the response of FA profiles to seasonal changes in water temperature and to comparisons with diet composition, of shrimp reared in ponds (Middleditch et al., 1980). Seasonal changes in lipid composition of the whole body as well as of the gonads, hepatopancreas and muscles have also been attributed to redistribution of nutrient reserves concomitant with gonadal maturation in males as well as in females, as in the shrimp Pleoticus muelleri (Jeckel et al., 1991a,b).

1.3.2.4 Diet

Feeding studies conducted with several decapod crustaceans have demonstrated the effect of (a) natural oils or ingredients in practical diets, or (b) purified FAs in semi-purified diets, on the FA profiles of the animals. In general, supplementation of diets with (n-3) FAs results in increased proportions of (n-3) series FAs in the PLs and

NLs of prawn (Penaeus japonicus) Kanazawa et al. (1978, 1979). Supplementation with 18:2(n-6) resulted an increase in the proportion of whole body 18:2(n-6) in the PLs and NLs of prawn (P. japonicus), but no other noticeable changes in FA composition, and no measurable conversion to 20:4(n-6) (1978, 1979). Similar results were attained with Homarus americanus (Castell & Boghen, 1979; D'Abramo et al., 1980). Lobsters incorporated dietary supplements of linoleic acid into their tissues, but there was no apparent bioconversion of linoleate to arachidonate.

1.4 THE NUTRITIONAL PHYSIOLOGY OF H. americanus AND OTHER DECAPOD CRUSTACEANS WITH RESPECT TO DIETARY FATTY ACIDS

1.4.1 Digestion, Emulsification and Absorption of Lipids

Feeding mechanisms and digestion in decapods is reviewed by Gibson (1982). Ingested food is triturated in the posterior, pyloric stomach with the assistance of a chitinous gastric mill. Extracellular digestion of the particles occurs by the action of enzymes secreted by the ß-cells of the hepatopancreas (Gibson & Barker, 1979). A review and model of the dynamics of lipid metabolism in decapods, including digestion, absorption, storage and mobilization of lipids is presented by Harrison (1990); excerpts are included in the summary which follows.

Dietary TAGs and phosphoglycerides (both may include EFAs and non-EFAs) are hydrolyzed in the foregut by lipases secreted from the hepatopancreas (O'Connor & Gilbert, 1968; Brockerhoff et al., 1970). Crustaceans do not produce bile salts. Instead, they produce fatty acyl-sarcosyl-taurines (Vonk, 1969; Lester et al. 1975). These non-cholesterol emulsifiers originate from the hepatopancreas and facilitate the action of the lipases and esterases. The emulsion of gastric juices and particles is sorted such that particles less than 100 nm in diameter pass into the hepatopancreas (Hopkin & Nott, 1980).

The remaining material passes into the midgut which is the only section of the digestive tract not lined with chitin (McLaughlin, 1983; Dall & Moriarty, 1983.).

Although the principal site of absorption into the blood stream is the hepatopancreas, there may be immediate absorption (within 1 to 3 hours after ingestion) of free FAs and mono- and di-acylglycerols into the epithelial cells lining the midgut, i.e. the brush border (Gibson, 1982). In the midgut epithelia, the TAGs are resynthesized and converted to PLs, then subsequently absorbed along with dietary PLs into the hemolymph as HDLs (with a protein to lipid ratio of approximately 1.2 to 1.0). Absorption of fats may be dependent on both chain length and degree of unsaturation of the component FAs. In vertebrates, short chain length FAs and PUFAs are the most readily absorbed.

The monoacylglycerols and free FAs, which are passed along with the fluid digesta into the hepatopancreas, are taken up by R-cells, specialized in resorption (or absorption) and storage of lipids and carbohydrates. Unlike vertebrates, FAs from the hepatopancreas are converted to PLs, and exported as the major lipid component of HDLs (Teshima et al., 1980); these enable solubilization and transport of lipids in the aqueous hemolymph.

There appear to be at least two typical modes of HDL export from the hepatopancreas and an additional mode during vitellogenesis (for details, see Harrison, 1990). Routine, post prandial absorption from the hepatopancreas of FAs from the diet occurs within 12 to 24 hours. TAGs which are stored in the hepatopancreas require breakdown to diacylglycerols (DAGs), subsequent conversion to PLs, and synthesis of carrier proteins, prior to export into the hemolymph as HDLs.

During maturation, certain R-cells of the hepatopancreas specialize as vitellogenocytes and synthesize the principal egg yolk protein, vitellin. The vitellins are apparently combined with polyunsaturated PLs, which may originate from the hepatopancreatic PL reserves, or may be available from dietary FAs. The combined vitellin

protein and PL is termed vitellogenin (a form of HDL), and may be complexed with glycogen and/or carotenoid pigments (which originate from the diet) for export via the hemolymph to the ovaries.

1.4.2 Lipid Mobilization

The net result of lipid mobilization and export as HDLs is a decrease in the total lipid, protein, and glycogen in the hepatopancreas, and an increase in hemolymph PLs. The magnitude of the increase of PLs in the hemolymph varies among species, but in general is not large, as it appears there is rapid uptake of the PLs and proteins by the tissues, particularly the ovaries.

Several important aspects of crustacean lipid metabolism and mobilization, including the location and characterization of the key enzymes, have yet to be resolved. In addition, determining the actions or affects of hormones on lipid storage and mobilization, particularly with respect to the cyclical changes in ecdysial hormones and the hormones involved in gonadal maturation, is required for a better understanding of crustacean lipid nutrition and metabolism.

The question of transfer of hepatopancreas lipid reserves to the ovary during induced maturation of female Penaeus japonicus was investigated by Teshima et al. (1988b) using a double tracer experiment. A single diet application containing labeled palmitic acid, [^{3}H]-16:0, and linolenic acid, [1-^{14}C]-18:3(n-3), was fed to 12 intermolt prawns. Tissue lipids of 3 animals were analyzed 24 hours after feeding to determine immediate fate of dietary FAs. The labels were primarily distributed in the phosphatidylcholines and free FAs of the hepatopancreas, and in the muscle phosphatidylcholines. Of the remaining labeled shrimp, unilateral eyestalk ablation was performed on 3 and 3 were maintained as controls. The distribution of labeled FAs 5 days after induced ovarian maturation was compared with the distribution in non-maturing

adults. Concomitantly, there was a reduction of proportion of label in the hepatopancreas lipids and an increase in label in the ovarian FAs. There was an apparent partitioning of the two labels among the ovarian lipid fractions, with labeled (n-3) FAs primarily in PLs (primarily phosphatidylcholine) and the non-essential, labeled palmitic acid and metabolites, distributed primarily in the TAGs. Although the fate, during maturation, of labeled FAs can be seen from this study, the fraction of labeled to non-labeled FAs in each tissue compartment are not reported, therefore the mobilization of FAs relative to tissue stores (e.g. in the hepatopancreas) can not be determined. Additionally, since the prawn were maintained on short necked clams (Tapes philippinarium), presumably rich in polyunsaturate (n-3) FAs, the relative contributions of the diet and hepatopancreas reserves can not be determined. It would be interesting to repeat this study with second, differently labeled, diet administered one week after the first, to compare the simultaneous relative incorporation of dietary FAs and previously labeled hepatopancreatic FAs, in the NL and PL fractions of mature ovaries.

1.5 NUTRITIONAL REQUIREMENTS FOR FATTY ACIDS IN Homarus americanus AND OTHER DECAPOD CRUSTACEANS

Dietary fats are nutritionally important for supplying energy and EFAs, and as carriers of fat-soluble vitamins. They may also be important in the emulsification and absorption of fat-soluble vitamins, as in vertebrates, but this has not been investigated in crustaceans. Fats are also an important constituent of diets because they impart flavors and affect texture, which in turn affect the attractability, palatability and pelletability of crustacean feeds (for a review of the roles and properties of dietary fats, see Kinsella, 1988). Because fats, particularly PUFAs, are prone to oxidative rancidity natural antioxidants (such as vitamin E) or manufactured antioxidants (such as BHA and BHT) are added to prolong the shelf-life of diets. Storage at cooler temperature and in sealed

containers also reduce the incidence of lipid oxidation. Lipid quality and antioxidant levels are important to consider when evaluating the effect of fats (in prepared diets) on animals. It is also important to consider (and eliminate) trace lipids from all non-fat ingredients in studies to determine EFA requirements.

The nutrient requirements of crustaceans, including lipid requirements, have been reviewed by Conklin (1982) and D'Abramo & Conklin (1985), for Homarus; Sandifer & Smith (1985), for Macrobrachium; and Akiyama & Dominy (1989), for Penaeus. EFA requirements of decapod crustaceans were reviewed by Castell (1982).

1.5.1 Energy

While dietary carbohydrates and proteins as well as lipids can be metabolized by crustaceans for energy (Cuzon et al., 1980; Claybrook, 1983; Akiyama & Dominy; 1989; Lim & Persyn, 1989), carbohydrates and proteins provide only 4-5 Cal/g, while more energy-dense lipids supply ~9 Cal/g. In addition, the efficiency of utilization of each of these substrates, and the optimal dietary balance may vary among crustacean species. Clifford & Brick (1983) review substrate metabolism and the hierarchy of substrate utilization in post-metamorphic crustaceans.

FAs from both triacylglycerides and phosphoglycerides can be cleaved from the glycerol backbone and catabolized for energy. Chapelle (1973) demonstrated the existence of all the enzymes implicated in FA oxidation (with the exception of acyl-CoA synthetase) in the muscle of two crustaceans, the Chinese crab (Eriorcheir sinensis) and the lobster (Homarus vulgaris). Further details of FA oxidation will not be addressed in this review. It should be noted however, that since dietary FAs are an important energy source, studies designed to determine EFA requirements must supply sufficient non-essential fats in the diet to satisfy energy requirements, and thus spare metabolism of EFA for energy.

1.5.2 Essential Fatty Acids

Since the late 1970's, there have been many studies to investigate the optimal lipid levels and FA requirements of decapod crustaceans. The early studies were based on practical diets containing natural oils (e.g. fish oils, vegetable oils) or natural (fresh and frozen) feed ingredients to supply the desired FAs. These studies indicated the importance of lipids, especially (n-3) PUFAs, to decapods for growth (Guary et al., 1976 (Penaeus japonicus); Sandifer & Joseph, 1976, (Macrobrachium rosenbergii). Castell & Covey (1976) demonstrated greater weight gain of adult lobsters (Homarus americanus) fed semi-purified diets supplemented with cold liver oil (high in (n-3) PUFAs) as compared to lobsters fed diets supplemented with corn oil (high in (n-6) PUFA) or hydrogenated coconut oil (which contains predominantly SFAs). Subsequently, semi-purified diets supplemented with purified (n-3) and/or (n-6) FAs were used to test EFA requirements. As discussed in Section 1.3.3, changes in FA composition as well as growth were assessed. These studies indicate that for penaeid shrimp and lobsters, 20:5(n-3) and 22:6(n-3) are EFAs and that their precursor FA, 18:3(n-3), may partially spare the dietary requirement for (n-3) PUFAs. Freshwater species may have a greater requirement for dietary (n-6) FAs (as well as a requirement for (n-3)), based on their FA profiles and the composition of their natural diets.

There has been substantial indirect evidence of the importance of lipids, especially (n-3) FAs, for maturation in decapod crustaceans (Middleditch et al., 1979; Brown et al., 1980; Lawrence et al., 1980). Information on broodstock FA requirements has been deduced from the changes in composition with maturation of the parental organs, especially the gonads and hepatopancreas, and from the composition of the spermatophores, eggs, and larvae from wild caught animals (Pillay & Nair, 1973; Read & Caulton, 1980; Teshima & Kanazawa, 1983; Teshima et al., 1989; Jeckel et al., 1989 a,b; Castille & Lawrence,

1989). Other clues to broodstock FA requirements are provided by research on lipid metabolism during maturation (Kanazawa, 1986; Teshima et al., 1988a), and on the endocrinology of reproduction and vitellogenesis (Charniaux-Cotton, 1985; Quackenbush, 1989). From the combined evidence of the presence of (n-3) and (n-6) PUFAs in gonadal tissues, the established essentiality of these FAs to crustaceans (Zandee, 1966; Kanazawa & Teshima, 1977), and indications of the direct influence of dietary FAs on the FA composition of the gonads and eggs, the importance of a maturation diet high in PUFAs, especially (n-3) FAs, can not be overemphasized.

Natural diets containing marine lipids are unlikely to be deficient in the (n-3) PUFAs presumed essential for crustaceans. However, in captivity, crustaceans fed solely on manufactured diets, may be subjected to EFA deficiency. Further research in needed to determine the appropriate levels of supplementation of the specific FAs required at each developmental stage.

1.5.3 Dietary Phospholipids

Although lobsters (Homarus americanus) have been shown to have the ability to synthesize PLs (Shieh, 1969), a deficiency syndrome, termed "molt death" has been demonstrated by D'Abramo et al. (1981) and described by Bowser & Rosemark (1981). In addition to the inability of the lobsters to extricate from their exoskeletons at ecdysis, lobsters fed diets deficient in phosphatidylcholine have significantly decreased levels of hemolymph PLs and cholesterol. As explained in Section 1.1.4, while PLs may appear to be essential, based on the deficiency syndrome, it is likely that they compensate for other dietary deficiencies by preserving the integrity of the diet, and assisting in the emulsification and absorption of other nutrients, as well as sparing the requirement for certain FAs or choline.

1.6 RESEARCH STRATEGY

Several key issues regarding EFA metabolism and requirements in Homarus americanus and other crustaceans remain unanswered. First, in the previous sections evidence was presented from many researchers showing that diets deficient in (n-3) and and/or (n-6) FAs increase mortality, depress growth, alter FA composition, and possibly diminish reproductive capacity of crustaceans. However, the classical EFA deficiency symptoms and syndromes characterized in mammals and fish, have not been described for invertebrates, including crustaceans. For crustaceans in general, nutrition-related pathologies are poorly documented and not well understood. Identifying and understanding the nature of deficiency symptoms can aid in clarifying the specific mechanisms of action and the roles of EFAs in crustaceans. The experiments of this research program (Parts II and III) were designed and executed in order to observe and characterize specific symptoms characteristic of EFA deficiency in lobsters. In particular, several experiments were conducted in conjunction with the second feeding trial, to investigate the effects of (n-3) and/or (n-6) FA deficiency at different levels of biological organization, including whole-animal effects (survival, growth, molting), physiological or metabolic effects (respiration rate and substrate metabolism), structural effects (integrity of the hepatopancreas and gills), and compositional changes in the NL and PL fractions of the FAs of several tissues (muscles, gills, and hepatopancreas). In addition, radioisotope experiments were conducted to investigate the ability for bioconversion of C_{18} (n-3) FAs to C_{20} and C_{22} FAs.

A second issue, not previously investigated, was the effect of balance of dietary (n-3) and (n-6) FAs on Homarus americanus lobsters. Aspects of EFA balance and the results of the first feeding trial, designed to address this issue, are discussed in Part II.

The role of membrane FAs in temperature acclimation in poikilothermic animals is a third issue currently under investigation. Temperature has been shown to affect the FA composition of several crustaceans, as previously discussed in Section 1.3. However, the effect of temperature on dietary requirements of crustaceans for EFAs has not been previously reported. The second feeding trial (Part III) was conducted at 10°C and at 20°C to investigate the effect of temperature on EFA metabolism and requirements in H. americanus.

1.7 OVERALL OBJECTIVES

1. Investigate the fatty acid nutrition and metabolism in juvenile American lobster (Homarus americanus)

2. Investigate the effect of dietary fatty acids on fatty acid profiles of whole animals and tissues in H. americanus

3. Characterize physiological or pathological effects of essential fatty acid deficiency based on FA profiles and deficiency symptoms

4. Investigate the effect of temperature on essential fatty acid requirements in H. americanus

5. Make recommendations regarding the inclusion of lipids in practical diets for lobster culture and in semi-purified diets for nutrition research

PART II

THE EFFECT OF THE LEVEL AND BALANCE OF DIETARY POLYUNSATURATED AND HIGHLY UNSATURATED (n-3) AND (n-6) FATTY ACIDS ON THE LOBSTER, H. americanus

GENERAL INTRODUCTION

The levels of inclusion of (n-3) and (n-6) fatty acids required in diets for good growth and survival have been determined for several crustacean species but have yet to be determined for the lobster Homarus americanus. Castell & Covey (1976) raised adult lobsters for ten months on semi-purified diets supplemented with either 1, 5, 10 or 15% cod liver oil [CLO, high in (n-3) FAs], 10% corn oil [CO, high in 18:2(n-6)], or 10% hydrogenated coconut oil (HCO, predominantly medium and short chain SFAs). They found that lobsters fed the CLO diets had the highest mean percent weight gain, molting rate and feed conversion ratio, and that the optimal level of inclusion was 5%. Performance of lobsters fed the CO diet exceeded that of lobsters fed the HCO diet, indicating that 18:2(n-6) may have some limited value (as essential fatty acids) to lobsters.

Using semi-purified diets and purified fatty acids, Castell & Boghen (1979) attempted to determine the requirements of juvenile lobsters for 18:3(n-3), 18:2(n-6), and 22:6(n-3). Unfortunately, there was high mortality and poor growth of lobsters on all diet treatments, attributed to an amino acid deficiency. The fatty acid profiles of surviving lobsters were unusual compared to those of other marine crustaceans, and interpretation was speculative. Most notable were the high levels of 16:1(n-3) and 20:3(n-3) which the authors suggested were the result of retroconversion from 18:3(n-3) and 22:6(n-3), as an acclimation response to the relatively high culture temperature (20°C) in comparison to much lower ambient temperatures in the wild. The results did not yield clear evidence as to

whether linolenate and/or linoleate, or docosahexaenoate are essential for juvenile lobsters. What was evident is that dietary fatty acids have an affect on whole animal fatty acid composition and that the inclusion of (n-3) or (n-6) fatty acids in the diet appeared to affect the metabolism of fatty acids from the alternate series. The authors recommended that the optimum dietary ratio of (n-6) to (n-3) fatty acids be determined.

The balance of dietary (n-3), (n-6), and (n-9) fatty acids is known to be important for many species. The biochemical basis for the critical nature of the balance is reviewed by Gurr & James (1980). In the body, oleate, linoleate and linolenate are metabolized along parallel biochemical pathways (Figure 2). The same enzymes are believed to act on equivalent substrates in each of the pathways to catalyze desaturation of the substrates, which alternates with 2 carbon elongation, to produce HUFA metabolites. Since these endproducts have varied roles and effectiveness as EFA, the rate and amount of their production (or direct intake by the animal) is critical.

The key regulatory enzyme in the metabolism of 18:2(n-6), 18:3(n-3), and 18:1(n-9) is Δ^6-desaturase. Desaturation is the rate-limiting step in the biosynthetic pathways of the (n-9), (n-6) and (n-3) series (Figure 2). In vertebrates, there is competition among oleate, linoleate and linolenate for this rate-limiting enzyme, with affinity of this enzyme for the substrates in the order 18:3 >18:2 >> 18:1 (i.e., there is a greater affinity of the desaturase enzymes for the most unsaturated fatty acid of a particular chain length). As a consequence, there is competitive inhibition by high levels of a substrate from one series on the metabolism of equivalent substrates from the other series. For this reason, not only the level of inclusion, but also the ratio of dietary fatty acids, is critical in satisfying EFA requirements. For example, a diet high in oleate can induce EFA deficiency by monopolizing available enzyme and reducing the level of (n-6) and (n-3) metabolites. In the same way, high levels of dietary (n-3) series fatty acids may reduce the desaturation of 18:2(n-6) and thus block the formation of arachidonate and the synthesis of prostaglandins

(Lands, 1991). As a result, dietary linoleate will accumulate, as well as 20:5(n-3) and 22:6(n-3) in species which are capable of their biosynthesis.

Lytle et al. (1990) and Harrison (1990) review evidence from studies on terrestrial vertebrates and on marine shrimp which suggests that the balance of dietary (n-3)/(n-6) FAs may be critical in inducing maturation of marine shrimp. While supplementation of diets with ingredients high in (n-3) fatty acids has been effective, Middleditch et al. (1979) suggested that arachidonic acid, 20:4(n-6), might be a key PUFA for shrimp maturation. This suggestion is based on high levels of arachidonate in the ovaries of wild caught shrimp, and on the well documented role of arachidonate in vertebrates as a precursor to prostaglandins. If arachidonate is important in crustacean reproduction, then excessive levels of (n-3) fatty acids may inhibit some reproductive activity (e.g. gonad maturation, oogenesis, spermatogenesis, spawning, etc.) unless there is a proper balance of (n-3) to (n-6) FAs in the diet.

Other dietary factors may also affect lipid metabolism. For example, the total level of dietary fat can affect the metabolism of essential fatty acids, since adequate energy levels (i.e. NADH, NADPH) are required for FA synthesis and for desaturation. During fasting, for example, there is a decrease in the synthesis of proteins, including desaturase enzymes, which in turn reduces the formation of PUFAs. Conversely, EFA-deficiency symptoms may be enhanced by a diet high in non-EFA fat, particularly saturated fat.

The level and quality of dietary proteins may also affect lipid metabolism in crustaceans, although these have not been investigated. For example, in vertebrates, lipoprotein formation is depressed on low protein diets. This results in lipemia (a build up of insoluble fatty acids in the blood). The same effect in crustaceans could impair absorption into the hemolymph and subsequent distribution of EFAs to the tissues, since the predominant form of lipid absorption and transport is as high density lipoproteins

(HDLs) (see Harrison, 1990, for review). ß-oxidation, or the utilization of dietary fats for energy, may depend on protein quality, since in vertebrates, it is depressed by diets deficient in methionine and lysine. Also in vertebrates, Δ^6-desaturase activity is enhanced by high protein diets. If this is true for crustaceans, then assessment of this ability to bioconvert (n-3) and (n-6) series PUFAs to HUFAs may also be limited by the protein level and quality of the test diets. It is evident from Castell & Boghen (1979) that protein quality can affect the determination of fatty acid requirements in crustaceans. Subsequent research by Boghen et al. (1982), Kean et al. (1985), and Castell et al. (1989), has investigated the efficacy of various sources of protein for crustacean test and reference diets. Purified rock crab protein (Cancer irroratus), at 40% (DWB), used in this study meets minimum protein and amino acid requirements for lobsters (Castell et al., 1989), although its effect on lipid metabolism remains untested.

To test the effect of the level and balance of dietary 18:3(n-3), 18:2(n-6), and 22:6(n-3) on juvenile lobsters, a feeding experiment was conducted. The diets and culture conditions are described in Chapter 1, in which the effect on survivorship and growth were examined. In Chapter 2, the effect of these diets on the FA composition of whole animal NLs and PLs was investigated.

CHAPTER 1

The Effect of the Balance of Dietary Fatty Acids on Growth, Molting and Survival

1.0 INTRODUCTION: PART II, CHAPTER 1

In this chapter, a feeding experiment is reported which tested the effect of 10 diets on the growth rate and survival of juvenile lobsters (H. americanus) reared at 20°C. The diets contained ~9% lipid as fatty acids, of which 8% was hydrogenated coconut oil (HCO) fatty acids and ~1% was either 18:3(n-3), 18:2(n-6), 22:6(n-3), or a mixture of these (n-3) and (n-6) fatty acids in various ratios.

1.1 OBJECTIVES

1. Compare the nutritional value for juvenile lobsters (Homarus americanus) of different levels and proportions of dietary (n-6) and (n-3) fatty acids by measuring the effects on growth and survival.

2. Compare the effect on growth and survival of a dietary source of longer chain, (n-3) highly unsaturated fatty acids (docosahexaenoate, 22:6(n-3)) with a precursor fatty acid (linolenate, 18:3(n-3)).

3. Determine the effect of a fat-free diet and an essential fatty acid-deficient diet on growth and survival of lobsters.

1.2 MATERIALS AND METHODS

1.2.1 SOURCE OF ANIMALS

Larvae were hatched from a single ovigerous female captured (authorized by special permit) off eastern Nova Scotia, Canada. Larvae from the 2nd, 3rd, and 4th days of hatching were reared in batches, at 20°C, at the Halifax laboratory of the Department of Fisheries & Oceans, Canada as described by Castell (1977). They were transferred to clean tanks and fed newly hatched Artemia nauplii, daily (Biomarine Aquafauna, Lot 644305). After approximately 10 days the larvae metamorphosed to the fourth

(postlarval)[5] stage and were transferred to the Aquatron seawater facility at Dalhousie University and acclimated overnight to allow recovery from shipping. (Permission for live animal holding and experimentation was granted by the Dalhousie University Animal Care Committee).

1.2.2 WET LABORATORY AND REARING CONDITIONS

Experiments were conducted in a wet lab at the Aquatron facility. The wet lab (Figure 3-A) was designed by Kim Harrison with the assistance of fellow graduate student Mr. Andrew Forsythe, and was constructed (carpentry and plumbing) by K. Harrison and fellow graduate student Mr. Kim Bell. Trays and tanks were supplied by J. D. Castell, DFO, Halifax, NS and by W. Patterson, president, Aquahealth, Ltd., PEI. The trays were manufactured to specifications by Maritime Plastics, Halifax, NS.

Triplicate sets of ten trays were randomly assigned one of ten test diets. Two additional trays held spare lobsters. The trays were immersed in fiberglass tanks (which were partitioned with a plexiglass divider to accommodate two trays per tank, with no water exchange between the two sections of the tank). Each tray was on legs to allow water flow through the screen on the tray floor. The trays were partitioned with plastic screens to form 20 compartments within each tray; the dimensions of each compartment were 1" long by 4" wide by ~3" of water depth above the tray floor (1" = 2.54 cm). Each compartment received separate inflow of continuously flowing, heated seawater (20°C; ~ 31 ppt) from a common header tank. Photoperiod was controlled by a clock timer set at 16 h light:8 h dark.

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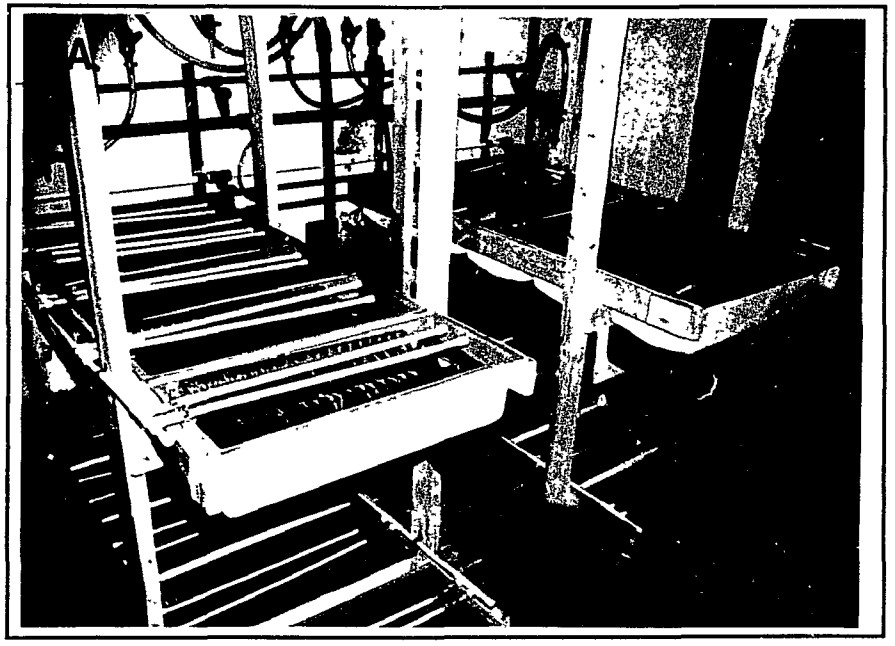
[5] Homarus americanus larvae metamorphose at the third molt into Stage IV (an adult-like form, bearing claws and exhibiting active swimming behavior), but remain predominantly planktonic and do not settle until after the fourth molt, into the benthic Stage V (termed juveniles in this treatise) (see discussion by Sasaki et al., 1986, regarding the debate in staging terminology).



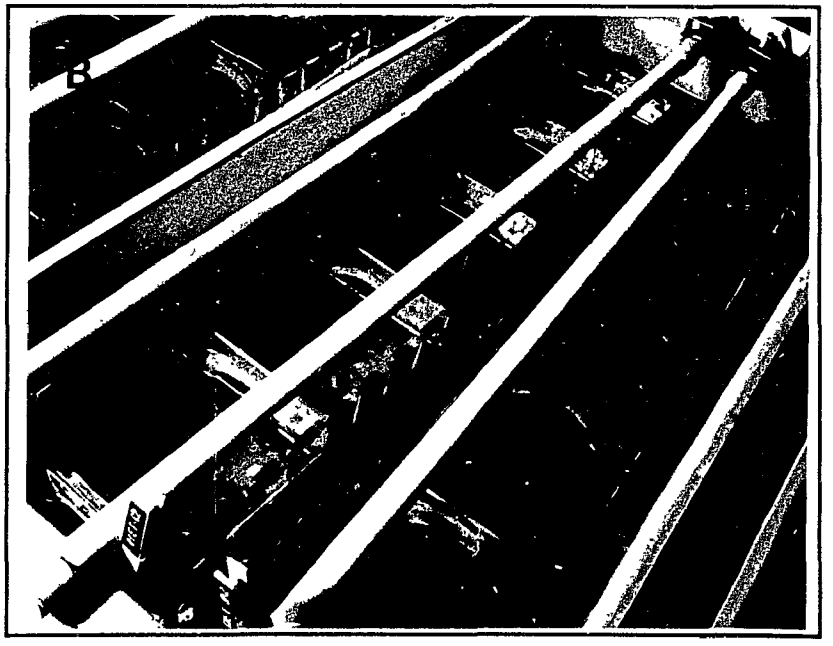


Figure 3. Wet Laboratory at the Dalhousie University Aquatron Facility, Halifax, NS.

A. View of wooden racks supporting four banks of five fiberglass tanks. Each tank is divided into three sections by a fitted plexiglass "T" partition; there is no mixing of water between the sections. The end section receives overflow from the tank sections and has a stand-pipe drain. B. View of tanks showing a plexiglass tray (4" x 4" x 20") in each section. Trays stand 1" off the bottom; water depth in tray,~3". Lobsters (a) are held in separate compartments (4" x 4", Part III, Ch.1) formed by plexiglass partitions with plastic screen windows. Each compartment receives separate seawater inflow (c) from 0.5"dia. pvc pipe, fed by one of two header tanks (set for 10°C or 20°C).
C. View of tray showing lobsters (a) and shed exuvia (b) in 1" x 4" compartments (Part II, Ch.1), and plastic screen tray bottoms.

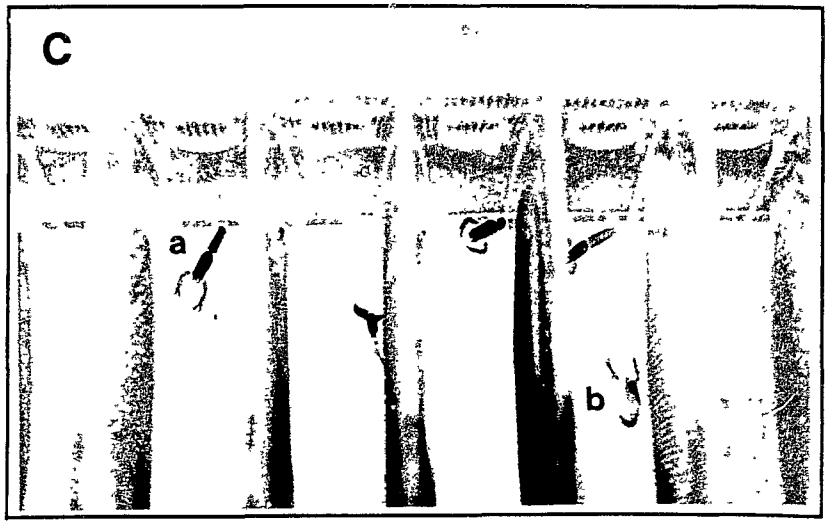


Photo credits: Cdr. J.L. Harrison Reprints: Mary Primrose

Individual, intact, injury-free, juvenile lobsters were selected, blotted dry, weighed to the nearest mg, and randomly allocated to one of 32 trays. Each individual was thereafter maintained in its separate coded compartment (Figure 3-B) within the plexiglass trays. The juvenile lobsters were maintained on the Halifax Crustacean Reference Diet (H-CRD) for approximately 10 days, until the start of the feeding experiment. They were then fed a fat-free 'conditioning' diet for 25 days in an attempt to deplete fat (and possibly EFA) reserves.

1.2.3 DIETS: INGREDIENTS, PREPARATION AND ANALYSIS

Nine semi-purified test diets were prepared, including a fat-free diet (Diet A), an EFA-deficient diet (Diet B) to which was added 9% hydrogenated coconut oil (HCO), and seven diets to which were added a lipid premix containing HCO and purified FA methyl esters. Lipid premix formulations are given in Table 1, and basal diet formulations and ingredient sources are given in Table 2. Diets were prepared separately, in sequence, from Diet A to Diet I.

To eliminate potential lipid contaminants, all glassware and mixing tools were rigorously washed and rinsed with hexane, then with acetone. Prior to inclusion in the basal diet, the gelatin, cornstarch, and α-cellulose were extracted with hot isopropanol (IPA) to remove any trace lipids and rinsed with ethanol to remove any residual IPA (which could be toxic to the lobsters), then dried (see details of method of extraction, Appendix 1-A). The vitamin mix (Appendix 1-B) was prepared and refrigerated, and the Bernhart-Tomarelli commercial mineral mix (Bernhart-Tomarelli, 1966) was finely milled.

The basal diet was mixed and finely milled. Each diet was then prepared separately. A portion of basal mix was weighed into a stainless steel mixing bowl. A lipid

Table 1.

Lipid Premix Formulations of Diets from Feeding Experiment I[1].

Diet	% Added (DWB)[2,3]					
	Vit E[4]	HCO[5]	18:2(n-6)	18:3(n-3)	22:6(n-3)	(n-3)/(n-6)
A	0.2	---------- Fat - Free Diet ----------				NA
B	0.2	9	0	0	0	NA
C	0.2	8	0	1	0	1/0
D	0.2	8	0.14	0.86	0	6/1
E	0.2	8	0.25	0.75	0	3/1
F	0.2	8	0.40	0.6	0	1.5/1
G	0.2	8	1	0	0	0/1
H	0.2	8	0	0	1	1/0
I	0.2	8	0.25	0	0.75	3/1

1. Lipid premixes constituted 9.2% of diets (DWB)
2. Source are cited in Table 2
3. Methyl ester derivatives of the fatty acids
4. Vitamin E is lipid soluble and a natural antioxidant
5. Hydrogenated coconut oil

Table 2.

Ingredient Composition of Diets from Feeding Experiment I.

Ingredient	% Dry Weight	Source[1]	Lot Number
Basal Dry Mix	90.8		
Crab Protein[2]	40.0		
Gelatin[3]	10.0	ICN	10578
Cornstarch[3]	15.0	ICN	7202
α-Cellulose[3]	16.8	ICN	25637
Mineral Mix[4]	5.0	USBC	36799
Vitamin Mix	2.0	Formulation	(see Appendix 1-B)
Cholesterol	1.0	JTB	051352
Choline Chloride	1.0	USBC	37900
Lipid Pre-Mixes	9.2		
DL-α-Tocopherol	0.2	USBC	34439
HCO[5]	(Table 1)	NBC	5756
18:2(n-6)[6]	(Table 1)	NCP	U-59M
18:3(n-3)[6]	(Table 1)	NCP	U-62A-MA14-4
22:6(n-3)[6]	(Table 1)	NCP	U-84A-F24-4

1. ICN (ICN Pharmaceuticals, Life Sciences Group, Cleveland, Ohio 44128), JTB (J. T. Baker, Philipsburg, N.J. 08865), NBC (Nutrition Biochemicals Corporation, Cleveland, Ohio 44128), NCP (Nu-Chek-Prep, P.O.Box 295, Elysian, MN 56028), USBC (U.S. Biochemicals Corporation, Cleveland, Ohio 44128).
2. Protein from rock crab, Cancer irroratus (Castell et al., 1989b)
3. Ingredient extracted by hot isopropanol, followed by an ethanol rinse, then dried and finely milled (extraction method, see Appendix 1-A)
4. Bernhart-Tomarelli salt mix, modified (Bernhart and Tomarelli, 1966) (Appendix 1-C)
5. Hydrogenated coconut oil
6. Methyl ester derivatives of the fatty acids

premix was then prepared under nitrogen atmosphere. The HCO (solid at room temperature) was weighed into a glass beaker, and heated at low temperature until melted. Vitamin E was added to the beaker, followed by the designated FA(s).

The mixing bowl (with basal diet) was placed on a hot plate and boiling water (3 parts) was stirred into 2 parts of the basal diet mix (i.e. ~60% moisture) to activate the binding properties of the gelatin. The lipid pre-mix was added quickly, and the diet was mixed with an electric hand mixer for ~ 1 minute.

Each diet was packed into a separate plastic container, which was then firmly tapped against the counter to eliminate air bubbles and air pockets. The diets were then refrigerated for 24 hours. Once set, a stainless steel cork borer (~ 1.2 mm dia. bore) was used to cut cylinders of the gelatenized diet. The cylinders were inserted into a manual spaghetti extruder, and compressed (by a hand lever) through a die, into strands.

Diet A (fat-free) and Diet B (EFA-deficient) were freeze-dried. After debate as to whether freeze-drying might affect the PUFAs added to Diets C through I, it was decided that they be frozen (at -20°C) in their moist state. After freezing, the strands of Diets C through I were stored in rubber ring-sealed glass canning jars at -20°C. The frozen strands were chopped, as needed, into ~3-5 mm length pieces. The pieces were fed to each lobster, using forceps which were washed between handling each diet.

Proximate composition was determined on duplicate samples of each diet (Table 3). Moisture was determined by drying samples to constant weight at 100°C. Samples were then ashed at 550°C for 5 hours. Kjeldahl analysis for protein content was performed by J. C. Kean, Department of Fisheries & Oceans Canada, Halifax Lab. Samples were analyzed on a Tecator Digestion System 40, 1016 Digester coupled with a Tecator Autostep; 1012 Controller. Total lipid was determined by a modified Bligh & Dyer (1959) (Appendix 2-

Table 3.

Proximate Composition of Diets from Feeding Experiment I[1].

Diet	% Moisture	% Dry Matter	% Ash[2]	% Lipid[2]	% Crude Protein[2]
A	8.0 ± 0.1	92.0	10.4 ± 0.0	1.1 ± 0.2	~50.0
B	6.0 ± 0.2	94.0	10.4 ± 0.2	10.3 ± 0.4	~50.0
C	50.2 ± 2.0	49.8	10.7 ± 0.4	10.2 ± 0.2	~50.0
D	50.1 ± 7.1	49.9	10.7 ± 0.2	10.4 ± 0.4	~50.0
E	51.8 ± 4.7	48.2	10.6 ± 0.0	10.4 ± 0.3	~50.0
F	53.4 ± 0.7	46.6	10.7 ± 0.0	10.4 ± 0.1	~50.0
G	51.3 ± 5.7	48.7	10.6 ± 0.3	10.5 ± 0.3	~50.0
H	56.2 ± 3.4	43.8	10.5 ± 0.2	10.4 ± 0.4	~50.0
I	56.8 ± 0.9	43.2	10.7 ± 0.2	10.4 ± 0.3	~50.0
J	9.1 ± 0.5	90.9	7.6 ± 0.0	10.4 ± 0.4	~50.0

1. Means of 2 replicates ± Std. Dev.

2. Calculated on dry matter basis

A). FA analysis of diets is reported in Section 1.3.1 and Table 4, methods are reported in Chapter 2.

1.2.4 MAINTENANCE AND MONITORING OF ANIMALS

Lobsters were fed twice daily at ~2.5% body weight/day. Trays were siphoned before each feeding to remove waste matter and uneaten food, the tanks were flushed and refilled rapidly. Mortalities, molts, and general observations were recorded at each feeding. Each compartment received separate water inflow from overhead pipes, and trays were rotated within each tank once daily, to equalize the possibility of "downstream" effects of water flow. Weekly, lobsters were transferred to clean trays and placed in a holding tank while their tanks were thoroughly hosed with hot tap water, and scrubbed with brushes.

All lobsters were weighed individually every 3-4 weeks. Lobsters from one of the triplicate sets of 10 trays (i.e. 200 maximum lobsters per set) were weighed each day (i.e. total weighing time was three days).

1.2.5 TERMINATION OF EXPERIMENTS

Lobsters were weighed then killed either when the mortality in the tray in which they were housed reached 50% (10 animals), or after 120 days (at which time the biomass of animals on the diet with best growth performance, i.e. Diet J, the H-CRD, had increased by 3-fold. Food was withheld from lobsters for 24 hours prior to killing to allow time for gut clearance. Lobsters were killed by freezing (in glass vials); they were stored frozen prior to lipid analysis.

Table 4.

Fatty Acid Composition of Diets from Experiment I (as % of Diet, DWB).

Fatty Acids	Diets									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
10:0	-	0.50	0.46	0.30	0.30	0.42	0.40	0.50	0.43	-
12:0	-	4.77	4.40	4.45	4.45	4.29	4.49	4.51	4.52	-
14:0	< 0.005	1.87	1.68	1.76	1.76	1.78	1.70	1.71	1.70	0.23
16:0	-	0.91	0.79	0.80	0.80	0.18	0.16	0.16	0.16	1.09
18:0	< 0.005	0.19	0.02	0.06	0.04	0.08	0.13	0.14	-	0.12
20:0	-									0.02
Σ SFA	-	8.24	7.35	7.37	7.35	6.75	6.88	7.02	6.81	1.46
14:1	-	-	-	-	-	-	-	-	-	0.03
16:1	-	-	-	-	-	-	-	-	-	0.53
18:1	-	0.62	0.52	0.55	0.52	0.58	0.50	0.50	0.50	2.33
20:1	-	-	-	-	-	-	-	-	-	0.61
22:1	-	-	-	-	-	-	-	-	-	0.35
Σ MFA		0.62	0.52	0.55	0.52	0.58	0.50	0.50	0.50	3.85
18:2(n-6)	< 0.005	0.12	0.13	0.32	0.34	0.55	0.84	0.12	0.33	2.31
20:2(n-6)	-	-	-	-	-	-	-	-	-	0.02
20:4(n-6)	-	-	-	-	-	-	-	-	-	0.02
Σ (n-6)	-	0.12	0.13	0.32	0.34	0.55	0.84	0.12	0.33	2.35
18:3(n-3)	-	-	0.83	0.68	0.61	0.43	-	-	-	0.06
20:5(n-3)	-	-	-	-	-	-	-	-	-	0.60
22:5(n-3)	-	-	-	-	-	-	-	-	-	0.03
22:6(n-3)	-	-	-	-	-	-	-	0.66	0.54	0.67
Σ (n-3)	-	0.00	0.83	0.68	0.61	0.43	0.00	0.66	0.54	1.36
Others	-	0.01	0.02	0.02	0.04	-0.09	0.03	0.04	0.04	0.38
U:S	-	0.09	0.20	0.21	0.20	0.23	0.19	0.18	0.20	5.18
P:S	-	0.01	0.13	0.14	0.13	0.15	0.12	0.11	0.13	2.54
(n-3)/(n-6)	-	-	6.38	2.13	1.79	0.78	-	5.50	1.64	0.58

U:S = Ratio of Σ Unsaturated FAs to Σ SFAs
P:S = Ratio of Σ PUFAs to Σ SFAs
(n-3)/(n-6) = Ratio of Σ (n-3) fatty acids to Σ (n-6) fatty acids

1.3 RESULTS: DIET COMPOSITION, SURVIVAL AND GROWTH

1.3.1 DIET COMPOSITION

The total lipid contents of the semi-purified test diets (Diets B through I) and the Crustacean Reference Diet (H-CRD) were 10.4 -10.5% DWB (Table 3). In addition to the HCO and FA methyl esters, added at a total of ~ 9%, other lipid sources are the fat-soluble vitamins, A, D, E, and K, contributing approximately 0.24%, and cholesterol, added at 1%. The analyzed total lipid level of the "fat-free" diet (Diet A) was 1.1% DWB.

A summary of the FA analysis of diets (DWB) is reported in Table 4. Appendices 1-D and 1-E give the means and standard deviations of FAs as a percentage of dietary FAs and as a dry weight percentage, respectively. Although lipid was first extracted from the appropriate basal diet ingredients, the "fat-free" diet nevertheless contained trace amounts of 18:2(n-6), likely originating from the 1% cholesterol. However, 18:2(n-6) (~36.5% of trace FAs) was estimated to be $\leq$ 0.005% of diet, DWB. Also, the HCO, which was added at 9% DWB to Diet B and at 8% DWB to Diets C-I, contributed a background level of 18:2(n-6) to these diets at 0.12 - 0.13% of dry weight. Thus, the response to a diet totally deficient in linoleate could not be assessed. Due primarily to this background level of linoleate, and partially to the difficulty of mixing and adding the appropriate amounts of purified FAs to the mix, the desired (n-3) to (n-6) ratios were not attained for all the designated diets. Nevertheless the range of intended levels and intended ratios were tested, with the exceptions of not producing a 18:2(n-6)-free diet containing 18:3(n-3), and a totally EFA-free diet.

In summary, Diet A contained ~1.1% lipid, and was deficient in (n-3) or (n-6) FAs (< 0.005% of diet as linoleate). Diets B contained ~9% FAs, which constituted 8.25% SFAs, 0.62% C_{18} MFAs, and 0.12% 18:2(n-6) in the diet on a dry weight basis. Diets C

through I contained ~ 8% FAs, of which ~7.4 - 7.7% was SFAs, 0.5 - 0.6% was C_{18} MFAs, and 0.8 - 1.0% PUFA. The (n-3) to (n-6) ratio of Diets C - G, containing 18:3(n-3) and 18:2(n-6), was Diet C - 6.4, Diet D - 2.1, Diet E - 1.8, Diet F - 0.8, and Diet G - 0.1. Diets H and I, containing 22:6(n-3) and 18:2(n-6), had (n-3) to (n-6) ratios of 5.5 and 1.6, comparable to Diets C and E, respectively, but substituting an (n-3) HUFA for the (n-3) PUFA. The crustacean reference diet, Diet J, contained 10.5% lipid, of which an estimated 9% was FAs. The FAs constituted ~1.47% SFAs, 3.82% monoenes, and 3.37% PUFA (2.31% 18:2(n-6) and 0.96% C_{20} and C22 (n-3) FAs).

1.3.2 SURVIVAL

After three weeks on a fat-free diet and an additional seven weeks on one of ten test diets, there was less than 3% mortality among the 600 juvenile lobsters in the experiment. The mortality rate among lobsters fed the EFA-deficient diet increased after 10 weeks to ~3%/day, and reached 50% cumulative mortality in the 14^{th} week. Among lobsters fed the fat-free diet, mortality commenced after 11 weeks and reached 50% in the 15^{th} week.

Survival was 90% or greater among lobsters fed diets supplemented with ~1% of (n-3) and/or (n-6) FAs (Table 5). Although not statistically different, survival was lower among lobsters on Diets C, G, and H, which were supplemented with predominantly a single PUFA (18:3(n-3), 18:2(n-6) and 22:6(n-3), respectively, having (n-3) to (n-6) ratios of 6.4 to 1, 1 to 10.5, and 5.5 to 1) than among lobsters fed Diets D, E, F and I, to which an intermediate balance of (n-6) and (n-3) were added. The highest survival rate was among lobsters fed diet J (98%) the H-CRD.

1.3.3 GROWTH

There was high variability among the initial weights of individuals in each treatment (Table 6). Analysis of variance of the initial weights showed that there was no significant

Table 5.

Weight Gain and Mortality Data from Lobsters Reared at 20°C for 100 Days on One of Ten Diets
The initial n of 60 represents 3 trays of 20 lobsters/tray (in separate compartments).

Diet		A*1	B*1	C	D	E	F	G	H	I	J
Fatty Acid	18:2(n-6)	0	0.12	0.13	0.32	0.34	0.55	0.84	0.12	0.33	2.31
Supplement	18:3(n-3)	0	0	0.83	0.68	0.61	0.43	0.08	0	0	0.06
(% of diet)	22:6(n-3)	0	0	0	0	0	0	0	0.66	0.54	0.67
	(n-3)/(n-6)	NA	NA	6.4	2.1	1.8	0.8	0.1	5.5	1.6	0.5
Initial wt	Mean*2	59	59	60	62	57	58	59	59	58	59
(mg)	SD	±7	±9	±9	±10	±9	±9	±9	±8	±8	±9
	n	60	60	60	60	60	60	60	60	60	59
Final wt	Mean	211	165	221	242	222	218	213	306	315	428
(mg)	SD	±60	±38	±60	±64	±62	±63	±49	±75	±63	±105
	SEM	17	11	8	9	8	9	7	11	9	14
	n	12	11	52	55	54	51	53	51	54	56
% Weight*3	Mean	362	272	372	401	388	370	366	526	539	737
gain	SD	±102	±38	±97	±118	±97	±89	±70	±139	±96	±173
% Mortality		>50%	>50%	10	3	5	3	7	10	5	2
% Biomass increase*4		216	149	319	360	352	322	321	440	477	691

*1. Two of three replicate trays of 20 initial lobsters were terminated when mortality reached 50%. Final weight data, % Weight gain, and % Biomass increase are based on survivors in one tray.

*2 Means represent all lobsters from the 3 replicate trays per treatment. There was no sig. dif. between trays , $p \leq 0.05$.

*3 % Weight gain = [(final wet weight - initial wet weight)/initial wet weight] x 100

*4 % Biomass increase = [(total final weight)-(total initial weight)/total initial weight] x 100

difference in mean weight of lobsters among the 30 trays (overall mean was 0.46 g ± 0.10).

ANOVA of final weights after 120 days showed a significant effect of diet. Lobsters fed Diet B, the EFA-deficient diet, grew significantly less than lobsters on all other diets, including the fat-free diet, Diet A, and lobsters fed the H-CRD showed significantly greater weight gain than lobsters fed all other diets. There was no significant difference of mean final weight among lobsters fed fat-free Diet A or Diets C-G which contained ~1% of 18:3(n-3), 18:2(n-6) or a combination of both FAs (Figure 4). There was a significant growth enhancement effect of Diets H and I, which contained 22:6(n-3), as compared to diets containing only C_{18} PUFAs.

Regressions of the mean wet weight with time for lobsters in each diet treatment are shown in Figure 5. Regression equations of growth and growth curves plotted separately for each diet are presented in Appendix 3. ANOVA of weight gain of individuals of each treatment, (log transformed, to stabilize the variance) showed a significant effect of diet. This can be attributed to the significantly greater weight gain of lobsters fed Diet J than lobsters from all other diet treatments ($p = 0.0025$). There was greater than three-fold increase in biomass, in 120 days, of lobsters fed the reference diet.

1.4 DISCUSSION

It is not surprising that the highest survival and growth rates were among lobsters fed the reference diet. Although it has the same total lipid content and the same protein source (purified rock crab protein) as the semi-purified test diets, it may contain nutrients other than FAs (or a different balance of nutrients and FAs) which are life supporting and growth enhancing. In particular, there are several differences regarding the FA composition, as compared to the test diets, which may account for the superior performance

Table 6.

ANOVA of Initial Weights

Source of Variation	DF	SS	MS	F-Value	P
Between Diets	9	16061.993	1784.666	1.149	0.326
Within Diets	590	916042.400	1552.614		
Total	599	932104.393			

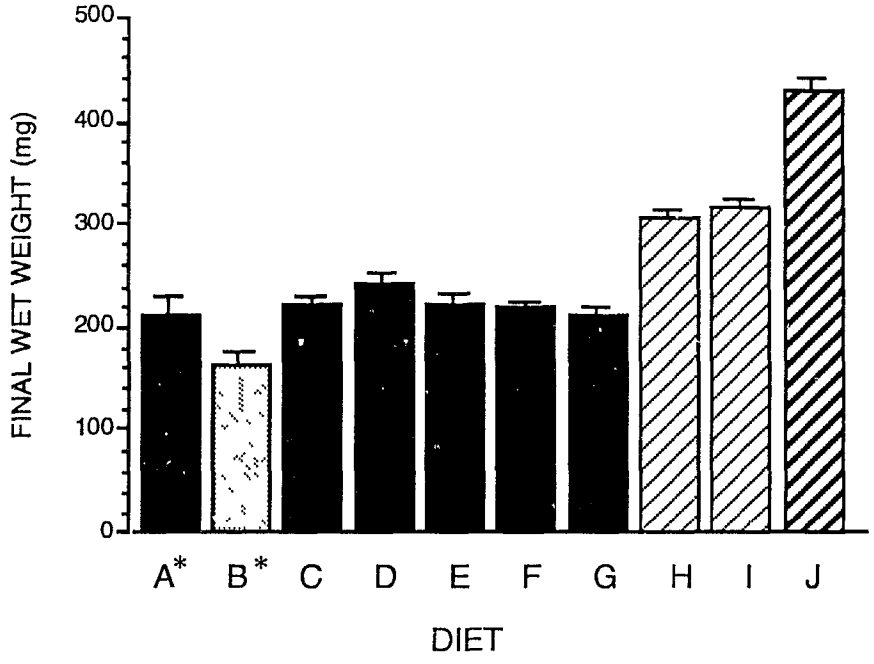


Figure 4. Final Wet Weight of Lobsters in Feeding Experiment I.

Values are means ±SEM of all surviving lobsters from three replicate trays per diet treatment. Columns with the same pattern are not significantly different at $p \leq 0.05$. * (For Diets A and B, SEM is calculated from surviving lobsters from one replicate group; survivors of the second and third replicates were terminated when mortality of each replicate reached 50%.)

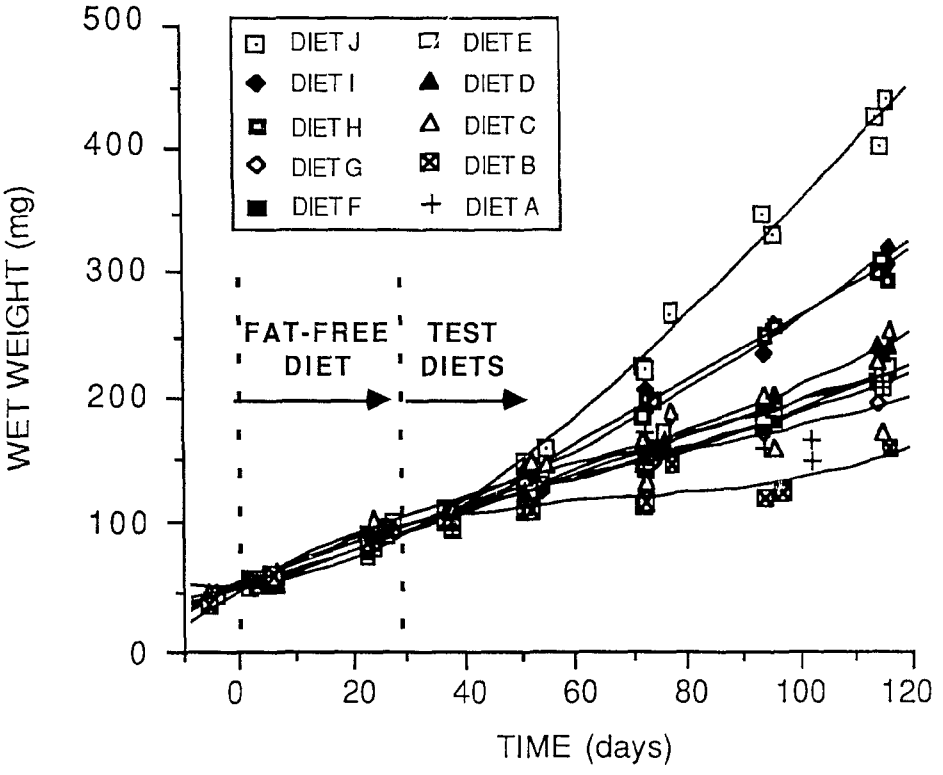


Figure 5. Regressions of Mean Growth of Lobsters in Feeding Experiment I.

Lobsters were reared at 20°C and fed one of ten test diets which differed in fatty acid composition.

Values plotted are mean weights of survivors in one of three replicate trays of twenty lobsters per diet treatment. Curves plotted are best fit polynomial regressions of mean weights of each replicate group for each diet on each weigh day (Cricket Graph v. 1.3.1). Regression equations are given in Appendix 3-A; separate plots of each regression are presented in Appendices 3-B through 3-C. See Table 5 and Figure 4 for comparison of final weights.

of lobsters fed this diet. These include: 1) a five fold lower level of SFAs, 2) a 3.4 fold higher level of total PUFA, 3) an approximately 1% level of C_{20} and C_{22} (n-3) FAs, and 4) an intermediate balance of (n-3) and (n-6) FAs, although the level of (n-6) exceeds (n-3) by 2 to 1. In addition, the levels of oleic acid and 18:2(n-6) are 5 times and 3 to 18 times higher, respectively, in the reference diet. These factors will be discussed in greater detail, with reference to results from the test diets. The effect of this high level of 18:2(n-6) was not examined among the semi-purified test diets in this study.

Because it was formulated to satisfy the basic nutritional requirements of a broad range of crustacean species, the reference diet is not used, in the strictest sense, as a control. Its value is as a standard diet to compare performance of animals within and between experiments. In this experiment it serves as a reference for comparison of the top performance of lobsters fed the test diets, with a better performance they are physiologically capable of achieving under the general conditions of the experiment. In this study it indicates inadequacies or deficiencies in even the optimum performance test diets (those containing 22:6(n-3)), and these deficiencies may alter lipid metabolism in the animals and the true response to, or requirements for, dietary FAs.

Although a previous study which examined the EFA requirements of juvenile lobsters tested four diets with similar inclusions of FAs to diets in this study (Castell & Boghen, 1979), comparisons of the results of these two studies may be obscured by the apparent stress and lethality of the early diets, which were attributed to amino acid deficiencies. Growth was not reported in the previous study; however, survival was substantially higher among lobsters fed the fat-free and EFA-deficient Diets A and B in this study than lobsters fed diets containing 1% 18:3(n-3), or 1% 22:6(n-3), or a 1% mix of 2 to 1 of 18:3(n-3) to 18:2(n-6) in the earlier study. The first study pre-dated the availability of the reference diet which would have served as a useful comparison between these

studies to eliminate or implicate other experimental factors (such as viability of the cohort or water quality) which (in addition to poor protein quality of the basal diet) may have affected the earlier studies.

The requirement for (n-3) or (n-6) PUFA for survival of juvenile lobsters is evident from the high mortality rate among juveniles fed the fat-free or EFA-deficient diets. This is particularly dramatic in contrast to the 95% or greater survival of lobsters fed diets supplemented with both (n-3) and (n-6) FAs.

In this experiment, with the exception of the fat-free diet, the level of non-EFA lipid in the diet was constant, and based on previous studies, supplied sufficient energy to spare the need to catabolize dietary EFA. However, the high level of SFAs may have been detrimental to the lobsters. EFA deficiency (as evaluated by growth and survival) was apparently exacerbated by the relatively high levels of dietary SFAs. Growth performance and survival were higher among lobsters fed a fat-free diet than among lobsters fed an EFA-deficient diet having 9% FAs, of which 8.25% were C_8 to C_{18} SFAs. The reference diet was low in SFAs, having a P:S ratio (PUFA to SFA ratio) of 2.3 (weight % basis), compared to the levels of 0.11 to 0.13 for the nine test diets.

The detrimental effects of diets high in SFAs have been well documented for warm-blooded vertebrates and fish. EFA deficiency symptoms are enhanced by a diet high in non-EFA fat (e.g. hydrogenated oils as the sole source of fat). A high fat diet can result in degenerative fatty liver disease (due to impaired catabolism of fats within the liver) and in high fat content in the blood (exceeding the ability of the liver to clear, or remove, fat from the blood). The excess fat is transported to extra-hepatic sites (e.g. adipose). Although the effect on growth and survival may be similar in vertebrates and crustaceans, the mechanisms of impairment of SFAs on fat metabolism may be different. As reviewed in the general introduction (Part I), absorption of dietary fats occurs quite differently in

crustaceans than in vertebrates. In contrast to being absorbed across the intestinal wall and accumulating in the blood prior to uptake by the liver with subsequent export to the tissues (or to the adipose tissue for storage), the route of absorption (and storage) of dietary fats in crustaceans is reversed from that in the vertebrates. Absorption is predominantly by the hepatopancreas, prior to release of FAs into the hemolymph for transport to the tissues. SFAs may accumulate in the hepatopancreas and exceed the ability of the absorptive cells to convert them to phospholipids for export into the hemolymph as high density lipoproteins. Vertebrates are able to absorb high quantities of neutral lipids (which may be high in SFAs) into the blood stream by the formation of chylomicrons in the intestinal mucosal cells. It would be interesting to test whether diets high in SFAs result in increased levels of SFAs in the hepatopancreas, to test the effect on hemolymph lipid composition and FA profiles, and also to examine the fat content of the feces to see if overall uptake by the hepatopancreas is reduced. This latter occurrence could result in a concomitant loss of fat-soluble nutrients such as vitamins A, D, E, and K, which could, in turn, enhance the apparent symptoms of EFA deficiency.

Several studies on penaeid shrimp have reported the growth-enhancement effects of dietary inclusion of natural oils containing 18:3(n-3) and/or 18:2(n-6) (Kanazawa et al., 1977a, 1979) or of semi-purified diets containing purified linolenic and/or 18:2(n-6) (Shewbart & Mies, 1973; Kanazawa et al., 1977b, 1979c; Read, 1981). Some studies have indicated that 18:3(n-3) has a greater nutritional value for marine crustaceans than does 18:2(n-6) (Guary et al., 1976; Kanazawa et al., 1977b). While the inclusion of ~1% of linolenic and/or 18:2(n-6) to the diets of juvenile lobsters in this study significantly improved growth and survival, there was no discernable difference in survival or growth in response to different ratios of these two FAs. D'Abramo & Sheen (1991) obtained similar results for the freshwater prawn, Macrobrachium rosenbergii. They found no significant differences in weight gain between prawns fed diets containing 0.15, 0.3, or 0.6% 18:3(n-

3) vs. equivalent levels of 18:2(n-6). The absence of differences in both studies could indicate an equivalent value of these FAs in satisfying EFA requirements, however, it is possible that differences might become apparent over a longer period of time. Differences between treatments may be masked in the short term by the high intraspecific variability in growth rate. Also, although growth and survival can indicate EFA requirements, they are not necessarily impaired by levels of EFA which may be required to satisfy other essential functions.

Although not statistically significant, there was a trend of higher survival rate (after 120 days) of lobsters on the five diets receiving a balanced dietary supplement of both (n-3) and (n-6) FAs (in a ratio of 0.8 - 2.1), as compared to those on the three diets containing predominantly a single PUFA (ratios of 5.5 - 6.4 to 1 or 1 to 10, of (n-3) to (n-6)). This may indicate that both (n-6) and (n-3) PUFA are essential and that a disproportion of one family of FAs may interfere with metabolism of the other. The performance of lobsters fed Diet C (< 0.005% 18:2(n-6)) indicates this level may be sufficient to partially satisfy an essential but minimal requirement for linoleate. This supports earlier speculation that (n-6) PUFA may be essential for juvenile lobsters (Castell & Boghen, 1979).

Growth was significantly higher among lobsters fed diets supplemented with 22:6(n-3). Similar results were obtained with supplements of 20:5(n-3) or 22:6(n-3) in diets fed to marine penaeid shrimps (Kanazawa et al., 1978, 1979d), and more recently for the freshwater prawn Macrobrachium rosenbergii (D'Abramo & Sheen, 1991). This indicates that C_{20} and C_{22} HUFA have a higher EFA value than C_{18} PUFA. The appropriate level of HUFA inclusion may be affected by the level of inclusion of (n-6) FAs, although in this study the ratio inclusion of 18:2(n-6) in a test diet having 22:6(n-3) did not affect growth.

1.5 CONCLUSIONS

1. Diets deficient in (n-3) and (n-6) fatty acids reduce survival and growth rates in juvenile lobsters; juvenile lobsters reared at 20°C can survive at least 10 to 11 weeks on a fat-free or polyunsaturated fatty acid-free diet, after which time there is rapid mortality.

2. Diets high in saturated fatty acids enhance the effect of (n-3) and (n-6) fatty acid deficiency.

3. Substitution of (n-3) polyunsaturated fatty acids with (n-6) polyunsaturated fatty acids does not improve growth in juvenile lobsters.

4. Either (n-3) fatty acids or (n-6) fatty acids are essential for growth and survival of juvenile lobsters.

5. For juvenile lobsters, 22:6(n-3) has a higher value as an essential fatty acid than does 18:3(n-3) or 18:2(n-6). Supplementation of 18:2(n-6) in addition to 22:6(n-3) does not further enhance growth.

CHAPTER 2

The Effect of the Balance of Dietary Fatty Acids on Whole-Animal Fatty Acid Composition

2.0 INTRODUCTION: PART II, CHAPTER 2

In this chapter, the effects of dietary FAs on the FA composition of juvenile lobsters cultured at 20°C were examined. The lobsters were fed a reference diet or one of nine semi-purified test diets which varied in the amount and ratio of inclusion of 18:3(n-3), 18:2(n-6), and 22:6(n-3). After assessment of the effects of these diets on growth and survival (Chapter 1), total lipid (TL) was extracted from whole lobster, and the neutral lipid (NL) and polar lipid (PL) fractions were analyzed for FA composition.

2.1 OBJECTIVES

1. Examine the effect of dietary fatty acid composition on the whole-animal fatty acid profiles of juvenile lobster Homarus americanus.

2. Examine the effect of different proportions of (n-6) and (n-3) fatty acids in the diet on whole-animal fatty acid composition, and investigate possible metabolic interactions of dietary (n-6) and (n-3) fatty acids.

3. Evaluate the ability of lobsters to desaturate and chain elongate linolenic acid, 18:3(n-3), to more highly unsaturated (n-3) fatty acids.

2.2 MATERIALS AND METHODS

2.2.1 GENERAL LIPID METHODS

Glassware was carefully washed and rinsed before use by the following the procedure of Sasaki (1984): (in sequence) soap, tap water, 10% HCL (2X), distilled water (3X), methanol (2X), acetone (2X), and oven or air dried. As necessary, glassware was soaked overnight in a concentrated solution of KOH-ethanol or 10% HCl.

In general, techniques of lipid analysis and appropriate precautions were followed, according to Christie (1982). All procedures were conducted in glass vessels and

glassware caps were lined with teflon or aluminum foil. Procedures involving lipids were conducted under an atmosphere of nitrogen, to prevent auto-oxidation of the lipids. An N-Evap nitrogen evaporator was used to evaporate small quantities of solvents from vials and tubes; the water bath was set at 45°C. Large volumes of solvent were evaporated from flasks using a rotary flash evaporator, under reduced pressure, and a water bath set at 45°C. Sample were stored at -20°C for intermediate storage or at -40°C for longer term storage.

2.2.2 EXTRACTION OF LIPIDS FROM DIETS AND LOBSTERS

FA composition was determined on lobsters at the start of the test diet feeding trial. The feeding history from hatching (see Section 1.2.1, Chapter 1) included two weeks on newly hatched Artemia nauplii (until metamorphosis), two weeks on the crustacean reference diet (H-CRD) (for FA composition, see Diet J, Table 4), and three weeks on a fat-free diet (Diet A, Table 4). A subsample of lobsters was analyzed at that point to determine the "initial" FA composition of whole body TLs (see "INIT", Table 7). Diet A lobsters were continued on the fat-free diet while all other lobsters began feeding on the other test diets or the reference diet. Lobsters were terminated for FA analysis after an additional ten weeks.

TL was extracted from diet samples and from pooled carcasses (3 individuals/sample) by a procedure modified from Bligh & Dyer (1959) (Appendix 2-A). Samples were homogenized, with solvents, in a Beckman Polytron. (Figure 6 is a schematic diagram of general procedures of lipid analysis.).

Although lipid extracts were prepared from replicate groups of 3 lobsters per diet treatment, due to several problems in this initial study, FA analyses of replicate samples are not available. Also FA analyses are not available for the following treatments: PLs of lobsters fed Diet E, and NLs of lobsters fed Diets A, C, E, or F. Several analyses were

Table 7.

Fatty Acid Composition of the Major Neutral Lipids of Whole Lobster (Weight %).

Fatty Acids	INIT TL	Diet B	D	G	H	I	J
12:0	8.3	0.5	9.8	12.6	11.9	4.7	0.1
14:0	4.1	16.1	19.6	10.2	33.1	5.5	3.4
16:0	20.1	33.5	23.6	19.3	18.7	6.6	12.1
18:0	4.1	6.9	3.7	3.3	2.0	trace	0.9
20:0	trace	n.d.	n.d.	n.d.	n.d.	n.d.	0.0
Σ SFA	36.6	57.0	56.7	45.3	65.7	16.8	16.6
16:1(n-9)	3.4	2.4	4.3	0.9	1.7	0.8	1.1
16:1(n-7)	5.0	10.3	3.8	3.8	5.6	3.3	6.9
18:1(n-9)	14.2	18.7	17.6	10.6	14.7	4.6	19.1
18:1(n-7)	7.8	2.0	1.3	1.1	1.5	1.0	6.3
20:1(n-9)	0.7	1.3	0.6	?	0.4	n.d.	3.6
Σ MFA	31.1	34.6	27.5	16.4	23.9	9.7	37.0
18:2(n-6)	6.8	1.7	4.6	26.6	2.0	11.9	26.0
20:2(n-6)	1.1	n.d.	0.4	2.7	0.3	0.5	1.4
20:4(n-6)	n.d.	n d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ (n-6)	7.9	1.7	5.0	29.2	2.3	12.4	27.4
18:3(n-3)	1.4	n.d.	6.0	?	trace	0.2	0.8
20:3(n-3)	0.3	n.d.	0.8	?	trace	trace	0.1
20:5(n-3)	8.3	n.d.	0.1	2.3	0.6	7.4	4.1
22:5(n-3)	n.d.	1.2	0.2	0.2	0.7	26.1	0.8
22:6(n-3)	4.2	< 0.1	< 0.1	0.6	4.9	20.3	5.7
Σ (n-3)	14.2	1.2	7.1	3.2	6.2	54.1	11.6
Others	10.2	5.5	3.7	5.9	1.8	7.1	7.4
(n-3)/(n-6)	1.8	0.7	1.4	0.1	2.7	4.3	0.4

n.d. = Not detected; s= shoulder (peak not integrated)

? = Identification uncertain (peak integration unreliable)

trace = Peak barely visible and not integrated

Values from pooled extracts of three lobsters

Totals (Σ's) may reflect fatty acids not listed

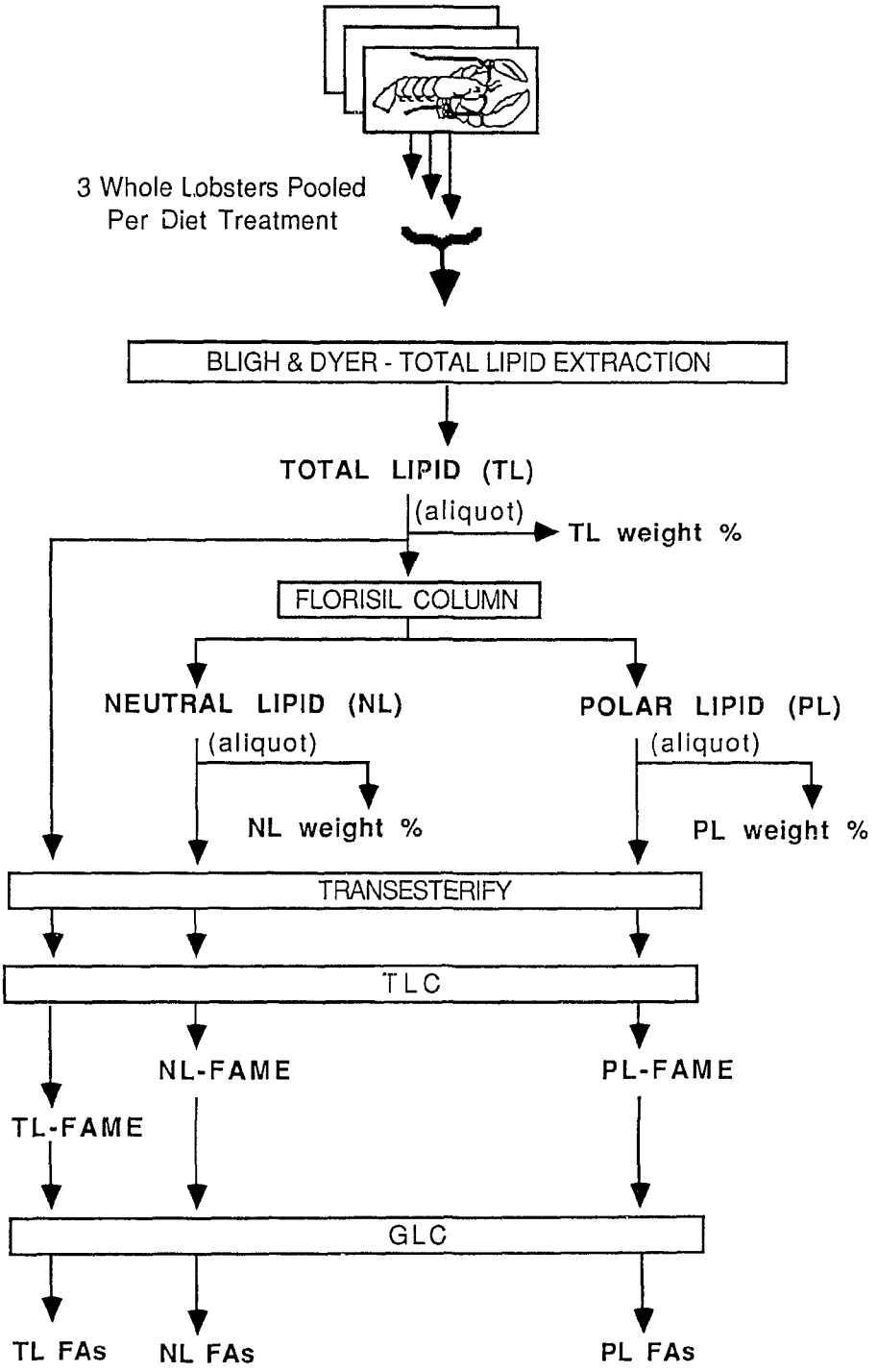


Figure 6. Schematic Diagram of Procedures of Lipid Analysis of Whole Lobsters.

lost or discarded due to errors in the programming of the GLC and integrator. Others were lost or discarded due to apparent dilution errors in samples prior to injection (i.e. too little sample was applied to the column for reliable identification of component fatty acids). Most unfortunate was that the samples were erroneously discarded prior to completion of analysis of the chromatograms, and the opportunity to test or retest remaining samples was lost. Due to lack of dupicate samples, these FA data are considered as preliminary; however, each sample represents the mean value of three whole lobsters and is thus comparable to much of the literature on crustacean and fish fatty acids published previously and at the time of this experiment.

2.2.3 SEPARATION OF NEUTRAL AND POLAR LIPIDS

The TL samples were separated into NL and PL fractions by column chromatography. The TL samples were dissolved in chloroform and passed through a Pasteur pipette plugged with glass wool and packed with acid washed Florisil (activated silicic acid) (Carroll, 1961; Christie, 1982). NLs were eluted with 5 ml of chloroform; PLs (together with glycolipids, GLs) were eluted with 5 ml of methanol.

Note that the FA profile of PLs of the "initial" lobsters is reported for a different group of three lobsters than the FA profile of TLs of "initial" lobsters.

2.2.4 CONVERSION OF FATTY ACIDS TO METHYL ESTERS

Fatty acid methyl esters (FAME) of the diet TL samples and the whole animal TL, NL and PL samples were prepared for GLC analysis by transesterification following a modification of the method of Morrison & Smith, (1964) (Appendix 2-C). The lipid samples were dissolved in 2 ml hexane, 0.5 ml benzene and refluxed in 2 ml of 7 % boron trifluoride in methanol for 60 minutes at 100°C (in a boiling water bath). The FAME were

extracted using hexane (3 X 2 ml), washed with distilled water, dried over anhydrous Na_2SO_4, and filtered through Whatman No.1 filter paper.

2.2.5 PREPARATIVE TLC

FAME were separated from other lipid components of the whole-animal TL samples by thin layer chromatography on silica gel plates ("Prekote" plates, 20 cm X 20 cm, 200 μm particle size, Applied Science Laboratories, College Park, PA, USA). Before use the plates were cleaned by developing in ethyl acetate and activated by heating at 110°C for 30 minutes. Lanes were scored on the plates to prevent lateral diffusion of samples and standards.

FAME samples were dissolved in chloroform and 10-50 μg/spot was applied with a Hamilton 10 μl syringe. Standards or standard mixtures were also applied to the plates to compare Rf values.

Plates were developed in glass tanks lined with chromatographic paper saturated with the developing solvent (hexane : diethyl ether : acetic acid, 85:15:1; v/v/v). Plates were air-dried, then sprayed with a 0.2% solution of 2',7'-dichlorofluorescene in ethanol, and observed under UV light.

The FAME bands were identified, scraped from the plates, and extracted (3 X 10 ml) with chloroform:hexane (1:1, v/v), and filtered through Whatman No. 1 paper.

2.2.6 GLC ANALYSIS

FA compositions of the TL or NL and PL fractions were determined by gas-liquid chromatography (GLC) of the FAME (conducted at the Canadian Department of Fisheries and Oceans Halifax Lab). Aliquots of 1 to 3 μl containing approximately 50 μg, of FAME in diethyl ether were injected into a Perkin-Elmer capillary gas chromatograph Model 3920

equipped with a hydrogen flame ionization detector (FID) and a polar column (a Silar 5 CP, fused silica, 45 m in length x 0.25 mm ID). The injection temperature was 200°C, the oven temperature was 180°C. The pressure of the carrier gas, hydrogen, was set at 30 psig. Total run time to elute 22:6(n-3) was ~ 2.5 hours.

Identifications of FA peaks were made by comparison to retention times of identified peaks of cod liver oil FAME, prepared as a secondary standard, and menhaden oil FAME standard (courtesy R.G. Ackman, CIFT, TUNS). Semilog plots of retention time (relative to 18:0) vs. carbon chain length were made of the sample FAME and standards, and Type II separation factors were calculated to aid the identification of FAs (Appendix 2-G) (Ackman, 1963).

Identifications were also made in comparison to the retention times of lipid standards obtained from Nu-Chek-Prep, Inc. Elysian, MN., USA, Supelco, Inc., Bellefonte, PA, USA and Serdary Research Laboratories, London, Ontario.

2.2.7 QUANTITATION OF FATTY ACIDS

The retention time and magnitude of each peak were recorded on a Laboratory Data Control Model 303 Computer Integrator.

Peak areas were converted to weight percent of the total amount of identified FAME using a modified computer program, originally developed by Ackman & Eaton (1978).

Preliminary compilation of FA data was made using the relational data base program Paradox (Copyright, 1988, Borland International) and IBM PC compatibles. Summary statistics and tables were produced with an Apple Macintosh computer, using the spreadsheet and database program Microsoft Excel 1.5, 2.0, & 2.2 (Microsoft Corporation, 1985).

2.3 RESULTS

Proximate and FA analyses of the diets was presented in Part II, Chapter 1, Section 1.3.1 and in Tables 3 and 4, respectively. The FA analysis of the NL and PL fractions of whole lobster lipids (pooled from 3 lobsters/sample) are given in Tables 7 and 8. Because replicate samples and the NL samples from several diet treatments were erroneously discarded, these results are based on single samples only. The reported FA profiles, however are a valuable indicator of fatty acid metabolism of lobsters under the experimental conditions, thereby providing a basis for further experiments. [It should be noted that at the time of this study, much of the published literature on fatty composition and nutrition reported analysis of only single samples from either single animals per treatment or of the means of replicate analysis of single samples pooled from few to several animals per treatment; only in the recent literature are results based on two or more samples per treatment (and these are typically not on single animals, but on pooled tissues or pooled whole animals and therefore individual variation is obscurred].

2.3.1 INITIAL FA COMPOSITION OF LOBSTERS ("INIT")

The FA composition of TLs of lobsters at the start of the test diet feeding trial is reported as "INIT TL" in Table 7. Saturated fatty acids (SFAs) and monounsaturated fatty acids (MFAs) comprised approximately 37% and 31%, respectively, of whole body TL of the lobsters. Palmitic acid, 16:0, was the major saturate (~20% of TL) and oleic acid, 18:1(n-9), was the principal monoene (~14% of TL). The sums of (n-3) FAs and (n-6) FAs were ~14% and ~8%, respectively, for an (n-3)/(n-6) ratio of 1.8. Of the (n-6) FAs, linoleic acid (18:2(n-6)) comprised ~7% of TL, and arachidonic acid (20:4(n-6)) comprised ~1%. The major (n-3) FA was 20:5, at ~8% of TL. 22:6(n-3) and 18:3(n-3) constituted ~4% and 1% of TL, respectively.

Table 8.

Fatty Acid Composition of the Major Polar Lipids of Whole Lobster (Weight %).

Fatty Acids	INIT PL	Diet A	B	C	D	F	G	H	I	J
12:0	0.3	0.7	0.9	7.9	1.8	0.8	6.6	1.2	24.5	0.1
14:0	6.5	2.5	6.0	5.3	7.0	4.5	8.2	8.6	12.2	1.2
16:0	44.1	25.4	29.4	5.0	27.2	18.2	15.4	23.2	9.6	11.9
18:0	6.7	5.0	4.6	0.3	5.6	2.6	2.5	3.4	0.9	0.9
20:0	n.d.	0.1	n.d.	trace	0.1	0.1	0.1	0.0	trace	trace
Σ SFA	57.6	33.7	41.0	18.5	41.6	26.1	32.8	36.5	47.2	14.1
16:1(n-9)	3.7	0.9	1.0	0.4	0.7	1.0	1.1	0.3	0.3	0.7
16:1(n-7)	4.3	13.7	12.2	2.8	3.7	3.1	2.6	4.8	3.5	3.8
18:1(n-9)	8.1	28.1	24.0	4.8	18.4	16.0	12.1	16.8	7.1	9.0
18:1(n-7)	3.7	4.5	5.2	0.7	2.3	2.0	12.5	2.5	2.2	2.2
20:1(n-9)	0.7	0.8	1.7	trace	0.6	0.3	0.4	0.6	0.3	0.5
Σ MFA	20.5	48.0	44.0	8.7	25.6	22.4	28.7	25.0	13.3	16.2
18:2(n-6)	1.5	4.2	4.5	5.0	10.3	19.1	27.8	5.1	10.0	19.6
20:2(n-6)	0.4	0.5	0.7	n.d.	1.0	1.8	2.4	0.8	0.8	1.3
20:4(n-6)	1.2	0.6	0.3	0.8	0.2	0.5	0.3	0.3	0.2	0.7
Σ (n-6)	3.1	5.3	5.5	5.9	11.4	21.3	30.5	6.1	11.0	21.5
18:3(n-3)	1.0	0.5	0.3	45.1	11.4	18.0	0.3	0.2	0.2	0.8
20:3(n-3)	0.4	0.0	n.d.	4.5	1.5	2.8	0.0	0.1	0.0	n.d.
20:5(n-3)	5.3	3.8	1.5	6.6	0.9	3.6	2.5	8.2	4.7	20.8
22:5(n-3)	n.d.	0.5	0.7	1.2	n.d.	0.5	0.3	1.4	0.3	3.4
22:6(n-3)	2.3	2.3	0.9	3.4	0.3	2.4	2.1	18.3	20.3	16.1
Σ (n-3)	8.9	7.0	3.5	60.8	14.2	27.4	5.3	28.2	25.6	41.1
Others	9.8	6.0	6.1	6.1	7.2	2.8	2.8	4.3	2.9	7.2
(n-3)/(n-6)	2.9	1.3	0.6	10.4	1.2	1.3	0.2	4.6	2.3	1.9

n.d. = Not detected; s = shoulder (peak not integrated)

? = identification uncertain (peak integration unreliable)

trace = Peak barely visible and not integrated

Values from pooled extracts of three lobsters

Totals (Σ's) may reflect fatty acids not listed.

The FA composition of initial lobster PLs (INIT PLs) is given in Table 8. The predominant FA was 16:0 acid, comprising ~44% of PLs. This contributed to SFAs comprising the largest fraction of FAs, at ~60% of PLs. The proportion of 12:0 was <1% of initial lobster PL, as compared to ~8% of initial lobster TL. Similarly, the proportion of the C_{12} MFAs in the PL and TL were <1% and ~4%, respectively. Proportions of C_{14} and C_{16} MFAs were similar in the PL and TL samples, but the proportions of 18:1(n-9)and 18:1n-7 in the TLs (~14% and 8%, respectively) were nearly double their levels in the PLs (~8% and 4%, respectively). This indicates that 12:0 and 18:1 FAs in the TLs are contributed primarily by the NL fraction.

Of the PL PUFAs of initial lobsters, the proportion of (n-3) FAs (~11%) is more than 3 times the proportion of (n-6) FAs (~3.5%), for an (n-3)/(n-6) ratio of 3.2. This is more than double the ratio in the TL sample. Linoleic acid, 18:2(n-6), comprised only 1.5% of PL, compared to ~7% of TL. The predominant PL (n-3) FAs were 20:5 and 22:6, at ~5% and 2% of PL, respectively.

2.3.2 LOBSTERS FED THE "FAT-FREE" DIET (DIET A)

The proportion of 14:0 and 16:0 in the PL of lobsters fed the fat-free diet for 13 weeks (~2.5% and ~25%, respectively) appeared to be reduced to nearly 50% of the proportion found in the PLs of lobsters fed the fat free diet for the initial 3 weeks. Concomitantly, the proportion of total 16:1 and total 18:1 PL FAs more than doubled, from ~8 and 12.5%, respectively, in initial lobsters to 14.5% and 32.5% in lobsters after the additional ten weeks. The proportion of 18:0 remained the same.

The total (n-3) PUFA of the PL did not change substantially from the initial PL proportions, while the proportion of the principal (n-6) PUFA, 18:2(n-6), appeared to have

doubled. Thus, the (n-3) to (n-6) ratio dropped to ~1.6 from 3.2, while the sum of the proportions of (n-3) plus (n-6) PUFA remained unchanged at roughly 15% of PLs.

2.3.3 LOBSTERS FED THE EFA-DEFICIENT DIET (DIET B)

There was a background level of 18:2(n-6) acid (at ~0.12% of diet) in the HCO - based diets. The diet was also comprised of ~8.25% SFAs (0.5% 10:0, 4.8% 12:0, 1.9% 14:0, 0.9% 16:0, and 0.2% 18:0) and 0.62% of 18:1.

After 13 weeks, the proportions of the individual MFAs and SFAs in the PLs were similar to those of lobsters on the fat-free diet. The proportion of SFAs in the NLs was approximately 40% higher than in the PLs of the same animals, and the MFAs is lower by ~16%.

The levels of 18:2(n-6) and 20:4(n-6) in whole body PL were ~4.5% and < 0.5%, equivalent to those of PL in lobsters fed the fat-free diet for 13 weeks. The proportion of 18:2(n-6) in the NL (~2%) was less than half that of the PL, however there was ~2% of 16:2(n-6) in the NLs, which was not detected in the PLs.

The proportion of (n-3) HUFA in whole lobster PL of lobsters fed Diet B was reduced as compared to the proportion in the PLs of initial lobsters and to the proportion in the PLs of lobsters fed the fat-free diet. Eicosapentaenoate and docosapentaenoate, 20:5(n-3) and 22:6(n-3), comprised ~1.5% and ~1% of PL, respectively, approximately half of the proportion in the PL of lobsters fed the fat-free diet, and 20:5(n-3) was approximately 30% the proportion of 20:5(n-3) in the PL of lobsters initially.

2.3.4 LOBSTERS FED DIETS SUPPLEMENTED WITH 18:3(n-3) &/or 18:2(n-6).

Analysis of the FA composition of Diets C, D, F, and G shows a consistent background level of 18:2(n-6) at ~0.12% of dry diet. The effect of dietary 18:3(n-3) in the

absolute absence of dietary 18:2(n-6) was therefore not tested, although 0.1% of diet can be considered "deficient". The ratios of 18:3(n-3) to 18:2(n-6) in the diets ranged from 0.78 to 6.38 (Table 4). There was an apparent direct relationship between dietary (n-3) to (n-6) ratio and the (n-3) to (n-6) ratio in whole lobster PL (Figure 7-C; R=0.93).

The proportion of 18:2(n-6) in whole animal PL varied directly with its inclusion in the diet, (for all test diets, dietary levels ranging from 0.13% to 0.84%) (Diets A, B, C, D, F, G, H, and I as % of dry diet), irrespective of the dietary level of 18:3(n-3) (Figure 7-B; R= 0.99). The proportions of 18:2(n-6) in lobster PLs ranged from 4% to 28%.

The levels of 18:3(n-3) and total (n-3) FAs in whole lobster PL were higher in lobsters fed Diet C than in lobsters on all other diets. In the absence of 18:3(n-3), only ~0.2 - 0.4% is present in tissue PL.

The proportion of 16:0 in lobsters fed the (n-3) &/or (n-6) test diets ranged from 5% to 27% of PL and the Σ18:1 ranged from ~5% to ~25%.

2.3.5 LOBSTERS FED DIETS SUPPLEMENTED WITH 22:6(n-3) (DIETS H&I)

The level of 22:6(n-3) in Diets H and I was 0.66% and 0.54% of diet, respectively, and 18:3(n-3) was also present at 0.12% and 0.33%, respectively, yielding (n-3) to (n-6) ratios of 5.5 and 1.6.

The level of 22:6(n-3) in whole lobster PL was highest in the lobsters fed Diets H and I (~18 and ~20 % of PL, respectively), than in the PL of lobsters on all other test diets (< 0.5- to - 2.5 % of PL). The level of 20:5(n-3) was 8% and 5% of PL in lobsters fed Diets H and I, respectively, equivalent to the level in lobsters fed Diet C, which contained 0.83% 18:3(n-3) and had an (n-3) to (n-6) ratio of 6.4. This proportion was also equivalent to the proportion of 20:5(n-3) in the PLs of initial whole lobsters and was two to five times higher than the proportion of 20:5(n-3) in lobsters fed Diets B, D, F, and G.

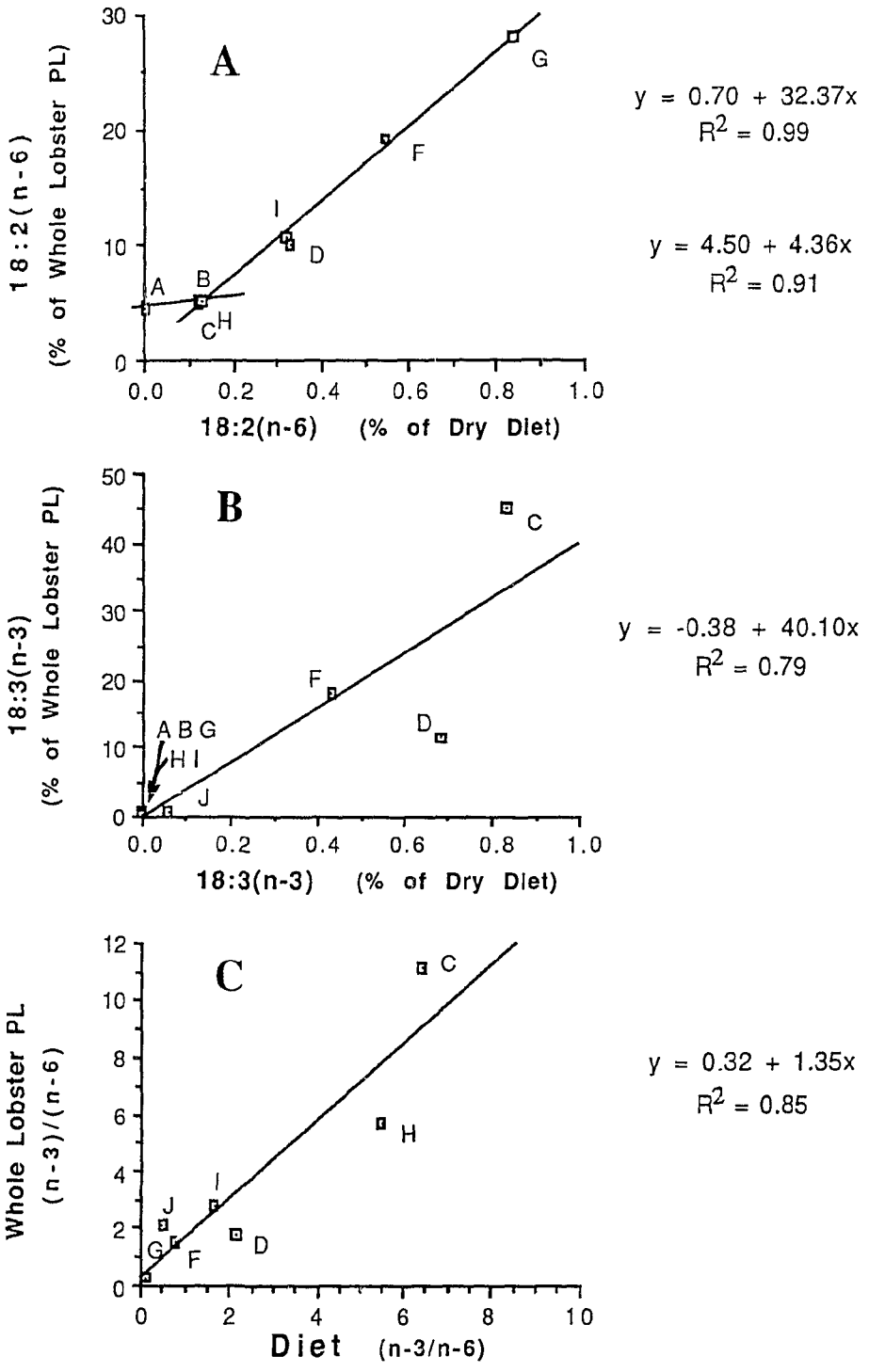


Figure 7. Regression Equations of the Relationships Between Levels of Dietary Fatty Acids and Proportions of those Fatty acids in Whole Body Polar Lipids.

A. 18:2(n-6), B. 18:3(n-3), and C. The Ratio of (n-3) to (n-6) Fatty Acids. Values for each diet are designated by the corresponding letter (see Table 4).

In the NLs of lobsters fed Diet H, the proportions of (n-3) HUFAs was substantially lower than in the PLs. For Diet I lobsters, however, the proportions of 22:5(n-3) and 22:6(n-3) were unusually high (~26% and 20%, respectively) compared to the NLs of all other lobsters, and compared to the proportion of 22:5(n-3) in the PLs of all lobsters tested.

As with lobsters fed Diets C through G, 18:2(n-6) was present in the lobsters in direct proportion to its levels in Diets H and I, in both the PLs (5% and 10%, respectively) and the NLs (2% and 12%, respectively) (Figure 7-A).

There was an unusually high proportion of 12:0 in the PL of lobsters fed Diet I (24.5%). This was comparable to the proportion of 16:0 in the PL of lobsters fed Diet H. However, the proportion of total SFAs was ~30% higher in Diet I lobster PLs than in those of Diet H lobsters. While the levels of C_{14} and C_{16} MFAs were comparable, the proportion of 18:1(n-9) was twice as high for Diet H lobster PLs than for Diet I lobster PLs (~17% and ~7%, respectively).

The proportion of total SFAs is markedly higher for lobsters fed Diet H (~66%) than for lobsters fed all other diets, owing to the unusually high proportion of 14:0 (~33%). By contrast, the NLs of Diet I lobsters had the lowest proportion on SFAs than all other test diets, but the level was equivalent to that of H-CRD fed lobsters (~17%). The proportions of C_{16} and C_{18} MFAs of Diet H and I lobsters was similar between the NLs and the PLs.

2.3.6 LOBSTERS FED THE CRUSTACEAN REFERENCE DIET (H-CRD)

The PLs of lobsters fed the H-CRD contained the lowest proportion of SFAs (~12.5%), and among the highest proportions of 18:2(n-6) (~19.5%) and of 22:6(n-3) (~16%) compared to other lobsters in this study. The most notable difference in the FA

profile of lobsters fed the H-CRD (the only diet which contained 20:5(n-3)) was the high proportion of 20:5(n-3) in the PLs (~21% of PLs). Also in H-CRD fed lobsters, there was a high proportion of 18:2(n-6) in the NLs (~26%), which was higher than in the PLs and equivalent to the proportion in the NLs of lobsters fed Diet G.

2.4 DISCUSSION

The lobsters in this study were raised at 20°C, the standard culture temperature for lobsters. While this is at the high end of the range of ambient temperatures in natural lobster habitats (1°C - 25°C), (Scarratt, 1979; Van Olst et al., 1980), it is within the range of thermal tolerance ($\geq$ 0°C - $\leq$ 31°C) (Mc Leese, 1956) and most importantly, it has been determined to be an optimal temperature for growth enhancement (Hughes et al., 1980). For this reason, the high culture temperature was selected. The effects of nutritional deficiencies are usually exacerbated in young, rapidly growing animals, due to a high requirement of nutrients for tissue manufacture and the turnover and depletion of nutrient reserves. Concomitantly, growth depression is more evident and is apparent in a shorter period of time.

It should be kept in mind, however, that temperature may affect FA metabolism in lobsters (see Part III). For example, as an acclimation response to high temperature, normal desaturase activity to form PUFA and HUFA may be depressed. This occurs in some organisms as an attempt to reduce the level of unsaturation in membrane PLs, and thereby counteract the natural tendency for increased permeability of membranes with increased temperature. Therefore, while the results and discussion refer to lobsters in general, it is implicit that the conclusions are applicable to juvenile lobsters at 20°C and may not necessarily represent the FA biochemistry of lobsters at colder temperatures.

The proportions of palmitic acid (16:0), palmitoleic acid (16:1n-7), and oleic acid (18:1(n-9)), in whole lobster PL were highest in lobsters fed fat-free and EFA-deficient

diets. Conversely, they were lowest in the PLs of lobsters fed dietary (n-3) and (n-6) FAs. This may indicate the suppression of FA synthesis in lobsters fed a high fat diet which includes ~1 % PUFA or HUFA. The evidence is not conclusive, however, since the amount of TL, and the proportion of NL and PL fractions, are not reported. It is also possible that, quantitatively, tissue levels of SFAs and MFAs remain constant but appear reduced due possibly to a relative selective incorporation and accumulation of dietary PUFA relative to dietary MFAs and SFAs. SFAs ranged from 36 - 66 % in the NL fraction of whole animals; and from 20 - 47 % of the PL fraction.

The proportion of 18:2(n-6) (an EFA for mammals) in the whole animal lipids of juvenile lobsters, was maintained at a minimum level of 4.1 to 5.0% of PLs in those fed diets deficient in (n-6) FAs (i.e. 0.12 - 0.13% of diet, DWB) and even in the virtual absence of dietary (n-6) and (n-3) FAs (i.e. Diet A). A single exception was in the PLs of initial animals. While this could otherwise indicate either de novo synthesis ability in lobsters to produce linoleate, or an unidentified source (e.g. gut microbes, or water born sources such as algae or microbes), in light of other evidence it is most likely that lobsters have an ability to selectively retain linoleate and also have a high capacity to incorporate it from diets containing trace levels. This may indicate the importance of linoleate in some functional aspect of tissue PLs. The ability of tissues to "tenaciously" retain important lipids and the concomitant difficulty of demonstrating deficiency of those lipids (by FA compositional analysis) is discussed by Lands (1991), in his review on the relationship between dietary (n-3)/(n-6) FAs and tissue response (in mammals). He advocated a need to establish better criteria for determining threshold levels of dietary deficiency and threshold levels of detrimental dietary excess.

A linear relationship was demonstrated between increased 18:2(n-6) in tissue PLs with increased levels of dietary 18:2(n-6), up to 1% of dry diet (Figure 7-A). Lands (1991) reviews studies showing a similar, (linear) relationship of increasing 18:2(n-6) in

the tissue TAGs with increasing dietary 18:2(n-6), but notes that in the tissue PLs (of rats), with increasing dietary levels, a "saturation" level is reached. This PL saturation may be attained in lobsters with diets containing 18:2(n-6) in excess of 1% of dry diet. This is indicated by the results of Diet J, which contained 18:2(n-6) at ~2.3% of diet (DWB). However, the lower proportion of 18:2(n-6) in the tissue PLs of lobsters fed Diet J is influenced by the uptake of (n-3) HUFAs from that diet. A linear relationship (although with fewer data points) was also demonstrated between dietary 18:3(n-3) and the proportion of 18:3(n-3) in whole lobster PLs (Figure 7-B). However, unlike studies with mammals in which the slope of the 18:3(n-3) relationship is approximately one half that for 18:2(n-6), in this study with lobsters, the slope for 18:3(n-3) is approximately 30% higher than that for 18:2(n-6). Lands, in citing Clouet et al. (1989), postulated that a 2-fold faster rate of mitochondrial oxidation of 18:3(n-3) may account for the difference in slopes in the mammalian studies. The results from this study may therefore reflect a relatively greater importance of (n-3) than (n-6) FAs to lobsters (and possibly other marine poikilotherms).

Arachidonate, 20:4(n-6), an EFA for mammals, is present at ~0.2-1.1% of whole animal PLs regardless of dietary inclusion of, or deficiency of, 18:2(n-6) (from < 0.005 % of diet, to 0.84% of diet in the absence of 18:3(n-3)), and at tissue PL levels of 18:2(n-6) ranging from 5 to 28%. Instead, there is an accumulation (in the PLs) of the two carbon elongation product of 18:2(n-6) (i.e. 20:2(n-6)), in direct proportion to dietary and tissue levels of 18:2(n-6) (Table 8). D'Abramo et al. (1980) also found less than 1% 20:4(n-6) in the tissues of juvenile lobsters despite high dietary levels of 18:2(n-6) (from corn oil). However, in the D'Abramo et al. (1980) study, they did not distinguish (by GLC) between 18:2(n-6) and 18:3(n-3), so they are reported together, for both diets and tissues. It is possible that 18:3(n-3) (possibly contributed by fish oils in the diets) competed with 18:2(n-6) for available desaturase. Nevertheless, their findings and those in this experiment, indicate a lack of Δ^6-desaturase activity for the (n-6) series FAs. The level of

20:4(n-6) would also be low if arachidonate was metabolized as rapidly as it was produced (e.g. in the manufacture of prostaglandins), however that would likely result in a depletion of available 18:2(n-6), which was not shown. Castell & Boghen (1979) reported 10 times the proportion of 20:4(n-6) in the TL of whole juvenile lobsters than were found in the lobsters in this study, and 25% to 50% of the level of 18:2(n-6) in lobsters fed a 2 to 1 mix of linolenate to linoleate.

In the absence of dietary 18:3(n-3), only ~0.2 to 0.5% was retained in lobster whole body PLs. However, since 20:5(n-3) is retained at ~1.5% to 2.5% of PL and 22:6(n-3) at ~1 to 2 %, on diets deficient in (n-3) PUFA or HUFA, the 18:3(n-3) may be depleted due to desaturase activity. Conversely, addition of 18:3(n-3) at 0.4 to 0.7% of diet results in its accumulation at 12 - 18%, and in 2C elongation to form 20:3(n-3), eicosatrienoic acid. The 20:3(n-3) accumulates at levels of 1.5 to 3% of PL, in direct proportion to 18:3(n-3) (as with 20:2(n-6) to 18:2(n-6)). Eicosatrienoate is not otherwise found in detectable amounts. Castell & Boghen (1979) reported unusually high levels of both 20:3(n-3) (10 to 21% of TL) and 16:1(n-3) (up to 13% of TL) in the juvenile lobsters in their study. In this study, the weight percentage of a peak which is not positively identified, but elutes where 16:1(n-3) would, theoretically (pers. comm. J. D. Castell) (Appendix 4) was never detectable at more than 0.3% of PL or NL, and 20:3(n-3) was found in detectable levels (in the PLs) only in lobsters fed the three diets which contained a minimum of 0.6% 18:3(n-3). The effect of 1% inclusion of 22:6(n-3) on accumulation of 20:3(n-3) as reported by Castell & Boghen (1979) was also not evident in the lobsters in this study. They attributed their findings to "reversible reactions of chain elongation and desaturation of (n-3) series FAs", i.e. retroconversion from 22:6(n-3). While the level of 20:5(n-3) (5 - 8% of PL) maintained by lobsters fed 22:6(n-3) is not inconsistent with possible retroconversion, it may also be explained by retention of 20:5(n-3), facilitated by a sparing effect by dietary 22:6(n-3) (i.e. metabolized in place of 20:5(n-3)). There is not

strong evidence in this study in support of active chain shortening of (n-3) FAs. Although peaks occur at the theoretical retention times of 16:3(n-3) and 14:3(n-3) (pers. comm. J. D. Castell) (Appendix 4) these FAs are not positively identified, and the levels at which they occur are both low and inconsistent, particularly in contrast to the levels reported by Castell & Boghen (1979).

In lobsters fed the H-CRD, the only diet which contained 20:5(n-3) (at 0.60% of diet, DWB), there was an apparent accumulation of 20:5(n-3) at ~20.8% of PLs (and ~4% of NLs). By contrast, this FA did not exceed 5 to 8% of FAs in lobsters fed any other diet treatment. The NLs and PLs of the initial lobsters contained ~8.3% and ~5.3% of 20:5(n-3), respectively. Considering that the proportion of 20:5(n-3) was < 2.5% of the NLs and < 1 to 3.8% of PLs in lobsters subsequently fed Diets A, B, D, F, G, and H, it appears that 20:5(n-3) may have still been retained in the initial animals after uptake from the H-CRD, fed prior to the three weeks of feeding the lobsters the fat-free diet. This may indicate that the three weeks on the fat-free diet was not sufficient to fully deplete the EFA reserves in juvenile lobsters. In fact, mortality among lobsters fed the fat-free and EFA-deficient diets did not commence for 10 to 11 weeks (Part II, Chapter 1). This somewhat "prolonged" retention of PUFAs or HUFAs may account for the high levels of 20:5(n-3) (10-19% of TLs) reported by Castell & Boghen (1979) for the juvenile lobsters fed one of four semi-purified diets which all lacked 20:5(n-3). Prior to feeding them the test diets, the lobsters had been fed Artemia for two weeks. This was a likely a high source of dietary 20:5(n-3) and other HUFA (Jackson et al., 1991). Due to high mortality of lobsters, it was necessary for Castell & Boghen to terminate the feeding trial and analyze the lobsters for FA composition after only 6 weeks, which may not have been sufficient time to deplete 20:5(n-3) levels and possibly other FAs taken up from the Artemia.

D'Abramo et al. (1980) also report (but do not discuss) high levels of 20:5(n-3) in

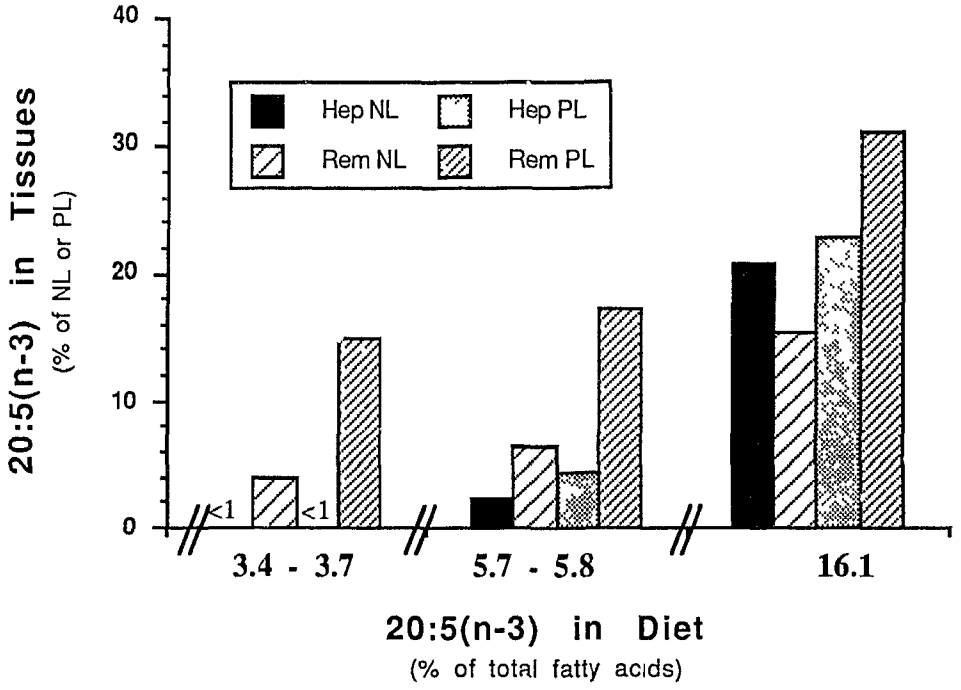


Figure 8. The Relationship Between Dietary Levels of 20:5(n-3) and Levels in the Hepatopancreas (Hep) and Whole Body Remains (Rem).

Adapted from data presented in Table by D'Abramo et al. (1980).

juvenile lobsters fed diets high in that FA. It appears from their data that the percentage of 20:5(n-3) in both the NLs and PLs of the hepatopancreas and the remaining tissues was directly proportional to the level of 20:5(n-3) in the diet (Figure 8, adapted from their data).

For all diet treatments, the proportion of 20:5(n-3) was higher in the PLs than in the NLs and was higher in the "remaining parts" (i.e. whole carcass without the hepatopancreas) than in the hepatopancreas.

Eicosapentaenoate, 20:5(n-3), comprised ~ 8% of PLs in lobsters fed Diet C (high in 18:3(n-3) and with only minimal 18:2(n-6)). This apparent buildup of 20:5(n-3) in the PLs, although minimal compared to the 18:3(n-3) in the PLs of Diet C lobsters (~44%), may demonstrate at least a limited capacity of juvenile lobsters for desaturation of (n-3) series FAs to form HUFA. Also, the ~5 to ~8 % proportion of 20:5(n-3) in the PLs of lobsters fed Diets H and I (both high in 22:6(n-3)), may indicate a capacity for retroconversion in order to maintain a desirable level of 20:5(n-3). There is further evidence of a small degree of retroconversion to form shorter chain (n-3) fatty acids, in lobsters fed Diet C.

The apparent active accumulation of 20:5(n-3) from dietary sources and its depletion from the NLs (primarily) and also from the PLs may indicate it has role as a metabolic precursor (e.g. of prostaglandins, etc.). Its retention (in the PLs), even at low levels, may indicate that it also fulfills a role in the membrane structure or function.

In the previous attempt by Castell & Boghen (1979) to determine lobster EFA requirement, there was high mortality within six weeks on the test diets, attributed to amino acid deficiencies. It is possible that the unusual FA profiles in the surviving lobsters (which have not been reported for other crustaceans and were not found in the lobsters fed similar diets in this study) may also have been at least partially due to amino acid deficiency

and concomitant interference with lipid metabolism. In the vertebrates, carnitine, synthesized from methionine (Met) and lysine (Lys), is required to shuttle fatty acyl groups across the mitochondrial membranes, and especially into the inner mitochondria, where ß-oxidation occurs. Since Met and Lys are essential amino acids (cannot be manufactured in the body), the deficiency in the diet of Met or Lys can result in fatty liver and tissues since the fatty acids taken up by the tissues can not be oxidized in the mitochondria. Methionine is also important in the PL metabolism of decapod crustaceans. The incorporation of ^{3}H-ethanolamine into the PC in the hepatopancreas of Carcinus maenas (Chapelle et al., 1982a), of (methyl-^{14}C)-S-adenosyl-L-methionine into whole lobster PC (Shieh, 1969), and of 1-methyl-^{14}C-methionine into hepatopancreas PC of Eriorcheir sinensis, indicate the occurrence of the N-methylation step in PL synthesis. While these details in fatty acid metabolism have not been fully elucidated for crustaceans, the deficiency of methionine in the Castell & Boghen diets may have impaired metabolism in those lobsters, giving rise to the unusual fatty acid profiles.

In summary, the results from this study provide consistent evidence for active chain elongation but only minimal desaturation of C_{18} (n-3) or (n-6) FAs and therefore minimal bioconversion to HUFAs in juvenile lobsters.

2.5 CONCLUSIONS

The following conclusions are suggested trends based on GLC analysis of single samples of three pooled, homogenized, whole lobsters per diet treatment.

1. 16:1(n-7) and 18:1(n-9) accumulate at ~36 to 42% of phospholipids in lobsters fed fat-free or essential fatty acid -deficient (hydrogenated coconut oil -based) diets, indicating Δ^{9}-desaturase activity.

2. The accumulation of 18:1(n-9) fatty acids in tissue phospholipids, with no detectable level of C_{20} or C_{22}, indicates a lack of Δ^{6}-desaturase activity on the (n-9) series fatty acids.

3. A minimum level of ~4.1 - 5% of 18:2(n-6) in the whole animal phospholipids of lobsters fed diets containing only < 0.005 to 0.13% 18:2(n-6) indicates:

   a) 18:2(n-6) is 'selectively' retained in the phospholipids,
   b) there is a high capacity for uptake from dietary sources, and
   c) there may be an essential role of 18:2(n-6) in lobster phospholipids.

4. The accumulation of 18:2(n-6) in whole lobster phospholipid (at levels from 5 to 28 % of phospholipids and 2 to 27% of neutral lipids) in proportion to its inclusion in the diet (from 0.13 to 0.84% of dry diet) demonstrates a high capacity for incorporation of 18:2(n-6) from dietary sources and indicates specific retention of this fatty acid relative to dietary saturated fatty acids and monounsaturated fatty acids.

5. The low levels of 20:4(n-6) acid (~0.2-1.1% of phospholipid) in the presence of high dietary and tissue levels of 18:2(n-6) and low 18:3(n-3) indicates a lack of Δ^6-desaturase activity for the (n-6) series fatty acids.

6. In the absence of a dietary source of 18:3(n-3), it is depleted from lobster whole body phospholipids. Conversely, addition of 18:3(n-3) at 0.4 to 0.8% of diet results in its accumulation at 11 - 45 % of phospholipid, and 2-C elongation to form 20:3(n-3).

7. There is apparent but minimal bioconversion of 18:3(n-3) to C_{20} and C_{22} (n-3) fatty acids, indicating limited activity of Δ^6 and other desaturase activity on the (n-3) series fatty acids; limited retroconversion of (n-3) fatty acids is indicated by minimal bioconversion of 22:6(n-3) to 20:5(n-3)

8. Highly unsaturated C_{20} and C_{22} fatty acids are readily incorporated from dietary sources into the phospholipids.

9. Based on conclusions 2, 5 and 7, there is limited Δ^6-desaturase activity in juvenile lobsters raised at 20°C.

PART III

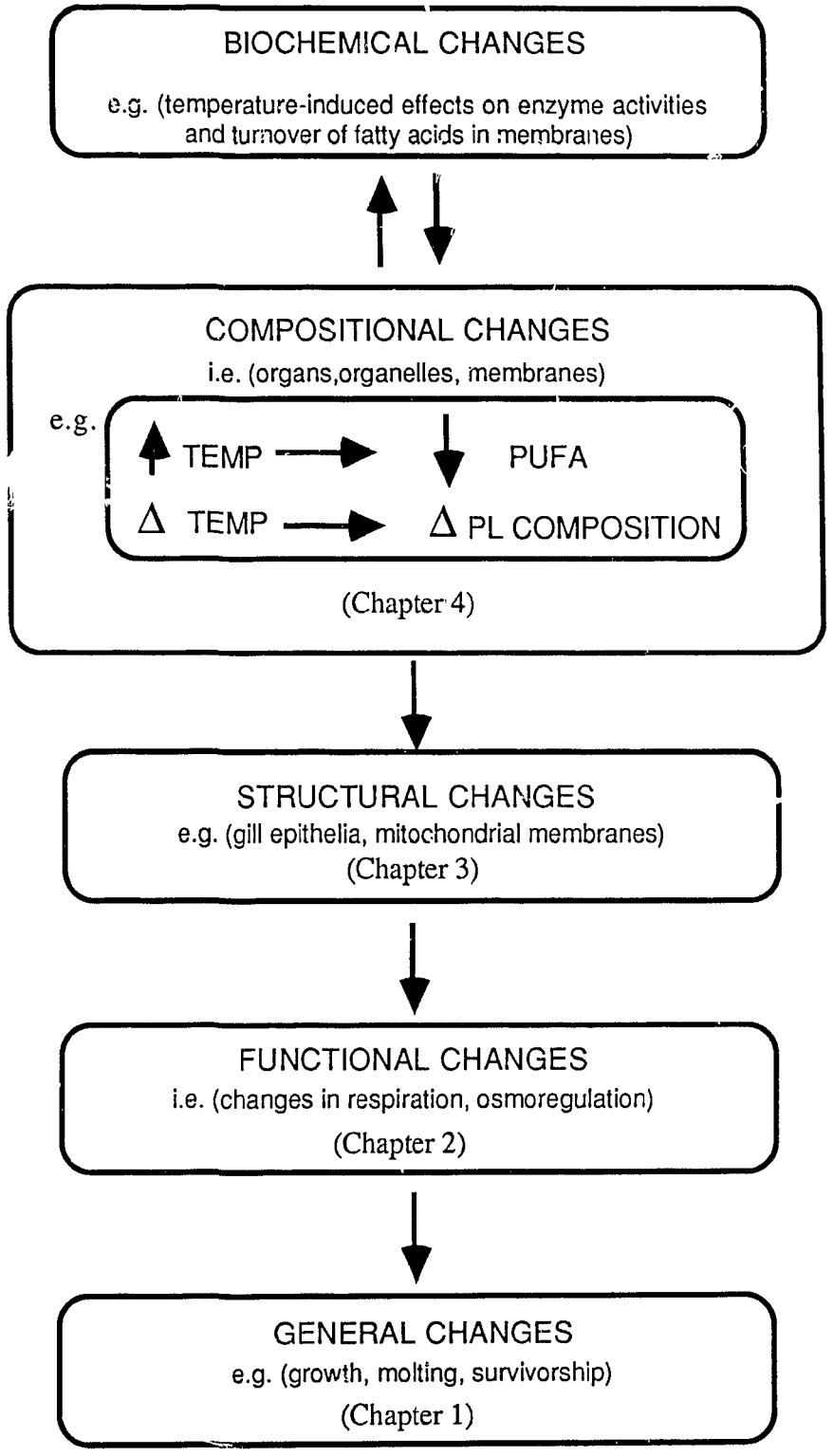
THE COMBINED EFFECTS OF TEMPERATURE AND DIETARY FATTY ACIDS ON THE LOBSTER, Homarus americanus

1.0 GENERAL INTRODUCTION

The lobster Homarus americanus, a decapod crustacean, is able to survive and function at widely different habitat temperatures. Since its body temperature fluctuates directly with the temperature of the environment. it is classified as a eurythermal poikilotherm (Prosser, 1973; Hazel & Prosser, 1974). Many poikilotherms respond to changes in temperature by acclimation or acclimatization. These processes are the result of physiological adjustments or compensations, which have been reported for all levels of biological organization (Hochachka &Somero,1974).

The experiments of each chapter in Part III of this thesis examine the effect of temperature, on the response to dietary deficiency of (n-3) or (n-6) fatty acids, at different levels of biological organization (Figure 9). The rationale behind this approach, with respect to possible changes in fatty acid requirements with changes in temperature, is as follows (refer to Figure 9): Temperature has been shown to induce biochemical changes in organisms (e.g. changes in enzyme kinetics and concomitant changes in rates of reactions can alter the 'normal' pathways of fatty acid metabolism). Alterations of this nature can affect changes in the fatty acid composition at various levels (membrane, organelle, whole organ). Changes in fatty acid composition may induce structural changes in the affected organs. Structural changes, in turn, may impact the physiological function of the organism

Figure 9. Effect of Temperature on Fatty Acid Metabolism and Consequential Changes at Various Levels of Biological Organization



(e.g. respiration, osmoregulation). These changes may then be manifested in altered general aspects of the organism (e.g. growth, molting frequency, survivorship).

This multi-leveled approach facilitates integrating information from general observations of growth, molting and survival with evidence of more specific effects on function (metabolic rate), structure, composition, and biochemistry in examining fatty acid metabolism in the lobster, and assessing the functions of and requirements for various fatty acids based on deficiency symptoms at the various levels.

CHAPTER 1

The Effects of Dietary PUFA Deficiencies on Growth, Molting and Survival of Lobsters Reared at 10°C and 20°C

1.0 INTRODUCTION: PART III, CHAPTER 1

This chapter examines the effects of four test diets with selected FA deficiencies, and a reference diet, on the growth, molting and mortality rates of juvenile lobsters reared at 10°C or 20°C. The lower temperature was selected for this feeding trial based on the findings of Hughes & Matthiessen (1962) that molting in Homarus americanus seldom occurs below 10°C. Growth below 10°C is thus concomitantly slower. Therefore, 10°C was the lowest practical rearing temperature to assess the combined effects of dietary (n-6) and/or (n-3) FA deficiencies at low temperature on normal physiological processes (i.e. growth and molting, as well as survival). Aiken & Waddy (1976, 1986) report that molting rate in H. americanus is a function of accumulated degree-days. For example, under constant temperature conditions, if intermolt period of a given stage lobster would take 30 days at 20°C, at 10°C, intermolt could be expected to last 60 days. (The real benefit of the degree-days relationship is that molting frequency and thus an estimate of growth rate can be determined under natural conditions, with fluctuating temperatures).

The upper rearing temperature of 20°C was selected to give a ten degree temperature differential between warm-acclimated and cold-acclimated lobsters in order to evaluate the effect of temperature on FA metabolism and EFA requirements. Secondly, it had been established by that maximum growth rate (with high survival) for H. americanus occurs at 21-22°C (Templeman, 1936; Botsford et al., 1974; Van Olst, 1975; reviewed by Van Olst et al., 1980), while Hughes & Mattheissen (1962) reported that maximum molting frequency of lobsters occurred in the 15-20°C range, and decreased at temperatures above 20°C. In addition, based on these findings, the standard rearing temperature in lobster nutrition research experiments has been established at 20°C in several laboratories (pers. comm. J. D. Castell, Department of Fisheries & Oceans, Halifax, NS; D. E. Conklin, Univ. of California, Davis & Bodega Bay, Calif.).

The diets in this study contained ~ 9% lipid as FAs of which - 8% of diet was commercial methyl oleate, 18:1(n-9), and ~1% was a PUFA (either 18:3(n-3) or 18:2(n-6), or a mixture of the two). A PUFA-deficient (EFA-deficient) diet and the Halifax Crustacean Reference Diet (H-CRD) were tested for comparison.

1.1 OBJECTIVES

1. Determine the effect of temperature on survival of juvenile lobsters fed diets deficient in (n-3), (n-6) or both types of fatty acids.

2. Determine the effect of temperature on growth of juvenile lobsters fed diets deficient in (n-3), (n-6) or both types of fatty acids.

3. Determine the effect of temperature on molting rate of juvenile lobsters raised on diets deficient in (n-3), (n-6) or both types of fatty acids.

1.2 MATERIALS AND METHODS

This experiment was designed to examine various aspects of the combined effects of temperature and FA deficiency not only on whole animals (which were pooled for analyses in the previous experiment) but also on separate tissues of individual lobsters. It was therefore necessary to conduct the feeding trial so as to ensure that, given the worst case scenarios, there would be sufficient mass of tissues isolated from individual lobsters (after a suitable time period) to permit FA analysis of both total lipid and lipid fractions.

The effects on growth and survival at 20°C were estimated by comparison with data from the previous experiment, as lobsters were fed diets with similar basal diet composition and FA deficiencies, but with a different source of non-essential FAs. However, it was anticipated that possibly no growth, limited growth or even weight loss and high mortality could occur among lobsters at the low temperature (10°C). Of the three tissues examined for evidence of FA deficiency (hepatopancreas, abdominal muscles and

gills), it was presumed that the tissue and lipid mass of the gills would be the critical limiting tissue. Based on literature values of gill lipids, it was decided that juveniles of approximately 4 to 5 months of age (past metamorphosis) would be the youngest (smallest) stage feasible to meet the above criteria. Due to space limitations in the wet lab, and the need to work with with larger lobsters (and the limited availability of lobsters at the required stage/size) the experiment was limited to 100 individuals. Therefore, the number of lobsters tested per replicate and the number of replicates were reduced from the previous experiment (the number of treatments selected remained at 10, 2 temperatures and 5 diets).

1.2.1 SOURCE OF ANIMALS

Full sibling juvenile lobsters were obtained from a commercial aquaculture facility, Advanced Lobster Technology (ALT), P.E.I., Canada. The lobsters had been spawned from a female captured from Northumberland Strait, off Nova Scotia, Canada. The larvae were reared in batches until they metamorphosed to the fourth (postlarval) stage. Individuals were then maintained in separate compartments and fed the Marine Lobster Farms Crustacean Lab Practical Diet (H-EXD) for approximately 4 months. H-EXD was developed by J. D. Castell, DFO, Halifax, and E. Mason, Marine Lobster Farms, P.E.I.).

1.2.2 WET LABORATORY AND REARING CONDITIONS

Experiments were conducted in a separate wet lab at the Aquatron seawater facility of Dalhousie University. In the laboratory, the lobsters were placed into individual, coded compartments (4 in. x 4 in. x ~2.5 in water depth) within plexiglass trays (Figure 3-C, Part II, Ch. 1). The trays were immersed in fiberglass tanks which received flow-through, heated seawater (20°C; 31 ppt) from a common header tank. Photoperiod was controlled by a clock timer set at 16 h light : 8 h dark.

1.2.3 STOCKING METHOD (Distribution of Lobsters to Treatments)

Neither molting nor weight gain records of these individuals had been kept at the ALT facility, so molt stage at the start of this experiment was not known. After arrival of the lobsters at the Aquatron laboratory, wet weight was determined for all individuals. Due to the large size range of the lobsters (0.62 g - 4.45 g wet weight, mean = 1.91, n = 114), the largest 100 animals were ranked by weight into 5 classes of twenty individuals. To equalize initial mean weight and size range for all 20 treatments and replicates (i.e. 2 temperatures x 5 diets x 2 replicate trays of 5 lobsters), one lobster from each weight class was randomly distributed into individual compartments in each of the 20 trays. One-way ANOVA (SPSS, v.9.0) of the mean weights of the twenty groups verified there was no significant difference among them (p= 0.9971). Trays and compartments were numerically and color coded to facilitate record-keeping of individuals throughout all experiments.

The juvenile lobsters were acclimatized to the system, maintained at 20°C, and fed the Halifax crustacean reference diet (H-CRD) for the subsequent 4 weeks, until the lobsters molted to next stage . A semi-purified fat-free diet was then fed to the lobsters for 3 weeks, in an attempt to reduce their lipid reserves. ANOVA and Student Newman-Keuls Multiple Comparison Tests showed no significant difference ($p < 0.05$) among mean weights of lobsters from the twenty trays after this three-week period, and Chi-Square analysis showed no significant difference in the number of lobsters with molted in each tray. The water temperature of lobsters in ten of the twenty trays was gradually reduced from 20°C to 10°C over 48 hours, and maintained at 10°C for the remainder of the experiment. A summary of the diet and temperature history of the test lobsters is shown in Figure 10.

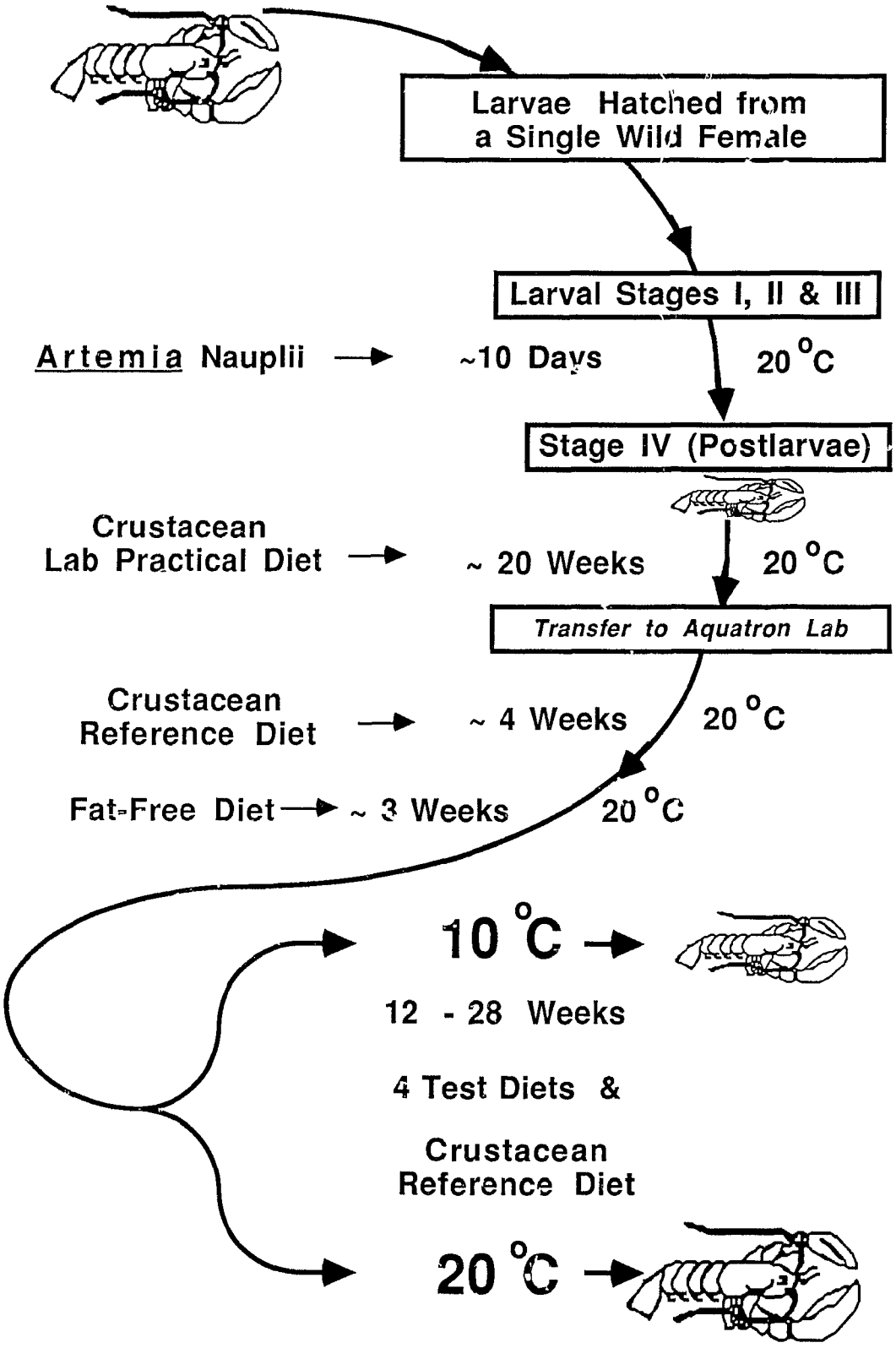


Figure 10. Diet and Temperature History of the 100 Lobsters in Feeding Experiment II (and the Associated Experiments in Chapters 1-5, Part III).

1.2.4 ASSIGNMENT OF TEST DIETS

In this experiment, lobsters were fed one of four semi-purified test diets or the H-CRD. The diets were randomly assigned to two trays of five lobsters at each temperature. Trays and diets were coded by letter and color.

1.2.5 DIETS AND DIET PREPARATION

Diets for Experiment Series II were prepared in the Halifax Laboratory, Department of Fisheries and Oceans, Canada, as in Part II, Chapter 1, Section 1.2. The composition and ingredient sources of the basal diet (90.2% of dry diet) are listed in Table 9. Trace lipids were extracted from appropriate basal diet ingredients (see method, Appendix 1-A). The vitamin mix formulation is listed in Appendix 1-B. The commercial mineral mix composition is cited in Bernhart-Tomarelli (1966). Lipid premix ingredient sources are listed in Table 9, and the lipid composition of the four semi-purified test diets are shown in Table 10. Lipids were added in the form of purified methyl esters. Methyl oleate was added to all diets at ~8% of dry weight; the EFA-deficient diet was supplemented with an additional 1% of methyl oleate only. The remaining 3 diets received 1% of either methyl linoleate, methyl linolenate, or a 1% mixture of both.

1.2.6 MAINTENANCE AND MONITORING OF LOBSTERS

Twice daily the condition of health and "appetite" (estimate of food consumption) of all individual lobsters was noted, excess food was removed by siphon, each compartment was flushed with a hose, and animals were fed pre-weighed amounts of the diets. Initially, lobsters were fed at ~2% body weight/day. This was gradually increased to match consumption. After ~55 days on the test diets, lobsters at 20°C would consume ~10% of body weight/day.

Table 9.

Ingredient Composition of the Semi-Purified Diets from Feeding Experiment II.

Ingredient	% Dry Weight	Source[1]	Lot Number
Basal Dry Mix	90.8		
Crab Protein[2]	40.0		Spring'84
Gelatin[3]	10.0	ICN	10578
Cornstarch[3]	15.0	USBC	26990
α-Cellulose[3]	16.8	USBC	39303
Mineral Mix[4]	5.0	USBC	36799
Vitamin Mix	2.0	Formulation	(see Appendix 1-B)
Cholesterol	1.0	JTB	051352
Choline Chloride	1.0	USBC	36912
Lipid Pre-Mixes	9.2		
DL-α-Tocopherol	0.2	USBC	38854
18:1(n-9)[5]	(Table 10)	NCP	U-46M-D3-5
18:2(n-6)[5]	(Table 10)	NCP	U-59M-AU23-4
18:3(n-3)[5]	(Table 10)	NCP	U-62M-D3-5

1. ICN (ICN Pharmaceuticals, Life Sciences Group, Cleveland, Ohio 44128), JTB (J. T. Baker, Philipsburg, N.J. 08865), NBC (Nutrition Biochemicals Corporation, Cleveland, Ohio 44128), NCP (Nu-Chek-Prep, P.O.Box 295, Elysian, MN 56028), USBC (U.S. Biochemicals Corporation, Cleveland, Ohio 44128).
2. Protein from rock crab, Cancer irroratus (Castell et al., 1989b)
3. Ingredient extracted by hot isopropanol, followed by an ethanol rinse, then dried and finely milled (extraction method, see Appendix 1-A)
4. Bernhart-Tomarelli salt mix, modified (Bernhart & Tomarelli, 1966)
5. Methyl ester derivatives of the fatty acids

Table 10.

Lipid Premix Formulations of Diets from Feeding Experiment II[1].

Ingredient[2,3]	Diet			
	A	B	C	D
DL-α-tocopherol (Vit E)[4]	0.2	0.2	0.2	0.2
Oleic acid[5], 18:1(n-9)	9	8	8	8
Linoleic acid, 18:2(n-6)	0	1	0	0.25
Linolenic acid, 18:3(n-3)	0	0	1	0.75

1. Lipid premixes constituted 9.2% of diets (DWB).
2. Source are cited in Table 9.
3. Methyl ester derivatives of the fatty acids.
4. Vitamin E is lipid soluble and a natural antioxidant.
5. Fatty acid analysis of methyl oleate yielded the following composition: 16:0, 0.01%; 16:1(n-7), 0.02%; 16:3(n-4), 0.1%; 18:0, 0.55%; 18:1(n-9 + n-7), 95.54%; 18:1(n-5), 3.03%; 18:2(n-6), 0.78%; 20:1(n-9), 0.01%.

1.2.7 MEASUREMENTS

All lobsters were examined twice daily for shed exuvia or mortalities, to establish molting rate and survival rate. Wet weight of all individuals was measured approximately every three to four weeks, as reported in results section.

1.3 RESULTS

1.3.1 DIET COMPOSITION

The proximate compositions of the semi-purified test diets (Diets A, B, C and D) and Diet E, the Halifax Crustacean Reference Diet (H-CRD) are given in Table 11. The total lipid levels of the diets ranged from 10.3 to 10.7% DWB. In addition to the FA methyl esters, added at a total of ~9% of diet (DWB), other lipid sources were the fat-soluble vitamins, A, D, E, and K (which contributed approximately 0.24%) and cholesterol (added at 1%).

A summary of the FA content of diets (as percentage of dry diet) is given in Table 12. As in Experiment I, although basal diet ingredients were extracted with hot IPA to remove trace lipids, it was evident from Diets A and C, (which were supposedly free of linoleate), that there was a background level of ~0.04 -0.05% of 18:2(n-6) in the diets. GLC analysis of the commercial methyl oleate (guaranteed analysis 99.99% pure) revealed that it contained ~0.78% 18:2(n-6).

The level of SFAs in the test Diets, A-D was ~0.01 to 0.02% of diet, and MFAs constituted ~7.9 to 8.9% of the diets, DWB. Diet B contained 0.88% 18:2(n-6) and no 18:3(n-3), Diet C contained 0.89% 18:3(n-3) and < 0.1% 18:2(n-6), and Diet D contained 0.25% 18:2(n-6), and 0.66% 18:3(n-3), for an (n-3) to (n-6) ratio of 2.5. The

Table 11.

Proximate Composition of Diets from Experiment Series II[1].

Diet	% Moisture	% Dry Matter	% Ash[2]	% Lipid[2]	% Crude Protein[2]
A	59.5 ± 0.1	40.5 ± 0.9	11.9 ± 0.6	10.3 ± 0.2	~50.0
B	59.8 ± 0.2	40.2 ± 1.3	11.7 ± 0.1	10.7 ± 0.2	~50.0
C	61.8 ± 2.0	38.2 ± 0.2	11.7 ± 0.2	10.3 ± 0.1	~50.0
D	60.0 ± 7.1	39.4 ± 0.1	11.8 ± 0.1	10.3 ± 0.4	~50.0
E	10.3 ± 4.7	89.7 ± 0.1	7.0 ± 0.1	10.6 ± 0.1	~50.0

1. Means of 2 replicates ± Std. Dev.
2. Calculated on dry matter basis

Table 12.

Fatty Acid Composition of the Diets from Experiment II (as % of Diet, DWB).

Fatty Acid	Diet				
	A	B	C	D	E
14:0	---	---	---	---	0.43
16:0	0.01	0.01	0.02	0.01	1.04
18:0	---	---	---	---	0.07
16:1	---	---	---	---	0.58
18:1	8.87	8.05	7.94	7.98	2.00
20:1	---	---	---	---	0.77
22:1	---	---	---	---	0.75
18:2(n-6)	0.04	0.88	0.05	0.25	2.36
18:3(n-6)	0.01	0.01	0.01	0.01	0.01
18:3(n-3)	0.01	---	0.89	0.66	0.05
18:4(n-3)	---	---	---	---	0.09
20:4(n-3)	---	---	---	---	0.01
20:5(n-3)	---	---	---	---	0.32
22:5(n-3)	---	---	---	---	0.23
22:6(n-3)	---	---	---	---	0.30
(n-3)/(n-6)	---	---	---	2.5	0.42

H-CRD contained ~1.5% SFAs, 4% MFAs, ~2.4% 18:2(n-6), ~0.05% 18:3(n-3), and ~0.95% (n-3) HUFA, and had an (n-3) to (n-6) ratio of 0.42.

1.3.2 SURVIVAL

There was a significant effect of temperature on survival of lobsters fed EFA-deficient diets, under the rearing conditions of this feeding experiment. At the elevated culture temperature (20°C), mortality commenced within 115 days and reached 60% within 130 days (see n-values in Table 13 for number of lobsters surviving each treatment at 130 days). By comparison, mortality was only 20% after 130 days among lobsters fed EFA-deficient diets at 10°C.

There was a trend in the effect of diet treatment on survival. After 130 days, there was no mortality among lobsters fed either the H-CRD or the semi-purified test diet supplemented with 1% mixed EFA (0.66% 18:3(n-3) + 0.25% 18:2(n-6)). After 130 days, mortality commenced among lobsters fed semi-purified Diets B and C, each contained only a single EFA (1% 18:2(n-6) or 1% 18:3(n-3), respectively).

1.3.3 GROWTH

There was a significant effect of temperature on growth. Lobsters reared at 20°C grew faster than those reared at 10°C (Figure 11).

ANOVA of final weights of lobsters showed no significant difference among diet treatments at each temperature, and high individual variability (Table 14). However, ANOVA of weight gain of individuals in each treatment, (log transformed, to stabilize the variance) showed a significant effect of diet among lobsters reared at 20°C ($p < 0.05$) There was a greater than three-fold increase in biomass of lobsters fed the reference diet

Table 13.

Means of Wet Weight (g) of Juvenile Lobsters Reared at 10°C or 20°C for 1, 28, 58, 88, 103, and 130 Days*1. n = Number of Surviving Lobsters per Treatment*2.

		Diet*3											
		A			B			C			D			E		
		(n-3) and (n-6) Deficient			0.9% 18:2(n-6)			0.9% 18:3(n-3)			0.25% 18:2(n-6) 0.66% 18:3(n-3)			H-CRD		
Temp.	Day	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
10°C	1	1.565	± 0.721	10	1.549	± 0.577	10	1.564	± 0.659	10	1.549	± 0.659	10	1.553	± 0.697	10
	28	1.911	± 0.747	10	1.835	± 0.508	10	2.116	± 0.972	10	1.897	± 0.597	10	1.917	± 0.698	10
	58	1.929	± 0.698	10	1.986	± 0.585	10	2.227	± 0.966	10	2.050	± 0.760	10	2.037	± 0.753	10
	88	1.989	± 0.877	9	1.966	± 0.607	10	2.206	± 0.975	10	2.055	± 0.773	10	2.058	± 0.828	10
	130	2.028	± 0.913	8	2.110	± 0.640	8	2.229	± 1.000	10	2.101	± 0.784	10	2.111	± 0.840	10
20°C	1	1.570	± 0.745	10	1.489	± 0.768	10	1.564	± 0.676	10	1.558	± 0.615	10	1.472	± 0.567	10
	28	2.005	± 0.858	10	1.920	± 0.723	10	1.724	± 0.683	10	2.100	± 0.575	10	1.700	± 0.585	10
	58	2.164	± 0.981	10	2.204	± 0.968	10	2.139	± 0.802	10	2.456	± 0.845	10	2.149	± 0.662	10
	88	2.229	± 0.905	10	2.295	± 0.939	10	2.170	± 0.870	10	2.575	± 0.816	10	2.513	± 0.777	10
	103	2.013	± 0.682	7	2.338	± 0.893	10	2.315	± 0.936	10	2.653	± 0.846	10	2.861	± 0.886	10
	130	2.315	± 0.748	4	2.661	± 0.885	9	2.366	± 1.050	9	2.821	± 0.854	10	3.316	± 1.090	10

1. Means represent weights of all animals in each treatment group
2. Each treatment group consists of two replicate trays of 5 individually compartmentalized lobsters.
3. Fatty acid composition values given for each diet are expressed as % of diet, DWB

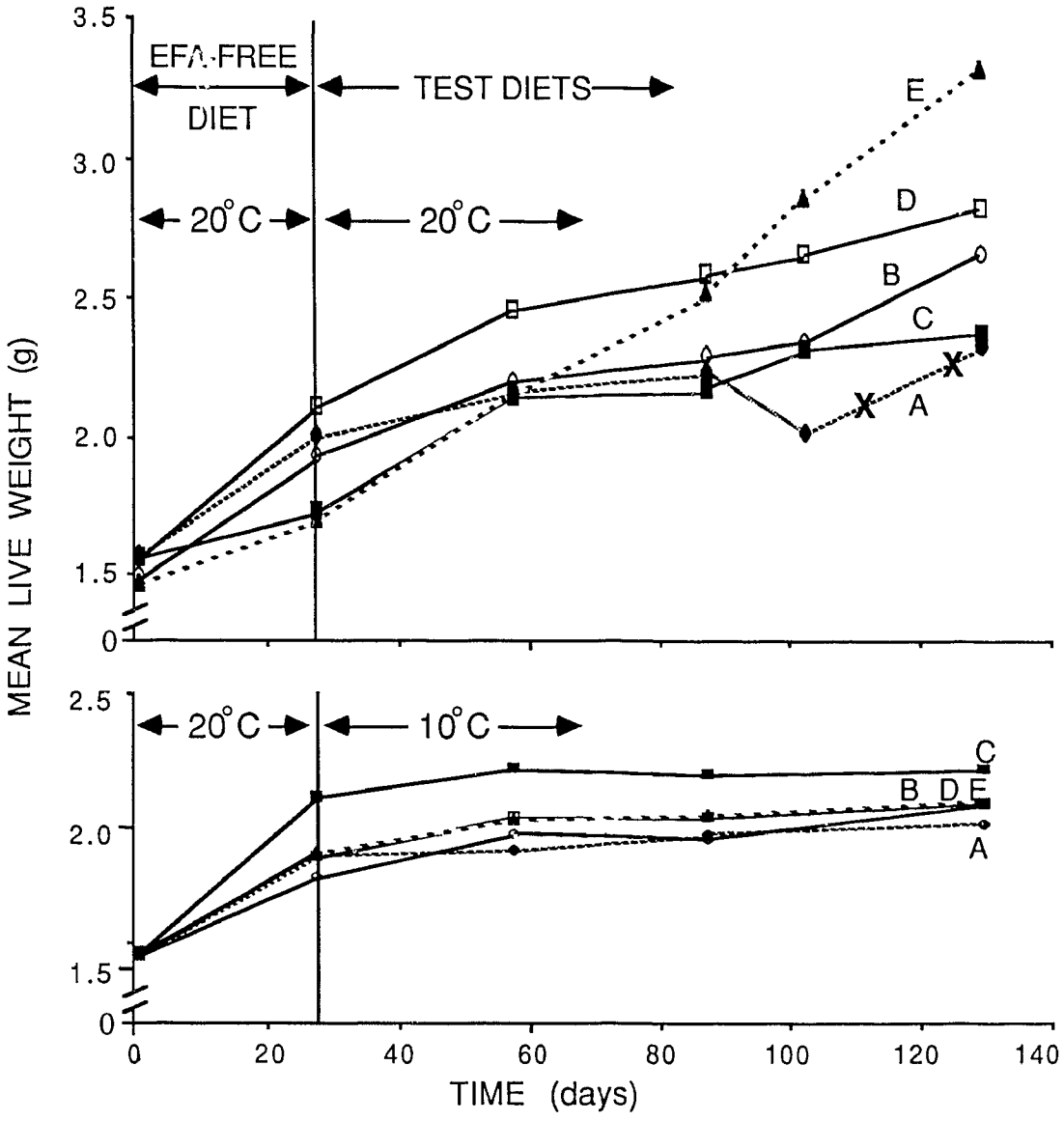


Figure 11. Effect of Temperature and Diet on Growth of Lobsters, Homarus americanus.

Diet treatments are A, EFA-deficient; B, ~1%18:2(n-6); C, ~1% 18:3(n-3); D, 0.25% 18:2(n-6) & 0.66% 18:3(n-3); E, Crustacean Reference Diet. X denotes > 50% mortality of 1 replicate tray of lobsters. *Despite higher mean weight of Diet C lobsters at day 25, there were large standard deviations, and by ANOVA, there was no significant difference between treatment means ($p \leq 0.05$).

Table 14.

Final Mean Wet Weight (g) of Lobsters.

Temperature	Diet	n	Mean* SD	SEM	Min.	Max.
10°C	A	8	2.03 ± 0.91	0.32	1.08	3.52
	B	8	2.10 ± 0.64	0.23	1.17	2.96
	C	10	2.23 ± 1.00	0.32	1.07	4.40
	D	10	2.10 ± 0.78	0.25	0.86	3.74
	E	10	2.11 ± 0.84	0.27	0.95	3.80
	Total	46	2.12		0.86	4.40
20°C	A	4	2.32 ± 0.75	0.37	1.25	3.00
	B	9	2.66 ± 0.88	0.29	1.44	4.38
	C	9	2.37 ± 1.05	0.35	1.06	4.31
	D	10	2.82 ± 0.85	0.27	1.39	3.94
	E	10	3.32 ± 1.09	0.34	1.03	4.56
	Total	42	2.76		1.03	4.56

SNK: No significant difference at $p \leq 0.05$ between groups within each temperature.

* These values (means and SDs) are the same as those reported in Table 13, except that they are rounded-off in this table.

(H-CRD), and weight gain of lobsters fed the H-CRD was significantly greater than the weight gain of lobsters from all other diet treatments ($p = 0.0025$) (Table 13).

1.3.4 MOLTING FREQUENCY

There was a significant effect of temperature on molt frequency. The intermolt period of lobsters at 10°C was longer than for lobsters reared at 20°C (Figure 12).

The effect of diet on molt frequency was significant at each temperature (analysis by ANOVA and Least Significant Differences, LSD). At both 10°C and 20°C, lobsters fed the H-CRD had a significantly higher molting rate than lobsters fed all other diets. Conversely, evaluation by Student Newman Keuls test, demonstrated that the intermolt time (log transformed) of lobsters fed H-CRD was significantly less than for lobsters fed all other diets (Tables 15, 16). At 20°C, the EFA-deficient diet (Diet A) had a significantly reduced molting frequency compared to all other diet treatments; the molting frequency of lobsters fed Diets B, C and D were not significantly different from each other. At 10°C, lobsters fed Diets A (EFA-deficient) and Diet B ((n-3)-free), had a significantly lower molting frequency than those on all other diets. Lobsters fed Diet C (containing 0.9% 18:3(n-3) and only 0.15% 18:2(n-6)) had a marginally (but significantly) higher molting rate than lobsters fed Diet B (containing 0.9% 18:2(n-6) as the only EFA).

1.4 DISCUSSION

The effect of temperature on weight gain (after 100 days) was predictable, with faster growth at the 20°C as compared to 10°C (Figure 11). Hughes et al. (1972) found that growth of lobsters (Homarus americanus) can be accelerated by rearing them at sustained, elevated temperatures (20-22°C). Faster growth of lobsters reared at elevated temperatures was also reported by Bartley et al. (1980).

Table 15.

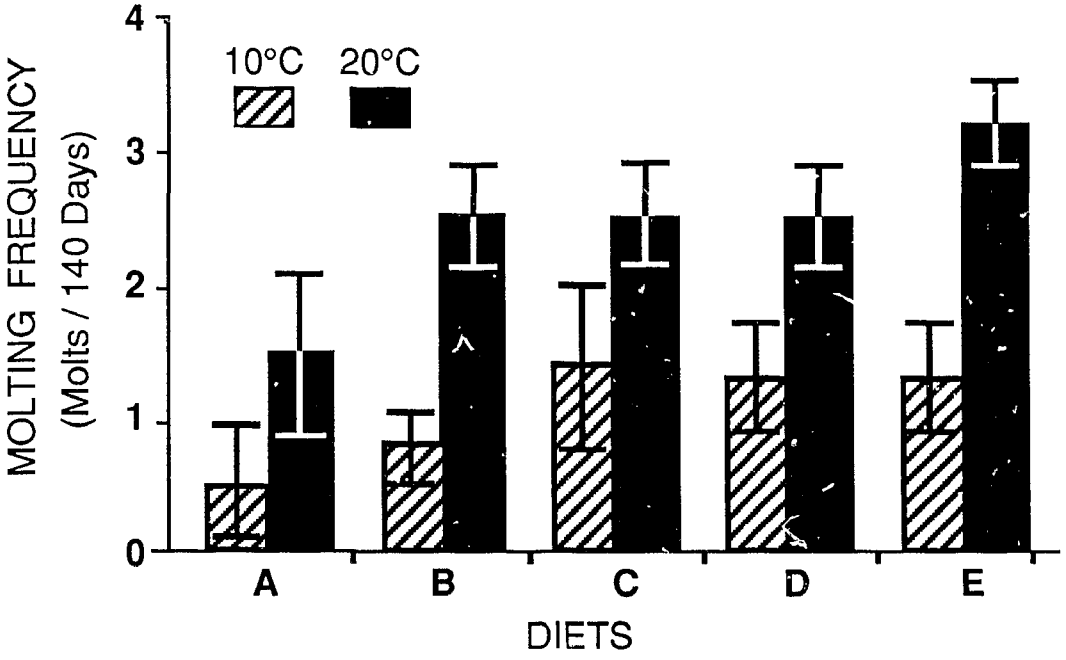
Duration of Intermolt (in days) of Lobsters at 20°C.

Diet	n	Mean	Log of Mean Days Intermolt	S.D.	S.E.M.
A	6	58	1.75	± 0.13	0.05
B	15	52	1.71	± 0.09	0.02
C	16	54	1.73	± 0.08	0.02
D	15	52	1.71	± 0.05	0.01
E	22	43	1.63	± 0.09	0.02
Total	74	50	1.69		

Table 16.

ANOVA of Intermolt Data.

Source of Variation	D.F.	SS	M.S.	F Value	P
Between Group	4	0.15	0.037	5.202	0.001
Within Groups	69	0.496	0.007		
Total	73	0.646			



FATTY ACID	% INCLUSION OF FATTY ACIDS IN DIETS				
	A	B	C	D	E
18:1(n-9)	8.8	8.0	7.9	8.00	H-CRD (see Table 12)
18:2(n-6)	0	0.9	0	0.25	
18:3(n-3)	0	0	0.9	0.66	

Figure 12. Molting Frequency of Juvenile Lobsters in Feeding Experiment II.

Lobsters were raised at 10°C or 20°C for 140 days and fed one of five semi-purified diets. Values plotted are means ± S.D. Fatty acid supplements given in table. H-CRD is a reference diet. Complete fatty acid composition of diets in Table 12. Values plotted are means ± S.D.

Koshio (1985) attributed faster growth rates in H. americanus reared at 20°C as compared to those reared at 15°C to shorter intermolt periods at the higher temperature. Results from this study support Koshio's findings, as there was a significantly higher molting frequency at 20°C than at 10°C over the 140 days it was monitored. In fact, although based on a small data set, these results are generally consistent with the degree-days concept of Aiken & Waddy, 1976, 1986) in that molting frequency at 20°C was nearly double that at 10°C. The results most closely fitting this model are those for lobsters fed Diet A (i.e. lobsters under the greatest nutritional stress - or poorest conditions) and for lobsters fed Diet E (i.e. under relatively optimal dietary conditions). Size increase per molt was not measured in this study. According to Mauchline (1977), molting increment is not affected by temperature, while Lellis & Russel (1990) report that molting increment as well as molting rate contributed to increased growth of spiny lobsters (Panulirus argus) at higher temperatures (30°C vs. 20 or 27°C).

Growth of juvenile lobsters under the experimental conditions was not a sensitive indicator of dietary (n-3) or (n-6) FA deficiency. At 10°C, growth was slower among lobsters on all diets, and therefore possible dietary requirements of lobsters for EFA might not be manifested as differences in growth. Mortality among lobsters fed deficient diets at 10°C commenced before treatment differences on growth were demonstrated, indicating that (n-3) and/or (n-6) FAs have greater value in satisfying physiological or biophysical roles than for growth enhancement.

The delay in growth response, among lobsters reared at 20°C, to dietary deficiencies in (n-3) and/or (n-6) FAs may be partly due to the "reserve" levels of EFA maintained in the body, and the apparent conservation of specific FAs (as demonstrated in Part II, Ch. 2). Moreover, the 8 to 9% dietary inclusion of oleic acid could effectively "spare" the catabolism of tissue PUFA to meet energy needs. The highest mean final

weight among lobsters fed Diets A-D was attained at 20°C (as compared to 10°C) and fed both (n-3) and (n-6) PUFA. The lowest weight was of lobsters fed the diet deficient in both (n-3) and (n-6) PUFA. However, differences in growth due to diet may have been masked by the high individual variability in weight among lobsters on the same treatments. D'Abramo et al. (1980) also attributed lack of growth response of juvenile lobsters to diets containing various combinations of oils (with different FA profiles) to high [genetic] variability in growth among individuals of the same treatment.

Growth rates were highest among lobsters reared at 20°C and fed the reference diet (H-CRD), a semi-purified diet which contained the same protein source as the test diets. As noted in Part II, Ch. 1 the H-CRD contains natural oils (fish oil and corn oil), which provide a broad spectrum of FAs (including ~2.4% by weight of 18:2(n-6) and ~1% by weight of (n-3) HUFAs; Table 12). The oils may also contribute nutrients other than FAs and provide a different balance of fatty acids and nutrients than the test diets, which could account for the superior performance of lobsters fed this diet. However, several studies have shown growth enhancement by inclusion of 20:5(n-3) and/or 22:6(n-3) in diets for crustaceans (Kanazawa et al., 1978, 1979d; D'Abramo & Sheen, 1991; see also, results Part II, Chapter 1).

1.5 CONCLUSIONS

1. Growth rate of juvenile lobsters, H. americanus, is accelerated at 20°C as compared to 10°C.

2. Mortality rate of juvenile lobsters fed a diet deficient in (n-3) and (n-6) polyunsaturated fatty acid occurs sooner and is higher at 20°C than at 10°C.

3. Survival is greater among lobsters fed a diet containing a polyunsaturated fatty acid supplement (~1% of dry diet) which contains both 18:(n-3) and 18:2(n-6) at > 0.3% each, than among lobsters fed

diets with a polyunsaturated fatty acid supplement containing 18:2(n-6) as the sole polyunsaturated fatty acid or with 18:3(n-3) as the sole polyunsaturated fatty acid (18:2(n-6) inclusion at < 0.2% of diet).

4. Growth and molting frequency are independently affected by diet and temperature.

5. (n-3) and (n-6) fatty acids enhance growth and survival of lobsters acclimated to 20°C.

CHAPTER 2

The Effects of Dietary PUFA Deficiencies on The Physiological Condition of Lobsters at Two Acclimation Temperatures, 10°C and 20°C: Assessment by Oxygen Consumption and Ammonia Excretion Measurements

2.1 OBJECTIVES: PART III, CHAPTER 2

1. Determine the effect of (n-3) and (n-6) deficient diets on the routine metabolic rate of juvenile lobsters.

2. Determine the effect of temperature on the respiratory response to essential fatty acid-deficient diets.

3. Evaluate the effect of essential fatty acid-deficient diets on substrate metabolism and the physiological state of juvenile lobsters.

2.2 METHODS AND MATERIALS

2.2.1 GENERAL PROTOCOL

Juvenile lobsters, H. americanus, (1.9 g to 3.0 g wet wt) were those reared under conditions previously described in Part III, Chapter 1, Section 1.2. Animals were maintained on five diets at two temperatures (10°C and 20°C). The diet formulations, proximate composition, and FA composition are given in Tables 9, 10, 11, and 12, respectively, in Part III, Chapter 1. Lobsters were fed in excess, twice daily approximately every 12 hours (at noon and midnight). The light cycle was approximately 16:8 h light:dark. Filtered, heated sea water was used.

After lobsters had been fed for 10 weeks on the test diets, 5 lobsters in the intermolt stage from each of the 10 diet-temperature treatments were selected for the respirometry trials. Although it has been found that rates of ammonia excretion in juvenile lobsters, Homarus americanus, are not significantly affected by photoperiod (Hawkins et al., 1986), they reported a marked daily rhythmic pattern of ammonia excretion. To reduce possible effects of food intake or daily metabolic rhythm on the metabolic rate of test animals, the timing and time of day of both of pre-test feeding and fasting, and of respirometry trials

were standardized for all lobsters. On the day prior to the respirometry, selected test animals were deprived of the noon feeding (and remaining food removed), and fed for approximately two hours (~2100h -~2300h), after which the relative amount of consumption was noted and excess food was removed. The gut passage rate for juvenile lobster has been reported to be 12 h (Bordner & Conklin, 1981); therefore, the test animals in this study were considered to be in the post-absorptive state. Respirometry was conducted at approximately the same time of day (1200 h - 1800 h). These hours of testing bracketed 1500 h, the time of day at which lobsters exhibit minimum motor activity (Reynolds & Casterlin, 1979). All animals from a given diet-temperature regime were tested on the same day.

The closed, plexiglass respirometry chamber (ca. 490 ml) was designed for this experiment by T.G. West (Department of Biology, Dalhousie University). The threaded cover (which screwed into the base) featured (i) a groove in which was fitted a rubber O-ring, to facilitate a tight seal upon closure, (ii) ports for an oxygen probe and a thermometer, and (iii) a concave 'ceiling' to facilitate the flushing out of air bubbles through the probe ports. A plastic screen matting on the floor of the chamber provided a substrate for traction by the lobsters. For each respirometry trial, the chamber was filled and a lobster was added, and the top (without the probe) was screwed on. Sea water was added to the chamber from a side port, to fill the chamber and flush any air bubbles out the top funnel (this was facilitated by the concave inner surface of the chamber lid). The oxygen probe was then inserted through a top port, displacing excess water, and a tight seal was formed. Individual lobsters were acclimated to the respirometry chamber (Figure 13) for 30 minutes prior to testing (Capuzzo & Lancaster, 1979). The chamber was immersed in a temperature-controlled water bath, which was adjusted to the rearing temperature (± 0.2°C) of the individual being tested. A black plastic screen was used to block direct florescent lab lighting and to seclude the chamber and thus minimize

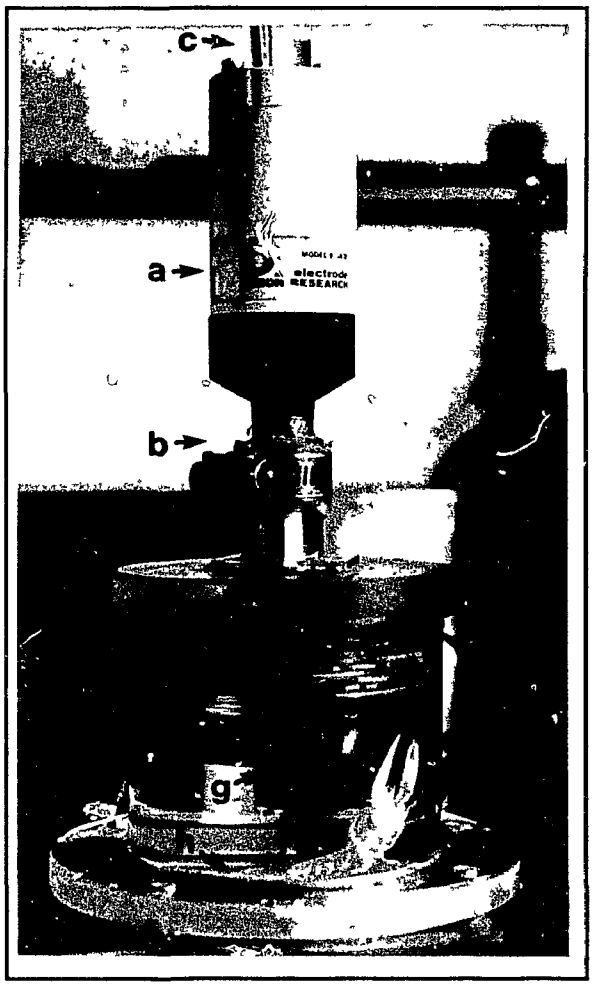


Figure 13. Respirometry Chamber for Juvenile Lobsters, and Oxygen Electrode.

Plexiglass construction; concave top screws into base. a, Oxygen electrode; b,oxygen probe port: spillover from probe insertion displaces trapped air bubbles; c, thermometer; d, O-ring seal; e, screw threads; f-1 & f-2, rubber stoppered ports which permit flushing of the chamber; g, stir bar; h,. plastic screen "mat"; i, juvenile lobster. Note: depigmentation of lobsters occurs with successive molts for lobsters on all test diets due to absence of dietary carotenoid pigments.

Respirometer: Timothy West Photo credit: Mary Primrose Reprints: Mary Primrose

disturbance of the lobsters from lab activity, yet it allowed observation of each animal's activity from a distance. Following acclimation of the lobster, the chamber was gently flushed with sea water (through the side port) and sealed. Simultaneous routine oxygen consumption and ammonia excretion rates were measured. Following each respirometry trial, the lobster was removed from the chamber and weighed, and the remaining liquid volume of the chamber (i.e. without that displaced by the lobster) was measured.

2.2.2 OXYGEN CONSUMPTION

Routine oxygen consumption[6] , measured as MO_2, (mg $O_2 \cdot h^{-1}$), was determined over a one hour period using an Orion oxygen electrode (Figure 12). Measurements were converted to molar values and weight-specific respiration rate, QO_2, in μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ wet weight. Calibration of the oxygen electrode was checked by Winkler titrations. The probe had a captive magnetic stir bar below the probe tip which facilitated mixing of the media for uniform oxygen distribution (Model 97-08, Orion Research Inc., Cambridge, Mass., U.S.A.). Oxygen depletion of the media was monitored on a Fisher Omniscribe 5000 chart recorder. Oxygen depletion never exceeded 60% saturated oxygen tension, well in excess of the critical oxygen tension level at which oxygen consumption becomes oxygen-dependent (Barclay et al., 1983; McLeese, 1956, reports on lethal limits of oxygen for Homarus americanus, as a function of temperature, salinity, size and molting). Blank trials with an unoccupied chamber were also run for a measure of background variation in oxygen concentration due to microbiota or electronic drift of the probe.

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[6] Measurement of routine oxygen consumption is made on animals under uncontrolled but minimal locomotor activity (Fry, 1971).

2.2.3 AMMONIA EXCRETION

Ammonia excretion rate (Q_N, nmol NH_4^+-N·h^{-1}·g^{-1} wet weight) was measured from two 50 ml aliquot samples of the media following each one-hour respirometry trial, and compared with control blanks (from trials with unoccupied chambers). Samples were immediately frozen at -20°C for not more than 5 days. Ammonia production was measured by the modified colorometric indophenol-blue method of Ivancic & Degobbis (1984). Samples were analyzed at 640 nm, using a Perkin-Elmer Lambda 3 UV/VIS Spectrophotometer.

2.2.4 ATOMIC O:N RATIO

The O:N ratio is an indicator of which substrates are being catabolized by an organism. It is a measure of the ratio, in atomic equivalents, of the oxygen consumed, to the ammonia-N excreted (Lucas & Beninger, 1985). Values greater than 50 indicate a high rate of lipid and carbohydrate metabolism (Conover & Corner, 1968; Capuzzo and Lancaster, 1979). Values less than 30 indicate a high rate of protein catabolism (i.e. ammonia-N release), and values less than 20 may indicate low food consumption or starvation (i.e. catabolism of muscle). The theoretical lower limit is 8. The values, because they are proportions, were arcsine transformed (to normalize the variances) prior to statistical analysis.

2.2.5 ANALYSIS OF DATA

To assess the effect of temperature, diet, and temperature-diet interactions, data was analyzed by two-way analysis of variance. Student Newman-Keuls post-hoc test was used to test for difference among means (Sokal & Rohlf, 1980). Calculations were made on MINITAB and SPSS on a mainframe computer.

2.3 RESULTS: RESPIROMETRY

A summary of mean wet weight and SEM of the five lobsters from each diet-temperature treatment which were subjected to respirometry trials is given in Table 17.

2.3.1 OXYGEN CONSUMPTION

The mean weigh-specific oxygen consumption rate ± SEM of lobsters from each of the ten treatments is given in Table 17 and shown in Figure 14-A. QO_2 ranged from 2.2 to 4.7 μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ wet weight. ANOVA statistics, summarized in Table 18, indicate a significant effect of temperature ($p = 0.019$) and diet ($p = 0.005$), and no significant interaction in the effects of temperature and diet.

Student Newman-Keuls test for difference among means indicates that at 20°C there are no significant differences in oxygen consumption among animals fed the 5 test diets (Appendix 3-A). The highest rates of oxygen consumption are among lobsters reared at 10°C and fed Diet D or Diet E. These rates are significantly higher than those of lobsters reared at 10°C and fed Diet A or reared at 20°C and fed Diet A. The QO_2 of lobsters fed Diet D at 10°C is also significantly higher than that of lobsters fed Diet D at 20°C.

2.3.2 AMMONIA EXCRETION

The major nitrogenous waste product of lobsters is ammonia (Logan, 1975). Table 17 summarizes the mean ammonia excretion rates of lobsters reared under the ten diet-temperature regimes. Mean rates ± SEMs are presented in Figure 13-B and ranged from 288 to 499 nmol NH_4^+-$N \cdot h^{-1} \cdot g^{-1}$ wet weight. ANOVA testing of the data shows no significant effects of temperature or diet on the ammonia excretion rates of lobsters reared under the conditions of these experiments (Table 18). ANOVA further indicated that there were no significant overall effects of ammonia excretion rate attributed to an interaction of

Table 17.

Mean Values for Wet Weight, Oxygen Consumption Rate, Ammonia Excretion Rate and O:N Ratio for Lobsters Fed One of Five Diets and Raised and Tested at 10°C or 20°C.

		Wet Weight g		Q O2 µ mol/g/h		Q NH4-N n mol/g/h		O:N Ratio	
Temperature	Diet	Mean*	S.E.M.	Mean*	S.E.M.	Mean*	S.E.M.	Mean*	S.E.M.
10°C	A	2.627	± 0.305	2.53	± 0.35	498.6	± 93.5	11.2	± 2.4
	B	2.282	± 0.345	3.69	± 0.92	464.4	± 90.3	16.2	± 3.7
	C	2.104	± 0.307	3.53	± 0.58	310.0	± 96.1	26.3	± 4.1
	D	1.911	± 0.178	4.67	± 0.33	288.0	± 94.4	48.0	± 14.6
	E	2.477	± 0.389	4.31	± 0.44	308.0	± 46.2	29.7	± 4.0
20°C	A	2.208	± 0.310	2.22	± 0.36	461.2	± 66.0	10.3	± 2.2
	B	2.336	± 0.200	2.98	± 0.32	300.7	± 61.2	28.1	± 11.5
	C	2.438	± 0.283	3.42	± 0.34	480.4	± 89.8	16.5	± 3.3
	D	2.583	± 0.367	2.69	± 0.21	386.8	± 32.0	14.1	± 0.8
	E	3.034	± 0.541	3.85	± 0.23	470.0	± 81.9	17.6	± 1.7

* n=5

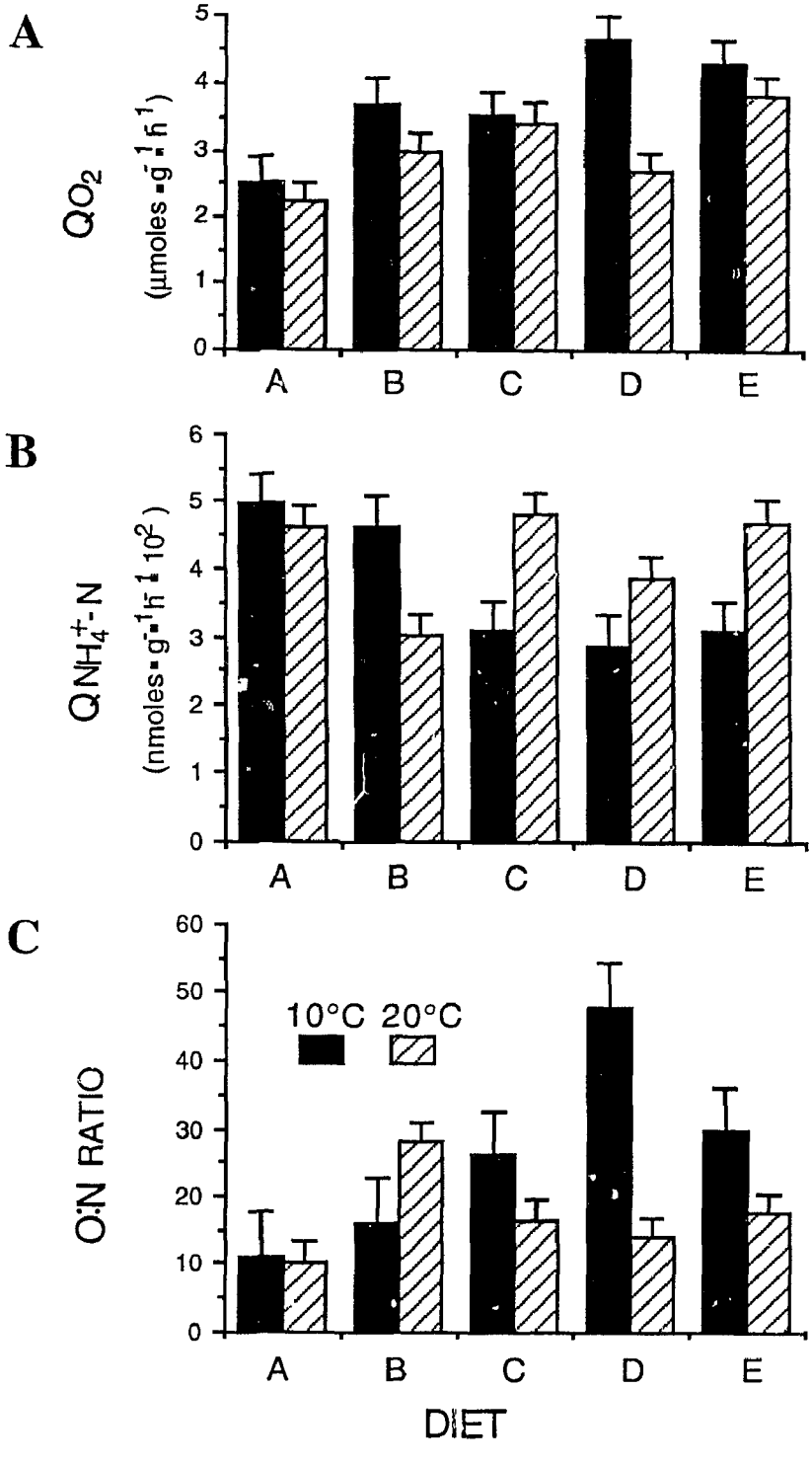

Figure 14. Weight-Specific Metabolic Rates and O:N Ratios of Juvenile Lobsters.

A. Oxygen consumption rates, B. Ammonia excretion rate, and C. Atomic O:N Ratio of lobsters acclimated for 10 weeks to 10°C or 20°C and fed one of five diets (A-E) which varied in fatty acid composition. Values plotted are means ± SEM; n = 5 (individual, intermolt lobsters tested).

Table 18.

ANOVA Statistics for Oxygen Consumption, Ammonia Excretion and O:N Ratio of Juvenile Lobsters on One of Ten Diet-Temperature Regimes.

Parameter	Source of Variation	SS	DF	MS	F-Value	P	Significance*1
Oxygen Consumption	Temp	5.511	1	5.511	5.986	0.019	*
	Diet	15.931	4	3.983	4.326	0.005	*
	Temp x Diet	6.316	4	1.579	1.715	0.166	N S.
	Error	36.825	40	0.921			
Ammonia Excretion	Temp	15523.220	1	15523.220	0.509	0.480	N.S.
	Diet	120500.880	4	30125.200	0.988	0.425	N.S.
	Temp x Diet	268952.080	4	67238.020	2.204	0.086	N.S.
	Error	1220016.000	40	30500.400			
O:N Ratio*2	Temp	113.583	1	113.583	3.891	0.055	*
	Diet	703.476	4	175.869	6.025	0.001	*
	Temp x Diet	187.178	4	46.795	1.603	0.192	N.S.
	Error	1167.558	40	29.189			

1. * Denotes significance at $p \leq 0.05$; N.S. denotes 'not significant'
2. Data were arcsine transformed prior to statistical evaluation

diet and temperature. In post hoc tests of differences among mean values of Q_N for each treatment, gave results of $p > 0.05$, indicating no significant differences. Possible differences among treatment means are likely obscured by high standard deviations.

2.3.2 AMMONIA EXCRETION

The major nitrogenous waste product of lobsters is ammonia (Logan, 1975). Table 17 summarizes the mean ammonia excretion rates of lobsters reared under the ten diet-temperature regimes. Mean rates ± SEMs are presented in Figure 13-B and ranged from 288 to 499 nmol NH_4^+-N•h^{-1}•g^{-1} wet weight. ANOVA testing of the data shows no significant effects of temperature or diet on the ammonia excretion rates of lobsters reared under the conditions of these experiments (Table 18). ANOVA further indicated that there were no significant overall effects of ammonia excretion rate attributed to an interaction of diet and temperature. In post hoc tests of differences among mean values of Q_N for each treatment, gave results of $p > 0.05$, indicating no significant differences. Possible differences among treatment means are likely obscured by high standard deviations.

2.3.3 O:N RATIO

The means and SEMs of the O:N ratios are shown in Table 17 and Fig. 13-C. ANOVA statistics (Appendix 5-B and Table 18) show an effect of temperature and of diet, and no interaction effect. At both 10°C and 20°C, the lowest mean O:N values are among lobsters fed Diet A. Student Newman-Keuls post hoc comparisons of means show the effects of Diet A at 10°C and at 20°C are significantly different from those of Diets C, D, and E at 10°C. At 20°C, Diet E was marginally different from Diet A ($p = 0.06$).

2.4 DISCUSSION

The lowest rates of oxygen consumption of lobsters reared at 10°C or at 20°C were among those fed Diet A, which was deficient in both (n-3) and (n-6) PUFAs. This significant depression of metabolic rate can be correlated to observations of the behavior of those lobsters. Diet A lobsters became sluggish and limp in comparison to lobsters from the other treatments. Before dying, Diet A lobsters were typically quiescent, and were apparently without sufficient strength to move about or lift their large chelipeds, and eventually collapsed, unable to hold their bodies up over their legs. This is consistent with the lowered metabolic rate in these animals. The lowest growth rate and highest mortality (Part III, Ch. 1) also occurred among Diet A lobsters and the hepatosomatic index of Diet A lobsters was significantly lower than that of lobsters on all other diet treatments (Part III, Ch. 3).

In contrast, Diet E lobsters, which had the highest metabolic rates, were the largest, fastest growing, most active lobsters, and appeared to be the most vigorous. The rate of oxygen consumption for Diet A lobsters at 10°C is 58% of the rate of Diet E lobsters at 10°C, and the rate for Diet A lobsters at 20°C is 58% of the rate of Diet E lobsters at 20°C. While the overall significant effect of temperature on oxygen consumption rate can likely be attributed to highest mean rates occurring in lobsters fed Diets D and E at 10°C as compared to the lowest mean rate occurring in lobsters at 20°C raised on Diet A, it should be noted that the mean Q_{O_2} of lobsters on each diet treatment are slightly lower among those reared at 20°C than at 10°C, in all cases (Table 19).

In general, there is minimal temperature effect on metabolic rate for lobsters on a given diet. This may be attributed to the long (10 week) acclimation period, which would have provided adequate time for temperature acclimation. Alternatively, 10°C and 20°C may be on either side of a thermal optimum for lobster in terms of oxygen uptake, activity,

Table 19.

Summary of Comparisons of Probabilities of Differences Between Diet-Temperature Treatments in Oxygen Consumption and O:N Ratios.

Ranked Oxygen Consumption Rates: (high to low)									
D10	E10	E20	B10	C20	C10	B20	D20	A10	A20

Ranked O:N Ratios: (high to low)									
D10	E10	C10	B20	E20	D20	B10	C20	A10	A20

Treatments are designated by letters A-E for diets and 10 or 20 for temperature (°C). Treatments not sharing the same underline are significantly different at $p \leq 0.05$. Values are given in Table 17; SNK pairwise comparisons of probabilities of differences are presented in Appendixes 3-A & 3-B.

food intake, etc. Reynolds & Casterlin (1979) and McLeese & Wilder (1958) showed that activity of lobsters (Homarus americanus) measured as light-beam interruptions or walking rate, respectively, increased from 10°C to 15°C and decreased from 15°C to 20°C. The metabolic rate of those animals should reflect activity, and based on their results, lobsters at 10°C and 20°C would have similar rates, as found in this study. Reynolds & Casterlin (1979) further demonstrated that adult lobsters introduced at 10-15°C to a thermal gradient demonstrated a thermal preferendum of 15-21°C, with a mean of 17°C, which did not vary between day and night for the first 6 days of testing. However, in the second 6 days of testing, thermal preference ranged from 10°C to 29°C. They attribute this to a "decrease in thermoregulatory precision" after several days. The metabolic rate data in this study are consistent with the findings of McLeese & Wilder (1958) and Reynolds & Casterlin (1979), that the lobster (H. americanus) has a broad range of thermal tolerance.

The overall rate of ammonia excretion was not significantly affected by diet or temperature treatments, or interactions between these factors ($p > 0.05$ in all cases). It appears, however, that for lobsters fed diets C, D, and E the mean rates of ammonia

excretion are lower at 10°C than at 20°C, despite the higher mean rate of oxygen consumption at the lower temperature among those lobsters. This may partially be attributed to a specific dynamic action affect. It is possible that at the lower temperature, gut passage rates may be lower (i.e. those lobsters may not be post-pradial), therefored, due to ongoing oxidative digestive processes, the oxygen consumption rate may be relatively higherm while the nitrogen excretion rate may not yet be elevated (pers. comm. O'Dor).

The rates for juvenile lobsters in this study, which ranged from ~250 to 500 $nmol \cdot h^{-1} \cdot g^{-1}$ wet weight, were somewhat less than those reported by Koshio (1985) for sub-adult lobsters. He measured ammonia excretion rates of 10-15 $ug \cdot h^{-1} \cdot g^{-1}$ for lobsters weighing ~2 g, which is equivalent to 556 to 833 $nmol \cdot h^{-1} \cdot g^{-1}$ wet weight . The lower rates found in this study, compared to those determined by Koshio may be partly attributed to the high fat content of the diets in this study, even though dietary protein levels were relatively high (~50% of diet, DWB).

In general there is a tendency of increased O:N ratio, in lobsters acclimated to each temperature, from those fed Diet A to Diet E. The effect is more evident at 10°C (as with oxygen consumption rates). The lobsters with the higher O:N would be relying less on amino acids for maintenance energy production (i.e. while protein appears to be the important energy substrate for lobsters fed Diets C, D, and E at 10°C, they may actually be sparing dietary protein compared to those fed Diets A and B). Lobsters at 10°C fed Diets D and E demonstrate the most active metabolism yet have higher O:N ratios. This may indicate that they are using more labile fuels (i.e. fats and carbohydrates) for routine activity, and sparing protein (which is then available for maintenance and growth). This is consistent with the higher growth rate of Diet E lobsters. This would suggest poorer utilization of nutrients by lobsters on diets deficient in (n-3) and/or (n-6) PUFA. The low O:N ratio of Diet A lobsters, combined with the results of depressed growth and increased

mortality, may indicate protein utilization derived from catabolism of hemolymph proteins, or muscle proteins.

Dall & Smith (1986) noted a reduction of metabolic rate with starvation in the prawn, Penaeus esculentus. They claim the reduction enables the sparing of nutrient reserves of the body (otherwise depleted for energy) and thus provides a mechanism for prolonging survival. They also reported that muscle protein is used preferentially as a source of energy during starvation in the carnivorous prawn, Penaeus esculentus. Their findings also indicate that dietary protein is the major energy substrate in fed prawns. This seems to be the case also for H. americanus.

2.5 CONCLUSIONS

1. Polyunsaturated fatty acid deficiency in diets for lobsters reared at 10°C or 20°C results in depressed weight-specific oxygen consumption rates compared to lobsters fed diets containing (n-3) and/or (n-6) polyunsaturated fatty acid. This correlates with an observed lower activity among lobsters fed polyunsaturated fatty acid-deficient diets.

2. The rate of ammonia excretion in juvenile lobsters reared at 10°C or at 20°C is not significantly affected by diets deficient in polyunsaturated fatty acids

3. O:N ratios indicate that protein is the principal substrate for energy metabolism in juvenile lobsters.

4. Dietary polyunsaturated fatty acid deficiency results in lowered O:N ratios; this is consistent with possible starvation and/or autolysis of body tissues in juvenile lobsters acclimated to 10°C or 20°C, as indicated in Part III, Chapter 3.

5. Dietary polyunsaturated fatty acid appeat to be essential to juvenile lobsters in order to maintain normal metabolic function and efficient utilization of the diet.

CHAPTER 3

The Effects of Dietary PUFA Deficiencies at 10°C and 20°C on the Mass, Structure and Integrity of the Hepatopancreas and Gills

3.0 INTRODUCTION: PART III, CHAPTER 3

For many vertebrate species, nutritional deficiencies have been correlated with tissue pathologies. These may occur at various levels of biological organization, including gross morphometric changes, tissue lesions and degeneration, cell and organelle abnormalities, etc. For the Crustacea, however, there has been little documentation of nutrition-related pathologies. "Black death syndrome" in penaeid shrimp, is attributed to ascorbic acid deficiency and characterized by a light-colored hepatopancreas and melanization of haemocytic lesions in collagenous tissues (Lightner et al., 1977, 1979). In lobsters, "molt-death syndrome" occurs with nutritional deficiencies, but the requisite nutrients to alleviate the syndrome are still not known, although it has been correlated with the absence of phospholipids in certain semi-purified diets (Bowser & Rosemark, 1981) as discussed in Part I of this thesis. Only recently has there even been a comprehensive description of normal structure and ultrastructure of crustacean tissues (Bell & Lightner, 1989), to serve as a comparison with presumed abnormal states.

Physical symptoms of EFA deficiency in crustaceans have not been described. Presumed EFA deficiency has been primarily determined on the basis of retarded molting and growth rates, and poor survival of crustaceans fed diets deficient in various PUFAs or HUFAs. Changes in FA composition have also been correlated with PUFA deficiencies, but these have not previously been related to tissue structure and function, although impaired integrity and function of tissues might be expected to accompany changes in fatty acid composition which result from PUFA deficiency.

The essentiality of certain fats in the diet and physical symptoms of deficiency in rats were first described by Burr and Burr (1929, 1930). Subsequently, an array of physical and physiological symptoms have been attributed to EFA deficiency in terrestrial vertebrates. In addition to weight loss, symptoms include several aspects of epidermal

ailments, including dermititis (rough, scaly, sloughing skin), tail necrosis, poor wound healing, depigmentation of skin, and susceptibility of skin irritants). Internally, EFA deficiency is manifested in the liver (it becomes pale, and there is accumulation of fat), the kidneys & spleen (characterized by organ enlargement and hemorrhagic spots), and in erythrocyte fragility, among other symptoms.

Similar physical symptoms of EFA deficiency in an aquatic species, rainbow trout (Salmo gairdneri), were described by Castell (1970) and Castell et al. (1972a,b). The symptoms included depressed growth, poor food conversion efficiency, loss of scales and deterioration of fins and decreased hemoglobin levels. Bell et al. (1985) were the first to demonstrated gross changes in gill structure in fish fed diets deficient in certain FAs. They found that the gill epithelium of the turbot fish (Scophthalmus maximus) is a sensitive indicator of EFA deficiency. Gross structural changes of the gills of fish fed diets deficient in 22:6(n-3) for 16 weeks included epithelial damage ('sloughing off' of the cells along the primary and secondary gill filaments and an accumulation of cellular debris in the inter-lamellar spaces), leading ultimately to the disintegration of gill tissue, and the disappearance of chloride cells. They also reported that some fish on the two PUFA-deficient diets bled from the edge of the fins and from the operculum.

In this chapter the effects of PUFA-deficient diets on the juvenile lobsters reared at 10°C or 20°C in feeding Experiment II are investigated. The intact lobster, hepatopancreata and the gills from a subset of the lobsters which were subjected to the four semi-purified test diets [which were (n-3) and/or (n-6) PUFA-deficient] or the reference diet were examined for symptoms of EFA deficiency.

The hepatopancreas was selected for examination based on its central role in the digestion, absorption, storage, and processing of lipids in crustaceans (as discussed in Part I), and based on the susceptibility of the corresponding organ of terrestrial and aquatic

vertebrates (i.e. the liver) to EFA deficiency, as noted earlier. Also, since this experiment was initiated, several studies have been reported on the hepatopancreas as "a monitor organ for the nutritional value of diets" (Storch et al., 1984; Vogt et al., 1985, 1986; Anger et al., 1985).

The gills were selected as the second organ to examine for signs of EFA deficiency, based on their importance of gill structure (and in particular, permeability of the gill epithelia) to three principal physiological functions in crustaceans: respiratory gas exchange, ion-osmoregulation, and nitrogen excretion. The gills from lobsters on each of the ten diet-temperature regimes, and from "control" lobsters of similar age which had been fed a crustacean practical diet were preserved for histological evaluation. Preliminary descriptions and analysis of a representative histological samples are presented in this chapter. Although absence of funding has precluded completion of the histological portion of the study, the preserved samples have been stored for examination at a later date.

3.1 OBJECTIVES: PART III, CHAPTER 3

1. Evaluate the effect of (n-3) and (n-6) deficient diets on the mass and structure of the hepatopancreas of juvenile lobsters
2. Evaluate the effects of diets deficient in (n-3) and (n-6) fatty acids on gill ultrastructure
3. Characterize physical pathologies and abnormalities associated with essential fatty acid deficiency in juvenile lobsters

3.2 MATERIALS & METHODS

The diets and maintenance protocol of lobsters examined in this part of feeding trial II were described in Part III, Ch. 1. The gills of an additional set of lobsters of approximately the same age, which had been reared on the Halifax Crustacean Lab Practical

Diet (EXD) were also histologically examined and served as a "control" (i.e. approximately normal condition).

3.2.1 PROTOCOL FOR TERMINATION AND DISSECTION OF LOBSTERS

Live lobsters were swiftly decapitated with a sharp scalpel blade and placed in a petri dish, on ice. The dorsal cephalothorax was opened to expose the hepatopancreas, its condition of which was noted, and then it was photographed in place. It was subsequently removed from the body (taking care to first remove the overlying heart, often still beating), placed in a petri dish (on ice) and photographed again. The entire hepatopancreas was then blotted, and placed in a small, pre-weighed glass vial. Excess surface moisture was removed from the tissue by a stream of nitrogen gas. The vial was closed, then weighed to determine tissue wet weight. Sufficient chloroform:methanol (2:1) was then added to the vial to submerse the tissue, the vial was flushed with nitrogen gas and stored at -40°C until lipid analysis (Part III, Ch. 4).

The hepatosomatic index (HSI)[6] was determined and gross morphology of the hepatopancreata was described. Regression analysis (SPSS) of the effects of temperature and diet on the hepatopancreas wet weight in proportion to body wet weight was conducted. Hepatopancreata of additional lobsters from each diet-temperature treatment were preserved for future histopathological examinations, following the procedures in Figure 15.

The entire abdominal muscle mass was removed from the exoskeleton, blotted, weighed and stored for lipid analysis, as above. The branchial chamber of selected specimens was then exposed and the 5 gill sets (associated with the pereiopods) from one half of the body were isolated from anterior to posterior according to their associated

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[6] HSI is the ratio of the wet weight of the hepatopancreas to the wet weight of the whole body (including hepatopancreas) x 100.

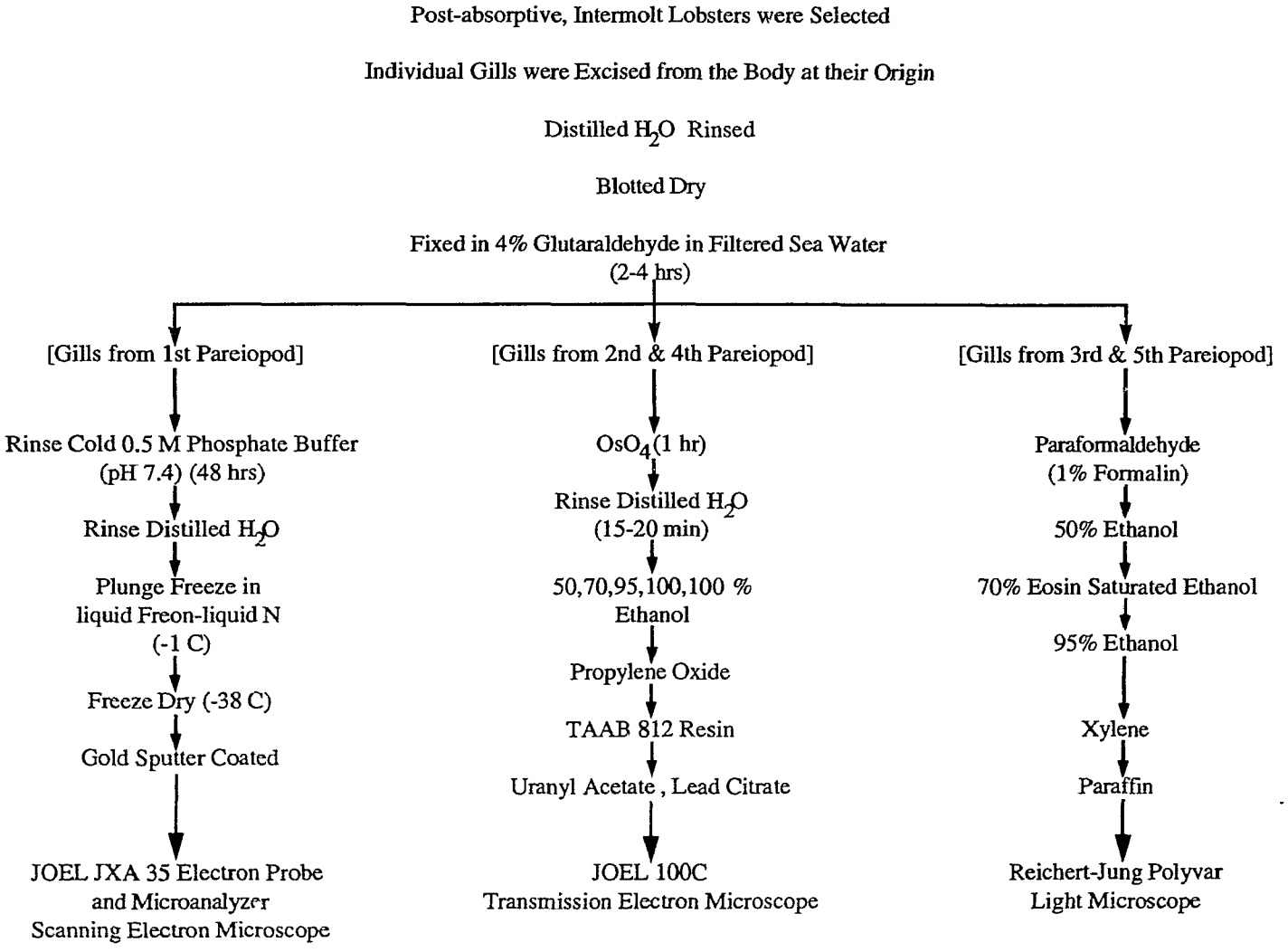


Figure 15. Flow Chart of Histological Preparation of Tissue Samples.

pereiopod, rinsed briefly in distilled water (to remove any adhering sea salts), blotted, and fixed for light microscopy, TEM or SEM; the gills from the other half of the chamber were segregated. blotted dry, then weighed and stored (as above) until lipid analysis. For other lobsters, the entire complement of gills was removed, rinsed, weighed, and prepared for FA analysis (Part III, Ch. 4).

3.2.2 Histology

Initial preparation of tissue samples for histology was conducted under the direction of Dr. Paul Odense, (now retired) at the National Research Council of Canada, Atlantic Regional Laboratory, Halifax, NS. Technical assistance was provided by Ms. J. McKenna and Ms. M. McInerney-Northcott. The procedure of sample preparation for light microscopy (Light M), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of gill tissue is shown in Figure 15; results of TEM examination of gills are not presented in this text. Evaluation and descriptions of gill histological sections, were provided by Dr. Gwyneth Jones.

3.3 RESULTS

3.3.1 GENERAL PHYSICAL CONDITION

Lobsters fed Diet A (deficient in both (n-3) and (n-6) PUFA) had an unusually thin, soft exoskeleton compared to lobsters on other diets. The exoskeleton was white, but fairly transparent, and was easily compressed by fingertip pressure. [It should be noted that the test diets in this study do not contain carotenoid pigments, which are required in the diet for normal pigmentation. Therefore, when lobsters shed their exoskeleton at ecdysis, there is a net loss of pigment (which can be partially regained by consumption of the exuvia)]. Of special note was an isolated event which occurred at ecdysis of a lobster reared on Diet B (deficient in (n-6) PUFA) at 20°C. Approximately 20 weeks into the

feeding trial the lobster had lost a cheliped (by autolysis) when being handled for weighing. Upon its subsequent molt, the regenerated cheliped was deformed, with abnormal exoskeleton and muscle tissue (Figure 16). The muscle tissue could be seen through the uncharacteristically transparent exoskeleton, appeared to be atrophied and, in fact, barely existent. However, since in newly molted crustaceans, the tissue mass would take up a small proportion of the volume of the new exoskeleton of the dactyl, and is not visible by routine observation, a comparison can not be made. The newly formed exoskeleton was unusually thin, scaly or blotchy in color, and did not harden as quickly as the exoskeleton covering the rest of the body. Presumably its permeability properties were altered, since the cheliped "bloated" to an unusual extent, and the intersegmental arthrodial membranes were bulging. While this was a unique occurrence, it would be interesting in future studies to specifically examine the effect of PUFA deficiency on regeneration.

3.3.2 VISUAL OBSERVATIONS OF THE HEPATOPANCREATA

The most dramatic effect on the hepatopancreas was among lobsters fed the diet deficient in both (n-3) and (n-6) fatty acids. The degenerate or atrophied state of the hepatopancreas of lobsters fed the EFA-deficient diet is visually apparent in Figures 17 C & D. The tissue was pale (white to clear and nearly transparent), whereas the tissue of lobsters fed semi-purified diets supplemented with 18:3(n-3) and/or 18.2(n-6) were brownish and more similar to the hepatopancreas of lobsters fed the reference diet, which contains natural fish oil and corn oil (Figure 17 A & B).

For comparison, the hepatopancreata of several juvenile lobsters reared on a crustacean practical diet were examined. The general pigmentation of these lobsters was similar to juveniles from the wild. In these animals, the tissue appeared more dense, far more pigmented (deep brown and green), and has a more substantial mass relative to the body cavity.

Figure 16. Photograph of Lobster with Impaired Limb Regeneration.
(Ruler values in cm, smallest grading in mm)

Photo credit: Dr. Paul Odense Reprints: Mary Primrose

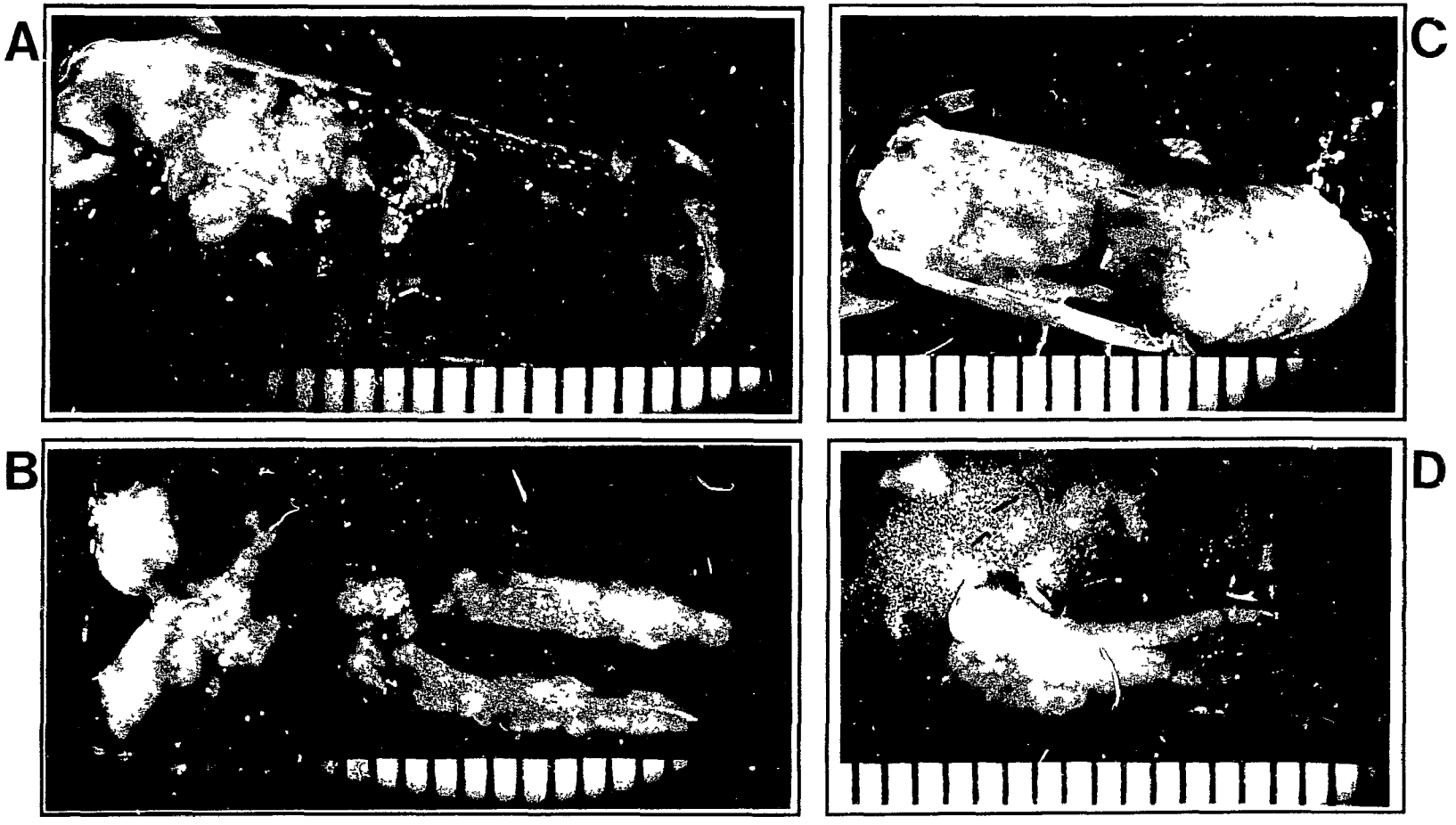


Figure 17. The Hepatopancreata of a Lobster Reared on a Control Diet and of a Lobster Reared on a Diet Deficient in Polyunsaturated Fatty Acids.

Specimens featured were sibling, intermolt juveniles, reared at 20°C. (Ruler grading = 1mm).

A. Dorsal section of carapace removed to expose hepatopancreas in body. Specimen reared on Diet D (containing 18:2(n-6) and 18:3(n-3). B. Hepatopancreas (shown in A), isolated from body, dorsal view. C. Same view as A; specimen was reared on Diet A, deficient in (n-6) and (n-3) fatty acids. D. Hepatopancreas (shown in C), isolated from body.

Photo credit: Dr. Paul Odense Reprints: Mary Primrose

3.3.3 HEPATOSOMATIC INDEX (HSI)

For lobsters acclimated to 10°C, there was insufficient growth over the duration of the experiment to detect proportional differences in organ weight, and there was no significant difference in HSI between lobsters fed the four test diets. However, for 10°C-acclimated lobsters, the HSI was significantly different (greater) for H-CRD animals than for those on the other four diets (Tables 20 and 21).

There was a significant proportional increase in the hepatopancreatic wet weight with increasing body weight of lobsters reared at 20°C. Deducting the effect of body weight on hepatopancreatic weight (which accounts of ~62% of the HSI difference between treatments), there is also a significant effect of diet on HSI. Post hoc analyses showed that the effects of Diets A and E on HSI were significantly different than the effects of all other test diets. There was no significant difference among lobsters fed semi-purified diets supplemented with 18:3(n-3) and/or 18:2(n-6). The HSI is greatest for lobsters fed the H-CRD and the slopes of plots of hepatopancreatic wet weight vs. body fresh weight (Figure 18) are significantly different, showing that for a given body weight, lobsters fed an EFA-deficient diet (A) have a smaller hepatopancreas than lobsters fed semi-purified diets containing one or more (n-3) or (n-6) PUFAs.

3.3.4 GILL HISTOLOGY

3.3.4.1 Normal histology

Lobster gills are trichobranchiate structures, with numerous finger-like filaments arising from a central elongate stalk. Figure 19 A is a scanning electron micrograph, ventral view, of an isolated gill. The gill surface is covered by a very thin layer of cuticle, as seen in the scanning electron micrographs of the gill filament and the filament wall (Figures 19 B & C). Beneath the cuticle lies a single, thin layer of flattened epithelial cells

Table 20.

Hepatosomatic Index of Lobsters Reared at 10°C or 20°C and Fed One of Five Diets

Temperature	Diet	n	Mean	SD	SEM	Min.	Max.
10°C	A	5	2.2	± 1.1	1.0	1.0	3.0
	B	5	3.1	± 1.0	0.9	2.0	4.0
	C	5	3.1	± 1.0	0.8	2.0	4.0
	D	5	3.0	± 1.0	0.7	2.0	4.0
	E	5	5.5	± 1.0	1.0	4.0	7.0
	Total	25	3.0			1.0	7.0
20°C	A	6	1.8	± 1.0	0.8	1.0	3.0
	B	6	3.5	± 1.0	0.8	2.0	4.0
	C	6	3.0	± 2.0	1.9	1.0	6.0
	D	5	3.4	± 1.0	1.1	2.0	5.0
	E	4	5.2	± 1.0	0.5	5.0	6.0
	Total	27	3.0			1.0	7.0

Table 21.

ANOVA Statistics of Hepatosomatic Index Data

Temperature	Source of Variation	SS	DF	MS	F	P	SIG.*1
10°C	Between Diets	0.003	4	0.001	9.137	0.000	*
	Within Diets	0.002	20	0.000			
	Total	0.005	24				
20°C	Between Diets	0.003	4	0.001	5.249	0.004	*
	Within Diets	0.003	22	0.000			
	Total	0.006	26				

1. * Denotes significance at $p \leq 0.05$

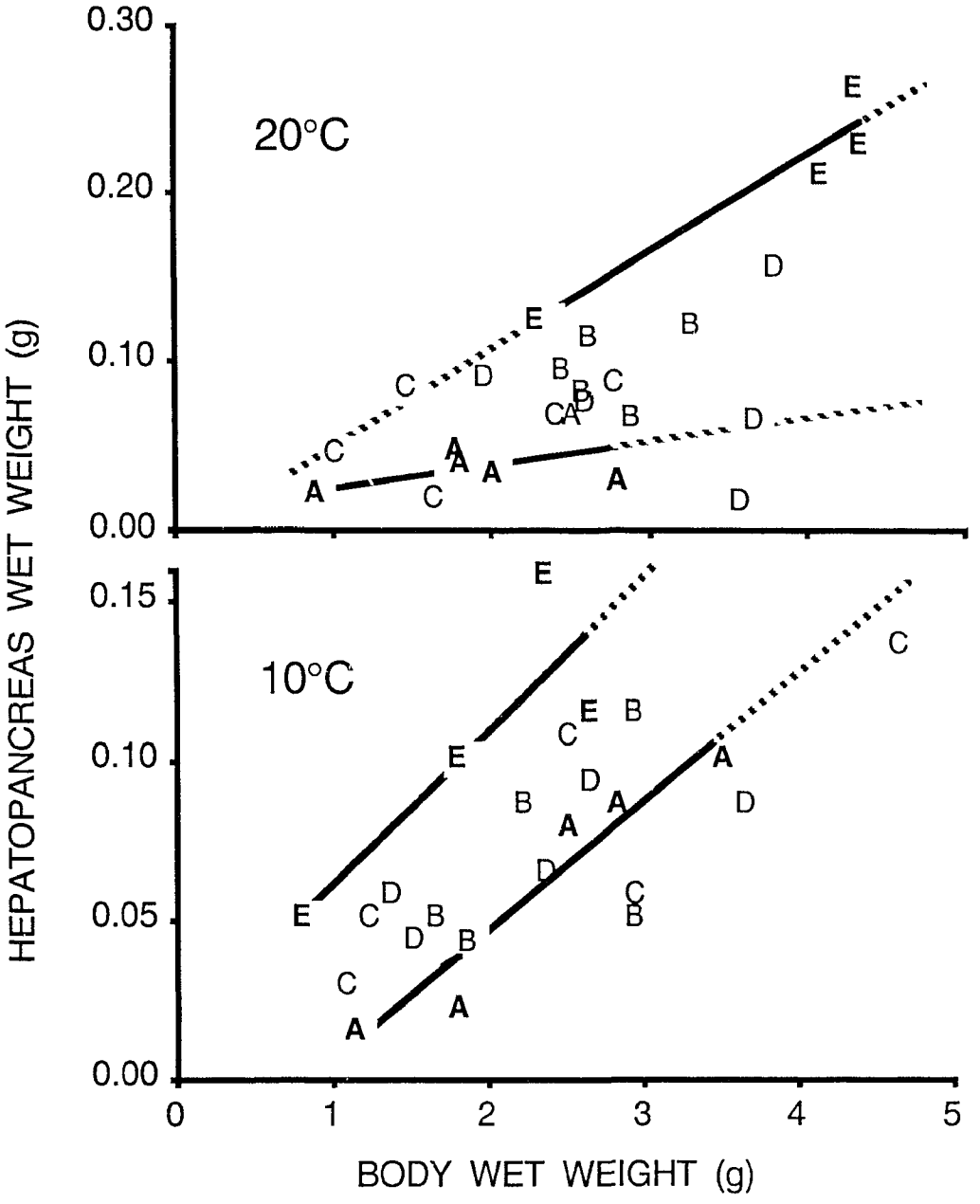


Figure 18. Relationship of Hepatopancreas Wet Weight to Body Wet Weight in Juvenile Lobsters.

Lobsters were reared at 10°C or 20°C and fed one of five diets (A-E). Letters plotted denote diet treatment; each marks the value for an individual lobster. Regression lines are plotted for Diet A lobsters (EFA-free) and Diet E lobsters (reference diet). Note difference in scale on y axes.

3.3.4 GILL HISTOLOGY

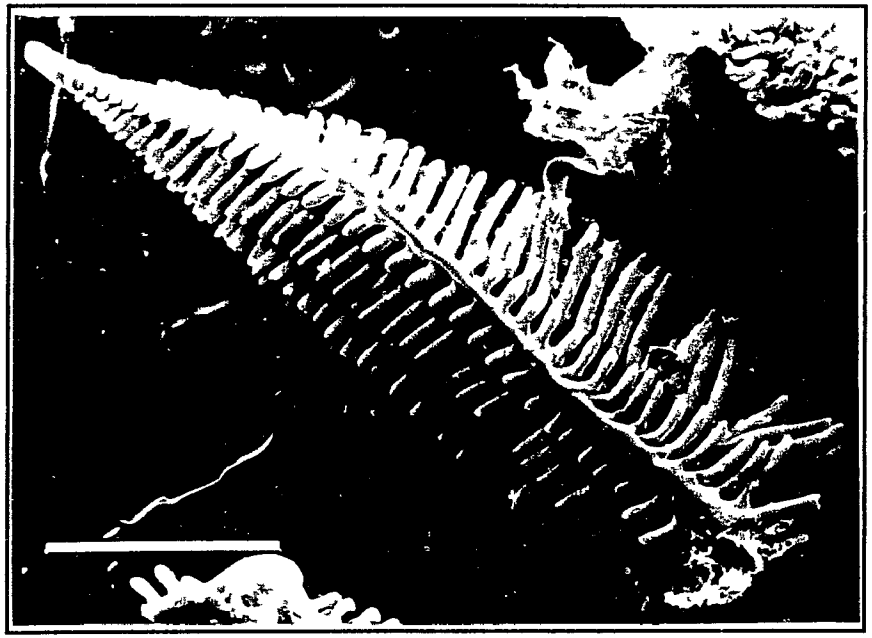
3.3.4.1 Normal histology

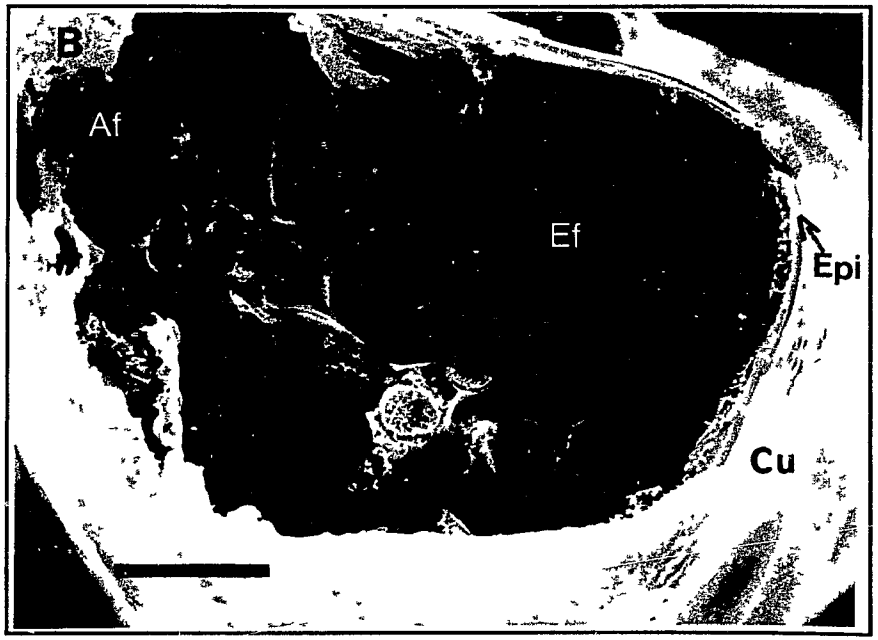
which border an extensive haemal space. Fine strands of loose connective tissue cross the haemal space, subdividing it into major haemal channels and smaller lacunae, all of which are filled with haemolymph and may contain haemocytes.

The gill stalk is similar in construction, although with thicker cuticle and a more prominent longitudinal septum of connective tissue than in the filaments. Groups of cells, including nephrocytes (fixed phagocytes), are localized within the stalk, resulting in a more complex histological appearance than in the filaments and for current purposes attention is concentrated on the filaments themselves.

This type of gill is similar in may respects to those of the freshwater crayfish, e.g. Pacifastacus (Morse et al., 1970). However, there are differences, particularly in the location of nephrocytes and in the organization of connective tissue septa in the filaments. Direct comparisons could therefore be misleading, since the complex functions of gills include ion transport in addition to gas-exchange and may be reflected in morphological or ultrastructural differences between the marine and freshwater gill (Martin & Odense, 1974), and no comparative studies have been made.

The gill filaments are oval to rounded in cross-section, or elongate as they arise from the stalk. There is little regional variation along the length of a filament. Beneath the overlying cuticle the epithelium forms a continuous cellular sheet which is closely applied to the cuticle and was often difficult to resolve in paraffin sections. This squamous epithelium is very thin (2μm or less), except where the perikaryon bulges inwards to contain the prominent nucleus. Fine strands of cellular material run across the haemal





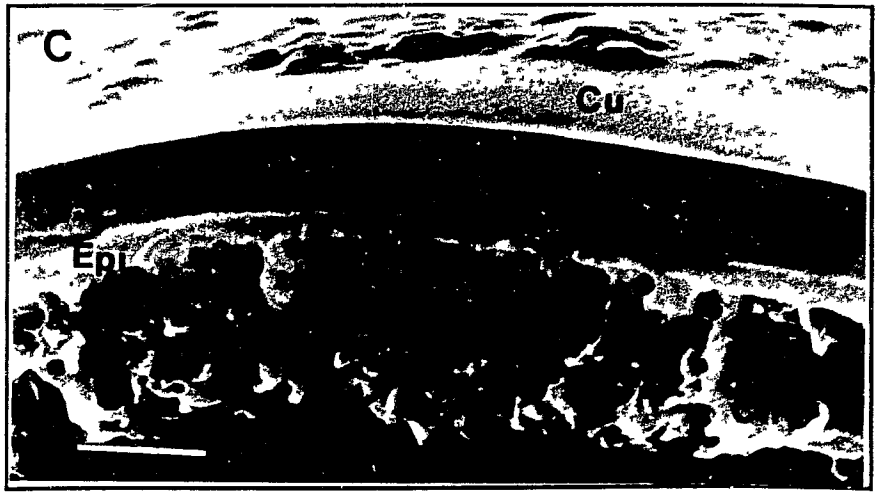


Figure 19. Scanning Electron Micrographs of Gills of the Lobster Homarus americanus. Specimens featured were intermolt juveniles, reared at 10°C.

A. Whole gill, ventral view. (bar = 1mm). B. Gill filament, cross-section. (bar = 10μm). C. Gill filament, cross-section of wall. (bar = 1μm). [Af, afferent vessel; Ef, efferent vessel; Cp,capillaries; Epi, epidermis; Cu, cuticle; Cn, connective tissue]

Photo Credit. Dr. Paul Odense Reprints: Mary Primrose

space, connecting portions of the epithelial sheet and forming small lacunae adjacent to the free surface of the epithelium. Probably, as in Pacifastacus (Morse, 1970), the lacunae communicate with each other and with the main lumen of the filament to form a system of continuous channels.

Major haemal spaces in the filament lumen are broadly divided by septa of loose connective tissue. In the freshwater crayfish the septum is a single, distinct strand clearly separating the filament lumen into afferent and efferent channels (Morse et al., 1970; Burggren et al., 1974). Ultrastructurally this strand may appear to be double, perhaps arising from two separate connective tissue cells (Morse et al., 1970).

In the lobster there was no such clear distinction between the afferent and efferent haemal channels. Most cross-sectional profiles show a double septum, dividing the main lumen into three irregular spaces. The profiles illustrated by Martin & Odense (1974), while perhaps more oblique than transverse sections, also show two or three separate septa crossing the main lumen. Frequently, one (outer) edge of one strand appears smooth and well-defined while the others have a granular or a ragged appearance. This might suggest that the loose connective tissue of a single septum was seen in samples of lobsters fed Diet E, however, this is difficult to interpret. The functional significance of a less clearly defined septum, with perhaps less distinct separation between afferent and efferent flow, is not easy to interpret, and evidently more comparative studies or normal gill histology between marine and freshwater genera would be valuable.

The gill filament epithelium is separated from haemal spaces only by a basal lamina. This was not resolvable in haematoxylin and eosin stained paraffin sections, and was difficult to identify in the weakly-staining 1μm plastic sections.

Haemocytes are recognizable in the haemal spaces. Both eosinophilic granulocytes and a few non-granular hyaline haemocytes are present as unattached cells.

3.3.4.2 Histology of Gills of Lobsters Fed Fatty Acid Deficient Diets

(a) Lobsters Acclimated to 10°C

Both sample 62 (Diet D) and sample 54 (Diet B) show little or no difference in the histology of the gill filament in comparison with control sample 62 (Diet E). There is no evidence of epithelial sloughing, necrosis, or connective tissue disruption other than the double-stranded condition of the septum mentioned previously.

Samples 75 and 59 (Diet A) show some significant differences from all of these samples. The epithelial sheet remains intact, and there is insufficient resolution to determine whether the basal laminae are affected. The double septum is mostly intact in 75, but in 59 it is widely spaced, giving the impression of a single lumen. In both samples cross-connections with the epithelial sheet are sparse, resulting in few, large lacunae. Some nuclei are dense, with peripheral condensation of what appears to be chromatin, randomly scattered throughout the filament. Connective tissue strands, particularly in 59, appear ragged with short, spiky or broken processes or granular accretions. Many filament profiles in 59 are compressed, sickle-shaped in cross-section, with tightly contracted septa; these profiles were not seen in 75, and may be a localized response in the distal part of the gill in 59.

Sample 59 shows more evident abnormalities than 75, but the variation between them suggests that responses may not be the same throughout even a single gill, or between animals. Nevertheless, it appears that some response is present, particularly in loose connective tissue, at the light microscopic level in animals receiving Diet A. Diets B and D do not induce these changes. Diet C animals were not observed.

(b) Lobsters Acclimated to 20°C

Interpretation of 20°C samples is complicated by the appearance of conurol 34 (Diet E) gill filaments; in these the connective tissue septum is expanded, and quite broadly connected to the epithelial sheet, leaving a single irregular lumen. In contrast, sample 40 (Diet B) resembles the control, B and D samples at 10°C.

In sample 26 (Diet EXD) the septa are distorted and filaments filled with loose connective tissue. Sample 46 (Diet A) shows contracted rings or clumps of connective tissue, with poorly defined septa. None of the samples show evidence sloughing or breakage of the epithelial sheet.

3.4 DISCUSSION

The visually apparent atrophic state of the hepatopancreas and the significantly lower HSI of Diet A lobsters than of lobsters fed diets containing (n-3) and/or (n-6) PUFA may indicate depletion of lipid reserves in the hepatopancreas or degeneration (autolysis or catabolism) of the hepatopancreatic tissue. Recently, Watanabe et al. (1989) described the effects of EFA-deficient diets on the hepatocytes of the white fish, Coregonus lavaretus maraena. Although they found no marked difference in HSI between treatment groups, EFA deficiency was evident from abnormally-shaped and disorganized hepatocytes with atrophied nuclei.

Anger et al. (1985) and Vogt et al.(1985) describe a "point of no return" (PNR) in the degeneration of the ultrastructure of the hepatopancreas of starved larval lobster (Homarus americanus) and adult shrimp (Penaeus monodon), respectively. In the larval lobsters, for example, the PNR is attributed to structural changes in the R-cells (those hepatopancreatic cells which function to absorb and metabolize lipids) which result in an inability to restore lipid reserves utilized during imposed starvation. Anger et al. (1985)

report that these changes include swollen mitochondria, reduced lipid inclusions, an increased number of residual bodies indicating autolysis, and a reduction in microvillous processes. The findings in this thesis indicate that (n-6) and/or (n-3) PUFA are required to maintain normal structural integrity of the hepatopancreas of juvenile lobsters. Thus, a similar PNR and associated conditions may have been attained in lobsters fed Diet A.

Lobsters in this feeding trial which were fed Diet A also exhibited depressed growth, molting and survival rates (Part III, Ch. 1) and depressed metabolic rate (Part III, Ch. 2). This abnormal, degenerate condition of the hepatopancreas may be expected to impair normal functioning of this important organ. As a possible consequence, absorption of dietary fats (and fat-soluble nutrients, such as vitamins A, D, E, and K) into cells lining the hepatopancreas may be reduced. This could lead to secondary effects, which in turn, could contribute to or accentuate symptoms of dietary EFA deficiency. For example, depigmentation and soft exoskeleton in penaeid shrimp have been postulated to be symptoms of deficiencies of vitamins A, D and E (and are accompanied by reduced growth and increased mortality) (Akiyama & Dominy, 1989). Thus, the occurrence of these conditions in the lobsters fed the PUFA-deficient diet may have been a secondary effect of the degenerative hepatopancreas as a possible consequence of impaired absorption.

Another possible consequence of a degenerate hepatopancreas could be impairment of the normal processes of metabolism of dietary fats and their reformation into HDL-phospholipids for export into the hemolymph and subsequent distribution to the extrahepatopancreatic tissues. In such a situation, sufficient amounts of lipid (to meet energy requirements) or specific fatty acids (to satisfy essential functions) may not be available at the tissues where they are required, despite dietary total lipid levels which would be sufficient under "normal" conditions. In this scenario the dietary lipid either may be poorly absorbed into the hepatopancreas and could thus be detected in the feces, or may accumulate in the hepatopancreas and result in a "fatty liver" condition similar to that which

occurs which occurs in vertebrates. This would also be consistent with the significantly higher O:N ratios of lobsters fed Diet A (Part III, Ch. 2). That is, despite adequate total lipid in the diet, there was a higher than usual proportion of protein catabolized for energy in those lobsters. This could reflect catabolism of the structural proteins (i.e. of the hepatopancreas and possible muscles or other tissues), or hemolymph proteins, which has been demonstrated to occur in lobsters (Stewart et al., 1967a) and other crustaceans during times of fasting, starvation, or nutritional deficiencies. In turn, catabolism of the oxygen-carrying blood proteins could lead to the sluggish behavior and significantly depressed metabolic rates of lobsters fed the PUFA-deficient diet (Part III, Ch. 2). Future studies on EFA deficiency should therefore examine the level of hemolymph proteins and the FA composition of the hemolymph.

The effect of PUFA-deficient diets on the morphology and untrastructure of lobster gills is not as obvious nor dramatic as those effects reported to occur in the gills of fish (Bell et al, 1985). Unlike the exposed, external layer of epithelial cells in fish gills, the epithelia in crustacean gills is protected from the environment by a waxy cuticle (described earlier). There are indications, however, that epithelial damage does occur in the lobster gills as a result of dietary PUFA deficiency. Further examination of preserved samples should prove interesting.

3.5 CONCLUSIONS

1. Externally, deficiency of dietary (n-3) and (n-6) polyunsaturated fatty acid s in juvenile lobsters is characterized by an exoskeleton which is thin, transparent and soft (easily compressible to the touch).

2. The hepatopancreas in juvenile lobsters fed semi-purified diets deficient in (n-3) and (n-6) polyunsaturated fatty acid s becomes pale (creamy white-colored) and visibly atrophied. The hepatosomatic index is significantly lower in these lobsters than in those fed semi-purified diets containing (n-6) polyunsaturated fatty acid and (n-3) highly unsaturated

fatty acid . This is the first documentation in crustaceans of degenerative hepatopancreas as a consequence of dietary polyunsaturated fatty acid deficiency.

3. Morphology and ultrastructure of the gills of "normal" juvenile lobsters has been documented. Preliminary comparison with the gills of lobsters fed diets deficient in polyunsaturated fatty acid s indicates that severe epithelial damage (as occurs in fish gills) does not occur in this crustacean. However, there is evidence that response to dietary fatty acids varies among individuals and possibly among gill filaments in the gills of individual specimens, and there are indications that limited structural damage does occur in the gills of lobsters fed a diet deficient in both (n-3) and (n-6) polyunsaturated fatty acid .

4. These results are consistent with evidence presented in the two previous chapters and further indicates that (n-3) and/or (n-6) fatty acids are essential for juvenile lobsters, and further, that they play a role in maintaining structural integrity of the hepatopancreas and possibly of the gills.

5. The effect of diets deficient in polyunsaturated fatty acid on limb regeneration in crustaceans should be examined.

CHAPTER 4

The Effects of Temperature and Dietary Fatty Acids on the Levels and Fatty Acid Composition of the Neutral and Polar Lipids of the Hepatopancreas, Abdominal Muscles, and Gills

4.0 INTRODUCTION: PART III, CHAPTER 4

Temperature has a direct effect on the physical and kinetic properties of membrane components and thus on the structural order and functional integrity of biological membranes. Lower temperatures induce a greater cohesiveness and rigidity of the macromolecules which comprise membranes, and conversely, higher temperatures (i.e. higher thermal energy) promote a greater fluidity. In crustaceans as in other poikilotherms, the maintenance of membrane properties (such as permeability) and of the activity of membrane-bound or imbedded enzymes, independent of changes in the temperature of the environment, is achieved by the alteration of the lipid composition of the tissues and/or organelles. This acclimatory response was first described for the bacterium E. coli (Sinensky, 1974) and termed "homeoviscous adaptation", i.e. adjustments made to maintain a constant viscosity or "fluidity " of the membranes. For most species investigated, a principal strategy in counteracting the effects of temperature change on membranes involves altering both the ratio of unsaturated FAs to SFAs (U/S ratio) and changing the degree of unsaturation of the FAs (the unsaturation index) of the PLs of cell

and organelle membranes. These changes are such that at lower temperatures there is an overall tendency for a higher degree of unsaturation. The double bonds introduced (by desaturation) in the FAs of membrane phospholipids restrict rotation about the carbon-carbon bonds along the fatty acyl chains, disrupting the alignment of the chains and, concomitantly, the van der Waals bonds between them. In this way, desaturation counteracts the effects of lowered temperature.

Although the metabolic basis and time course of the restructuring of membrane FAs in response to different temperatures are only partially understood, three general mechanisms have been identified:

(i) temperature-dependent incorporation of selected FAs during de novo synthesis of membrane phospholipids (in crustaceans, enhanced production of PUFAs at low temperatures has been demonstrated in the crab Carcinus maenas (Chapelle, 1978) and in crustacean plankton (Farkas, 1979); and in the freshwater crayfish Procambarus clarkii Farkas & Nevenzel (1981) demonstrated a significant incorporation of [1-^{14}C]-acetate into long chain PUFAs of total PLs at lower temperatures)

(ii) turnover of selected FAs of membrane phospholipids in response to temperature (i.e. selective deacylation by phospholipases and/or selective reacylation by acyltransferases), and

(iii) direct desaturation of the acyl chains of membrane phospholipids in response to low temperature (demonstrated in fish by Hagar & Hazel (1985) and Neas & Hazel (1985).

These selectivities (in all three mechanisms) may involve differential modifications of the relative amount and composition of various phosphatides. In the crab Carcinus maenas, for example, an increased proportion of PE and a decreased proportion of PC at low temperature has been shown by Chapelle et al. (1977, 1978), Brichon et al. (1980), and Chapelle et al. (1982a) (for reviews see Hazel & Sellner, 1980; and Chapelle, 1986).

The restructuring of membranes by selective incorporation of (n-6) and/or (n-3) long-chain PUFA, either during de novo synthesis of membrane lipids or by reacylation during membrane phospholipid turnover, is dependent on the availability of those FAs. If the FAs required for restructuring are EFAs, then the ability of the crustaceans to acclimate to lower temperature, for example, will be affected by dietary supply and tissue lipid "reserves". Thus, EFA deficiency may be manifested as low temperature intolerance. On the other hand, at higher temperatures, growth and molting rate are accelerated and thus there is a greater demand for appropriate FAs for the manufacture of new tissue. In this case, EFA deficiency may be manifested as depressed growth and lowered molting rates, molt impairment, and altered FA composition of the tissues.

Although previous studies on crustaceans have demonstrated that the FA profiles of the tissues are affected by short term temperature changes and by seasonal changes in temperature (and/or diet), the roles of diet and tissue lipid "reserves" (and intracellular pools of free-fatty acids, FFAs) in providing EFAs for temperature acclimation have not been clearly differentiated. Longer term feeding trials conducted to establish EFA requirements of crustaceans have not concomitantly examined the effects of thermal stress, and temperature acclimation studies conducted to examine changes in FA metabolism and composition seldom control diet history or report dietary FA composition, and have not examined the effect of diet on temperature acclimation. Thus, the independent and/or synergistic affects of temperature and dietary FAs on tissue composition, temperature acclimation and EFA deficiency have not previously been determined.

In this study, the combined effects of long term (14 weeks) temperature acclimation and diets with selected FA deficiencies on a marine crustacean were investigated. Laboratory-hatched, sibling lobsters, Homarus americanus, were raised from 4th stage to 12th stage at 20°C on defined diets. They were subsequently reared at 10°C or 20°C and

fed one of four semi-purified test diets (containing purified FAMEs as the sole source of dietary lipid), or the Halifax Crustacean Reference Diet. After 14 weeks, a set of 4 to 5 intermolt lobsters from each of the ten diet-temperature treatments was sacrificed, and the FA profiles of the NLs and PLs of the hepatopancreata, abdominal muscles and gills from individual lobsters were analyzed.

4.1 OBJECTIVES: PART III, CHAPTER 4

1. Compare the effects of dietary fatty acids and fatty acid deficiencies on the composition of juvenile lobsters.

2. Determine the effect of temperature (10°C vs. 20°C) on the fatty acid composition of juvenile lobsters Homarus americanus.

3. Examine tissue-specific fatty acid profiles in juvenile lobsters; compare the effects of dietary fatty acid composition and temperature on the fatty acid profiles of the hepatopancreas, abdominal muscles and gills.

4. Compare the lipid class fatty acid profiles (NL vs. PL fractions) of the hepatopancreas, abdominal muscles and gills and compare the effects of dietary fatty acids and temperature on the fatty acid profiles of the NLs and PLs.

4.2 MATERIALS AND METHODS

4.2.0 TEST ANIMALS

Full sibling juvenile lobsters, H. americanus (1.8 to 3.6 g wet weight) were from those reared at 10°C or 20°C under conditions previously described in Part III, Chapter 1, Section 1.2. They were fed a reference diet or one of four semi-purified test diets were either PUFA-deficient or enriched with 18:2(n-6) &/or 18:3(n-3) at 1% of diet (DWB). Diet and temperature history of the lobsters and FA composition of the diets are reported in Part III, Chapter 1 (Figure 9 and Table 12, respectively).

Intermolt lobsters were selected for FA analysis after 14 weeks on the diet-temperature treatments. Selected animals were not fed (and remaining food removed) for one day prior to their termination. Wet weight and orbit length were determined just prior to sacrifice.

4.2.1 PROTOCOL FOR KILLING AND DISSECTION OF LOBSTERS

(see section 3.2.1)

4.2.2 EXTRACTION OF LIPIDS FROM DIETS AND TISSUES

General precautions were followed as stated in Part II, Chapter 2, Section 2.2.1. (See schematic diagram, Figure 20, for general procedures of lipid analysis).

Total lipid was extracted from the samples by a procedure modified from Folch et al. (1957) (Appendix 2-B). Diet samples and tissue samples (entire hepatopancreata, abdominal muscles or gills of individual lobsters) were homogenized, with the solvents, using an Ultra-Turrax homogenizer). Samples were evaporated to dryness under nitrogen and brought up to 500 µl with chloroform. Replicate 10 to 50 µl aliquots were measured with a Hamilton syringe, and expelled onto ~12.2 mm diameter pre-weighed aluminum foil discs, placed on a heating plate to evaporate the solvent, cooled, and re-weighed using a Cahn model 29 Automatic Electrobalance (0.1 µg - 25 mg range).

4.2.3 SEPARATION OF NEUTRAL, GLYCO-, AND POLAR LIPIDS

The remainder of each tissue TL sample (after weighing) was separated by the method of Cook (1978) into neutral, glyco- and polar lipid fractions (NL, GL, and PL) by column chromatography as follows. The TL samples were dissolved in chloroform and passed through a pasteur pipette plugged with glass wool and packed with ~0.5 g of 100-200 mesh Unisil (activated silicic acid, Clarkson Chemical Company, Inc., Williamsport,

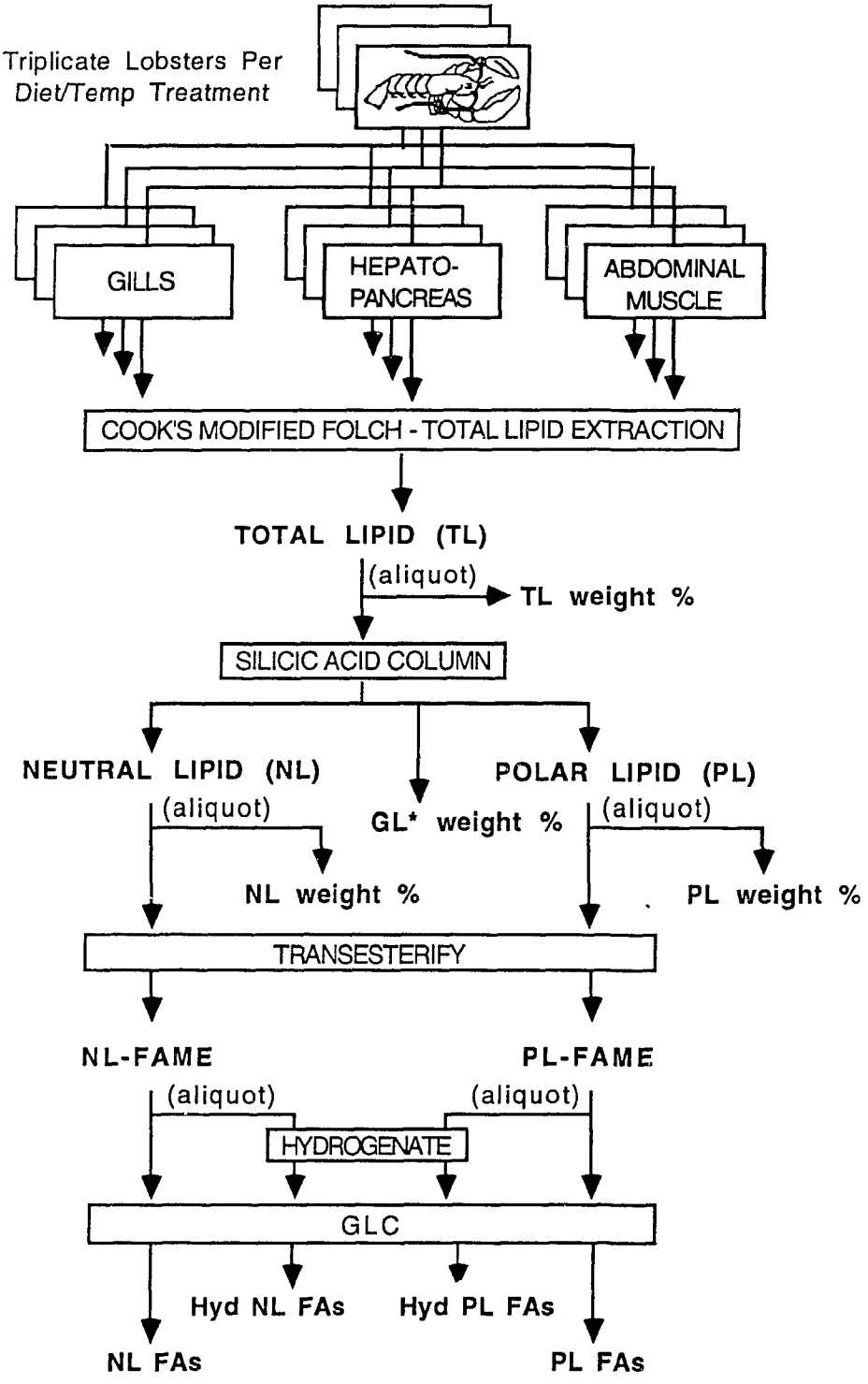


Figure 20. Schematic Diagram of Procedures of Lipid Analysis of Lobster Tissue Samples. *(GL = glycolipid)

Pa.) (~ 1 g/10 mg lipid). Before use, the Unisil was washed with chloroform, then methanol, hen acetone, and suction filtered through Whatman No. 1 paper, using a buchner funnel. NLs were eluted with 5 ml of chloroform; GLs were eluted with 2.5 ml of chloroform:acetone, 1:1 (v/v), followed by 2.5 ml acetone; PLs were eluted with 5 ml of methanol.

4.2.4 CONVERSION OF FATTY ACIDS TO METHYL ESTERS

Fatty acid methyl esters (FAME) of diet TL samples and tissue NL and PL samples were prepared for GLC analysis by transesterification following a modification of the method of Morrison & Smith, (1964) (Appendix 2-C). The lipid samples (0.5 mg - 32 mg) were dissolved in 2 ml hexane, 0.5 ml benzene and refluxed in 2 ml of 7% boron trifluoride in methanol (Esterification Kit, Alltech Assoc., Inc., Applied Science Labs, Deerfield, IL) for 60 minutes at 100°C (in a boiling water bath). The smaller quantities of NLs and PLs from gill extracts (10 µg - 50 µg/sample) were refluxed in 0.5 ml hexane, 0.5 ml benzene and 1 ml of 3.5% BF_3 in methanol (pers. com. N. Ratnayake, TUNS). The FAME were extracted using hexane (3 X 2 ml), washed with distilled water, dried over anhydrous Na_2SO_4, and decanted off or filtered through Whatman No.1 filter paper.

4.2.5 GLC ANALYSIS

GLC analyses were conducted in the Marine Lipids Laboratory of Dr. R. G. Ackman, Technical University of Nova Scotia. Fatty acid compositions of the FAME of the NL, PL, or TL fractions were determined using a Perkin-Elmer capillary gas chromatograph Model 8420. Aliquots of 1 to 3 µl containing approximately 50 µg to 17 µg/µl, respectively, of FAME in hexane were injected into the GLC which was equipped with a flame ionization detector (FID) and a polar column (Supelcowax 10 flexible fused silica capillary column; 30 m in length x 0.32 mm ID). The injection temperature was

250°C, the detector temperature was 270°C, and the oven temperature was programmed as follows: 195°C for 8 min, increasing at 3 °C/min to 240°C and holding for 7 minutes. A final stage with temperature increasing at 10°C/min to 250 and holding for 23 min was used to clear any residual material from the column. Total run time was 56 min. The pressure of the carrier gas, helium, was set at 11 psig; gas flow through the column was 1.24 ml/minute.

Identifications of FA peaks were made by comparison to retention times of identified peaks of a menhaden oil FAME standard (Western Gulf Composite Sample 1985, courtesy R. G. Ackman, Canadian Institute of Fisheries Technology, Technical University of Nova Scotia). Identifications were also made in comparison to the retention times of lipid standards obtained from Nu-Chek-Prep (Elysian, MN, USA), Supelco, Inc. (Bellefonte, PA, USA) and Serdary Research Laboratories (London, Ontario). Fifty-seven FA peaks were identifiable in the lipid extracts from lobster tissues.

Aliquots of the menhaden oil reference standard were tested in triplicate at the standard concentration (50 μg/μl) and at two dilutions (17 μg/μl and ~9μg/μl) to assure precision and accuracy of the analysis of dilute samples. To accommodate the small quantities of gill NL and PL FAME, the split-ratio of the GLC injection port was adjusted to allow a greater proportion of injected sample to load onto the column. The menhaden oil reference standard also was run at the reduced split-ratio.

4.2.6 HYDROGENATION OF METHYL ESTERS

Selected NL and PL samples from each tissue were hydrogenated, using platinum IV oxide catalyst (PtO_2), for further verification of peak identifications (Ackman & Burgher, 1964) (see details of method in Appendix 2-E).

4.2.7 QUANTITATION OF FAs AND STATISTICAL EVALUATION

The retention time and magnitude of each peak was recorded by a Perkin-Elmer LCI-100 Laboratory Computing Integrator. These values and the corresponding chromatograms were printed on thermal paper with a Perkin-Elmer G.P. 100 graphics printer. Peak areas were converted to weight-percent (and molar-percent) of the total amount of identified FAME using a modified computer program, originally developed by Ackman & Eaton (1978).

Preliminary compilation of FA data was made using the relational data base program Paradox (Copyright, 1988, Borland International) and IBM PC compatible computers. Summary tables of data and statistics were produced with an Apple Macintosh computer, using the spreadsheet and database programs Microsoft Excel 1.5, 2.0, and 2.2 (Microsoft Corp., 1985).

The FA composition of samples is calculated as the relative percentage contribution of each FA (based on weight-percent or molecular weight-percent) as a fraction of all identified FAs. Proportional values are not normally distributed, and therefore cannot be subjected to conventional analysis of variance (ANOVA). Percentage data are therefore arcsine transformed (arcsine $X^{1/2}$) to ensure normal distribution, prior to statistical analysis. The normalized data were then subjected to ANOVA, using SAS/STAT (Version 6, 4th ed., vol 2) General Linear Model on a mainframe computer. Post-hoc tests to identify differences among treatment means ($P < 0.05$) included Tukey's and Bonferroni's. (SAS Institute, 1990) (Appendices 10-A thru 10-R).

4.3 RESULTS

4.3.1 LIPID LEVELS AND LIPID CLASS COMPOSITION OF TISSUES

After 14 weeks, four lobsters were sacrificed from each of the ten diet-temperature treatments, from among those lobsters which were in the intermolt stage. The mean weights, orbit lengths, tissue wet weights (of the hepatopancreata, abdominal muscles and gills), and wet tissue weights expressed as a percentage of body wet weight (% BW), of the sacrificed lobsters used for FA analysis are given in Table 22. Mean live weights of this sample of lobsters ranged from 1.7 to 3.0 g for those fed Diets A-D and from 1.8 to 3.6 g for those fed the H-CRD. Corresponding mean orbit lengths ranged from 3.5 to 4.2 cm for those on Diets A-D and 3.8 to 4.5 cm for those fed the H-CRD. The mean amounts of NL and PL in the hepatopancreas, abdominal muscles, and gills of intermolt lobsters from each of the ten treatments are presented as mg NL or PL/lobster in Appendix 6-A, as mg-percent (i.e. µg/100 mg tissue) in Appendix 6-B, and as mg/g body wet weight in Appendix 6-C.

4.3.1.1 THE HEPATOPANCREATA

The mean wet weights of the hepatopancreata of lobsters reared at 10°C ranged from 2.6 to 3.1% BW of those fed Diets A-D and 5.3 to 5.7% BW of lobsters reared on the H-CRD (Table 22). Of the lobsters grown at 20°C, the mean hepatopancreas wet weight comprised 1.9% BW for lobsters fed Diet A, ranged from 3.2 to 3.6% BW for lobsters on Diets B-D and was 5.3% for lobsters fed the H-CRD. [The hepatosomatic indices (HSI) of all surviving lobsters on the ten treatments were presented in Part III, Chapter 3].

The TL content and lipid class composition (NL, PL and GL fractions) of the hepatopancreata of lobsters from each diet-temperature treatment are presented Table 23.

Table 22.

Orbit Length, Whole Body Wet Weight and Wet Weight of Isolated Tissues of Lobsters Raised for 14 Weeks at 10°C or 20°C and Fed One of Five Diets.

Temp	Diet		Whole Lobster		Hepatopancreas		Muscle		Gills	
°C			Wet Wt. (g)	Orbit L (cm)	Wet Wt. (mg)	% BW	Wet Wt. (mg)	% BW	Wet Wt. (mg)	% BW
10	A	Mean	2.48	4.03	69.33	2.56	292.76	11.64	15.30	0.56
		SD	1.01	0.70	38.07	0.86	134.97	1.41	9.43	0.27
	B	Mean	2.29	3.99	66.10	2.88	258.52	11.25	14.37	0.64
		SD	0.70	0.36	33.05	0.94	86.19	1.17	3.97	0.11
	C	Mean	2.81	4.21	88.08	3.09	340.41	11.37	15.67	0.60
		SD	1.51	0.59	54.70	0.86	161.47	0.57	6.18	0.10
	D	Mean	2.57	4.01	73.02	2.91	272.87	10.51	16.22	0.64
		SD	0.89	0.47	20.22	0.42	108.21	0.68	5.01	0.12
	E	Mean	1.83	3.78	103.46	5.66	218.01	11.95	13.50	0.67
		SD	0.72	0.47	44.57	0.77	91.30	0.36	7.93	0.25
20	A	Mean	2.02	3.75	36.37	1.85	246.94	11.98	17.65	0.76
		SD	0.87	0.69	22.35	0.71	115.08	0.79	14.97	0.45
	B	Mean	2.79	4.18	90.00	3.22	319.86	11.43	28.70	0.98
		SD	0.37	0.18	22.46	0.64	62.57	1.47	23.65	0.69
	C	Mean	1.73	3.54	56.80	3.55	216.23	11.99	13.53	0.82
		SD	0.75	0.48	30.86	2.02	136.26	2.74	7.19	0.51
	D	Mean	2.97	4.19	94.78	3.33	384.15	13.01	34.45	1.06
		SD	0.87	0.50	39.51	1.29	115.61	1.14	34.44	0.89
	E	Mean	3.57	4.45	187.73	5.27	435.10	12.09	34.33	0.91
		SD	1.14	0.48	57.12	0.12	152.34	0.65	27.98	0.54

Table 23.

Total Lipid Content and Lipid Class Composition (NL, PL & GL Fractions) of the Hepatopancreata (H) of Lobsters Fed One of 5 Diets at 10°C or 20°C for 14 Weeks.

Temp °C	Diet		TL (% of H)	NL (% of TL)	PL (% of TL)	GL (% of TL)	NL/PL	NL (% of H)	PL (% of H)
10	A	Mean	3.2	65.5	31.9	10.1	2.1	2.1	1.0
		SD	1.1	19.0	14.5	0.8			
		n	4	3	4	2			
	B	Mean	3.5	57.2	21.0	25.4	2.7	2.0	0.7
		SD	1.7	7.0	7.2	4.8			
		n	4	4	3	2			
	C	Mean	3.5	69.7	16.6	13.7	4.2	2.4	0.6
		SD	1.4	4.5	3.4	1.9			
		n	4	3	3	3			
	D	Mean	3.9	62.3	26.0	15.9	2.4	2.4	1.0
		SD	3.1	0.9	8.1	6.0			
		n	4	3	3	2			
	E	Mean	12.9	73.1	5.5	24.9	13.3	9.4	0.7
		SD	3.7	3.5	1.7	0.3			
		n	4	4	4	2			
20	A	Mean	2.1	54.2	25.8	25.2	2.1	1.1	0.5
		SD	0.6	7.5	3.5	0.9			
		n	4	4	3	2			
	B	Mean	4.8	69.5	19.0	21.4	3.7	3.3	0.9
		SD	2.1	8.3	7.6	15.5			
		n	4	3	3	2			
	C	Mean	3.5	79.8	23.9	7.2	3.3	2.8	0.8
		SD	1.2	10.4	9.0	1.1			
		n	4	3	4	2			
	D	Mean	4.1	65.4	22.1	15.4	3.0	2.7	0.9
		SD	0.8	6.5	3.4	4.6			
		n	3	3	3	2			
	E	Mean	21.1	80.1	3.9	6.9	20.7	16.9	0.8
		SD	2.7	17.1	0.9	5.4			
		n	3	3	3	2			

The percentage of TL composition of the hepatopancreata (WWB) was lowest among Diet A lobsters at each temperature (3.2% and 2.1% for lobsters at 10°C and 20°C, respectively). The mean TL level (as percentage of hepatopancreas) ranged from 3.5 to 4.8% among lobsters reared on Diets B-D, regardless of temperature, and was 12.9% and 19.1% for H-CRD lobsters at 10°C and 20°C, respectively.

Among lobsters fed Diets A-D, NL constituted 54 to 80% of TL, PL was 17 to 32% of TL, and GL ranged from 7 to 25% of TL. The NL/PL ratios ranged from 2.1 (Diet A lobsters), to 2.4 to 4.2 for lobsters on Diets B - D. The mean NL/PL ratios of lobsters fed the H-CRD were 13 and 21 for lobsters reared at 10°C and 20°C, respectively. NL and PL comprised ~2% and ~1%, respectively, of hepatopancreata wet weight among lobsters on Diets A-D at 10°C, ~1% and 0.5%, respectively, for Diet A lobsters at 20°C, ~3% and 1%, respectively, for Diet B-D lobsters at 20°C, and for lobsters fed the H-CRD, were ~9% and ~1%, respectively, at 10°C and ~17% and ~1%, respectively, at 20°C.

4.3.1.2 THE ABDOMINAL MUSCLES

Abdominal muscle wet weight constituted 11% to 13% BW of lobsters reared on all diet-temperature treatments (Table 22). The percent TL, NL, PL, and GL content of the abdominal muscles (WWB) are presented in Table 24. Levels were consistent for all diet-temperature treatments. Muscle tissue contained 0.7 to 1.1% TL. NL accounted for 0.3% of muscle for lobsters on all diet-temperature treatments, and PL comprised 0.4 to 0.7% of muscle; the NL/PL ratios ranged from 0.4 (Diet A lobsters at 20°C) to 0.8 (Diet D lobsters at 20°C).

4.3.1.3 THE GILLS

Table 22 presents the mean wet weights of the gills which ranged from 0.6 to 0.7% of body weight of lobsters reared at 10°C, and 0.8 to 1.1% of body weight of

Table 24.

Total Lipid Content and Lipid Class Composition (NL, PL & GL Fractions) of the Muscle (M) of Lobsters Fed One of 5 Diets at 10°C or 20°C for 14 Weeks.

Temp °C	Diet		TL (% of M)	NL (% of TL)	PL (% of TL)	GL (% of TL)	NL/PL	NL (% of M)	PL (% of M)
10	A	Mean	0.9	33.1	66.6	5.2	0.5	0.3	0.6
		SD	0.2	10.8	6.0	0.0			
		n	4	4	4	2			
	B	Mean	0.8	35.5	62.2	6.1	0.6	0.3	0.5
		SD	0.1	8.0	6.4	2.5			
		n	4	4	4	2			
	C	Mean	0.9	32.8	63.2	3.9	0.5	0.3	0.6
		SD	0.3	1.2	0.7	0.5			
		n	2	2	2	2			
	D	Mean	0.8	30.8	57.9	5.6	0.5	0.3	0.5
		SD	0.3	0.2	9.7	0.5			
		n	3	3	3	2			
	E	Mean	0.9	36.1	60.5	7.2	0.6	0.3	0.6
		SD	0.2	4.7	5.5	2.4			
		n	4	4	4	2			
20	A	Mean	1.1	26.2	63.1	5.7	0.4	0.3	0.7
		SD	0.3	6.6	1.9	0.1			
		n	4	4	4	2			
	B	Mean	0.8	33.5	58.5	6.5	0.6	0.3	0.5
		SD	0.1	9.4	10.1	3.0			
		n	4	4	4	3			
	C	Mean	0.8	30.9	54.4	8.3	0.6	0.3	0.4
		SD	0.3	4.1	4.4	2.4			
		n	4	4	4	3			
	D	Mean	0.7	39.7	51.3	8.9	0.8	0.3	0.4
		SD	0.1	2.0	3.2	2.0			
		n	4	4	4	3			
	E	Mean	0.9	33.1	55.9	11.0	0.6	0.3	0.5
		SD	0.1	11.4	11.0	0.9			
		n	3	3	3	3			

lobsters reared at 20°C. The percentage contribution of TL, NL, PL, and GL to the gill wet weight of lobsters on each of the ten treatments are reported in Table 25. The relatively high standard deviations reflect both the difficulty in obtaining accurate wet weight measurements of gills and the small quantities of gill lipids. TL constituted 4 to 6% of gill wet weight among lobsters at 10°C and from 2 to 5% of gill wet weight among lobsters at 20°C. Of lobsters reared at 10°C, NL and PL were 2.2 to 2.5% and 1.0 to 1.4% of gill wet weight, respectively, among lobsters fed Diets B-E and were 4.0 and 1.7% of gill wet weight of Diet A lobsters; the NL/PL ratio ranged from 1.8 to 2.4. Among lobsters reared at 20°C, NLs and PLs ranged from 1.1 to 2.7% and from 0.5 to 1.5% of gill wet weight, respectively, and the NL/PL ratios ranged from 1.6 to 2.6.

4.3.2 TREATMENT EFFECTS ON FA PROFILES

Fifty-seven FAs were identified from the NLs and PLs of the hepatopancreata, abdominal muscles, and gills of juvenile lobsters. The FAs and the categories which they comprise are listed in Appendix 2-F.

The results presented in Sections 4.3.2 - 4.3.5 are comparisons of the FAs (as percentage of NL or PL fractions) of lobsters fed the semi-purified test diets, A-D (which contained purified FAMEs; for Diet composition, see Table 12). Comparisons of the effects of the reference diet, E (H-CRD) on juvenile lobsters with the effects of the other semi-purified diets are discussed separately. H-CRD contained a broad spectrum of FAs including (n-3) HUFA from marine fish oil, and a high proportion of 18:2(n-6), from inclusion of corn oil (Table 12); this complicated direct comparisons with the test diets (as in Part II, Chapter 2).

Table 25.

Total Lipid Content and Lipid Class Composition (NL, PL & GL Fractions) of the Gills (G) of Lobsters Fed One of 5 Diets at 10°C or 20°C for 14 Weeks.

Temp °C	Diet		TL (% of G)	NL (% of TL)	PL (% of TL)	GL (% of TL)	NL/PL	NL (% of G)	PL (% of G)
10	A	Mean	6.2	63.8	27.5	~24	2.3	4.0	1.7
		SD	4.8	8.2	8.2				
		n	4	3	3				
	B	Mean	3.7	59.6	32.3	~24	1.8	2.2	1.2
		SD	1.5	17.5	17.5				
		n	4	4	4				
	C	Mean	4.5	56.9	32.0	22.2	1.8	2.5	1.4
		SD	2.6	13.7	16.0	14.1			
		n	4	4	4	2			
	D	Mean	4.2	58.2	28.2	27.2	2.1	2.5	1.2
		SD	3.5	15.0	6.6	2.2			
		n	4	4	4	2			
	E	Mean	3.7	62.5	26.1	22.8	2.4	2.3	1.0
		SD	2.3	20.9	8.4	22.0			
		n	4	4	4	2			
20	A	Mean	4.5	53.8	26.3	~28	2.0	2.4	1.2
		SD	3.2	14.8	14.8				
		n	4	4	4				
	B	Mean	3.2	60.8	23.7	31.0	2.6	1.9	0.7
		SD	1.6	3.9	17.1	0.7			
		n	4.0	4.0	4.0	2.0			
	C	Mean	4.8	55.2	30.8	28.1	1.8	2.7	1.5
		SD	2.9	21.2	19.4	21.9			
		n	4	4	4	2			
	D	Mean	2.6	53.5	32.8	27.5	1.6	1.4	0.8
		SD	1.5	2.1	16.2	1.2			
		n	4	4	4	2			
	E	Mean	2.0	54.6	27.2	27.2	2.0	1.1	0.5
		SD	0.6	2.7	18.0	1.0			
		n	3	3	3	2			

4.3.2.1 EFFECT OF TEMPERATURE

There was a significant effect of temperature of acclimation (10°C vs. 20°C) on the FA composition of juvenile lobsters. In particular, there was a significant effect of temperature on the proportion of 14:0, 16:0, 16:1, 18:1, total MFAs, 18:2(n-6), 20:4(n-6), 20:5(n-3), 22:6(n-3), total (n-3) FAs, and total PUFAs. Total (n-3) and total PUFA were significantly higher at the lower temperature, attributable in these two cases primarily to the effect of temperature on the proportion of 20:5(n-3) (Figure 21). However, of the major fatty acids, the proportions of 18:0, total SFAs, total (n-6) PUFA, 18:3(n-3), 22:5(n-3), and the ratio of (n-3) to (n-6) FAs were not significantly different between lobsters reared at 10°C and 20°C.

The effect of temperature was most noticeable in the gill PLs; at 10°C the mean percent contribution of 20:5(n-3) was 9.7 - 12.4% of PLs, more than twice as high as in gill PLs at 20°C (1.7 - 5.3%). The proportion of total PUFA in muscle PLs was higher in lobsters at 10°C (52.7 to 53.5% of PLs) than in muscle PLs at 20°C (44.1% to 53.1% of PLs) for lobsters on Diets B, C, and D. In contrast, while the level of total SFAs was not affected by temperature, the mean level (results for all diet treatments combined) of palmitate (16:0) was significantly higher in the tissues of lobsters reared at 20°C (12.7%) than at 10°C (10.9%), attributable principally to differences among the treatment means of PLs (which were lower in 16:0 than were the NL, within all tissues for all diet treatments) (Figure 22). This three way interaction of diet x tissue x lipid was significant at $P \leq 0.05$ (Appendix 10-D).

4.3.2.2 EFFECT OF DIET (Semi-purified Diets A-D)

There was a significant effect of diet on the proportion of all major FAs and FA groupings (SFAs, MFAs, PUFAs), and on the proportions of the (n-3) and (n-6) series of FAs (Appendices 7-B thru 7-R). Lobsters fed Diet A, deficient in (n-3) and (n-6) FAs,

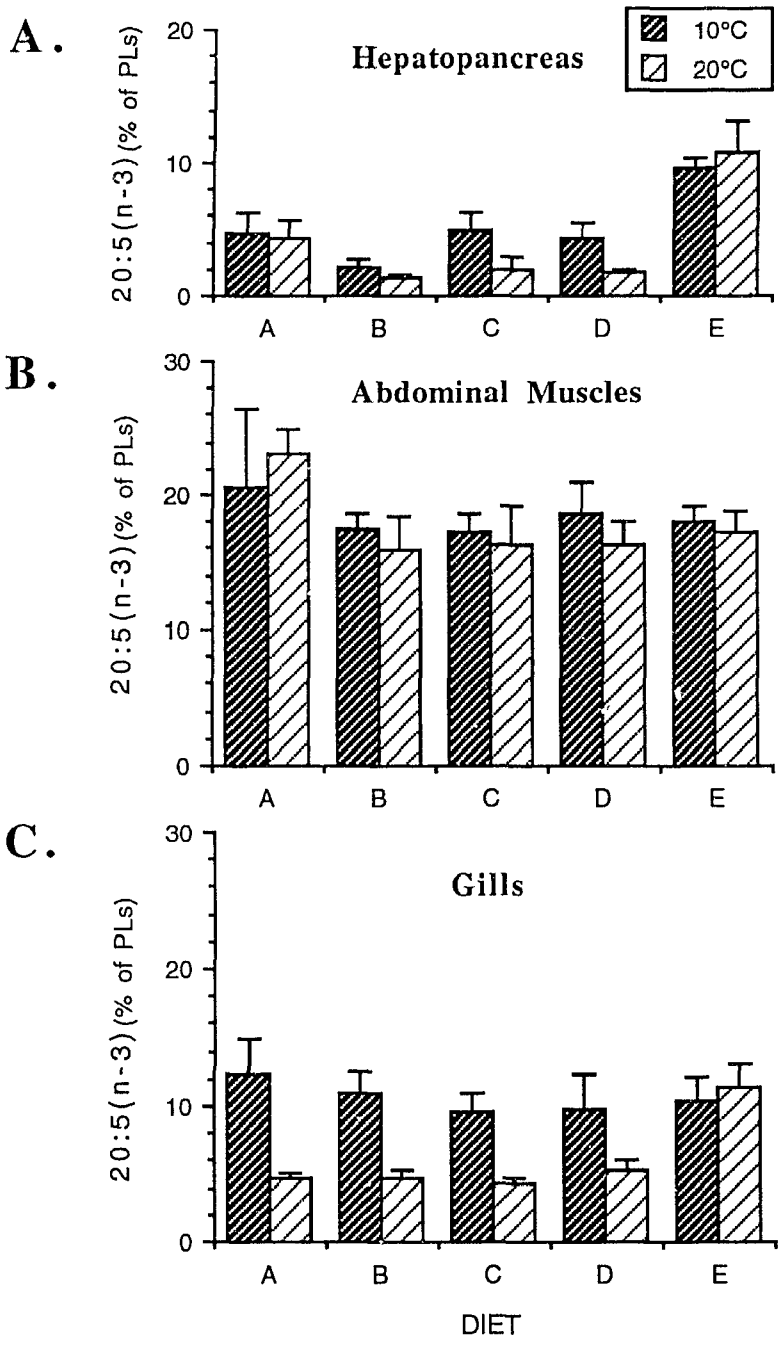


Figure 21. The Proportion of 20:5(n-3) in the Polar Lipids of Lobster Tissues.

Lobsters were reared at 10°C or 20°C and fed one of five diets (A-E) for 14 weeks. Values are means of whole tissue from 2 - 4 lobsters ± SEM.

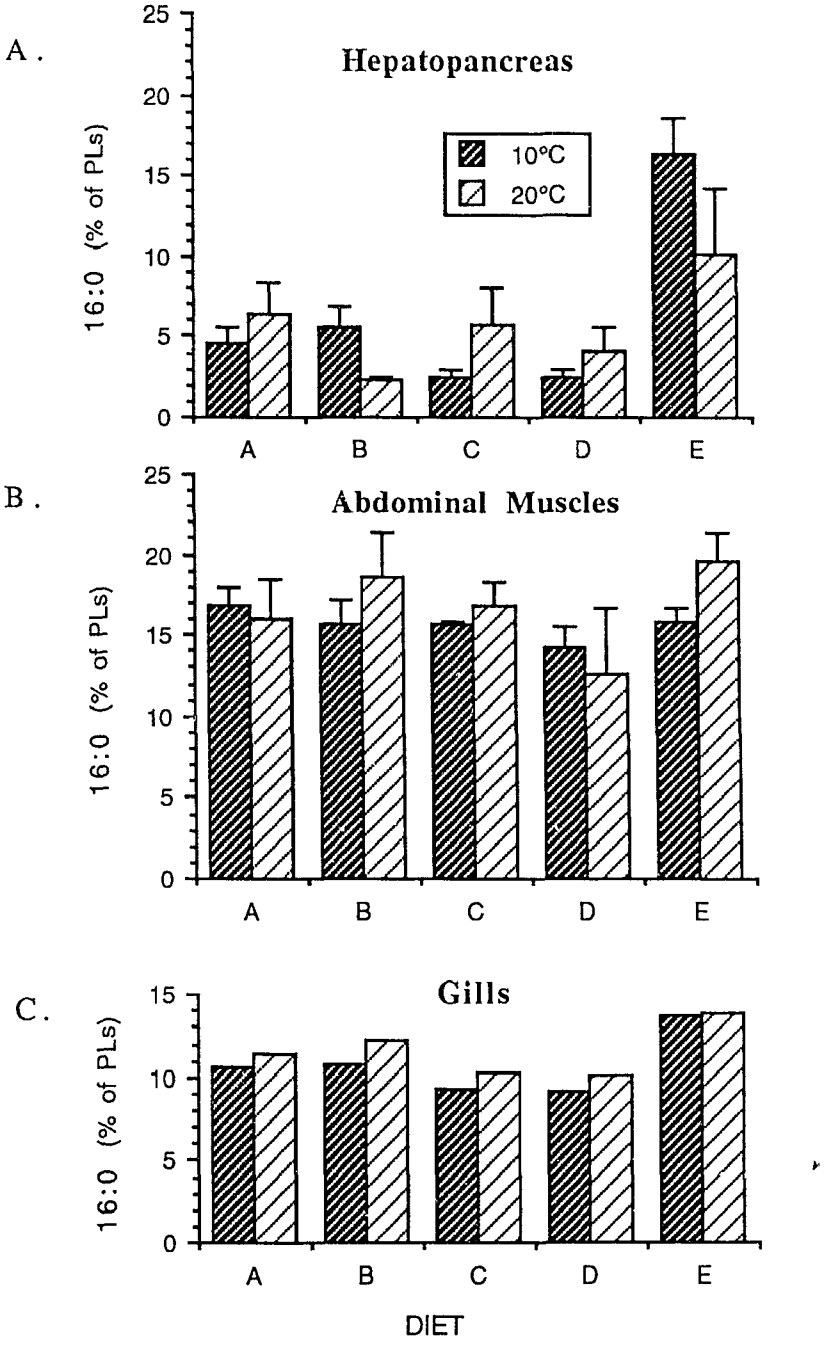


Figure 22. The Proportion of 16:0 in the Polar Lipids of Lobster Tissues.

Lobsters were reared at 10°C or 20°C and fed one of five diets (A-E) for 14 weeks. Values are means of whole tissue from 2 - 4 lobsters ± SEM.

had significantly different tissue levels of 14:0, 16:0, 18:0, total saturates, 16:1(n-7), 18:2(n-6), 18:3(n-3), 20:5(n-3), and 22:6(n-3) than lobsters fed all other semi-purified diets. The proportion of total PUFA did not vary significantly among lobsters fed the semi-purified diets, including lobsters fed Diet A.

The tissues of lobsters fed Diet B (which contained ~1% 18:2(n-6), but was deficient in (n-3) FAs) were similar to those of lobsters fed Diets C and D (which contained 18:3(n-3)), with respect to proportions of 14:0, 16:0, 18:0, total saturates, 16:1, 18:1, total monoenes, 20:4(n-6), 20:5(n-3), 22:6(n-3), and total PUFA. Diet B lobsters had significantly higher proportions of 18:2(n-6) than those fed Diets A, C and D. The proportion of 18:3(n-3) in Diet B lobsters was not significantly different from lobsters fed Diet A, also deficient in that FA.

Lobsters fed Diets C and D, which contained 18:3(n-3), had significantly elevated levels of 18:3(n-3) in the hepatopancreas (4.8 to 7.0 of NLs and 10.1 to 12.5% of PLs) compared to lobsters receiving diets without supplemental 18:3(n-3) (0.1 to 0.2% of NLs and < 0.1 to 0.4% of PLs) (Tables 26 and 27). This apparent accumulation of 18:3(n-3) was evident but not as dramatic in the muscle NLs (Tables 28 and 29) or in the gill PLs (Tables 30 & 31).

The proportion, in the tissues, of all major saturates, monoenes, and (n-3) FAs were similar among lobsters fed Diets C and D. There were significantly higher levels of 18:2(n-6) and total (n-6) in the hepatopancreata and some lipid classes of the gills and muscles of lobsters fed Diet D, which contained 18:2(n-6).

Though 18:2(n-6) in muscle and gil stended to be higher than in the hepatopancreas the levproportion appeared to be less influenced by diet in these tissues than in the hepatopancreas.

Table 26. Principal NL and PL Fatty Acids (Wt %) of the Hepatopancreata of Juvenile Lobsters Reared at 10°C for 14 Weeks.

Diet		A		B		C		D		E	
Lipid Fraction		NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
Fatty Acid	14:0	1.15	0.84	0.59	0.81	0.14	0.62	0.21	0.63	2.63	1.09
	16:0	8.54	4.74	7.12	4.50	3.32	2.43	4.15	2.53	10.26	16.34
	18:0	2.64	1.78	0.97	1.64	0.40	1.00	0.70	1.08	0.76	6.08
	Σ SFAs	13.23	10.03	9.24	8.88	3.84	5.59	5.20	5.56	13.95	28.12
	16:1(n-7)	7.68	3.49	5.16	3.14	2.92	1.32	3.56	1.40	4.58	1.30
	18:1	64.76	53.01	65.51	50.62	78.26	47.02	79.00	45.05	22.91	6.63
	20:1	1.49	2.37	1.71	1.81	1.49	2.76	1.63	2.16	11.96	5.90
	22:1	0.45	0.62	0.67	0.58	0.17	0.83	0.34	0.79	8.58	3.62
	Σ MFAs	76.38	61.73	73.98	58.13	87.11	53.02	84.84	50.34	19.89	18.12
	18:2(n-6)	2.39	2.42	10.86	12.73	0.92	1.44	3.15	3.55	24.46	10.08
	20:4(n-6)	0.16	0.93	0.16	0.57	0.05	0.73	0.07	0.77	0.13	0.48
	Σ (n-6)	4.49	5.08	12.92	16.98	1.53	4.29	3.92	6.14	25.60	13.48
	18:3(n-3)	0.20	0.16	0.21	0.37	5.75	8.72	4.76	7.59	0.61	0.41
	20:5(n-3)	0.60	5.78	0.37	2.70	0.21	4.86	0.29	4.27	3.62	9.61
	22:5(n-3)	0.33	0.47	0.28	0.48	0.16	0.60	0.16	0.48	0.37	1.48
	22:6(n-3)	0.56	3.65	0.45	2.18	0.20	3.92	0.27	3.81	3.76	14.50
	Σ (n-3)	2.82	10.80	2.08	6.67	7.12	18.96	5.61	17.13	9.13	29.49
	Σ PUFAs	9.54	24.84	16.32	28.91	8.92	38.14	9.81	40.28	35.17	47.69
	(n-3)/(n-6)	0.52	2.16	0.16	0.40	5.73	4.59	1.49	2.88	0.36	2.29

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

Table 27. Principal NL and PL Fatty Acids (Wt %) of the Hepatopancreata of Juvenile Lobsters Reared at 20°C for 14 Weeks.

Diet		A		B		C		D		E	
Lipid Fraction		NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
Fatty Acid	14:0	0.91	0.51	0.50	0.20	0.30	0.56	0.24	0.30	2.24	0.82
	16:0	10.91	6.33	7.01	2.31	5.53	5.24	5.53	4.01	14.68	16.69
	18:0	2.02	1.53	0.77	0.60	0.33	1.73	0.49	1.27	0.84	3.77
	Σ SFAs	14.72	10.12	8.54	3.70	6.34	8.19	6.34	6.19	18.07	22.26
	16:1(n-7)	8.59	5.89	6.01	1.46	6.40	2.31	5.68	1.97	5.64	1.25
	18:1	62.29	51.50	75.08	47.74	74.47	49.59	77.53	46.27	24.27	9.17
	20:1	1.97	1.95	1.42	2.67	1.69	2.88	1.21	2.99	11.50	8.97
	22:1	1.11	0.79	0.28	0.72	0.40	0.94	0.06	0.66	6.23	4.03
	Σ MFAs	75.87	62.40	78.88	54.41	84.07	53.16	84.51	52.74	51.40	21.44
	18:2(n-6)	2.67	2.21	9.58	16.12	0.83	1.76	2.66	5.60	21.78	15.92
	20:4(n-6)	0.16	1.13	0.08	0.48	0.06	0.35	0.06	0.55	0.12	0.62
	Σ (n-6)	4.38	7.78	10.80	19.24	1.25	4.60	3.09	7.43	23.45	19.13
	18:3(n-3)	0.11	0.16	0.09	0.04	7.00	12.54	4.94	10.11	0.39	1.59
	20:5(n-3)	1.05	4.28	0.22	1.33	0.10	1.15	0.06	1.74	2.32	16.60
	22:5(n-3)	0.21	0.21	0.13	0.54	0.27	0.43	0.07	0.17	0.28	1.27
	22:6(n-3)	1.11	2.11	0.25	1.14	0.05	0.73	0.18	1.50	2.59	14.08
	Σ (n-3)	3.10	10.40	0.84	4.63	7.43	16.80	5.54	15.11	6.32	37.90
	Σ PUFA	8.62	23.00	12.24	35.61	9.17	30.80	9.02	34.00	30.31	59.54
	(n-3)/(n-6)	0.70	1.46	0.07	0.24	6.00	4.75	1.85	2.05	0.27	2.02

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

Table 28. Principal NL and PL Fatty Acids (Wt %) of the Abdominal Muscles of Lobsters Reared at 10°C for 14 Weeks.

Diet		A		B		C		D		E	
Lipid Fraction		NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
Fatty Acid	14:0	2.38	0.25	4.04	0.34	2.84	0.25	2.21	0.31	2.58	0.36
	16:0	23.54	16.95	18.86	15.73	17.53	15.69	17.41	14.17	19.62	15.91
	18:0	4.48	3.58	4.44	3.67	5.02	2.72	4.55	3.60	5.12	3.33
	Σ SFAs	32.37	21.61	30.60	20.81	29.18	19.86	26.88	19.24	29.96	20.42
	16:1(n-7)	6.69	1.37	11.09	1.17	8.75	1.40	5.44	1.71	7.39	1.74
	18:1	29.99	15.68	22.37	15.57	24.37	17.10	24.22	16.76	19.67	14.56
	20:1	1.47	2.55	1.81	3.15	1.96	3.41	2.77	3.36	3.76	3.82
	22:1	1.27	1.52	0.93	0.83	1.44	0.87	1.18	0.71	1.48	0.62
	Σ MFAs	43.02	24.61	41.32	25.60	41.37	27.03	39.36	26.64	37.62	25.10
	18:2(n-6)	4.67	8.56	4.43	10.98	2.48	8.12	6.66	9.94	8.11	12.02
	20:4(n-6)	0.89	1.24	0.62	1.20	0.96	1.59	0.69	0.89	0.86	1.04
	Σ (n-6)	9.07	12.97	7.77	15.80	6.28	13.44	11.07	14.01	12.16	16.07
	18:3(n-3)	0.16	0.12	0.15	0.27	1.58	1.40	1.24	0.88	0.58	0.61
	20:5(n-3)	6.34	20.66	5.71	17.46	5.29	17.21	6.11	18.60	6.48	17.99
	22:5(n-3)	0.96	0.74	2.02	0.60	0.45	0.79	1.28	0.51	1.24	0.57
	22:6(n-3)	3.08	17.81	0.85	16.89	3.26	17.53	2.99	17.62	3.58	17.42
	Σ (n-3)	13.61	40.08	14.26	36 46	15.97	38.19	14.57	38.42	14.86	37.43
	Σ PUFA	22.80	53.47	24.88	53.14	26.31	52.64	30.84	53.21	30.36	54.24
	(n-3)/(n-6)	1.33	3.09	1.83	2.32	2.53	2.84	1.33	2.85	1.27	2.35

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table

Table 29. Principal NL and PL Fatty Acids (Wt %) of the Abdominal Muscles of Lobsters Reared at 20°C for 14 Weeks.

Diet		A		B		C		D		E	
Lipid Fraction		NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
Fatty Acid	14:0	2.78	0.27	1.96	0.25	2.64	0.24	1.48	0.29	1.81	0.33
	16:0	23.69	17.78	22.99	18.66	18.27	16.77	21.93	16.32	21.42	19.62
	18:0	4.53	4.05	5.31	3.04	4.48	3.41	3.40	2.72	4.93	3.34
	Σ SFAs	30.34	21.78	32.31	22.81	28.19	21.29	28.21	19.77	31.29	23.98
	16:1(n-7)	5.94	1.33	5.16	1.62	7.05	1.60	5.46	1.68	3.91	1.87
	18:1	25.06	16.43	28.25	24.78	38.61	24.61	35.90	21.98	10.91	13.29
	20:1	2.73	2.58	2.43	2.27	1.71	2.88	2.24	2.85	4.79	4.15
	22:1	1.44	0.85	1.20	0.49	0.61	0.63	1.15	0.55	2.03	0.54
	Σ MFAs	35.64	24.44	40.90	32.62	47.17	33.47	48.51	30.45	25.61	24.22
	18:2(n-6)	3.27	5.70	5.66	9.41	3.18	5.47	5.67	7.22	8.00	12.83
	20:4(n-6)	1.08	1.19	0.82	0.62	0.80	0.92	0.36	0.76	1.65	0.59
	Σ (n-6)	11.03	10.13	9.31	13.29	6.93	9.04	8.04	11.42	21.32	16.64
	18:3(n-3)	0.20	0.09	0.18	0.11	2.84	2.55	2.94	1.84	0.96	0.27
	20:5(n-3)	4.61	23.17	6.23	15.94	3.51	16.26	4.27	15.18	4.95	17.33
	22:5(n-3)	0.33	0.53	1.37	0.29	0.42	0.33	0.60	0.47	2.13	0.64
	22:6(n-3)	4.76	17.38	2.18	12.66	3.04	13.90	4.36	15.82	2.96	14.72
	Σ (n-3)	11.93	41.83	13.26	29.71	12.12	34.44	12.77	35.90	15.45	34.04
	Σ PUFAs	27.05	53.10	24.48	44.12	22.63	44.67	22.02	49.48	40.92	51.39
	(n-3)/(n-6)	1.01	4.16	1.43	2.31	1.92	3.79	1.6	3.15	0.75	2.06

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

Table 30. Principal NL and PL Fatty Acids (Wt %) of the Gills of Lobsters Reared at 10°C for 14 Weeks.

Diet		A		B		C		D		E	
Lipid Fraction		NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
Fatty Acid	14:0	4.39	1.81	3.10	2.27	3.28	1.26	3.76	2.29	4.69	1.46
	16:0	17.40	10.61	17.66	10.82	17.00	9.27	16.38	9.14	18.07	13.80
	18:0	4.66	3.22	5.92	3.64	4.90	3.19	4.88	2.85	5.32	4.12
	Σ SFAs	29.91	17.75	29.83	19.57	27.93	15.97	27.83	16.29	31.34	21.67
	16:1(n-7)	6.99	4.41	6.11	4.07	7.08	2.74	6.49	3.30	7.03	3.72
	18:1	31.18	31.90	28.34	26.06	29.88	31.92	31.38	33.82	19.90	16.61
	20:1	1.82	2.32	1.58	2.40	1.57	2.53	2.03	3.13	2.15	3.04
	22:1	2.15	1.20	1.89	0.98	1.65	0.94	1.09	0.80	1.95	1.44
	Σ MFAs	46.28	44.94	42.39	39.77	44.08	44.11	46.32	45.01	36.99	31.53
	18:2(n-6)	2.61	4.26	5.46	7.13	3.07	4.24	4.07	5.24	6.55	12.28
	20:4(n-6)	0.50	1.55	0.58	1.25	0.61	1.15	0.61	1.22	0.61	0.92
	Σ (n-6)	7.39	10.11	10.01	13.22	7.34	11.79	8.55	10.55	10.68	18.04
	18:3(n-3)	0.74	0.57	0.70	0.66	1.21	2.68	1.36	2.49	0.90	0.61
	20:5(n-3)	2.41	12.42	2.31	10.94	2.44	9.67	2.13	9.81	2.80	10.45
	22:5(n-3)	1.96	1.74	1.47	1.02	2.04	1.60	1.11	1.38	2.41	1.95
	22:6(n-3)	0.78	3.26	1.15	2.86	1.22	3.25	0.69	3.01	1.06	4.18
	Σ (n-3)	8.93	21.38	9.86	17.84	11.62	19.18	8.40	18.90	10.80	19.96
	Σ PUFAs	19.84	34.33	24.63	35.58	24.50	35.67	22.18	34.98	27.38	43.16
	(n-3)/(n-6)	1.24	2.15	1.00	1.37	1.63	1.82	1.03	1.79	1.09	1.11

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

Table 31. Principal NL and PL Fatty Acids (Wt %) of the Gills of Lobsters Reared at 20°C for 14 Weeks.

Diet		A		B		C		D		E	
Lipid Fraction		NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
Fatty Acid	14:0	4.42	3.16	3.80	1.25	5.41	1.33	2.98	1.40	3.8	1.58
	16:0	18.80	11.42	16.74	12.28	18.19	10.27	15.57	10.10	17.64	13.92
	18:0	5.27	3.63	4.91	2.60	5.52	3.15	4.28	3.03	4.61	4.32
	Σ SFAs	32.23	22.59	28.19	17.98	32.75	17.30	25.36	16.26	28.77	22.55
	16:1(n-7)	9.95	6.97	7.63	3.83	8.47	4.37	8.35	3.28	7.85	1.95
	18:1	24.35	25.56	29.99	37.27	17.51	32.71	31.92	41.94	17.12	13.17
	20:1	1.77	2.35	1.72	2.22	1.48	2.63	1.41	3.31	30.10	3.30
	22:1	1.65	1.37	2.61	0.79	1.66	1.55	1.33	1.18	1.94	1.61
	Σ MFAs	41.98	41.68	45.34	48.40	33.95	46.32	46.77	54.05	34.78	25.54
	18:2(n-6)	2.67	2.18	4.78	9.97	2.65	2.47	2.81	4.22	9.35	12.07
	20:4(n-6)	0.32	0.61	0.57	0.61	0.54	0.68	0.50	0.91	0.76	0.72
	Σ (n-6)	6.93	7.12	9.89	15.11	9.27	8.04	8.17	8.06	14.55	17.68
	18:3(n-3)	0.69	0.15	0.44	0.27	0.83	3.48	1.48	3.20	0.60	0.54
	20:5(n-3)	2.09	1.68	1.82	4.67	1.87	4.26	1.54	5.35	4.71	11.32
	22:5(n-3)	1.87	4.33	2.08	0.84	2.50	2.92	1.46	1.56	1.34	1.95
	22:6(n-3)	1.05	2.20	2.03	2.05	1.61	2.23	0.57	1.77	2.55	4.92
	Σ (n-3)	9.19	13.26	10.52	10.24	12.67	16.63	7.94	14.10	11.23	22.71
	Σ PUFAs	22.29	30.18	24.62	30.98	27.41	29.96	25.11	26.57	33.16	46.43
	(n-3)/(n-6)	1.35	1.88	1.04	0.67	1.40	2.07	1.05	1.76	0.80	1.28

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

4.3.3 TISSUE-SPECIFIC FA COMPOSITION

There were no major FAs for which the proportions were equivalent among the three tissues examined (the hepatopancreata, abdominal muscles and gills). However, the proportions of 18:0, total SFAs, 16:1 and 20:4(n-6) were similar in the gills and muscles.

Although the overall proportions of 16:1(n-7) in the muscles and hepatopancreata were not significantly different, there were several differences between the organs with respect to diet, temperature and lipid type; in particular, there were higher levels in muscle NL at 10°C than in hepatopancreas NL at 10°C, of lobsters fed Diets B, C, and D.

4.3.3.1 HEPATOPANCREAS FA COMPOSITION

The principal FAs (treatment means, weight percentage basis) of the hepatopancreata of lobsters reared at 10°C and 20°C are reported in Tables 26 and 27 , respectively. These tables are expanded in Appendices 4-A & 4-B, to include n (number of individuals) and standard deviations. The hepatopancreata had significantly higher percentages of MFAs than the gills or muscle (in both the NL and PL fractions). This was attributed almost entirely to the proportions of 18:1(n-9), which ranged (by diet treatment) from means of 62.3 to 79.0% in the NLs and from 45.1 to 53.0% in the PLs. These were the highest proportions of any single FA in the NL or PL fraction of any tissue, for all diet treatments.

The proportions of total SFAs in the NL and PL fractions of the hepatopancreata were one-half to one-third of the proportions found in the gill and the muscle NL and PL. The pattern was consistent for 14:0, 16:0 and 18:0, with the largest differences among means occurring for 16:0; in both the NL and PL fractions of the hepatopancreata, the proportions were 3.3 to 10.9% and 2.3 to 6.3%, respectively, less than half of the proportions found in the gill and the muscle NL and PL.

The proportions of 18:3(n-3) and 18:2(n-6) in the hepatopancreata vary significantly with dietary levels of each of these FAs.

20:5(n-3) in PL of Diet D at 20°C was significantly higher than in PL or NL, at 20C or at 10C in any other hepatopancreas sample. There was a similar, but lesser, trend in NL 20:5(n-3) of Diet D lobsters at 20°C.

4.3.3.2 ABDOMINAL MUSCLE FA COMPOSITION

The major FAs (treatment means, weight percentage basis) of the abdominal muscle tissue are given in Tables 28 and 29 for lobsters acclimated to 10°C and 20°C, respectively. These tables are expanded in Appendix 7-C and 7-D, to include n (number of individuals) and standard deviations. The most notable difference in muscle FA composition as compared to that of the gills and especially to the hepatopancreas was in the proportion of 22:6(n-3), which ranged from 16.9 to 17.8% of muscle PLs of lobsters at 10°C and from 12.7 to 17.4% of PLs in lobsters at 20°C, irrespective of diet.

Muscle PLs also contained among the highest levels of 18:2(n-6) regardless of diet. Means ranged from 8.1 to 11.0% for lobsters at 10°C and were lower in muscle PLs (5.5 to 9.4%) for lobsters at 20°C. The NLs of muscle contained higher levels of 18:2(n-6) than the NLs of the hepatopancreas and the gills, for lobsters fed Diets A, C , and D.

Although the proportion of 20:4(n-6) is quite low for all treatments (≤ 1.6% of NLs or PLs), it was significantly higher in the muscles than in the gills or hepatopancreas, and both the proportion and the amount of 20:4(n-6) were greatest in the muscle PLs (Appendices 8-I and 8-I, respectively).

The muscle PLs of both 10°C and 20°C-acclimated lobsters had the highest proportions of 20:5(n-3) (15.2 - 23.2% of PL), 22:6(n-3) (12.7 -17.8% of PL) and total (n-3) PUFA (29.7% -41.8%) compared to both NLs and PLs of the three tissue-types examined.

4.3.3.3 FA COMPOSITION OF THE GILLS

Tables 30 and 31 give the principal FAs (treatment means, weight percentage basis) of the gill tissue of lobsters acclimated to 10°C and 20°C, respectively. These tables are expanded in Appendices 7-E and 7-F, to include n (number of individuals) and standard deviations. The major FAs of the gill NLs and PLs of lobsters fed Diets A-D was 18:1(n-9); 28.3 - 31.4% of NLs and 26.1 - 33.8% of PLs at 10°C, and 17.5 - 31.9% of NLs and 25.6 - 41.9% of PLs at 20°C. At 10°C, the proportions of MFAs were similar in gill NL and PL for all diet treatments (39.8 - 46.3%), although 16:1(n-7) was higher in the NL than in the PL and in general 18:1(n-7) was higher in the PL than in the NL for all diet and temperature treatments. Palmitate (16:0) was the second most abundant FA in the gill NLs, and the means ranged from 15.6 to 18.8% of NLs and 9.1 - 12.3% of PLs. Total SFAs ranged from 275.4 to 32.7% of NLs and from 16.0 to 22.6% of PLs, and were not significantly influenced by diet or temperature.

There was a significant response of the gill PL composition to temperature, primarily in the proportions of 16:0, 18:2(n-6) and 18:3(n-3). At 10°C, 16:0 constituted 9.1 - 10.8% of PL while at 20°C the means in the PL were higher (10.1-12.3%). The (n-3) HUFA, 20:5, was present at 9.7 - 12.4% of PL at 10°C, even in the absence of dietary (n-3) and in the presence of dietary (n-6); this was nearly double the proportions present in the PL at 20°C (1.7% - 5.3%). The pattern was similar for the HUFA, 22:6(n-3), although at a much lower proportion of total PL FAs. It constituted 2.9 - 3.3% in the gill PL at 10°C, slightly, but consistently higher than in the gill PL at 20°C (1.8 - 2.2%) and higher

than in the gill NL at 10°C (0.6 - 1.1%) and 20°C (≤0.6%). Linoleate was also present at higher levels in the gill PL at 10°C than at 20°C (means of 4.3, 4.2, and 5.2% vs. 2.2, 2.5, and 4.2%, for Diets A, C, and D, respectively. The highest proportion of 18:2(n-6) occurred in the gill PL of lobsters fed Diet B, irrespective of culture temperature (7.1% at 10°C and 10.0% at 20°C).

4.3.4 FA COMPOSITION OF NL VS PL FRACTIONS OF LOBSTER TISSUES

The response of the FA composition of the NL and the PL fractions of juvenile lobsters to diet and temperature, and as affected by tissue type and lipid class, and the interactions between these various factors, singly and in combination, are summarized in Appendix 6-B. In general, NL and PL fractions were significantly different with respect to content of all major FAs. The difference in total SFAs, MFAs, and PUFAs between the NLs and the PLs of lobsters fed Diets A-D and Diet E are shown in Figure 23. A differential response to temperature between the NL and PL fractions is significant only for the proportions of total SFAs, total MFAs, 20:5(n-3), and 22:6(n-3). For example, the effect of temperature on the proportion of 20:5(n-3) is greater on the gill PLs than on the gill NLs, and accounts for a significant three-way interaction between lipid class, tissue type and temperature.

4.3.4.1 NEUTRAL LIPIDS

The NLs were considerably higher in SFAs than were the PLs in the gills and muscle (Figure 23), attributable primarily to 16:0, and also to 14:0; the difference was not as noticeable in the hepatopancreas. In the gills, the NLs were higher in 16:1(n-7) than the PL for all ten diet-temperature treatments. NLs of the muscles had higher proportions of MFAs (35.6 - 48.5%) than the muscle PLs (24.4 - 33.5%).

4.3.4.2 POLAR LIPIDS

The PLs were substantially higher in the proportion of PUFAs than the NLs for all tissues under all diet and temperature treatments (Figure 23). Conversely, proportion of MFAs was much lower in the PLs than in NLs in the hepatopancreata and muscles.

4.3.5 INDIVIDUAL, GROUPS AND SERIES OF FATTY ACIDS

The mean weight percent contributions of each principal FA, group of FAs and (n-3) and (n-6) series of FAs of the NL and PL fractions of the hepatopancreata, muscle, and gills of lobsters at each diet-temperature treatment are provided in separate tables in Appendix 8. Treatment means of the amount (in μg) of each FA in the above categories are given in separate tables in Appendix 9. A summary of the factors affecting the contribution of each FA and FA grouping is given in Appendix 10-B, and individual ANOVA and post-hoc analyses are given in Appendices 10-C thru 10-Q.

The proportion of total SFAs ranged from 3.8% to 32.7% of NL and 3.7 to 22.8% of PLs in the tissues of H. americanus (Appendix 8-D), and in general, was not significantly different between lobsters acclimated to high vs. low temperature (Appendices 10-B and 10-F_2). There was, however, a significant effect of diet on the proportion of total SFAs, and proportions were significantly different between tissue types and lipid types. The primary SFA, 16:0 (Appendix 8-B) comprised 14.2 to 26.8% of muscle NLs and PLs, 9.1 to 18.2% of gill NLs and PLs, and was lowest in the hepatopancreas NLs and PLs (2.3 to 10.9%). The second most abundant SFA was generally 18:0 (Appendix 8-C), which comprised 0.3 to 2.6% of NLs and PLs of the hepatopancreas, and 3.4 to 5.9% of the NLs and PLs of the muscles and gills.

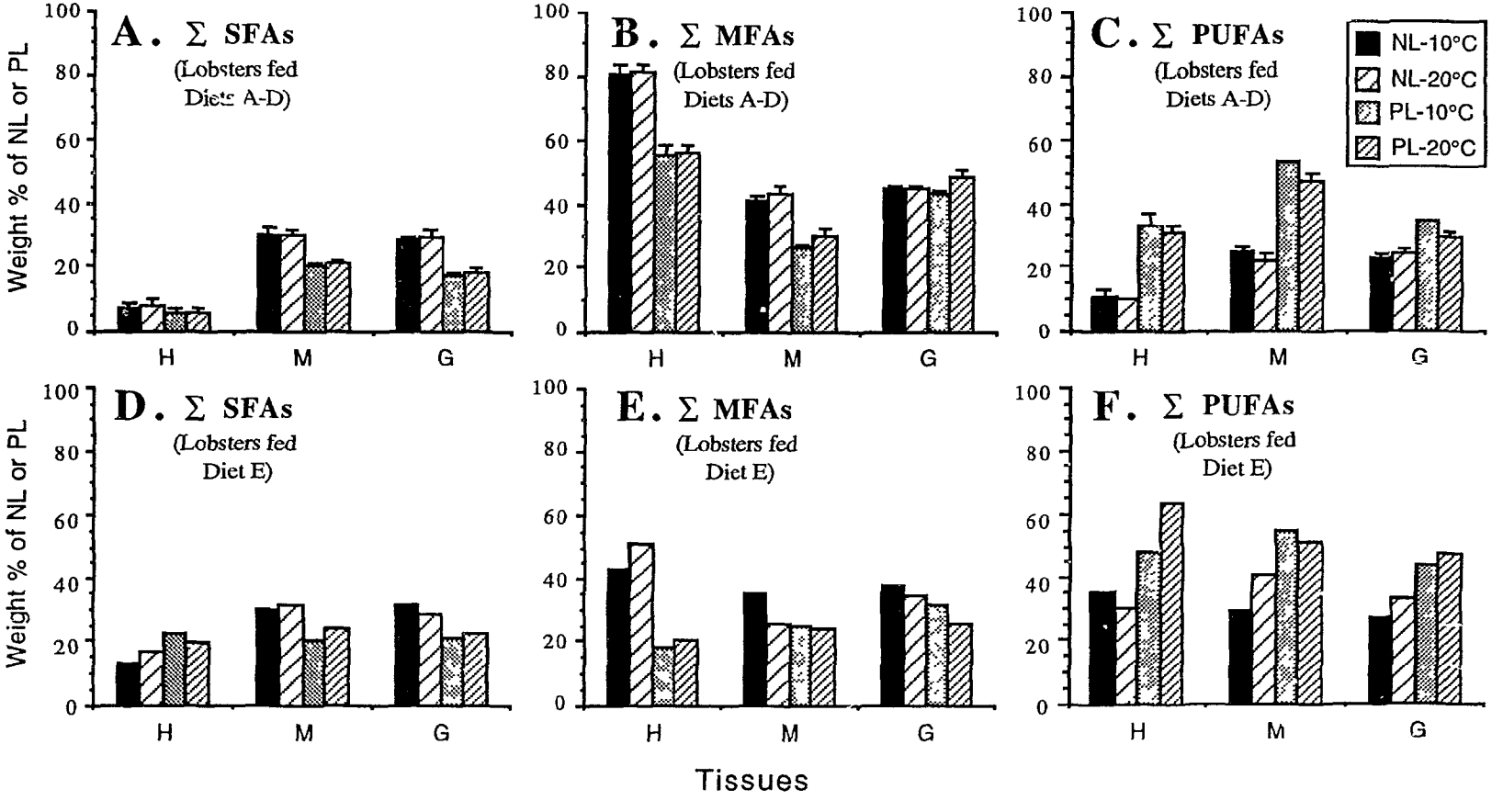


Figure 23. The Proportion of Saturated Fatty Acids (SFAs), Monounsaturated Fatty Acids (MFAs), and Polyunsaturated Fatty Acids (PUFAs) in the Tissues of Juvenile Lobsters.

Lobsters were reared at 10°C or 20°C and fed one of five diets, A-E. Values plotted in A-C are the means ± SEM of separate tissue samples from each of 2-4 lobsters from each of four Diets treatment, A-D, at each temperature; values plotted in D-F are the means of separate tissue samples from each of 3-4 lobsters fed the reference diet, Diet E, at each temperature.
NL, neutral lipids; PL, polar lipids; H, hepatopancreas; M, abdominal muscles; and G, gills.

The proportion of total SFAs ranged from 3.8% to 32.7% of NL and 3.7 to 22.8% of PLs in the tissues of H. americanus (Appendix 8-D), and in general, was not significantly different between lobsters acclimated to high vs. low temperature (Appendices 10-B and 10-F_2). There was, however, a significant effect of diet on the proportion of total SFAs, and proportions were significantly different between tissue types and lipid types. The primary SFA, 16:0 (Appendix 8-B) comprised 14.2 to 26.8% of muscle NLs and PLs, 9.1 to 18.2% of gill NLs and PLs, and was lowest in the hepatopancreas NLs and PLs (2.3 to 10.9%). The second most abundant SFA was generally 18:0 (Appendix 8-C), which comprised 0.3 to 2.6% of NLs and PLs of the hepatopancreas, and 3.4 to 5.9% of the NLs and PLs of the muscles and gills.

There was a significant effect of temperature, diet, tissue type and lipid type on the proportion of MFAs (Appendices 10-B and 10-I_1 - 10-I_5). The predominant MFA was oleate, 18:1(n-9). The proportion of oleate was highest in the hepatopancreas, where it comprised 62.3 to 79.0% and 45.0 to 53.0% of the NLs and PLs, respectively. It constituted 22.4 to 35.9% of NLs and 15.7 to 24.8% of PLs in the muscles, and 17.5 to 31.9% of NLs and 25.6 to 41.9% of PLs in the gills.

Linoleic acid, 18:2(n-6), accumulated in both NLs and PLs in lobsters which were fed diets supplemented with 18:2(n-6). The proportion of 20:4(n-6) in the three tissues examined did not exceed 1.6% of NL or PL fractions. It was not affected by diet composition nor was it affected by high levels of 18:2(n-6) accumulated in the tissue NL or PL fractions (even as high as 11% of NLs and 16% of PLs). In most cases, however, the proportion in the 20:4(n-6) in the PLs was twice that of the NLs and was generally twice as high in lobsters reared at 10°C than at 20°C.

The highest overall proportions of both 20:5(n-3) and 22:6(n-3) in the lobster were found in the PLs of muscle tissue and were slightly higher in 10°C-acclimated lobsters than

in those aclimated to 20°C (Appendices 8-L & 8-M, respectively; Figure 21). Although there was no apparent effect of dietary (n-6) FAs on the proportion of 20:5(n-3) & 22:6(n-3) in muscle PLs, the proportion of total (n-3) FAs was lowest in lobsters fed 18:2(n-6), in the absence of dietary (n-3) FAs.

The ratio of (n-3) to (n-6) FAs was not affected by temperature or by temperature interactions with diet, tissue type or lipid type. The ratio was, however significantly affected in both the NL and PL fractions of all three tissues by the presence of (n-3) and (n-6) fatty acids in the diet. The response was different among tissue types and between NLs and PLs.

Both 20:5(n-3) and 22:6(n-3) in the PL and NL of Diet D lobsters at 20°C were significantly higher than in Diet A-C lobsters.

4.4 DISCUSSION

4.4.1 LIPID CLASS COMPOSITION OF THE TISSUES

The hepatopancreata of lobsters which had been fed the H-CRD (a semi-purified diet which contained corn oil and cod liver oil) were ~5.3 to 5.7% of wet body weight (Table 23). This is in the range of values reported by D'Abramo et al. (1980) (4.8 to 6.2% BW) for lobsters fed similar semi-purified-type diets which contained natural oils (including corn oil, cod liver oil, and tuna oil). Compared to these lobsters which were fed the diets comprised of natural oils (and thus a variety of n-3 and n-6 PUFA and HUFA), the lobsters fed Diets A-D had lower HSI, attributable both to their smaller body size and to dietary FA deficiencies (see Part III, Chapter 3). TL of the hepatopancreas was also less among Diet A-D lobsters (2.1-4.8% of hepatopancreas, WWB), compared to that in lobsters fed natural oil diets, despite equivalent dietary lipid levels. TL was 12.9 to 21.1%

of hepatopancreas wet weight in lobsters fed H-CRD (this study) and was comparable to the level in lobsters in the D'Abramo et al. (1980) study (13.6 to 25.6%).

Temperature did not affect the TL of abdominal muscle in H. americanus. TL was 0.7 to 1.1% of wet weight regardless of temperature or dietary FA composition. Likewise, muscle TL of the shrimp Pleoticus muelleri comprised 1.1 to 1.3% of body wet weight regardless of seasonal temperature differences (Jeckel et al., 1991b). Muscle PLs, which ranged from 51.3 to 63.1% of TL in warm-acclimated lobsters, and were slightly higher in cold-acclimated lobsters (57.9 to 66.6%), were in general similar to levels reported to occur in several other decapods (53.9 to 68.7% in Penaeus japonicus (Teshima & Kanazawa, 1983); 62 to 70% in four species of marine prawns (Gopakumar & Nair, 1975); and 73.6% in the crab Carcinus maenas (Huggins & Munday, 1968). Higher levels have been reported for two other decapods (82.9 to 90.8% of TL in Pleoticus muelleri (Jeckel et al., 1991b), and 78.8% of TL in the queen crab Chionoecetes opilio (Addison et al., 1972). Glycolipids, which comprised 3.9 to 11% of muscle TLs in H. americanus (this study), were not detected in the muscle lipids of P. muelleri (Jeckel et al., 1991b).

Gill TL, which ranged from 2.0 to 6.2% of gill wet tissue weight, was an order of magnitude higher than values reported by Nevenzel et al. (1985) for the crayfish Procambarus clarkii, WWB (0.38 to 0.64%), but only ~13 to 40% of the values reported on a dry weight basis (15.5% of gill tissue). They were closer to the high end of the range reported by Dumont (1958) (0.85 - 3.63%, WWB). The high TL levels obtained in this study may reflect the precautions taken to protect the gill lipid from oxidizing; fresh gill tissue was blotted dry, added to a tared screw-capped vial, then any remaining excess moisture was eliminated by a stream of nitrogen gas prior to obtaining the fresh weight measurement. This would reduce the fresh weight measurement as compared to other studies and effectively increase the measurement of relative TL content. No other explanation is evident as to why lobster gills in particular might have a higher TL level than

the gills of other decapods, however, the NL/PL ratio is higher in H. americanus in this study compared to values reported for other crustaceans reflecting a higher amount of NL in the gills. This is possibly a consequence of high dietary lipid.

The NL/PL ratio may reflect an accumulation (storage) or depletion of TAGs and indicate a positive or negative energy balance. An excessively high ratio, in the hepatopancreas for example, may also indicate impaired tissue function, as in "fatty liver" condition associated with certain nutritional deficiencies. (D'Abramo et al., 1980). In this study, the NL/PL ratio of the hepatopancreata was lowest (2.1) for those fed the (n-3)- & (n-6)-deficient diet, Diet A. A low NL/PL ratio seems to be a symptom of EFA deficiency, orresponding also to the lowest hepatopancreatic TL levels, the lowest HSI (Part III,Ch. 3) and an atrophied condition of the hepatopancreata of the EFA-deficient lobsters (Part III, Ch. 3). Since the hepatopancreas is the principal storage and processing organ of lipids in crustaceans (see Part I, Introduction), impaired function may be expected to affect the absorption of dietary lipids into the hemolymph and subsequent distribution to the tissues. Although this is not apparent in the lipid composition of the abdominal muscle, it may have contributed to depressed growth rate among Diet A lobsters.

The NL/PL ratio ranged from 2.4 to 4.2 for the hepatopancreas of lobsters fed Diets B, C, and D, similar to the ratio (3.4) reported by D'Abramo et al. (1980) for juvenile lobsters also fed sem-purified diets. The ratio was substantially higher in lobsters fed the reference diet (13.3 for lobsters at 10°C, and 20.7 for 20°C-acclimated lobsters, but does not appear to be detrimental as these lobsters had the fastest molting and growth rates (Part II, Ch. 1).

In the muscles, the NL/PL ratio was somewhat higher in the juvenile lobsters from this study (0.4 to 0.6) and in the body (without hepatopancreas) of lobsters from the D'Abramo et al. (1980) study, (ratio = 0.6), than in muscles of wild caught shrimp,

Pleoticus muelleri (Jeckel et al., 1991b). This reflects the higher percentage of NL in the lobsters (0.3%, regardless of diet or temperature) than in the shrimp (0.1 - 0.2% in winter, spring and summer, and 0.3-0.4% in the autumn). The greater NL deposition in the muscles of the lobsters from both laboratory studies is likely attributable to the high total lipid level in the artificial diets. The unusually high levels of 18:1(n-9) accumulated in the lobsters, from this study, which received diets high in 18:1(n-9) (as discussed in Section 4.4.2.2) provides evidence of this. In Pleoticus muelleri, a similar response to diet is shown by the NL deposition subsequent to summer feeding.

4.4.2 EFFECT OF DIETARY FAs ON LOBSTER FA COMPOSITION

4.4.2.1 EFA DEFICIENCY

EFA deficiency in vertebrates results in the accumulation of Mead's acid (eicosatrienoic acid), 20:3(n-9) and a relative decrease in the levels of (n-3) and/or (n-6) HUFA. This results from the action of Δ^6- and Δ^5-desaturase on (n-9) series FAs, in the absence of competing (n-6) or (n-3) substrates (see Figure 2, p.9). Thus, the ratio of 20:3(n-9) / 20:4(n-6) in mammals, and the ratio of 20:3(n-9) / 22:6(n-3) in freshwater fish such as rainbow trout (Castell et al., 1972), common carp (Takeuchi & Watanabe, 1977), chum salmon (Takeuchi & Watanabe, 1982), and channel catfish (Satoh et al., 1989) serve as useful indicators of EFA status. However, these are not useful indicators for either marine or freshwater crustaceans thus far examined, since 20:3(n-9) does not appear to accumulate as a consequence of (n-3) or (n-6) deficiency despite other indicators of EFA deficiency, such as depressed growth or increased mortality. For the marine shrimp Penaeus japonicus, Kanazawa et al. (1979e) found only very low levels of 20:3(n-9), and it was not reported to occur in the study conducted with P. japonicus by Deshimaru et al. (1979). Likewise, Sheen & D'Abramo (1991) suggested that the lack of 20:3(n-9) in

freshwater prawn Macrobrachium rosenbergii fed EFA-deficient diets is further evidence of the non-existence or inefficiency of elongase and desaturase enzymes in crustaceans.

Eicosatrienoic acid was not identified among the FAs of H. americanus fed (n-3) &/or (n-6) deficient diets in this study, consistent with the previous findings for other decapods. It was also not reported among the major FAs to occur in H. americanus whole body total lipids after 5-6 weeks of feeding (n-3) & (n-6) deficient diets (Castell & Boghen, 1979) or in the hepatopancreas or remaining body NLs or PLs of H. americanus fed EFA-deficient diets for 12 weeks (D'Abramo et al., 1980).

4.4.2.2 ACCUMULATION of DIETARY FAs

Chapelle (1986) noted that in crustaceans (as in other organisms) differences in PUFA contents of the various tissues probably reflect a combination of dietary influence and specific EFA requirements of each organ. Such is likely the case in this study, since dietary FA composition significantly influenced the FA profiles of the tissues examined, but the effect of diet varied with tissue type. The abdominal muscles and gills were least affected by differences in dietary FAs, although proportions of 18:2(n-6) and 18:3(n-3) in the tissues were higher for lobsters fed diets containing those FAs. In contrast, there was a significant and obvious impact of dietary FAs on the FA profile of the hepatopancreas. Inclusion of 18:2(n-6) and/or 18:3(n-3) (at a total of only 1% of diet, DWB) substantially increased their amount and proportion in the NLs and PLs of the hepatopancreas. Although the hepatopancreas was only 2.6 to 5.7% of the body weight (WWB), compared to 10.5 to 13.0% for the abdominal muscle mass, it contributed 2 to 6 times as much TL in Diet A-D lobsters and 14 to 23 times as much TL in Diet E lobsters as the muscles, and therefore would influence whole body TL composition. Considering the relatively constant FA composition of the muscles, it is likely that the direct, linear relationship of dietary inclusion of 18:2(n-6) and/or 18:3(n-3) and whole body levels as

seen in Part I, Chapter 2 (Figures 7-A and 7-B) can be attributed primarily to the influence of diet on the composition of the hepatopancreata.

The four test diets in this experiment contained a high level of 18:1(n-9) (8 or 9%, DWB), and an accumulation of 18:1(n-9) was evident in all tissues examined of the lobsters fed these four diets. The highest proportions of 18:1(n-9) were in the hepatopancreatic NLs, of which it comprised 57 to 79%. It also comprised 45 to 57% of hepatopancreatic PLs, 18.0 to 33.8% of muscle NLs and 24.5 to 38.6% of muscle PLs of lobsters fed Diets A-D. By contrast, the reference diet, H-CRD, contained only 2%, DWB of 18:1(n-9), and lobsters fed this diet contained 18:1(n-9) as 6.6 to 19.0% and 22.9 to 27.8% of hepatopancreatic PLs and NLs, respectively and 13.3 to 18.4% and 10.9 to 21.9% of muscle PLs and NLs, respectively. Therefore, although there is an obvious accumulation of this FA which may be attributed to de novo synthesis as well as to accumulation from the diet, the tissue levels are substantially less than in lobsters fed diets which had high levels of 18:1(n-9). Similar results were obtained with lobsters fed test diets from Experiment I (Part II, Chapter 2, which contained 18:1(n-9) at only 0.5 to 0.6% of diet (DWB). With one exception, values ranged from 14.2 to 19.1% of NLs and from 4.8 to 28.1% of PLs of whole lobsters.

The selective accumulation of dietary (n-3) HUFA is also evident in the tissues of lobsters fed the reference diet. The H-CRD contained 20:5(n-3) and 22:6(n-3) at 0.32 and 0.30% of diet (DWB), respectively. Docosahexaenoate accumulated at 14.1 to 14.5% of hepatopancreas PLs and at 2.6 to 3.8% of hepatopancreas NLs, compared to proportions of less than 4% and 1% of PLs and NLs, respectively in lobsters fed the four test diets, which were deficient in (n-3) HUFA. However, the level of 22:6(n-3) was only slightly elevated in the gill PLs of H-CRD lobsters (4 to 5% of PLs) as compared to Diet A-D lobsters (1.8 to 3.3% of PLs). Similar results were seen for whole body PLs and NLs

in Experiment I lobsters (Part II, Ch.2), fed the H-CRD or one of two test diets which contained 22:6(n-3) as compared to the FA profiles of lobsters fed all other diets.

4.4.2.3 De Novo SYNTHESIS VS. RETENTION OF IMPORTANT FAs

The capacity of lobsters for de novo synthesis of non-essential FAs was not directly tested for in this feeding experiment, particularly since preformed 18:1(n-9) was fed to all lobsters at a level presumed sufficient to meet energy requirements for maintenance and growth (and stress).

Lands (1991) reports that arachidonate is "retained tenaciously" by mammalian tissues and that it is difficult to obtain a deficiency, owing to the importance of this FA as a precursor of mammalian hormones. Such is not the case for arachidonate in the lobster H. americanus or in most other marine crustaceans. Instead, it appears (based on proportional distribution in the tissues) that (n-3) HUFAs may replace (n-6) HUFAs in fulfilling many important EFA functions in these marine poikilothermic animals. In this study, despite 14 -17 weeks on diets deficient in (n-3) FAs, high proportions of 20:5(n-3) were found in the muscle and the gill PLs, and 20:5(n-3) contributed at least 1.1 to 2.7% of hepatopancreas PLs (Figure 15). Although de novo synthesis was not tested in this study, previous nutrition studies and radioisotope experiments have demonstrated a lack of de novo synthesis of (n-3) and (n-6) PUFA in lobsters and other decapods, as in all other animals examined to date (Gurr and James, 1980) (see Part I, Introduction, Sections 1.1.1 - 1.1.3). Therefore, the high levels of (n-3) HUFA are most likely attributable to retention of (n-3) HUFAs. The magnitude of the retention or the rate of depletion can not be quantified since "spare" sibling lobsters were not available at the start of the feeding trial to establish initial proportions and quantities of tissue FAs. Since all lobsters had been fed the reference diet (H-CRD) prior to the experiment, it might be assumed that the FA profile of lobsters at the start of the feeding trial was similar to the profiles reported for H-CRD -fed

lobsters in the results section. Also, the 8 to 9% level of 18:1(n-9) in the diet may have protected tissue reserves of these FAs. A FA-free or low fat treatment would have provided an interesting 'negative control' to indicate whether these possible EFAs would be retained (i.e. selectively spared) in the event that body fats needed to be catabolized for energy.

Nonetheless, it appears, especially for Diet A lobsters, that (n-3) HUFA are "tenaciously retained" in the gill and muscle PLs, so it may therefore be inferred that they perform important functions. It must also be recognized that growth of those lobsters acclimated to 20°C on the (n-3)- and (n-6)-deficient diets was stunted compared to H-CRD -fed lobsters (Figures 5 & 10), and that growth of 10°C-acclimated lobsters on all diet treatments was minimal (Figure 10). Had substantial growth occurred, retention of (n-3) HUFA at the current levels would not have been possible on (n-3)- or (n-6)-deficient diets, since an increase in tissue mass would mandate an increase in quantity of those FAs in order to maintain the same proportional distribution. This could only be obviated by an alternate exogenous source (as in gut bacteria synthesizing B vitamins), endogenous synthesis (which has been ruled out), or bioconversion from other FAs (this appears to be negligible; see next section), once body reserves have been depleted. These arguments are also assuming that the essential nature of the (n-3) HUFA in these tissues is structural and that they are not "consumed" in fulfilling their role, as in bioconversion to eicosanoids, or other metabolites.

In summary, high proportions of (n-3) HUFA were retained in the PLs of the gills and muscles of lobsters fed diets deficient in (n-3) FAs, indicating they have an important function. Either it was possible to maintain the high proportions in the tissues for the duration of the experiment because there was minimal increase in tissue mass or, conversely, the dietary deficiency may have contributed to a restriction in growth in order to maintain functional levels of those FAs in the tissues. That is, although the level of total

dietary FAs was sufficient to meet the energy demands for growth, the level (or absence of) (n-3) HUFA may limited growth or other essential functions and could therefore be considered essential for the lobster.

4.4.2.4 ELONGATION and/or DESATURATION OF FAs

Compared to the accumulation of 18:2(n-6) in the muscle NLs and PLs of lobsters fed diets supplemented with 18:2(n-6), there is minimal accumulation of 18:3(n-3) in the NLs or PLs of lobsters fed diets supplemented with 18:3(n-3). Considering also the high proportions of 20:5(n-3) and 22:6(n-3) in the muscle PLs of lobsters on all diet-temperature regimes, this could be accounted for by greater activity of Δ^6-, Δ^5-, and Δ^4-desaturase enzymes on (n-3) series FAs in the muscles than in the hepatopancreas (see Figure 2). It may also indicate greater Δ^4-desaturase activity in the muscles than in the gills, since the gills accumulate high proportions of 20:5(n-3) (especially at the lower temperature of acclimation) but have substantially lower proportions of 22:6(n-3) than the muscles .

Although the proportion of 20:4(n-6) varied among tissues, between NLs and PLs within tissues, and with temperature of acclimation, treatment means did not exceed 1.6% of lipid class. In addition, the level of arachidonate was not affected by high levels of 18:2(n-6) in the diet or accumulated in the tissues. This is consistent with the results of the experiment in Part II, Chapter 2 where the proportion of arachidonate ranged from ~0.2 to 1.1% of whole lobster PLs regardless of dietary inclusion of, or deficiency of, 18:2(n-6), and in feeding trials with juvenile lobster conducted by D'Abramo et al. (1980), in which the level in the tissues did not exceed 1% of TL for lobsters fed ample dietary 18:2(n-6). These results further indicate a lack of Δ^6-desaturase activity for the (n-6) series FAs. Although this may appear contrary to the results of Castell & Boghen (1979), it is possible that the high levels of 20:4(n-6) they reported to occur in the TLs of

juvenile lobsters had been accumulated from a dietary source prior to their ~ 5 week feeding trial, and were not yet depleted. Sasaki (1984) found that the FA profiles of 4th stage lobsters (H. americanus) reflected dietary FA composition. He analyzed the FA composition of various lots of Artemia used in his feeding trials and found that they contained 20:4(n-6) at 1.0 to 4.0% of FAs. The proportion of 20:4(n-6) in larval lobsters fed Artemia ranged from 2.1 to 4.1% of NLs, 4.6 to 5.3% of PE, and 5.0 to 5.8% of PC in whole, 4th stage lobsters (1.5 to 2.3µg.NL/individual, 1.7 to 2.0 µg PE/ind., and 4.7 to 5.4 µg PC/ind., respectively). Arachidonate was incorporated into phosphatidylcholine at a higher proportion than in the Artemia (feed). These are comparable to the levels reported by Castell & Boghen (1979) for ~ 5th -6th Stage lobsters which had previously been fed Artemia. In contrast, the level of 20:4(n-6) in 4th stage lobsters which had been starved from hatching, were 0.17 µg NL/individual, 0.43 -0.52 µg PE/individual, and 0.66-1.2 µg PC/individual Sasaki (1984).

4.4.2.5. COMPETITION BETWEEN (n-3), (n-6),and (n-9) SERIES FAs

In lobsters fed dietary 18:2(n-6) and/or 18:1(n-9), the relative proportions of 20:5(n-3) and 22:6(n-3) are depressed in the hepatopancreas PLs. This is not inconsistent with a possible competition of these FAs with 18:3(n-3) for Δ^6-desaturase. However, since neither 20:4(n-6) nor 20:3(n-9) accumulate in the tissues of (n-3) deficient lobsters, the depressed tissue levels of (n-3) HUFA are likely accounted for by the relative accumulation of 18:2(n-6) and 18:1(n-9) in lobsters fed those FAs. Again, it appears that Δ^6-desaturase activity, if it exists, is minimal in these animals.

4.4.3 EFFECT OF TEMPERATURE ON FA PROFILES OF THE TISSUES

Results from this study demonstrate that temperature has a significant influence on the FA composition of different tissues of the lobster, H. americanus and that the response to a 10°C temperature differential varies with organ and with the lipid classes within each

organ. As in previous studies (Chapelle, 1978; Farkas, 1979; see Introduction, this chapter), there is in general a greater proportion of PUFA and a lower proportion of 16:0 in the PLs at the lower acclimation temperature. This is discussed in the next sections, with respect to individual tissues.

4.4.4 THE ROLE OF FAs IN THE TISSUES OF THE LOBSTER

Of the three tissues examined, the FA composition of the muscle tissue was the most constant among lobsters on the ten diet-temperature regimes, the gill tissue was most affected by temperature, and the hepatopancreas was most affected by dietary fatty acid composition. These trends are likely indicative of the different roles FAs play in each of the tissues.

For example, Chapelle (1986) notes a general tendency in the total PLs of decapod crustaceans for the proportion of 20:5(n-3) to be greater than 22:6(n-3). Although that was generally true in this study, it was found that not only the amounts (as mg-percent of tissue, WWB), but also the relative amounts of 20:5(n-3) and 22:6(n-3) vary with tissue, and for a given tissue type (O, for organ), vary with temperature (T) and diet (D) (Appendix 10-N and 10-O). This three way interaction of T x D x O is significant at $p < 0.05$. Based on their relatively high proportion of total FAs, these (n-3) HUFA are apparently important in both the muscles and the gills of the lobster, but given the differences in FA profiles, in particular the ratio of 20:5(n-3) to 22:6(n-3) and the different response of these profiles to diet and temperature, it seems that these (n-3) HUFA may serve different aspects of membrane function in these two tissue, as discussed in the next sections.

4.4.4.1 HEPATOPANCREAS

In the hepatopancreas the proportion of SFAs, (16:0 in particular) and MFAs (especially 18:1(n-9)) were noticeably lower and higher, respectively, than in the muscle and the gills. In addition, the proportion of 18:1(n-9) in the hepatopancreas was dramatically higher (and the proportion of 16:0, substantially lower) than the values reported by D'Abramo et al. (1980) for hepatopancreas NLs and PL in juvenile lobsters fed semi-purified diets or a more natural diet of mussels. They found 16:0 comprised 17.3 to 19.5% of NLs and 17.5 to 26.3% of PLs, compared to 3.3 to 10.5% of NLs and 2.4 to 8.3% of PLs, found in this study for lobsters fed the test diets. The proportion of 16:0 in the hepatopancreata of reference diet lobsters, however, was in the higher range (10.3 to 14.7% of NLs and 16.3 to 16.7% of PLs). The proportion of 18:1(n-9) in the hepatopancreas of lobsters in the D'Abramo study was 15.5 to 16.8% of NLs and 11.5 to 17.0% of PLs in lobsters fed their semi-purified test diets and was lower in lobsters fed mussels (10.8% of NLs and 10.7% of PLs), which they attributed to either the high lipid content of the test diets (6% oils plus 8% soy lecithin) or to lipid deposition and a resultant "fatty liver" condition due to possible nutritional deficiencies. By comparison, 18:1(n-9) ranged from 57 to 79 % of NLs and 45 to 57% of PLs in the lobsters fed test diets in this study.

The differences may be attributed to the role of the hepatopancreas in lipid synthesis and storage as discussed in detail in Part I. The level and proportion of 18:1(n-9) in the test diets of this experiment were unnaturally high for lobsters, compared to the composition of their natural food organisms. It appears that dietary 18:1(n-9) is absorbed and incorporated as is into the hepatopancreas NLs and PLs, with minimal subsequent oxidation and conversion to 16:0 for storage. With substantial lipid reserves in the form of 18:1(n-9), and a positive energy balance due to the high total caloric level in the diet, de novo synthesis of 16:0 was likely suppressed.

4.4.4.2 ABDOMINAL MUSCLES

The high proportions of both 20:5 and 22:6 in the muscles (together, 30 - 40% of muscle PLs and 10 - 15% of muscle NLs) remained stable regardless of diet or temperature, even among surviving lobsters maintained on the (n-3) and (n-6)-deficient diet. This may indicate an importance of these FAs in the muscle cell membranes for the maintenance of a membrane structure conducive to the mechanical, locomotory function of the muscles and without compensation for temperature. Similar results were recently reported by Jeckel et al. (1991b) for the marine shrimp Penaeus muelleri. They examined the FA profiles of the muscles of male and female shrimp (both mature and immature) captured during each of the four seasons and found that the FA composition remained constant throughout the year, despite seasonal changes in temperature and diet. The proportions of 20:5(n-3) and 22:6(n-3) in the PLs ranged from 13.9 to 20.4% and 11.0 to 16.9%, respectively, comparable to the values for H. americanus in this study. They attributed this constancy in composition to the structural role of PLs in muscle membranes. They also reported NL values of 9.9 to 17.5% and 9.7 to 16.5% of NLs, respectively, for 20:5(n-3) and 22:6(n-3), somewhat higher than those in the lobster.

The abdominal muscles of the freshwater prawn, Macrobrachium rosenbergii, are also reportedly high in long chain PUFAs, which are retained "during long periods of lipid mobilization" (Reigh & Stickney, 1989). They suggest these FAs are protected during fasting, until the onset of muscle catabolism, indicating their importance in muscle membrane structure. Sheen and D'Abramo (1991b) reported that (n-3) to (n-6) ratios were less variable in the abdominal tissue than in the hepatopancreas for M. rosenbergii fed diets which varied in the level of inclusion of (n-3) and (n-6) PUFA and HUFA, also reflecting the relatively stable FA composition of muscle.

In contrast, other researchers have reported an affect of temperature on muscle phospholipids of decapod crustaceans. In the muscle PLs of crayfish, Austropotamobius pallipes Cossins (1976) reported a greater degree of unsaturation in cold-acclimated than in warm-acclimated animals. Martin & Ceccaldi (1977) examined the FA composition of the abdominal muscle of the prawn Paleamon serratus acclimatized for one month at 9°C, 15°C, 18°C, or 25°C. They found that the proportions of the following PUFAs (18:2(n-6), 20:5(n-3), 20:3(n-6), and 22:6(n-3)) were highest at the lowest temperature. The proportion of PUFAs ranged from 28.9% of TLs at 25°C to 41.5% of TLs at 9°C; SFAs ranged from 34.3% at 25°C to 26.6% if TLs at 9°C.

4.4.4.3 GILLS

In the gill tissue of the lobsters in this study, 20:5(n-3) seems to play a dominant role , more so than 22:6(n-3), as indicated by the relative proportions retained or accumulated in the PLs. Unlike the muscle tissues, the proportion of 20:5(n-3) in gills PLs is significantly affected by temperature. Thus it appears that there is a compensatory response to the affect of temperature in order to maintain certain properties of the gill membranes, which are different from those in the muscles.

In crustaceans, the gills serve three important functions: (i) oxygen uptake from the environment to the hemolymph, (ii) ionic & osmotic exchange between the environment and the hemolymph, and (iii) nitrogen waste elimination, as ammonia. There is a division of labor (and structure) between the anterior gills (specialized for gaseous exchange) and the posterior gills (specialized for salt and water transport) (see review by McMahon & Wilkens, 1983). The gills are characterized by an extensive surface area (Bergmiller & Bielawski, 1970) which is a single layer of epithelial cells, protected on the exterior (the apical side) by a cuticle and bathed at the basal surface by the hemolymph (for review, see Mantel & Farmer, 1983; Johnson, 1980). There may be microvilli at the apical

surface of the epithelial cells, and extensive infolding at the lateral and basal surfaces; the cells contain smooth and rough ER, microfilaments, vesicles, and may contain numerous mitochondria at the basement membrane.

A critical aspect of the ultrastructure and physiological functions of these transporting epithelia is the contribution of lipid content and composition to fluidity and permeability, enzyme activity (e.g. ATPases), and energy production. Several researchers have investigated compensatory changes in the content and FA composition of the PLs of crustacean gills in response to changes in environmental salinity (Whitney, 1974; Chapelle et al., 1976; Morris et al., 1982; Dawson et al., 1984) and temperature (Dawson et al., 1984; Morris et al., 1987). Further, the FA profiles of various PL classes (PC and PE) of the mitochondrial and microsomal fractions of anterior and posterior gills of the crab Eriorcheir sinensis have been described by Chapelle & Zwingelstein (1985), and correlated with differences of membrane microviscosity (i.e. lipid fluidity).

In this study, the gill TLs, which ranged from 2.0 - 6.2% of gill fresh tissue (Table 25) were higher than those reported for freshwater crayfish (Procambarus clarkii), 0.4 to 0.6% of gill wet weight (Nevenzel et al., 1988), and for crabs (Eriorcheir sinensis) which had been starved and acclimated for two weeks at 14°C (0.96 - 2.5% of fresh tissue) (Chapelle et al., 1976). Chapelle and co-workers (1976) further reported a higher PL content (as percentage of fresh tissue) in the posterior gills (1.5 - 2.1%) as compared to the anterior gills (0.71 - 0.75%). The PL content for the total gill complement from Homarus americanus in this study was in the same range, and somewhat higher in lobsters acclimated to 10°C (1.0 - 1.7% of fresh tissue) than in those at 20°C (0.5 - 1.5%) (Table 25). Whitney (1974) and Chapelle et al. (1976) noted that the concentration of PLs in gills increases when Callinectes sapidus and E. sinensis, respectively, are acclimated to fresh water (both are osmoregulating crabs). In fact, Whitney, using [1-^{14}C]-acetate, demonstrated rapid biosynthesis of PE and PS in the gills of C. sapidus, when the crabs

are exposed to dilute media. On the other hand, no change in PL content was found in the crab Libinia emarginata, an osmoconformer. Hazel (1985) reported significantly higher proportions of PE (than PC) in gill extracts of 5°C-acclimated trout (Salmo gairdneri) as compared to 20°C-acclimated trout. The higher PL content reported here for cold-acclimated lobsters may indicate a similar acclimatory compensation, in response to temperature.

In the gills of the crab Eriocheir sinensis, arachidonate is presumed to be important to osmoregulatory function, based on the effect of salinity on its proportion in the PLs of the posterior gills (13.7% of PLs in freshwater-acclimated crabs, and 17.1% of PLs in saltwater-acclimated crabs) (Chapelle et al., 1976). Although the proportion of 20:4(n-6) in the lobster gills was substantially lower than those in the crab gills, the proportion was twice as high in the gill PLs of lobsters acclimated to 10°C (ranging from 1.1 to 1.6% of PLs) than in those of 20°C acclimated lobsters (ranging from 0.6 to 0.9% of PLs). This temperature effect may indicate that despite these low levels, arachidonate may also have an important function in the lobster gills. It would be interesting to examine the FA profiles of the various phospholipid classes in the gills to determine whether 20:4(n-6) is associated with a particular phosphatide. This could provide further information as to its importance. For example in marine fish, although the level of 20:4(n-6) in the total PLs is relatively low, it is a major component of PI, and thus presumed to have a specific important function (Bell et al., 1983).

In summary, in contrast to the presumed contribution of 20:5(n-3) and 22:6(n-3) to a structural role in the muscles, these FAs likely contribute to the permeability properties of the gills and thus may be critical (and essential in the diet) for proper gill function (i.e. transport of gases, solutes and water).

The possible roles of EFAs for crustaceans as precursors of eicosanoids such as prostaglandins or leukotrienes cannot be assessed from this study and have not been

investigated in other crustaceans. Considering the multitude of eicoasanoid-mediated effects in vertebrates, which impact on all major organ systems, this could be a fascinating field of research with crustaceans. More specifically, this could provide insight into the possible dietary requirement for (n-6) FAs.

4.5 CONCLUSIONS

Based on differences in the fatty acid composition of the neutral lipids and phospholipids of the hepatopancreas, abdominal muscles and gills of juvenile Homarus americanus reared at 10°C or 20°C and fed semi-purified diets containing purified fatty acids, the following conclusions are indicated:

1. In juvenile lobsters, dietary deficiency of both (n-3) and (n-6) fatty acids results in diminished tissue mass and altered lipid content and composition of the hepatopancreas. The neutral lipid content, and as a result, the TL content and the neutral lipid/phospholipid ratio, of the hepatopancreas are depressed in lobsters fed a polyunsaturated fatty acid -deficient diet.

2. There is a significant effect of long term temperature acclimation (at 10°C vs. 20°C) on the proportions of 20:5(n-3) and 16:0 in the tissues of juvenile lobsters:
   a) At the lower temperature, there is a significantly higher proportion of 20:5(n-3), which attributes to significantly higher proportions of both total (n-3) fatty acids and a total polyunsaturated fatty acids in the three tissues examined.
   b) The response to temperature is greatest in the gill phospholipids.
   c) At the lower temperature, there is significantly higher level of 16:0, particularly in the phospholipids.
3. The most notable effect of temperature is on the proportion of 20:5(n-3) in the gill phospholipids

4. There is a significant impact of dietary fatty acid composition on the fatty acid profiles of lobster tissues. The hepatopancreas is affected more than the muscles and the gills.

5. Accumulation of 18:2(n-6), 18:3(n-3) and 18:1(n-9) in the hepatopancreas is directly proportional to levels of those fatty acids in the diet.

6. The gills and the abdominal muscles are less affected by levels of dietary fatty acids; (n-3) and (n-6) highly unsaturated fatty acid appear to be "tenaciously retained" despite dietary deficiency of those fatty acids. Also, (n-3) to (n-6) ratios were less variable in the abdominal tissue than in the hepatopancreas, reflecting the relatively stable fatty acid composition of muscle.

7. Docosahexaenoic acid, 22:6(n-3), is highest in the muscle phospholipids compared to the phospholipids of gills and hepatopancreata. The proportion of 22:6(n-3) is significantly affected by temperature, and is not affected by diet. Mean levels in the muscle phospholipids range from 16.9 to 17.8% of phospholipids at 10°C, and are lower in 20°C acclimated lobsters, means ranging from 12.7 to 17.4% of phospholipids.

8. There is no accumulation of 20:3(n-9) in the tissues of lobsters fed diets deficient in (n-3) or (n-6) fatty acids (a characteristic of essential fatty acid deficiency in vertebrates), even in the presence of dietary 18:1(n-9). Instead, unusually high levels of dietary 18:1(n-9) accumulates in the hepatopancreas. This indicates lack of desaturase activity.

9. The low levels of arachidonic acid, 20:4(n-6), in the presence of high dietary and tissue levels of 18:2(n-6) and low 18:3(n-3) indicates a lack of Δ^6-desaturase activity for the (n-6) series fatty acids

10. Based on conclusions 2, 5 and 7, there is limited Δ^6-desaturase activity in juvenile lobsters raised at 20°C, these results suggest a limited ability of chain elongation and desaturation from C18 to C20 to C22 fatty acids.

CHAPTER 5

Incorporation and Metabolism of [1-^{14}C]-18:3(n-3) in the Hepatopancreas and Abdominal Muscle

5.1 OBJECTIVE: PART III, CHAPTER 4

Evaluate the capacity for bioconversion of [1-14C]-18:3(n-3) to 20:5(n-3) and 22:6(n-3) in juvenile lobster, H. americanus.

5.2 MATERIALS AND METHODS

Radioisotope experiments (injection and incubation of lobsters, dissections, and tissue lipid extractions) were conducted in designated radioisotope rooms in the Biology Department, Life Sciences Center, Dalhousie University, following the guidelines of the Dalhousie University Radiation Safety Manual. These experiments were made possible through the cooperation and radioisotope licence of Dr. T. H. MacRae, Department of Biology, Dalhousie University.

Analysis of radiolabeled lipid samples was conducted in the laboratories of the Atlantic Research Center for Mental Retardation, Dalhousie University. Equipment and supplies were made available through Dr. H. W. Cook.

5.2.1 TEST ANIMALS AND INCUBATION APPARATUS

5.2.1.0 TEST ANIMALS

Intermolt juvenile lobsters were selected from those reared in the temperature-diet trial (Part III, Chapter 1). Test animals were fasted for 12 hours following a scheduled feeding and were then transported to the radioisotope lab. Wet weight of each animal was measured in order to calculate the appropriate dose of radioisotope. Orbit length[8] was also measured. Each lobster was then acclimated in an individual incubation flask to experimental conditions for ~22 hours at which time the water was changed (2 hours prior to injection of isotope).

[8] Orbit length is the length from the posterior edge of the eye socket to the tip of the telson.

5.2.1.0 INCUBATION APPARATUS

Each incubation vessel was a glass 3 liter Erlenmeyer flask with a rubber stopper through which two glass tubes were fitted. One tube was connected on the outside of the flask to the air hose from a portable aquarium air pump; within the flask, the same tube was connected to airline tubing and an air stone, which was submersed in the millipore-filtered sea water. The second tube was a vent for atmospheric gas from the flask. The vented air was bubbled through a series of three 250 ml Erlenmeyer flasks containing ~ 200 ml of a 4% $Ba(OH)_2$ solution, to trap expired $^{14}CO_2$. This is similar to the methods reported by Kanazawa et al. (1979) and Dall (1965). The incubation flasks were set in water baths to minimize temperature fluctuation. Flasks with lobsters previously reared in 20°C sea water were placed in a table top water bath (~20-22°C). The incubation flasks of lobsters reared in 10-12°C sea water were set in a water bath within an incubation chamber, regulated at ~ 10°C.

5.2.1.2 RADIOISOTOPE AND INJECTION OF TEST ANIMALS

The radiolabelled lipid, linolenic acid, 9,12,15-[1-^{14}C] (NEC-779, sp. act. 54.8 mCi/mmol; NEN Research Products, Boston, Mass., U.S.A.), was received as an ethanol solution (0.05mCi; 0.26mg in 0.5ml). Using a Hamilton glass microsyringe, the isotope ethanol solution was injected into the lobsters through the interarticular membrane at the base of the pereiopods (legs), and into the ventral sinuses. Dose was calculated on a wet weight basis. After preliminary trials, the effective dose was determined to be ~0.8µl/g wet weight, of the solution concentrated, under N_2, to 2.5 µg/µl. After injection, the lobsters were incubated for ~ 24 hours, without food.

5.2.1.3 TERMINATION AND DISSECTION OF LOBSTERS

Lobsters were blotted dry and held in a -20°C freezer until immobile (approx. 30 min.). They were then weighed to the nearest mg, decapitated, and dissected as in Part III, Chapter 3, Section 3.2.1. The body parts (hepatopancreas, abdominal muscles and gills) were rinsed with distilled water, blotted dry and weighed. They were placed in teflon-lined screw cap vials in 2:1 chloroform:methanol, flushed with N_2, sealed, and stored at -20°C.

5.2.2 EXTRACTION, FRACTIONATION AND ANALYSIS OF RADIO-LABELED FATTY ACIDS

The general procedures for handling lipid samples as detailed in Part II, Chapter 2, Section 2.2.1 were followed. Schematic diagram of procedures used in this chapter is given in Figure 24.

5.2.2.1 EXTRACTION OF LIPIDS FROM TISSUES

The hepatopancreas, abdominal muscles, and gills from individual animals were homogenized with an Ultra-Turrax polytron-type homogenizer. The total lipid sample from individual tissue homogenates was extracted by a modified Folch method (1957) (Part III, Chapter 4; Appendix 2-B) and TL was measured.

5.2.2.2 SCINTILLATION COUNTING

Tissue total lipid samples were prepared and tested for level of ^{14}C by the following procedure. Each sample was concentrated in 1 ml chloroform:methanol (2:1) and a 5% (50µl) aliquot was added to 5 ml of HP scintillation cocktail and vortexed. Samples were analyzed on a Beckman LS 7800 liquid scintillation counter.

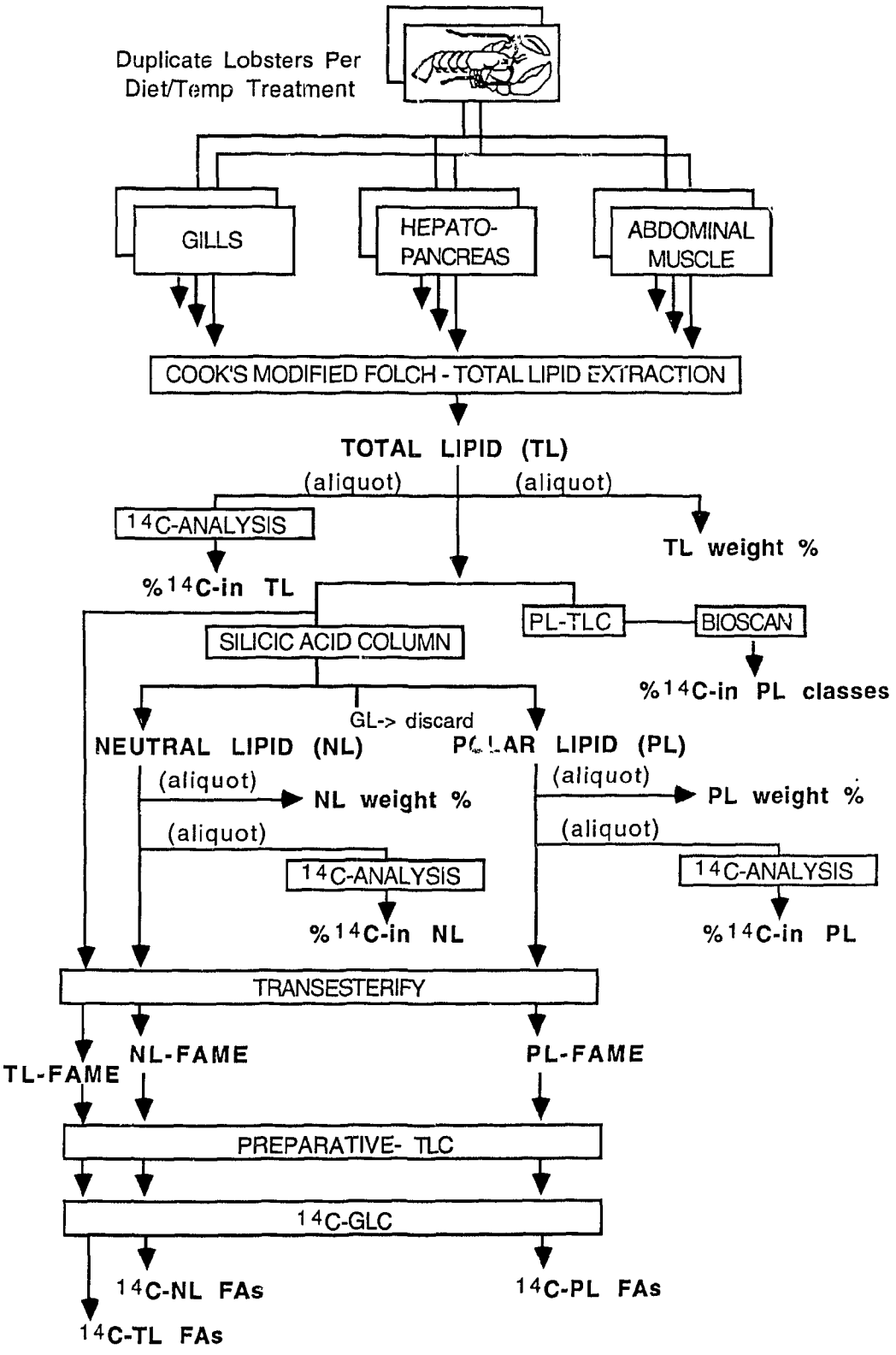


Figure 24. Schematic Diagram of Procedures of Lipid and C-14 Lipid Analyses of Lobster Tissue Samples. (GL = glycolipid)

5.2.2.3 SEPARATION OF TL INTO NL AND PL FRACTIONS

NLs and PLs were separated by column chromatography as in Part III, Chapter 3. A glass pasteur pipette was packed with Unisil (silicic acid) and lipid fractions were eluted as follows: the NLs, with chloroform; then the GLs, with chloroform:acetone (1:1), then acetone; and finally, PLs were eluted with methanol. NL and PL samples were weighed, then analyzed for level of radioactivity as detailed for TL samples.

5.2.2.4 SEPARATION OF TL, NL AND PL COMPONENTS BY TLC

Thin layer chromatography was performed on two brands of TLC plates (20cm x 20cm; SIL G25, precoated with 0.25mm silica gel with fluorescent indicator, UV_{254}; Macherey-Nagel, West Germany) or (Prekote silica gel plates; Applied Sciences Laboratories, College Park, PA, USA). TLC plates were cleaned before used by developing in ethyl acetate, then dried. Plates were heat activated in a drying oven at 100°C for ~ 30 minutes, then scored to form 8 lanes. Samples were dissolved in chloroform and a 10 µl Hamilton glass syringe was used to apply 10 to 50 µl to each lane, to deliver ~0.2 to 0.5 ml of standard, or ~50,000 counts of tissue lipid extract. Samples were compared to an α-18:3(n-3) free acid standard, a mixed PL standard (cholesterol, PE, PC, lyso PC; Supelco, Inc. 47000) or mixed calf brain standard.

The TLC solvent system for separating PL components (pers. comm. H. Cook) was: $CHCl_3$: EtOH : H_20 : TEA (4:5:1:4.), development time, at room temperature, was ~ 1 hour 40 minutes. TLC solvent system for separating neutral lipid fractions was as follows: Hexane: Diethyl Ether: Acetic Acid (85:15:1). UV sensitive plates were examined and marked under UV light. Non-UV plates were sprayed with rhodamine 6-G (pink) or molybdate (purple), or 1% 2',7-dichlorofluorescein solution in ethanol, then observed

under UV light. A ^{14}C-TLC plate scanner (Bioscan Imaging Scanner System 20IBM with Autochanger; Bioscan, Inc., Washington, D.C., USA) was used to examine some plates.

For preparative TLC, the bands from some plates were scraped and dissolved in chloroform:methanol (2:1), filtered, and an aliquot was analyzed for radioactivity by liquid scintillation counting.

5.2.2.5 CONVERSION OF FAs TO METHYL ESTER DERIVATIVES

A modified BF_3-methanol method was used to convert sample TL or NL and PL samples to their methylated derivatives (for details, see Part III, Chapter 3).

5.2.2.6 GAS-LIQUID CHROMATOGRAPHY

TL, NL, or PL FAME were analyzed for ^{14}C fatty acid fractions by use of a Perkin-Elmer Sigma FID Gas Chromatograph, with a ^{14}C-Activity Peak Integrator and FAME Mass Integrator (Hewlett Packard, Model HP3390A) (Cook and Spence, 1974). The stainless steel wide bore column allows application of samples of sufficient mass (up to 1.5 mg) to detect radioactivity in individual fatty acids. Samples were dissolved in carbon disulfide and concentrated to contain 40,000 to 50,000 counts in a 5-10 µl aliquot injection. The carrier gas was Helium at a flow rate of 103 ml/min.; column pressure was 160 K Pascals (~24 psi), and the injector and detector temperatures were 250°C.

5.3 RESULTS AND DISCUSSION

Lack of funding precluded analyzing all the samples in the experimental temperature x diet x tissue x lipid type matrix. Therefore, only selected results are reported below; further results will be discussed in subsequent papers. The remainder of the samples were stored in in teflon-lined, screw-capped glass vials in solvent (hexane), under nitrogen gas atmosphere, at -80°C.

5.3.1 INCORPORATION OF 18:3(n-3) INTO TL, NL AND PL FRACTIONS OF MUSCLE AND HEPATOPANCREAS FAs:

5.3.1.1 LOBSTERS FED THE H-CRD AND REARED AT 20°C:

In the muscle NL and the hepatopancreas NL, 95-96% of the incorporated label was in its original form, 18:3(n-3). In the NL and the PL of the muscle, ~2.8-2.9% of incorporated label was in 20:3(n-3).

There was minimal desaturation to 20:5(n-3) and no measurable desaturation and elongation to 22:6(n-3). This could indicate a dietary requirement for either of these fatty acids known to fulfill essential functions in other organisms.

In the hepatopancreas NLs, only 2-C chain elongation from 18:3(n-3) to 20:3(n-3) , and no desaturation was evident.

5.3.1.2 LOBSTERS REARED AT 20°C AND FED A SEMI-PURIFIED DIET CONTAINING 18:3(n-3) AND 18:2(n-6):

In the muscle neutral lipid and muscle polar lipid fractions, 96 to 98% of the incorporated label was in the original form, 18:3(n-3), and ~ 3.8% was in the 2C chain-

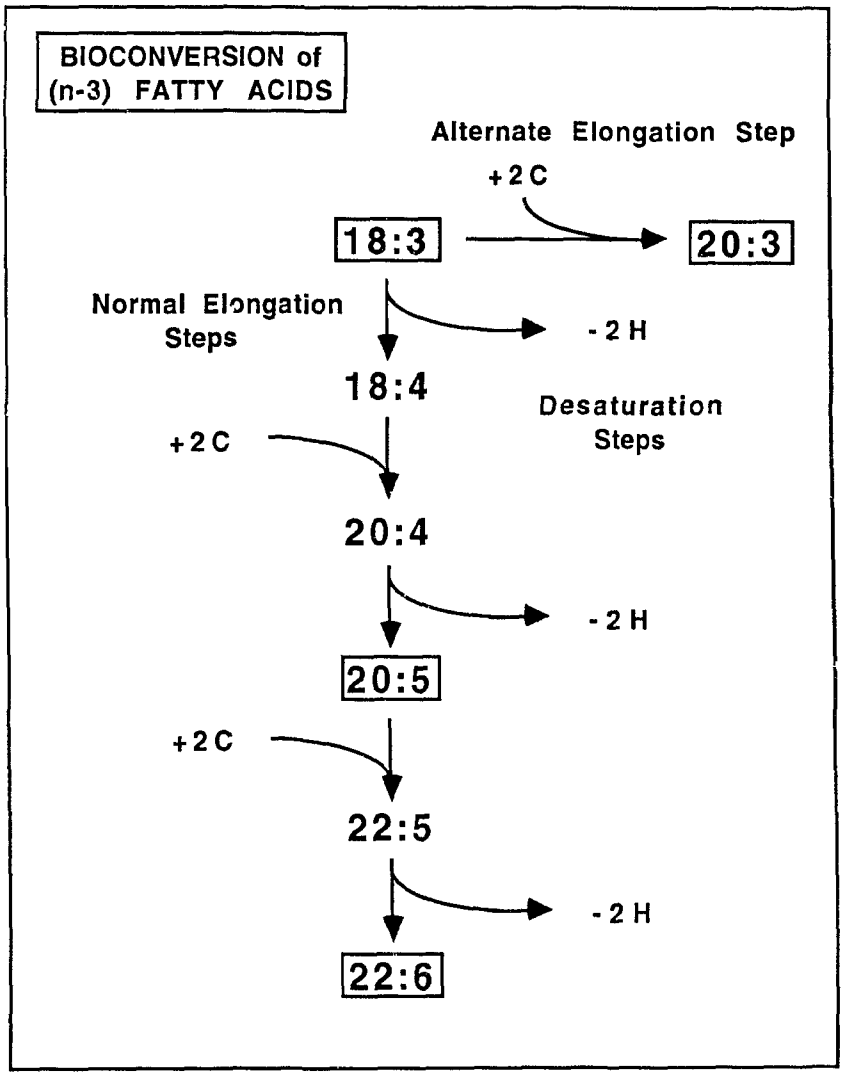


Figure 25. Pathways of Bioconversion of (n-3) Fatty Acids.

Vertical pathway depicts normal elongation and desaturation steps from linolenic acid, 18:3(n-3), to eicosapentaenoic acid, 20:5(n-3) and to docosahexaenoic acid, 22:6(n-3). Alternate pathway (horizontal) shows 2C elongation of 18:3(n-3) to 20:3(n-3), presumably a "dead-end" product which has no known function.

elongation product, 20:3(n-3). This fatty acid represents a biochemical "dead end", regarding known physiological function (see Figure 25).

In the hepatopancreas, TL ~94-96% of the incorporated label remained as 18:3(n-3) and ~ 3% was the chain-elongated product, 20:3(n-3). Approximately 1 to 3% of the incorporated label was in 16:1. This can indicate ß-oxidation of the injected 18:3(n-3) and subsequent incorporation of the label through fatty acid synthesis. It may also indicate degredation (although minimal) of the stored sample.

5.3.2 PHOSPHOLIPID CLASSES:

Incorporation of label into different phospholipid classes is consistent for muscle and hepatopancreas PL. The majority of label was in the PC fraction (40-45%), and was nearly double the relative incorporation into PE (~ 20% of label). Label was also detected in the PI fraction (5-10%) and in an unidentified (UNK) fraction (Figure 26).

From the available data, rates of incorporation or turnover of phospholipids cannot be assessed since the proportion of unlabeled FAs in each lipid class was not determined; i.e. it cannot be determined whether the majority of label appearing in the PC fraction is due to higher proportion of available PC or whether the rate of 18:3(n-3) incorporation under the prevailing conditions was greater in PC than for the other PL classes, or both. However, it is apparent that incorporation of label into the phospholipid classes was similar for muscles and hepatopancreas.

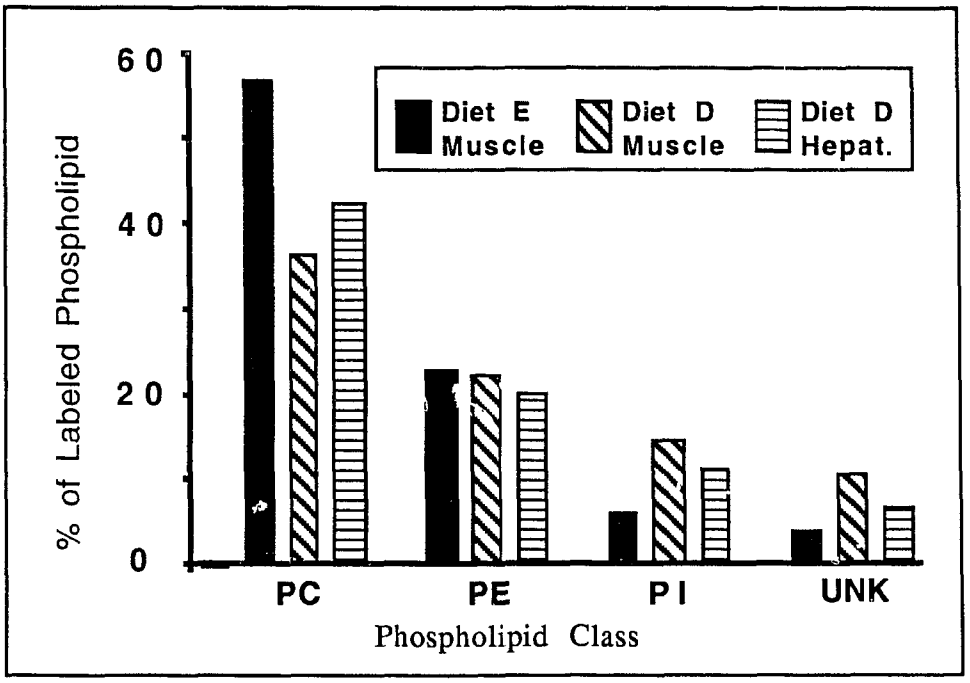


Figure 26. Distribution of Injected [1-14]C18:3(n-3).

Relative amount incorporated into lipid classes (reported as percentage of label in total phospholiids) of the abdominal muscles and the hepatopancreas of juvenile lobsters, H. americanus. Lobsters were acclimated to 20°C and previously fed Diet D or Diet E. Abbreviations are as follows: Hepat = hepatopancreas; PC = phosphatidylcholine; PE = phosphaatidylethanolamine; PI = phosphatidyl inositol; Unk = unknown.

In larval Homarus americanus, P. ethanolamine (PE) appears to be more tightly regulated (with respect to FA composition) (Sasaki, 1984). This is consistent with the findings of Brokerhoff and Hoyle (1967) that lobster PE has predominantly PUFA esterified to the B-carbon and SFAs or MFAs esterified to the α-carbon. If there is already much 18:3(n-3) or other PUFA present in the PLs (as probably is the case for both Diet D and Diet E), then the likely B-carbons already have PUFAs, so labeling would have to depend on "routine" turnover, especially at the warmer acclimation temperature (20°C), rather than to switching over from a more saturated state at a cooler temperature.

5.5 CONCLUSIONS

1. Muscle and hepatopancreas phospholipids contain phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol.

2. The majority of injected 18:3(n-3) which is incorporated into muscle and hepatopancreas phospholipids occurs in phosphatidylcholine (40 to 60%). Only half as much label is esterified to phosphatidylethanolamine. These results indicate turnover of hepatopancreatic and muscle phosphatides, and incorporation of 18:3(n-3)

3. There is formation of 20:3(n-3) from 18:3(n-3) in the hepatopancreas and muscle, indicating elongase activity in those tissues, but only minimal formation of 20:5(n-3), and virtually no bioconversion to 22:6(n-3). indicating lack of desaturase activity.

4. Based on the evidence in the previous chapters as to the depressed growth, depressed metabolic rate, degenerate hepatopancreas, and retention of (n-3) highly unsaturated fatty acids in the tissues, and this further evidence indicating a limited ability of bioconversion of 18:3(n-3) to (n-3) highly unsaturated fatty acids, and uptake of circulating 18:3(n-3), 20:5(n-3) and/or 22:6(n-3) are essential for juvenile lobsters.

IV. SUMMARY

This thesis examined the effects of dietary (n-3) and (n-6) fatty acids on the lobster (Homarus americanus). Specifically, an attempt was made to establish which fatty acids are essential for lobsters and to determine symptoms or disorders associated with EFA deficiency. Three overall advancements were made with this research program.

(1) This was the first documentation of the effects of EFA deficiency in lobsters, owing to a newly formulated semi-purified basal diet which was made available for this research program by J.D. Castell. The formulation was based on lobster nutrition research conducted at the Department of Fisheries and Oceans, Halifax Lab. This basal diet satisfied (for the first time in lobster studies) the "imperative prerequisites" for EFA deficiency studies: It was well-defined, EFA-deficient, and provided sufficient sustenance to conduct a feeding trial over several months. Despite the use of this basal diet, however, (and the rigorous extraction of basal diet ingredients), trace amounts of linoleic acid were introduced in the diets in both feeding trials; in the first trial, with the hydrogenated coconut oil, and in the second trial, with the purified oleic acid. Thus for future studies, it can not be overemphasized that all diet ingredients (even those for which analyses are provided) be analyzed for trace levels of polyunsaturated fatty acids.

(2) The second general advancement in the understanding of crustacean lipid nutrition and metabolism is the documentation, in a single cohort of lobsters, of EFA-deficiency effects at several levels of biological organization. This multidisciplinary approach permits the correlation of general effects (e.g. growth and survival) with more specific effects (e.g. altered metabolic rate, tissue condition, fatty acid composition, etc.) in assessing the possible roles, functions or general importance of specific dietary fatty acids.

(3) The third new area investigated in this research program was the combined effects of dietary EFA deficiency and controled temperature. Previously, studies which examined the effects of temperature on fatty acid composition of crustaceans, typically did not control or define dietary intake of fatty acids; conversely; most previous research on the nutritional requirements of various crustaceans do not also examine the effect of long-term temperature acclimation.

Two long-term feeding trials were conducted, for which lobsters were reared on semi-purified test diets supplemented with or deficient in selected (n-6) or (n-3) polyunsaturated fatty acids (PUFA) or the highly unsaturated fatty acid (HUFA), 22:6(n-3). The first feeding study specifically investigated the nutritional value of different levels and proportions of dietary (n-3) and (n-6) polyunsaturated fatty acids, by measuring growth, monitoring survival, and analysing whole body fatty acid composition.

Although the juvenile lobsters could survive 10-12 weeks on diets deficient in both (n-3) and (n-6) PUFA, growth and molting rate were depressed, and rapid mortality occured after this critical time. A diet high in saturated fatty acids enhanced these deficiency effects. There was no discernable difference in the benefits of 18:3(n-3) vs. 18:2(n-6); each alone improved growth, molting rate and survival. There was an additional significant improvement in these factors by diets supplemented with 22:6(n-3) or with mixed HUFAs.

In the second feeding trial, 100 sibling juveniles were reared at 10°C or 20°C and fed one of five semi-purified test diets. A multi-disciplinary approach was used to evaluate deficiency-related pathologies and to investigate the role of these nutrients in the lobster at the following levels of biological organization: (a) general: growth, molting, survivorship, (b) functional: metabolic rate, (c) structural: tissue and membrane histology, (d)

compositional: tissue-specific fatty acid composition and (e) biochemical: fate of radio-labelled fatty acids.

The oxygen consumption rate of lobsters fed the diet deficient in PUFA was significantly lower than that of lobsters fed diets with supplemental (n-3) or (n-6) PUFA. There was no affect of dietary fatty acids on ammonia excretion rates. The low O:N ratios indicated protein catabolism. The significantly lower O:N ratios of lobsters fed the PUFA-deficient diet indicated a starvation state and a possible degenerative condition or catabolism of body tissues for energy. These lobsters were notably lethargic prior to death.

Lobsters fed the PUFA-deficient diet had a significantly lowered hepatosomatic index (HSI). In addition, this is the first time evidence of degradative hepatopancreas associated with PUFA deficiency has been documented in crustaceans.

There were significant (and independent) effects of dietary fatty acids and temperature on lobster fatty acid composition. The major response to temperature was significantly lower proportions of 20:5(n-3) and significantly higher proportions of 16:0 in the polar lipids (PLs) of lobsters at 20°C as compared to those at 10°C. The response to diet and temperature varied with tissue type. The gill PLs were most responsive to changes in temperature, the muscle PLs were relatively unaffected by changes in diet or temperature, and the hepatopancreas PLs were primarily affected by diet but also by temperature. Although diet has a significant affect on the fatty acid composition of selected tissues of the lobster, the shifts in fatty acid composition in response to temperature appear to result from physiological acclimatization, and are independent of diet.

Incorporation of injected ^{14}C-18:3(n-3) into the phospholipids (predominantly PC, and also PE and PI) of the muscles and the hepatopancreas was demonstrated, indicating both uptake of this fatty acid from the hemolymph and short term turnover of membrane phospholipids. There was elongation of ^{14}C-18:3(n-3) to ^{14}C-20:3(n-3), negligible

bioconversion of ^{14}C-18:3(n-3) to ^{14}C-20:5(n-3), and no evidence of bioconversion to ^{14}C-22:6(n-3). These data (and evidence from the effect of dietary fatty acids on the fatty acid composition of the tissues) indicate elongase activity and a lack of desaturase activity.

In conclusion, (n-3) and/or (n-6) fatty acids are essential for juvenile lobsters and HUFAs [e.g. 22:6(n-3)] have a higher EFA value than 18-C PUFAs. EFA deficiency in the lobster is manifested at each level of biological organization; symptoms include changes in fatty acid composition, degenerative tissues (hepatopancreas), depressed metabolic rate and tissue catabolism, lethargy, poor growth and depressed molting rates, and mortality.

APPENDICES

Appendix 1.

Diets and Diet Preparation

Appendix 1-A.

Extraction of Trace Lipids from Basal Diet Ingredients.

Equipment	Supplies	Reagents[1]
Blender	Whatman No.1 filter paper	500 ml IPA
Buchner funnel	(to fit buchner funnel)	(isoproponal)
Filter flask	Aluminum foil drying pan	200 ml EtOH
Aspirator	Boiling chips	(ethanol)
Heating mantles		
Round bottom flask (RBF)		
Drying oven or fume hood		
Asbestos gloves		

1. Bring IPA and EtOH to boiling in separate RBFs over heating manifolds (use boiling chips to reduce bumping)
2. To blender, add: 100 g of dry ingredient
   200 ml of Hot IPA
3. Filter through Whatman No. 1 filter paper with buchner funnel while adding:
   100 ml Hot IPA
4. Scrape off filtrate into blender; add:
   200 ml Hot IPA Blend ~ 1 minute.
5. Filter, adding: 200 ml Hot EtOH through filtrate, stirring carefully (to avoid tearing filter paper).
6. Dry filtrate at low temperature (~ 60°C) on aluminum pan or foil; "chop up" finely and stir occasionally to aid in drying.

1. For 100 g of dry ingredient to be extracted

Appendix 1-B.

Vitamin Mix of Diets from Feeding Experiments I & II.

Ingredient	% Dry Weight	Source[1]	Lot Number	
			I	II
Celite 545[2]	77.78	JTB	339702	339702
Inositol (myo inositol)	12.50	USBC	36927	42509
Niacinamide	2.56	ICN	3448	3448
PABA	2.00	NBC	6344	6344
D-Ca-Pantothenate	1.41	USBC	20704	30117
BHA	0.75	NBC	5390	9286460D
BHT	0.75	ICN	8823	8823
Riboflavin	0.72	USBC	19264	19264
Vit A Acetate (500,000 IU/g)	0.500	ICN	3531	7253
Thiamin (HCl)	0.320	ICN	11988	11988
Cobalamine	0.266	ICN	8042	10791
Pyridoxine HCl	0.240	USBC	13291	13291
Folic Acid	0.096	ICN	38505	1003
Menadione (Vit K)	0.080	NBC	4662	15700
Calciferol (850,000 IU/g)	0.025	ICN	5102	5102
D-Biotin (1% trituration in Calcium Phosphate Dibasic)	0.008	USBC	36648	36648

1. ICN (ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio 44128), JTB (J. T. Baker, Philipsburg, N.J. 08865), NBC (Nutrition Biochemicals Corporation, Cleveland, Ohio 44128), USBC (U.S. Biochemicals Corporation, Cleveland, Ohio 44128).

2. Celite, an inert filler, is used in substitution for α-cellulose (Celufil) since celite is gritty and may aid in thorough mixing of the vitamin mix.

3. Note: Ascorbic Acid (Vitamin C), generally added at 6.0%, was not added to this vitamin mix. Dejardins et al. (1983) demonstrate that Homarus americanus can synthesize it de novo; it was substituted by celite in this mix.

Appendix1-C.

Fatty Acid Composition of Diets (as % of Dietary FAs).

Fatty Acids	Diets									
	A		B		C		D		E	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10:0	1.93	± 0.74	5.60	± 0.03	5.10	± 0.00	3.55	± 0.47	3.28	± 0.07
12:0	2.17	± 1.18	52.97	± 1.20	48.90	± 0.04	48.62	± 1.30	49.42	± 0.21
14:0	3.25	± 0.08	20.80	± 0.06	18.67	± 0.04	19.42	± 0.06	19.53	± 0.24
16:0	22.82	± 1.03	10.13	± 0.40	8.76	± 0.01	9.06	± 0.23	8.93	± 0.05
18:0	2.08	± 0.45	2.15	± 0.11	1.84	± 0.00	1.92	± 0.07	1.85	± 0.03
20:0	-	-	-	-	-	-	-	-	-	-
Σ SFAs	32.24	± 1.85	91.64	± 0.66	83.27	± 0.01	82.57	± 1.41	83.01	± 0.46
14:1	-	-	-	-	-	-	-	-	-	-
16:1	-	-	-	-	-	-	-	-	-	-
18:1	8.17	± 0.78	6.85	± 0.55	5.81	± 0.01	6.10	± 0.36	5.82	± 0.01
20:1	-	-	-	-	-	-	-	-	-	-
22:1	-	-	-	-	-	-	-	-	-	-
Σ MFAs	8.17	± 1.56	6.85	± 0.55	5.81	± 0.01	6.10	± 0.36	5.82	± 0.01
18:2(n-6)	36.52	± 2.37	1.39	± 0.13	1.42	± 0.03	3.53	± 0.06	3.82	± 0.21
20:2(n-6)	-	-	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-
22:5(n-6)	-	-	-	-	-	-	-	-	-	-
Σ (n-6)	36.52	± 3.89	1.39	± 0.13	1.42	± 0.03	3.53	± 0.06	3.82	± 0.21
18:3(n-3)	-	-	-	-	9.26	± 0.08	7.58	± 1.13	6.77	± 0.01
20:5(n-3)	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-
Σ (n-3)	-	-	-	-	9.26	± 0.08	7.58	± 1.13	6.77	± 0.01
Others	-		0.13		0.25		0.23		0.59	
(n-3)/(n-6)					6.52		2.15		1.77	

(Continued)

Appendix 1-C. (Continued)

Fatty Acid Composition of Diets (as % of Dietary FAs).

Fatty Acids	Diets									
	F		G		H		I		J	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10:0	4.63	± 1.05	4.50	± 1.21	5.55	± 0.83	4.80	± 0.70	0.04	± 0.02
12:0	47.66	± 1.84	49.93	± 1.95	50.14	± 0.37	50.17	± 0.76	0.03	± 0.01
14:0	19.79	± 0.63	18.93	± 0.76	18.97	± 0.91	19.00	± 0.40	2.51	± 0.04
16:0	9.53	± 0.66	8.45	± 0.21	8.81	± 0.62	8.61	± 0.62	12.08	± 0.33
18:0	2.03	± 0.18	1.75	± 0.09	1.80	± 0.04	1.77	± 0.00	1.35	± 0.02
20:0	-	-	-	-	-	-	-	-	0.18	± 0.02
Σ SFAs	83.64	± 0.69	83.56	± 1.20	85.26	± 1.09	84.35	± 0.40	16.17	± 0.37
14:1	-	-	-	-	-	-	-	-	0.11	± 0.01
16:1	-	-	0.25	± 0.00	-	-	-	-	5.68	±
18:1	6.44	± 0.49	5.60	± 0.33	5.59	± 0.16	5.53	± 0.13	25.85	± 0.70
20:1	-	-	-	-	-	-	-	-	6.76	± 0.11
22:1	-	-	-	-	-	-	-	-	3.84	± 0.19
Σ MFAs	6.44	± 0.04	5.85	± 0.06	5.59	± 0.01	5.53	± 0.01	42.23	± 0.25
18:2(n-6)	6.16	± 0.56	9.35	± 0.74	1.31	± 0.01	3.65	± 0.06	25.65	± 0.64
20:2(n-6)	-	-	-	-	-	-	-	-	0.19	± 0.08
20:4(n-6)	-	-	-	-	-	-	-	-	0.22	± 0.01
22:5(n-6)	-	-	-	-	-	-	-	-	0.12	± 0.04
Σ (n-6)	6.16	± 0.05	9.35	± 0.07	1.31	± 0.00	3.65	± 0.01	26.17	± 0.00
18:3(n-3)	4.79	± 0.35	0.09	± 0.01	-	-	-	-	0.63	± 0.04
20:5(n-3)	-	-	-	-	-	-	-	-	2.55	± 2.94
22:5(n-3)	-	-	-	-	-	-	-	-	0.37	± 0.29
22:6(n-3)	-	-	-	-	7.38	± 0.95	6.05	± 0.18	7.39	± 0.27
Σ (n-3)	4.79	± 0.03	0.09	± 0.01	7.38	± 0.95	6.05	± 0.18	10.93	± 2.88
Others	-1.03		1.15		0.46		0.42		4.51	
(n-3)/(n-6)	0.78		0.01		5.63		1.66		0.42	

Appendix 1-D.

Fatty Acid Composition of Diets (as % of Diet, DWB).

Fatty Acids	Diets									
	A		B		C		D		E	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10:0	-	-	0.50	± 0.00	0.46	± 0.00	0.30	± 0.04	0.30	± 0.01
12:0	-	-	4.77	± 0.11	4.40	± 0.04	4.45	± 0.12	4.45	± 0.02
14:0	-	-	1.87	± 0.01	1.68	± 0.04	1.76	± 0.01	1.76	± 0.02
16:0	-	-	0.91	± 0.04	0.79	± 0.01	0.80	± 0.02	0.80	± 0.00
18:0	-	-	0.19	± 0.01	0.17	± 0.00	0.17	± 0.01	0.17	± 0.00
20:0	-	-	-	-	-	-	-	-	-	-
Σ SFAs	-	-	8.24	± 0.05	7.50	± 0.00	7.48	± 0.13	7.48	± 0.04
16:1	-	-	-	-	-	-	-	-	-	-
18:1	-	-	0.62	± 0.05	0.52	± 0.01	0.55	± 0.03	0.52	± 0.00
20:1	-	-	-	-	-	-	-	-	-	-
22:1	-	-	-	-	-	-	-	-	-	-
Σ MFAs	-	-	0.62	± 0.05	0.52	± 0.00	0.55	± 0.03	0.52	± 0.00
18:2(n-6)	-	-	0.12	± 0.01	0.13	± 0.00	0.32	± 0.01	0.34	± 0.02
20:2(n-6)	-	-	-	-	-	-	-	-	-	-
20:4(n-6)	-	-	-	-	-	-	-	-	-	-
22:5(n-6)	-	-	-	-	-	-	-	-	-	-
Σ (n-6)	-	-	0.12	± 0.00	0.13	± 0.00	0.32	± 0.01	0.34	± 0.02
18:3(n-3)	-	-	-	-	0.83	± 0.01	0.68	± 0.10	0.61	± 0.00
20:5(n-3)	-	-	-	-	-	-	-	-	-	-
22:5(n-3)	-	-	-	-	-	-	-	-	-	-
22:6(n-3)	-	-	-	-	-	-	-	-	-	-
Σ (n-3)	-	-	-	-	0.83	± 0.01	0.68	± 0.10	0.61	± 0.00
Others	-		0.02		0.02		-0.03		0.05	
(n-3)/(n-6)	-		-		6.38		2.13		1.79	

(Continued)

Appendix 1-D. (Continued)

Fatty Acid Composition of Diets (as % of Diet, DWB).

Fatty Acids	Diets									
	F		G		H		I		J	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10:0	0.42 ±	0.09	0.40 ±	0.11	0.50 ±	0.08	0.43 ±	0.06	-	-
12:0	4.29 ±	0.17	4.49 ±	0.18	4.51 ±	0.03	4.52 ±	0.07	0.06 ±	0.00
14:0	1.78 ±	0.06	1.70 ±	0.07	1.71 ±	0.08	1.71 ±	0.04	0.23 ±	0.00
16:0	0.86 ±	0.06	0.76 ±	0.02	0.79 ±	0.06	0.77 ±	0.00	1.09 ±	0.03
18:0	0.18 ±	0.02	0.16 ±	0.01	0.16 ±	0.00	0.16 ±	0.01	0.12 ±	0.00
20:0	-	-	-	-	-	-	-	-	0.02 ±	0.00
Σ SFAs	7.53 ±	0.06	7.51 ±	0.11	7.67 ±	0.10	7.59 ±	0.04	1.50 ±	0.03
16:1	-	-	0.04 ±	0.00	-	-	-	-	0.53 ±	0.02
18:1	0.58 ±	0.04	0.50 ±	0.03	0.50 ±	0.01	0.50 ±	0.01	2.33 ±	0.06
20:1	-	-	-	-	-	-	-	-	0.61 ±	0.01
22:1	-	-	-	-	-	-	-	-	0.35 ±	0.02
Σ MFAs	0.58 ±	0.04	0.54 ±	0.06	0.50 ±	0.01	0.50 ±	0.01	3.82	0.08
18:2(n-6)	0.55 ±	0.05	0.84 ±	0.07	0.12 ±	0.00	0.33 ±	0.01	2.31 ±	0.06
20:2(n-6)	-	-	-	-	-	-	-	-	0.02 ±	0.01
20:4(n-6)	-	-	-	-	-	-	-	-	0.02 ±	0.00
22:5(n-6)	-	-	-	-	-	-	-	-	0.01 ±	0.00
Σ (n-6)	0.55 ±	0.05	0.84 ±	0.07	0.12 ±	0.00	0.33 ±	0.01	2.36 ±	0.00
18:3(n-3)	0.43 ±	0.03	-	-	-	-	-	-	0.06 ±	0.00
20:5(n-3)	-	-	-	-	-	-	-	-	0.42 ±	0.00
22:5(n-3)	-	-	-	-	-	-	-	-	0.03 ±	0.03
22:6(n-3)	-	-	-	-	0.66 ±	0.09	0.54 ±	0.02	0.67 ±	0.02
Σ (n-3)	0.43 ±	0.03	0.00 ±	0.00	0.66 ±	0.09	0.54 ±	0.02	1.18 ±	0.00
Others	-0.09		0.11		0.05		0.04		0.14	
(n-3)/(n-6)	0.78		0.00		5.50		1.64		0.50	

Appendix 2.

Lipid Methods

Appendix 2-A.

Modified Bligh & Dyer Procedure for Total Lipid Extraction*.

Equipment	Supplies	Reagents
Homogenizer	Whatman No.1 filter paper	20 ml Chloroform
Homogenizer tube	N_2 gas	20 ml MeOH
Buchner funnel	glass pipettes	distilled H_2O
Vortexer	glass wool	Na_2SO_4
Centrifuge (opptional)	test tubes	
Aspirator	test tube rack	

1. Weigh accurately, 1 - 3 g of sample; transfer to homogenizer tube
   (Estimated minimum lipid content 100 - 200 mg)
2. Add to the tube:
   a. 5 ml Chloroform
   b. 10 ml methanol
   c. Homogenize thoroughly (approx. 2 - 3 minutes)

   Add to the tube:
   a. ~ 4 ml water; blend for 30 seconds.

   [initial ratio of $CHCl_3$: MeOH: H_2O should be 1 : 2 : 0.8; include water in sample]
3. Filter homogenate through Whatman #1 filter paper, using a buchner funnel.
4. Scrape residue into homogenizer tube for reextraction

   Add: 5 ml chloroform
   10 ml MeOH — Homogenize 1 - 2 minutes.

   Filter homogenate; rinse polytron & residue with
   5 ml chloroform
5. Add to combined filtrates:
   14 ml water — Vortex 1 minute.

   [final ratio of $CHCl_3$: MeOH: H_2O should be 2:2:1.8]
6. Centrifuge or allow sufficient time for total separation of layers
7. Remove (aspirate) upper alcholic layer & interfacial residue; discard.

   Add Na_2SO_4 to remaining chloroform & lipids layer (to remove water)

* personal communication: Dr. J.D. Castell

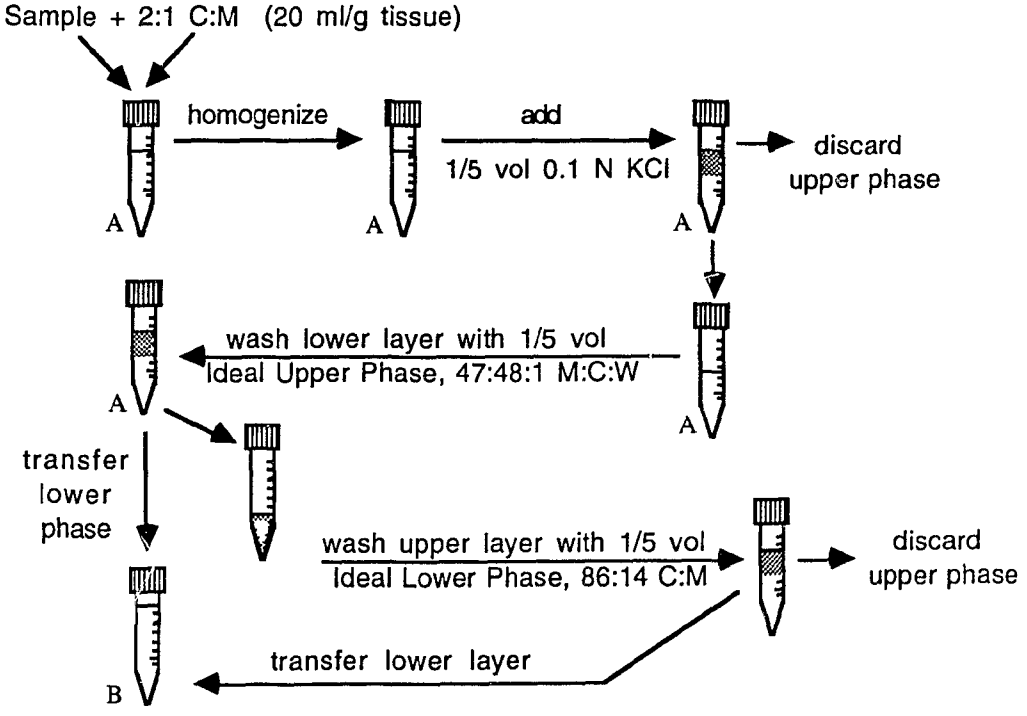
Appendix 2-B.

Modified Folch Procedure for Total Lipid Extraction*.

Equipment	Supplies	Reagents
Homogenizer	Watman No.1 filter paper	20 ml Chloroform [C]
Homogenizer tube	N_2 gas	20 ml MeOH [M]
Buchner funnel	glass pipettes	0.1 N KCl [W]
Suction filtration apparatus	glass wool	Na_2SO_4
Vortexer	screw cap conical bottom centrifuge tubes	
Centrifuge (opptional)		

1. Weigh sample accurately; transfer to homogenizer tube.
2. Homogenize in 20 vol (20 ml/g tissue) of 2:1 [C:M]
   (reserve some C:M to rinse off probe tip after homogenization)
3. Make two phase separation with 1/5 vol. 0.1 N KCl
   Vortex ~ 1 min. Remove upper phase (discard)
4. Wash lower phase with 1/5 vol Ideal Upper Phase, 47:48:1, [M:C:W]
5. Reextract upper phase with 1/5 vol Ideal Lower Phase, 86:14, [C:M]
6. Add Na_2SO_4 to remaining chloroform & lipids layer (to remove water)

* personal communication Dr. H. Cook



Appendix 2-C.

Procedure for Transesterification of Fatty Acids*.

Equipment	Supplies	Reagents
Heating Block or	Watman No.1 filter paper	Hexane
Boiling Water Bath with	10 ml screw-capped centrifuge tubes	Benzene
test tube rack	N_2 gas	7% BF_3 in MeOH
Vortexer	glass pipettes & bulbs	Na_2SO_4
test tube rack	Distilled H_2O	

1. Add lipid sample* to 10 ml tube with 1 ml Hexane. *[~5-50 mg lipid]
2. Add 1 ml Benzene; Vortex;
   Add 1 ml BF_3 in MeOH; flush with N_2, swirl.
3. Put on heating block (100°C) for 60 min. (in in test tube rack in boiling water bath).
4. Cool. Add 3 ml H_2O. Vortex (1 min.).
5. Remove top organic layer (Benzene & FAME) with disposable pipette; put into a second 10 ml screw cap vial.
6. Extract esters from H_2O layer 3 X 2 ml Hexane .
7. Wash organic/hexane/benzene layer (2nd vial) with 3 ml H_2O.
   Vortex. Remove H_2O layer (bottom) and discard.
8. Dry (remove residual H_2O) with excess Na_2SO_4.
   Let stand 5 - 10 minutes in refigerator (under N_2).
9. Transfer dried solution to a 3rd vial, washing Na_2SO_4 with Hexane.
10. Evaporate to dryness under N_2. Add appropriate solvent for GC; concentrate to ~50mg/ml.

Notes:

Use with teflon-lined screw caps (three tubes per sample). Use N_2 at every step.

Be certain to SEAL-TEST the screw caps by checking for evaporation of ~2 ml of MeOH or other solvent from the tube after 15 min. in heating block or water bath.

Sodium sulfate should be heat treated by muffle furnace. (If not sufficiently pure, it may lead to mysterious GC peaks).

For small samples: use 0.5 ml Hexane + 0.5 ml Benzene + 1 ml 3.5% BF_3 in MeOH.

* pers. comm.: T. Farquaharson, Dr. R.G. Ackman's Marine Lipids Lab, CIFT, TUNS.

Appendix 2-D.

Procedure for Thin Layer Chromatography*[1].

Equipment	Supplies	Reagents	
UV light box	TLC plates (UV-type, optional)	Ethylacetate	Hexane
		Diethyl ether	Chloroform
chromatography tank	screw cap test tubes	Anhydrous CaCl2	Acetic Acid
Oven	Hamilton microsyringe	Distilled Water	Ethanol
Vortexer	glass pipettes & bulbs	Triethyl amine (TEA)	
Plate streaker (optional)	test tube rack	2,7-Dichlorofluorescin (0.2% in EtOH)	
	glass funnel(s)		
	Watman No.1 filter paper		

1. Preparation of TLC plates
   a. Wash silica plates in chromatographic tank with 200 ml ethylacetate (~ 30 min; until solvent reaches top of plate)
   b. Air dry (~10 min)
   c. Oven dry at 100°C to activate plates (~30 - 60 min)
   d. Store in desiccator over anhydrous $CaCl_2$ until required (use within one day of activation)
2. Spot or streak plates ~2.5 cm from bottom of the plate (~15 mg/streak, in chloroform; 10-50 µg in chloroform/spot)
3. Develop plates in 200 ml of one of the following:
   a) Hexane : Diethyl ether : Acetic Acid [85 : 15 : 1] for total lipid samples*[2]
   b) Petroleum Ether: Diethyl ether: Acetic Acid [70 : 30 : 1] for neutral lipid separation*[3]
   c) Chloroform : Methanol : Water [65 : 25 : 4] for polar lipid samples
   d) Chloroform : Ethanol: H_20: TEA [2 : 5 : 1 : 4] for phospholipid class separation*[3]

   Take out of tank when solvent from is 1-2 cm from top of plate. Air dry.
4. Detection:

   Observe plates under UV

   Identify and mark bands with edge of glass pipette

   Make a permanent trace of the plate, noting origin, solvent from, and lipid bands.
5. Scrape & extract bands 3X with 10 ml Chloroform: Hexane [1:1]

   Filter through Watman No. 1 filter paper

   Evaporate to dryness under N2

   Store in hexane at -30°C, under nitrogen.

*1 general methods are found in Christie (1982)
*2 pers. comm.: T. Farquaharson, Dr. R.G. Ackman's Marine Lipids Lab, CIFT, TUNS.
*3 pers. comm. H. Cook, ACMR

Appendix 2-E.

Hydrogenation of Fatty Acid Methyl Esters*1.

Equipment	Supplies	Reagents
125 ml round-bottomed flask (RBF)	Whatman No.1 filter paper	25 ml 96% MeOH
125 ml separatory funnel	N_2 gas	(if not avail. use 95%)
Magnetic stir bar	H_2 gas	~2 mg PtO_2 or Pd
Rotary flash evaporator	glass pipettes	40 ml Hexane
Vortexer		25 ml distilled H_2O
50 ml glass test tube		Na_2SO_4

1. a. Take 1 ml FAME (~25 ml sample upper limit) and put into 125 ml RBF
   b. Evaporate to dryness under N_2 (g)
2. a. Add ~20-25 ml of 96% MeOH (if not available, use 95%)
   b. Add ~ 2 mg of PtO_2 or Pd catalyst (just a pinch)
   c. Flush the flask with H_2 (g), then continue a low flow for 30 to 60 minutes, stirring the solution with a magnetic bar.
3. a. Filter, using 2 fluted filter papers (prewashed).
   b. Rinse through filter with ~ 20 ml hexane.

OR: (for samples ≤ 0.1 mg, to avoid losses in the filter paper)

   a. Evaporate to near dryness under N_2 (g) using a rorary flash evaporator (water bath temperature ~ 45°C).
   b. Rinse with hexane (3X) into test tube, vortex, let layers separate, remove top, hexane layer (catalyst will float on bottom, MeOH-H_2O layer)

4. a. Put combined solutions (Hexane and MeOH) into 125 ml separatory funnel.
   b. Add 10-20 ml Hexane
   c. Wash with 25 ml distilled H_2O.
   d. Rinse hexane layers with H_2O.
5. a. Put into 50 ml tube with Na_2SO_4 (to dry)
   b. Vortex and let sit 10 minutes
6. Pipette into 10 ml tube and evaporate to dryness under N_2 (g).

*1. Christie (1982)

Appendix 2-F.

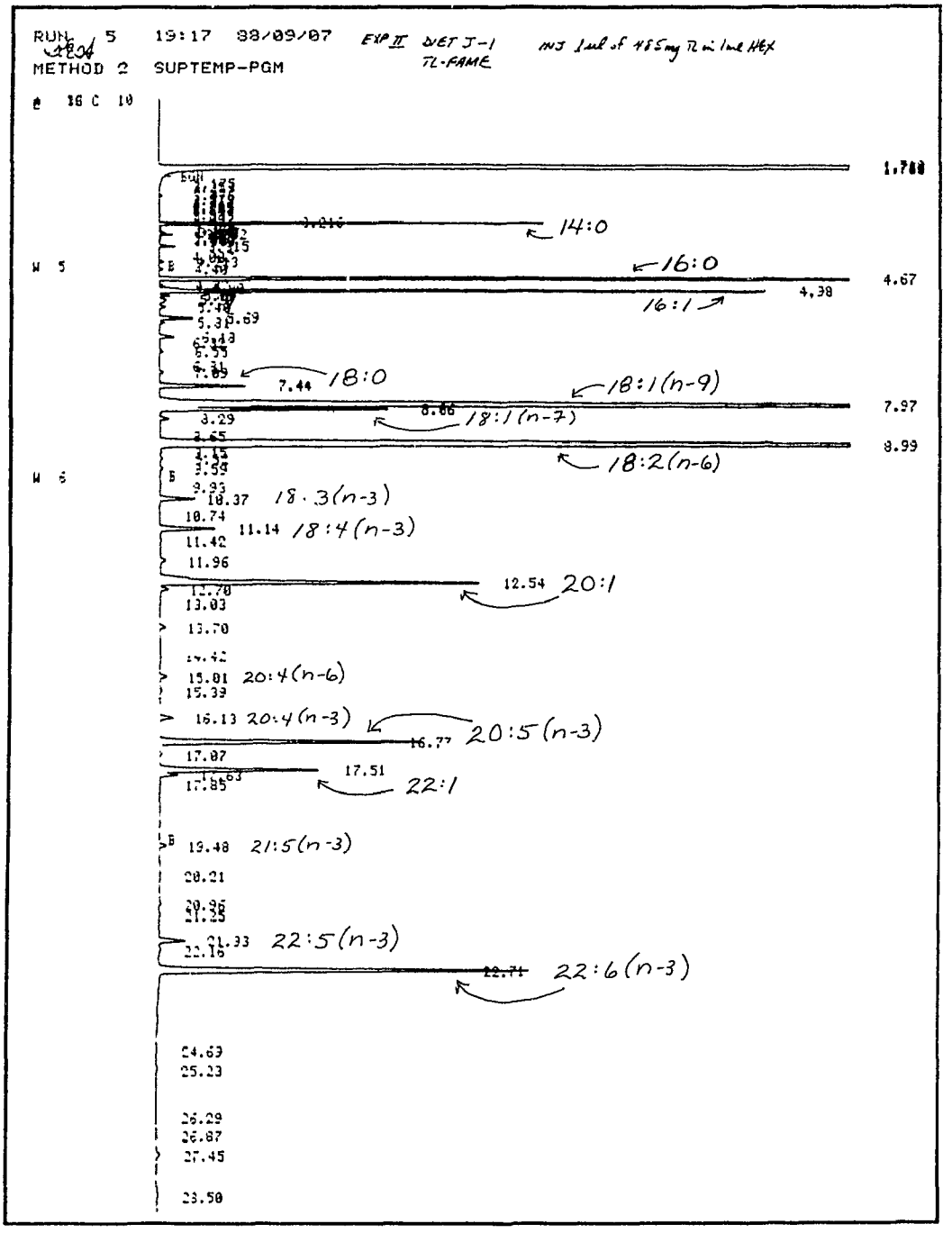
Fatty Acids Identified in Experiment II and the Categories in Which They are Included.

Peak Name	SFA	MFA	(n-6)	(n-3)	PUFA	HUFA	Peak Name	SFA	MFA	(n-6)	(n-3)	PUFA	HUFA
14:0	X						19:1		X				
14:1(n-7)		X											
15:0	X												
15:1		X					20:0	X					
									20:1				
16:0	X						20:1(n-11)		X				
Ante 16							20:1(n-9)		X				
Iso 16							20:1(n-7)		X				
		16:1					20:1(n-5)		X				
16:1(n-11)		X											
16:1(n-9)		X					20:3NMIT						
16:1(n-7)		X					20:2(n-6)			X		X	
16:1(n-5)		X					20:3(n-4)					X	
							20:3(n-6)			X		X	
16:2(n-4)							20:4(n-3)				X	X	X
16:2(n-6)			X				20:4(n-6)			X		X	X
16:2(n-7)							20:5(n-3)				X	X	X
16:3(n-3)				X	X								
16:3(n-4)					X		21;5(n-3)				X	X	X
16:3(n-6)			X		X								
16:4(n-1)					X	X	22:0	X					
16:4(n-3)				X	X	X			22:1				
							22:1(n-11&15)		X				
17:0	X						22:1(n-9)		X				
							22:1(n-7)		X				
18:0	X						22:1(n-5)		X				
		18:1											
18:1(n-11)		X					22:2(n-6)			X		X	
18:1(n-9)		X					22:4(n-3)				X	X	X
18:1(n-7)		X					22:4(n-6)			X		X	X
18:1(n-5)		X					22:5(n-3)				X	X	X
							22:5(n-6)			X		X	X
18:2(n-4)					X		22:6(n-3)				X	X	X
18:2(n-6)			X		X								
18:2(n-7)					X								
18:2(n-9)					X								
18:3(n-3)				X	X								
18:3(n-4)					X								
18:3(n-6)			X		X								
18:4(n-1)					X	X							
18:4(n-3)				X	X	X							
18:4(n-6)			X		X	X							
18:5(n-3)				X	X	X							

Appendix 2-G.

Chromatogram of TL-FAME Sample.

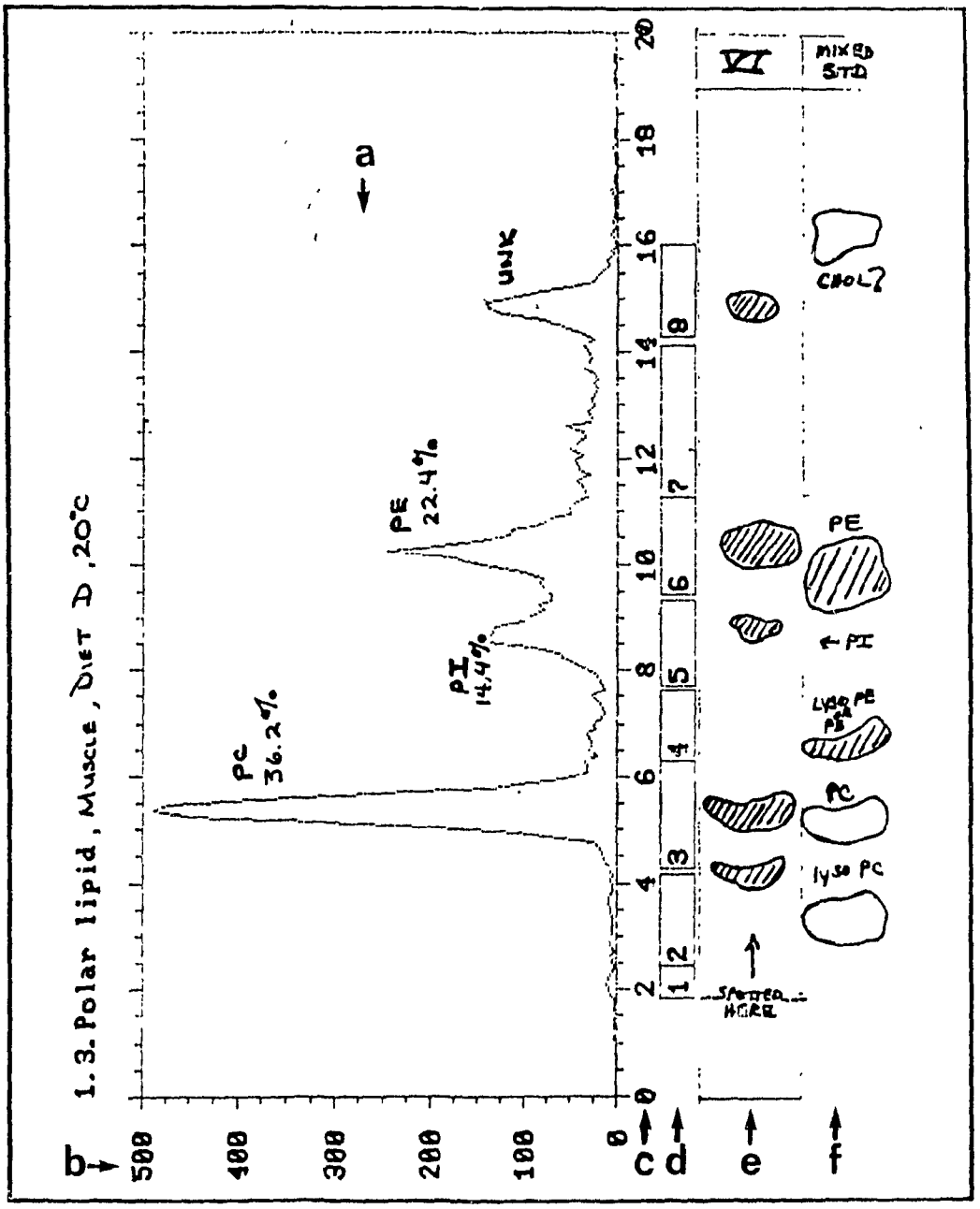
(Example shown is a TL extract from the Halifax Crustacean Reference Diet)



Appendix 2-H.

TLC Chromatogram Showing the Distribution of 14C in Phospholipid Classes of Lobster Muscle.

Example shown is from a polar lipid extract of muscle from a lobster reared at 20°C and fed Diet D. Features on the chromatogram: a. Separation and migration of sample phospholipids from a spot application to a 2 cm lane on the TLC plate; b. Y-axis is the 14C counts from phospholipids located along c; c. X-axis in cm from origin (bottom) to terminal edge (top) of TLC plate; d. Manual demarcation of peak upper and lower limits (automatic integration of the proportional radioactivity, ie. relative peak areas, for each region 1-8); e. Migration of PLs in Standard VI; f. same for Mixed Standard.



Appendix 3.

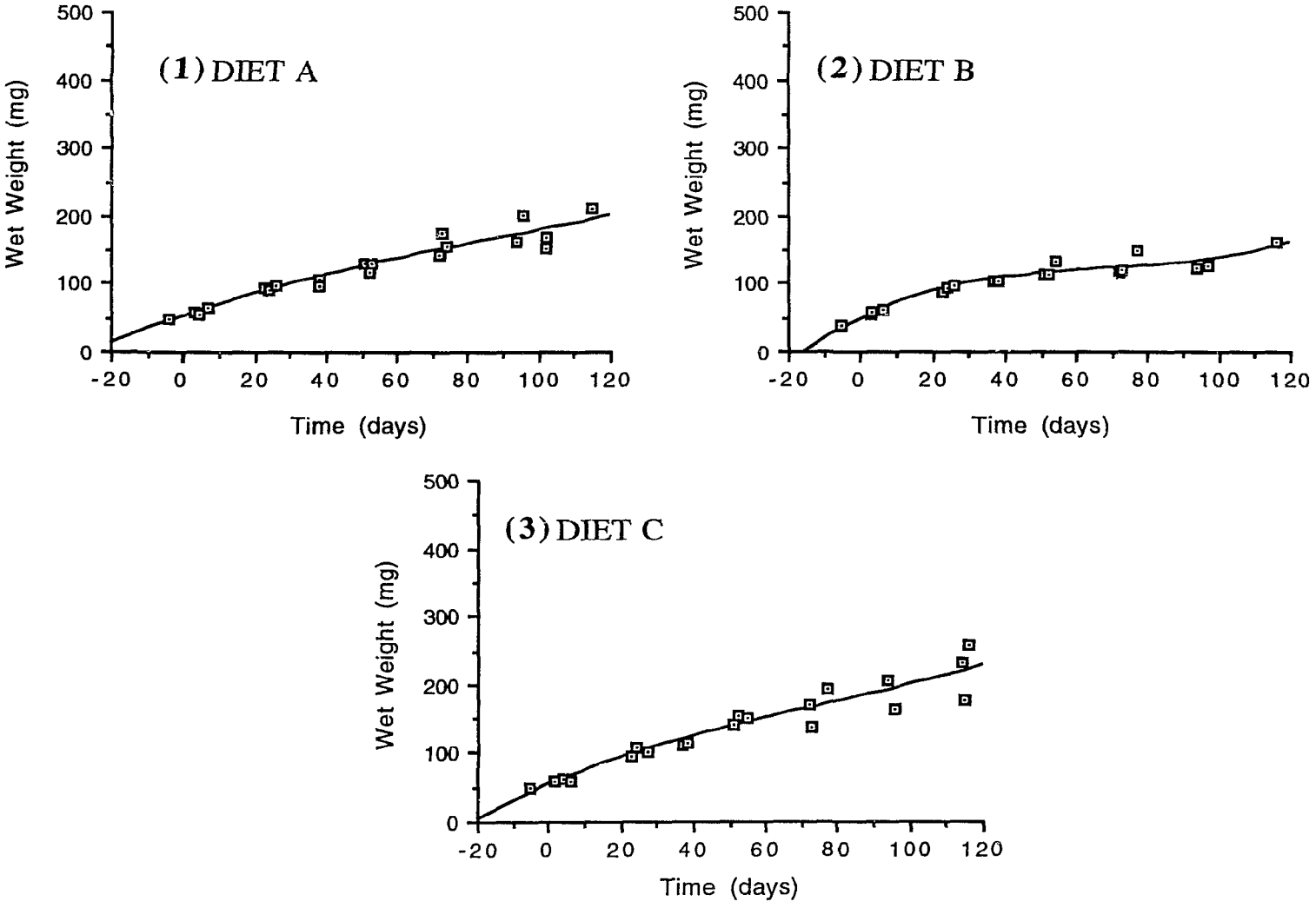
Regression Analysis of Growth of Lobsters Raised at 20°C and Fed One of Ten Semi-Purified Diets Which Varied in Fatty Acid Composition.

Appendix 3-A

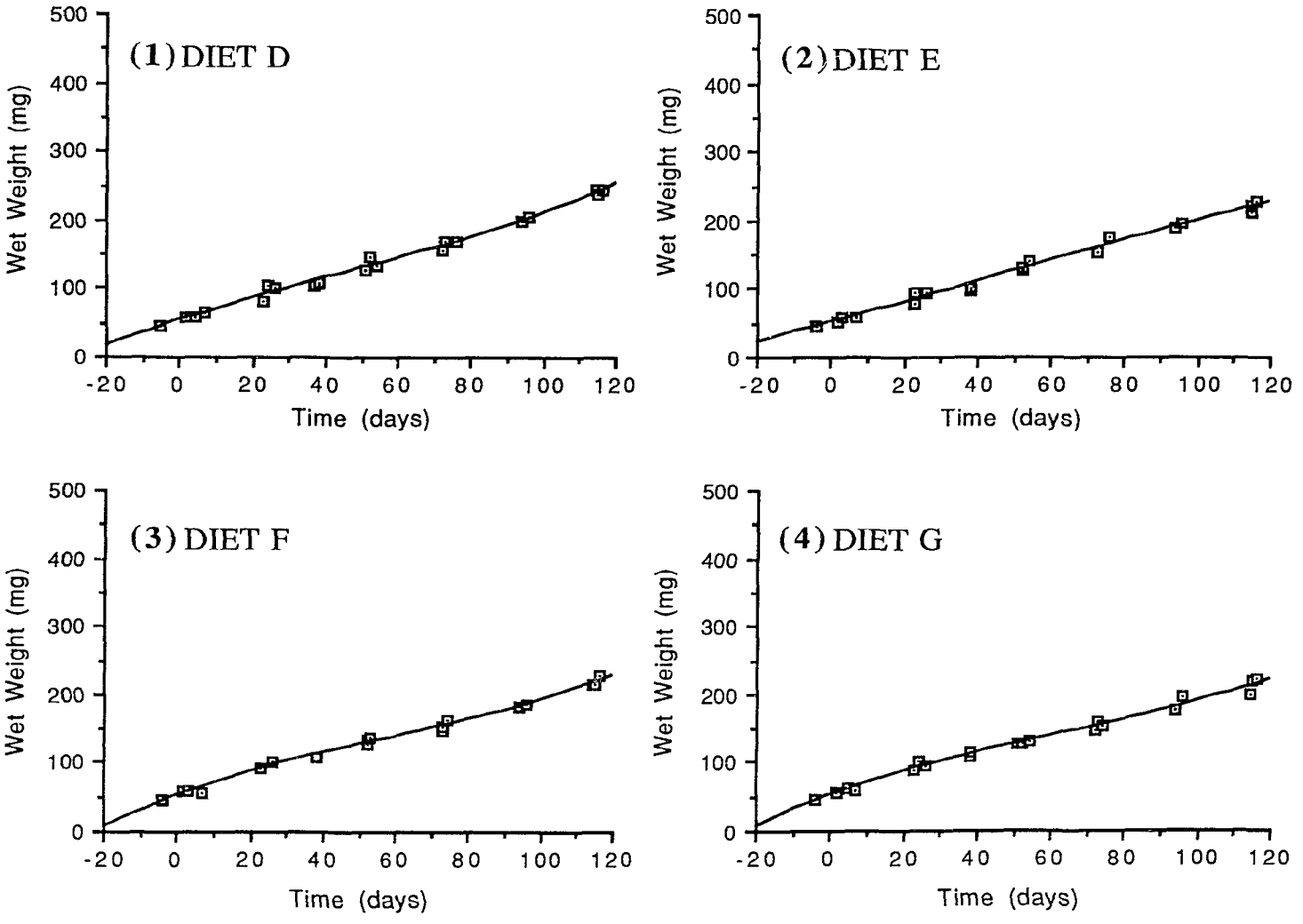
Regression Equation of Mean Growth of Lobsters from Three Replicate Groups of Twenty Lobsters on Each Diet

The following sigmoid growth curves were the best fit equations describing growth of lobsters on each diet (Cricket Graph v. 1.3.1). Regressions for each diet are plotted on separate graphs in Appendixes 3-B through 3-D, and together in one graph in Figure 5. The values plotted are the means of 10 to 20 lobsters surviving in each of three replicate group of lobsters weighed on a given day (note: not all replicate groups were weighed at each weigh time). Survival of lobsters after 100 days on the test diets is given in Table 5.

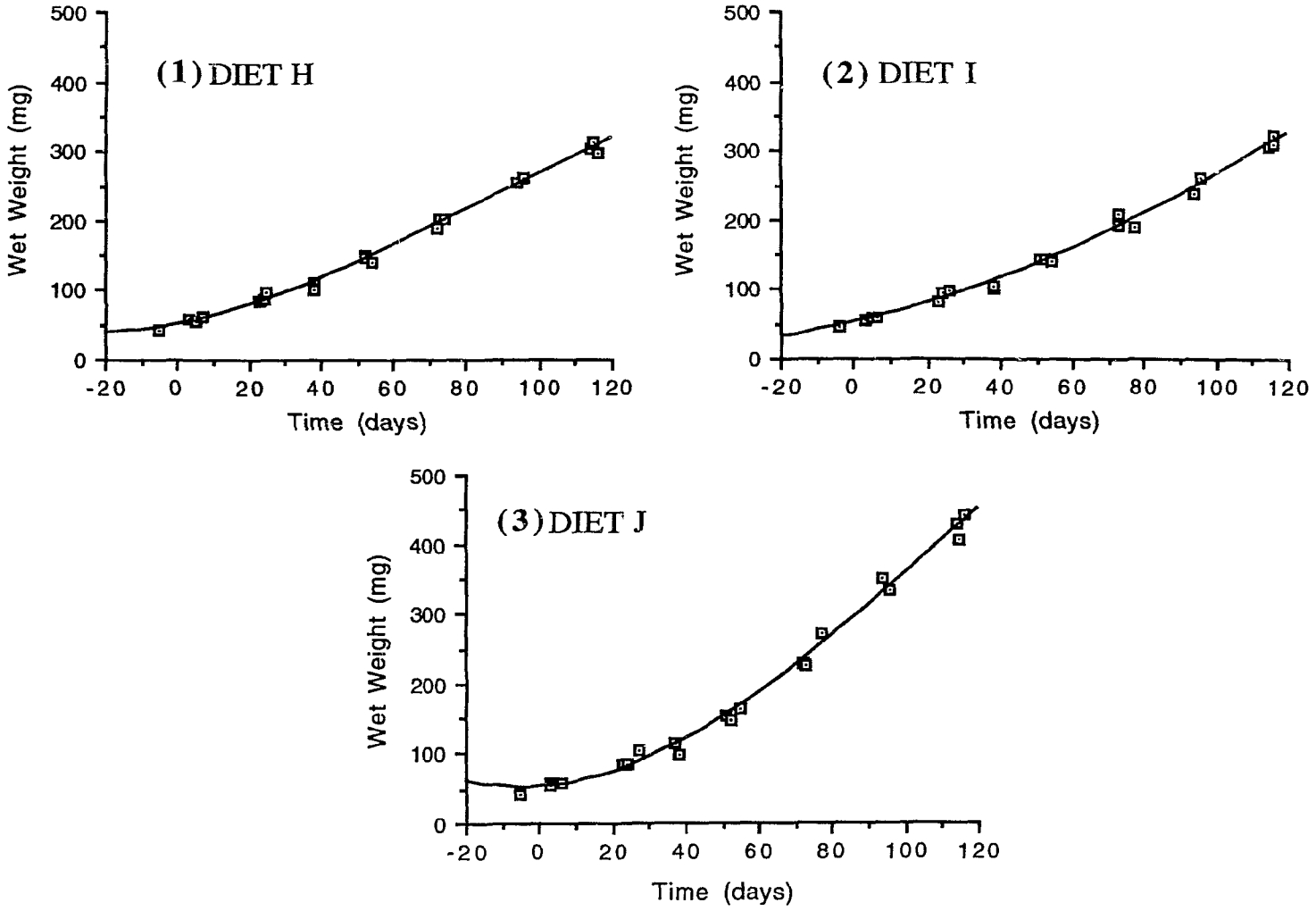
Diet A	$Y = 1.78\ X - (7.69 \times 10^{-3})\ X^2 + (2.66 \times 10^{-5})\ X^3 + 52.98$	$R^2 = 0.93$
Diet B	$Y = 2.59\ X - (3.33 \times 10^{-2})\ X^2 + (1.64 \times 10^{-4})\ X^3 + 49.19$	$R^2 = 0.94$
Diet C	$Y = 2.21\ X - (1.38 \times 10^{-2})\ X^2 + (6.23 \times 10^{-5})\ X^3 + 53.73$	$R^2 = 0.91$
Diet D	$Y = 1.71\ X - (6.04 \times 10^{-3})\ X^2 + (4.72x\ 10^{-5})\ X^3 + 54.29$	$R^2 = 0.99$
Diet E	$Y = 1.43\ X + (2.06 \times 10^{-3})\ X^2 - (1.44 \times 10^{-5})\ X^3 + 51.50$	$R^2 = 0.99$
Diet F	$Y = 2.02\ X - (1.40 \times 10^{-2})\ X^2 + (8.01 \times 10^{-5})\ X^3 + 51.80$	$R^2 = 0.99$
Diet G	$Y = 1.93\ X - (1.19 \times 10^{-2})\ X^2 + (6.37 \times 10^{-5})\ X^3 + 51.95$	$R^2 = 0.99$
Diet H	$Y = 1.02\ X + (1.85 \times 10^{-2})\ X^2 - (7.04 \times 10^{-5})\ X^3 + 51.67$	$R^2 = 1.00$
Diet I	$Y = 1.22\ X + (9.97 \times 10^{-3})\ X^2 - (8.10 \times 10^{-6})\ X^3 + 52.01$	$R^2 = 0.99$
Diet J	$Y = 0.33\ X + (3.80 \times 10^{-2})\ X^2 - (1.09 \times 10^{-4})\ X^3 + 53.56$	$R^2 = 0.99$



Appendix 3-B1 to B3. Weight Regressions of Lobsters Fed Diets A, B or C. Values plotted are mean weights of 10-20 lobsters from each of three replicate trays (which had twenty lobsters each at the start of the feeding trial).



Appendix 3-C1 to C4. Weight Regressions of Lobsters Fed Diets D, E, F or G. Values plotted are mean weights of 10-20 lobsters from each of three replicate trays (which had twenty lobsters each, at the start of the feeding trial.)



Appendix 3-D1 to D3. Weight Regressions of Lobsters Fed Diets H, I, or J. Values plotted are mean weights of 10-20 lobsters from each of three replicate trays (which had twenty lobsters each, at the start of the feeding trial.)

Appendix 4-A.

Fatty Acid Composition of Whole Lobster Neutral Lipids (Weight %).

RRT	Fatty Acids	Initial TL	Diet B	D	G	H	I	J
0.12	**12:0**	8.34	0.49	9.78	12.55	11.92	4.69	0.12
0.14	**12:1n-7**	4.42	n.d.	n.d.	n.d.	n.d.	0.37	n.d.
0.145	**12:1n-5**	0.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.15	**12:1n-3**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.21	**12:3n-3**	n.d.	n.d.	n.d.	n.d.	?	0.94	0.08
0.24	**14:0**	4.11	16.07	19.62	10.23	33.07	5.45	3.43
0.25		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12
0.26	**14:1n-9**	trace	n.d.	0.26	trace	n.d.	n.d.	0.03
0.27	**14:1n-7**	0.69	0.69	0.68	trace	0.26	0.50	0.10
0.28		0.14	n.d.	n.d.	trace	0.06	n.d.	0.34
0.29		0.05	0.53	0.29	0.15	0.10	n.d.	0.08
0.33	**14:2n-6**	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.35	**15:0**	1.01	1.69	0.94	0.20	0.50	n.d.	0.27
0.43		0.79	n.d.	0.05	1.62	?	2.32	n.d.
0.50	**16:0**	20.14	33.51	23.60	19.27	18.72	6.61	12.11
0.53	**16:1n-11**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09
0.54	**16:1n-9**	3.42	2.39	4.26	0.92	1.67	0.76	1.05
0.55	**16:1n-7**	4.96	10.25	3.77	3.77	5.56	3.33	6.92
0.56	**16:1n-5**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11
0.61		0.08	n.d.	0.24	n.d.	0.07	0.48	0.10
0.67	**16:2n-6**	1.25	1.93	0.05	1.36	0.64	n.d.	0.28
0.69	**16:2n-4**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12
0.71	**17:0**	0.02	0.43	0.35	n.d.	0.05	0.78	0.32
0.79	**16:3n-4**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.23
0.86		n.d.	n.d.	n.d.	0.53	?	1.05	n.d.
1.00	**18:0**	4.05	6.91	3.65	3.28	2.02	trace	0.94
1.10	**18:1n-11**	s.	2.75	n.d.	n.d.	s.	n.d.	0.94
1.11	**18:1n-9**	14.17	18.65	17.59	10.57	14.69	4.58	19.12
1.14	**18:1n-7**	7.84	2.00	1.31	1.13	1.53	0.98	6.29
1.33	**18:2n-6**	6.80	1.73	4.64	26.55	2.02	11.93	26.03
1.64	**18:3n-3**	1.43	n.d.	6.00	?	trace	0.23	0.84
2.01	**20:0**	trace	n.d.	n.d.	n.d.	n.d.	n.d.	0.02
2.23	**20:1n-9**	0.67	1.26	0.61	?	0.44	n.d.	3.61
2.65	**20:2n-6**	1.12	n.d.	0.36	2.67	0.31	0.51	1.35
2.93	**20:3n-6**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3.09	**20:4n-6**	1.11	n.d.	n.d.	0.28	trace	0.23	0.15
3.26	**20:3n-3**	0.31	n.d.	0.81	?	trace	trace	0.11
3.82	**20:5n-3**	8.29	n.d.	0.12	2.33	0.60	7.43	4.13
5.56	**22:4n-6**	n.d.	n.d.	n.d.	0.35	n.d.	n.d.	0.16
6.66	**22:5n-6**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03
	22:5n-3	n.d.	1.20	0.17	0.24	0.74	26.10	0.82
8.16	**22:6n-3**	4.18	< 0.1	< 0.1	0.60	4.90	20.31	5.69

(see next page)

Appendix 4-B.

Fatty Acid Composition of Whole Lobster Polar Lipids (Weight %).

RRT	Fatty Acids	INIT PL	Diet A	B	C	D	F	G	H	I	J
0.12	12:0	0.33	0.67	0.94	7.92	1.75	0.80	6.59	1.24	24.50	0.1
0.14	12:1n-7	0.10	n.d.	n.d.	n.d.	n.d.	n.d.	0.04	n.d.	0.04	0.04
0.150		n.d.	0.44	n.d.	n.d.	0.06	?	n.d.	n.d.	0.57	n.d.
0.21		0.14	0.12	n.d.	0.26	0.06	?	0.05	0.03	0.03	0.18
0.24	14:0	6.48	2.54	6.04	5.33	6.96	4.49	8.19	8.59	12.16	1.17
0.26	14:1n-9	0.32	n.d.	n.d.	n.d.	n.d.	0.05	0.07	n.d.	n.d.	n.d.
0.27	14:1n-7	0.62	0.43	0.21	0.07	0.17	0.06	0.14	0.03	0.04	0.07
0.28		0.52	n.d.	0.23	0.03	0.05	0.09	0.11	n.d.	n.d.	0.07
0.29		0.53	0.14	0.14	0.06	0.05	0.11	0.10	0.05	0.43	0.06
0.33	14:2n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.32	0.11	n.d.	n.d.
0.35	15:0	1.84	0.35	0.57	0.05	0.22	0.29	n.d.	n.d.	0.05	0.19
0.43		0.76	1.03	2.19	3.30	2.47	1.57	1.23	1.93	1.02	2.09
0.50	16:0	44.11	25.35	29.43	4.97	27.17	18.15	15.39	23.20	9.64	11.9
0.53	16:1n-11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.36	0.02	0.06
0.54	16:1n-9	3.73	0.90	0.95	0.35	0.69	1.04	1.11	0.31	0.33	0.67
0.55	16:1n-7	4.27	13.65	12.23	2.80	3.65	3.05	2.57	4.81	3.48	3.79
0.56	16:1n-5	0.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.61		0.22	n.d.	0.27	n.d.	n.d.	0.28	0.12	0.05	n.d.	0.18
0.67	16:2n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11
0.70	17:0	0.92	0.12	0.40	0.03	0.12	0.05	0.08	n.d.	n.d.	0.07
0.79	16:3n-4	n.d.	0.55	0.16	n.d.	0.10	0.05	0.06	n.d.	n.d.	0.07
0.86		0.60	0.36	0.59	2.03	1.28	0.73	0.52	0.69	0.66	1.66
1.00	18:0	6.71	5.01	4.57	0.32	5.61	2.63	2.54	3.40	0.87	0.94
1.10	18:1n-11	0.66	n.d.	0.62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.43
1.11	18:1n-9	8.13	28.12	23.99	4.82	18.40	16.02	12.09	16.76	7.05	9.01
1.14	18:1n-7	3.68	4.52	5.16	0.70	2.32	2.03	12.54	2.52	2.22	2.17
1.33	18:2n-6	1.53	4.15	4.48	5.04	10.27	19.05	27.75	5.05	10.02	19.57
1.64	18:3n-3	1.04	0.46	0.33	45.11	11.41	18.01	0.31	0.20	0.21	0.81
2.01	20:0	n.d.	0.12	n.d.	trace	0.14	0.05	0.06	0.03	trace	trace
2.23	20:1n-9	0.69	0.80	1.66	trace	0.55	0.30	0.38	0.60	0.26	0.51
2.65	20:2n-6	0.35	0.50	0.72	n.d.	0.95	1.77	2.40	0.79	0.80	1.26
2.93	20:3n-6	0.37	0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3.09	20:4n-6	1.23	0.61	0.28	0.83	0.21	0.52	0.34	0.27	0.22	0.69
3.26	20:3n-3	0.35	0.03	n.d.	4.50	1.53	2.77	0.04	0.05	0.02	n.d.
3.82	20:5n-3	5.28	3.81	1.53	6.60	0.94	3.64	2.51	8.22	4.72	20.81
	22:5n-3	n.d.	0.48	0.73	1.24	n.d.	0.53	0.30	1.40	0.26	3.37
8.16	22:6n-3	2.27	2.27	0.87	3.36	0.30	2.40	2.10	18.31	20.34	16.08

Values from pooled extracts of three lobsters
n.d. = Not detected
s. = Shoulder (peak not integrated)
? = identification uncertain (peak integration unreliable)
trace = Peak barely visible and not integrated

Appendix 5-A.

Newman-Keuls Multiple Comparisons Matrix of Pairwise Comparisons of Probabilities of Differences in Oxygen Consumption Rates.

Temp		20°C					10°C				
	Diet	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
20°C	A	1.000									
	B	0.594	1.000								
	C	0.367	0.748	1.000							
	D	0.717	0.635	0.627	1.000						
	E	0.157	0.614	0.766	0.414	1.000					
10°C	A	0.615	0.735	0.581	0.786	0.330	1.000				
	B	0.214	0.649	0.661	0.477	0.799	0.405	1.000			
	C	0.405	0.612	0.829	0.588	0.796	0.592	0.788	1.000		
	D	0.008	0.106	0.262	0.043	0.376	0.027	0.386	0.231	1.000	
	E	0.033	0.269	0.475	0.136	0.455	0.093	0.574	0.463	0.554	1.000

Appendix 5-B.

Newman-Keuls Multiple Comparisons Matrix of Pairwise Comparisons of Probabilities of Differences in O:N Ratios.

Temp		20°C					10°C				
	Diet	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
20°C	A	1.000									
	B	0.120	1.000								
	C	0.268	0.734	1.000							
	D	0.345	0.361	0.914	1.000						
	E	<u>0.064</u>	0.696	0.617	0.560	1.000					
10°C	A	0.737	0.178	0.223	0.379	0.107	1.000				
	B	0.380	0.435	0.969	0.897	0.479	0.461	1.000			
	C	<u>0.046</u>	0.809	0.571	0.542	0.824	0.083	0.499	1.000		
	D	<u>0.005</u>	0.504	0.175	0.178	0.625	<u>0.011</u>	0.183	0.593	1.000	
	E	<u>0.018</u>	0.703	0.367	0.360	0.775	<u>0.035</u>	0.349	0.349	0.604	1.000

Values that are underlined show significance at $p \leq 0.05$.

Appendix 6.

Lipid Content of Lobster Tissues

Appendix 6-A.

Amount of Neutral and Polar Lipids (mg/animal) in the Hepatopancreata, Muscles, and Gills of Intermolt Lobsters Fed One of 5 Diets at 10°C or 20°C.

Temp °C	Diet		Hepat NL (mg)	Hepat PL (mg)	Muscle NL (mg)	Muscle PL (mg)	Gill NL (mg)	Gill PL (mg)
10	A	Mean	1.36	0.61	0.75	1.59	0.029	0.030
		SD	1.12	0.44	0.15	0.41	0.015	0.009
		n	3	4	4	4	3	3
	B	Mean	1.56	0.53	0.75	1.33	0.035	0.016
		SD	1.59	0.27	0.20	0.37	0.017	0.012
		n	4	3	4	4	4	4
	C	Mean	2.78	0.59	1.18	2.27	0.033	0.018
		SD	2.09	0.42	0.26	0.39	0.010	0.008
		n	3	3	2	2	4	4
	D	Mean	1.21	0.46	0.89	1.59	0.036	0.017
		SD	0.53	0.04	0.36	0.81	0.032	0.013
		n	3	3	3	3	4	4
	E	Mean	10.18	0.71	0.69	0.94	0.022	0.030
		SD	5.39	0.38	0.24	0.27	0.000	0.015
		n	4	4	4	4	4	4
20	A	Mean	0.50	0.24	0.64	1.68	0.050	0.090
		SD	0.52	0.25	0.23	0.90	0.011	0.027
		n	4	3	4	4	4	4
	B	Mean	3.89	0.63	0.83	1.46	0.043	0.070
		SD	1.65	0.27	0.22	0.43	0.030	0.016
		n	3	3	4	4	4	4
	C	Mean	1.51	0.44	0.50	0.86	0.029	0.000
		SD	1.37	0.26	0.25	0.37	0.060	0.060
		n	3	4	4	4	4	4
	D	Mean	2.50	0.81	1.07	1.41	0.033	0.023
		SD	1.02	0.18	0.26	0.48	0.016	0.021
		n	3	3	4	4	4	4
	E	Mean	31.98	1.47	1.48	2.21	0.036	0.040
		SD	14.88	0.36	1.11	0.63	0.027	0.032
		n	3	3	3	3	3	3

Appendix 6-B.

Neutral and Polar Lipid Content (mg %) in the Hepatopancreata, Muscles, and Gills of Intermolt Lobsters Fed One of 5 Diets at 10°C or 20°C.

Temp °C	Diet		NL mg/ 100 mg H	PL mg/ 100 mg H	NL mg/ 100 mg M	PL mg/ 100 mg M	NL mg/ 100 mg G	PL mg/ 100 mg G
10	A	Mean	2.5	0.9	0.3	0.6	3.1	1.2
		SD	1.3	0.2	0.2	0.2	1.5	0.7
		n	3	3	4	4	3	3
	B	Mean	2.0	0.7	0.3	0.5	2.4	1.3
		SD	1.1	0.3	0.1	0.1	0.9	1.0
		n	4	3	4	4	4	4
	C	Mean	2.5	0.6	0.3	0.6	2.5	1.6
		SD	1.3	0.2	0.1	0.2	1.2	1.7
		n	3	3	2	2	4	4
	D	Mean	1.5	0.8	0.3	0.5	2.3	1.3
		SD	0.6	0.7	0.1	0.2	1.8	1.2
		n	3	3	3	3	4	4
	E	Mean	9.4	0.7	0.3	0.5	2.4	1.0
		SD	2.3	0.1	0.1	0.1	1.9	0.7
		n	4	4	4	4	4	4
20	A	Mean	0.3	0.0	0.3	0.7	2.6	1.8
		SD	0.2	0.0	0.1	0.2	2.5	0.8
		n	1	3	4	4	4	4
	B	Mean	0.1	0.0	0.3	0.5	1.9	0.7
		SD	0.1	0.0	0.1	0.1	0.9	0.5
		n	3	3	4	4	4	4
	C	Mean	0.2	0.0	0.3	0.4	2.9	1.1
		SD	0.1	0.0	0.1	0.2	2.5	0.4
		n	3	4	4	4	4	4
	D	Mean	0.2	0.0	0.3	0.4	1.4	0.9
		SD	0.2	0.0	0.1	0.0	0.8	0.8
		n	3	3	4	4	4	4
	E	Mean	0.2	0.1	0.3	0.5	1.1	0.5
		SD	0.2	0.1	0.2	0.1	0.4	0.3
		n	3	3	3	3	3	3

Appendix 6-C.

Proportion of Neutral and Polar Lipids (mg/g animal) in the Hepatopancreata, Muscles, and Gills of Lobsters Fed One of 5 Diets at 10°C or 20°C for 14 Weeks.

Temp °C	Diet		Hepat NL (mg/g body)	Hepat PL (mg/g body)	Muscle NL (mg/g body)	Muscle PL (mg/g body)	Gill NL (mg/g body)	Gill PL (mg/g body)
10	A	Mean	0.52	0.24	0.35	0.69	0.12	0.05
		SD	0.22	0.10	0.19	0.18	0.03	0.03
		n	3	4	4	4	3	3
	B	Mean	0.61	0.21	0.33	0.60	0.16	0.09
		SD	0.50	0.06	0.03	0.17	0.08	0.08
		n	4	3	4	4	4	4
	C	Mean	0.91	0.19	0.34	0.65	0.15	0.10
		SD	0.62	0.09	0.07	0.16	0.09	0.12
		n	3	3	2	2	4	4
	D	Mean	0.45	0.16	0.28	0.53	0.16	0.09
		SD	0.23	0.04	0.10	0.26	0.15	0.08
		n	3	3	3	3	4	4
	E	Mean	5.38	0.40	0.40	0.44	0.13	0.06
		SD	1.67	0.12	0.11	0.23	0.05	0.06
		n	4	4	4	4	4	4
20	A	Mean	0.23	0.12	0.33	0.84	0.14	0.13
		SD	0.18	0.08	0.04	0.25	0.06	0.08
		n	4	3	4	4	4	4
	B	Mean	1.38	0.21	0.30	0.52	0.15	0.06
		SD	0.41	0.07	0.08	0.10	0.06	0.05
		n	3	3	4	4	4	4
	C	Mean	1.16	0.27	0.28	0.50	0.21	0.08
		SD	0.96	0.14	0.05	0.07	0.12	0.04
		n	3	4	4	4	4	4
	D	Mean	0.77	0.25	0.37	0.48	0.11	0.08
		SD	0.31	0.09	0.08	0.09	0.04	0.06
		n	3	3	4	4	4	4
	E	Mean	9.08	0.42	0.40	0.62	0.10	0.06
		SD	2.95	0.07	0.21	0.03	0.06	0.07
		n	3	3	3	3	3	3

Appendix 7.

Fatty Acid Composition (in Weight % ± S.D.) of the NL and PL Fractions of the Hepatopancreata, Abdominal Muscles or Gills of Lobsters.

Juvenile lobsters were reared at 10°C or 20°C and fed one of four fatty acid controlled semi-surified diets (A - D) or the Halifax Crustacean Reference Diet (E).

Appendix 7-A. Fatty Acid Composition (Weight % ± S.D.) of the Hepatopancreata of Lobsters Reared at 10°C.

Diet	A		B		C	
Lipid Fraction	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**
Fatty Acid						
14:0	1.15 ± 1.19	0.84 ± 0.44	0.59 ± 0.48	0.81 ± 0.59	0.14 ± 0.11	0.62 ± 0.33
16:0	8.54 ± 4.39	4.74 ± 2.56	7.12 ± 3.24	4.50 ± 0.54	3.32 ± 1.52	2.43 ± 1.05
18:0	2.64 ± 3.51	1.78 ± 1.16	0.97 ± 0.16	0.64 ± 0.25	0.40 ± 0.23	1.00 ± 0.28
Σ SFAs	13.23 ± 10.08	10.03 ± 5.79	9.24 ± 4.90	8.88 ± 1.80	3.84 ± 1.93	5.59 ± 1.62
A+ISO 16	0.34 ± 0.39	2.26 ± 0.84	1.25 ± 2.23	2.56 ± 2.70	0.04 ± 0.05	2.54 ± 1.60
16:1(n-7)	7.68 ± 4.28	3.49 ± 1.92	5.16 ± 2.12	3.14 ± 1.21	2.92 ± 0.36	1.32 ± 0.42
18:1(n-9)	64.76 ± 24.65	53.01 ± 10.77	65.51 ± 8.89	50.62 ± 5.39	78.26 ± 11.79	47.02 ± 13.52
20:1	1.49 ± 0.49	2.37 ± 0.87	1.71 ± 0.23	1.81 ± 0.48	1.49 ± 0.90	2.76 ± 1.35
22:1	0.45 ± 0.45	0.62 ± 0.22	0.67 ± 0.39	0.58 ± 0.25	0.17 ± 0.10	0.83 ± 0.64
Σ MFAs	76.38 ± 21.28	61.73 ± 8.35	73.98 ± 6.71	58.13 ± 3.33	87.11 ± 2.71	53.02 ± 11.85
18:2(n-6)	2.39 ± 2.43	2.42 ± 1.95	10.86 ± 0.40	13.73 ± 2.60	0.92 ± 0.38	1.44 ± 0.24
20:4(n-6)	0.16 ± 0.14	0.93 ± 0.42	0.16 ± 0.04	0.57 ± 0.13	0.05 ± 0.01	0.73 ± 0.24
Σ (n-6)	4.49 ± 4.66	5.08 ± 2.24	12.92 ± 0.48	16.98 ± 2.76	1.53 ± 0.87	4.29 ± 1.18
18:3(n-3)	0.20 ± 0.31	0.16 ± 0.22	0.21 ± 0.16	0.37 ± 0.42	5.75 ± 1.07	8.72 ± 2.19
20:5(n-3)	0.60 ± 0.73	5.78 ± 2.52	0.37 ± 0.12	2.70 ± 0.06	0.21 ± 0.15	4.86 ± 2.95
22:5(n-3)	0.33 ± 0.46	0.47 ± 0.17	0.28 ± 0.43	0.48 ± 0.19	0.16 ± 0.11	0.60 ± 0.25
22:6(n-3)	0.56 ± 0.39	3.65 ± 1.31	0.45 ± 0.08	2.18 ± 0.45	0.20 ± 0.11	3.92 ± 2.14
Σ (n-3)	2.82 ± 3.57	10.80 ± 4.10	2.08 ± 0.98	6.67 ± 0.74	7.12 ± 1.03	18.96 ± 3.29
Σ PUFAs	9.54 ± 10.52	24.84 ± 4.12	16.32 ± 1.52	28.91 ± 3.87	8.92 ± 1.06	38.14 ± 9.02
(n-3)/(n-6)	0.52 ± 0.17	2.16 ± 0.12	0.16 ± 0.08	0.40 ± 0.10	5.73 ± 3.05	4.59 ± 0.87

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

(continued)

Appendix 7-A. (con't) FA Composition (Wt %) of the Hepatopancreata of Lobsters at 10°C.

Diet	D		E	
Lipid Fraction	**NL**	**PL**	**NL**	**PL**
Fatty Acid				
14:0	0.21 ± 0.09	0.63 ± 0.45	2.63 ± 0.36	1.09 ± 0.18
16:0	4.15 ± 1.66	2.53 ± 0.94	10.26 ± 0.93	16.34 ± 4.64
18:0	0.70 ± 0.42	1.08 ± 0.35	0.76 ± 0.15	6.08 ± 0.96
Σ SFAs	5.20 ± 2.26	5.56 ± 2.85	13.95 ± 1.13	28.12 ± 4.71
A+ISO 16	0.07 ± 0.03	3.05 ± 1.44	0.05 ± 0.01	3.26 ± 0.99
16:1(n-7)	3.56 ± 1.25	1.40 ± 0.66	4.58 ± 0.66	0.30 ± 0.44
18:1(n-9)	79.00 ± 4.89	45.05 ± 14.29	22.91 ± 0.62	6.63 ± 1.36
20:1	1.63 ± 0.93	2.16 ± 0.55	11.96 ± 1.81	5.90 ± 3.07
22:1	0.34 ± 0.29	0.79 ± 0.12	8.58 ± 2.14	3.62 ± 1.68
Σ MFAs	84.84 ± 3.76	50.34 ± 14.67	50.68 ± 3.06	19.89 ± 5.94
18:2(n-6)	3.15 ± 0.83	3.55 ± 0.61	24.46 ± 1.36	10.08 ± 2.59
20:4(n-6)	0.07 ± 0.05	0.77 ± 0.24	0.13 ± 0.03	0.48 ± 0.16
Σ (n-6)	3.92 ± 1.13	6.14 ± 0.90	25.60 ± 1.29	13.48 ± 2.61
18:3(n-3)	4.76 ± 0.60	7.59 ± 0.89	0.61 ± 0.17	0.41 ± 0.04
20:5(n-3)	0.29 ± 0.19	4.27 ± 2.34	3.62 ± 0.77	9.61 ± 1.63
22:5(n-3)	0.16 ± 0.13	0.48 ± 0.48	0.37 ± 0.03	1.48 ± 0.10
22:6(n-3)	0.27 ± 0.03	3.81 ± 1.35	3.76 ± 0.36	14.50 ± 1.83
Σ (n-3)	5.61 ± 0.81	17.13 ± 3.14	9.13 ± 1.36	29.49 ± 3.58
Σ PUFAs	9.81 ± 2.01	40.28 ± 10.90	35.17 ± 2.38	47.69 ± 2.33
(n-3)/(n-6)	1.49 ± 0.26	2.88 ± 0.92	0.36 ± 0.04	2.29 ± 0.77

Values and sums (Σs) are means of single samples from each of 2-4 lobsters.

Sums may reflect FAs not listed in this table.

Appendix 7-B. Fatty Acid Composition (Weight % ± S.D.) of the Hepatopancreata of Lobsters Reared at 20°C.

Diet	A		B		C	
Lipid Fraction	NL	PL	NL	PL	NL	PL
Fatty Acid						
	0.91 ± 0.50	0.51 ± 0.26	0.50 ± 0.49	0.20 ± 0.06	0.30 ± 0.12	0.56 ± 0.53
16:0	10.91 ± 4.86	6.33 ± 3.43	7.01 ± 3.44	2.31 ± 0.39	5.53 ± 1.07	5.24 ± 4.63
18:0	2.02 ± 1.30	1.53 ± 1.74	0.77 ± 0.75	0.60 ± 0.12	0.33 ± 0.02	1.73 ± 1.82
Σ SFAs	14.72 ± 6.88	10.12 ± 5.53	8.54 ± 5.00	3.70 ± 0.65	6.34 ± 1.02	8.19 ± 7.35
A+ISO 16	0.52 ± 0.55	3.74 ± 2.66	0.20 ± 0.16	6.78 ± 1.56	0.31 ± 0.22	4.25 ± 0.84
16:1(n-7)	8.95 ± 2.47	5.89 ± 1.74	6.01 ± 2.29	1.46 ± 0.72	6.40 ± 2.11	2.31 ± 0.78
18:1(n-9)	62.29 ± 15.39	51.50 ± 11.43	75.08 ± 3.31	47.74 ± 2.57	74.47 ± 4.16	49.59 ± 9.10
20:1	1.97 ± 0.91	1.95 ± 0.19	1.42 ± 0.25	2.67 ± 0.85	1.69 ± 0.25	2.88 ± 1.21
22:1	1.11 ± 1.25	0.79 ± 0.17	0.28 ± 0.30	0.72 ± 0.14	0.40 ± 0.37	0.94 ± 0.51
Σ MFAs	75.87 ± 10.61	62.40 ± 9.28	78.88 ± 7.18	54.41 ± 3.47	84.07 ± 1.77	53.16 ± 8.24
18:2(n-6)	2.67 ± 2.09	2.21 ± 0.18	9.58 ± 0.62	16.12 ± 1.60	0.83 ± 0.14	1.76 ± 0.23
20:4(n-6)	0.16 ± 0.14	1.13 ± 0.37	0.08 ± 0.10	0.48 ± 0.47	0.06 ± 0.02	0.35 ± 0.01
Σ (n-6)	4.38 ± 2.48	7.78 ± 4.21	10.80 ± 1.13	19.24 ± 1.18	1.25 ± 0.25	4.60 ± 2.56
18:3(n-3)	0.11 ± 0.11	0.16 ± 0.04	0.09 ± 0.13	0.04 ± 0.05	7.00 ± 0.92	12.54 ± 4.95
20:5(n-3)	1.05 ± 0.79	4.28 ± 2.40	0.22 ± 0.24	1.33 ± 0.39	0.10 ± 0.01	1.15 ± 0.26
22:5(n-3)	0.21 ± 0.40	0.21 ± 0.18	0.13 ± 0.09	0.54 ± 0.41	0.27 ± 0.44	0.43 ± 0.42
22:6(n-3)	1.11 ± 0.80	2.11 ± 0.93	0.25 ± 0.20	1.14 ± 0.71	0.05 ± 0.07	0.73 ± 0.09
Σ (n-3)	3.10 ± 1.69	10.40 ± 3.98	0.84 ± 0.74	4.63 ± 1.25	7.43 ± 1.05	16.80 ± 6.00
Σ PUFAs	8.62 ± 5.49	23.00 ± 4.76	12.24 ± 2.31	35.61 ± 2.82	9.17 ± 1.36	3.08 ± 1.68
(n-3)/(n-6)	0.70 ± 0.24	1.46 ± 0.42	0.07 ± 0.06	0.24 ± 0.07	6.00 ± 0.68	4.75 ± 3.95

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

(continued)

Appendix 7-B. (con't) FA Composition (Wt %) of the Hepatopancreata of Lobsters at 20°C.

Diet	D		E	
Lipid Fraction	**NL**	**PL**	**NL**	**PL**
Fatty Acid				
14:0	0.24 ± 0.05	0.30 ± 0.20	2.24 ± 0.19	0.82 ± 0.42
16:0	5.53 ± 1.35	4.01 ± 2.59	14.68 ± 1.10	16.69 ± 2.39
18:0	0.49 ± 0.17	1.27 ± 0.84	0.84 ± 0.14	3.77 ± 4.34
Σ SFAs	6.34 ± 1.36	6.19 ± 3.69	18.07 ± 1.46	22.26 ± 7.33
A+ISO 16	0.79 ± 0.27	0.54 ± 0.44	0.41 ± 0.43	0.05 ± 0.04
16:1(n-7)	5.68 ± 1.11	1.97 ± 0.54	5.64 ± 0.89	1.25 ± 0.35
18:1(n-9)	77.53 ± 1.50	46.27 ± 8.38	24.27 ± 1.25	9.17 ± 2.46
20:1	2.77 ± 1.17	3.36 ± 0.96	3.76 ± 1.07	3.82 ± 0.44
22:1	1.18 ± 0.44	0.71 ± 0.35	1.48 ± 0.67	0.62 ± 0.22
Σ MFAs	84.51 ± 1.25	52.74 ± 7.87	51.40 ± 2.57	21.44 ± 3.41
18:2(n-6)	2.66 ± 0.41	5.60 ± 0.37	21.78 ± 2.57	15.92 ± 5.15
20:4(n-6)	0.06 ± 0.01	0.55 ± 0.08	0.12 ± 0.08	0.62 ± 0.13
Σ (n-6)	3.09 ± 0.65	7.43 ± 0.48	23.45 ± 2.89	19.13 ± 5.38
18:3(n-3)	4.94 ± 0.71	10.11 ± 1.24	0.39 ± 0.05	1.59 ± 1.68
20:5(n-3)	0.06 ± 0.03	1.74 ± 0.29	2.32 ± 0.56	16.60 ± 4.41
22:5(n-3)	1.28 ± 0.69	0.51 ± 0.27	1.06 ± 0.52	0.57 ± 0.01
22:6(n-3)	0.18 ± 0.14	1.50 ± 0.72	2.59 ± 0.97	14.08 ± 2.93
Σ (n-3)	5.54 ± 0.44	15.11 ± 2.04	6.32 ± 1.54	37.90 ± 5.47
Σ PUFAs	9.02 ± 0.79	34.00 ± 4.92	30.31 ± 4.09	59.54 ± 9.85
(n-3)/(n-6)	1.85 ± 0 43	2.05 ± 0.39	0.27 ± 0.05	2.02 ± 0.28

Values and sums (Σs) are means of single samples from each of 2-4 lobsters.
Sums may reflect FAs not listed in this table

Appendix 7-C. Fatty Acid Composition (Weight % ± S.D.) of the Abdominal Muscles of Lobsters Reared at 10°C.

Diet	A		B		C	
Lipid Fraction	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**
Fatty Acid						
14:0	2.38 ± 1.23	0.25 ± 0.11	4.04 ± 0.09	0.34 ± 0.09	2.84 ± 0.37	0.25 ± 0.02
16:0	23.54 ± 2.39	16.95 ± 2.47	18.86 ± 3.64	15.73 ± 2.92	17.53 ± 5.95	15.69 ± 0.45
18:0	4.48 ± 1.33	3.58 ± 0.62	4.44 ± 0.94	3.67 ± 0.32	5.02 ± 0.93	2.72 ± 0.53
Σ SFAs	32.37 ± 4.75	21.61 ± 2.37	30.60 ± 3.55	20.81 ± 2.85	29.18 ± 7.33	19.86 ± 0.08
A+ISO 16	0.77 ± 0.38	0.50 ± 0.80	0.93 ± 0.09	0.11 ± 0.04	1.20 ± 0.18	0.04 ± 0.05
16:1(n-7)	6.69 ± 3.59	1.37 ± 0.26	11.09 ± 2.76	1.17 ± 0.69	8.75 ± 0.35	1.40 ± 0.60
18:1(n-9)	29.99 ± 4.00	15.68 ± 1.86	22.37 ± 2.73	15.57 ± 0.54	24.37 ± 4.38	17.10 ± 0.96
20:1	1.47 ± 0.35	2.55 ± 0.41	1.81 ± 0.66	3.15 ± 0.49	1.96 ± 0.21	3.41 ± 0.68
22:1	1.27 ± 0.64	1.52 ± 2.01	0.93 ± 0.01	0.83 ± 0.39	1.44 ± 0.62	0.87 ± 0.37
Σ MFAs	43.02 ± 0.66	24.61 ± 1.19	41.32 ± 2.99	25.60 ± 1.54	41.37 ± 3.96	27.03 ± 2.47
18:2(n-6)	4.67 ± 1.01	8.56 ± 0.67	4.43 ± 0.40	10.98 ± 1.05	2.48 ± 1.34	8.12 ± 0.37
20:4(n-6)	0.89 ± 0.37	1.24 ± 0.50	0.62 ± 0.04	1.20 ± 0.40	0.96 ± 0.02	1.59 ± 0.51
Σ (n-6)	9.07 ± 1.60	12.97 ± 0.24	7.77 ± 0.43	15.80 ± 1.43	6.28 ± 1.00	13.44 ± 0.22
18:3(n-3)	0.16 ± 0.03	0.12 ± 0.02	0.15 ± 0.11	0.27 ± 0.20	1.58 ± 1.00	1.40 ± 0.83
20:5(n-3)	6.34 ± 2.87	20.66 ± 0.92	5.71 ± 0.84	17.46 ± 2.19	5.29 ± 2.33	17.21 ± 1.89
22:5(n-3)	0.96 ± 0.54	1.32 ± 1.19	2.02 ± 1.06	0.60 ± 0.23	0.45 ± 0.64	0.79 ± 0.50
22:6(n-3)	3.08 ± 1.20	17.81 ± 1.02	0.85 ± 0.06	16.89 ± 2.08	3.26 ± 0.54	17.53 ± 0.54
Σ (n-3)	13.61 ± 3.76	40.08 ± 1.07	14.26 ± 2.04	36.46 ± 3.03	15.97 ± 3.63	38.19 ± 1.17
Σ PUFAs	22.80 ± 4.60	53.47 ± 1.48	24.88 ± 5.15	53.14 ± 3.50	26.31 ± 4.14	52.64 ± 1.82
(n-3)/(n-6)	1.33 ± 0.21	3.09 ± 0.07	1.83 ± 0.16	2.32 ± 0.28	2.53 ± 0.17	2.84 ± 0.04

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

(continued)

Appendix 7-C. (con't) FA Composition (Wt %) of the Abdominal Muscles of Lobsters at 10°C.

Diet	D		E	
Lipid Fraction	NL	PL	NL	PL
Fatty Acid				
14:0	2.21 ± 0.86	0.31 ± 0.20	2.58 ± 1.05	0.36 ± 0.19
16:0	17.41 ± 1.28	14.17 ± 2.42	19.62 ± 2.40	15.91 ± 1.52
18:0	4.55 ± 1.14	3.60 ± 0.43	5.12 ± 0.46	3.33 ± 0.60
Σ SFAs	26.88 ± 1.54	19.24 ± 2.58	29.96 ± 1.92	20.42 ± 2.18
A+ISO 16	0.79 ± 0.27	0.54 ± 0.44	0.41 ± 0.43	0.05 ± 0.04
16:1(n-7)	5.44 ± 0.53	1.77 ± 0.40	7.39 ± 0.71	1.74 ± 0.70
18:1(n-9)	24.22 ± 9.20	16.76 ± 1.99	19.67 ± 1.88	14.56 ± 1.49
20:1	2.77 ± 1.17	3.36 ± 0.96	3.76 ± 1.07	3.82 ± 0.44
22:1	1.18 ± 0.44	0.71 ± 0.35	1.48 ± 0.67	0.62 ± 0.22
Σ MFAs	39.36 ± 7.55	26.64 ± 0.47	37.62 ± 0.25	25.10 ± 0.94
18:2(n-6)	6.66 ± 3.17	9.94 ± 2.76	8.11 ± 3.19	12.02 ± 1.16
20:4(n-6)	0.69 ± 0.32	0.89 ± 0.20	0.86 ± 0.04	1.04 ± 0.30
Σ (n-6)	11.07 ± 2.67	14.01 ± 2.94	12.16 ± 2.21	16.07 ± 1.13
18:3(n-3)	1.24 ± 0.64	0.88 ± 0.21	0.58 ± 0.12	0.61 ± 0.37
20:5(n-3)	6.11 ± 2.26	18.60 ± 3.96	6.48 ± 0.42	17.99 ± 2.57
22:5(n-3)	1.28 ± 0.69	0.51 ± 0.27	1.06 ± 0.52	0.57 ± 0.01
22:6(n-3)	2.99 ± 0.36	17.62 ± 1.29	3.58 ± 0.31	17.42 ± 2.49
Σ (n-3)	14.57 ± 3.64	38.42 ± 4.21	14.86 ± 1.44	37.43 ± 4.29
Σ PUFAs	30.84 ± 6.61	53.21 ± 1.81	30.36 ± 1.18	54.24 ± 2.98
(n-3)/(n-6)	1.33 ± 0.23	2.85 ± 0.79	1.27 ± 0.39	2.35 ± 0.39

Values and sums (Σs) are means of single samples from each of 2-4 lobsters.

Sums may reflect FAs not listed in this table.

Appendix 7-D. Fatty Acid Composition (Weight % ± S.D.) of the Abdominal Muscles of Lobsters Reared at 20°C.

Diet	A		B		C	
Lipid Fraction	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**
Fatty Acid						
14:0	2.78 ± 1.57	0.27 ± 0.15	1.96 ± 0.77	0.25 ± 0.09	2.64 ± 1.08	0.24 ± 0.06
16:0	23.69 ± 2.05	17.78 ± 4.49	22.99 ± 1.58	18.66 ± 4.70	18.27 ± 2.72	16.77 ± 2.97
18:0	4.53 ± 0.71	4.05 ± 1.58	5.31 ± 1.01	3.04 ± 0.53	4.48 ± 1.47	3.41 ± 1.96
Σ SFAs	30.34 ± 5.83	21.78 ± 3.49	32.31 ± 2.40	22.81 ± 4.00	28.19 ± 5.03	21.29 ± 2.25
A+ISO 16	0.87 ± 0.63	0.32 ± 0.53	0.61 ± 0.16	0.12 ± 0.02	0.56 ± 0.18	0.24 ± 0.27
16:1(n-7)	5.94 ± 1.49	1.33 ± 0.20	5.61 ± 0.43	1.62 ± 0.49	7.05 ± 2.70	1.60 ± 0.47
18:1(n-9)	25.06 ± 11.59	16.43 ± 3.71	28.25 ± 5.09	24.78 ± 5.67	38.61 ± 7.75	24.61 ± 10.84
20:1	2.73 ± 1.52	2.58 ± 0.65	2.43 ± 0.51	2.27 ± 0.23	1.71 ± 0.39	2.88 ± 0.20
22:1	1.44 ± 0.99	0.85 ± 0.53	1.20 ± 0.44	0.49 ± 0.18	0.61 ± 0.24	0.63 ± 0.38
Σ MFAs	35.64 ± 9.69	24.44 ± 4.29	40.90 ± 5.31	32.62 ± 5.58	47.17 ± 8.23	33.47 ± 10.78
18:2(n-6)	3.27 ± 0.99	5.70 ± 0.69	5.66 ± 1.02	9.41 ± 2.08	3.18 ± 1.57	5.47 ± 0.90
20:4(n-6)	1.08 ± 0.68	1.19 ± 0.11	0.82 ± 0.60	0.62 ± 0.18	0.80 ± 0.54	0.92 ± 0.46
Σ (n-6)	11.03 ± 2.44	10.13 ± 0.61	9.31 ± 1.48	13.29 ± 2.36	6.93 ± 2.49	9.04 ± 1.16
18:3(n-3)	0.20 ± 0.13	0.09 ± 0.05	0.18 ± 0.09	0.11 ± 0.05	2.84 ± 1.31	2.55 ± 1.40
20:5(n-3)	4.61 ± 1.20	23.17 ± 3.83	6.23 ± 4.08	15.94 ± 4.41	3.51 ± 1.25	16.26 ± 5.87
22:5(n-3)	0.33 ± 0.37	0.53 ± 0.22	1.37 ± 0.57	0.29 ± 0.13	0.42 ± 0.45	0.33 ± 0.16
22:6(n-3)	4.76 ± 2.21	17.38 ± 1.61	2.18 ± 0.68	12.66 ± 4.20	3.04 ± 0.82	13.90 ± 3.98
Σ (n-3)	11.93 ± 1.92	41.83 ± 5.73	13.26 ± 3.08	29.71 ± 8.40	12.12 ± 1.42	34.44 ± 9.71
Σ PUFAs	27.05 ± 6.89	53.10 ± 5.92	24.98 ± 3.93	44.12 ± 8.14	22.63 ± 5.45	44.67 ± 11.03
(n-3)/(n-6)	1.01 ± 0.62	4.16 ± 0.75	1.43 ± 0.27	2.31 ± 0.82	1.92 ± 0.65	3.79 ± 0.91

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

(continued)

Appendix 7-D. (con't) FA Composition (Wt %) of the Abdominal Muscles of Lobsters at 20°C.

Diet	D		E	
Lipid Fraction	**NL**	**PL**	**NL**	**PL**
Fatty Acid				
14:0	1.48 ± 0.43	0.19 ± 0.05	1.81 ± 0.35	0.33 ± 0.25
16:0	21.93 ± 9.39	16.32 ± 4.41	21.42 ± 7.88	19.62 ± 3.11
18:0	3.40 ± 1.74	2.72 ± 0.38	4.93 ± 1.64	3.34 ± 0.24
Σ SFAs	28.21 ± 10.87	19.77 ± 4.11	31.29 ± 6.42	23.98 ± 3.49
A+ISO 16	0.32 ± 0.19	0.03 ± 0.03	0.56 ± 0.33	0.24 ± 0.39
16:1(n-7)	5.46 ± 1.18	1.68 ± 0.45	3.91 ± 0.57	1.87 ± 0.85
18:1(n-9)	35.90 ± 10.38	21.98 ± 3.06	10.91 ± 0.83	13.29 ± 0.59
20:1	2.24 ± 0.40	2.85 ± 0.54	4.79 ± 1.07	4.15 ± 0.68
22:1	1.15 ± 0.89	0.55 ± 0.30	2.03 ± 0.15	0.54 ± 0.17
Σ MFAs	48.51 ± 9.79	30.45 ± 3.28	25.61 ± 0.42	24.22 ± 1.61
18:2(n-6)	5.67 ± 1.25	7.22 ± 0.13	8.00 ± 1.20	12.83 ± 1.10
20:4(n-6)	0.36 ± 0.11	0.76 ± 0.21	1.65 ± 0.91	0.59 ± 0.10
Σ (n-6)	8.04 ± 1.60	11.42 ± 1.27	21.32 ± 6.41	16.64 ± 1.52
18:3(n-3)	2.94 ± 1.72	1.84 ± 0.97	0.96 ± 0.36	0.27 ± 0.14
20:5(n-3)	4.27 ± 2.17	15.18 ± 0.68	4.95 ± 0.03	17.33 ± 2.57
22:5(n-3)	0.60 ± 0.19	0.47 ± 0.29	2.13 ± 0.87	0.64 ± 0.09
22:6(n-3)	4.36 ± 4.43	15.82 ± 3.17	2.96 ± 0.11	14.72 ± 2.03
Σ (n-3)	12.77 ± 6.17	35.90 ± 6.15	15.45 ± 0.59	34.04 ± 4.68
Σ PUFAs	22.02 ± 6.32	49.48 ± 5.95	40.92 ± 6.69	51.39 ± 4.38
(n-3)/(n-6)	1.60 ± 0.67	3.15 ± 0.48	0.75 ± 0.20	2.06 ± 0.38

Values and sums (Σs) are means of single samples from each of 2-4 lobsters.

Sums may reflect FAs not listed in this table.

Appendix 7-E. Fatty Acid Composition (Weight % ± S.D.) of the Gills of Lobsters Reared at 10°C.

Diet	A		B		C	
Lipid Fraction	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**
Fatty Acid						
14:0	4.39 ± 1.80	1.81 ± 1.11	3.10 ± 0.73	2.27 ± 1.36	3.28 ± 0.86	1.26 ± 0.57
16:0	17.40 ± 3.70	10.61 ± 2.43	17.66 ± 2.82	10.82 ± 2.44	17.00 ± 3.19	9.27 ± 0.69
18:0	4.66 ± 0.75	3.22 ± 0.47	5.92 ± 0.77	3.64 ± 0.70	4.90 ± 0.52	3.19 ± 0.44
Σ SFAs	29.91 ± 6.34	17.75 ± 4.55	29.83 ± 3.95	19.57 ± 3.78	27.93 ± 4.68	15.97 ± 1.49
16:1(n-7)	6.99 ± 1.52	4.41 ± 2.23	6.11 ± 0.56	4.07 ± 1.23	7.08 ± 1.53	2.74 ± 0.88
18:1(n-9)	31.18 ± 8.80	31.90 ± 6.64	28.34 ± 5.63	26.06 ± 4.74	29.88 ± 4.02	31.92 ± 6.04
Σ MFAs	46.28 ± 9.07	44.94 ± 3.64	42.39 ± 4.87	39.77 ± 4.38	44.08 ± 3.36	44.11 ± 5.89
18:2(n-6)	2.61 ± 0.24	4.26 ± 0.21	5.46 ± 0.82	7.13 ± 2.46	3.07 ± 0.20	4.24 ± 2.05
20:4(n-6)	0.50 ± 0.21	1.55 ± 0.44	0.58 ± 0.14	1.25 ± 0.27	0.61 ± 0.29	1.15 ± 0.16
Σ (n-6)	7.39 ± 0.77	10.11 ± 0.98	10.11 ± 0.88	13.22 ± 2.63	7.34 ± 1.35	11.79 ± 5.22
18:3(n-3)	0.74 ± 0.64	0.57 ± 0.15	0.70 ± 0.53	0.66 ± 0.69	1.21 ± 0.30	2.68 ± 0.50
20:5(n-3)	2.41 ± 1.18	12.42 ± 5.23	2.31 ± 0.71	10.94 ± 3.34	2.44 ± 0.55	9.67 ± 2.51
22:5(n-3)	1.96 ± 1.11	1.74 ± 0.57	1.47 ± 0.86	1.02 ± 0.68	2.04 ± 1.29	1.60 ± 0.69
22:6(n-3)	0.78 ± 0.24	3.26 ± 1.19	1.15 ± 0.46	2.86 ± 0.28	1.22 ± 0.60	3.25 ± 0.80
Σ (n-3)	8.93 ± 2.55	21.38 ± 3.47	9.86 ± 1.45	17.84 ± 1.96	11.62 ± 2.65	19.18 ± 2.01
Σ PUFAs	19.39 ± 2.99	34.33 ± 2.83	24.63 ± 3.17	35.58 ± 4.38	24.50 ± 3.28	35.67 ± 5.07
(n-3)/(n-6)	1.24 ± 0.44	2.15 ± 0.52	1.00 ± 0.22	1.37 ± 0.17	1.63 ± 0.45	1.82 ± 0.61

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

(continued)

Appendix 7-E. (con't) FA Composition (Wt %) of the Gills of Lobsters at 10°C.

Diet	D		E	
Lipid Fraction	NL	PL	NL	PL
Fatty Acid				
14:0	3.76 ± 1.39	2.29 ± 1.34	4.69 ± 1.18	1.46 ± 0.43
16:0	16.38 ± 5.28	9.14 ± 2.09	18.07 ± 1.48	13.80 ± 2.25
18:0	4.88 ± 1.59	2.85 ± 0.93	5.32 ± 0.35	4.12 ± 0.60
Σ SFAs	27.83 ± 8.51	16.29 ± 4.99	31.34 ± 2.01	21.67 ± 3.15
16:1(n-7)	6.49 ± 2.59	3.30 ± 0.93	7.03 ± 0.68	3.72 ± 0.88
18:1(n-9)	31.38 ± 16.92	32.82 ± 8.97	19.90 ± 3.66	16.61 ± 2.57
Σ MFAs	46.32 ± 12.96	45.01 ± 5.00	36.99 ± 3.47	31.53 ± 2.30
18:2(n-6)	4.07 ± 1.11	5.24 ± 0.74	6.55 ± 1.77	12.28 ± 2.48
20:4(n-6)	0.61 ± 0.29	1.22 ± 0.09	0.61 ± 0.12	0.92 ± 0.18
Σ (n-6)	8.55 ± 2.19	10.55 ± 0.18	10.68 ± 3.05	18.04 ± 0.63
18:3(n-3)	1.36 ± 0.37	2.49 ± 0.53	0.90 ± 0.28	0.61 ± 0.24
20:5(n-3)	2.13 ± 0.65	9.81 ± 4.38	2.80 ± 0.32	10.45 ± 3.03
22:5(n-3)	1.11 ± 0.87	1.38 ± 0.83	2.41 ± 1.66	1.98 ± 0.76
22:6(n-3)	0.69 ± 0.05	3.01 ± 1.20	1.06 ± 0.33	4.18 ± 1.16
Σ (n-3)	8.40 ± 0.93	18.90 ± 4.62	10.80 ± 1.10	19.96 ± 3.25
Σ PUFAs	2.18 ± 4.02	34.98 ± 3.75	27.38 ± 3.01	43.16 ± 5.74
(n-3)/(n-6)	1.03 ± 0.29	1.79 ± 0.42	1.09 ± 0.38	1.11 ± 0.06

Values and sums (Σs) are means of single samples from each of 2-4 lobsters.

Sums may reflect FAs not listed in this table.

Appendix 7-F. Fatty Acid Composition (Weight % ± S.D.) of the Gills of Lobsters Reared at 20°C.

Diet	A		B		C	
Lipid Fraction	NL	PL	NL	PL	NL	PL
Fatty Acid						
14:0	4.42 ± 0.76	3.16 ± 0.43	3.80 ± 1.36	1.25 ± 0.23	5.41 ± 1.72	1.33 ± 0.56
16:0	18.80 ± 3.13	11.42 ± 2.34	16.74 ± 2.00	12.28 ± 1.70	18.19 ± 4.85	10.27 ± 1.20
18:0	5.27 ± 1.58	3.63 ± 0.88	4.91 ± 0.92	2.60 ± 0.48	5.52 ± 3.15	4.28 ± 0.99
Σ SFAs	32.23 ± 4.86	22.59 ± 3.48	28.19 ± 4.49	17.98 ± 1.77	32.75 ± 7.70	17.30 ± 1.12
16:1(n-7)	9.95 ± 4.50	6.97 ± 2.75	7.63 ± 1.87	3.83 ± 0.49	8.47 ± 0.18	4.37 ± 0.40
18:1(n-9)	24.35 ± 5.80	25.56 ± 4.77	29.99 ± 10.31	37.27 ± 4.84	17.51 ± 2.56	32.71 ± 5.53
Σ MFAs	41.98 ± 6.83	41.68 ± 2.62	45.34 ± 7.99	48.40 ± 4.04	33.95 ± 2.17	46.32 ± 4.44
18:2(n-6)	2.67 ± 0.81	2.18 ± 0.88	4.78 ± 0.98	9.97 ± 1.12	2.65 ± 0.85	2.47 ± 0.24
20:4(n-6)	0.32 ± 0.18	0.61 ± 0.64	0.57 ± 0.44	0.61 ± 0.25	0.54 ± 0.44	0.68 ± 0.20
Σ (n-6)	6.93 ± 0.86	7.12 ± 0.92	9.89 ± 1.85	15.10 ± 1.36	9.27 ± 0.38	8.04 ± 1.04
18:3(n-3)	0.69 ± 0.59	0.15 ± 0.31	0.44 ± 0.22	0.27 ± 0.08	0.83 ± 0.17	3.48 ± 0.70
20:5(n-3)	2.09 ± 0.89	1.68 ± 0.89	1.82 ± 0.51	4.67 ± 1.38	1.87 ± 1.09	4.26 ± 0.66
22:5(n-3)	1.87 ± 0.74	4.33 ± 1.01	2.08 ± 1.23	0.84 ± 0.33	2.50 ± 1.14	2.92 ± 0.86
22:6(n-3)	1.05 ± 1.03	2.20 ± 1.91	2.03 ± 0.87	2.05 ± 0.92	1.61 ± 0.37	2.23 ± 1.10
Σ (n-3)	9.19 ± 1.71	13.26 ± 1.73	10.52 ± 4.23	10.24 ± 2.52	12.67 ± 3.61	16.63 ± 2.50
Σ PUFAs	22.29 ± 4.23	30.18 ± 1.81	24.62 ± 6.52	30.98 ± 4.44	27.41 ± 7.46	29.96 ± 3.92
(n-3)/(n-6)	1.35 ± 0.31	1.88 ± 0.28	1.04 ± 0.32	0.67 ± 0.13	0.40 ± 0.16	2.07 ± 0.13

Values and sums (Σs) are means of single samples from each of 2-4 lobsters. Sums may reflect FAs not listed in this table.

(continued)

Appendix 7-F. (con't) FA Composition (Wt %) of the Gills of Lobsters at 20°C.

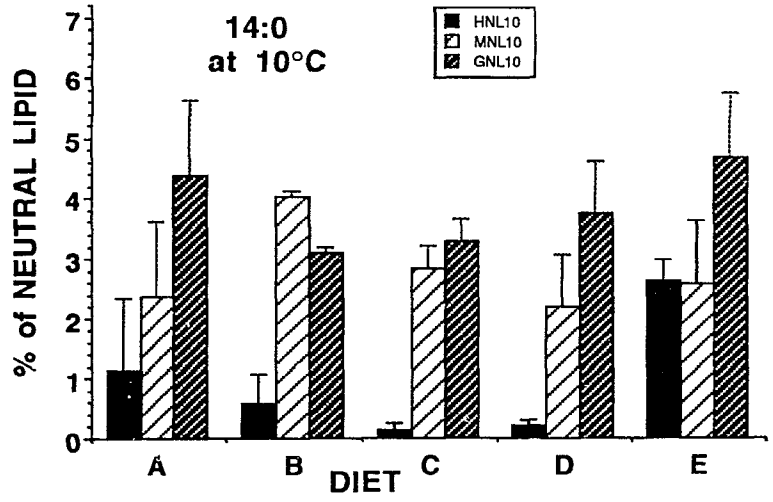
Diet	D		E	
Lipid Fraction	NL	PL	NL	PL
Fatty Acid				
14:0	2.98 ± 0.11	1.40 ± 0.47	3.84 ± 1.48	1.58 ± 0.57
16:0	15.57 ± 2.04	10.10 ± 0.68	17.64 ± 0.96	13.92 ± 0.69
18:0	4.28 ± 0.99	3.03 ± 0.86	4.61 ± 0.95	4.32 ± 1.36
Σ SFAs	25.36 ± 3.35	16.26 ± 1.63	28.77 ± 2.15	2.55 ± 1.66
16:1(n-7)	8.35 ± 0.54	3.28 ± 0.72	7.85 ± 2.21	1.95 ± 0.67
18:1(n-9)	31.92 ± 5.56	41.94 ± 6.72	17.12 ± 3.97	13.17 ± 3.17
Σ MFAs	46.77 ± 3.98	54.05 ± 4.52	34.78 ± 1.45	25.54 ± 1.07
18:2(n-6)	2.81 ± 0.08	4.22 ± 0.41	9.35 ± 3.65	12.07 ± 2.65
20:4(n-6)	0.50 ± 0.18	0.91 ± 0.19	0.76 ± 0.18	0.72 ± 0.37
Σ (n-6)	8.17 ± 2.15	8.06 ± 1.02	14.55 ± 4.02	17.68 ± 0.80
18:3(n-3)	1.48 ± 0.52	3.20 ± 0.38	0.60 ± 0.25	0.54 ± 0.53
20:5(n-3)	1.54 ± 0.39	5.35 ± 1.34	4.71 ± 1.76	11.32 ± 0.54
22:5(n-3)	1.46 ± 0.46	1.56 ± 0.73	1.34 ± 0.38	2.93 ± 1.70
22:6(n-3)	0.57 ± 0.30	1.77 ± 0.67	2.55 ± 0.07	4.92 ± 1.65
Σ (n-3)	7.94 ± 0.76	14.10 ± 0.63	11.23 ± 0.98	22.71 ± 1.34
Σ PUFAs	25.11 ± 3.62	26.57 ± 1.74	33.16 ± 2.51	46.43 ± 0.54
(n-3)/(n-6)	1.05 ± 0.43	1.76 ± 0.15	0.80 ± 0.18	1.28 ± 0.02

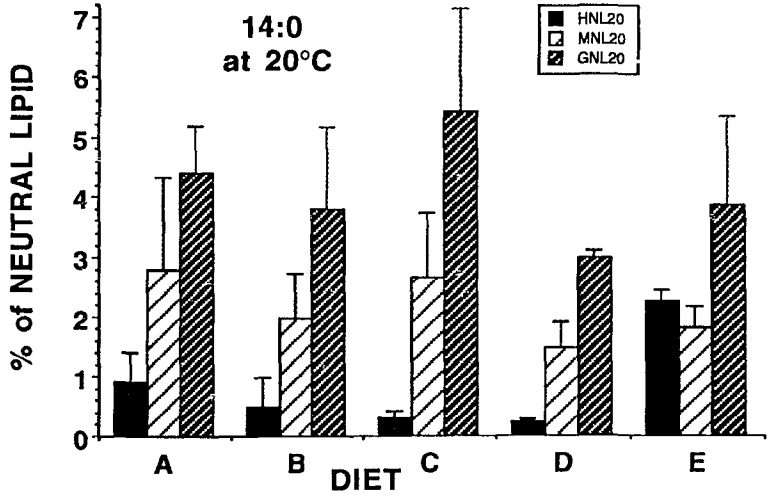
Values and sums (Σs) are means of single samples from each of 2-4 lobsters.

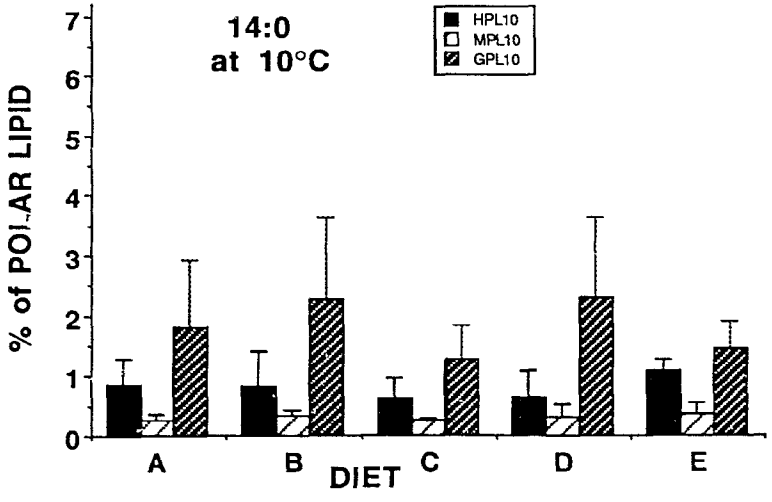
Sums may reflect FAs not listed in this table.

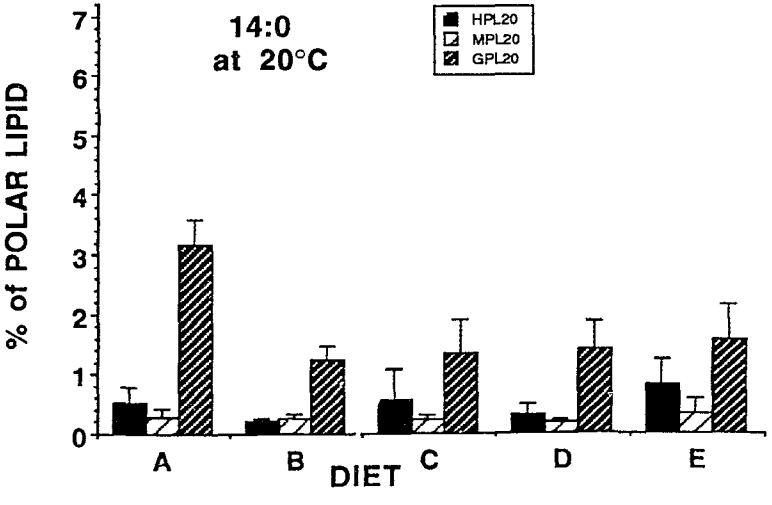
Appendix 8.

Percent Contribution of Each Fatty Acid (in Weight %) to the NL and PL Fractions of the Hepatopancreas, Muscles and Gills of Lobsters Reared at 10°C or 20°C and Fed Diet A, B, C, D, or E.

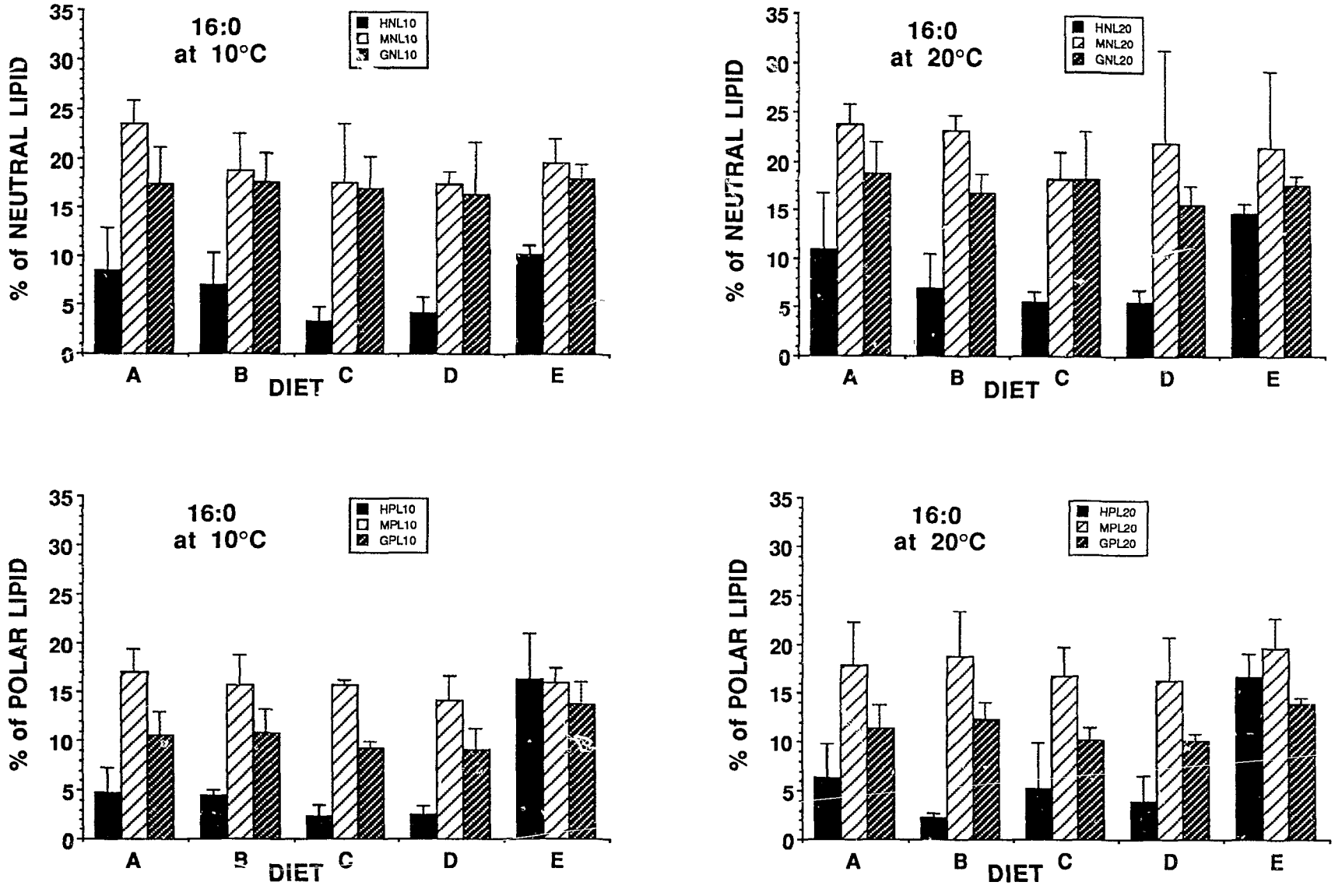




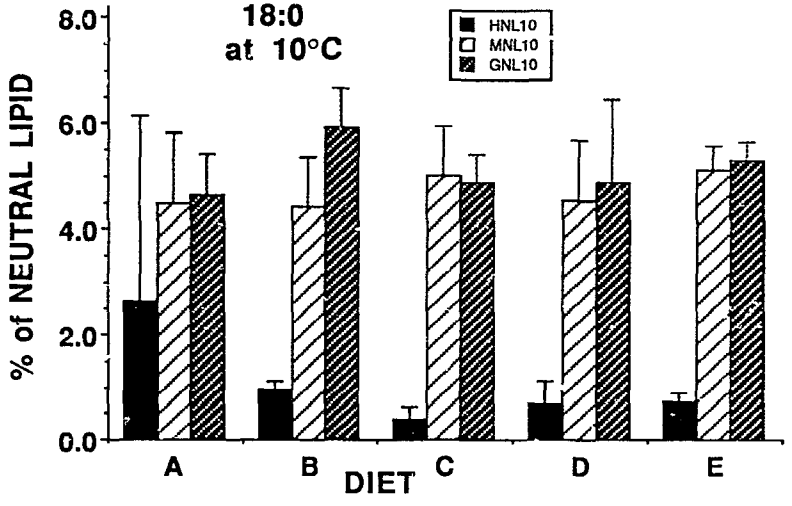


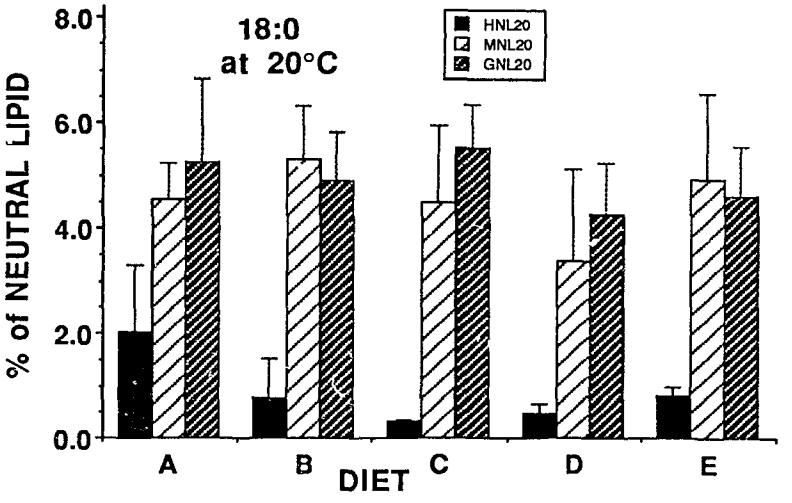


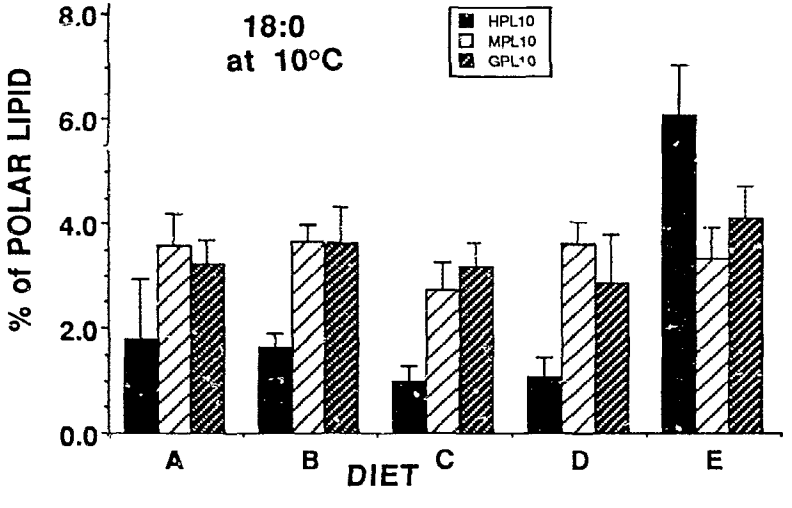
Appendix 8-A. Percent Contribution of 14:0 (in weight)

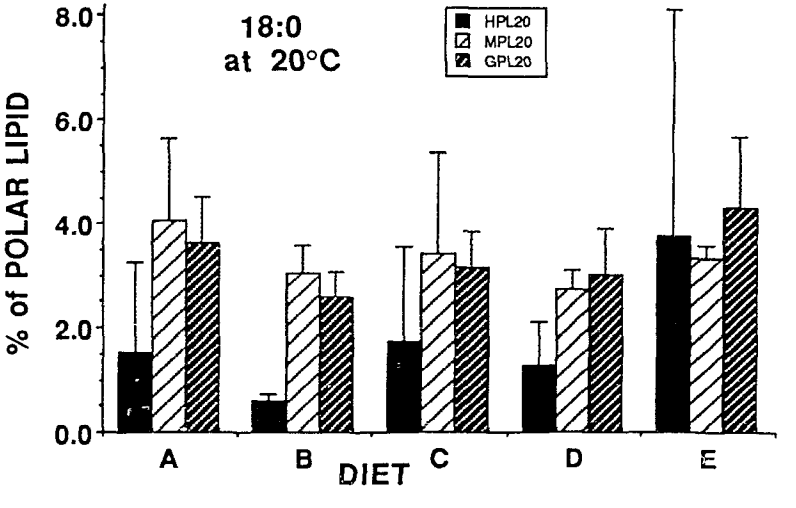


Appendix 8-B. Percent Contribution of 16:0 (in weight %).

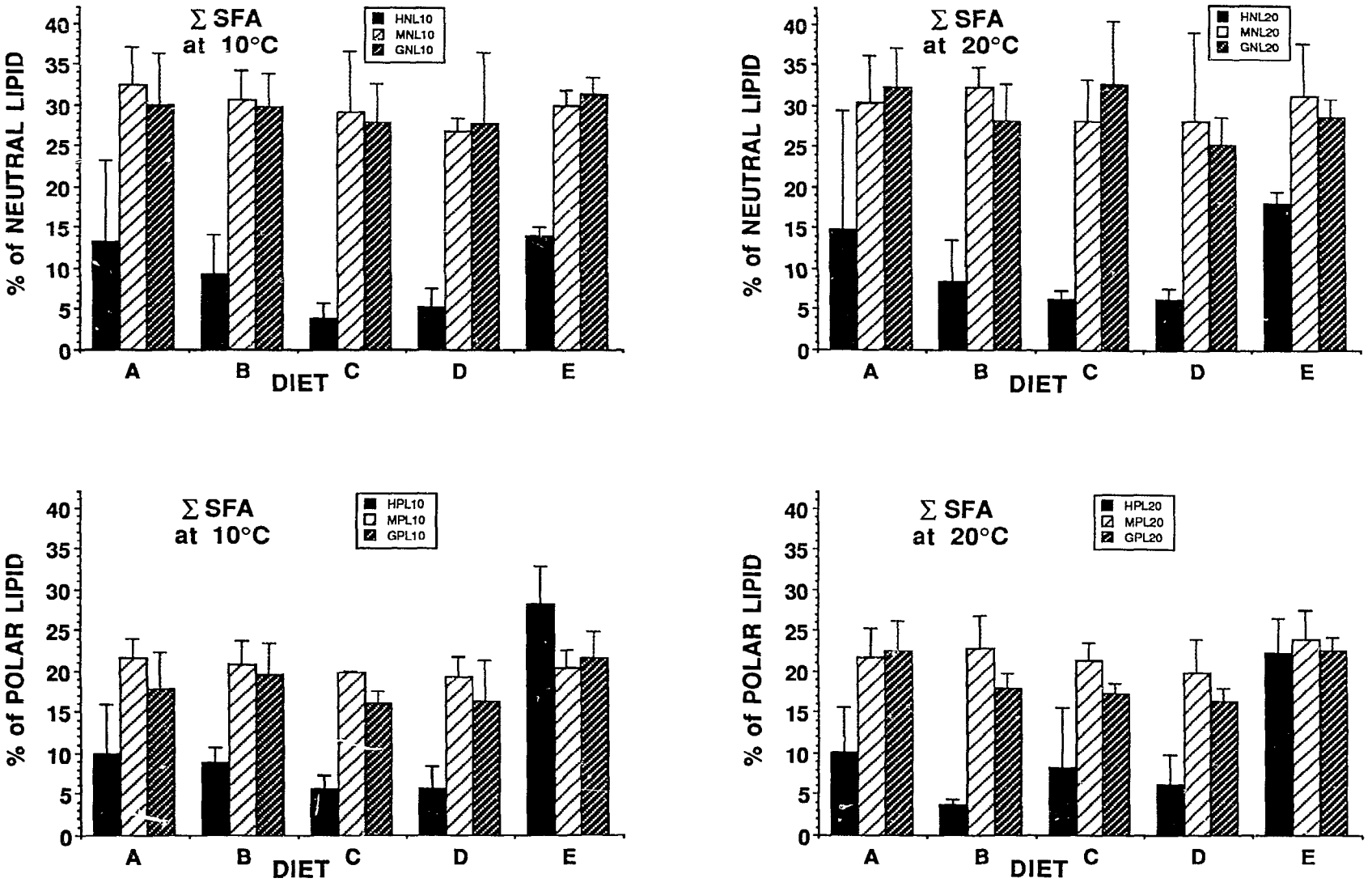




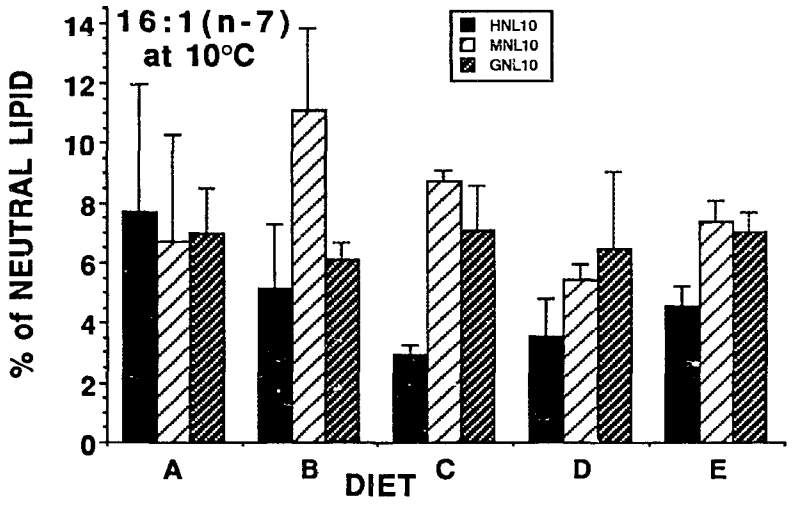


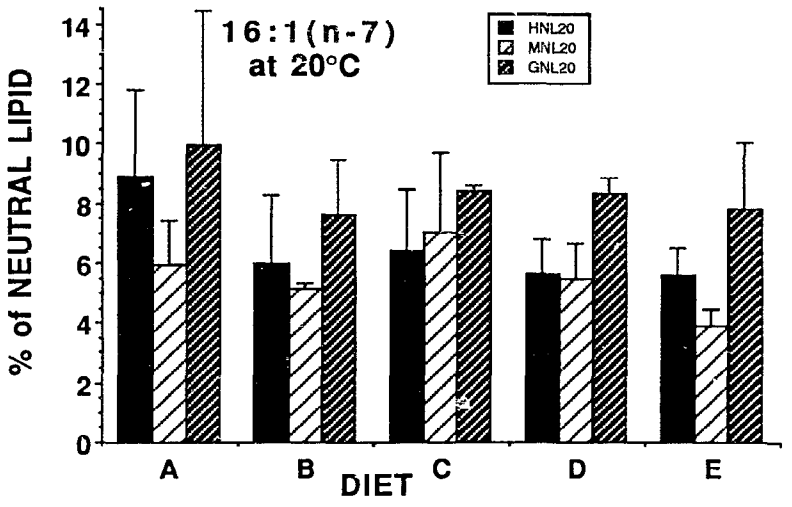


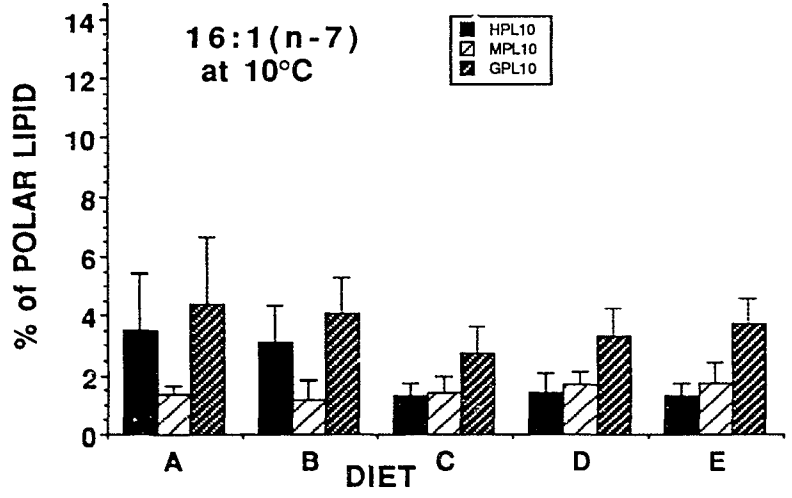
Appendix 8-C. Percent Contribution of 18:0 (in weight %).

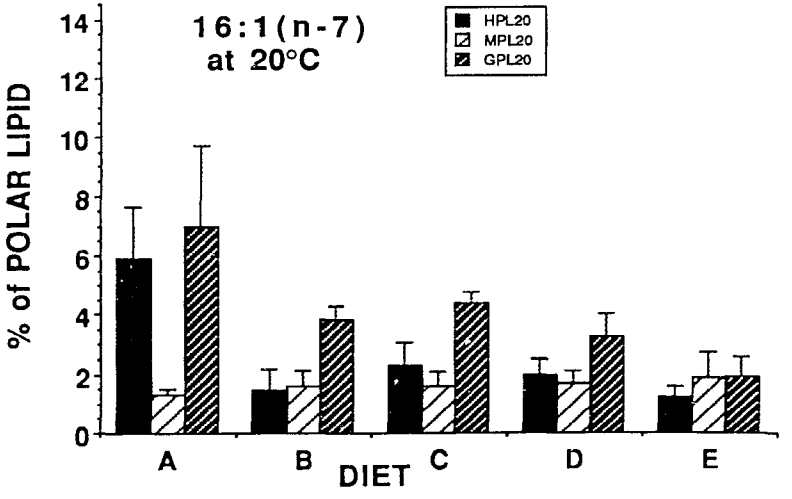


Appendix 8-D. Percent Contribution of ΣSFAs (in weight %).

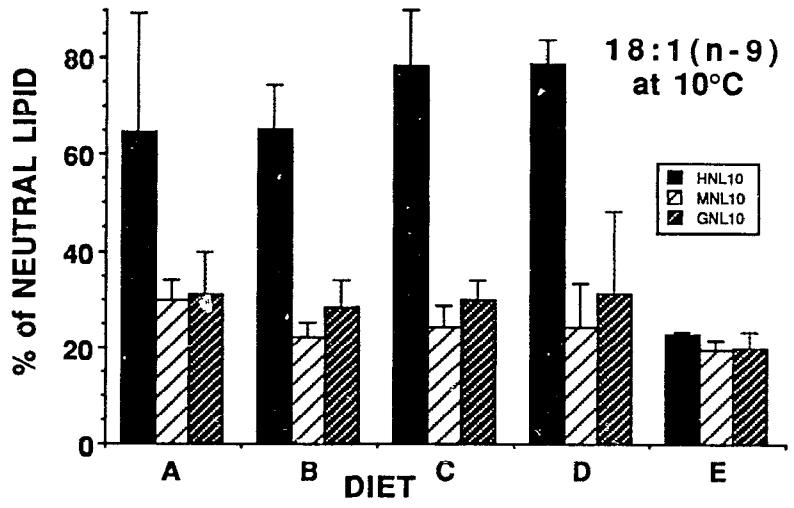


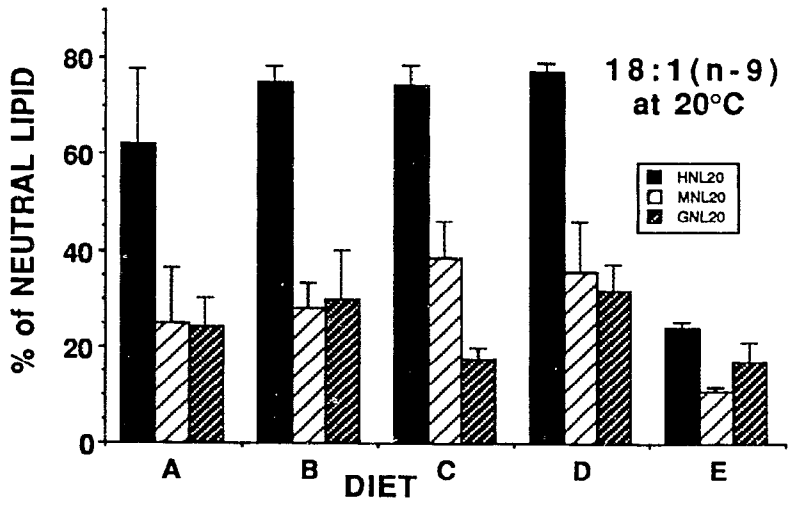


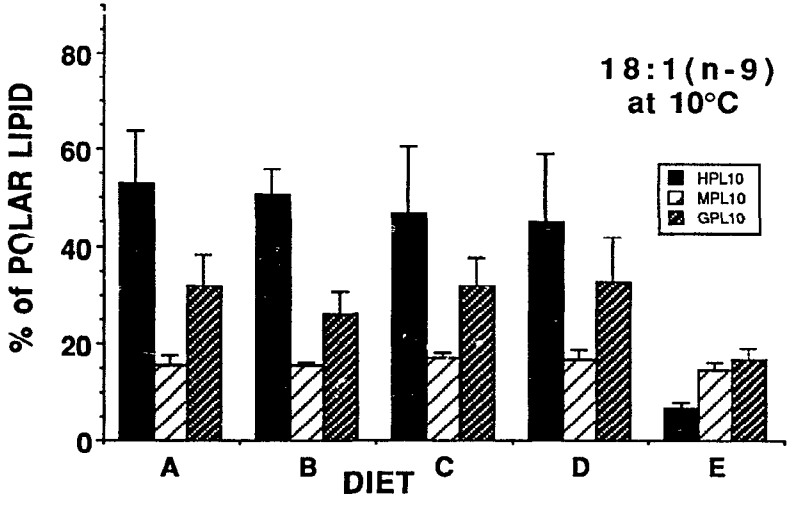


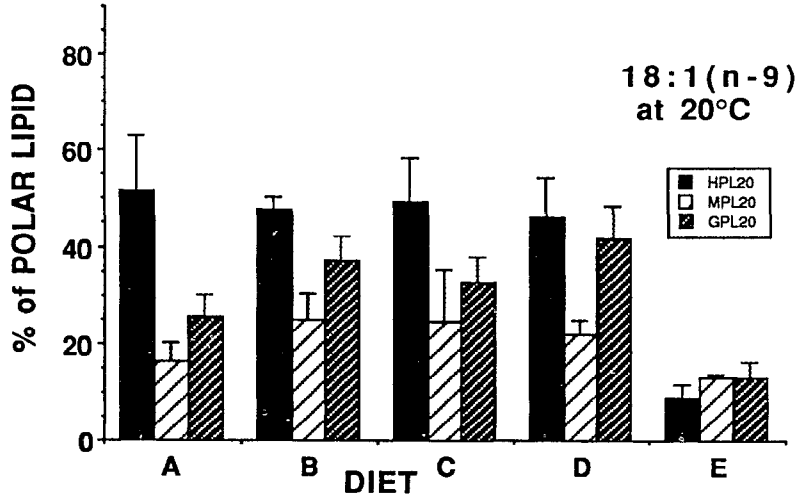


Appendix 8-E. Percent Contribution of 16:1(n-7) (in weight %).

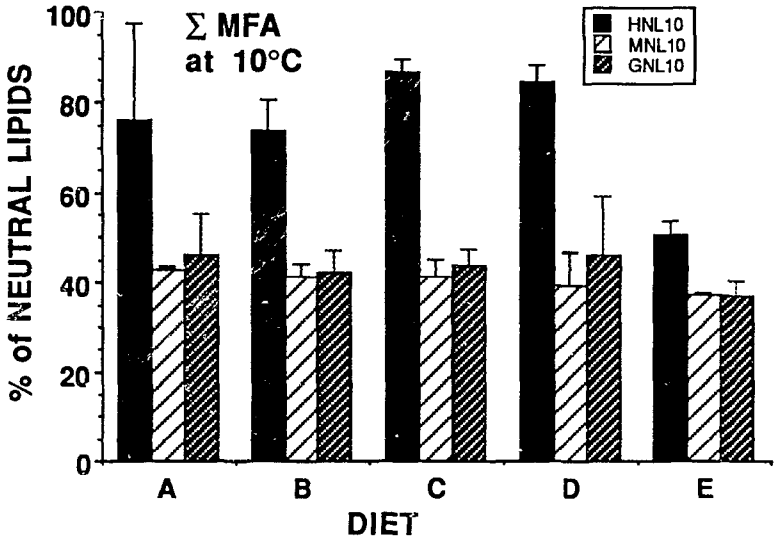


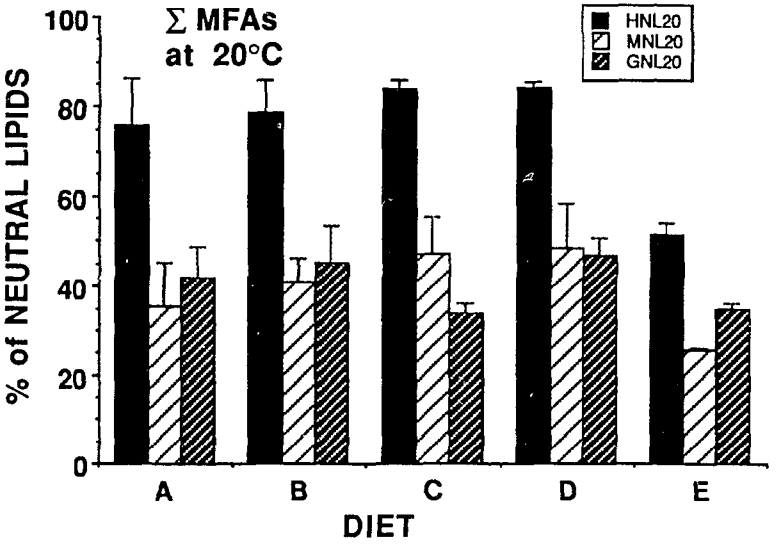


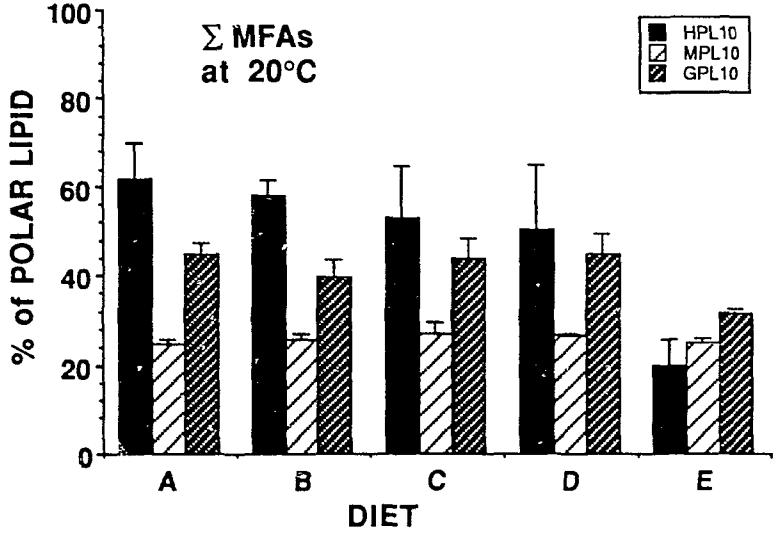


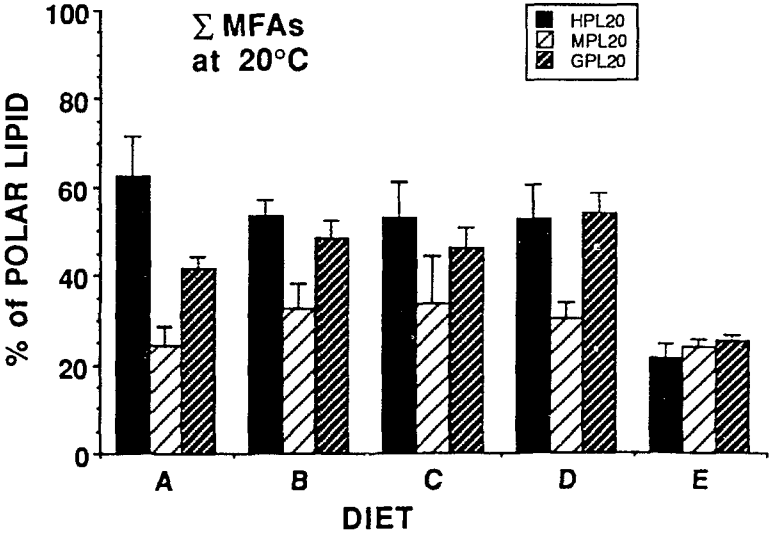


Appendix 8-F. Percent Contribution of 18:1(n-9) (in weight %).

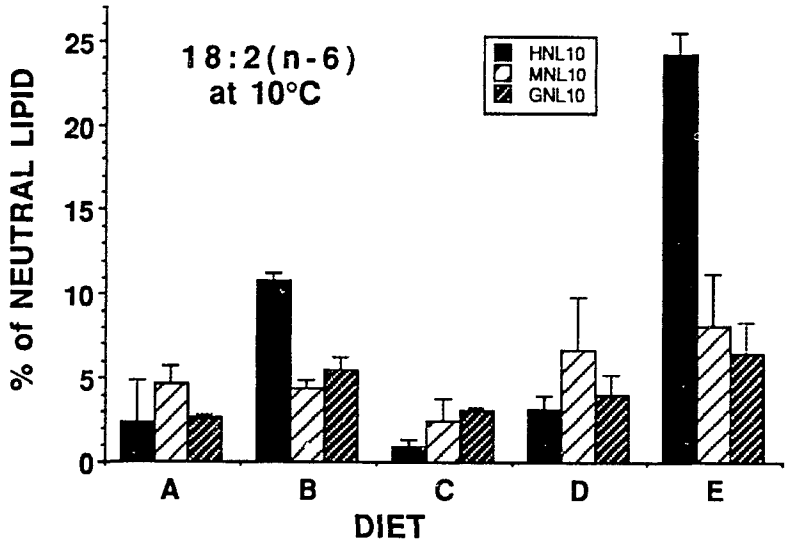


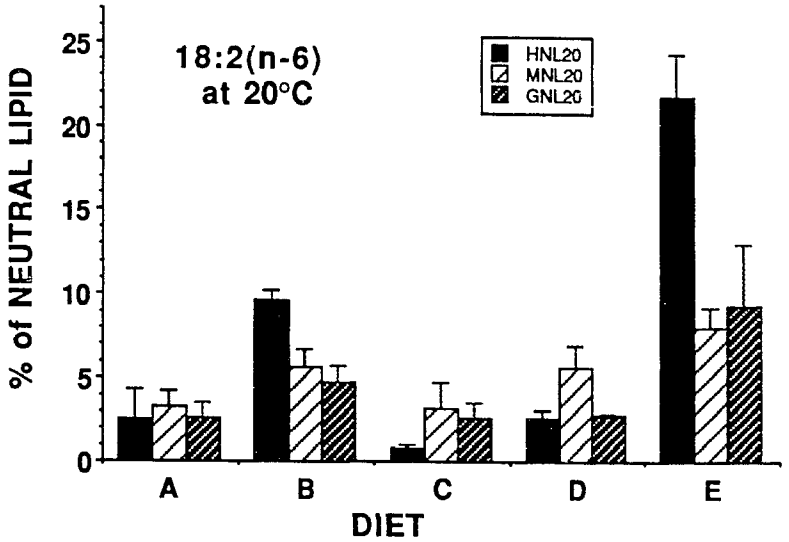


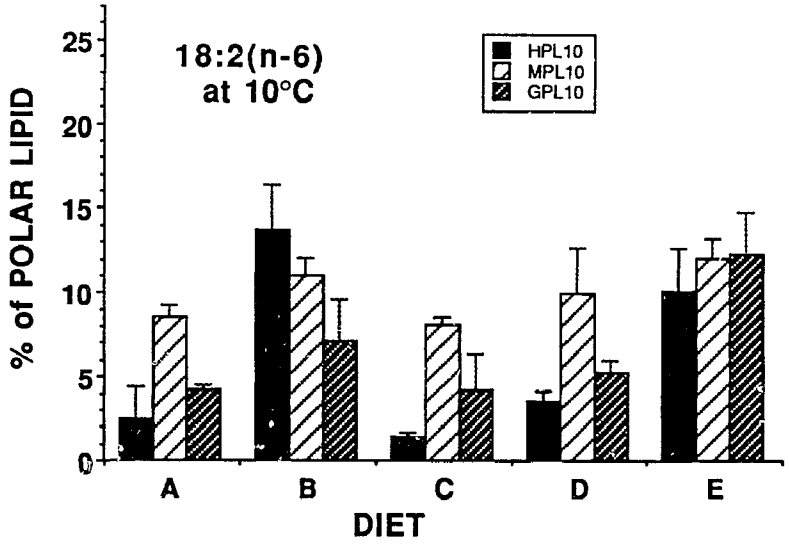


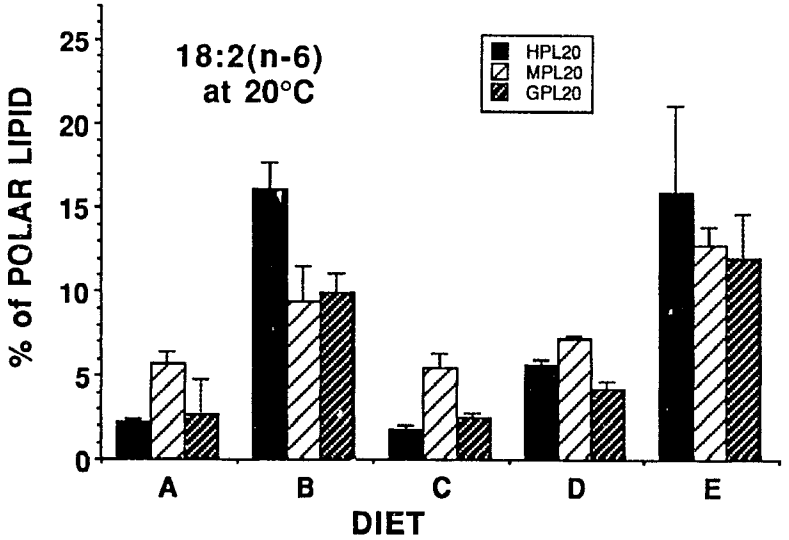


Appendix 8-G. Percent Contribution of Total MFAs (in weight %).

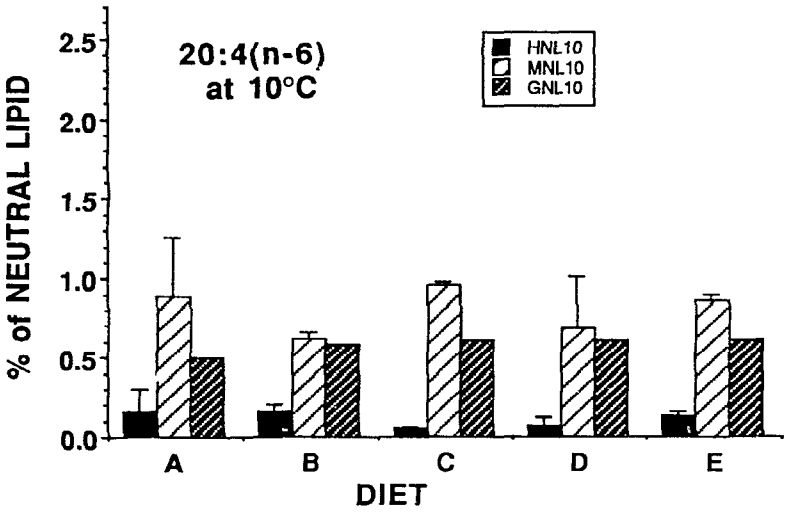


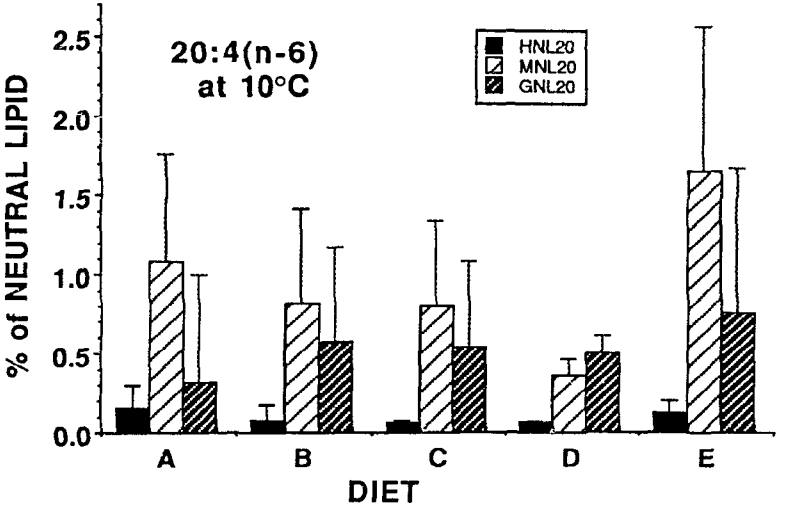


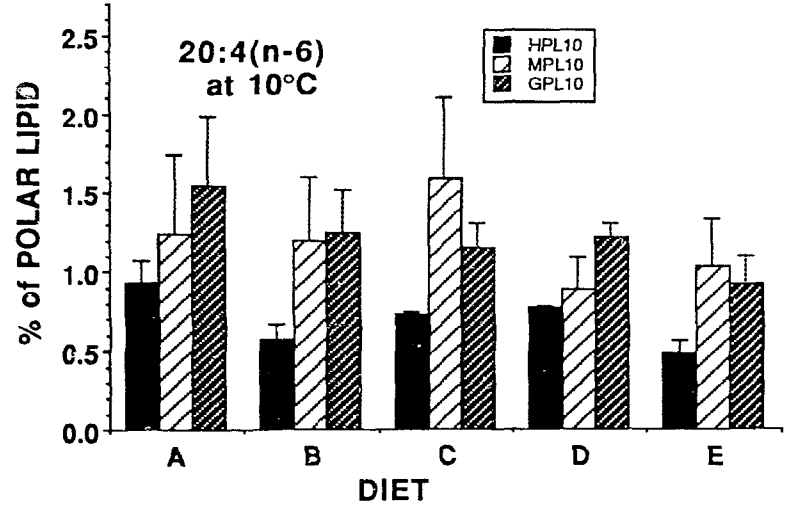


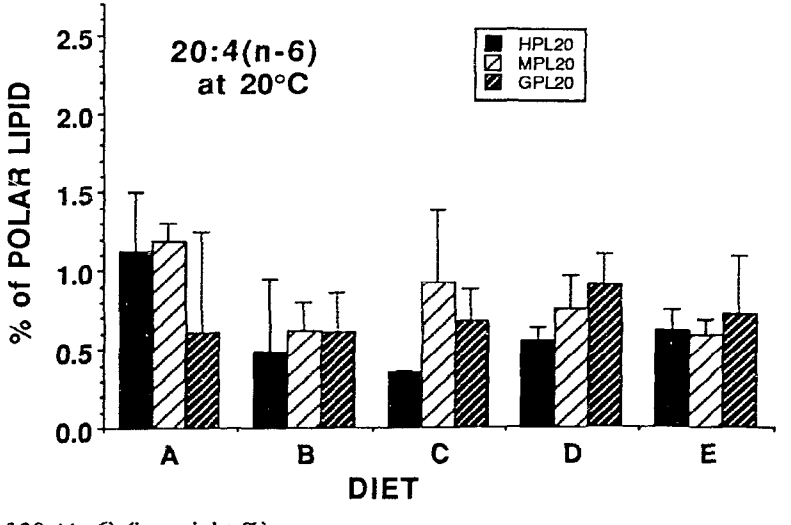


Appendix 8-H. Percent Contribution of 18:2(n-6) (in weight %).

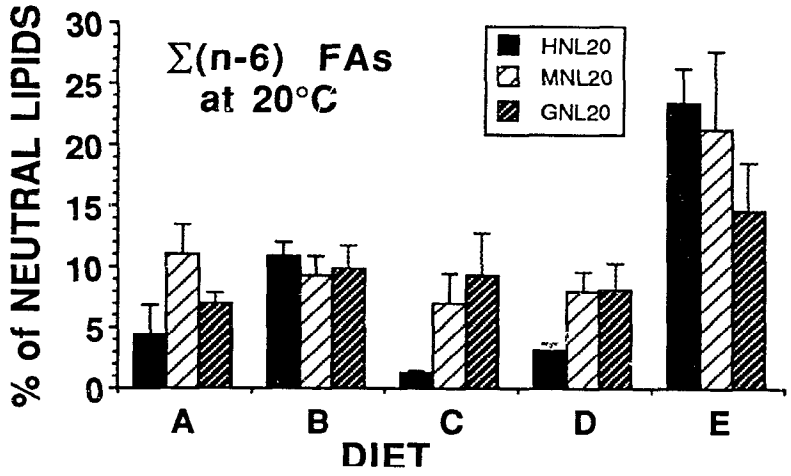


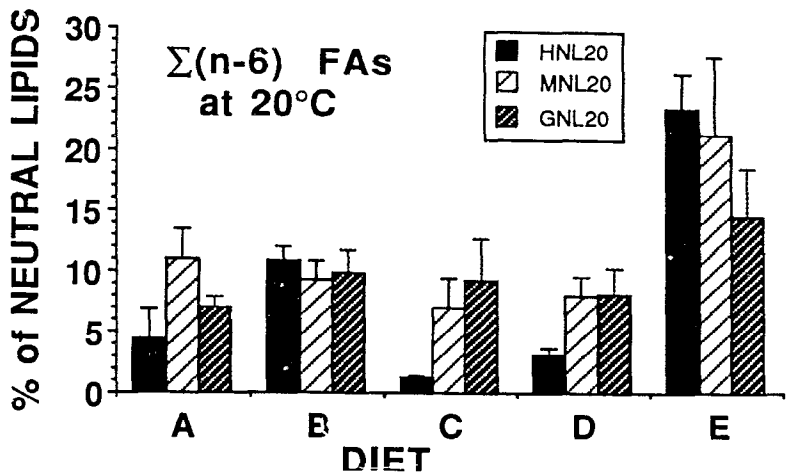


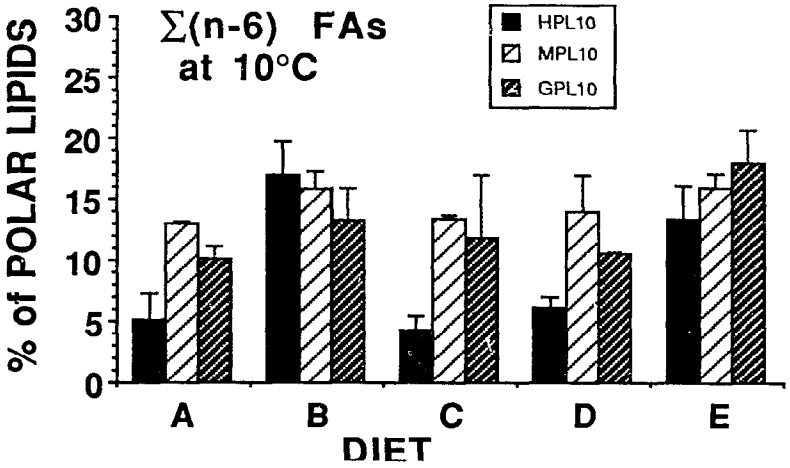


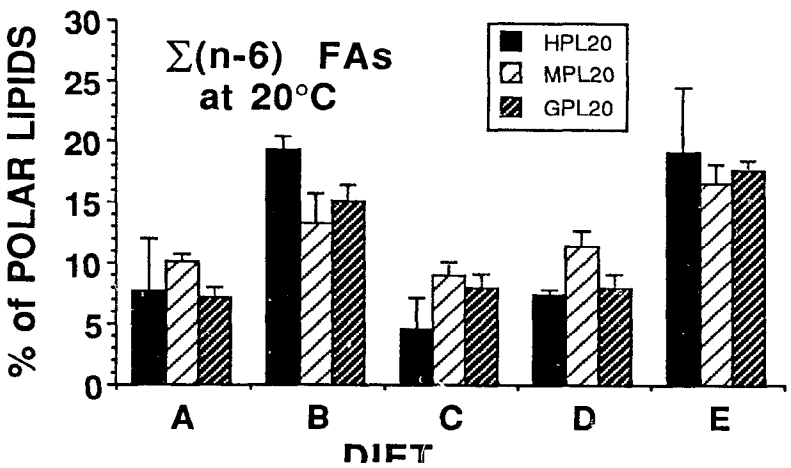


Appendix 8-I. Percent Contribution of 20:4(n-6) (in weight %).

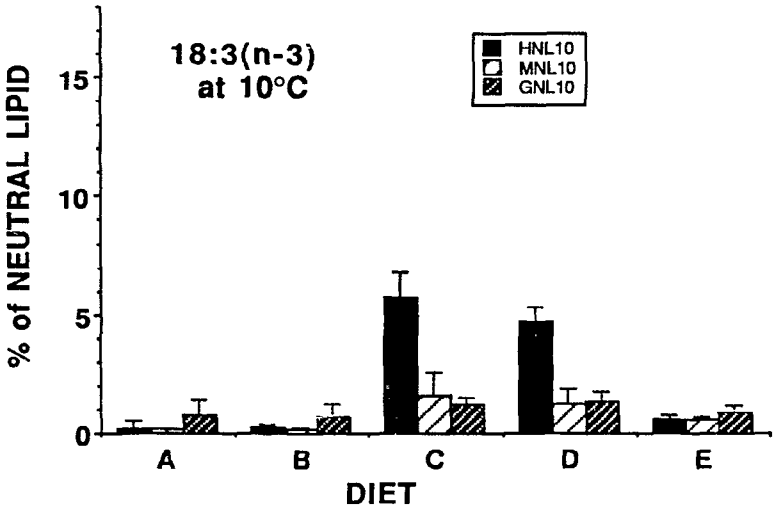


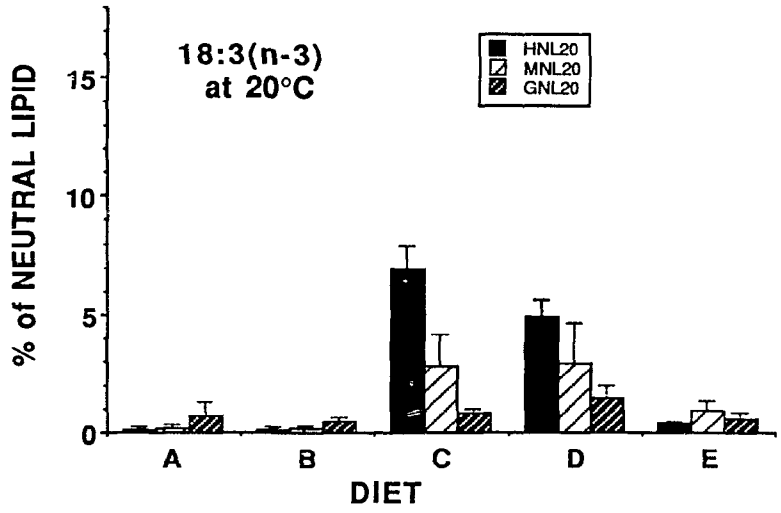


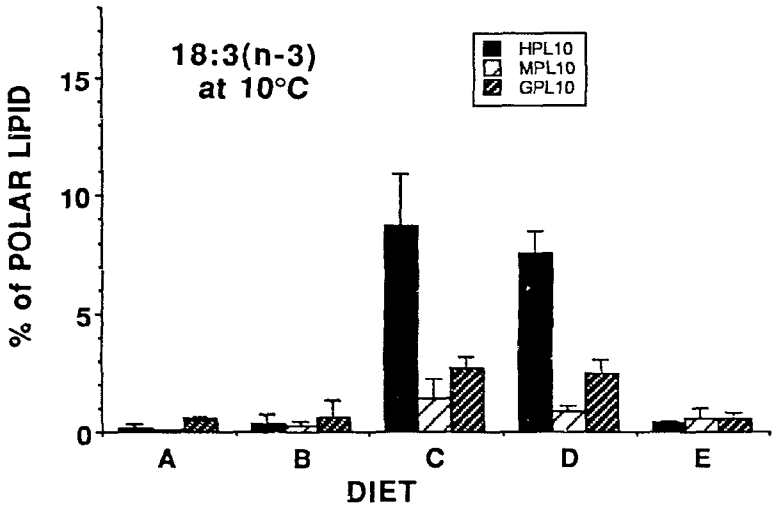


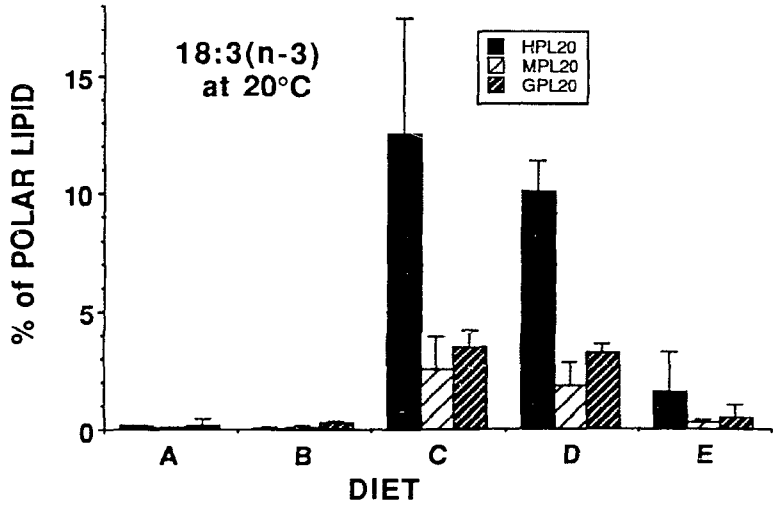


Appendix J. Percent Contribution of Total (n-6) Fatty Acids (in weight %).

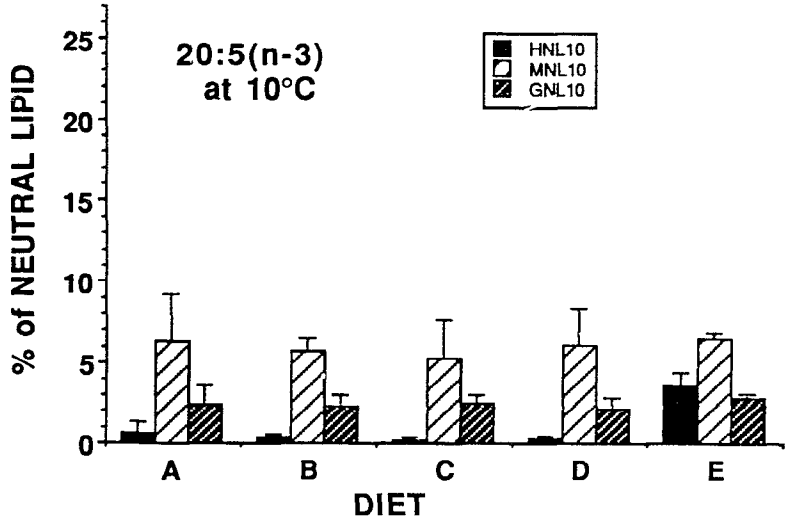


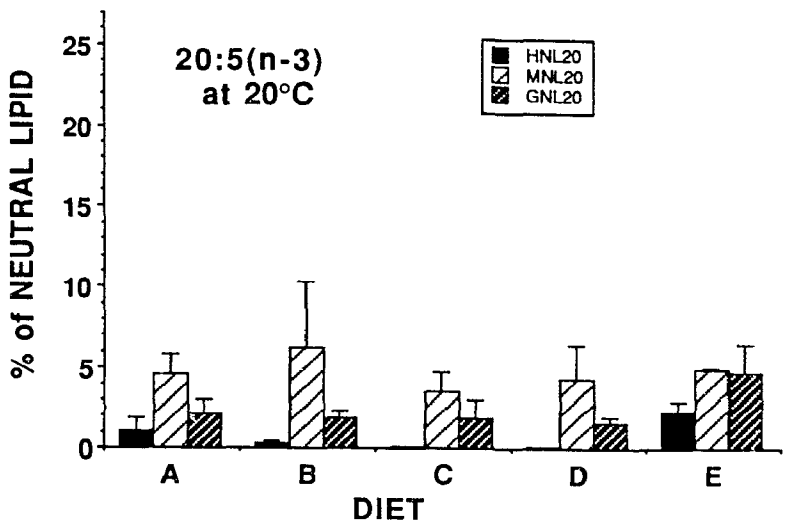


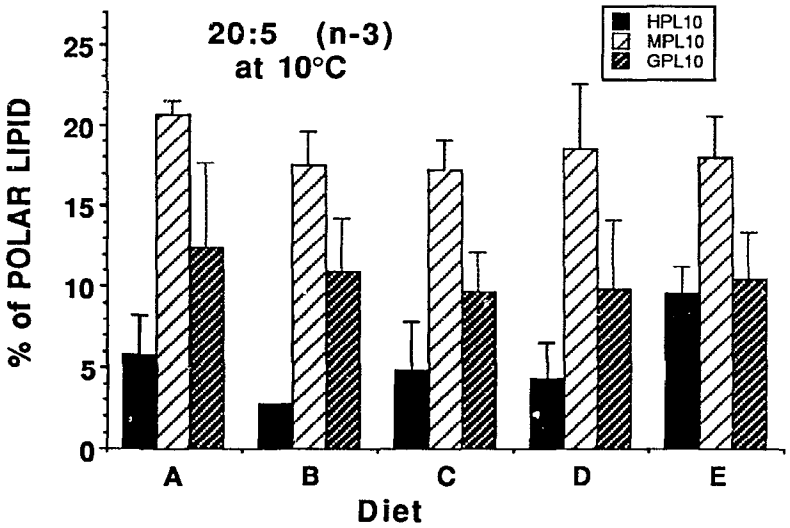


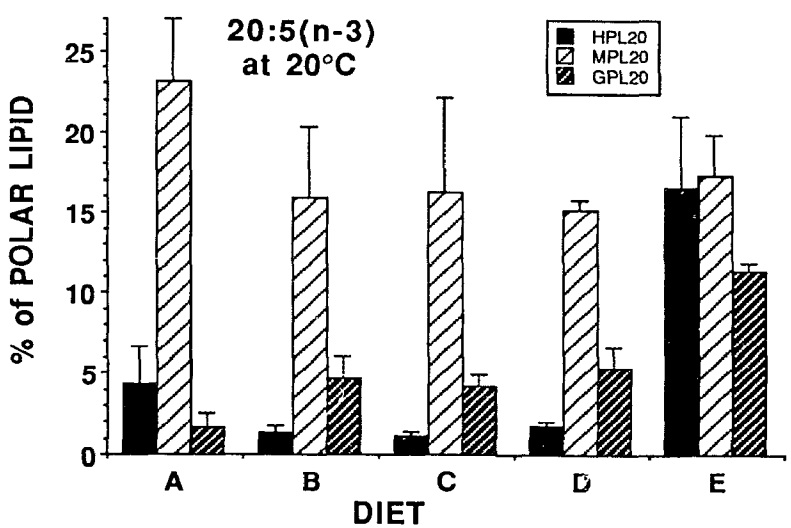


Appendix 8-K. Percent Contribution of 18:3(n-3) (in weight %).

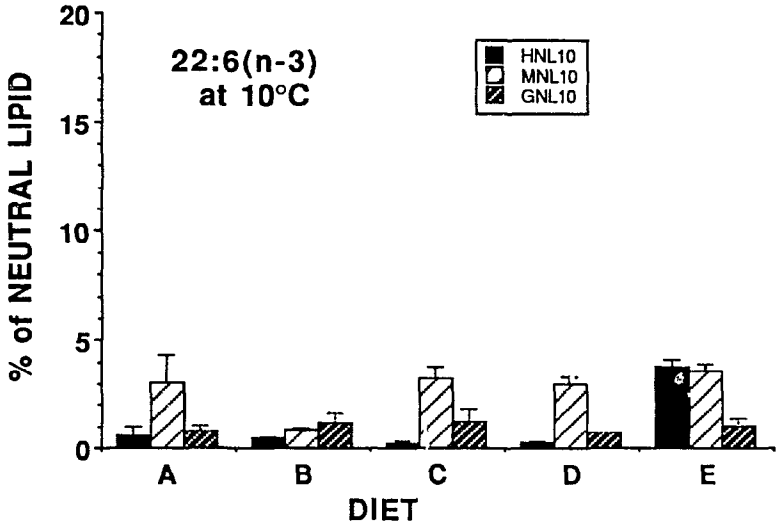


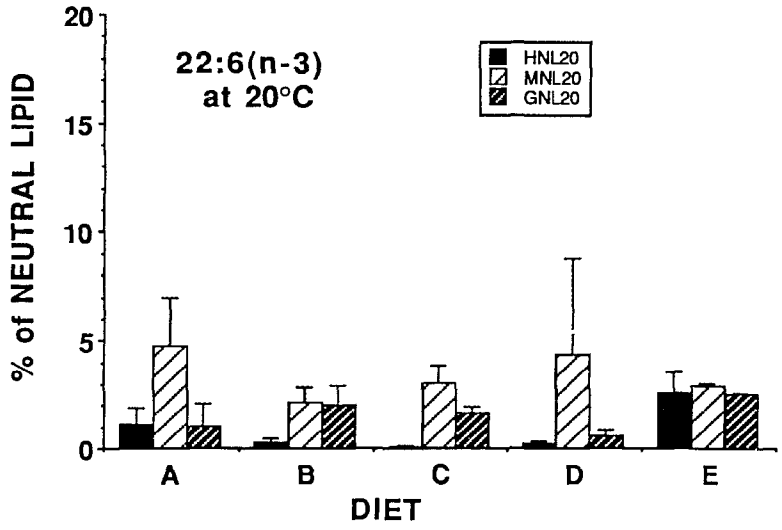


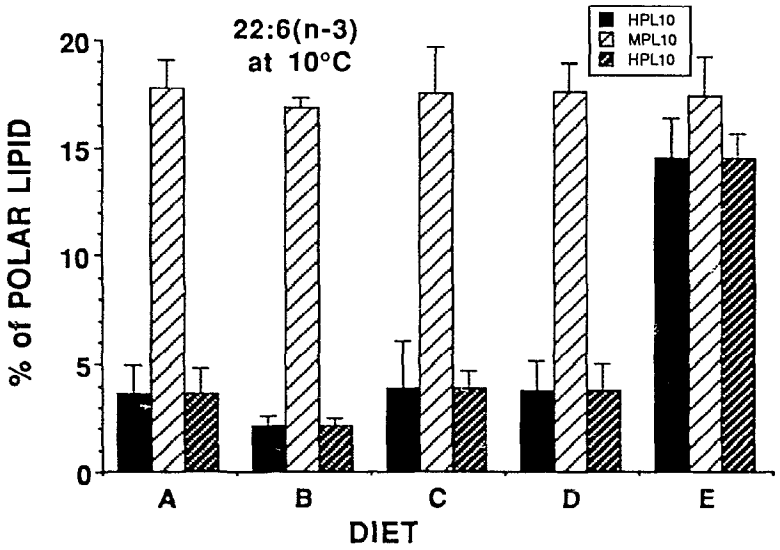


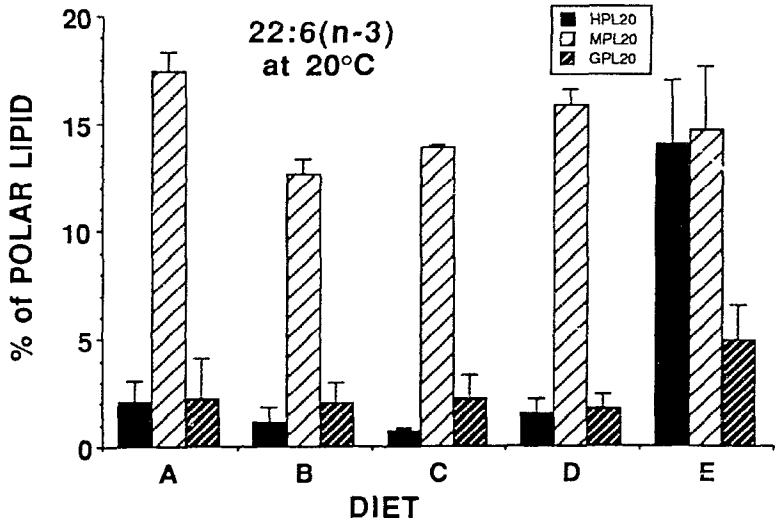


Appendix 8-L. Percent Contribution of 20:5(n-3) (in weight %).

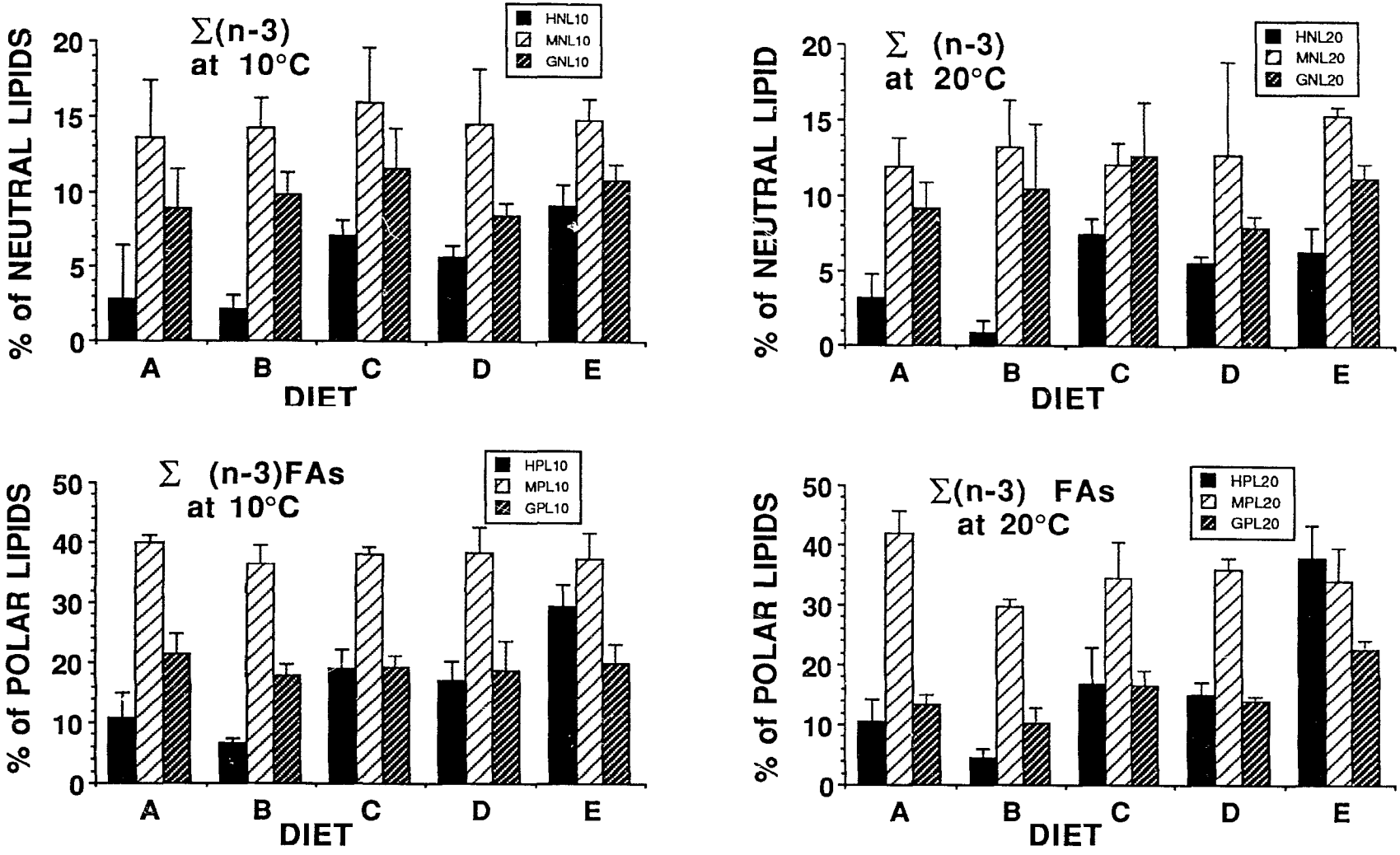




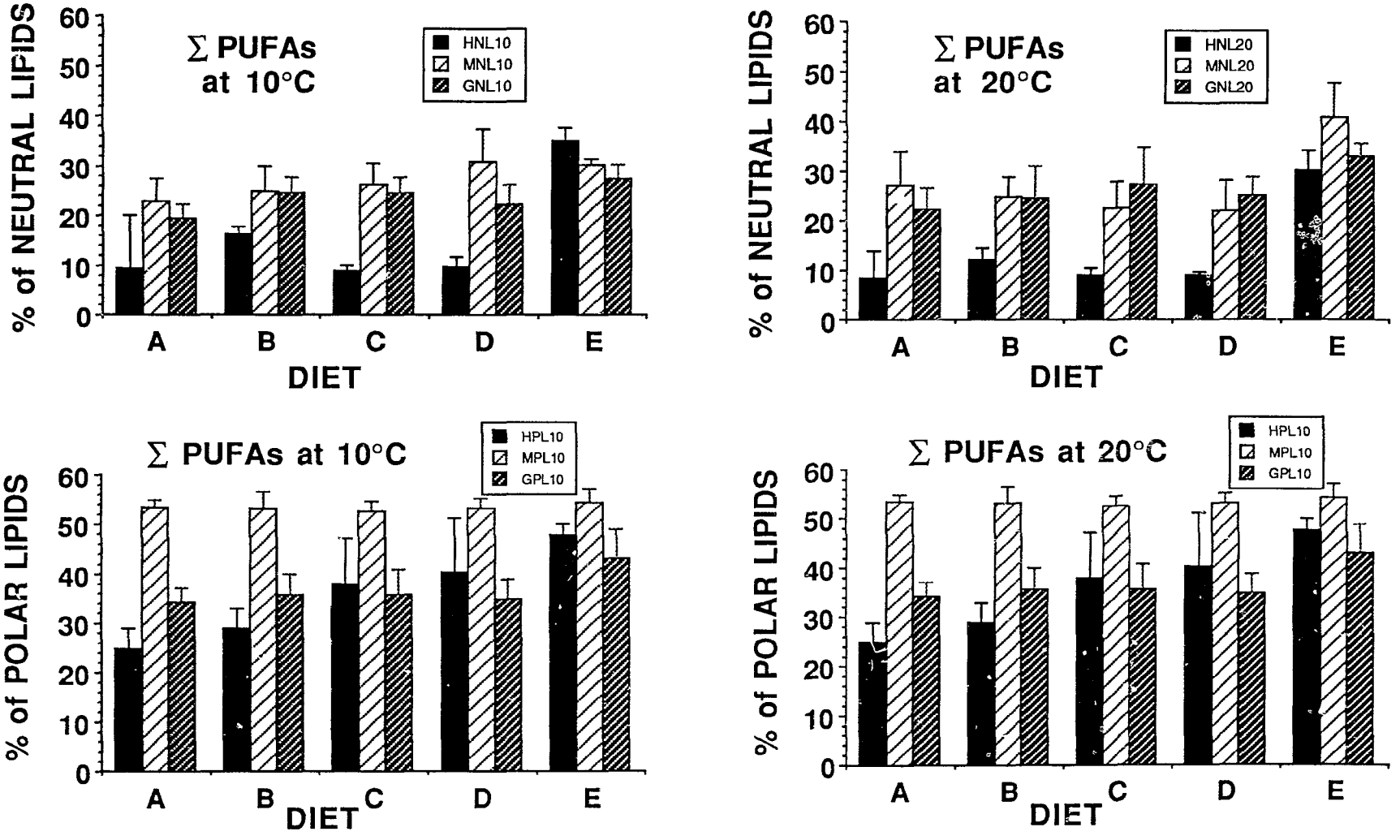




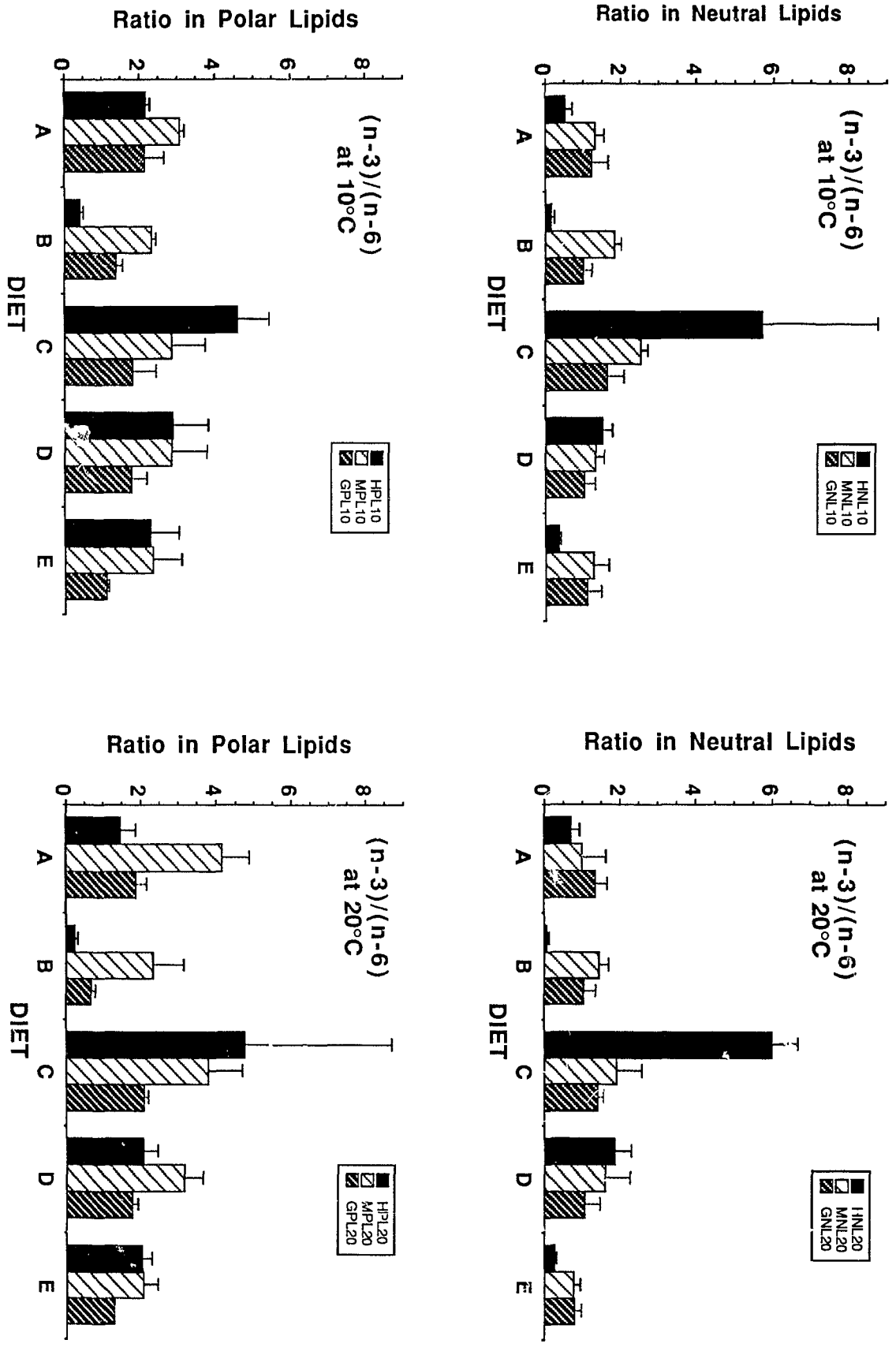
Appendix 8-M. Percent Contribution of 22:6(n-3) (in weight %).



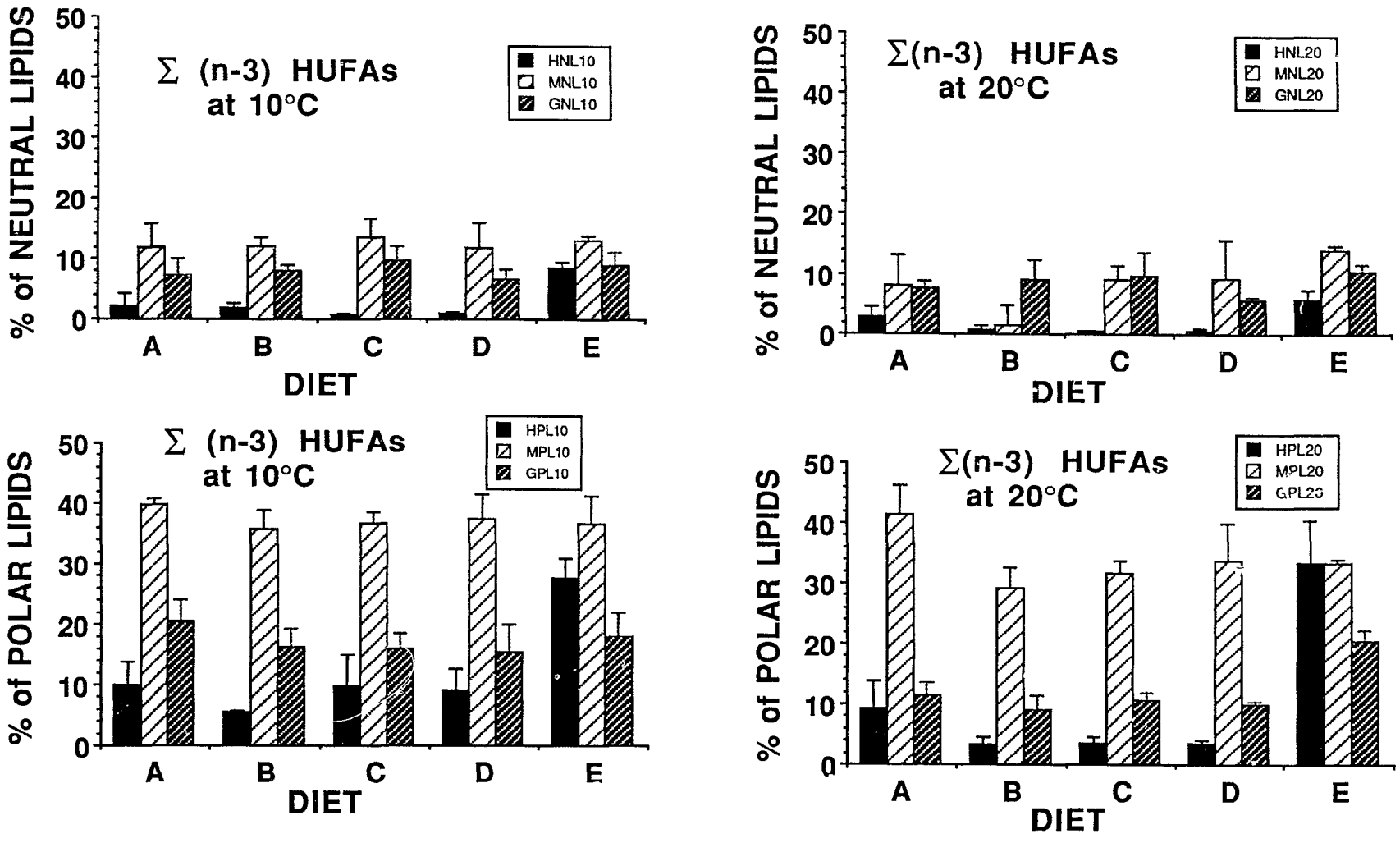
Appendix N. Percent Contribution of Total (n-3) Fatty Acids (in weight %).



Appendix O. Percent Contribution of Total PUFAs (in weight %).



Appendix 8-P. Ratio of (n-3)/(n-6) FAs



Appendix Q. Percent Contribution of Total (n-3) HUFAs (in weight %).

Appendix 9.

Amount of Each Fatty Acid (in μg) in the NL and PL Fractions of the Hepatopancreas, Muscles and Gills of Lobsters Reared at 10°C or 20°C and Fed Diet A, B, C, D, or E.

Appendix 9-A.

Amount (μg) of 14:0 in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-A for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		12.7	2.4	15.6	5.1	24.9	4.0
	B		10.9	3.6	9.2	4.3	30.3	4.5
	C		10.8	2.3	3.9	3.7	33.5	5.7
	D		13.5	4.0	2.5	2.9	19.7	4.9
	E		10.3	1.9	267.7	7.7	17.8	3.4
20	A		11.1	9.2	4.6	1.2	17.8	4.5
	B		16.3	2.1	19.5	1.3	16.3	3.7
	C		15.7	1.9	4.5	2.5	13.2	2.1
	D		9.8	3.2	6.0	2.4	15.8	2.7
	E		13.8	3.8	716.4	12.1	26.8	7.3

Appendix 9-B.

Amount (μg) of 16:0 in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-B for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		13.5	4.2	35.9	9.6	37.3	100.8
	B		20.7	5.8	15.1	8.7	33.3	48.8
	C		16.2	5.7	11.1	5.9	59.2	61.7
	D		17.6	5.0	8.5	5.0	40.5	57.2
	E		11.7	5.4	77.4	43.2	35.3	31.3
20	A		12.4	10.5	10.3	4.9	29.1	57.3
	B		21.1	4.4	30.0	3.8	44.1	44.4
	C		16.0	4.4	5.0	7.6	22.4	29.3
	D		14.1	13.8	12.3	10.3	36.4	38.4
	E		16.6	10.4	268.6	55.4	73.0	73.8

Appendix 9-C.

Amount (μg) of 18:0 in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-B for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		13.5	4.2	35.9	9.6	37.3	100.8
	B		20.7	5.8	15.1	8.7	33.3	48.8
	C		16.2	5.7	11.1	5.9	59.2	61.7
	D		17.6	5.0	8.5	5.0	40.5	57.2
	E		11.7	5.4	77.4	43.2	35.3	31.3
20	A		12.4	10.5	10.3	4.9	29.1	57.3
	B		21.1	4.4	30.0	3.8	44.1	44.4
	C		16.0	4.4	5.0	7.6	22.4	29.3
	D		14.1	13.8	12.3	10.3	36.4	38.4
	E		16.6	10.4	268.6	55.4	73.0	73.8

Appendix 9-D.

Amount (μg) of SFAs in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-D for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		86.7	23.1	148.2	50.3	260.5	343.6
	B		104.4	31.3	129.0	38.3	229.5	276.8
	C		92.2	28.7	95.6	26.5	344.2	450.8
	D		100.2	28.3	54.5	20.7	239.2	305.9
	E		68.9	28.2	1342.7	163.0	206.7	191.9
20	A		80.6	65.5	63.5	20.6	202.8	365.9
	B		121.2	30.6	301.9	19.5	268.2	333.0
	C		95.0	24.2	90.8	28.4	141.0	183.1
	D		83.7	37.4	146.3	39.9	301.8	278.8
	E		103.6	54.1	5510.2	143.3	463.1	530.0

Appendix 9-E.

Amount (μg) of 16:1(n-7) in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-E for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		20.3	5.7	104.4	22.5	50.2	21.8
	B		21.4	6.5	80.5	16.6	83.2	15.6
	C		23.4	4.9	81.2	7.8	103.3	31.8
	D		22.8	5.7	43.1	6.4	48.4	27.2
	E		15.5	4.8	466.2	9.2	51.0	16.4
20	A		27.9	20.2	42.7	14.1	38.0	22.3
	B		32.8	6.5	233.8	9.2	42.8	23.7
	C		24.6	6.1	96.6	10.2	35.3	13.8
	D		27.6	7.5	142.0	16.0	58.4	23.7
	E		28.3	4.7	1803.7	18.4	57.9	41.3

Appendix 9-F.

Amount (μg) of 18:1(n-9) in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-F for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		90.4	39.8	880.7	347.0	219.3	286.0
	B		99.2	41.7	1022.0	268.3	183.8	254.7
	C		98.6	57.5	2175.6	277.4	314.6	473.3
	D		78.1	58.8	955.9	207.2	242.7	322.6
	E		43.8	21.5	2332.2	47.1	151.3	173.3
20	A		65.4	80.2	284.9	128.0	160.5	299.2
	B		137.1	66.1	2762.7	304.9	234.5	361.8
	C		55.7	49.3	1062.4	220.0	193.1	218.1
	D		111.9	101.0	1938.3	380.1	384.1	309.9
	E		73.4	32.7	8887.2	278.7	161.5	293.7

Appendix 9-G.

Amount (μg) of MFAs in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-D for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		134.2	58.4	1037.3	374.2	327.2	391.3
	B		148.4	63.6	1153.2	306.7	309.9	345.3
	C		145.5	79.4	2421.7	312.4	488.2	613.6
	D		166.8	78.3	1024.9	231.2	350.3	423.6
	E		81.4	41.0	4343.8	128.7	259.6	235.9
20	A		105.0	120.9	378.4	149.0	228.1	410.6
	B		195.0	82.3	3064.9	336.4	339.5	476.3
	C		98.5	64.8	1295.4	250.4	235.9	287.8
	D		154.3	124.3	2112.8	427.2	519.1	429.3
	E		125.2	61.3	14618.1	293.3	378.9	535.3

Appendix 9-H.

Amount (μg) of 18:2(n-6) in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-H for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		7.6	5.6	13.5	14.0	34.7	136.1
	B		19.1	11.4	169.4	67.5	33.2	146.0
	C		10.1	7.6	25.6	8.5	29.3	184.3
	D		14.7	9.1	38.1	16.3	59.3	158.0
	E		14.4	16.0	2469.7	71.6	56.0	113.0
20	A		5.9	6.3	12.6	5.3	20.9	95.8
	B		20.6	16.9	372.7	101.6	47.0	137.4
	C		7.7	3.5	12.5	7.7	15.9	47.0
	D		9.3	9.7	66.5	45.4	60.7	101.8
	E		33.7	29.0	6965.2	246.8	118.4	283.5

Appendix 9-I.

\mount (μg) of 20:4(n-6) in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-I for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		1.5	2.0	2.2	5.1	6.8	19.7
	B		2.0	2.0	2.5	3.0	8.1	16.0
	C		2.0	2.1	1.4	4.3	11.3	36.1
	D		2.2	2.1	0.8	3.5	6.1	14.2
	E		1.3	1.2	13.2	3.4	5.9	9.8
20	A		0.8	1.8	1.0	2.7	6.9	20.0
	B		2.5	1.0	3.1	3.0	6.8	9.1
	C		1.6	1.0	0.9	1.5	4.0	7.9
	D		1.7	2.1	1.5	4.5	3.9	10.7
	E		2.7	1.7	38.4	9.1	24.4	13.0

Appendix 9-J.

Amount (μg) of (n-6) FAs in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-J for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		21.4	13.1	61.1	31.0	70.7	206.2
	B		35.0	21.2	201.6	90.0	58.3	210.3
	C		24.2	21.2	42.5	25.3	74.1	305.1
	D		30.8	18.4	47.4	28.2	98.5	222.8
	E		23.5	23.5	2606.1	95.7	83.9	151.1
20	A		17.3	20.6	21.9	18.7	70.6	170.2
	B		42.5	25.7	420.1	121.5	77.3	194.0
	C		26.9	11.3	18.9	15.4	34.7	77.7
	D		27.0	18.5	77.3	60.2	86.0	161.0
	E		52.4	42.5	7499.3	310.6	315.5	367.7

Appendix 9-K.

Amount (μg) of 18:3(n-3) in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-K for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		2.1	0.7	2.7	1.2	1.7	1.9
	B		2.5	1.1	3.3	2.0	1.1	3.6
	C		4.0	4.8	159.9	51.4	18.6	31.8
	D		4.9	4.3	57.6	27.6	11.0	14.0
	E		2.0	0.8	62.1	2.9	4.0	5.7
20	A		1.9	0.4	0.5	0.4	1.3	1.5
	B		1.4	0.5	3.5	0.3	1.5	1.6
	C		2.4	4.9	105.7	55.2	14.7	15.8
	D		4.9	7.4	123.5	81.9	31.5	25.9
	E		1.4	1.3	124.7	23.4	14.2	2.7

Appendix 9-L.

Amount (μg) of 20:5(n-3) in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-L for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		7.0	16.1	8.2	35.3	49.3	328.5
	B		8.1	17.5	5.8	14.3	42.8	232.2
	C		8.1	17.4	5.8	28.7	62.4	390.7
	D		7.7	17.1	3.5	19.6	54.4	295.7
	E		6.2	13.6	368.5	68.2	44.7	169.1
20	A		5.2	4.9	4.1	10.3	29.5	361.2
	B		7.8	7.9	8.6	8.4	51.7	232.7
	C		5.4	6.0	1.5	5.1	17.6	139.8
	D		5.1	12.3	1.5	14.1	45.7	214.0
	E		17.0	27.2	741.9	244.0	73.3	383.0

Appendix 9-M.

Amount (μg) of 22:6(n-3) in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-M for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		2.3	4.2	7.6	22.3	26.7	283.2
	B		4.0	4.6	7.0	11.6	6.4	224.6
	C		4.0	5.9	5.6	23.1	38.5	397.9
	D		2.5	5.2	3.3	17.5	26.6	280.2
	E		2.3	5.4	382.8	103.0	24.7	163.7
20	A		2.6	6.4	5.5	5.1	30.5	292.0
	B		8.7	3.5	9.7	7.2	18.1	184.8
	C		4.7	3.1	0.3	3.2	15.2	119.5
	D		1.9	4.1	4.5	12.2	19.7	223.1
	E		9.2	11.8	828.3	207.0	43.8	325.3

Appendix 9-N.

Amount (μg) of (n-3) FAs in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-N for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		25.9	27.8	38.4	66.3	102.1	637.3
	B		34.5	28.5	32.4	35.4	98.9	484.9
	C		38.3	34.5	197.9	111.9	147.1	866.9
	D		30.2	32.9	67.9	78.8	129.7	610.9
	E		23.8	25.9	929.4	209.4	102.5	351.8
20	A		23.0	38.5	15.5	25.0	76.4	702.7
	B		45.2	17.4	32.7	29.2	110.1	433.8
	C		36.7	23.3	112.2	71.7	60.6	296.2
	D		26.2	32.4	138.5	122.4	109.7	506.2
	E		40.4	54.5	2021.1	587.4	228.7	752.3

Appendix 9-O.

Amount (μg) of PUFAs in the Neutral and Polar Lipids of Three Tissues. (Values are means of separate extracts from 2 - 4 individual lobsters; see Append. 8-O for n values)

Treatment		Lipid Extract						
T°C	Diet	Tissue	Gill		Hepatopancreas		Muscle	
		Lipid Fraction	NL	PL	NL	PL	NL	PL
10	A		57.5	45.1	129.9	150.3	182.0	847.8
	B		87.5	57.6	254.9	153.5	174.7	703.7
	C		82.1	64.8	248.0	225.4	254.9	1198.6
	D		81.6	61.0	118.7	185.8	265.5	844.4
	E		58.9	56.6	3582.3	340.1	202.8	508.9
20	A		55.0	86.8	43.2	55.2	163.8	890.1
	B		106.7	53.3	476.5	225.7	203.2	641.1
	C		81.4	42.9	139.2	127.0	101.8	381.7
	D		73.4	61.9	225.5	275.8	203.4	679.3
	E		119.9	113.3	9705.9	933.5	600.6	1133.5

Appendix 10.

ANOVA Tables of Fatty Acid Data.

Appendix 10-A. ANOVA Class Level Information.

CLASS	LEVELS	VALUES
TEMPERATURE	2	10°C, 20°C
DIET	5	A, B, C, D, E (H-CRD)
TISSUE (Organ)	3	Hepatopancreas, Muscle, Gills
TYPE (Lipid)	2	Neutral Lipid, Polar Lipid

Number of observations in data set = 300 separate GLC chromatographs, with up to 53 fatty acids identified for each run.

Appendix 10-B.

Sources of Variation in the Fatty Acid Composition of the Neutral and Polar Lipid Fractions of the Hepatopancreas, Abdominal Muscles and Gills of Juvenile Lobsters Reared at Two Temperatures on One of Five Diets.

Fatty Acids	Single Factors				2-Way Interactions						3-Way Interactions			
	T	D	O	L	T x D	T x O	T x L	D x O	D x L	O x L	T x D x O	T x D x L	T x O x L	D x O x L
14:0	*	*	*	*	NS	NS	NS	*	*	*	NS	NS	NS	*
16:0	*	*	*	*	NS	NS	NS	*	*	*	*	NS	NS	*
18:0	NS	*	*	*	NS	NS	NS	NS	*	*	NS	NS	NS	NS
Σ SFAs	NS	*	*	*	NS	NS	*	*	NS	*	NS	NS	*	NS
16:1(n-7)	*	*	*	*	*	*	NS	*	NS	*	NS	NS	*	NS
18:1(n-9)	*	*	*	*	*	NS	NS	*	NS	*	*	NS	NS	*
Σ MFAs	*	*	*	*	*	NS	*	*	NS	*	NS	NS	NS	*
18:2(n-9)	NS	*	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
18:2(n-6)	*	*	*	*	NS	*	NS	*	*	*	NS	*	*	*
20:4(n-6)	*	*	*	*	NS	NS	NS	*	NS	*	NS	*	*	NS
Σ (n-6)	NS	*	*	*	*	NS	NS	*	*	NS	NS	*	*	*
18:3(n-3)	NS	*	*	*	*	NS	NS	*	*	*	NS	NS	NS	*
20:5(n-3)	*	*	*	*	*	*	*	*	NS	*	*	*	*	NS
22:5(n-3)	NS	*	*	NS	NS	*	NS	*	*	*	NS	*	NS	NS
22:6(n-3)	*	*	*	*	*	*	*	*	*	*	NS	NS	NS	*
Σ (n-3)	*	*	*	*	*	NS	NS	*	*	*	NS	*	NS	*
Σ PUFA	*	*	*	*	*	NS	NS	*	*	*	NS	NS	NS	*
(n-3)/(n-6)	NS	*	*	*	NS	NS	NS	*	*	*	NS	NS	*	*

Significance at 0.05 level denoted by "*"; NS denotes "Not Significant".

Abbreviations are as follows: T (temperature), D (diet), O (organ, tissue type), L (neutral or polar lipid).

Appendix 10 C. Myristic Acid, 14:0

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-C_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	0.63252	0.01240	18.20	0.0001	*
Temp (T)	1	0.00224	0.00224	3.29	0.0716	
Diet (D)	4	0.02955	0.00739	10.84	0.0001	*
Organ (O)	2	0.28043	0.14021	205.78	0.0001	*
Lipid Type (L)	1	0.16380	0.16380	240.39	0.0001	*
T x D	4	0.00620	0.00155	2.27	0.0640	
T x O	2	0.00258	0.00129	1.89	0.1542	
T x L	1	0.00087	0.00087	1.28	0.2599	
D x O	8	0.02926	0.00366	5.37	0.0001	*
D x L	4	0.00841	0.00210	3.09	0.0178	*
O x L	2	0.08334	0.04167	61.16	0.0001	*
T x D x O	8	0.00249	0.00031	0.46	0.8842	
T x D x L	4	0.00250	0.00062	0.92	0.4566	
T x O x L	2	0.00360	0.00180	2.64	0.0745	
D x O x L	8	0.01724	0.00215	3.16	0.0025	*
Error	148	0.10084	0.00068			
Corr. Total	199	0.73336				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 14:0 in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'

Appendix 10-C_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	98	1.39	A[2]
20	102	1.23	A

1. Based on arcsine transformation of the relative amount of 14:0 in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-C_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	T	NS	\		
4	*	NS	NS	\	
5	NS	*	*	*	\

1. Based on arcsine transformation of the relative amount of 14:0 in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level by Tukey's and Bonferroni's Tests; T denotes pairs significantly. different. at the $P \leq 0.05$ level by Tukey's Test only.

Appendix 10 C_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of 14:0 in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-C_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	2.08	A[2]
PL	101	0.72	B

1. Based on arcsine transformation of the relative amount of 14:0 in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 D Palmitic Acid, 16:0

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1]

Appendix 10-D_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	1.81110	0.03551	20.94	0.0001	*
Temp (T)	1	0.04183	0.04183	24.67	0.0001	*
Diet (D)	4	0.23232	0.05808	34.25	0.0001	*
Organ (O)	2	1.11466	0.55733	328.65	0.0001	*
Lipid Type (L)	1	0.16994	0.16994	100.21	0.0001	*
T x D	4	0.00647	0.00162	0.95	0.4352	
T x O	2	0.00459	0.00229	1.35	0.2617	
T x L	1	0.00222	0.00222	1.31	0.2544	
D x O	8	0.12134	0.01517	8.94	0.0001	*
D x L	4	0.02148	0.00537	3.17	0.0157	*
O x L	2	0.02124	0.01062	6.26	0.0025	*
T x D x O	8	0.03259	0.00407	2.40	0.0183	*
T x D x L	4	0.00480	0.00120	0.71	0.5879	
T x O x L	2	0.00371	0.00185	1.09	0.3379	
D x O x L	8	0.03385	0.00423	2.50	0.0144	*
Error	147	0.24929	0.00170			
Corr. Total	198	2.06032				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 16:0 in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'

Appendix 10-D_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	99	10.86	A[2]
20	100	12.73	B

1. Based on arcsine transformation of the relative amount of 16:0 in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-D_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	NS	\		
4	*	NS	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of 16:0 in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-D_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of 16:0 in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-D_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	13.60	A[2]
PL	101	10.10	B

1. Based on arcsine transformation of the relative amount of 16:0 in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 E. Stearic Acid, 18:0

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-E_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	0.73637	0.01444	3.90	0.0001	*
Temp (T)	1	0.00598	0.00598	1.62	0.2057	
Diet (D)	4	0.07571	0.01893	5.12	0.0007	*
Organ (O)	2	0.32709	0.16354	44.20	0.0001	*
Lipid Type (L)	1	0.03007	0.03007	8.13	0.0050	*
T x D	4	0.00710	0.00177	0.48	0.7506	
T x O	2	0.01938	0.00969	2.62	0.0762	
T x L	1	0.00158	0.00158	0.43	0.5146	
D x O	8	0.05418	0.00677	1.83	0.0756	
D x L	4	0.05424	0.01356	3.67	0.0071	*
O x L	2	0.03519	0.01760	4.76	0.0100	*
T x D x O	8	0.05062	0.00633	1.71	0.1004	
T x D x L	4	0.01486	0.00372	1.00	0.4074	
T x O x L	2	0.00165	0.00083	0.22	0.8003	
D x O x L	8	0.05871	0.00734	1.98	0.0522	
Error	148	0.54757	0.00370			
Corr. Total	199	1.28394				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 18:0 in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'

Appendix 10-E_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	99	3.16	A[2]
20	101	2.79	A

1. Based on arcsine transformation of the relative amount of 18:0 in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-E_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	NS	\		
4	*	NS	NS	\	
5	NS	NS	NS	NS	\

1. Based on arcsine transformation of the relative amount of 18:0 in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-E4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Hepatopancreata	Muscle
Gills	\		
Hepatopancreata	*	\	
Muscle	NS	*	\

1. Based on arcsine transformation of the relative amount of 18:0 in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-E5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	0.70	A[2]
PL	101	2.60	B

1. Based on arcsine transformation of the relative amount of 18:0 in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 F. Saturated Fatty Acids, (SFAs)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-F_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	2.94011	0.05765	24.68	0.0001	*
Temp (T)	1	0.00379	0.00379	1.62	0.2049	
Diet (D)	4	0.20910	0.05228	22.38	0.0001	*
Organ (O)	2	2.13090	1.06545	456.19	0.0001	*
Lipid Type (L)	1	0.27487	0.27487	117.69	0.0001	*
T x D	4	0.02170	0.00542	2.32	0.0594	
T x O	2	0.00705	0.00352	1.51	0.2246	
T x L	1	0.00920	0.00920	3.94	0.0490	*
D x O	8	0.10748	0.01343	5.75	0.0001	*
D x L	4	0.00776	0.00194	0.83	0.5074	
O x L	2	0.06890	0.03445	14.75	0.0001	*
T x D x O	8	0.03634	0.00454	1.95	0.0575	
T x D x L	4	0.01352	0.00338	1.45	0.2214	
T x O x L	2	0.03789	0.01895	8.11	0.0005	*
D x O x L	8	0.01160	0.00145	0.62	0.7595	
Error	146	0.34099	0.00234			
Corr. Total	197	3.28110				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of SFAs in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-F_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	97	16.32	A[2]
20	101	16.97	A

1. Based on arcsine transformation of the relative amount of SFAs in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-F_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	NS	\		
4	*	NS	NS	\	
5	NS	*	*	*	\

1. Based on arcsine transformation of the relative amount of SFAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-F_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	NS	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of SFAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-F_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	98	19.55	A[2]
PL	100	14.01	B

1. Based on arcsine transformation of the relative amount of SFAs in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 G. Palmitoleic, 16:1(n-7)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-G_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	0.77624	0.01522	13.59	0.0001	*
Temp (T)	1	0.01131	0.01131	10.10	0.0018	*
Diet (D)	4	0. 03910	0.00978	8.73	0.0001	*
Organ (O)	2	0.12498	0.06249	55.80	0.0001	*
Lipid Type (L)	1	0.45616	0.45616	407.32	0.0001	*
T x D	4	0.01191	0.00298	2.66	0.0351	*
T x O	2	0.01833	0.00916	8.18	0.0004	*
T x L	1	0.00027	0.00027	0.24	0.6258	
D x O	8	0.04577	0.00572	5.11	0.0001	*
D x L	4	0.00573	0.00144	1.28	0.2788	
O x L	2	0.02567	0.01284	11.46	0.0001	*
T x D x O	8	0.00647	0.00081	0.72	0.6716	
T x D x L	4	0.00122	0.00030	0.27	0.8958	
T x O x L	2	0.01338	0.00669	5.98	0.0032	*
D x O x L	8	0.01592	0.00199	1.78	0.0860	
Error	147	0.16463	0.00112			
Corr. Total	198	0.94087				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 16:1(n-7) in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-G_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	97	3.74	A[2]
20	101	4.33	B

1. Based on arcsine transformation of the relative amount of 16:1(n-7) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-G_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	NS	\		
4	*	NS	NS	\	
5	*	NS	NS	NS	\

1. Based on arcsine transformation of the relative amount of 16:1(n-7) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-G4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	NS	\	
Hepatopancreata	*	NS	\

1. Based on arcsine transformation of the relative amount of 16:1(n-7) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-G5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	98	6.24	A[2]
PL	101	2.35	B

1. Based on arcsine transformation of the relative amount of 16:1(n-7) in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 H. Oleic Acid, 18:1(n-9)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-H_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	4.87823	0.09565	29.27	0.0001	*
Temp (T)	1	0.01374	0.01374	4.20	0.0421	*
Diet (D)	4	1.29420	0.32355	99.02	0.0001	*
Organ (O)	2	2.13143	1.06571	326.17	0.0001	*
Lipid Type (L)	1	0.26112	0.26112	79.92	0.0001	*
T x D	4	0.08500	0.02125	6.50	0.0001	*
T x O	2	0.01609	000804	2.46	0.0888	
T x L	1	0.01148	0.01148	3.51	0.0629	
D x O	8	0.58135	0.07267	22.24	0.0001	*
D x L	4	0.01274	0.00318	0.97	0.4234	
O x L	2	0.32361	0.16181	49.52	0.0001	*
T x D x O	8	0.06658	0.00832	2.55	0.0125	*
T x D x L	4	0.00456	0.00114	0.35	0.8444	
T x O x L	2	0.00249	0.00125	0.38	0.6834	
D x O x L	8	0.07386	0.00923	2.83	0.0060	*
Error	149	0.48683	0.00327			
Corr. Total	200	5.36507				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 18:1(n-9) in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-H_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	100	2.72	A[2]
20	101	2.91	B

1. Based on arcsine transformation of the relative amount of 18:1(n-9) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-H_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	T	NS	\		
4	*	NS	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of 18:1(n-9) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-H_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of 18:1(n-9) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-H_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	97	31.95	A[2]
PL	104	25.03	B

1. Based on arcsine transformation of the relative amount of 18:1(n-9) in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-I. Monounsaturated Fatty Acids (MFAs)

The Relative Amount of in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix $10\text{-}I_1$. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	3.07392	0.06027	29.8[illegible]	0.0001	*
Temp (T)	1	0.00274	0.00274	1.36	0.2460	
Diet (D)	4	0.61987	0.15497	76.67	0.0001	*
Organ (O)	2	1.26651	0.63325	313.28	0.0001	*
Lipid Type (L)	1	0.44411	0.44411	219.71	0.0001	*
T x D	4	0.03179	0.00795	3.93	0.0046	*
T x O	2	0.00353	0.00177	0.87	0.4198	
T x L	1	0.01085	0.01085	5.37	0.0219	*
D x O	8	0.32094	0.04012	19.85	0.0001	*
D x L	4	0.01827	0.00457	2.26	0.0654	
O x L	2	0.27454	0.13727	67.91	0.0001	*
T x D x O	8	0.02567	0.00321	1.59	0.1331	
T x O x L	2	0.00009	0.00005	0.02	0.9770	
D x O x L	8	0.05130	0.00641	3.17	0.0024	*
Error	148	0.29916	0.00202			
Corr. Total	199	3.37308				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Table __ .
2. Based on arcsine transformation of the percent contribution of MFAs in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-I_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	98	37.52	A[2]
20	102	37.98	A

1. Based on arcsine transformation of the relative amount of MFAs in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-I_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	NS	\			
3	NS	NS	\		
4	*	NS	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of MFAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-I4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of MFAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-I5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	42.81	A[2]
PL	101	33.18	B

1. Based on arcsine transformation of the relative amount of MFAs in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 J. Linoleic Acid, 18:2(n-6)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-J_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	1.59376	0.03125	32.92	0.0001	*
Temp (T)	1	0.00508	0.00508	5.35	0.0220	*
Diet (D)	4	0.99674	0.24918	262.51	0.0001	*
Organ (O)	2	0.06265	0.03133	33.00	0.0001	*
Lipid Type (L)	1	0.05565	0.05565	58.62	0.0001	*
T x D	4	0.00793	0.00198	2.09	0.0849	
T x O	2	0.00721	0.00361	3.80	0.0245	*
T x L	1	0.00092	0.00092	0.97	0.3267	
D x O	8	0.29157	0.03645	38.39	0.0001	*
D x L	4	0.02867	0.00717	7.55	0.0001	*
O x L	2	0.03566	0.01783	18.78	0.0001	*
T x D x O	8	0.00539	0.00067	0.71	0.6822	
T x D x L	4	0.01626	0.00407	4.28	0.0026	*
T x O x L	2	0.01465	0.00733	7.72	0.0006	*
D x O x L	8	0.06536	0.00817	8.61	0.0001	*
Error	154	0.14618	0.00094			
Corr. Total	205	1.73994				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 18:2(n-6) in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-J_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	102	5.99	A[2]
20	104	5.53	B

1. Based on arcsine transformation of the relative amount of 18:2(n-6) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix10-J_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	*	\		
4	*	*	*	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of 18:2(n-6) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-J4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of 18:2(n-6) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-J5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	101	4.97	A[2]
PL	105	6.57	B

1. Based on arcsine transformation of the relative amount of 18:2(n-6) in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 K. Arachidonic Acid, 20:4(n-6)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-K_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	0.16407	0.00322	3.19	0.0001	*
Temp (T)	1	0.00848	0.00848	8.39	0.0043	*
Diet (D)	4	0.01369	0.00342	3.39	0.0110	*
Organ (O)	2	0.04256	0.02128	21.07	0.0001	*
Lipid Type (L)	1	0.02550	0.02550	25.25	0.0001	*
T x D	4	0.00529	0.00132	1.31	0.2691	
T x O	2	0.00084	0.00042	0.42	0.6605	
T x L	1	0.00019	0.00019	0.19	0.6665	
D x O	8	0.01826	0.00228	2.26	0.0261	*
D x L	4	0.00417	0.00104	1.03	0.3932	
O x L	2	0.00528	0.00264	2.61	0.0767	*
T x D x O	8	0.00836	0.00104	1.03	0.4130	
T x D x L	4	0.01032	0.00258	2.55	0.0414	*
T x O x L	2	0.00684	0.00342	3.39	0.0365	*
D x O x L	8	0.01431	0.00179	1.77	0.0870	
Error	149	0.15048	0.00101			
Corr. Total	200	0.31455				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 20:4(n-6) in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-K_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	100	0.74	A[2]
20	1041	0.53	B

1. Based on arcsine transformation of the relative amount of 20:4(n-6) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-K_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	T	\			
3	NS	NS	\		
4	*	NS	NS	\	
5	NS	NS	NS	NS	\

1. Based on arcsine transformation of the relative amount of 20:4(n-6) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ by Tukey's and Bonferroni's Tests; T denotes pairs significantly different at the $P \leq 0.05$ by Tukey's Test only.

Appendix 10-K_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	NS	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of 20:4(n-6) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-K_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	101	0.46	A[2]
PL	105	0.83	B

1. Based on arcsine transformation of the relative amount of 20:4(n-6) in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 L. (n-6) Fatty Acids

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-L_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	1.32800	0.02604	24.01	0.0001	*
Temp (T)	1	0.00291	0.00291	2.69	0.1033	
Diet (D)	4	0.69081	0.17270	159.23	0.0001	*
Organ (O)	2	0.08704	0.04352	40.13	0.0001	*
Lipid Type (L)	1	0.08480	0.08480	78.19	0.0001	*
T x D	4	0.02053	0.00513	4.73	0.0013	*
T x O	2	0.00176	0.00088	0.81	0.4454	
T x L	1	0.00076	0.00076	0.70	0.4026	
D x O	8	0.29157	0.03645	33.60	0.0001	*
D x L	4	0.03974	0.00994	9.16	0.0001	*
O x L	2	0.00031	0.00016	0.14	0.8654	
T x D x O	8	0.01304	0.00163	1.50	0.1609	
T x D x L	4	0.01029	0.00257	2.37	0.0550	
T x O x L	2	0.02290	0.01145	10.56	0.0001	*
D x O x L	8	0.06152	0.00769	7.09	0.0001	*
Error	148	0.16052	0.00108			
Corr. Total	199	1.48852				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of (n-6) FAs in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-L_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	98	9.89	A[2]
20	102	9.44	A

1. Based on arcsine transformation of the relative amount of (n-6) FAs in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-L_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	*	\		
4	NS	*	*	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of (n-6) FAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-L_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of (n-6) FAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-L_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	8.42	A[2]
PL	101	10.95	B

1. Based on arcsine transformation of the relative amount of (n-6) FAs in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 M. Linolenic Acid, 18:3(n-3)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-M_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	1.24131	0.02434	33.75	0.0001	*
Temp (T)	1	0.10675	0.00072	1.26	0.2643	
Diet (D)	4	0.80564	0.20141	279.25	0.0001	*
Organ (O)	2	0.08585	0.04292	59.51	0.0001	*
Lipid Type (L)	1	0.00761	0.00761	10.55	0.0014	*
T x D	4	0.01667	0.00417	5.78	0.0002	*
T x O	2	0.00247	0.00123	1.71	0.1846	
T x L	1	0.00000	0.00000	0.00	0.9600	
D x O	8	0.24945	0.03112	43.23	0.0001	*
D x L	4	0.02296	0.00574	7.96	0.0001	*
O x L	2	0.01630	0.00815	11.30	0.0001	*
T x D x O	8	0.00389	0.00049	0.67	0.7140	
T x D x L	4	0.00449	0.00112	1.56	0.1892	
T x O x L	2	0.00364	0.00182	2.52	0.0839	
D x O x L	8	0.02143	0.00268	3.71	0.0006	*
Error	148	0.10675				
Corr. Total	199	1.34806				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 18:3(n-3) in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-M_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	97	1.10	A[2]
20	103	1.01	A

1. Based on arcsine transformation of the relative amount of 18:3(n-3) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-M_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	NS	\			
3	*	*	\		
4	*	*	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of 18:3(n-3) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-M_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of 18:3(n-3) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-M_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	101	0.94	A[2]
PL	99	1.17	B

1. Based on arcsine transformation of the relative amount of 18:3(n-3) in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10 N. Eicosapentaenoic Acid, 20:5(n-3)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-N_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	2.89572	0.05678	37.32	0.0001	*
Temp (T)	1	0.05469	0.05469	35.95	0.0001	*
Diet (D)	4	0.16738	0.04184	27.51	0.0001	*
Organ (O)	2	1.27435	0.63718	418.85	0.0001	*
Lipid Type (L)	1	1.05133	1.05133	691.10	0.0001	*
T x D	4	0.02175	0.00544	3.57	0.0082	*
T x O	2	0.01371	0.00685	4.51	0.0126	*
T x L	1	0.01607	0.01607	10.56	0.0014	*
D x O	8	0.12376	0.01547	10.17	0.0001	*
D x L	4	0.00737	0.00184	1.21	0.3084	
O x L	2	0.06074	0.03037	19.96	0.0001	*
T x D x O	8	0.02749	0.00344	2.26	0.0263	*
T x D x L	4	0.01914	0.00478	3.14	0.0163	*
T x O x L	2	0.03380	0.01690	11.11	0.0001	*
D x O x L	8	0.02415	0.00302	1.98	0.0522	
Error	147	0.22362	0.00152			
Corr. Total	198	3.11934				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 20:5(n-3) in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-N_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	99	5.67	A[2]
20	100	4.23	B

1. Based on arcsine transformation of the relative amount of 20:5(n-3) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-N_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	NS	\		
4	*	NS	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of 20:5(n-3) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-N_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of 20:5(n-3) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-N_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	8.70	A[2]
PL	101	2.15	B

1. Based on arcsine transformation of the relative amount of 20:5(n-3) in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-O. Ratio of (n-3) to (n-6) Fatty Acids

The Ratio in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-O_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	0.40914	0.00802	20.41	0.0001	*
Temp (T)	1	0.00030	0.00030	0.77	0.3801	
Diet (D)	4	0.14018	0.03504	89.14	0.0001	*
Organ (O)	2	0.03272	0.01636	41.61	0.0001	*
Lipid Type (L)	1	0.06700	0.06700	170.43	0.0001	*
T x D	4	0.00320	0.00080	2.04	0.0922	
T x O	2	0.00044	0.00022	0.56	0.5723	
T x L	1	0.00001	0.00001	0.03	0.8674	
D x O	8	0.11332	0.01417	36.03	0.0001	*
D x L	4	0.01636	0.00409	10.40	0.0001	*
O x L	2	0.01162	0.00581	14.78	0.0001	*
T x D x O	8	0.00123	0.00015	0.39	0.9237	
T x D x L	4	0.00222	0.00055	1.41	0.2332	
T x O x L	2	0.00492	0.00246	6.26	0.0024	*
D x O x L	8	0.01561	0.00195	4.96	0.0001	*
Error	148	0.05818	0.00039			
Corr. Total	199	0.46732				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the ratio of (n-3) to (n-6) FAs in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-O_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	98	1.63	A[2]
20	102	1.57	A

1. Based on arcsine transformation of the relative amount of the ratio of (n-3) to (n-6) FAs in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-O_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	*	\		
4	NS	*	*	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the ratio of (n-3) to (n-6) FAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-O_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	NS	*	\

1. Based on arcsine transformation of the ratio of (n-3) to (n-6) FAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-O_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	1.15	A[2]
PL	101	2.10	B

1. Based on arcsine transformation of the ratio of (n-3) to (n-6) FAs in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-P. Docosahexaenoic Acid, 22:6(n-3)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-P_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	2.68099	0.05257	58.42	0.0001	*
Temp (T)	1	0.01425	0.01425	15.83	0.0001	*
Diet (D)	4	0.18224	0.04556	50.63	0.0001	*
Organ (O)	2	1.16959	0.58479	649.85	0.0001	*
Lipid Type (L)	1	0.81287	0.81287	903.30	0.0001	*
T x D	4	0.00987	0.00247	2.74	0.0308	*
T x O	2	0.01414	0.00707	7.86	0.0006	*
T x L	1	0.01904	0.01904	21.16	0.0001	*
D x O	8	0.16948	0.02119	23.54	0.0001	*
D x L	4	0.02414	0.00604	6.71	0.0001	*
O x L	2	0.23043	0.11521	128.03	0.0001	*
T x D x O	8	0.01212	0.00151	1.68	0.1072	
T x O x L	2	0.00262	0.00131	1.46	0.2366	
D x O x L	8	0.01660	0.00208	2.31	0.0234	*
Error	144	0.12958	0.00090			
Corr. Total	195	2.81057				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of 22:6(n-3) in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-P_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	99	3.83	A[2]
20	97	3.20	B

1. Based on arcsine transformation of the relative amount of 22:6(n-3) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-P_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	NS	\		
4	*	NS	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of 22:6(n-3) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-P_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	NS	*	\

1. Based on arcsine transformation of the relative amount of 22:6(n-3) in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-P_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	96	1.40	A[2]
PL	100	6.40	B

1. Based on arcsine transformation of the relative amount of 22:6(n-3) in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-Q. (n-3) Fatty Acids

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-Q_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	3.76831	0.07389	48.16	0.0001	*
Temp (T)	1	0.01864	0.01864	12.15	0.0006	*
Diet (D)	4	0. 29291	0.07323	47.73	0.0001	*
Organ (O)	2	1.32208	0.66104	430.85	0.0001	*
Lipid Type (L)	1	1.49016	1.49016	971.25	0.0001	*
T x D	4	0.02329	0.00582	3.80	0.0057	*
T x O	2	0.00008	0.00004	0.02	0.9755	
T x L	1	0.00478	0.00478	3.11	0.0797	
D x O	8	0.29852	0.03731	24.32	0.0001	*
D x L	4	0.05462	0.01365	8.90	0.0001	*
O x L	2	0.17960	0.08980	58.53	0.0001	*
T x D x O	8	0.01199	0.00150	0.98	0.4563	
T x D x L	4	0.01844	0.00461	3.01	0.0203	*
T x O x L	2	0.00871	0.00435	2.84	0.0618	
D x O x L	8	0.04448	0.00556	3.62	0.0007	*
Error	147	0.22554	0.00153			
Corr. Total	198	3.99384				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of (n-3) FAs in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-Q_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	98	14.61	A[2]
20	101	13.26	B

1. Based on arcsine transformation of the relative amount of (n-3) FAs) in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-Q_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	*	\			
3	*	*	\		
4	NS	*	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of (n-3) FAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-Q_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of (n-3) FAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-Q_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	98	8.19	A[2]
PL	101	20.65	B

1. Based on arcsine transformation of the relative amount of (n-3) FAs in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-R. Polyunsaturated Fatty Acids (PUFAs)

The Relative Amount in the Neutral or Polar Lipid Fractions of the Hepatopancreata, Muscles, or Gills of Lobsters Reared at 10°C or 20°C and Fed One of Five Test Diets[1].

Appendix 10-R_1. ANOVA.[2]

Source of Variation	df	SS	MS	F	P	Sig.[3]
Model	51	3.21449	0.06303	31.21	0.0001	*
Temp (T)	1	0.01596	0.01596	7.90	0.0056	*
Diet (D)	4	0.42636	0.10659	52.77	0.0001	*
Organ (O)	2	0.60532	0.30266	149.85	0.0001	*
Lipid Type (L)	1	1.56986	1.56986	777.23	0.0001	*
T x D	4	0.04274	0.01068	5.29	0.0005	*
T x O	2	0.00128	0.00064	0.32	0.7286	
T x L	1	0.00387	0.00387	1.92	0.1682	
D x O	8	0.24237	0.03030	15.00	0.0001	*
D x L	4	0.02760	0.00690	3.42	0.0105	*
O x L	2	0.19979	0.09990	49.46	0.0001	*
T x D x O	8	0.00923	0.00115	0.57	0.8000	
T x D x L	4	0.01425	0.00356	1.76	0.1392	
T x O x L	2	0.00719	0.00359	1.78	0.1724	
D x O x L	8	0.04866	0.00608	3.01	0.0037	*
Error	148	0.29893	0.00202			
Corr. Total	199	3.51343				

1. The NL and PL fractions were obtained from the tissues of two to five lobsters, see fatty acid tables for N; neither lipid fractions nor tissue samples were pooled. Diet Composition: Tables 11 & 12.
2. Based on arcsine transformation of the percent contribution of PUFAs in the NL or PL fractions of each tissue.
3. Significance at the $P \leq 0.05$ level is indicated by '*'.

Appendix 10-R_2. Bonferroni/Tukey Comparisons of the Effect of Temperature. [1]

Temperature	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
10	98	27.67	A[2]
20	102	26.08	B

1. Based on arcsine transformation of the relative amount of PUFAs in the NL or PL fraction of each tissue.
2 Means with the same letter are not significantly different at $P \leq 0.05$.

Appendix 10-R_3. Bonferroni/Tukey Comparisons of the Effect of Diet.[1,2]

Diet	1	2	3	4	5
1	\				
2	NS	\			
3	NS	NS	\		
4	NS	NS	NS	\	
5	*	*	*	*	\

1. Based on arcsine transformation of the relative amount of PUFAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$.

Appendix 10-R_4. Bonferroni/Tukey Comparisons of Tissues.[1,2]

Organ	Gills	Muscle	Hepatopancreata
Gills	\		
Muscle	*	\	
Hepatopancreata	*	*	\

1. Based on arcsine transformation of the relative amount of PUFAs in the NL or PL fraction of each tissue.
2. The symbol '*' denotes pairs significantly different at the $P \leq 0.05$ level.

Appendix 10-R_5. Bonferroni/Tukey Comparison of the Neutral and Polar Lipids[1]

Lipid Fraction	N	Mean (% of Lipid Fraction)	Bonferroni/Tukey Grouping
NL	99	19.15	A[2]
PL	101	35.16	B

1. Based on arcsine transformation of the relative amount of PUFAs in the NL or PL fraction of each tissue.
2. Means with the same letter are not significantly different at $P \leq 0.05$.

APPENDIX 11

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