OPTIMIZATION OF HATCHERY CULTURE OF THE SEA SCALLOP, PLACOPECTEN MAGELLANICUS (GMELIN, 1791): DIETARY LIPID QUALITY AND FATTY ACID REQUIREMENTS

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OPTIMIZATION OF HATCHERY CULTURE OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS (GMELIN, 1791)*: DIETARY LIPID QUALITY AND FATTY ACID REQUIREMENTS

by

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ABSTRACT

Different species of live algal cultures varying broadly in essential fatty acid content – eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and arachidonic acid (ARA; 20:4n-6) – were used in exploratory experiments to evaluate the impact of dietary lipid quality on broodstock conditioning and larval culture of the sea scallop (*Placopecten magellanicus*). Egg biochemical composition was relatively stable when diets were fed to animals in a partially conditioned state, but varied more for wild eggs compared to lab-conditioned animals. When diet treatments were initiated earlier in a second experiment, differences among the fatty acid composition of gonad samples were more apparent.

In larval feeding trials, fatty acids in neutral lipids, and to lesser extent phospholipids, of larvae reflected the composition of the algae they were fed. C_{20} and C_{22} PUFA were preferentially accumulated, particularly DHA which plays a specific structural role in Pectinids. Growth results suggest increasing dietary ARA, or possibly 22:5n-6.

Last, variability in lipid quality was examined in aging batch cultures of a strain of *Pavlova* sp. (CCMP459). Lipid, mainly TAG, as a proportion of dry weight, more than doubled over the experimental period. As cultures aged, the (n-3)/(n-6) series fatty acid ratio was reduced. By controlling algal harvest strategies or culture conditions, the biochemical composition of algae may be optimized to best meet the nutritional needs of a particular bivalve species or developmental stage.

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LIST OF ABBREVIATIONS

ARA	Arachidonic Acid (20:4n-6)
AMPL	Acetone-Mobile Polar Lipids
ALC	Aliphatic Alcohols
CN	Carbon, Nitrogen
DAG	Diacylglycerols
DHA	Docosahexaenoic acid (22:6n-3)
DMA	Dimethyl Acetals
EFA	Essential Fatty Acid
EPA	Eicosapentaenoic Acid (20:5n-3)
Et-KET	Ethyl Ketones
FA	Fatty Acid
FAME	Fatty Acid Methyl Esters
FFA	Free Fatty Acids
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detection
GS-MS	Gas Chromatography-Mass Spectrometry
GE	Acylated Glyceryl Ethers
HC	Aliphatic Hydrocarbons
ME	Methyl Esters
Me-KET	Methyl Ketones
MONO	Monounsaturated fatty acids
NL	Neutral Lipids
NMID	Non-Methylene Interupted Dienes

PCA	Principal component analysis
PL	Phospholipids
PUFA	Polyunsaturated Fatty Acids
RCB	Randomized Complete Block
SAT	Saturated fatty acids
SD	Standard deviation
SE	Steryl Esters
ST	Sterols
TAG	Triacylglycerols
T-Iso	*Isochrysis* sp. (Prymnesiophyte alga) Tahitian strain or clone T-ISO
TLC-FID	Thin Layer Chromatography-Flame Ionization Detection
UNSAT	Unsaturated fatty acids
WE	Wax Esters
wt %	weight-percent

CHAPTER 1 – INTRODUCTION AND OVERVIEW

1.1 The sea scallop as a candidate species for aquaculture

Research into sea scallop (*Placopecten magellanicus*) aquaculture has been ongoing for the past 25 years (Couturier, 1990). Commercial interest has depended on fluctuations in market price, but is presently high in both the northeast U.S. and Canadian Maritimes. Coincidental with a gradual decline in fisheries (Robinson, 1993) a number of start-up operations and a handful of more established ones are in existence.

The sea scallop is a temperate-cold water species indigenous to the northwest Atlantic and can be grown in offshore waters where gear and other competing use conflicts can be minimized (R. Taylor, personal communication). Potential farm sites include areas off Maine, Nova Scotia, Quebec, Prince Edward Island, New Brunswick, and Newfoundland – regions that have large areas of coastline and are in need of economic activities to support coastal communities in the wake of declining fisheries and a lack of alternative employment.

The sea scallop fishery is over 100 years old. Because of its status as one of the most important commercial bivalves in North America, an established market and infrastructure already exist. A recent economic analysis reported that sea scallop farming has profit potential, but links it to fluctuating market prices, economies of scale, seed price and grow out protocols (Ford, 1998). There is also interest in large-scale enhancement and cooperative fisheries employing bottom seeding (Naidu et al., 1991, Nadeau and Cliche, 1997).

1.2 General biology

The sea scallop is distributed in the northwest Atlantic from the north shore of the Gulf of St. Lawrence to Cape Hatteras, North Carolina at depths between 15 and 110 m (Posgay, 1957). It commonly reaches sizes between 10-15 cm and up to 21 cm and thrives at temperatures from 5-15°C.

Development is typical of many bivalves. *Placopecten magellanicus* is dioecious, although a small incidence of hermaphrodism has been noted (Naidu, 1970). One female can produce up to 90 million eggs. Fertilization occurs in the water column and the embryo progresses through a trochophore, veliger (prodissoconch I and II), and pediveliger stage before undergoing metamorphosis into a juvenile. Spawning in Newfoundland occurs in August and September, corresponding to a drop in water temperature, although the timing varies with latitude (Naidu, 1970; MacDonald and Thompson, 1988).

1.3 Hatchery production as a constraint on mariculture development

Wild set has provided seed for past sea scallop mariculture projects, but success of natural spat collection is extremely variable. Currently, seed supply is cited as the main biological constraint to continued commercial development (Dabinett and Couturier, 1994). In addition to solving seed availability problems, hatcheries employ strict environmental controls that can be used to manipulate sea scallops to initiate gametogenesis and spawn earlier than would occur in the wild. Thus the total time

required for animals to reach market size (normally 3-4 years) can be reduced, adding to profitability and reducing risk in grow-out operations.

Couturier et al. (1994) point out that the numerous fishing plants no longer operating in Atlantic Canada could be converted to scallop hatcheries relatively inexpensively. The water temperature required for rearing larval *P. magellanicus* (13-15°C) is appropriate for culture operations during autumn months. In Maine, sea scallop seed production could extend the season of existing bivalve hatcheries and diversify them – thus offsetting fixed costs.

Sea scallops are easy to spawn (Desrosiers and Dubé, 1993), and culture methods are similar to those for other bivalves, but scallops appear to be more vulnerable to stresses encountered in the hatchery. Production is still sporadic and research focusing on increasing hatchery yields will serve to reduce seed costs. This need applies to bivalve hatcheries of all types, but is especially important when rearing sea scallops due to their 28-40 day larval period (compared to just 8 days for the bay scallop, *Argopecten irradians*). The early developmental stages of bivalves are stress points during the life history of these animals (*i.e.*, development from egg to D-stage larvae and metamorphosis into juveniles). Intensive culture exacerbates these stresses and great losses can occur, but they can be minimized through the optimization of hatchery routines.

1.4 Links to bivalve nutrition

Numerous factors affect growth and survival of bivalves, including temperature, salinity, current speed, dissolved oxygen, suspended sediments, container size, animal density, genetics, and diet (Dupuy, 1975; reviewed by Rice and Pechenik, 1992). Lannan et al. (1980) showed, through a large number of reciprocal crosses, that much of the variability in yield encountered when rearing bivalves is due to factors other than genetics. Thus, yield may be optimized through control of environmental variables in a bivalve's culture environment. Furthermore, some evidence suggests that diet, or specifically food quantity and quality, may account for much of this variability in growth (Wikfors et al., 1992), and a substantial body of work exists concerning suitable diets for bivalves.

1.5 Evolution of bivalve nutritional studies

Past reviews on bivalve nutrition have pointed to a lack of basic understanding despite significant research (Ukeles, 1969). The field of nutrition is complex and multidisciplinary, involving chemistry, biochemistry, genetics, microbiology, endocrinology, statistics, physiology, and biophysics (Lloyd et al., 1978). A renaissance in this area has occurred over the last decade due to breakthroughs in culture techniques and analytical methods.

In addition to being a prerequisite to the success of *Placopecten magellanicus* as a commercially farmed species, hatchery production facilitates the study of bivalve nutrition by allowing for controlled diets and the ability to correlate nutritional factors

with growth and culture vigor. It also makes available suitable numbers of larvae for biochemical analyses. The increase in papers published on nutritional requirements of bivalves over the last twenty years has both driven and been supported by the proliferation of hatcheries and controlled culture techniques. Carriker (1988) remarks on the shift in emphasis from field studies to closed system molluscan mariculture - characterizing it as a stimulus to creative research (although see Mann (1988) for a dissenting view advocating a return to ecological studies). The development of microanalytical techniques (Holland and Gabbott, 1971) and the use of gas chromatographic analyses of fatty acid moieties (Parrish, 1988) has also contributed to the approach in these studies. Though still of importance, ecological and biomechanical (*e.g.*, feeding processes) and simple feeding trial studies are being replaced by multivariate experimental approaches aimed at resolving fundamental biochemical mechanisms (Ukeles, 1969). These trends have offered opportunities, but information is still sparse on *specific* nutritional requirements of bivalves. Study has been complicated by the inherent variability of the major food source for most bivalves – phytoplankton.

1.6 Role of phytoplankton in bivalve nutrition

Although the potential food sources of bivalves are diverse, including dissolved organic substances, detritus, protozoans, seaweed fragments, and even zooplankton (De Pauw and De Leenheer, 1980; Shumway et al., 1987), phytoplankton comprises the bulk of the sea scallop's diet. Despite continued efforts to develop alternative food sources in hatcheries and an increased recognition of the possible importance of dissolved organic

matter and bacteria in bivalve nutrition, live microalgae remain the mainstay of nearly all commercial hatcheries (Couteau and Sorgeloos, 1992). Algal production is also the most expensive component of these hatcheries. While other aspects of algal diets such as cell size, morphology, exudates, cell toxicity and digestibility, and food concentration and feeding frequency are of importance (Nell, 1992), in this study the focus is on the link between biochemical composition of algae in diets and nutritional requirements of bivalves.

1.7 Nutrition overview – the current perspective

Like all food, algae consist of gross constituents - protein, lipid, and carbohydrate, with their sub-components, amino-acids, lipid classes, fatty acid moeities and sugars. Minor constituents include vitamins and minerals. Minerals and some vitamins can be absorbed directly from seawater, as can amino acids, fatty acids, and glucose (Nell et al., 1983). These dissolved nutrients may be important when particulates are scarce, but with the possible exception of vitamins required in trace amounts, their overall significance in the diet is relatively small (Manahan (1983) suggests that dissolved amino acids may contribute at most between 2.0-9.5% of the protein requirements of oyster larvae).

Bivalves can satisfy much of their nutritional requirements endogenously through biosynthesis from basic chemical building blocks. Maternal reserves passed on through the eggs also support early larvae. However, in addition to the raw components, some specific nutrients must be supplied through the diet. These constituents are "essential nutrients" and include certain amino acids, fatty acids, and sterols.

It is desirable for the diet to be appropriately "balanced" (provided in proportion to the animal's requirements) in order to minimize the energy required for biosynthesis and increase the scope for growth. This "balance" becomes more critical in aquaculture when trying to maximize feed conversion of expensive algal cultures and maintain optimal growth and health under the inherently stressful conditions of high density bivalve cultures.

1.7.1 Protein

Protein (amino acids) is an important macronutrient for bivalves, mainly as a structural component, though it can also be catabolized for energy. Vitellins, complexes of lipids and polypeptides found in invertebrate eggs, are involved in lipid transport and are important in developing embryos (Lee and Heffernan, 1991). Protein sources presented in artificial diets have been shown to affect juvenile growth in *Crassostrea virginica* (Langdon and Siegfried, 1984), but there is little interspecific variation in microalgal protein levels (Enright et al., 1986a) and only minor variations in amino acid compositions (Webb and Chu, 1983). However, protein content can vary intraspecifically due to culture conditions of algae and this can be an issue for nutrient balance (Fabregas et al., 1986; Utting, 1986; Wikfors et al., 1992).

1.7.2 Carbohydrate

Correlative evidence suggests that carbohydrate may be a limiting factor in some bivalve diets (Wikfors et al., 1984; Whyte et al., 1989). Glycogen is often the major

energy store for adult bivalves, but it is less important than lipid for larvae and spat. Likewise, carbohydrate is only a minor component of bivalve eggs, indicating that it is not a reserve source for development (Holland, 1978). Carbohydrate composition of algae may also be important (Chu et al., 1982), *e.g.*, cellulose is difficult for some bivalves to digest, due to a lack of cellulase activity, and should be minimized in the diet (Langdon and Newell, 1990).

1.7.3 Lipid

Lipids are a diverse class of biochemical compounds that are soluble in non-polar solvents such as chloroform, but not in water. They are important physiologically as membrane components (phospholipids), as precursors to hormone-like signal molecules (prostaglandins and eicosanoids) that play a variety of metabolic roles, and energetically as storage components in the form of triacylglycerols (and wax esters in some invertebrates such as copepods).

Lipids are compact and efficient fuel molecules making up the major reserve material of bivalves in early stages of development (Holland, 1978). Manning (1986) showed that *Placopecten magellanicus* has a lipid-based metabolism up to at least nine months of age. Lipids may also serve a secondary role in maintenance of buoyancy in larvae.

Because lipids are so important to developing bivalves, the present study focuses on this diet component. A number of authors have suggested that increased lipid levels in the diet can aid growth (*e.g.*, Pillsbury (1985) for queen conch larvae; Parrish et al.

(1993) for juvenile sea scallops), but beyond a certain level other constituents (protein or carbohydrate) may become limiting. Gallager and Mann (1986) suggest that a minimum amount of lipid reserve in the animal is a predictor of culture success, but they found no clear correlation with growth beyond a threshold level.

1.8 Lipid and fatty acid structure

1.8.1 Lipid classes

Lipids can be divided into classes based on structure (Parrish, 1988). These include (in order of increasing polarity): aliphatic hydrocarbons (HC), wax esters (WE), steryl esters (SE), acylated glyceryl ethers (GE), triacylglycerols (TAG), free fatty acids (FFA), free aliphatic alcohols (ALC), sterols (ST), diacylglycerols (DAG), acetone mobile polar lipids (AMPL; an analytical grouping consisting of monoacylglycerols glycolipids, and pigments) and phospholipids (PL) (Figure 1.1). AMPL and PL are collectively called "polar lipids" and the less polar classes are "neutral lipids". Lipid classes important in bivalve nutrition include TAG among the neutral classes and sterols and phospholipids among the polar classes.

1.8.2 Fatty acid methyl esters (FAME)

Free fatty acids and lipids containing esterified fatty acids are "acyl lipids" from which fatty acid methyl esters can be derived (FAME in Figure 1.1). Fatty acids display a wide variety of structures, but in marine animals are commonly made up of even numbered carbon chained compounds containing between 14 and 22 carbon atoms with

varying numbers of double bonds (unsaturation). The terms "saturated" (SAT), "monounsaturated" (MONO), and "polyunsaturated" (PUFA) describe fatty acids with no double bonds, one double bond, and more than one double bond, respectively. Three systems of nomenclature for fatty acids are found in the literature: systematic names, trivial names and short hand notation (Table 1.1). The short hand notation is used primarily here, but systematic and trivial names referred to in this report are clarified in Table 1.1. By combining fatty acid data with lipid class composition, a great deal of information is obtained about the nutritive status of a sample (Parrish, 1988).

1.9 Lipid and fatty acid metabolism

A thorough discussion of fatty acid metabolism and synthesis is beyond the scope of this thesis, but a brief review is necessary. Most of the published work has been done on mammalian systems, but the general mechanisms are also applicable to bivalves.

1.9.1 Fatty acid degradation

Fatty acids in eukaryotes are catabolized by the sequential removal of two-carbon units. This process is called β-oxidation and results in the maintenance of predominantly even-numbered carbon chain fatty acids in animals. Oxidation of unsaturated fatty acids requires accessory enzymes because progression of the normal biochemical pathway is blocked by the presence of a double bond. This may result in a small inefficiency in oxidation of PUFAs as opposed to saturated fatty acids. Some authors argue that this can have a measurable effect on animal growth when PUFA are provided in excess of the

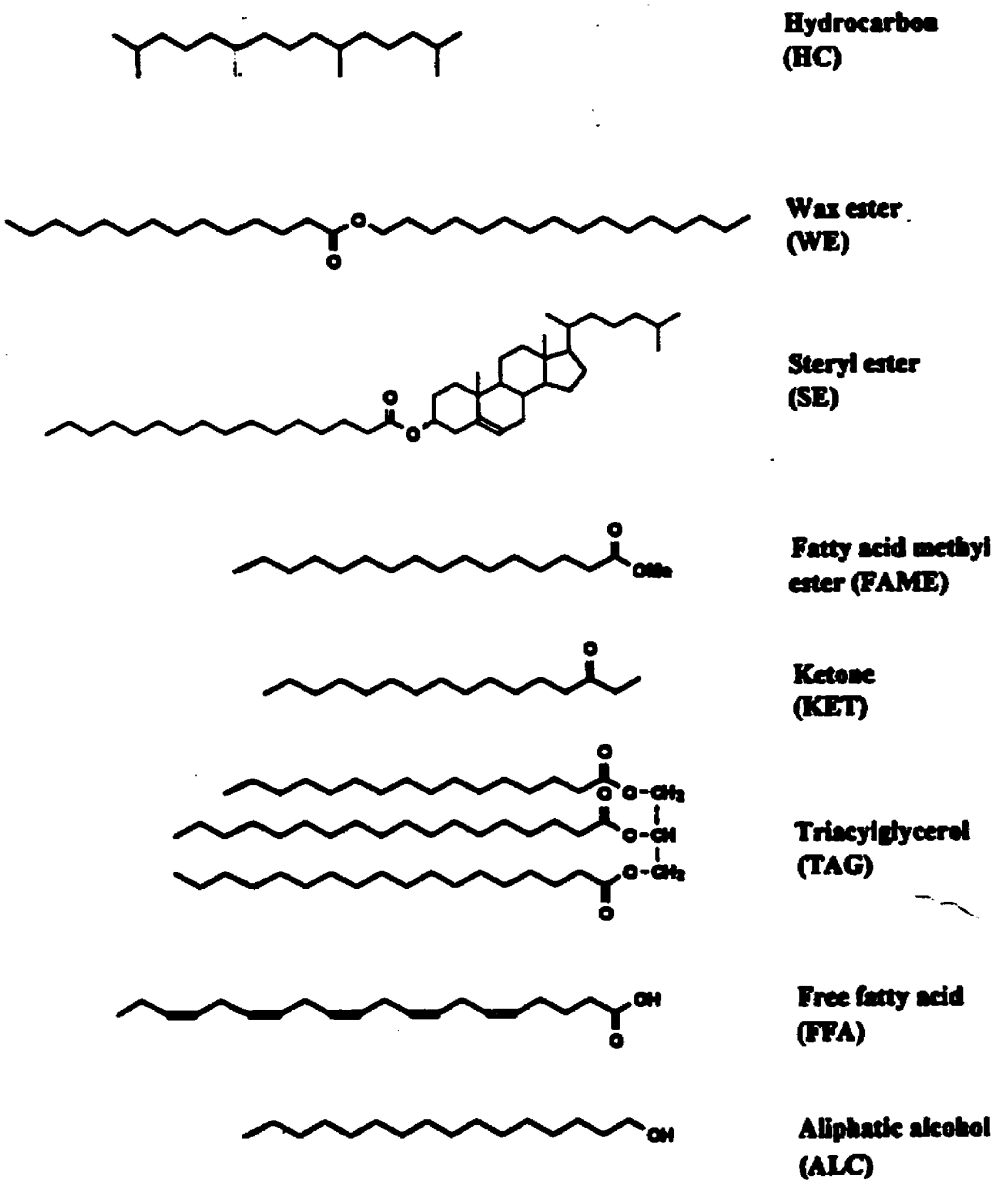


Figure 1.1. Representative structures of common marine lipid classes. Adapted from Budge (1999).

Table 1.1. Glossary of fatty acid nomenclature.*

Common name	Systematic name	Notation**	Abbreviation
Saturated			
Myristate	Dodecanoate	14:0	
Palmitate	Hexdecanoate	16:0	
Stearate	Octadecanoate	18:0	
Arachidate	Eicosanoate	20:0	
Monoenoic			
Palmitoleic	9-hexdecenoic	16:1n-7	
Oleic	9-octadecenoic	18:1n-9	
Vaccenic	11-octadecenoic	18:1n-7	
Gadoleic	9-eicosenoic	20:1n-11	
Dienoic			
Linoleic	9,12-octadecadienoic	18:2n-6	
Trienoic			
a-Linolenic	9,12,15-octadecatrienoic	18:3n-3	
y-Linolenic	6,9,12-octadecatrienoic	18:3n-6	
Dihomo-y-linolenic	8,11,14-eicosatrienoic	20:3n-6	
Tetraenoic			
Arachidonic	5,8,11,14-eicosatetraenoic	20:4n-6	ARA
Parinaric	9,11,13,15-octadecatetraenoic	18:4n-3	
Adrenic	7,10,13,16-docosatetraenoic	22:4n-6	
Pentaenoic			
Timnodonic	5,8,11,14,17-eicosapentaenoic	20:5n-3	EPA
Docosapentaenoic	4,7,10,13,16-docosapentaenoic	22:5n-6	
Clupanodonic	7,10,13,16,19-docosapentaenoic	22:5n-3	
Hexaenoic			
Cervonic	4,7,10,13,16,19-docosahexaenoic	22:6n-3	DHA

* Adapted from: Stubbs and Smith (1984) and Stryer (1993).

** The abbreviated form denotes the number of carbon atoms in the acyl chain (22:6n-3) followed by the number of double bonds (22:6n-3) and the position of the first double bond from the methyl end (22:6n-3).

The position of the remaining double bonds can be determined since the abbreviated notation is used only for methylene interrupted double bonds.

Unless otherwise stated- all double bonds are in the *cis* configuration.

animal's requirements (Whyte et al., 1990).

1.9.2 Fatty acid synthesis and essential fatty acids

The pathways for fatty acid synthesis are separate from those of degradation. Palmitate (16:0) is the major product of the fatty acid synthetase system in animals, and C_{14} - C_{22} FA are predominant in most animals (Stryer, 1993). A variety of mono and polyunsaturated fatty acids can be synthesized *de novo* through a combination of elongation and desaturation enzymes. Elongation occurs by the addition of 2-C units at the carboxyl end of the chain and requires NADPH or NADH. Thus, the series structure (position of the first double bond from the terminal end) is maintained. However, like higher animals, bivalves lack enzymes to introduce double bonds distal to C-9 and cannot synthesize fatty acids of the (n-3) or (n-6) series. Thus they require linolenate (18:3n-3) and linoleate (18:2n-6) in the diet. Plants have more robust fatty acid synthesis systems and are the ultimate source of these compounds.

Normally these fatty acids are the starting points for synthesis of other long chain (n-6) and (n-3) PUFA (Figure 1.2). However, in bivalves and other marine animals, there is evidence of a lack of bioconversion of 18:3n-3 and 18:2n-6 into longer C_{20} and C_{22} chain PUFA, presumably because the necessary enzyme systems are lacking or inefficient. Waldock and Holland (1984) concluded through radiolabelling experiments that only limited elongation and desaturation occurred in *Crassostrea gigas* juveniles fed diets deficient in one or more of these PUFA. Chu and Greaves (1991) likewise found only limited elongation of 18:3n-3 and 18:2n-6 and no desaturase activity for *C.*

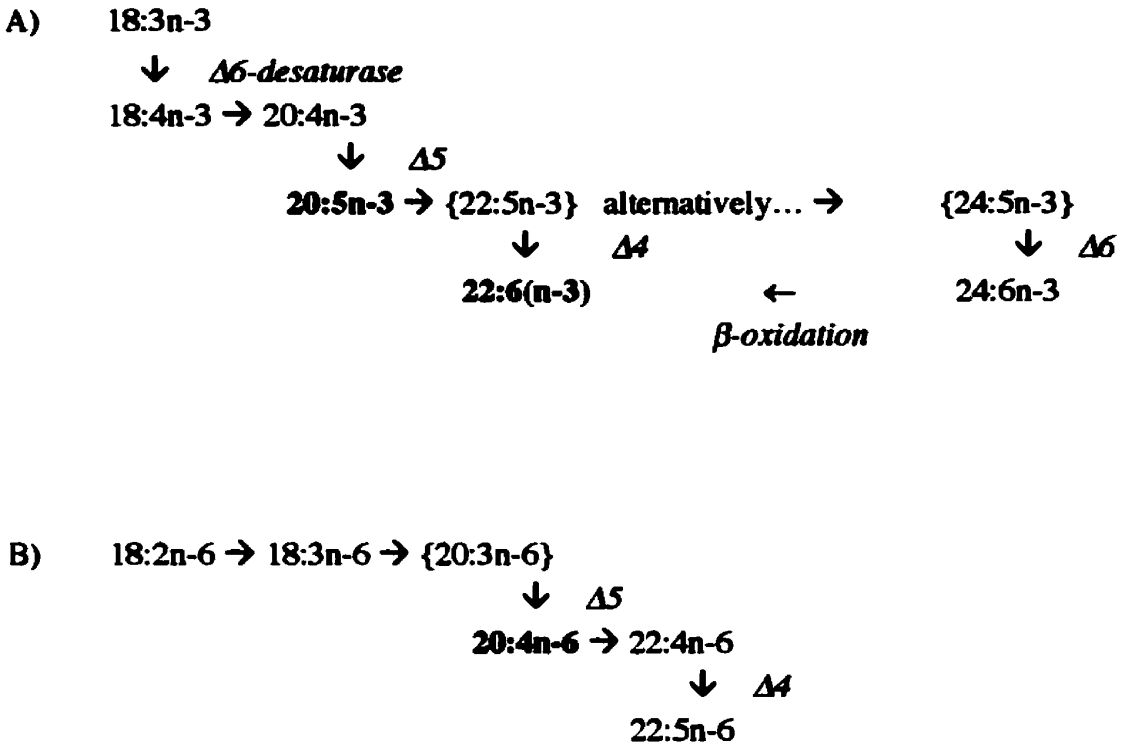


Figure 1.2. Major elongation and desaturation pathways of: a) linolenic (18:3n-3) and b) linoleic (18:2n-6) to long chain (C_{20} and C_{22}) PUFAs after Chu and Greaves (1991) and Voss et al., (1991) (alternative n-3 pathway). Essential fatty acids result from a lack of, or insufficient, activity in any of the desaturases along the pathway. Important EFAs for bivalves are shown in bold. Intermediate structures are in brackets.

virginica. In *Mytilus edulis*, Zhukova (1991) did show the presence of a Δ-5 desaturase which led to the production of unusual (though common to bivalves) C_{20} and C_{22} non-methylene interrupted dienes (20:2 NMID, 22:2 NMID) *de novo*. However, the enzyme was specific for only monounsaturated fatty acids and no conversion into 20:4n-6, 20:5n-3, or 22:6n-3 occurred. An inability to convert linoleic or linolenic acid to longer chain PUFA is also found in certain other animals. For example, cats cannot synthesize 20:4n-6 from 18:3n-6 (Urich, 1994).

The fatty acids 20:5n-3 (eicosapentaenoic acid or EPA) and 22:6n-3 (docosahexaenoic acid or DHA) occur in significant quantities in bivalves and many other marine organisms. These compounds have important roles in membrane physiology, including maintenance of membrane fluidity. There is a general inverse relationship between the melting point of a fatty acid and its double bond number. This is important in ectotherms, and a number of mechanisms exist to modify membrane composition to regulate membrane fluidity in response to environmental temperatures (Stubbs and Smith, 1984). A deficiency of (n-3) PUFA in the diet inhibits growth, reproduction, and health of many marine bivalves, other invertebrates, and fish (Watanabe, 1993; Le Pennec et al., 1998).

Arachidonic acid (ARA; 20:4n-6) is also present in varying but significant quantities in marine organisms. It is important as a precursor for prostaglandins and consequently is involved in reproductive and other processes. There is some evidence that this (n-6) fatty acid must also occur in suitable levels in the diet in order to maintain optimal growth and health (Delaunay et al., 1993). Thus EPA, DHA, and ARA are

generally considered "essential" nutrients in most bivalves. A few species, however, such as the manila clam, *Tapes philippinarum*, may synthesize longer chain fatty acids from C_{18} PUFA (Laing et al., 1990).

1.10 Controversy over the importance of "essential fatty acids"

One or more of these fatty acids are often found in phytoplankton, but hatchery diets usually consist of only a few algal species. Thus, hatchery diets may vary more in quality than natural phytoplankton communities and deficiencies in EFAs may limit bivalve growth or affect health. Algae do show species-specific and class-specific fatty acid patterns unlike with other biochemical constituents (Viso and Marty, 1993), but relative proportions of these fatty acids vary with environmental and endogenous conditions (Brown et al., 1993).

It has been well established that algal species lacking all these fatty acids, such as *Dunaliella tertiolecta*, are "poor" nutritionally (Webb and Chu, 1983). However, some authors contend that (as with total lipid), EFA content of microalgae beyond a threshold level has little or even a negative effect on culture vigor (Whyte et al., 1989). Furthermore, the relative importance of EPA and DHA needs to be established, as some authors contend that either one or the other is sufficient to meet dietary needs (Langdon and Waldock, 1981; Pillsbury, 1985). Work with fish has benefited from standard pre-formulated diets and greater inroads have been made with regard to defining correct EPA/DHA and (n-6)/(n-3) ratios in the diet of various species. Also poorly understood in bivalve nutrition and overshadowed in much of the literature by discussion of (n-3)

PUFA is the role of (n-6) PUFA and arachidonic acid in particular (Sargent et al., 1997).

From a brief survey of bivalve nutritional studies it is apparent that there is little consensus on what constitutes an ideal bivalve diet (Table 2.1). Some discrepancies are probably due to species-specific differences in nutritional requirements. For example, the quahog, *Mercenaria mercenaria*, appears to have less stringent EFA requirements than three other bivalve species (Helm and Laing, 1987). Differences in the relative efficiencies of various mechanisms to maintain optimal membrane composition, interspecific differences in the optimal composition itself and/or membrane fatty acid turnover rates may account for these varied nutritional requirements.

The fatty acid composition of bivalves often reflects their diet, and in this respect fatty acids are also useful as biomarkers in food-web studies. However, bivalves have some ability to modify PUFA to suit their requirements through either selective accumulation and/or retention of these fatty acids and possibly through limited biosynthesis. Some studies suggest a strong diet effect on animal biochemical composition with only a modest ability (or need) to modify assimilated fatty acids (Waldock and Holland, 1984). Other studies report little diet effect on subsequent animal composition. For example, Watanabe and Ackman (1974) state that *Crassostrea virginica* and *Ostrea edulis* rapidly converts exogenous fatty acids from algal diets into a species-oriented profile.

Nutritional requirements for fatty acids may also be related to genetic or environmental factors. PUFA may be more important to a cold water species such as *Placopecten magellanicus* due to an increased need to maintain membrane fluidity in

Table 1.2. Survey of selected studies on bivalve nutrition demonstrating wide ranging results depending on species, algal treatments, life history stage, and experimental approach.

* = listed nutrient had no significant effect or negative correlation with animal growth or survival for the particular experimental diets.

Author	Species	Life stage	Algae	Nutritional requirement	No effect *
Pillabury (1985)	*Strombus gigas* (Queen conch)	larvae	*Isochrysis galbana, Prorocentrum* sp. *Emiliania huxleyi, Heterocapsa pygmacea*	High lipid EPA and/or DHA	
Helm and Laing (1987)	*Mercenaria mercenaria*	larvae			EPA, mixed diets
Helm and Laing (1987)	*Crassostrea gigas, C. rhizophorae Tapes semidecussata*	larvae	*Isochrysis* sp. (T-Iso) *Chaetoceras calcitrans*	EPA Balanced FA	
Whyte et al. (1990)	*Crassadoma gigantea* (Rock Scallop)	larvae	*I. galbana, C. calcitrans Thallasiosira pseudonona, Skeletonema costatum*	carbohydrate	Lipid, protein Fatty acid composition
Waldock and Nascimento (1979)	*C. gigas*	larvae		TAG reserves in larvae	Dietary Lipid
Thompson et al. (1994)	*Patinopecten yessoensis*	larvae	*Chaetoceros simplex, Pavlova lutheri* Light modified cultures	Saturated fatty acids	EPA, DHA – negatively correlated
Thompson and Harrison (1992)	*C. gigas*	larvae	*Thalassiosira pseudonona* Light modified cult	DHA Saturated FA (14:0, 16:0)	EPA- negatively correlated
Whyte et al. (1989)	*Patinopecten yessoensis*	larvae	*I. galbana, C. calcitrans* Log and Stationary cultures	carbohydrate	
Epifanio (1979)	*C. virginica*	spat	*Tetraselmis suecia,, I. galbana*	DHA	
Langdon and Waldock (1981)	*C. gigas*	spat	*P. lutheri, Dunaliella tertiolecta, T. suecia,* Lipid microcapsules	DHA	Dietary Lipid
Knauer and Southgate (1997)	*C. gigas*	spat	*D. tertiolecta* EPA/DHA microcapsules	EPA	DHA
Waldock and Holland (1985)	*O. edulis*	spat		TAG reserves	
Enright et al. (1986a)	*O. edulis*	juvenile	16 species	EPA,DHA, Carbohydrate	amino acids, protein
Wikfors et al. (1996)	*C. virginica*	juvenile	*Tetraselmis* spp., T-Iso, *Chlamydomonas*	EPA, Sterol	
Parrish et al. (1999)	*Placopecten magellanicus*	juvenile	T-Iso *Chaetoceras muelleri*	20:4n-6, carbohydrate n-3/n-6, PUFA/SAT ratio	
Wikfors et al. (1984)	*C. virginica*	juvenile	range	High lipid	
Kreeger and Langdon (1993)	*Mytilus edulis trossulus*	juvenile	T-Iso modified by culture conditions	protein	
Wikfors et al. (1992)	*Mercenaria mercenaria*	juvenile	19 algae species	Protein and lipid balance	
Enright et al. (1986b)	*Ostrea edulis*	juvenile	*C. calcitrans*- culture modified	DHA, carbohydrate	
Berntsson et al. (1997)	*O. edulis*	broodstock		PUFA and DHA in egg	PUFA and DHA - diet
Le Pennec et al. (1998)	*Argopecten purpuratus, Pecten maximus*	broodstock	Hatchery surveys	ARA, DHA, 20:1n-9, sterols	
Robinson (1992a,b)	*C. gigas*	broodstock	Lipid supplements	(n-3) fatty acids	
Soudant et al. (1996)	*P. maximus*	broodstock	T-Iso, PTSC mix, *C. calcitrans*	DHA	

response to colder temperatures. Napolitano et al. (1992) found scallops collected at greater depths (and hence colder temperatures) to be higher in DHA.

Both the fatty acid requirement and the ability to cope with dietary deficiencies are also related to life stage. Larvae, juveniles, and adults are very different biologically and physiologically. Younger animals have fewer biochemical reserves to draw on and possibly less developed enzyme systems to deal with fatty acid deficient diets. The maintenance or accumulation of fatty acids largely absent in the diet is indicative of an underlying physiological significance; it is important to distinguish among these fatty acids that may affect growth, health, and development and simple dietary biomarkers.

1.11 Experimental approaches

Different experimental approaches and problems inherent in the study of bivalve nutrition may account for the conflicting results often reported for a given species at the same developmental stage. Nutritional studies have gradually evolved in complexity from simple evaluations of growth in response to different live algal diets to correlating the biochemical composition of the diet and of the animal with its growth and development.

To test for nutritional requirements, some authors have employed algae modified biochemically by manipulating culture conditions. Other advances have been made through the use of controlled artificial diets and lipid microcapsules (Langdon and Siegfried, 1984; Heras et al., 1994), but these diets tend to be less well accepted by bivalves than are live microalgae, and at present are less suitable for commercial

applications. Another interesting approach compares hatchery-cultured animals with those reared in the natural environment (Martinez et al., 1992). Soudant et al. (1999) compared the fatty acid compostition of field-cultured *Crassostrea gigas* with that of hatchery reared animals. Given the assumption that animals *in situ* display a more optimal fatty acid composition under most wild conditions due to availability of a broader array of food species, suitable hatchery diets were suggested. Other studies involving radioactive tracers and fatty acid studies resolved to detailed polar lipid classes are shedding light on the fundamental mechanisms of fatty acid metabolism (Soudant et al., 1996).

1.12 Objectives

Previous work with *Placopecten magellanicus* has focused more on gross biochemical composition (Couturier, 1986; Manning, 1986; Ryan, 1999) and later life stages (juveniles and adults; Parrish et al., 1993; Coutteau et al., 1996), although work on fatty acid requirements of other pectinids (such as *Pecten maximus*, a temperate species) has been carried out (Soudant et al., 1996). These studies serve as useful comparisons to this study on the essential fatty acid requirements of *P. magellanicus*.

In this research, the impact of dietary fatty acids and lipids on broodstock conditioning (Chapter 2) and larval culture (Chapter 3) was investigated. Different species of live algal cultures varying broadly in levels of essential fatty acids are compared with an *Isochrysis* sp. (clone T-Iso) reference diet. Culture performance (hatching success and larval growth) is evaluated in relation to diet and animal

composition. In Chapter 4, the effects of culture conditions on a "new" species of algae used as a treatment in the larval feeding trials presented in this thesis are reported and changes in its biochemical composition in relation to optimal harvest strategies discussed. The overall results will help define the essential fatty acid requirements of this important bivalve in the context of other bivalve nutrition studies and provide some commercially relevant recommendations for optimizing hatchery culture.

CHAPTER 2 – BROODSTOCK CONDITIONING – EFFECTS OF DIETARY LIPID QUALITY ON *PLACOPECTEN MAGELLANICUS* EGG BIOCHEMICAL COMPOSITION

2.1 Introduction

Broodstock conditioning is the first step in hatchery production of bivalve molluscs. From a commercial standpoint, it is desirable to produce larvae as early in the hatchery season as possible in order to increase total production and make use of high water temperature and food availability conditions in the spring and summer. The gametogenic cycle in bivalves is characterized by an accumulation of nutritional reserves followed first by gonad growth and differentiation of gametes, then by vitellogenesis (the accumulation of nutrients within gametes), and culminating in one or more spawning events before a final quiescent period. Animals are artificially conditioned by simulating conditions that would occur *in situ* (*i.e.*, manipulating water temperature to a threshold level that initiates gametogenesis). Nutrients are deposited in eggs through a variety of pathways including transfer of lipids from the digestive gland (Vassallo, 1973), glycogen and *de novo* synthesis of lipids from the adductor muscle (Barber and Blake, 1985), recycling of nutrients from egg atresia within the gonad, and intestinal loop transfer (Le Pennec et al., 1991). These mechanisms depend on the initial nutritive state of the animal (endogenous reserves) and on dietary inputs during the conditioning process. Thus, food is a major factor affecting gametogenesis, although environmental and other factors such as photoperiod, water quality, temperature (Utting and Millican, 1997), stress (Bayne et

al., 1975), and length of the conditioning period (*i.e.*, degree days, Helm et al., 1991) may also have an impact.

The quantity of food provided to broodstock is important for both economic and nutritional reasons. Too small a ration can produce poor results (Chaparro, 1990) while a suitable ration can boost egg lipid levels above that of eggs from unfed brood (Manning, 1986; Muranaka and Lannan, 1984). An excessive ration decreases the absorption efficiency in feeding bivalves and wastes costly algae.

Lipid quality may also affect fecundity. Millican and Helm (1994) found a large decrease in the number of larvae released from *Ostrea edulis* fed a diet lacking PUFA (*D. tertiolecta*) compared with control diets. However, other than broadly differing treatments such as fed and non-fed, the mean lipid content of eggs is often unrelated to dietary lipid quantity or quality. There is evidence that bivalves may control fecundity in order to maintain egg quality in terms of lipid content (MacDonald and Thompson, 1985). Obtaining enough eggs is rarely a problem for hatcheries, due to the high fecundity of most bivalves. Furthermore, the number of eggs released is often unrelated to the number of D-stage larvae produced (Le Pennec et al., 1998) and beyond a threshold level necessary for embryogenesis, initial egg lipid reserves have little effect on subsequent larval growth (Gallager and Mann, 1986).

The influence of diet quality on egg PUFA composition may be of greater importance. Because most bivalves cannot synthesize long chain PUFA (especially EPA and DHA), these compounds are incorporated into eggs ultimately from dietary sources, and variation in these EFA in the diet can impact subsequent egg composition (Soudant,

1996). Some studies have linked the PUFA composition of eggs to hatch rates and subsequent larval vigor (Soudant, 1996; Berntsson et al., 1997).

This study examines the important link between dietary fatty acid composition and egg biochemical composition in *Placopecten magellanicus*, focusing on the "essential" fatty acids EPA, DHA, and ARA. In two experiments broodstock were conditioned on diets consisting of equal dry weight rations of live microalgae varying in EPA and DHA content. Lipid composition and fatty acid profiles of the resulting eggs were compared with those of the diet, and performance evaluated as hatching rates. In the first experiment, the fatty acid composition of the digestive gland neutral lipids and adductor muscle were also determined to examine dietary labelling and possible nutrient transfer from these tissues. Fatty acid profiles of eggs from naturally conditioned broodstock served as a reference group for comparisons. An improved basic understanding of egg fatty acid composition and the impacts of dietary lipids should lead to significant improvements in conditioning protocols and diet optimization in hatcheries.

2.2 Methods

2.2.1 Algal culture

Isochrysis sp. (Tahitian strain, clone T-Iso, CCMP 1324), *Dunaliella tertiolecta* (CCMP 1320; Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow, ME), and *Tetraselmis chui* (strain PLY429; NMFS Milford Laboratory, Milford, CT), were grown in 80-L and 200-L batch cultures. Culture water was filtered nominally to 0.45 μm and treated with chlorine (20 ppm) for at least two hours. After

neutralizing the chlorine with sodium thiosulfate, culture water was fertilized (Guillard's F/2 media) and inoculated from 2-L flasks (in the case of 80-L culture tubes) or 20-L carboys (200-L culture tubes). Cultures were grown under continuous fluorescent light and supplemented with air (filtered to 0.45 μm) for mixing and approximately 1% CO_2 as a carbon source. Cultures were staggered and harvested in late logarithmic growth phase. Cell concentrations were determined daily on a Coulter Multisizer™ and health of cultures monitored by microscopic observation. Primary cultures were originally axenic and treated aseptically, but no attempt was made to maintain the large batch cultures in a bacteria-free state.

Cultures were sampled (as described below) five times during the first experiment to establish the mean gross composition as defined by the C:N ratio and the lipid content, lipid class composition, and fatty acid profile.

2.2.2 Broodstock conditioning experiment #1

2.2.2.1 Experimental design

Adult scallops were collected by SCUBA divers on July 23, 1998 from North Harbour, Placentia Bay, Newfoundland, Canada and taken to the Ocean Sciences Centre, St. John's, Newfoundland (located on Logy Bay). The experiment was set up as a completely randomized block design. Three blocks of adjacent water baths (1 m x 1 m x 0.5 m) consisted of four 70-L tanks each, arranged in a square configuration. One of four treatments was randomly assigned to each of the four tanks within each block. Random assignment of the treatments was accomplished by assigning a number from 1-4 *a priori*

to each treatment and then generating random integers from 1 to 4 (without replacement) for each tank position and in each block represented in an Excel™ spreadsheet using the random function. Scallops were sexed visually, marked with nail polish, and three adult female scallops (ranging in size from 98 to 130 mm) placed in each tank for a total of 36 animals (9 per treatment).

Treatments consisted of algae commonly cultured for feeding bivalves and represented rations characterized by a wide range of EPA:DHA fatty acid ratios. They were:

1) *Isochrysis* sp. (T-Iso) (Prymnesiophyceae)- high DHA, low EPA treatment
2) *Tetraselmis chui* (PLY429) (Prasinophycae) – low DHA, high EPA treatment
3) *Isochrysis* sp./*T. chui* mix (50:50 by dry weight) – moderate DHA and EPA treatment
4) *Dunaliella tertiolecta* (Chlorophyceae) – low DHA, low EPA treatment

As in previous studies on suitable conditioning rations (Utting and Millican, 1997), scallops were drip-fed a daily ration equivalent to approximately 6% of the dry meat weight of the scallops in each tank ($8x10^9$ cells/animal/day for *Isochrysis* sp.). Seawater (1 μm nominal filtered) was exchanged daily and animals were kept at a temperature of approximately 16°C by slow exchange of a water bath of ambient seawater. Tanks were thoroughly cleaned weekly.

After four weeks of diet treatments, animals were placed in individual containers and spawned by thermal shock. On initiation of spawning, each female was placed separately in a new container containing 1 μm filtered seawater. Egg samples (fresh, non-fertilized) from these females (6-8 animals for each treatment) were collected for

determination of lipid content, lipid class and fatty acid composition, dry and ash weight, and egg diameter.

Naturally conditioned scallops, of a similar size range, freshly collected from the same site were also spawned and egg samples taken as a reference for biochemical comparison.

2.2.2.2 Hatch rates

Hatch rates represent the percent yield of healthy D-stage veliger larvae (after 2-3 days of development for *Placopecten magellanicus*) from the spawned eggs. Hatch rates were determined by fertilizing a portion of the eggs from three individual spawners (selected in an arbitrary manner) from each diet treatment (excluding naturally conditioned spawners). Eggs were fertilized by sperm pooled from 6 males held separately from the females. Eggs were counted and sized using a Coulter Multisizer™ and fertilized eggs were distributed and incubated in 20-L buckets filled with 1 μm filtered seawater (approximately 16°C) at a concentration of approximately 50 eggs/mL. The resulting D-stage larvae were concentrated by screening (35-μm-mesh) the water in these 20-L tanks after 2.5 days, transferring the larvae in 1 L containers, and taking three replicate 1 mL samples from each container for counting on a Sedgewick rafter cell using a microscope.

2.2.2.3 Tissue dissection and analyses

After spawning, three scallops from each algal treatment were selected without bias for lipid and fatty acid analysis of the digestive gland and adductor muscle. The digestive gland and adductor muscles were excised and the total individual tissue wet weights determined on a Sartorius™ electronic balance. A small section from each organ was cut from the tissue and the wet weights of each of the two pieces determined. Each small piece was placed immediately in a test tube containing 2 mL chloroform, sealed under nitrogen, and stored at –20°C for later biochemical analysis. The remainder of the organ was frozen and lyophilized. Dry weight of this sample was determined after lyophilization and a wet weight to dry weight ratio calculated for each organ sampled. The dry weight of the subsample taken for lipid and fatty acid analysis could then be calculated using these ratios and total lipid and lipid class determinations of the sample expressed as mg/g dry tissue weight.

Total lipids extracted from the digestive gland were separated into neutral and polar lipids (see below) after lipid class determination on a Mark V Iatroscan TLC/FID. FAMEs were derived by transesterification and fatty acid analyses conducted on the total, neutral, and polar lipid portions of the digestive gland. Only data for the fatty acid composition of the neutral (primarily TAG) fraction is presented here because of the high proportion of this lipid class in the digestive gland and its relevance in the context of food quality. Adductor muscles consisted almost entirely of polar lipids and sterols so FAMEs were derived and analyzed only for the total lipids of this tissue, but these are representative of the polar lipid fraction as well.

2.2.3 Biochemical analyses

2.2.3.1 Algae

Algal cultures were sampled five times over the course of the experiment in order to obtain a mean biochemical composition (lipid content, lipid class determination, fatty acid analysis, and dry and ash weight) for each broodstock diet. Carbon and nitrogen were also determined for three of these samples with an elemental analyzer (Perkin Elmer 2400) calibrated with acetanilide. First, cell densities and cell sizes of each sample were determined using a Coulter Multisizer™. The sample was then divided into three subsamples for CN analysis, determination of dry and ash weight, and lipid extraction. The two subsamples for CN and dry and ash weight determination were further divided into three more subsamples so that the data for each sampling date represents the mean of these three analytical replicates. Cells were collected under gentle vacuum pressure on a precombusted Whatman GF/C filter. Two filter blanks were included to use as correction factors in each CN and dry/ash weight determination, and one filter blank consisting of an equal sample volume of 0.45 μm filtered seawater was included with each sample for lipid extraction as a check for lipid contamination.

2.2.3.2 Eggs

Eggs were gently passed through a coarse filter (300 μm) and collected on a screen (20 μm) and gently rinsed into a 1 L container with 1 μm filtered seawater. Eggs were counted and sized on a Coulter Multisizer™ and subsamples of $1 \times 10^4 - 10 \times 10^4$ collected on precombusted Whatman GF/C filters under gentle vacuum for lipid

extraction and dry and ash weight determination. The eggs for lipid extraction (one subsample) were placed immediately in a vial containing 2 mL of chloroform, the head-space flushed with nitrogen, and the vial stored at –20°C. Samples for dry weight determination were rinsed with isotonic (3%) ammonium formate solution to rid the samples of excess salts, dried at 45°C for approximately 72 hours and stored in a dessicator with activated silica gel prior to weighing on a Mettler™ microbalance. They were then combusted overnight at 200°C and cooled in a dessicator before reweighing. Three subsamples of eggs from each spawner were taken and data used in analyses represent the means of these three analytical replicates.

2.2.3.3 Lipid analyses

Lipid extraction, quantification, fractionation, and fatty acid analysis are detailed in Chapter 4 (section 4.2). Procedures used here were identical with the exception that no lipid or fatty acid standard was added to samples prior to analysis. Briefly, lipids were extracted using a modified Folch procedure, total lipid and lipid classes determined on a Mark V Iatroscan TLC/FID system, FAMEs derivatized by reacting acyl lipids with BF_3-methanol, followed by GC and comparing peak retention times with known standards. Lipid extracts from selected egg samples (approximately 2 per conditioning treatment) were separated into neutral and polar components, using a simple column fractionation technique, for a total (versus between treatment) comparison of these two fractions. Estimates of total fatty acids and absolute quantities of fatty acids in the diet were calculated using an Excel™ spreadsheet program (see Chapter 3, section 3.2.4.3).

2.2.4 Statistical analyses

Treatment differences in total lipid, lipid class, fatty acids (as a weight percent of total fatty acids), hatch rates, and egg organic weight were tested using the GLM function of Systat™. The experiment was originally set up as a randomized complete block design, and accordingly the block x diet treatment interaction term was suppressed in the analysis (Sokal and Rohlf, 1995). Block effects were found to be insignificant, so the data were pooled. This allowed the inclusion of biochemical data from the naturally conditioned spawners that were not included in the block design and increased the power of the test. Untransformed data was subjected to analysis by a model I ANOVA (diet treatments were considered as fixed factors) using Statview™, followed by Fisher's LSD as a post-hoc test to compare treatment means. For all statistical analyses $\alpha = 0.05$. Only fatty acids comprising more than 1% of total fatty acids were included in statistical analyses.

2.2.5 Broodstock conditioning experiment # 2

A second conditioning experiment was initiated on June 25, 1999. Here, only three diet treatments were used: *Isochrysis* sp. (T-Iso), *Tetraselmis chui*, and *Dunaliella tertiolecta* (no mix or naturally conditioned treatments). Diets consisted of the same live algal cultures and ration per scallop as before, but included the use of algal pastes (Reed Mariculture, Santa Cruz, CA; www.seafarm.com as viewed on June 1, 1999) as partial diet replacements for live *Isochrysis* sp. and *T. chui*, to reduce the requirement for live

algae (pastes were the same strain designation as live food). These diets were also fed on an equal dry weight basis.

Scallops for experiment #2 were from the same collection trip as scallops used in the first experiment and were held over the winter in a flow-through tank. Two 150-L conical tanks were allocated to each treatment (6 tanks total). Three scallops were placed in each tank and hung in a three-tiered pearl net assembly (one scallop per tier), for a total of 18 scallops (3 scallops x 2 tanks x 3 treatments). Seawater (1 μm nominal filtered) was changed approximately every other day. Approximately weekly, the pearl net assemblies were rotated to different tanks to minimize possible tank and position effects on conditioning. Scallops were conditioned for a total of 10 weeks. The purpose of this second experiment was to initiate diet treatments earlier and to extend the conditioning period in comparison with experiment #1.

Due to poor temperature control in the tanks and other factors discussed later, scallops from this experiment did not ripen fully and failed to spawn. Thus, instead of egg samples, gonads were excised and the distal portion sampled for lipid and fatty acid analyses. Three scallops from each treatment were selected in an arbitrary manner for this analysis and each scallop treated as a replicate. Lipid and fatty acid analysis and statistical procedures were conducted as above, but data were expressed in terms of wet weight of the gonad. In this experiment, gross changes in gonad EFA composition (mainly EPA and DHA) were the primary concern, so algae and algal pastes were not sampled for lipid and fatty acid analysis and it was assumed that the species-specific characteristics of the algae were maintained.

2.3 Results

2.3.1 Diet composition

2.3.1.1 Gross composition and lipid content

The C:N ratio (wt/wt) of the diets ranged from a low of 5.7 for *Dunaliella tertiolecta* to 6.9 for *Tetraselmis chui* (Table 2.1). Lipid content per cell was highest in *T. chui* (28.5 pg/cell vs. 5.8 pg/cell for *Isochrysis* sp., but when standardized per unit dry weight, lipid was lower in *T. chui* (11.6 %) compared with both *D. tertiolecta* (19.3%) and *Isochrysis* sp. (21.3%).

2.3.1.2 Lipid classes

TAG, AMPL, and PL were the major lipid classes (>10% of total lipid) in all three algal species with HC, ST, and DAG present in quantities from approximately 2 - 7% (Table 2.2). Ethyl and methyl ketones comprised a significant proportion of lipids in *Isochrysis* sp. (10% combined) but were present in only trace levels in *T. chui* and at less than 2% in *D. tertiolecta*. FFA were present at relatively low concentrations from 0.3% to 1.5% of total lipid. TAG was twice as high in *D. tertiolecta* (20.5%) as the other species, but TAG content was variable in both *T. chui* and *D. tertiolecta* with standard deviations approaching the mean relative content.

2.3.1.3 Fatty acids

Fatty acid analyses of the diet reflected the species-specific fatty acid charcteristics for which the treatments were chosen (Table 2.3). In terms of EFA content,

Table 2.1. Gross (proximate) biochemical composition of the diets expressed as carbon and nitrogen content, C:N ratio, cell dry and organic weight and percent lipid (mean ± standard deviation, n=5).

Diet		*Isochrysis* sp.	*Tetraselmis chui*	*Dunaliella tertiolecta*
Carbon	*(pg/cell)*	12.7 ±1.9	96.0 ±26.7	31.5 ±1.7
Nitrogen	*(pg/cell)*	2.0 ±0.4	14.7 ±6.3	5.5 ±0.5
C:N ratio	*(weight ratio)*	6.5 ±0.7	6.9 ±1.5	5.7 ±0.2
Dry weight	*(pg/cell)*	26.3 ±2.5	244.7 ±5.4	67.1 ±6.5
Org content	*(pg/cell)*	25.4 ±1.5	208.5 ±2.0	61.9 ±3.5
Lipid	*(pg/cell)*	5.8 ±1.7	28.5 ±8.5	12.9 ±2.7
Lipid	*(% dry weight)*	21.3 ±6.6	11.7 ±3.6	19.3 ±3.5
Lipid	*(%AFDW)*	22.0 ±6.8	13.7 ±4.2	20.9 ±3.7
Cell diameter	*(μm)*	3-5	14-23	6-9

Table 2.2. Total lipid and lipid class composition (> 1%) of broodstock diets as pg/cell (top) and as a relative proportion of total lipid (bottom) (mean ± standard deviation, n=5).

Diet	*Isochrysis* sp.	*Tetraselmis chui*	*Dunaliella tertiolecta*
Total Lipids (pg/cell)	5.8 ±1.7	28.5 ±8.5	12.9 ±2.7
HC	0.1 ±0.1	0.8 ±0.3	0.4 ±0.2
Et-KET	0.3 ±0.2	0.0 ±0.0	0.0 ±0.1
Me-KET	0.3 ±0.3	0.1 ±0.1	0.0 ±0.0
TAG	0.6 ±0.1	2.5 ±2.0	2.5 ±2.8
FFA	0.1 ±0.1	0.1 ±0.2	0.2 ±0.2
ST	0.2 ±0.2	1.0 ±0.7	0.5 ±0.4
DAG	0.4 ±0.2	2.1 ±2.7	0.4 ±0.5
AMPL	1.5 ±0.5	8.6 ±5.9	4.7 ±2.7
PL	2.3 ±0.8	13.2 ±3.2	3.8 ±0.7
Percent of total lipid (%)			
HC	2.2 ±1.1	2.5 ±1.0	3.2 ±2.1
Et-KET	5.7 ±4.5	0.0 ±0.0	1.5 ±2.5
Me-KET	4.3 ±5.2	0.3 ±0.7	0.0 ±0.0
TAG	9.6 ±2.5	8.0 ±7.0	20.5 ±21.0
FFA	1.5 ±1.0	0.3 ±0.6	1.2 ±1.2
ST	4.5 ±1.8	3.1 ±1.8	3.7 ±2.2
DAG	7.3 ±2.9	5.8 ±7.6	2.6 ±3.2
AMPL	25.3 ±2.1	34.0 ±9.5	35.2 ±16.5
PL	38.8 ±2.6	45.2 ±8.0	29.6 ±5.8

Isochrysis sp. had a relative DHA level of 12% while *T. chui* and *D. tertiolecta* contained none. EPA was present at 6.5% in *T. chui* but only at 0.4% in *Isochrysis* sp. while *D. tertiolecta* contained no detectable amount (<0.1%). Arachidonic acid (ARA) was present in significant quantities in only the *T. chui* diet (1.9%) although *Isochrysis* sp. had similar quantities of the longer chain 22:5n-6.

Other fatty acid characteristics of the diets included the following: *Isochrysis* sp. had high levels of the saturated FA 14:0 (13.2%). This was nearly equivalent to the level of the other major saturated fatty acid 16:0 (12.8%). Both *T. chui* and *D. tertiolecta* contained 16:0, almost exclusively, as the major saturated fatty acid (18.1% and 17.7%, respectively) and only small amounts (ca. 1%) of 14:0. Correspondingly, *Isochrysis* sp. had higher levels of total saturates at 26.8% versus 19.4% and 19.9% for *T. chui* and *D. tertiolecta*, respectively.

The major monounsaturate in all three species was 18:1n-9, but levels were highest in *Isochrysis* sp. (15.2% vs. 6.5% for both *T. chui* and *D. tertiolecta*). Content of this fatty acid showed a relatively high degree of variability intraspecifically among samples with coefficients of variation (CV) from 33% (*Isochrysis* sp.) to approximately 85% (*D. tertiolecta* and *T. chui*). The fatty acids 16:1n-7 and 18:1n-7 were also present in significant quantities (> 2%). The mean level of monounsaturates was highest in the *Isochrysis* sp. diet at 22.3% followed by 14.7% for *T. chui* (although variable) and 11.0% for *D. tertiolecta*.

Other than C_{20} and C_{22} PUFA differences described above, the *T. chui* and *D. tertiolecta* diets were characterized by a diversity of both C_{16} PUFA and C_{18}PUFA,

Table 2.3. Relative fatty acid composition (wt % of total FA) of broodstock diets (mean % ± standard deviation, n=5).

Diet	*Isochrysis* sp. %	*Tetraselmis chui* %	*Dunaliella tertiolecta* %
14:0	13.2 ±1.2	0.9 ±0.9	1.0 ±0.5
15:0	0.5 ±0.1	- -	- -
16:0	12.8 ±3.2	18.1 ±2.0	17.7 ±4.9
17:0	- -	0.0 ±0.1	1.3 ±0.2
18:0	0.6 ±0.0	0.3 ±0.5	- -
Total SAT	26.8 ±2.8	19.3 ±2.5	19.9 ±4.8
16:1n-9	1.0 ±0.6	0.6 ±1.8	- -
16:1n-7	4.0 ±0.8	3.0 ±1.0	2.5 ±1.7
18:1n-9	15.2 ±5.7	6.6 ±5.6	6.5 ±4.5
18:1n-7	2.2 ±0.6	3.2 ±1.5	1.9 ±1.3
20:1n-11	0.6 ±0.5	0.3 ±0.4	- -
20:1n-9	- -	1.1 ±1.0	- -
Total MONO	22.3 ±3.7	14.7 ±7.9	11.0 ±4.0
16:2n-4	1.1 ±0.5	- -	- -
16:2n-6	- -	2.8 ±1.7	- -
16:3n-6	- -	2.4 ±2.8	- -
16:3n-3	- -	0.3 ±0.6	2.7 ±1.0
16:4n-3	- -	13.5 ±2.4	14.2 ±2.4
18:2n-6	3.5 ±1.5	7.7 ±2.6	5.7 ±2.1
18:3n-6	1.2 ±0.4	1.7 ±1.4	4.0 ±0.7
18:3n-3	5.9 ±1.8	11.8 ±1.5	35.4 ±3.5
18:4n-3	21.5 ±4.7	12.3 ±3.0	1.2 ±0.6
18:4n-1	- -	0.2 ±0.5	- -
20:2n-6	- -	0.1 ±0.4	- -
20:4n-6	tr -	1.9 ±0.8	- -
20:4n-3 (ARA)	- -	0.0 ±0.1	- -
20:5n-3 (EPA)	0.4 ±0.1	6.5 ±2.1	- -
22:5n-6	1.5 ±0.4	- -	- -
22:6n-3 (DHA)	12.1 ±1.5	- -	- -
Total PUFA	44.8 ±5.7	61.1 ±8.8	63.1 ±6.1
branched	1.1 ±0.7	3.7 ±1.7	4.3 ±2.9
unidentified	2.5 ±2.0	1.2 ±1.3	1.6 ±2.2
C_{20} and C_{22} PUFA	13.9 ±1.7	12.4 ±9.1	- -
sum (n-9)	16.2 ±6.4	10.7 ±7.2	6.5 ±4.5
sum (n-7)	5.8 ±1.9	3.7 ±1.5	4.5 ±2.4
sum (n-6)	5.1 ±1.8	16.7 ±7.0	9.7 ±1.8
sum (n-3)	39.8 ±5.8	44.4 ±6.0	53.4 ±6.4
(n-6)/(n-3)	0.13 ±0.06	0.38 ±0.18	0.19 ±0.05
sat/unsat	0.40 ±0.05	0.26 ±0.04	0.27 ±0.07

whereas *Isochrysis* sp. contained almost no C_{16} PUFA. The main C_{16} PUFA in both *T. chui* and *D. tertiolecta* was 16:4n-3, which comprised approximately 14% of total fatty acids in both species. Lipids of *Isochrysis* sp. contained remarkably high levels of 18:4n-3 (21.5%). This fatty acid was also at a high level in *T. chui* (12.3%) but at less than 2% in *D. tertiolecta*. Levels of 18:3n-3 were lowest in *Isochrysis* sp. (5.9%), intermediate in *T. chui* (11.8%), and extremely high in *D. tertiolecta* (35.4%). PUFA of the (n-6) series was highest in the *T. chui* diet (16.7%) due to significant quantities (ca. 2%) of 16:2n-6, 16:3n-6, and 18:3n-6 in addition to 20-4n-6. Linoleic acid, 18:2n-6, was present in the profiles of all three species at levels between 3.5- 7.7%. Despite a lower amount of $C_{20,22}$ PUFA in *T. chui* and a complete lack of these PUFA in *D. tertiolecta*, both algal species were high in total PUFA (>60%) in both the (n-6) and (n-3) series and contained (on average) less saturated and monounsaturated fatty acids. These characteristics are reflected in the saturated/unsaturated and (n-6/n-3) fatty acid ratios.

2.3.2 Broodstock conditioning experiment # 1

2.3.2.1 Hatch rates, egg size and weight

Neither egg organic weight nor size differed significantly among conditioning treatments (p =0.3071 and 0.9906, respectively, Table 2.4), although mean organic weight was lowest in the *D. tertiolecta* treatment (36.6 ng/egg) and highest in the *Isochrysis* sp./ *T. chui* mixed diet (48.5 ng/egg). Hatch rates were extremely variable within treatment groups ranging between a mean of 13.5% to 21.5%, and differences between groups were not significant (p=0.7187).

Table 2.4. Total lipids and lipid classes of *Placopecten magellanicus* eggs from adults conditioned on four diet treatments and in the wild (experiment #1) expressed as egg content (pg/egg) and a percent of total lipid (mean ± standard deviation).

Diet	***Isochrysis* sp.**	***T-Iso/Tetraselmis chui* mix**	***Tetraselmis chui***	***Dunaliella tertiolecta***	***Wild***
replicates (n)	(n=6)	(n=6)	(n=8)	(n=8)	(n=8)
Egg Content *(pg/egg)*					
Total Lipids	7864 ±472	8260 ±2053	7652 ±1393	7479 ±1297	7802 ±849
TAG	4158 ±452	4483 ±1170	4078 ±938	4036 ±885	3891 ±269
PL	2393 ±220	2297 ±501	2245 ±347	2209 ±346	2340 ±177
AMPL	567 ±317	532 ±128	501 ±174	461 ±112	512 ±256
ST	283 ±41	328 ±93	296 ±86	266 ±50	325 ±176
SE	130 ±23	147 ±50	159 ±38	124 ±33	128 ±26
FFA	114 ±53	162 ±96	94 ±42	106 ±86	181 ±265
DAG	66 ±60	107 ±93	120 ±109	84 ±57	130 ±129
Relative composition *(% tot lipids)*					
TAG	52.6 ±3.1	54.3 ±2.8	52.9 ±3.9	53.6 ±4.3	51.4 ±7.7
PL	30.5 ±2.4	28.1 ±2.1	29.6 ±3.1	29.9 ±4.3	30.1 ±1.0
AMPL	7.4 ±4.5	6.5 ±0.7	6.6 ±2.2	6.2 ±1.4	6.4 ±2.5
ST	3.6 ±0.5	4.0 ±0.5	3.8 ±0.6	3.6 ±0.6	4.0 ±2.9
SE	1.7 ±0.3	1.8 ±0.2	2.1 ±0.3	1.6 ±0.3	1.6 ±0.3
FFA	1.5 ±0.7	1.8 ±1.0	1.3 ±0.6	1.4 ±1.0	2.1 ±3.0
DAG	0.8 ±0.7	1.2 ±1.0	1.6 ±1.6	1.1 ±0.7	1.7 ±1.0
AFDW *(ng/egg)*	42.3 ±10.1	48.5 ±13.8	46.6 ±11.6	36.6 ±5.0	45.3 ±12.7
Egg diameter *(μm)*	66.8 ±0.3	65.7 ±0.6	66.3 ±1.3	67.5 ±0.4	67.7 ±0.5
Hatch rate (%)*	15.2 ±11.5	13.5 ±5.8	18.5 ±9.6	21.5 ±19.0	no data

* n=4

2.3.2.2 Lipid classes

Total lipid per egg ranged from a low of 7479 pg/egg in the *D. tertiolecta* treatment to 8261 pg/egg in the *Isochrysis* sp./ *T. chui* mixed diet, but no significant differences were detected in the ANOVA for this variable (p=0.8293; Table 2.4). Lipid class composition was extremely stable between the conditioning treatments. No significant differences were found for TAG (p=0.8673) or PL (p= 0.8092). In order of dominance within total egg lipid: TAG comprised approximately 50-55% of total lipids followed by PL (28-31%). AMPL and sterol comprised 6-7% and 3.5-4% respectively. SE, FFA, and DAG were all present in minor quantities (generally <2%). Neither Et-KET nor Me-KET were detected indicating that they were present at <0.1%.

2.3.2.3 Egg fatty acids

The fatty acid profiles of eggs (total lipids) from the 4 diet treatments and naturally conditioned broodstock were similar both qualitatively and quantitatively (Table 2.5; complete fatty acid profiles and standard deviations in Appendix 2.1). Eggs contained mostly 16:0, 20:5n-3 (EPA), and 22:6n-3 (DHA). These three fatty acids alone contributed nearly 50% of the total fatty acids (approximately 17%, 20%, and 10%, respectively). Saturated fatty acids included small but significant quantities of 14:0 (ca 2%) and 18:0 (3.5%). Monounsaturates were mostly 18:1n-7 (7.5%) followed by 16:1n-7 (5%),18:1n-9 (4-5%), and 20:1n-11 (2.5%). The (n-9) and (n-7) series 20:1 fatty acids were also present as minor constituents (<1%). PUFA was composed almost entirely of C_{18} - C_{22} fatty acids. Only 16:4n-1 (which was not a significant dietary PUFA) was

present at levels greater than 1% in eggs from lab conditioned animals. Other than EPA and DHA, 18:4n-3 was the only major PUFA at 5-6% of total FA. The fatty acids 18:3n-3 and 18:2n-6 were present at >2% and 20:4n-6 appeared in lesser but consistent proportions between 1.1% and 2.1%. PUFA present in small amounts (0.5% - 1%) included 21:5n-3, 22:5n-6, and 22:5n-3.

Eggs were rich in total PUFA (46-48%) followed by SAT (23.3% - 25.9%) and MONO (20.9% - 22.2%; Table 2.5). The proportion of these major groups and fatty acid series groups were also relatively stable. Long chain PUFA (C_{20} - C_{22}) comprised most of the total PUFA due mainly to the high levels of EPA and DHA (ca. 35%). Fatty acids of the (n-3) series were the dominant group in *P. magellanicus* eggs (ca. 40%) followed by (n-7) at 13%. FA of the (n-9) series came mainly from 18:1n-9. The (n-6) series showed the most variability among groups, ranging from 3.73% in eggs from the natural conditioning treatment to 7.10% in eggs from the *T. chui* diet treatment. The (n-6)/(n-3) ratio reflected this. The saturated/unsaturated FA ratio was consistent between the cultured algae treatments (0.33-0.34) and slightly higher in wild eggs (0.38).

2.3.2.4 Treatment differences

Proportions of fatty acids varied significantly between lab conditioned diet treatments for only a few fatty acids, and naturally conditioned broodstock varied more than lab conditioned animals (Table 2.5). Within the lab conditioned groups only 20:2n-6 and 20:4n-6 varied significantly, with higher levels of both found in the *T. chui* treatment. This was reflected in the (n-6)/(n-3) ratio, which was also significantly higher

Table 2.5. Variation in proportions of selected (>0.2%) fatty acids (as a wt % of total FA) in eggs of *Placopecten magellanicus* fed four different diets (experiment #1) (mean ± standard deviation, replicates, n, as indicated).

Diet	*Isochrysis* sp. (n=7)	*T-Iso/ Tetraselmis chui* (n=6)	*Tetraselmis chui* (n=8)	*Dunaliella tertiolecta* (n=8)	Wild (n=9)
14:0	2.0	1.9	1.6 a	1.8	2.2 b
16:0	16.7	17.0	17.0	17.2	18.7
18:0	3.5	3.4	3.5	3.4	4.1
Total SAT	23.3 a	23.5 a	23.3 a	23.5 a	25.9 b
16:1n-7	5.1 a	5.0 a	4.8 a	5.3	5.9 b
18:1n-9	4.9	4.6	4.1	4.1	4.3
18:1n-7	7.7	7.8	7.9	7.2	7.7
20:1n-11	2.4	2.3	2.7	2.4	2.5
20:1n-9	0.8	0.8	0.7	0.6	0.5
20:1n-7	0.5	0.5	0.4	0.5	0.2
Total MONO	22.2	21.9	21.4	20.9	21.9
16:4n-1	0.2	0.2	0.3	0.3	0.1
18:2n-6	2.3 ac	2.5 ac	2.8 a	2.1 c	1.7 bc
18:2n-4	0.5	0.5	0.5	0.6	0.6
18:3n-6	0.4 a	0.5 a	0.6 a	0.6 a	0.1 b
18:3n-3	3.1 a	3.5 a	3.2 a	3.4 a	1.7 b
18:4n-3	6.2	6.3	5.5	5.1	5.6
20:2n-6	1.0 ac	1.0 a	1.2 b	0.9 a	0.7 c
20:4n-6 (ARA)	1.4 ac	1.5 a	2.1 b	1.6 a	1.1 c
20:3n-3	0.5	0.8	0.7	0.8	0.3
20:5n-3 (EPA)	19.4	19.5	20.4	20.8	23.2
21:5n-3	0.7	0.6	0.7	0.7	0.8
22:5n-6	0.7 a	0.5	0.4	0.6 a	0.2 b
22:5n-3	0.7	0.6	0.6	0.7	0.7
22:6n-3 (DHA)	10.5	9.4	8.4	10.1	9.7
Total PUFA	47.5	47.5	47.3	48.1	46.1
C_{20} and C_{22} PUFA	35.0	34.2	34.6	36.3	36.5
EPA/DHA	1.9	2.1	2.5	2.2	2.5
sum (n-9)	5.7	5.5	4.8	4.7	4.8
sum (n-7)	13.2	13.3	13.1	13.0	13.8
sum (n-6)	5.8 a	6.1 ac	7.1 c	5.7 a	3.7 b
sum (n-3)	40.7	40.4	39.2	41.3	41.6
(n-6)/(n-3)	0.14 a	0.15 ac	0.18 b	0.14 a	0.09 c
sat/unsat	0.33	0.34	0.34	0.34	0.38

* complete fatty acid profile in Appendix 2.1

Fatty acids designated with the same letter are not significantly different ($p<0.05$)

in the *T. chui* treatment. Eggs from field collected scallops showed significant differences from the other groups, having a slightly higher proportion of 16:0 and total SAT, and16:1n-7 (compared with all but the *D. tertiolecta* treatment). Levels of 18:2n-6, 18:3n-6, 18:3n-3, and 22:5n-6 were also slightly lower. Because of these decreased amounts in the (n-6) series fatty acids, eggs from naturally conditioned scallops also displayed a lower total (n-6) level and (n-6)/(n-3) ratio than the other groups.

Despite the few significant differences, trends in the mean levels of certain fatty acids (14:0, 18:1n-9, 18:4n-3, 20:5n-3, 22:6n-3, and 22:5n-6) do indicate minor deviations in egg composition which coincide with dietary fatty acid inputs (Figures 2.1, 2.2; Table 2.5). In Figure 2.1, dietary fatty acids are expressed in absolute amounts (pg/ equivalent dry weight ration based on the *Isochrysis* sp. reference diet). This depiction is more representative of dietary fatty acids available for assimilation. The standard deviations in egg EPA proportions (Figure 2.2, Appendix 2.1) were higher in eggs from diet treatments lacking EPA (*Isochrysis* sp. and *D. tertiolecta*) than in diets with substantial levels of EPA (mixed and *T. chui*). Variability of EPA in eggs from naturally conditioned broodstock was also high, although mean levels were substantially greater than in any of the lab conditioned eggs. Standard deviations for the proportion of DHA were similar among treatments. These apparent trends were the impetus for the second broodstock conditioning experiment.

Table 2.6. Relative fatty acid composition (wt % of total FA) of neutral and polar lipid in selected egg samples (mean ± standard deviation, n=8).

	Egg- Neutral Lipid (%)	Egg- Polar Lipid (%)	
14:0	2.6 ±0.4	1.2 ±0.5	*
15:0	0.4 ±0.1	0.3 ±0.7	
16:0	18.5 ±1.7	19.7 ±4.0	
17:0	0.5 ±0.1	0.9 ±0.7	
18:0	3.2 ±0.5	7.7 ±1.4	*
Total SAT	25.2 ±1.9	30.0 ±5.7	*
16:1n-7	6.8 ±0.8	2.2 ±0.8	*
16:1n-5	0.3 ±0.2	1.9 ±0.5	*
18:1n-9	4.6 ±0.8	3.4 ±0.5	*
18:1n-7	9.5 ±0.8	5.1 ±1.7	*
18:1n-5	0.5 ±0.2	0.0 ±0.0	
20:1n-11	0.8 ±1.2	2.9 ±1.4	*
20:1n-9	0.5 ±0.2	1.1 ±0.3	
20:1n-7	0.7 ±0.1	1.1 ±0.2	
Total MONO	23.7 ±1.5	17.6 ±3.5	*
16:4n-1	0.3 ±0.3	0.1 ±0.1	
16:4n-3	0.4 ±0.1	tr -	
18:2n-6	2.3 ±0.7	1.5 ±0.9	
18:2n-4	0.6 ±0.1	1.7 ±0.8	*
18:3n-6	0.4 ±0.2	0.5 ±1.0	
18:3n-4	0.1 ±0.1	0.0 ±0.0	
18:3n-3	2.8 ±1.1	1.3 ±0.8	*
18:4n-3	6.5 ±1.3	2.6 ±0.6	*
20:2n-6	0.9 ±0.2	0.5 ±0.1	
20:3n-6	0.2 ±0.1	0.1 ±0.1	
20:4n-6 (ARA)	1.0 ±0.3	2.6 ±0.9	*
20:3n-3	0.7 ±0.2	0.4 ±0.7	
20:5n-3 (EPA)	20.3 ±1.8	18.7 ±2.1	
22:4n-6	0.8 ±0.1	0.3 ±0.2	
22:5n-6	0.4 ±0.2	0.8 ±1.3	
22:5n-3	0.5 ±0.1	0.6 ±0.2	
22:6n-3 (DHA)	8.4 ±1.1	11.9 ±2.8	*
Total PUFA	46.5 ±2.1	46.1 ±6.1	
branched	1.4 ±0.4	1.9 ±1.1	
DMA	-	4.0 ±1.3	
unidentified	2.3 ±0.5	0.9 ±0.2	
C_{20} and C_{22} PUFA	33.2 ±2.2	35.9 ±5.1	
EPA/DHA	2.5 ±0.4	1.6 ±0.4	*
sum (n-9)	5.9 ±1.6	8.2 ±1.6	*
sum (n-7)	16.9 ±0.9	7.5 ±2.0	*
sum (n-6)	6.1 ±1.2	6.3 ±3.4	
sum (n-3)	39.4 ±1.5	38.0 ±4.9	
(n-6)/(n-3)	0.16 ±0.03	0.17 ±0.09	
sat/unsat	0.36 ±0.03	0.47 ±0.11	*

* = significantly different ($p < 0.05$)

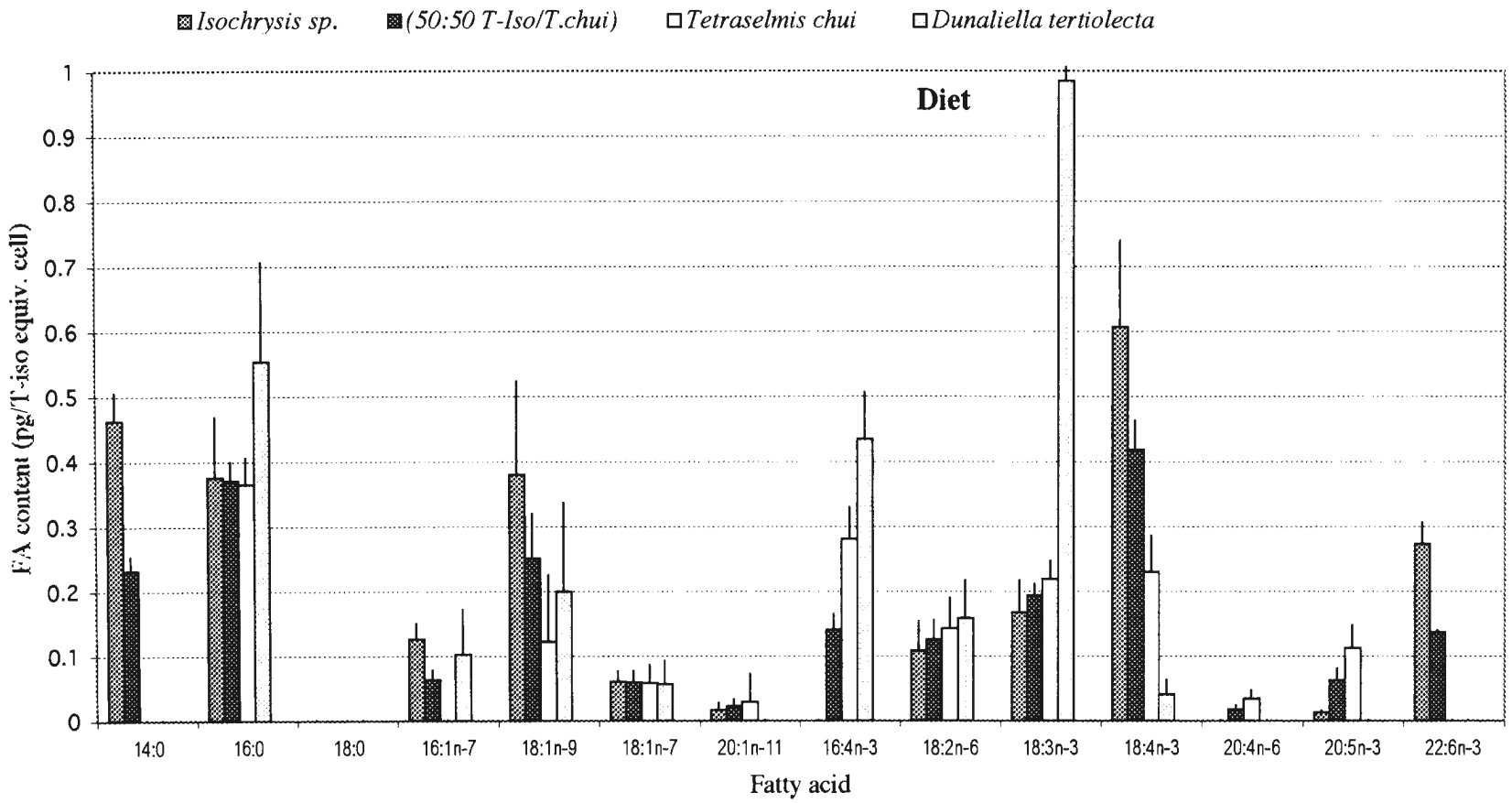


Figure 2.1. Fatty acid composition (pg/ T-Iso cell standardized by dry weight) of algae diet treatments in broodstock conditioning experiment #1. Error bars are + standard deviations.

Values calculated as: $FA_{STAND} = FA_{DIET}$ x ($DW_{T\text{-}ISO}$ / DW_{DIET}) where FA_{STAND} = fatty acid amount (pg) standardized to an equivalent T-ISO cell by dry weight, FA_{DIET} = fatty acid cell content of diet treatment (pg/cell) , $DW_{T\text{-}ISO}$ = mean cell dry weight of T-Iso, and DW_{DIET} = mean cell dry weight of diet treatment.

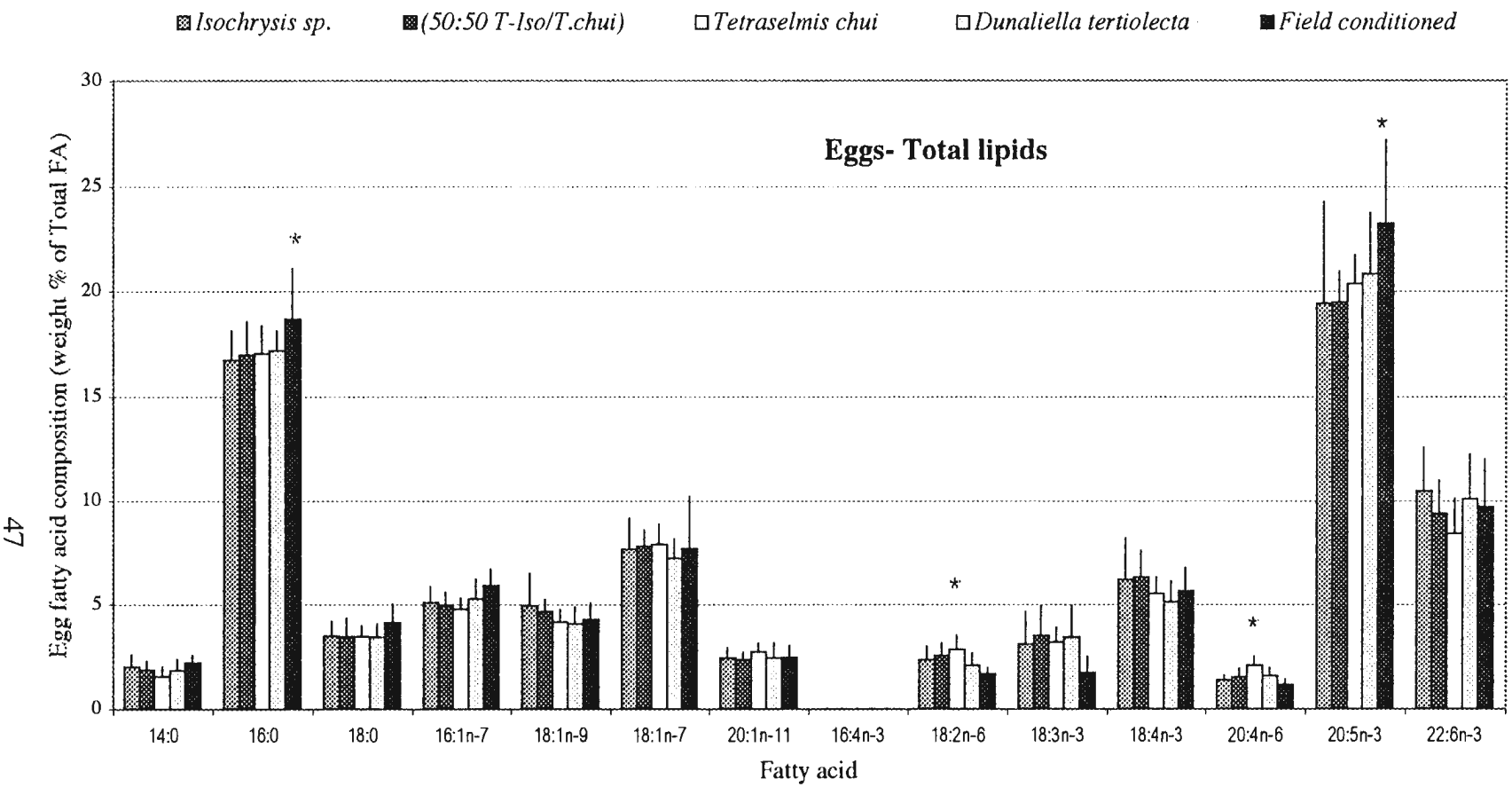


Figure 2.2. Variation in the relative fatty acid composition (wt % of total FA) of egg lipids from *Placopecten magellanicus* fed four different diets and field conditioned scallops (experiment # 1). Error bars are + standard deviations.

* = sig. diff. at $p<0.05$

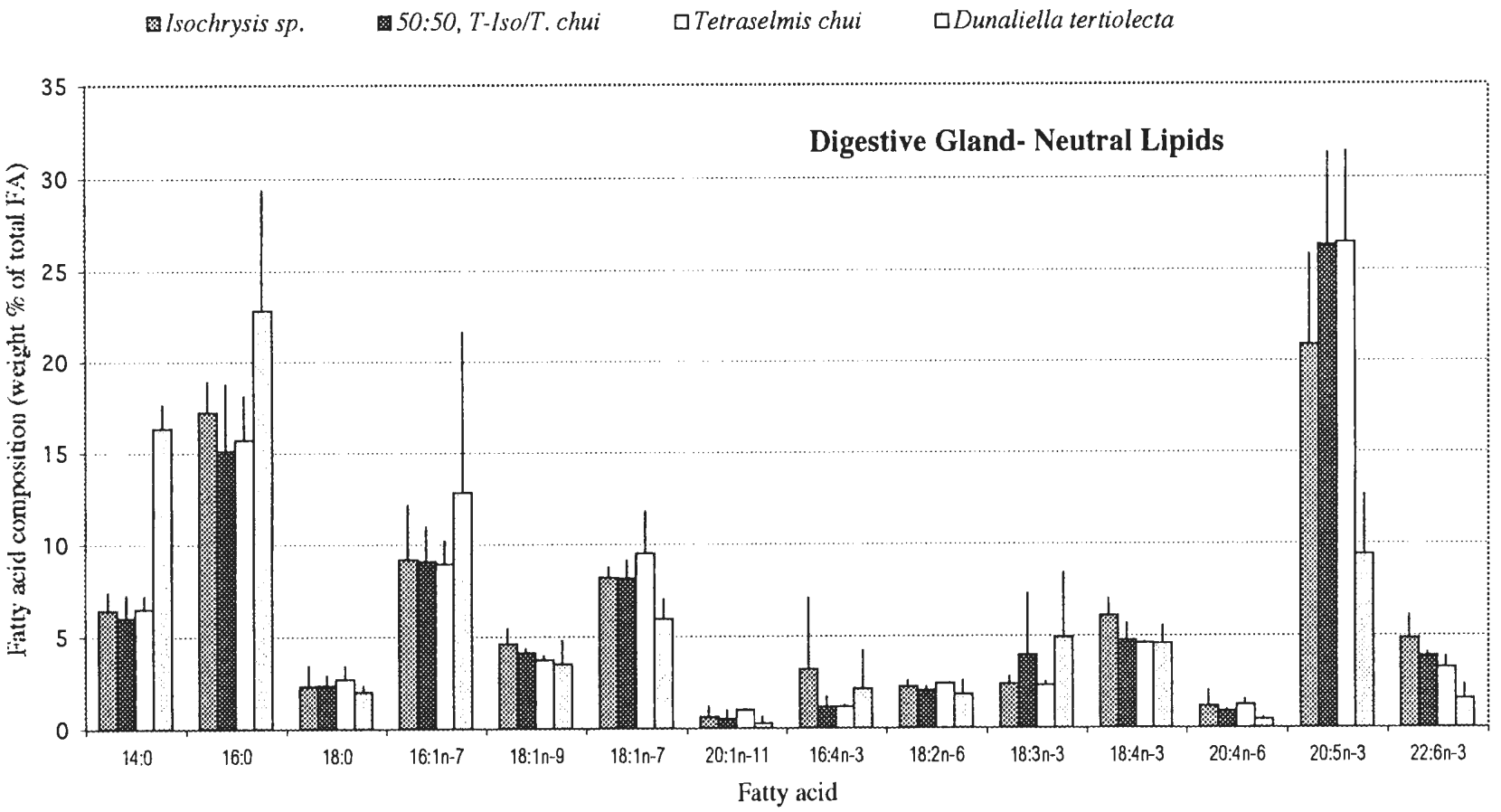


Figure 2.3. Variation in the relative fatty acid composition (wt % of total FA) of neutral lipids in digestive gland tissue of *Placopecten magellanicus* fed four different diets (experiment # 1). Error bars are +standard deviations.

2.3.2.5 Neutral and phospholipid FAMEs

Because only two egg samples per treatment were selected non-randomly for lipid fractionation, it was not statistically valid to make among treatment comparisons in fatty acid composition of neutral and polar lipids in experiment #1. Nonetheless, pooled data for NL and PL indicated that while fatty acids from these two fractions were qualitatively similar, they differed quantitatively in their relative proportions (Table 2.6). Polar lipids in eggs contained significantly less 14:0 (1.2% vs. 2.6%) and more 18:0 (7.8% vs. 3.2%). Monoenes were significantly lower in egg PL (17.6% vs. 23.7%) due to lower levels of 16:1n-7 (2.2% vs. 6.8%), 18:1n-9 (3.4% vs. 4.6%), and 18:1n-7 (5.1% vs. 9.5%). PL, however, was higher in longer chain MONO, primarily 20:1n-11, 20:1n-9, and 20:1n-7. PUFA in PL were characterized by higher DHA (11.9% vs. 8.4%) and hence a lower EPA/DHA ratio (1.6 vs. 2.6) and higher ARA (2.6% vs. 1.0%) compared to NL. The fatty acid 18:4n-3 was much higher in egg NL (6.5%) than PL (2.6%). Despite these differences, total PUFA was very high in both NL and PL fractions.

2.3.2.6 Adductor muscle tissue

Samples of adductor muscle taken from scallops in the different diet treatments displayed an organ-specific fatty acid profile but no differences in total lipid, lipid class or fatty acid composition. There were also no trends in wet:dry weight ratios (ca. 4.5:1) that would indicate large differences in glycogen transport from this tissue (Table 2.7). Lipid levels were relatively low in adductor muscle (32.2-38.1 mg/g) and comprised almost exclusively of PL and ST at approximately 85% and 14% respectively as a

Table 2.7. Total lipids and lipid classes in adductor muscle of *Placopecten magellanicus* fed four different diets (experiment #1). Data expressed as mg/g dry weight (top) and as a percent of total lipid (bottom) (mean ± standard deviation, n=3).

Diet treatment	*Isochrysis* sp.	*T-Iso/Tetraselmis chui* mix	*Tetraselmis chui*	*Dunaliella tertiolecta*
(mg/g dry wt)				
TL	38.1 ±2.8	32.2 ±4.6	35.0 ±5.9	32.5 ±2.4
ST	5.1 ±0.4	4.7 ±0.8	4.9 ±0.6	5.1 ±0.4
PL	33.0 ±2.7	27.5 ±3.8	30.1 ±5.4	27.4 ±2.2
(% total lipids)				
ST	13.5 ±1.2	14.6 ±0.4	14.0 ±1.0	15.8 ±1.4
PL	86.5 ±1.2	85.4 ±0.4	86.0 ±1.0	84.2 ±1.2
wet: dry weight ratio	4.5 ±0.1	4.3 ±0.2	4.6 ±0.2	4.5 ±0.1

Table 2.8 Mean relative fatty acid composition (wt % of total FA) of total lipids in adductor muscles of *Placopecten magellanicus* fed four different diets (experiment #1) (mean ± standard deviation). Major fatty acids (>2%) appear in bold.

Fatty Acid	Grand mean (%)
14:0	2.28 ±0.56
15:0	0.55 ±0.12
16:0	19.85 ±0.38
17:0	0.49 ±0.09
18:0	6.04 ±0.40
Total SAT	29.21 ±0.77
16:1n-7	2.27 ±0.25
16:1n-5	0.50 ±0.11
18:1n-9	1.84 ±0.39
18:1n-7	7.03 ±0.54
18:1n-5	0.17 ±0.14
20:1n-11	0.53 ±0.14
20:1n-9	1.29 ±0.29
20:1n-7	0.48 ±0.26
20:1n-5	0.14 ±0.23
Total MONO	14.24 ±0.73
16:3n-4	0.29 ±0.09
16:4n-1	0.19 ±0.18
18:2n-7?	0.11 ±0.09
18:2n-6	0.64 ±0.20
18:2n-4?	0.21 ±0.10
18:3n-3	0.64 ±0.21
18:4n-3	2.61 ±0.26
20:2NMID	0.17 ±0.26
20:2NMID	0.16 ±0.19
20:2n-6	0.88 ±0.27
20:3n-6	1.05 ±0.75
20:4n-6	2.35 ±0.15
20:3n-3	0.24 ±0.07
20:4n-3	0.36 ±0.13
20:5n-3	21.74 ±1.14
21:5n-3	1.09 ±0.15
22:5n-6	0.28 ±0.32
22:5n-3	1.45 ±0.23
22:6n-3	19.08 ±0.58
Total PUFA	53.54 ±1.00
C_{20} and C_{22} PUFA	48.85 ±1.27
EPA/DHA	1.14 ±0.06
sum (n-9)	3.13 ±0.11
sum (n-7)	9.89 ±0.49
sum (n-6)	5.21 ±1.03
sum (n-3)	47.20 ±1.79
(n-6)/(n-3)	0.11 ±0.03
sat/unsat	0.43 ±0.01
branched	0.76 ±0.75

proportion of total lipids (Table 2.7). The fatty acids consisted mainly of 16:0, 18:0, 18:1n-9, 20:5n-3, and 22:6n-3. The fatty acid 20:5n-3 was only slightly higher than 22:6n-3 at 21.7% and 19.1%. Other fatty acids comprising more than 2% of the total were 14:0, 16:1n-7,18:4n-3, and 20:4n-6. FA 20:1n-9 and 22:5n-3 were also present (1-2% of total). Overall means are shown in Table 2.8, with means split by treatment group in Appendix 2.2. Long chain C_{20}- C_{22} PUFA were nearly 50% of the total fatty acids with the (n-3) series making up 47.2%.

2.3.2.7 Digestive gland

The digestive gland samples from the same animals also revealed an organ-specific lipid and fatty acid composition (Tables 2.9, 2.10). There were no significant differences in total lipid concentration or lipid class composition, although mean levels of total lipid and TAG were highest in the mixed diet treatment. In contrast to adductor muscle tissue, the digestive gland was composed primarily of TAG (73.7%-80.2%) followed by PL (9%-10%) and AMPL (5%-6.5%). FFA were present at low but consistent levels (1.2%-1.7%), as were sterols (1.6-1.7%). Mean levels of ketone were highest in the *Isochrysis* sp. and mixed diet groups, undoubtedly due to presence of these compounds in the diets. Total lipid concentration was high (300-369 mg/g dry weight).

Fatty acid profiles of neutral lipids from the digestive gland did show treatment differences. The *D. tertiolecta* group showed a much higher proportion of 14:0 (16.3% vs. 6.0-6.5%) and total SAT (43% vs. 24-26%) with a concomitant decrease in total (28% vs. 45-47%) and long chain PUFA (12.2 vs. 30-34%). This was primarily due to

Table 2.9. Total lipids and lipid classes in digestive glands of *Placopecten magellanicus* fed four different diets (experiment #1) expressed as mg/g dry weight (top) and as a percent of total lipid (bottom) (mean ± standard deviation, n=3).

Diet	*Isochrysis* sp.	*T-Iso/Tetraselmis chui* mix	*Tetraselmis chui*	*Dunaliella tertiolecta*
(mg/g dry wt)				
TL	297.4 ±6.6	368.8 ±135.5	318.4 ±36.1	309.9 ±113.3
TAG	219.4 ±17.3	301.4 ±136.1	251.1 ±32.3	252.7 ±134.3
PL	28.9 ±2.1	30.3 ±10.0	31.8 ±2.5	28.1 ±10.8
AMPL	19.3 ±4.9	17.4 ±2.6	18.9 ±5.9	15.9 ±0.1
ST	6.1 ±1.6	5.7 ±1.2	5.1 ±0.8	4.7 ±2.7
FFA	4.9 ±1.6	5.2 ±1.7	5.0 ±0.0	2.7 ±3.9
KET	2.8 ±3.9	2.7 ±2.7	1.4 ±1.9	1.5 ±2.1
(% total lipid)				
TAG	73.7% ±4.2	80.2% ±6.8	78.8% ±1.2	78.9% ±14.5
PL	9.7% ±0.9	9.1% ±5.0	10.1% ±1.9	10.4% ±7.3
AMPL	6.5% ±1.8	4.9% ±1.0	5.9% ±1.2	5.5% ±2.0
ST	2.1% ±0.6	1.7% ±0.6	1.6% ±0.1	1.8% ±1.5
FFA	1.7% ±0.6	1.4% ±0.3	1.6% ±0.2	1.2% ±1.7
KET	0.9% ±1.3	0.9% ±0.9	0.4% ±0.6	0.6% ±0.9

Table 2.10. Variation in relative fatty acid composition (wt % of total FA) of neutral lipids of digestive glands of *Placopecten magellanicus* fed four different diets (experiment #1) (mean ± standard deviation, n=3).

Diet	*Isochrysis* sp. (%)		*T-Iso/Tetraselmis chui* mix (%)		*Tetraselmis chui* (%)		*Dunaliella tertiolecta* (%)	
14:0+TMTD?	6.4 ±1.0	a	6.0 ±1.2	a	6.5 ±0.7	a	16.3 ±1.3	b
16:0	17.2 ±1.7		15.1 ±3.6		15.7 ±2.4		22.8 ±6.6	
18:0	2.3 ±1.1		2.3 ±0.6		2.7 ±0.7		2.0 ±0.3	
Total SAT	25.9 ±1.9	a	24.0 ±3.0	a	25.4 ±3.8	a	43.0 ±3.0	b
16:1n-7	9.2 ±3.0		9.1 ±1.9		8.9 ±1.3		12.8 ±8.8	
16:1n-5	0.5 ±0.2		0.4 ±0.1		0.3 ±0.1		0.6 ±0.2	
18:1n-11	0.0 ±0.1		0.1 ±0.1		0.1 ±0.1		0.1 ±0.1	
18:1n-9	4.6 ±0.8		4.1 ±0.2		3.7 ±0.2		3.5 ±1.3	
18:1n-7	8.2 ±0.5	a	8.1 ±1.0	a	9.5 ±2.3	a	5.9 ±1.1	b
18:1n-5	0.1 ±0.2		0.3 ±0.1		0.2 ±0.2		0.2 ±0.2	
20:1n-11	0.6 ±0.5		0.5 ±0.4		1.0 ±0.0		0.2 ±0.3	
20:1n-9	0.4 ±0.3		0.2 ±0.3		0.0 ±0.1		0.6 ±0.4	
20:1n-7	1.8 ±1.9		1.2 ±0.2		1.7 ±0.4		0.5 ±0.3	
20:1n-5	0.0 ±0.0		0.1 ±0.1		0.1 ±0.0		0.1 ±0.2	
Total MONO	24.8 ±1.8		24.9 ±2.2		26.3 ±1.0		27.2 ±6.6	
16:4n-3	0.8 ±0.9		0.7 ±0.5		0.7 ±0.2		1.3 ±0.9	
16:4n-1	3.2 ±3.9		1.1 ±0.5		1.1 ±0.1		2.1 ±2.1	
18:2n-6	2.2 ±0.3		2.0 ±0.2		2.4 ±0.0		1.8 ±0.7	
18:3n-6	0.2 ±0.0		0.4 ±0.3		0.3 ±0.1		0.4 ±0.4	
18:3n-4	0.2 ±0.1		0.1 ±0.0		0.1 ±0.0		0.1 ±0.0	
18:3n-3	2.4 ±0.4		3.9 ±3.4		2.3 ±0.2		4.9 ±3.5	
18:4n-3	6.1 ±0.9		4.7 ±0.9		4.6 ±0.0		4.5 ±1.0	
20:2n-6	0.5 ±0.3		0.7 ±0.1		0.7 ±0.0		0.4 ±0.3	
20:3n-6	0.1 ±0.1		0.3 ±0.1		0.2 ±0.0		0.1 ±0.2	
20:4n-6	1.1 ±0.8	a	0.9 ±0.1		1.2 ±0.3	a	0.4 ±0.1	b
20:3n-3	0.3 ±0.3		1.1 ±1.1		0.5 ±0.1		0.8 ±1.0	
20:4n-3	0.4 ±0.3		0.6 ±0.1		0.5 ±0.2		0.4 ±0.1	
20:5n-3	20.9 ±4.9	a	26.3 ±5.0	a	26.5 ±4.9	a	9.4 ±3.2	b
21:5n-3	0.8 ±0.7		0.3 ±0.0		0.3 ±0.2		0.1 ±0.1	
22:5n-3	0.2 ±0.2		0.3 ±0.0		0.3 ±0.0		0.2 ±0.2	
22:5n-6	0.1 ±0.2		0.0 ±0.0		0.0 ±0.0		0.0 ±0.0	
22:6n-3	4.8 ±1.2	a	3.9 ±0.1	ac	3.2 ±0.5	c	1.5 ±0.8	b
Total PUFA	45.7 ±4.4	a	47.3 ±1.1	a	45.1 ±4.6	a	28.0 ±6.8	b
unidentified	3.0 ±1.6		3.8 ±0.7		3.4 ±0.3		2.5 ±1.5	
branched	1.2 ±1.0		0.7 ±0.2		0.5 ±0.2		1.2 ±0.2	
C_{20}-C_{22} PUFA	30.4 ±3.1	a	34.3 ±3.8	a	33.5 ±5.0	a	12.2 ±2.9	b
EPA/DHA	4.3 ±1.6		6.8 ±1.1		8.2 ±0.1		8.6 ±7.2	
sum (n-9)	5.0 ±0.9		4.4 ±0.5		3.8 ±0.3		4.0 ±1.7	
sum (n-7)	19.2 ±0.9		18.4 ±2.0		20.1 ±1.4		19.2 ±8.2	
sum (n-6)	4.9 ±0.7	a	4.3 ±0.5	a	4.9 ±0.4	a	2.8 ±0.9	b
sum (n-3)	39.3 ±4.1	a	42.2 ±2.0	a	39.3 ±5.2	a	23.7 ±6.1	b
(n-6)/(n-3)	0.1 ±0.0		0.1 ±0.0		0.1 ±0.0		0.1 ±0.1	
sat/unsat	0.4 ±0.1	a	0.3 ±0.1	a	0.4 ±0.1	a	0.8 ±0.3	b

also present (<1%): 15:0, 15:1, 17:0, 17:1, 22:1n-9

* treatments designated with different letters are significantly different

significant decreases in EPA, in addition to less DHA and 20:4n-6 (Table 2.10, Figure 2.3). These distinctions also affected the sum (n-6) and sum (n-3) groupings and SAT/UNSAT ratio, which also differed significantly in the *D. tertiolecta* treatment. The overall variability introduced by inclusion of the *D. tertiolecta* group in the ANOVA made post-hoc tests less sensitive to deviations in composition in the other treatment groups. DHA was significantly lower (3.2% vs. 4.8%) in the *T. chui* treatment compared with *Isochrysis* sp. Mean levels of EPA were also lower in the *Isochrysis* sp. group than in the mix and *T. chui* groups (20.9% vs. 26.3% and 26.5%), and mean EPA/DHA ratios corresponded to the diet treatments. However, the range of values was less than that of EPA/DHA in the diets (Table 2.10). These trends are evident when comparing Figure 2.3 with the diet composition (Figure 2.1).

TMTD (4,8,12-trimethyltridecanoic acid), an isoprenoid fatty acid breakdown product of chlorophylls, was not observed in the digestive gland samples, but may have co-eluted with 14:0 (Napolitano and Ackman, 1993).

2.3.3 Broodstock conditioning experiment #2

2.3.3.1 Gonad lipids

Total lipid and lipid class composition was not significantly different among gonad samples from any of the diet treatments (*Isochrysis* sp., *T. chui*, or *D. tertiolecta*), but variability in total lipid and major lipid classes (TAG and PL) within treatments was high. TAG was lower as a percent of total lipids (8.26-27.84%) compared to eggs from experiment # 1 (Table 2.11).

Table 2.11. Variation in total lipid and lipid class composition in gonads of *Placopecten magellanicus* fed three different diets (experiment #2) expressed as a percentage of gonad wet weight (top) and percent of total lipid (bottom) (mean ± standard deviation).

Diet treatment (% wet wt)	*Isochrysis* sp.	*Tetraselmis chui*	*Dunaliella tertiolecta*
TL	0.92 ±0.39	0.64 ±0.56	0.82 ±0.29
TAG	0.27 ±0.15	0.15 ±0.21	0.07 ±0.09
PL	0.50 ±0.16	0.37 ±0.24	0.59 ±0.25
AMPL	0.06 ±0.04	0.04 ±0.05	0.05 ±0.03
ST	0.08 ±0.03	0.07 ±0.04	0.10 ±0.03
(% TL)			
TAG	27.84 ±6.25	15.57 ±13.26	8.26 ±9.85
PL	56.19 ±7.67	65.12 ±12.80	71.48 ±11.26
AMPL	5.44 ±2.09	4.74 ±2.40	5.76 ±3.17
ST	9.02 ±0.62	12.18 ±2.70	13.22 ±2.17

2.3.3.2 Fatty acids from neutral and polar lipids and comparisons with healthy eggs

Fatty acid differences among the treatment groups were evident. Fatty acids of the neutral lipids corresponded to the diet treatments to a greater extent than that of phospholipids. However, even within the PL portion of gonad lipid samples, the influence of the diet could be seen in significant differences in C_{20} - C_{22} PUFA.

In the neutral lipids, levels of 14:0, 16:0, 18:1n-9, 18:4n-3, 20:5n-3, and 22:6n-3 differed among the treatments and reflected species characteristic dietary fatty acid inputs (Table 2.3 vs. Table 2.12; Figure 2.4). In addition, a number of the C_{16} PUFA present in the *T. chui* and *D. tertiolecta* diets appeared in neutral lipids of the gonads of scallops from these two treatments, but not in scallops fed *Isochrysis* sp. Mean levels of 18:2n-6, 18:3n-3, 20:4n-6, and 22:5n-6 also appeared to correspond with the diet, but variability was high (Table 2.12, Figure 2.4).

Proportions of total SAT, MONO, and PUFA, and the SAT/UNSAT ratio in gonad NL did not vary among treatments, and were also similar to proportions found in healthy eggs (experiment #1 samples; Table 2.6 vs. Table 2.12). Long chain PUFA were, however, substantially reduced compared with healthy eggs. This was due primarily to a drop in EPA levels in all treatment groups, but especially in the *Isochrysis* sp. and *D. tertiolecta* treatments, where EPA in the NL fraction dropped to less than 2% compared with a mean of over 20% in healthy eggs. DHA was substantially higher (16%) in the NL of gonads from the *Isochrysis* sp. group compared with both healthy eggs and the other 2 treatments, which accounted for a higher mean C_{20} - C_{22} PUFA. DHA was lower in the *T. chui* and *D. tertiolecta* treatment groups but maintained at levels greater than 5%.

EPA/DHA ratios were thus also greatly reduced here compared to healthy eggs. ARA (20:4n-6) was higher in neutral lipids of gonads from experiment #2 than in experiment #1 eggs. The sum of the (n-6) series fatty acids and the (n-6)/(n-3) ratio were also higher in experiment #2 NL.

Polar lipids of gonads showed a more stable fatty acid composition than neutral lipids, but significant differences were found in long chain PUFA- 20:4n-6, 20:5n-3, 22:5n-6, and 22:6n-3. Fatty acids of the *D. tertiolecta* group were fairly similar to the *T. chui* group. All significant differences were between *Isochrysis* sp. and the other two treatments. These differences included a decrease in the EPA content of *Isochrysis* sp. PL and an increase in DHA. The EPA/DHA ratio was lower in this group than the other two (0.59 vs 1.21 and 1.34; Table 2.13). As for the NL described previously, total levels of SAT, MONO, and PUFA were similar across treatment groups and compared with healthy eggs. Unlike the corresponding NL, total levels of C_{20} - C_{22} PUFA were largely conserved and also similar to that of healthy eggs despite deviations in the relative proportions of DHA and EPA. The levels of 20:4n-6, 20:1n-11, and 18:0 were also higher in experiment # 2 gonad PL compared with healthy eggs (Table 2.13; Figure 2.5).

Figures 2.4 and 2.5 compare the major fatty acid profiles of neutral and polar lipids. In general the trends found in the NL fraction mirrored mean levels in PL in their rank position, but, again, the proportions found in the PL were constrained to smaller relative differences. This narrower range was also evident in the fatty acid groupings of the gonad NL (Figure 2.6) and PL (Figure 2.7) fractions. Fatty acid ratios (Figure 2.8a and 2.8b) showed a similar trend, with the exception of SAT/UNSAT ratio, which was

Table 2.12. Variation in relative fatty acid composition (wt % of total FA) of neutral lipids extracted from gonads of *Placopecten magellanicus* fed three different diets (experiment #2) (mean ± standard deviation).

Diet	*Isochrysis* sp.		*Tetraselmis chui*		*Dunaliella tertiolecta*		*
14:0	7.6 ±1.1	*a*	2.2 ±3.2	*b*	2.7 ±0.4	*b*	
16:0	13.9 ±0.7	*a*	20.5 ±3.9	*b*	19.3 ±1.1	*b*	
18:0	1.9 ±1.5		2.5 ±0.4		1.9 ±1.0		
20:0	tr -		1.9 ±1.2		1.2 ±1.1		
Total SAT	23.4 ±2.5		27.1 ±1.5		25.1 ±1.8		
16:1n-7	7.2 ±1.8		2.8 ±2.3		5.3 ±2.4		
18:1n-9	10.7 ±1.2	*a*	5.6 ±0.9	*b*	7.9 ±1.2	*b*	
18:1n-7	8.1 ±1.6		7.7 ±0.7		6.0 ±0.4		
20:1n-11	- -		- -		1.0 ±1.7		
20:1n-9	1.3 ±0.5		2.3 ±0.9		0.5 ±0.9		
20:1n-7	0.2 ±0.3		- -		0.2 ±0.3		
Total MONO	27.6 ±0.7		18.4 ±3.0		20.9 ±3.9		
16:2n-4	1.1 ±0.8		2.0 ±1.5		3.3 ±2.1		
16:2n-6	- -	*a*	2.1 ±0.9	*b*	0.4 ±0.6	*a*	
16:3n-3	- -	*a*	1.6 ±1.0	*b*	1.2 ±0.2	*b*	
16:4n-3	- -	*a*	3.6 ±1.5	*b*	4.0 ±1.1	*b*	
18:2n-6	8.4 ±1.3		10.9 ±6.4		11.2 ±4.0		
18:3n-6	1.0 ±1.4		- -		1.1 ±1.1		
18:3n-3	5.6 ±1.7		6.9 ±1.9		12.2 ±8.1		
18:4n-3	8.6 ±1.8	*a*	7.1 ±1.9	*a*	3.6 ±1.5	*b*	
20:2n-6	1.5 ±0.4		1.8 ±0.5		2.2 ±0.4		
20:4n-6	1.3 ±1.0		3.9 ±3.3		1.7 ±1.1		
20:5n-3	1.8 ±0.3	*a*	4.2 ±0.9	*b*	0.6 ±0.6		
22:5n-6	3.2 ±0.8		0.9 ±1.3		1.4 ±1.3		
22:5n-3	0.3 ±0.5		0.8 ±1.2		- -		
22:6n-3	16.0 ±2.1	*a*	5.7 ±5.5	*b*	5.2 ±4.6	*b*	
Total PUFA	48.4 ±2.1		50.8 ±4.2		48.1 ±8.6		
branched	0.3 ±0.5		0.5 ±0.7		3.7 ±2.5		
unidentified	0.1 ±1.2		2.5 ±0.2		2.2 ±0.5		
C_{20}-C_{22} PUFA	24.0 ±3.5		17.3 ±12.7		11.1 ±6.2		
EPA/DHA	0.1 ±0.0	*a*	1.2 ±1.0	*b*	0.1 ±0.1	*a*	
sum (n-9)	12.1 ±1.5	*a*	7.9 ±0.0	*b*	8.4 ±0.5	*b*	
sum (n-7)	15.5 ±2.1	*a*	10.5 ±3.0	*b*	11.5 ±2.3	*b*	
sum (n-6)	15.5 ±2.8		19.9 ±2.2		18.0 ±1.9		
sum (n-3)	31.8 ±2.9		28.8 ±7.9		26.8 ±11.6		
(n-6)/(n-3)	0.49 ±0.1		0.73 ±0.3		0.79 ±0.44		
sat/unsat	0.31 ±0.0		0.39 ±0.0		0.37 ±0.05		

* treatments designated with different letters are significantly different at $p<0.05$

Table 2.13. Variation in relative fatty acid composition (wt % of total FA) of polar lipids in gonads of *Placopecten magellanicus* fed three different diets (experiment #2) (mean ± standard deviation).

Diet	*Isochrysis* sp.		*Tetraselmis chui*		*Dunaliella tertiolecta*		* *
14:0	0.5 ±0.6		tr -		0.5 ±0.9		
16:0	12.4 ±2.2		13.1 ±1.5		11.5 ±2.0		
18:0	10.2 ±2.8		14.0 ±4.2		14.4 ±1.8		
20:0	0.3 ±0.3		0.5 ±0.7		0.3 ±0.5		
Total SAT	22.6 ±4.4		28.5 ±1.7		26.8 ±1.1		
16:1n-7	1.1 ±1.0		1.3 ±1.8		1.1 ±1.0		
16:1n-5	0.8 ±0.6		- -		- -		
18:1n-9	5.4 ±1.3		3.3 ±0.5		4.9 ±0.7		
18:1n-7	4.9 ±1.1		3.5 ±0.8		3.9 ±0.2		
20:1n-11	6.8 ±3.4		5.9 ±2.3		7.6 ±2.3		
20:1n-9	2.2 ±0.8		2.2 ±0.5		2.3 ±0.3		
20:1n-7	3.4 ±1.3		3.0 ±0.4		2.8 ±0.6		
Total MONO	24.3 ±4.6		19.2 ±2.7		22.6 ±1.2		
16:4n-1	1.8 ±2.5		0.7 ±1.0		0.2 ±0.3		
18:2n-6	2.7 ±1.8		2.9 ±1.5		3.0 ±1.0		
18:2n-4	0.2 ±0.3		0.0 ±0.0		0.0 ±0.0		
18:3n-3	0.2 ±0.3		0.3 ±0.4		0.9 ±0.8		
18:4n-3	4.7 ±4.1		2.2 ±0.8		1.7 ±0.2		
20:2n-6	1.4 ±1.0		0.5 ±0.7		0.9 ±0.2		
20:4n-6	6.3 ±1.6	*a*	8.2 ±2.2	*b*	7.0 ±1.2	*b*	
20:5n-3	9.7 ±1.7	*a*	15.2 ±1.3	*b*	14.4 ±1.6	*b*	
22:5n-6	1.8 ±1.2	*a*	0.7 ±0.9	*b*	1.0 ±0.3	*b*	
22:5n-3	0.0 ±0.0		tr -		0.4 ±0.8		
22:6n-3	16.4 ±1.4	*a*	12.6 ±0.1	*b*	11.3 ±1.4	*b*	
Total PUFA	48.9 ±8.7		47.7 ±2.8		45.1 ±0.6		
branched	2.2 ±1.0		2.3 ±0.5		2.5 ±0.7		
DMA	4.2 ±0.9		4.1 ±1.2		4.1 ±0.7		
unidentified	1.8 ±2.2		1.4 ±2.3		1.4 ±1.7		
C_{20} and C_{22} PUFA	36.5 ±1.1		38.1 ±2.6		39.9 ±3.6		
EPA/DHA	0.6 ±0.0	*a*	1.2 ±0.2	*b*	1.3 ±0.2	*b*	
sum (n-9)	7.6 ±2.7		5.5 ±1.0		7.2 ±0.6		
sum (n-7)	9.6 ±0.1	*a*	7.8 ±1.1	*b*	7.8 ±0.3	*b*	
sum (n-6)	12.4 ±3.3		12.3 ±1.6		12.2 ±0.4		
sum (n-3)	33.5 ±1.3		31.9 ±1.2		29.7 ±1.0		
(n-6)/(n-3)	0.49 ±0.4		0.54 ±0.0		0.68 ±0.02		
sat/unsat	0.31 ±0.1		0.43 ±0.0		0.40 ±0.01		

* treatments designated with different letters are significantly different at $p<0.05$

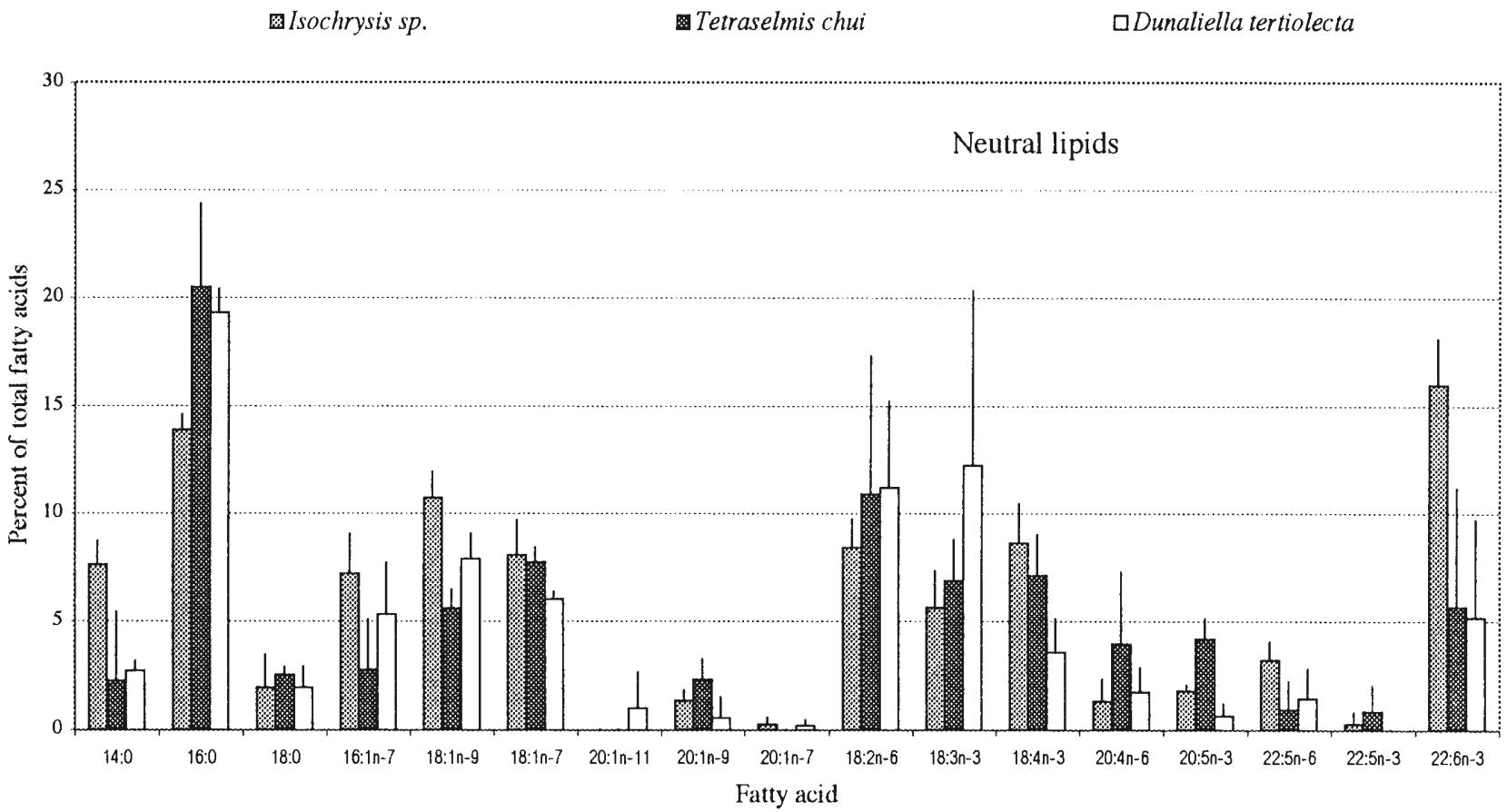


Figure 2.4. Variation in the relative fatty acid composition (wt % of total FA) of neutral lipids in gonad samples from *Placopecten magellanicus* fed three different diets (experiment #2). Error bars are + standard deviations.

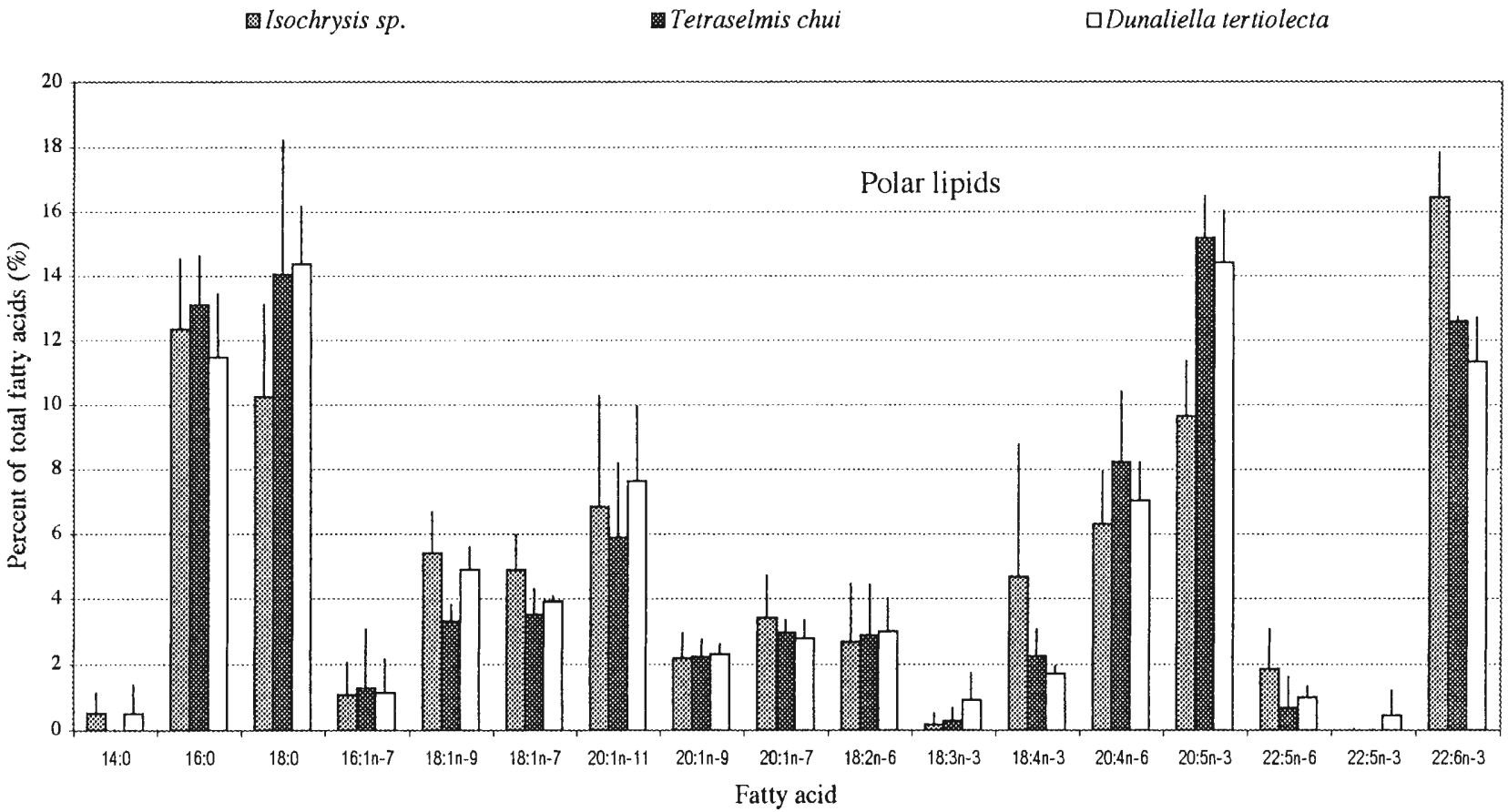


Figure 2.5. Variation in the relative fatty acid composition (wt % of total FA) of polar lipids in gonad samples from *Placopecten magellanicus* fed three different diets (experiment #2). Error bars are + standard deviations.

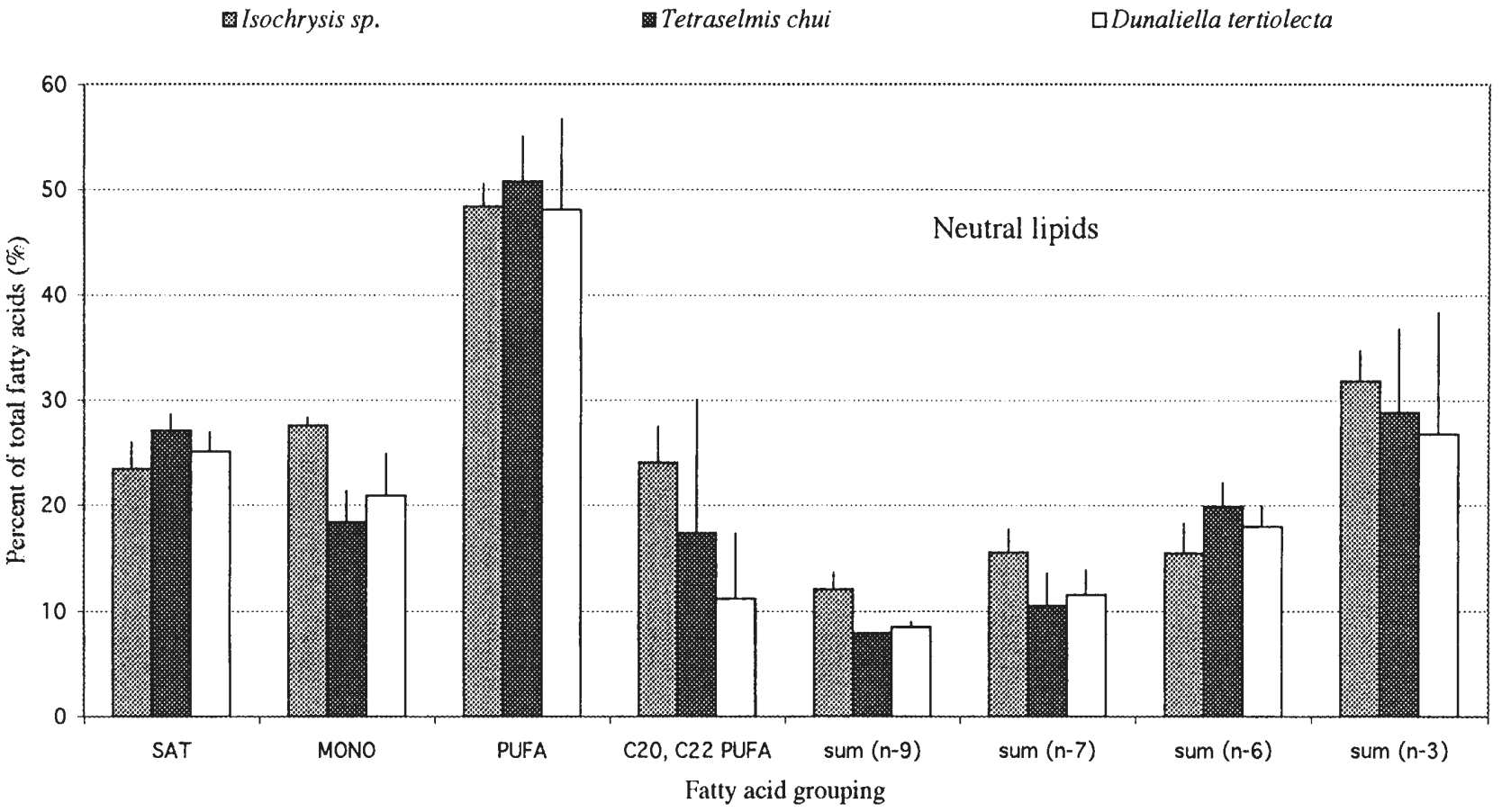


Figure 2.6. Fatty acid groupings for neutral lipids from experiment #2 gonad samples. Error bars are + standard deviations.

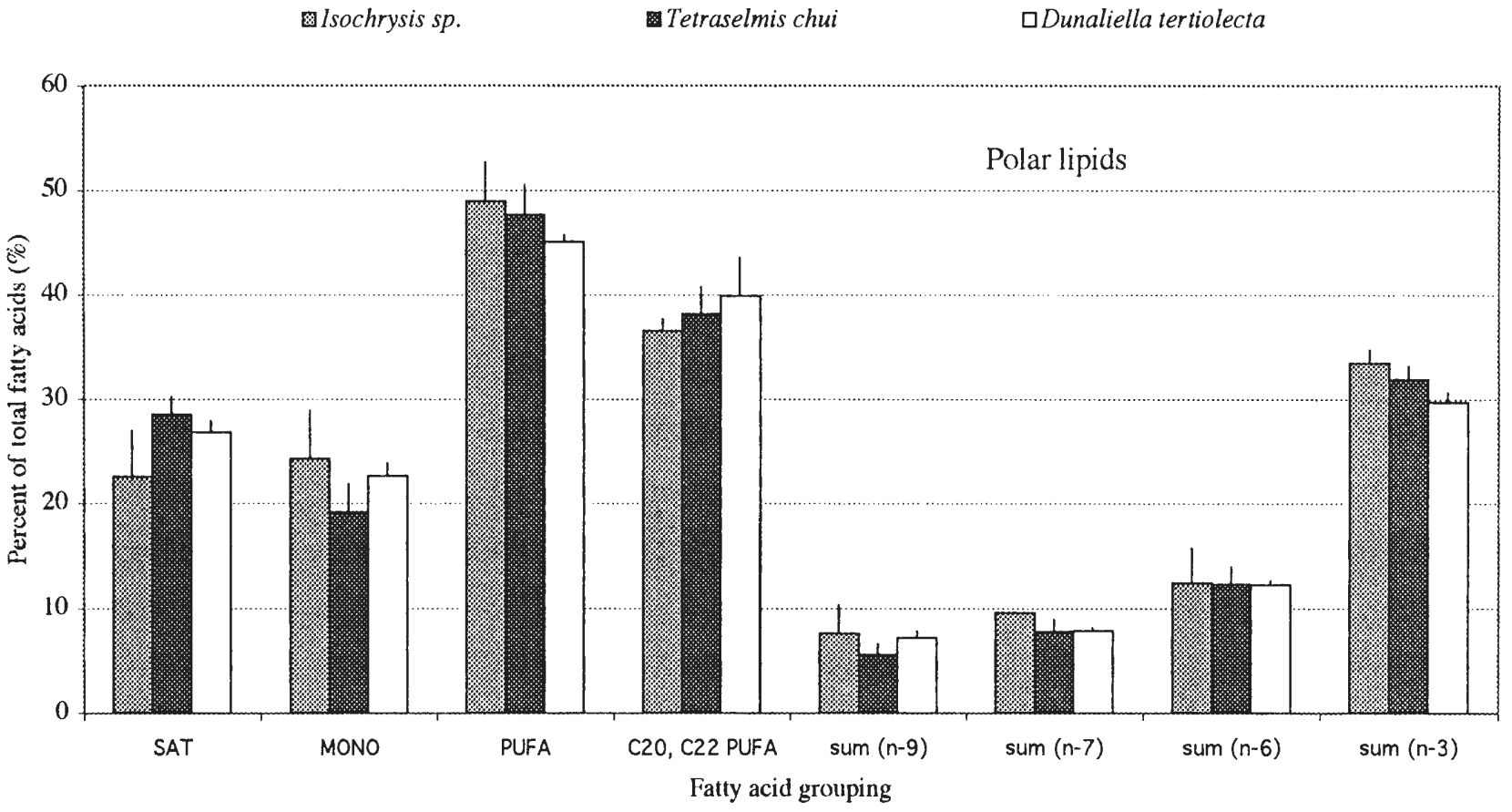
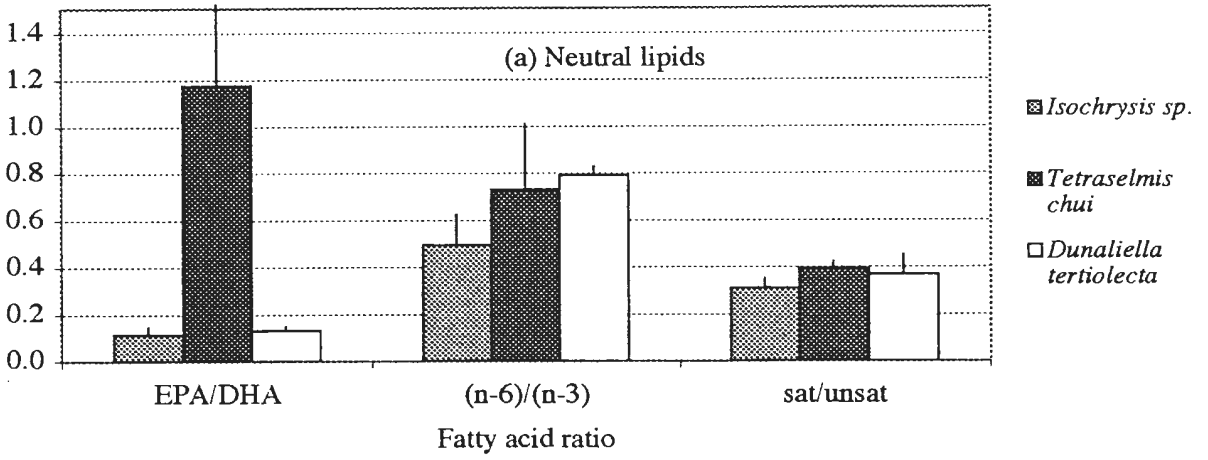


Figure 2.7. Fatty acid groupings for polar lipids from experiment #2 gonad samples. Error bars are + standard deviations.



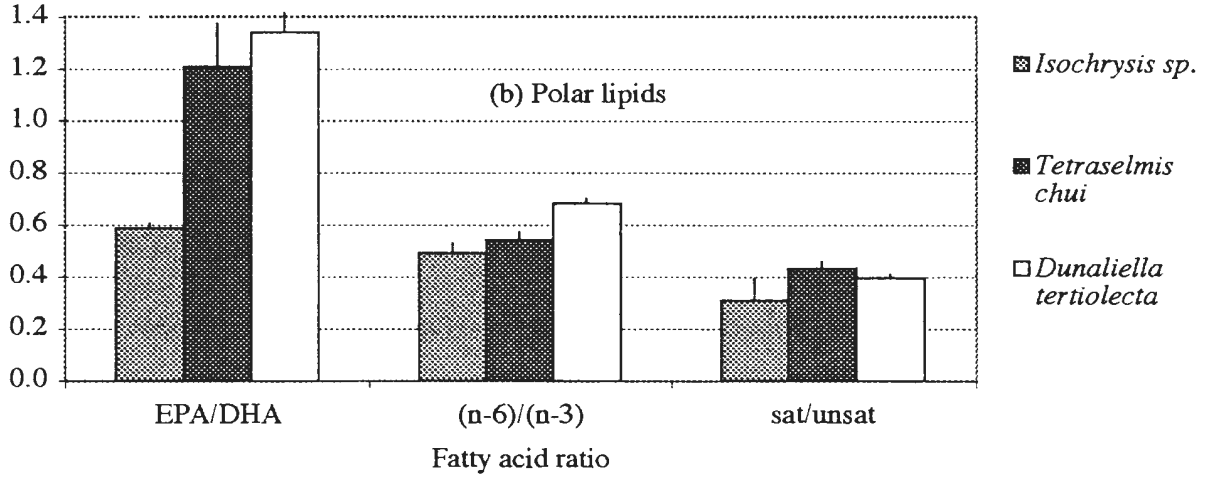


Figure 2.8. Fatty acid ratios for a) neutral lipids and b) polar lipids from experiment #2 gonad samples. Error bars are + standard deviations.

similarly constrained in both NL and PL fractions.

2.4 Discussion

2.4.1 Broodstock conditioning experiment #1

2.4.1.1 Stability of egg quality

The biochemical composition, in terms of lipid quantity and quality, of eggs from experiment #1 varied little in response to the diet treatments. Total lipids, lipid classes, and fatty acids of the *Placopecten magellanicus* eggs in this study were similar to those found in other *P. magellanicus* studies (Manning, 1986; Napolitano et al., 1992) and studies of some other pectinids (Soudant et al., 1996). The stability in egg quality is consistent with some studies but not with others. Berntsson et al. (1997) found that the PUFA and DHA content of newly released *Ostrea edulis* larvae correlated with subsequent growth rates. However, the initial fatty acid composition of early larvae was unrelated to the diet treatments provided to broodstock, suggesting that genetic or other sources of variability influenced larval biochemical composition more than the conditioning diet. In contrast, Soudant et al. (1996) found that fatty acids of *Pecten maximus* eggs were related to their proportions in the diet. The reason for this apparent discrepancy is probably the prior condition (*i.e.*, initial nutritive state) of broodstock. Scallops in experiment #1 were conditioned for only four weeks and were exposed to the spring phytoplankton bloom *in situ* before being transported to the lab. They were also collected from a relatively shallow site where food was probably abundant and may have experienced unusually warm temperatures early in the season. Berntsson et al. (1997)

also attributed the lack of treatment differences in their study to an adequate quantity and quality of endogenous reserves prior to initiation of conditioning. In Soudant et al.'s (1996) study, scallops were spawned on collection, thus draining most reserves, and held for a total of 12 weeks. Apparent egg quality stability in our study may have been aided by translocation of lipids and fatty acids from the digestive gland during conditioning (Vassallo, 1973). The total lipid concentration was not significantly reduced in digestive glands from scallops fed the diet lacking in long chain PUFA (*Dunaliella tertiolecta*), but the proportion of these PUFA in the neutral lipid portion was reduced to levels below one half of those found in the other diets. Whether this was due only to the impact of diet or, in part, to differential translocation of long chain PUFA in scallops from this group cannot be determined. Nonetheless, it is likely that both lipids formed *de novo* and dietary lipids are transported from the digestive gland to the gonad. Napolitano and Ackman (1993) found, in an ecological study, that a number of digestive gland fatty acids correlated with their corresponding levels in TAG of female gonads. A large drop in the Polyunsaturation Index (PUI; the sum of PUFA weight percents divided by the total number of double bonds) occurred in late summer and early autumn, corresponding with gonad development and indicating a possible translocation of PUFA. An autoradiographic or other labeling experiment would be helpful to examine this and to better understand the nutrient dynamics of the digestive gland and its contribution to the developing gonad. Comparisons of the biochemical composition of male and female digestive glands from a conditioning experiment similar to the one presented here may

also permit an examination of PUFA transport from the digestive gland to the gonad during gametogenesis.

The lack of differences in egg size, organic weight, and hatch rates also indicate that egg quality did not differ among treatments. The variability in hatch rates may suggest a significant genetic component to egg quality. However, mean hatch rates (ca. 15%) were lower than expected (although similar to hatch rates in Soudant et al.'s *Pecten maximus* study (1996), and may have resulted from logistical problems in the experiment. Eggs used in determination of hatch rates were all spawned in one day. Although efforts were made to standardize egg handling and incubation within replicates, the number of animals spawned made it difficult to monitor fertilization (polar body release) and to control the number of eggs in each sample prior to fertilization while still fertilizing within 15-25 minutes of spawning.

2.4.1.2 Partial diet effects on egg composition

Despite an overall lack of differences in fatty acid profiles from eggs in experiment #1, some of the data suggest at least a partial diet effect. Arachidonic acid (ARA) was significantly higher in the *Tetraselmis chui* diet and in eggs from this treatment. ARA is both a major component of some phospholipid classes and an important precursor for bioactive prostaglandins and eicosanoids (PGE_2, F_2, and D_2) which have been associated with gametogenesis and spawning in scallops (Osada et al., 1989). Soudant et al. (1996) found that ARA was the major PUFA (33-39% of total fatty acids) in the phosphatidylinositol fraction of *Pecten maximus* gonads. The

proportion of this fatty acid increased throughout gametogenesis even when it was found in low amounts in the diet. This fatty acid was also preferentially accumulated in fatty acids of gonad polar lipids in the current study. Furthermore, the increased variability in EPA in total lipids from eggs from the *Isochrysis* sp. treatment also suggests that the diet had a partial impact on egg composition.

Also of interest were the differences between wild conditioned compared to the lab-conditioned spawners. Some studies indicate a difference in biochemical composition between eggs obtained from wild stock and those conditioned in the laboratory, with the implication that eggs obtained from wild stock are superior. Helm et al. (1991) found a reduction in EPA in released larvae from hatchery-reared *Ostrea edulis* compared with *in situ* populations. Likewise, gonads of *Argopecten purpuratus* held in the ocean were higher in DHA and spawned earlier than those of animals held in the hatchery over the same time period. Our results also show fatty acid differences between eggs from wild and lab-conditioned animals to be greater than those of lab-conditioned animals under different diet regimes. Whether this relates to diet (presumably a more diverse diet is available in the wild than in the individual diet treatments chosen for this study), or to a stress response such as a greater incidence of egg atresia in captive scallops, is unclear and needs further investigation. The likelihood that the controlled lab diets were more variable in their fatty acid profiles than natural plankton stocks available to the naturally conditioned group suggests that factors other than diet were involved. Inclusion of hatch rates of the wild-conditioned animals and measures of fecundity would have been useful. An analysis of fatty acids in the D-stage larvae from the respective

treatments may have been helpful to establish differences due to atresic eggs that were spawned along with healthy eggs.

More significant differences may have been evident in neutral lipid portions if separations had been done on all of the egg samples to allow for a statistical comparison. In egg formation, since polar lipids are generally established before vitellogenesis (Sastry and Blake, 1971) is completed, fatty acids of the egg polar lipids, which not only form first but are known to vary less than egg neutral lipids, may have obscured treatment differences in neutral lipid composition.

2.4.1.3 Tissue analyses

The diet effect found for digestive gland lipids is not surprising due to this organ's association with ingested food. Digestive gland lipid is in the form of intracellular oil droplets contained in specialized tubular cells. Indeed, food particles themselves are present in the digestive cells due to pinocytosis (Napolitano et al., 1993).

The conservatism in the fatty acid profiles of adductor muscles suggests a slow turnover rate of lipids in this organ. Watanabe and Ackman (1974) reported that dietary fatty acids provided to *Crassostrea virginica* and *Ostrea edulis* adults were rapidly converted into a species-specific profile. Our results for adductor tissue in *P. magellanicus* indicated that fatty acids in organs that display less active lipid metabolism were difficult to alter through dietary means. However, even when changes in fatty acids in total tissue are not detected, specific organs that are actively involved in lipid metabolism such as the digestive gland and gonad may show alterations. Fatty acids in

the digestive gland may be used in ecological studies as tracers for large scale spatial variability in phytoplankton assemblages and possibly as indicators of the nutritive state of scallops. Napolitano and Ackman (1993) found that fatty acids of gut contents, and seasonal changes in available EPA and DHA in particular, appeared related to changes in the fatty acid composition of the digestive gland, although no statistics were done because samples were pooled. However, in natural environments where fatty acids of phytoplankton assemblages may be more diverse than in experimentally manipulated lab diets, changes in gonad composition may represent endogenous temporal variations in the state of gametogenesis (*i.e.*, selective deposition of certain PUFA in the gonad) rather than environmental inputs from phytoplankton (Chu et al., 1990; Napolitano and Ackman, 1993). The combination of dietary factors, differential metabolic control and metabolic rates, and organ-specific fatty acid distributions confounds fatty acid analyses of total tissues in adult organisms. However, analysis of fatty acids on an organ-specific basis and under experimental conditions can yield very useful information.

2.4.2 Broodstock conditioning experiment 2 – dietary effects on gonad composition

In contrast to eggs from experiment #1, lipids from gonads of scallops in the second conditioning experiment did suggest a dietary influence on gametogenesis for lab-conditioned animals fed algae varying widely in their fatty acids. The failure of these animals to spawn and the biochemical analyses show that broodstock from the second experiment were in a relatively poor reproductive condition. Total lipid and TAG concentrations were lower than would be expected based on the results of other studies

(Napolitano et al., 1992). This apparent poor condition may have been the result of an inadequate diet, as all the groups lacked one or both essential fatty acids DHA and EPA. A combination of factors may have also affected conditioning: 1) Tank temperature problems were experienced during the second experiment due to logistical problems. 2) Poor water quality may have resulted from the use of paste as a partial diet replacement in the *Isochrysis* sp. and *T. chui* treatments. 3) Endogenous reserves in the animals may have been depleted due to holding the animals in a potentially food-limited flow-through system during the winter. Animals were fed only a minimum ration approximately once a week as it was assumed that enough food would be naturally available from flow-through.

Gonad neutral lipid fatty acids reflected the diet to a greater extent than did fatty acids of the phospholipids (polar lipids). There is greater metabolic control over phospholipid formation due to the importance of fatty acid composition in membrane function. This is a common finding in fatty acid studies (Waldock and Holland, 1984). Variability was high in neutral lipid fatty acid composition (Figure 2.4) and the sample size was small, but mean differences clearly reflect the species-specific characteristics of the algae (Figure 2.1). Although greatly reduced compared with the *Isochrysis* sp. treatment group, significant levels of DHA were still present in neutral lipids of the *T. chui* and *D. tertiolecta* (diets with no DHA) treatments. Relative amounts of EPA also reflected the diet in terms of rank order, but were not as high in the *T. chui* treatment as was expected. Proportions of neutral lipid EPA across all treatments were substantially reduced compared to that of healthy eggs (experiment #1). One possible explanation is

that EPA, associated with storage lipid cannot be deposited within the eggs until egg polar lipids are formed. DHA is associated with egg polar lipids in pectinids and believed to be assigned a specific role in membranes that cannot be fulfilled by other PUFA (Soudant, 1996). Assuming the progression of gametogenesis described above, if DHA is limiting, vitellogenesis (and hence accumulation of EPA) may also be inhibited.

Levels of SAT, MONO, and PUFA were relatively stable in both NL and PL fractions and in both experiments. Maintenance of proportions of fatty acids in these groups represents a first stage in fatty acid metabolic control in scallops and indicates a physiological necessity for maintenance of membrane function (Soudant et al., 1996). NL lipids may share the same enzyme systems and hence be similarly controlled. The ratios may also be important during embryogenesis, when egg TAG pools may provide a supply of fatty acids for membrane formation in trochophores and early larvae before the shift from endotrophy to exotrophy.

Although ratios of SAT, MONO, and PL were consistent among treatments and between NL and PL, C_{20} and C_{22} PUFA were depleted in NL and selectively accumulated in the polar lipids. Mean levels of C_{20} and C_{22} PUFA in experiment # 2 gonad PL were only slightly lower (29-33%) than in healthy (experiment #1) eggs (36%), but EPA/DHA levels in gonad PL were over half as low as for healthy eggs. Thus a greater retention of DHA over EPA is shown. ARA was also higher in PL FA from experiment #2. Millican and Helm (1994) showed through a starvation experiment that DHA and ARA were selectively retained at the expense of EPA. Similarly, Soudant et al. (1996) showed a partial retention of EPA, but a greater retention of DHA. In the turbot (*Scophthalmus* sp.)

ARA and DHA were also selectively retained over EPA when animals were fed a diet totally deficient in PUFA (Bell et al., 1985; Castell et al., 1994). The retention of these two FA demonstrates their important, though still largely undefined, roles as PL components. The diet effects are consistent with the currently held view that scallops cannot synthesize these compounds in suitable amounts to meet their needs. Nevertheless, some metabolic control takes place in scallops fed deficient diets. With appropriate desaturases for synthesizing PUFA lacking, acyltransferases may be the chief mechanism behind this regulation (Marty et al., 1992).

2.4.3 Summary of experiments #1 and #2

Experiment #1 represents a conditioning scenario where spawners are brought into a hatchery in a semi-conditioned state before final conditioning with cultured algae, whereas experiment #2 represents broodstock conditioning from a more or less spent state (and possibly a partial depletion of endogenous reserves). Soudant et al. (1996) show that the fatty acid composition of eggs in *Pecten maximus* is determined mostly in the first 5 weeks of conditioning. Based on the combined results of the two experiments, this seems to be the case with *P. magellanicus* as well. However, certain PUFA such as EPA (associated with TAG) or ARA (which may be involved as a precursor to compounds involved in spawning) may accumulate later or throughout gametogenesis. The timing of conditioning, or "conditioning window", is important in terms of egg quality and hence hatchery management. It appears difficult to 'enhance' egg PUFA composition for broodstock brought in mid-season. Conversely, spawners partially

conditioned in the natural environment can be fed a sub-optimal diet without any deleterious effects on egg quality. Thus, using broodstock partially conditioned from the wild may free spawns from a dependence on hatchery food in terms of both quantity (Paon and Kenchington, 1996) and quality. However, when broodstock are collected in mid-winter, as is often the case, diet becomes more important. This is demonstrated by experiment #2 results where fatty acid composition of the gonads was influenced by diet, and diet regimes lacking in one or more EFAs yielded poor results. Endogenous reserves also play a role and a period of algal supplementation prior to initiating elevated temperatures may be crucial. Robinson (1992a,b) demonstrated a similar scenario for *Crassostrea gigas* broodstock. She found that when conditioning was initiated earlier in the year, broodstock supplemented with algae or lipid microspheres produced superior eggs with better hatch rates and subsequently better settlement rates. However, no differences were found when animals were conditioned later in the year. Dietary fatty acids from the conditioning treatments were not evident in oyster tissue but did influence egg composition.

2.4.4 Broodstock diets

Because of the stability in egg quality in experiment #1 and the poor conditioning results in experiment #2, it is difficult to recommend particular conditioning diets. A lack of EFA does appear to be detrimental when scallops are conditioned under stressful conditions or when previous reserves are depleted. DHA and ARA are more critical than EPA as essential components. Thus, inclusion of a high DHA microalga such as

Isochrysis sp. is important. Soudant et al. (1996) did an experiment, similar to the one presented here, with *Pecten maximus*, using *Isochrysis* sp., *Chaetoceros calcitrans* (a high EPA low DHA alga like *T. chui*), and a PTSC (*Pavlova lutheri*, *Isochrysis* sp., *Skeletonema costatum*, and *C. calcitrans*) mixed diet. They obtained slightly better hatching rates and fewer abnormal larvae with the *Isochrysis* sp. treatment, and recommended that the proportion of this alga in mixed diets be increased until the EPA/DHA ratio approaches 1. While this seems appropriate given their results, the fact that healthy eggs possess an EPA/DHA ratio closer to 2:1 suggests that this ratio would be better. Since ARA is also an important component, inclusion of this fatty acid in conditioning diets is also desirable (Le Pennec et al., 1998). Thus *T. chui* with its high EPA and comparatively higher ARA levels would be useful in a mixed diet with *Isochrysis* sp.. The high C_{16} PUFA content of *T. chui*, however, may be of little use in gametogenesis since these are only minor egg components. *D. tertiolecta*, although unsuitable because of its lack of EFAs, could be a source of other important nutrients such as glycogen in a mixed diet (Webb and Chu, 1983). It would be desirable to repeat experiment #1, initiating the diet treatments earlier in the spring and gathering more data on egg performance, including initial growth rates of the resulting larvae. However, Dorange et al. (1989), in a more comprehensive study on egg quality in *Pecten maximus*, found that viable larvae were produced from eggs varying substantially in their biochemical content. Thus other factors, such as the animals' prior nutritive state, stress, or temperature, in addition to the inclusion of EFAs in the diet, may be more important in conditioning than trying to optimize fatty acid ratios. Nonetheless, in a commercial

operation, a quick fatty acid assay of sample gonads prior to spawning could be useful to assess the condition of animals or diagnose early mortality as due to inadequate nutrition or some other cause (*i.e.*, bacterial).

2.5 Conclusions

1) Small dietary effects in egg fatty acid composition (mainly a higher relative percent of ARA in eggs from brood fed *Tetraselmis chui*) were apparent from broodstock partially conditioned using algal treatments varying broadly in their fatty acid composition. However, differences between wild and lab-conditioned scallops were greater than differences among algal treatments within the lab-conditioned group.

2) No differences were found in egg lipid level, lipid class proportions, hatch rates, organic weight, or size among treatments. Egg quality stability may be explained by: a) adequate prior endogenous reserves and possible transport of stored fatty acids from the digestive gland, b) accumulation of most egg fatty acids relatively early in the gametogenic cycle, prior to final maturation, c) a degree of metabolic control, including preferential incorporation of certain fatty acids (such as small amounts of EPA present in the *Isochrysis* sp. diet) into eggs, and d) possible differences in fecundity such that egg quality is maintained despite diet deficiencies.

3) Dietary effects were more apparent for female scallop gonads in a second experiment in which conditioning was initiated earlier. Neutral lipid fatty acids reflected diets to

a greater extent than polar lipids. Polar lipids showed deviations in C_{20} - C_{22} PUFA content in conjunction with EFA deficiencies in the diet, but despite diet deficiencies, levels of ARA, DHA, and EPA (to a lesser degree) were selectively retained.

4) Levels of total SAT, MONO, and PUFA were similar in all treatments and in healthy eggs from experiment #1 and female gonads from experiment # 2. Thus, maintenance of these proportions is probably important to membrane function.

5) Gonad (egg), digestive gland, and adductor muscle tissues displayed organ-specific fatty acid compositions. The fatty acid composition of adductor muscle was not influenced by the diet regimes, but organs with more active lipid metabolism (gonad, digestive gland) did reflect diet to a limited extent.

6) The poor conditioning results of experiment #2, where diets were deficient in one or more EFA, and the partial dietary impact exhibited in eggs from experiment #1 suggested that conditioning diet regimes in terms of lipid quality and EFA content were important. However, egg quality can be maintained and diet effects lessened through a number of mechanisms. Thus factors other than diet such as stress, prior nutritional reserves, and genetics may play a larger role in successful spawns than an "optimal" diet. While it has been established that batches of eggs can vary substantially in their lipid content and fatty acid composition, the effects on egg development and subsequent larval vigor are still unclear.

CHAPTER 3 – EFFECT OF DIETARY LIPID QUALITY ON *PLACOPECTEN MAGELLANICUS* LARVAL GROWTH AND BIOCHEMICAL COMPOSITION

3.1 Introduction

Lipids in bivalves have been extensively studied over the past 20 years, from a variety of perspectives, due to their dual role as a primary energy store in young bivalves and as a key structural component of cell membranes (Holland, 1978). Despite this research, specific lipid nutritional requirements of larvae are still poorly defined. Although the abundance of lipid reserves (mainly TAG) within larvae may be an indicator of larval health (Waldock and Nascimento, 1979; Gallager et al., 1986), few studies have found a correlation between total lipid or TAG in the diet and larval performance.

Recent work indicates that the quality of the diet, in terms of its fatty acid composition, may be nutritionally more important than gross composition. Lipid quality of microalgae varies substantially more than protein or carbohydrate composition (Webb and Chu, 1983), and many studies have demonstrated a link between the fatty acid composition of the diet and subsequent larval performance. The role of (n-3) series essential fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been given the most attention in the literature. although arachidonic acid (ARA), a C_{20} (n-6) series fatty acid, may also be important (Webb and Chu, 1983; Napolitano et al., 1990). Conversely, some authors contend that the role of essential fatty acids in bivalve

nutrition has been over-emphasized (Whyte et al., 1989; Thompson et al., 1994; Ackman and Kean-Howie, 1995) and that fatty acids need only be supplied at some minimal level.

The current practice of trying to ensure a 'balanced' diet simply by providing two or more species of algae may be inadequate. A better understanding of how dietary fatty acids are assimilated and how deficiencies in essential fatty acids affect larval performance is needed before determining if and how diets can be optimized to improve hatchery yields and efficiency.

Much of the work with essential fatty acids and bivalves has centered on species of importance to aquaculture such as oysters (*Ostrea edulis*, *Crassostrea virginica*, and *C. gigas*). Nutritional work with *P. magellanicus* has concentrated on gross composition (Manning, 1986; Ryan, 1999) or on juvenile stages (Parrish et al., 1993) with relatively few algal species used as treatments. An extension of this work to larval *P. magellanicus* is important to assess the generality of recent discoveries in bivalve nutrition, and the specific fatty acid requirements of this candidate aquaculture species.

In the present study, live algae with broad species-specific differences in EFA content are used for an exploratory experiment on dietary effects of lipid quality on larval fatty acid composition and growth. Three of the species chosen, *Isochrysis* sp., *Pavlova lutheri*, and *Thalassiosira pseudonana* (strain 3H) are commonly used in hatcheries. The fourth alga, *Pavlova* sp. (strain CCMP459) has not been examined in conjunction with *P. magellanicus* and serves as a useful comparison to *Pavlova lutheri*.

3.2 Methods

3.2.1 Algal culture

Batch cultures of *Isochrysis* sp. (Tahitian strain or T-Iso), *Pavlova lutheri*, *Pavlova* sp., and *Thalassiosira pseudonana* (strain 3H) (Table 3.1) were maintained in 2-L Erlenmeyer flasks. Ambient seawater (31 ppt salinity) was filtered to 0.45 µm (nominal rating), enriched with F/2 (and silicate for *T. pseudonana* cultures) and autoclaved prior to inoculation from line flasks. Algae were grown under continuous fluorescent light at a temperature of 24-27°C, and bubbled with 0.45 μm filtered air and a continuous 1% CO_2 supplement as a carbon source. Cultures were restarted every three days in order to stagger algal harvest and provide cells at a consistent point in late exponential growth phase. Culture condition was monitored by microscopic examination and a Coulter Multisizer™ used to measure cell density.

3.2.2 Larval culture

Wild conditioned sea scallops, collected from North Harbour, Newfoundland on August 1, 1998, were spawned by thermal shock. A portion of eggs pooled from 12 individual spawners were fertilized with a 2-3 mL of a sperm suspension from 6 males. Fertilized eggs were distributed in three 68-L square polyethylene tanks at a concentration of approximately 25 eggs/mL seawater and subsequently thinned after 2 days to 2-4 larvae/mL seawater. Larvae were cultured in these containers for eight days and fed once daily a diet of *Isochrysis* sp. and *P. lutheri* at a concentration of $2x10^4 - 3 x 10^4$ cells/mL. A flowing seawater bath around the tanks served to maintain a culture

temperature of 14-17°C. Cultures were provided with gentle aeration for mixing. Larvae were removed on screens every other day, all culture apparatus was cleansed, tanks were refilled with 1 μm filtered seawater and larvae redistributed after counting on a Sedgewick rafter cell and monitoring for health by microscopic observation.

3.2.3 Experimental design

Nine days after fertilization, larvae were pooled and redistributed equally into twelve 20-L cylindrical polyethylene tanks divided into three blocks consisting of adjacent water baths with treatments assigned randomly within each block (4 treatments x 3 block RCB design). Random assignment was accomplished by designating an integer from one to four for each respective treatment *a-priori* and then using the "random" function of Microsoft Excel™ to generate diet assignments for experimental units within each block position symbolized in the spreadsheet. The four treatments consisted of unialgal diets: *Isochrysis* sp., *Pavlova lutheri*, *Pavlova* sp. (CCMP459), and *T. pseudonana*. Larvae were fed an equal cell ration of 3 x 10^4 cells/mL daily and cultured as described above at a density of 1-2/mL. Samples of at least 50 larvae were taken periodically and videotaped for later growth measurements (shell length) and mortality estimates using NIH Image™ software for Macintosh™. The feeding trial was terminated after 9 days of feeding.

3.2.4 Biochemical analyses

3.2.4.1 Sampling of algae

Algal cultures were sampled aseptically every other day (four total samples), counted on a Coulter Multisizer™, and three subsamples collected on precombusted Whatman GF/C filters for subsequent lipid, fatty acid, CHN, and dry and ash weight determinations. Subsamples for lipid analysis were placed directly into chloroform, sealed under a nitrogen atmosphere, and stored at –20°C for later analysis.

3.2.4.2 Sampling of larvae

Samples of $2x10^4 - 4x10^4$ larvae were taken on day 2 (when embryos developed to the prodisoconch I veliger stage), day 9 (prior to the initiation of the diet trials), and on day 18 (at the termination of the feeding trial). The original eggs were also sampled prior to fertilization. Larvae were collected after water changes and prior to feeding to allow for gut evacuation and rinsed twice on a sieve with 0.45 μm filtered seawater. Control samples of the filtered seawater were taken to check for lipid contamination. Larvae were placed in a petri dish, videotaped and subsequently counted using NIH Image™ software. The entire sample was gently filtered on a precombusted Whatman GF/C filter and placed directly in chloroform, sealed under a nitrogen atmosphere, and stored at –20°C for later lipid and fatty acid analysis.

3.2.4.3 Lipid and FAME analyses

Lipid extraction, quantification, and separation into neutral and polar components, and fatty acid analysis are detailed in Chapter 4 (sections 4.2.3- 4.2.7). The same procedures were used here but no lipid or fatty acid internal standard was added to the samples prior to analysis. Estimates of total fatty acids in the sample total lipid and in each of the lipid classes were made with a spreadsheet program. The spreadsheet calculates an average fatty acid molecular weight based on FAME proportions from GC analysis and applies this to the Iatroscan lipid class data to obtain an estimated FAME weight for each class which is then summed for a total fatty acid weight (Budge, 1999).

3.2.4.4 Other analyses

CN and dry weight were also determined as described in Chapter 4. Following dry weight determination of algal samples, filters were combusted overnight at 450°C and stored in a drying chamber with activated silica gel prior to weighing on a Mettler™ microbalance. Ash-free dry weight (AFDW, organic content) per cell was then calculated.

Differential settling of algal cells within the larval tanks was measured in a separate experiment. Larval tanks used during the feeding trial were filled with seawater containing $3x10^4$ cells/mL of each treatment algae and cell densities determined at the start and after 24 hours with a Coulter Multisizer™. Data were examined by analysis of variance (ANOVA).

3.2.5 Statistical analyses

Growth was calculated as the slope of the linear regression line of shell length over the period of the feeding trial in days for each experimental unit. Cumulative percent mortality was estimated at the end of the feeding trial as the number of empty shells divided by the number of larvae in videotaped samples. Differences in fatty acid and lipid class profiles and larval growth in response to the diet treatments were tested by a single factor model I ANOVA (Statview™ for Macintosh™) followed by a post-hoc Tukey's multiple comparisons test (alpha = 0.05) (Sokal and Rohlf, 1995). In most tests it was unnecessary to use the arcsine transformation since percent values were confined to a relatively narrow range. Block and treatment effects were first tested using the GLM function of SYSTAT™ for Macintosh. In keeping with the original RCB design, the *block x treatment* interaction term was suppressed in the analysis (Sokal and Rohlf, 1995). Very little variance was explained by differences between blocks ($F_{2,6} = 0.513$, p= 0.623) so data were pooled and re-analyzed by ANOVA. Only fatty acids representing more than 1% of the total were tested. An exploratory analysis of dietary biochemical components associated with larval growth was carried out by Principal Components Analysis (PCA) using SYSTAT™ for Macintosh™. Not enough larvae were available for both dry weight determination of larvae and biochemical analysis, but a rough estimate of larval dry weight was obtained by Couturier's (1986) regression of log larval dry weight against shell length in healthy larval cultures of *P. magellanicus*:

$$W = 1.8681 \times 10^{-3} \times L^{2.4438} \quad (r^2=0.998)$$

where W = larval dry weight (ng) and L = shell length (μm).

3.3 Results

3.3.1 Algal biochemical composition

3.3.1.1 Gross composition

A simple estimate of gross composition of the algal cultures was obtained by CN analysis. The C:N ratio of the algae was similar with a low of 5.61 (± 2.24 SD) for *Pavlova lutheri* to 6.88 (± .79 SD) for *Thalassiosira pseudonana* (Table 3.2; p=0.735). Ash-free dry weight ranged from 26.3 pg/cell (±1.7 SD) for *Isochrysis* sp. to 38.0 pg/cell (±5.4 SD) for *T. pseudonana*, but these differences were not significant (Table 3.2; p=0.192).

3.3.1.2 Lipid and lipid class composition

Total lipids per cell were similar (4.1 to 5.3 pg/cell, Table 3.3), comprising from 14.0% – 18.7% of AFDW (Table 3.2). TAG was slightly higher per cell (overall ANOVA p= 0.0396) in *P. lutheri* than in *Isochrysis* sp. (p= 0.0304) and *T. pseudonana* (p= 0.0119). Ethyl and methyl ketones comprised an average of 10.2% of total lipids in *Isochrysis* sp. and a small percentage (3.0%) in *T. pseudonana*, but none were found in either of the *Pavlova* species. Other lipid class components were not significantly different either in terms of cell content or relative proportion of total lipids (Table 3.3).

3.3.1.3 Fatty acids

With regards to essential fatty acids, *Isochrysis* sp. was rich in DHA (11.47%) but contained only small amounts of EPA (0.46%). The two *Pavlova* species contained

Table 3.1. Growth rate of *Placopecten magellanicus* larvae and cumulative mortality over the feeding trial period 9 to18 (mean ± standard deviation, n=3). Diet treatment, essential fatty acid summary, and strain designation at left

Diet	Class	Strain Designation	Growth rate (K) (μm/day) SD	*	Est. cum. mortality % SD	*
Isochrysis sp. *HIGH DHA: LOW EPA*	Prymnesiophycea	CCMP1324 (T-ISO, NEPCC5)	1.43 ±0.13	*a*	2.28 ±0.77	*ns*
Pavlova lutheri (Droop) *HIGH DHA: HIGH EPA*	Prymnesiophycea	CCMP1325 (MONO, NEPCC601)	1.42 ±0.15	*a*	2.99 ±1.18	*ns*
Pavlova sp. *HIGH DHA:HIGH EPA: HIGH AA*	Prymnesiophycea	CCMP459 (IIF1, IIF1AX)	1.90 ±0.15	*b*	1.63 ±0.46	*ns*
Thalassiosira pseudonana (Hustedt) *LOW DHA:HIGH EPA*	Coscinodiscophyceae	CCMP1015 (3H, ARC5)	0.89 ±0.21	*c*	3.62 ±1.85	*ns*

* = treatments designated with different letters are significantly different at $p<0.01$

Table 3.2. Diet gross biochemical composition: Carbon and nitrogen content, C:N ratio, cell dry and organic weight, and percent lipid (mean ± standard deviation, n=4).

Diet		*Isochrysis sp.*	*Pavlova lutheri*	*Pavlova sp.*	*Thalassiosira pseudonana*
Carbon	*(pg/cell)*	11.52	11.37	11.82	14.49
		±2.96	±0.27	±1.24	±2.92
Nitrogen	*(pg/cell)*	1.71	1.67	1.97	2.11
		±0.39	±0.82	±0.07	±0.67
C:N ratio		6.71	5.61	6.01	6.88
		±0.18	±2.24	±0.86	±0.79
Dry wt/ cell	*(pg/cell)*	27.19	31.39	38.11	48.40
		±2.71	±11.1	±14.08	±7.60
AFDW	*(pg/cell)*	26.28	27.00	33.00	38.00
		±1.65	±8.30	±9.90	±5.40
Lipid	*(%AFDW)*	18.68	15.37	15.06	14.00
		±2.13	±6.89	±7.12	±3.97

Table 3.3. Total lipid and lipid class composition of algal diets as pg/cell (top) and as a relative proportion of total lipids (bottom) (mean ± standard deviation, n=4).*

Diet	*Isoxhrysis sp.*		*Pavlova lutheri*		*Pavlova sp.*		*Thalassiosira pseudonana*	
Total Lipids (pg/cell)	5.0 ±0.6		4.1 ±1.9		5.0 ±2.4		5.3 ±1.5	
HC	0.1 ±0.1		0.2 ±0.1		0.1 ±0.0		0.2 ±0.1	
SE/WE	0.0 ±0.0		0.0 ±0.1		0.2 ±0.4		0.0 ±0.1	
ME	0.1 ±0.1		0.0 ±0.0		0.1 ±0.1		0.1 ±0.2	
E.KET	0.4 ±0.1	*a*	0.0 ±0.0	*b*	0.0 ±0.0	*b*	0.0 ±0.0	*b*
M. KET	0.2 ±0.3	*a*	0.0 ±0.0	*b*	0.0 ±0.0	*b*	0.1 ±0.1	*a*
TAG	0.5 ±0.1	*b*	1.1 ±0.5	*a*	0.6 ±0.2	*ab*	0.4 ±0.3	*b*
FFA	0.1 ±0.0		0.2 ±0.1		0.1 ±0.1		0.2 ±0.2	
ALC	0.0 ±0.0		0.1 ±0.1		0.2 ±0.3		0.3 ±0.1	
ST	0.1 ±0.0		0.2 ±0.1		0.3 ±0.1		0.4 ±0.4	
DAG	0.3 ±0.2		0.3 ±0.2		0.4 ±0.5		0.4 ±0.1	
AMPL	1.2 ±0.2		0.7 ±0.5		1.2 ±0.9		1.1 ±0.2	
PL	1.9 ±0.4		1.4 ±1.1		1.8 ±0.7		2.1 ±0.4	
(%)								
HC	2.6 ±1.0		3.5 ±2.3		2.7 ±1.5		3.5 ±0.4	
SE/WE	0.2 ±0.2		0.3 ±0.8		2.9 ±4.5		0.9 ±1.6	
ME	1.2 ±2.1		0.0 ±0.0		1.5 ±2.3		2.5 ±4.4	
E.KET	7.6 ±3.0	*a*	0.0 ±0.0	*b*	0.0 ±0.0	*b*	0.0 ±0.0	*b*
M. KET	3.6 ±6.2	*a*	0.0 ±0.0	*b*	0.0 ±0.0	*b*	3.0 ±3.1	*a*
TAG	10.6 ±1.4	*b*	29.1 ±15.0	*a*	14.1 ±7.4	*ab*	6.5 ±4.0	*b*
FFA	2.0 ±0.4		4.5 ±3.2		2.1 ±2.6		3.1 ±2.2	
ALC	0.0 ±0.0		2.2 ±2.1		4.2 ±3.6		5.1 ±0.8	
ST	2.6 ±0.8		6.3 ±3.2		6.6 ±2.9		7.2 ±4.7	
DAG	6.3 ±3.5		7.2 ±3.2		7.5 ±9.6		7.0 ±2.3	
AMPL	24.8 ±2.2		15.5 ±2.8		21.5 ±6.4		21.4 ±2.3	
PL	38.4 ±2.8		31.2 ±11.7		36.7 ±5.1		39.6 ±4.0	

* lipid classes designated with different letters were significantly different at $p<0.01$

significant quantities of both DHA (10.2% for *P. lutheri* and 5.2% for *Pavlova* sp.) and EPA (16.6% and 16.7%, respectively). *T. pseudonana* contained similar levels of EPA (16.9%) but relatively little DHA (2.2%). This wide variation in essential (n-3) fatty acids is reflected in the EPA to DHA ratios: 0.04, 1.62, 3.98, and 8.36 for *Isochrysis* sp., *P. lutheri*, *Pavlova* sp., and *T. pseudonana*, respectively (Table 3.3).

Pavlova sp. was further distinguished from *P. lutheri* by a high content of long chain C_{20} - C_{22} (n-6) series fatty acids — ARA (20:4n-6; 5.9%) and 22:5n-6 (9.9%). The highamounts of these two fatty acids resulted in an unusually high (n-6):(n-3) ratio (0.70 vs. 0.14 and 0.12 for *Isochrysis* sp. and *P. lutheri*, respectively and 0.04 for *T. pseudonana*) and a high PUFA and long chain PUFA content (52.1% and 37.7%, respectively) compared with the three other species utilized (Table 3.4).

Other species-related fatty acid characteristics included a high amount of C_{18} fatty acids in *Isochrysis* sp., in which, 18:1n-9 was the prevalent monounsaturate compared with 16:1n-7 for the other species. The fatty acid 18:4n-3 was present at high levels (21.7%), and 18:2n-6 and 18:3n-3 also occurred in significant quantities (3.9% and 6.0%, respectively). The two *Pavlova* species contained proportionally more 16:0 and slightly less 14:0 compared with *Isochrysis* sp. and *T. pseudonana*.

Total proportions of saturated fatty acids were similar, ranging from 27.2% (*Isochrysis* sp.) to 32.1% for *P. lutheri*. Monounsaturated fatty acids were lowest in *Pavlova* sp. (15.5%) and highest in *T. pseudonana* (28.9%), followed by 26.8% for *P. lutheri* and 21.3% for *Isochrysis* sp.. Total fatty acids per cell were not significantly different among the diets, ranging from 2.7 to 2.8 pg/cell (Table 3.4).

Table 3.4. Relative fatty acid composition (wt % of total FA) of algal diets (mean %, ± standard deviation, n=4).

Diet	*Isochrysis sp.* (%)	*Pavlova lutheri* (%)	*Pavlova sp.* (%)	*Thalassiosira pseudonana* (%)
14:0	13.8 ±1.5	11.0 ±1.2	11.3 ±0.7	15.9 ±0.8
15:0	0.5 ±0.2	0.5 ±0.2	0.6 ±0.7	0.8 ±0.1
16:0	12.5 ±2.7	20.0 ±4.2	19.0 ±3.4	11.0 ±6.1
18:0	0.5 ±0.1	0.7 ±0.2	0.4 ±0.4	0.9 ±0.2
Total SAT	27.2 ±2.4	32.1 ±3.8	31.3 ±3.9	28.6 ±6.3
16:1n-9	1.2 ±0.6	0.6 ±0.1	0.5 ±0.5	2.1 ±1.3
16:1n-7	4.1 ±0.7	22.7 ±2.6	11.6 ±1.7	20.5 ±4.2
16:1n-5	- -	- -	2.1 ±0.4	0.0 ±0.0
18:1n-9	13.8 ±5.2	1.1 ±0.1	0.5 ±0.3	0.4 ±0.3
18:1n-7	2.2 ±0.4	2.7 ±1.0	0.9 ±0.6	5.3 ±3.4
18:1n-5	- -	- -	- -	0.3 ±0.6
20:1n-11	0.6 ±0.5	0.2 ±0.0	- -	- -
Total MONO	21.3 ±3.5	26.8 ±1.9	15.5 ±2.8	28.9 ±0.9
16:2n-7	- -	- -	- -	1.4 ±1.6
16:2n-4	1.1 ±0.4	1.0 ±0.2	1.4 ±0.2	2.4 ±1.1
16:3n-4	- -	- -	0.2 ±0.5	3.1 ±0.7
16:4n-3	- -	- -	- -	0.6 ±0.5
18:2n-6	3.9 ±1.4	1.0 ±0.3	3.5 ±0.8	0.1 ±0.1
18:2n-4	- -	1.3 ±0.9	- -	- -
18:3n-6	1.3 ±0.0	0.7 ±0.2	0.6 ±0.1	0.2 ±0.3
18:3n-4	- -	- -	- -	0.1 ±0.2
18:3n-3	6.0 ±1.5	0.8 ±0.1	3.0 ±1.0	0.2 ±0.3
18:4n-3	21.7 ±3.9	3.7 ±0.7	5.6 ±1.7	2.6 ±2.1
20:2n-6	- -	0.5 ±0.1	- -	- -
20:4n-6	- -	0.6 ±0.1	5.9 ±2.9	0.4 ±0.6
20:3n-3	- -	0.3 ±0.0	- -	- -
20:4n-3	- -	0.2 ±0.0	- -	- -
20:5n-3	0.5 ±0.1	16.6 ±2.8	16.7 ±4.0	16.9 ±2.6
22:5n-6	1.6 ±0.3	1.3 ±0.3	9.9 ±2.1	0.1 ±0.3
22:5n-3	0.3 ±0.0	0.5 ±0.2		0.5 ±0.6
22:6n-3	11.5 ±1.6	10.2 ±1.0	5.2 ±3.1	2.2 ±0.8
Total PUFA	45.0 ±4.6	37.8 ±4.2	52.1 ±5.8	30.1 ±3.5
Unidentified	4.9 ±1.3	3.6 ±2.5	0.4 ±0.3	4.9 ±2.8
Branched	1.3 ±0.7	1.7 ±0.9	0.2 ±0.3	6.1 ±2.9
Total fatty acids	2.7 ±0.6	2.8 ±0.5	2.8 ±0.7	2.8 ±0.9
C_{20}- C_{22}PUFA	13.4 ±1.6	29.4 ±3.4	37.7 ±4.7	20.1 ±3.0
EPA/DHA	0.0 ±0.0	1.6 ±0.2	4.0 ±1.9	8.4 ±3.3
sum (n-7)	6.0 ±1.6	25.4 ±2.3	12.5 ±2.1	25.9 ±0.9
sum (n-6)	5.4 ±1.6	3.8 ±0.4	19.9 ±3.5	0.8 ±1.0
sum (n-3)	39.5 ±4.71	31.7 ±4.5	30.5 ±7.6	22.4 ±4.3
(n-6)/(n-3)	0.14 ±0.05	0.12 ±0.02	0.70 ±0.24	0.04 ±0.04
sat/unsat	0.41 ±0.05	0.50 ±0.08	0.47 ±0.08	0.48 ±0.10

3.3.2 Settling and cell size

No significant differences in settling rate among algal species were found (p=0.57). Very little settling of cells occurred in lightly aerated tanks over 24 hours. Cell diameter for all species ranged from 3-6 μm.

3.3.3 Larval growth and mortality

Larval growth was significantly different between treatments (Table 3.1, Figure 3.1; p=0.0005). Treatments ranked in order of larval growth performance as determined by Tukey's multiple comparison test were:

Pavlova sp. > *Isochrysis* sp. = *P. lutheri* > *T. pseudonana*

Growth rates ranged from 0.89 μm/day for *T. pseudonana* to 1.90 μm/day for *Pavlova* sp. Cumulative mortality was variable and relatively low in all treatments with no significant differences found (Table 3.1).

3.3.4 Larval biochemical composition

3.3.4.1 Total lipid and lipid class composition

Lipid levels were high in eggs (7283 pg/egg), but fell to 3422 pg/larvae in day 2 veligers (Table 3.5). Total lipid increased, as the larvae grew, to 5383 pg/larvae in day 9 veligers. At the end of the feeding trial (day 18) lipid ranged from 5460 pg/larvae in the *T. pseudonana* treatment to 8249 in larvae fed *Isochrysis* sp. (Table 3.5). Despite this range, within-treatment variability was high and no significant differences were observed (p= 0.1530).

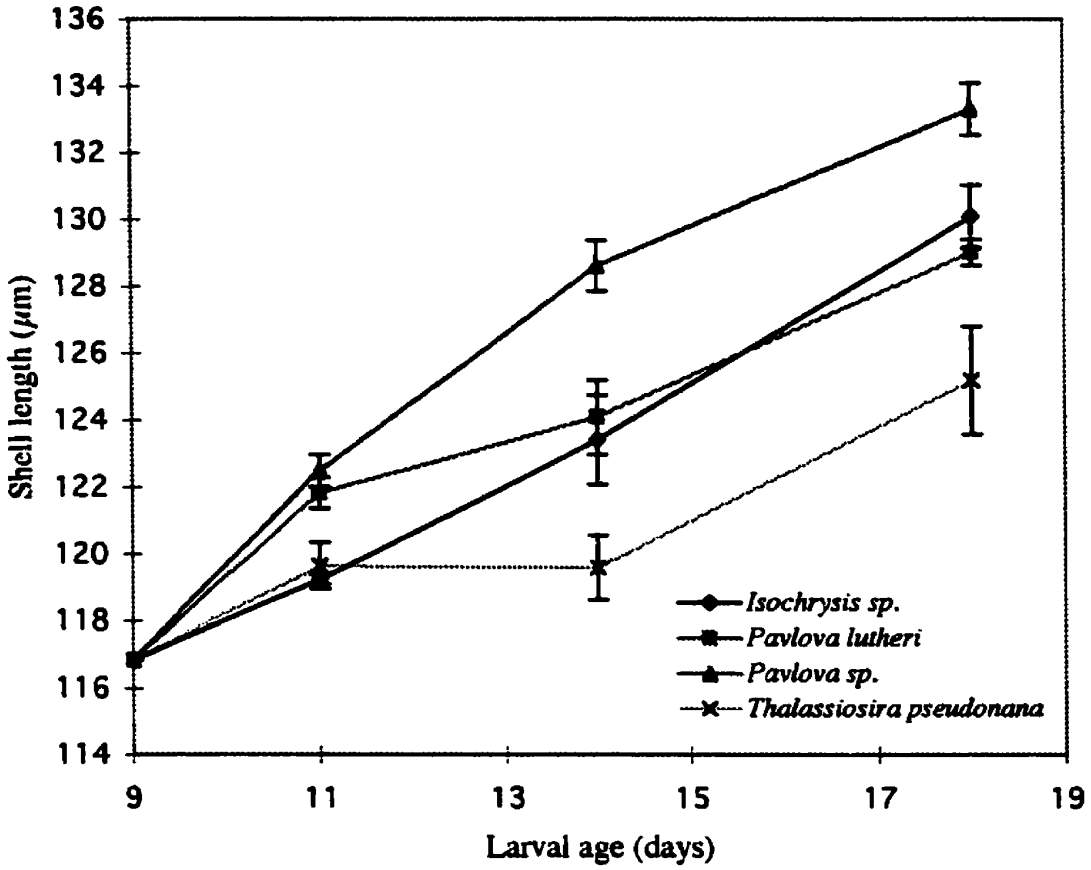


Figure 3.1. Larval growth, over the treatment period, in response to different algal diets.

Error bars are ± standard error.

Table 3.5. Total lipid and lipid class composition in *Placopecten magellanicus* eggs, day 2, and day 9 larvae prior to the start of the feeding trial with four algal species and of day 18 larvae at the end of the feeding trial. Data expressed as: pg/larvae (top) and as a relative proportion of total lipid (bottom) (mean±standard deviation, n=3).

Diet				*Isochrysis sp.*	*Pavlova lutheri*	*Pavlova sp.*	*Thalassiosira pseudonana*
Day	Egg	2	9	18	18	18	18
Total Lipids *(pg/larvae)*	7283 ±387	3422 ±431	5383 ±169	8249 ±1628	7109 ±3025	7382 ±2699	5460 ±762
HC	86 ±15	183 ±172	325 ±177	549 ±181	581 ±386	414 ±194	544 ±349
SE/WE	109 ±31	58 ±40	- -	- -	- -	- -	- -
ME	126 ±16	65 ±46	- -	- -	- -	108 ±58	7 ±11
M. KET	- -	- -	79 ±93	120 ±58	109 ±127	349 ±346	273 ±236
TAG	3026 ±174	1718 ±181	1422 ±169	958 ±423	369 ±295	768 ±491	273 ±236
FFA	121 ±33	34 ±51	33 ±29	590 ±461	137 ±50	86 ±30	0 ±0
ALC	128 ±27	151 ±29	118 ±50	308 ±80	362 ±314	149 ±62	229 ±152
ST	210 ±22	13 ±20	284 ±125	415 ±158	601 ±189	405 ±113	514 ±159
D.G.	137 ±3	0 ±0	0 ±0	723 ±243	275 ±158	397 ±163	210 ±75
AMPL	672 ±29	207 ±34	999 ±160	1316 ±319	1192 ±656	1245 ±757	981 ±132
PL	2113 ±60	994 ±153	2122 ±348	3269 ±394	3483 ±1921	3459 ±997	2633 ±601
(%)							
HC	1.2 ±0.9	5.2 ±4.5	6.1 ±3.4	6.5 ±1.0	7.5 ±2.2	5.6 ±1.1	10.4 ±6.8
SE/WE	1.6 ±0.4	1.9 ±1.3	- -	- -	- -	- -	- -
ME	1.5 ±0.6	1.9 ±1.3	- -	- -	- -	1.7 ±1.1	0.1 ±0.2
M. KET	- -	- -	1.4 ±1.7	1.6 ±1.1	1.4 ±1.8	4.1 ±2.8	1.1 ±1.9
TAG	53.6 ±4.6	50.4 ±3.4	26.4 ±3.0	11.2 ±3.2	5.0 ±3.9	9.6 ±3.7	4.7 ±4.1
FFA	1.4 ±1.1	0.9 ±1.4	0.6 ±0.5	6.7 ±5.0	2.0 ±0.1	1.3 ±0.6	0.0 ±0.0
ALC	3.6 ±0.6	4.5 ±1.1	2.2 ±0.9	3.8 ±0.8	5.4 ±4.2	2.3 ±1.1	4.0 ±2.1
ST	2.9 ±0.8	0.3 ±0.5	5.2 ±2.3	4.9 ±1.0	9.0 ±2.2	5.7 ±0.9	9.3 ±1.8
D.G.	3.4 ±0.0	0.0 ±0.0	0.0 ±0.0	8.9 ±3.0	5.0 ±4.1	6.0 ±3.0	3.8 ±1.1
AMPL	9.2 ±1.5	6.1 ±1.0	18.6 ±4.2	16.1 ±3.2	15.9 ±4.5	15.9 ±4.2	18.4 ±4.4
PL	29.0 ±4.6	29.0 ±1.4	39.4 ±6.1	40.3 ±5.6	48.8 ±11.8	47.8 ±4.0	48.2 ±8.5

Table 3.6. Total lipids: relative composition (wt % of total FA) of major fatty acids in *Placopecten magellanicus* eggs, day 2, and day 9 larvae prior to the start of the feeding trial with four algal species and in day 18 larvae at the end of the trial (mean %, ± standard deviation; n=3).

Diet				*Isochrysis sp.*	*Pavlova lutheri*	*Pavlova sp.*	*Thalassiosira pseudonana*
Days	Egg	2	9	18	18	18	18
	%	%	%	%	%	%	%
14:0	2.2	2.2	5.0	6.4	5.3	5.8	6.0
16:0	18.8	18.5	28.5	17.7	14.6	16.1	17.4
18:0	3.5	4.3	13.3	6.7	6.8	5.8	9.7
Total SAT	24.8	26.7	49.3	34.4	31.6 *a*	31.8 *a*	39.0 *b*
16:1n-7	7.5	6.5	3.0	3.5	4.4	4.1	3.4
18:1n-9	3.8	3.3	3.4	12.5 *a*	3.6 *b*	2.7 *b*	6.9 *c*
18:1n-7	9.4	9.3	2.3	.	5.1 *a*	3.9 *b*	4.1 *b*
20:1n-9+11	3.0	3.3	2.2	3.2	5.0	3.1	3.7
Total MONO	23.9	22.3	13.0	24.0 *a*	19.3 *b*	14.6 *c*	19.0 *b*
18:2n-6	1.4	1.2	1.5	3.6 *a*	1.3 *b*	2.9 *c*	2.3 *c*
18:3n-3	0.9	0.9	1.3	3.0 *a*	1.2 *b*	1.7 *c*	1.2 *b*
18:4n-3	4.5	3.6	2.6	6.1 *a*	2.8 *b*	2.2 *b*	2.0 *b*
20:2n-6	0.7	-	2.0	0.8	0.3	0.9	0.2
20:4n-6	1.1	1.2	3.7	1.4 *a*	2.7 *b*	6.8 *c*	2.6 *b*
20:5n-3	29.6	24.4	4.0	2.5 *a*	6.8 *b*	8.3 *c*	6.4 *b*
22:5n-6	tr	1.2	1.4	2.7 *a*	2.7 *a*	11.7 *b*	1.7 *a*
22:6n-3	9.1	9.0	7.4	15.4 *a*	16.7 *a*	10.3 *b*	12.8 *b*
Total PUFA	49.0	45.1	29.3	36.9 *a*	37.1 *a*	46.1 *b*	30.9 *c*
Total FA *(pg/larva)*	6088	2639	3243	4984	3642	3657	2651
Est. Dry wt.*(ng/larva)*				269.7	270.0	287.9	250.0
Total FA *(mg/g dw)*				18.5	13.5	12.7	10.6
C_{20}- C_{22} PUFA	42.3	38.0	19.3	22.0 *a*	28.9 *b*	37.1 *c*	23.5 *a*
EPA/DHA	3.3	2.7	0.5	6.2 *a*	2.5 *b*	1.2 *c*	2.0 *b*
sum (n-7)	17.0	15.8	5.5	8.3 *a*	10.6 *b*	8.8 *a*	8.4 *a*
sum (n-6)	2.7	4.7	8.6	8.5 *a*	7.1 *a*	22.3 *b*	6.7 *a*
sum (n-3)	45.6	40.3	15.8	27.9 *a*	28.4 *a*	23.3 *b*	23.7 *b*
(n-6)/(n-3)	0.1	0.1	0.5	3.3 *a*	4.0 *a*	1.0 *b*	3.6 *a*
sat/unsat	0.3	0.4	1.2	0.6 *a*	0.6 *a*	0.5 *a*	0.8 *b*

* different letters indicate fatty acid differences among day 18 larvae at $p<0.05$.

TAG, AMPL and PL were the primary lipid classes of larvae and eggs, but HC, ALC, Sterol, and DG were also present in significant quantities (2.0% – 9.3%), especially in older larvae. FFA, KET and HC represented from 1% - 10% of total lipid (Table 3.5). Mean TAG content in day 18 larvae ranged from 273 pg/larvae in *T. pseudonana* to 957.7 pg/larvae in *Isochrysis* sp. However, variability within the treatments was high, and no differences were observed among treatments (p=0.0828).

3.3.4.2 Fatty acid composition - changes with development

The fatty acid composition of D-stage day 2 veligers strongly resembled that of eggs but the larvae contained less than half the amount of total fatty acids of eggs (Table 3.6). EPA and DHA were consistently the prominent PUFA, 16:0 was the main SAT, and the MONO FA 16:1n-7, 18:1n-9, 18:1n-7, and 20:1n-9 appeared in varying amounts (Table 3.6). The DHA level in the neutral lipids of Day 2 veligers was lower than in the eggs (3.9% vs. 9.5%), and total PUFA in the neutral fraction fell from 45.9% in the eggs to 33.4% in the larvae (Table 3.7).

Day 9 veligers showed a large reduction in the level of EPA (4.0% compared with 24.4% in day 2 veligers) and a proportional increase in saturated fatty acids (mainly 16:0 and 18:0). The PUFA level was higher in the day 18 larvae, ranging from 30.9% – 37.1% compared with 24.1% in the day 2 larvae (Table 3.6).

3.3.4.3 Effect of diet on fatty acid composition

Significant differences among treatments were found for most major fatty acids in the larvae, largely reflecting the composition of the diet, especially for unsaturated fatty acids with chain lengths longer than C_{18}. The proportions of 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6, and 22:5n-6 in the larvae corresponded with amounts in the algal treatments (Figure 3.2). Fatty acids in neutral lipids were affected by diet to a greater extent than polar lipids, with more significant differences found in larval fatty acid composition (Tables 3.7, 3.8). Amounts of 18:4n-3, 18:2n-6, EPA, and DHA of neutral lipids possibly corresponded with diet, but only to a limited extent in polar lipids (Figure 3.2).

Figure 3.3 shows trends in fatty acid groupings (a) and ratios (b) in neutral (Figure 3.3b) and polar lipids (Figure 3.3c) of the larvae with respect to diet (Figure 3.3.1). Significant differences were found among the treatments in these groupings (Tables 3.6-3.8). The (n-6)/(n-3) and EPA/DHA ratio in neutral lipids of the larvae corresponded with levels in the diet, but the (n-6), (n-3), and (n-7) series fatty acids were conserved to some extent regardless of diet, especially in larval polar lipids. However, the high (n-6) series content of *Pavlova* sp. was transferred, even to the polar lipids of larvae (Figures 3.3a and 3.3b).

3.3.4.4 Fatty acids in neutral versus polar lipids

Polar lipids also displayed a consistently higher proportion of PUFA (31.5% to 37.8% in neutral and 40.7% to 57.3% in polar lipids; Tables 3.7 and 3.8) and lower

Table 3.7. Neutral lipids: relative composition (wt % of total FA) of major fatty acids in *Placopecten magellanicus* eggs, day 2, and day 9 larvae prior to start of feeding trial with four algal species and in day 18 larvae at the end of the trial (mean % ± standard deviation; n=3).*

Diet				*Isochrysis sp.*	*Pavlova lutheri*	*Pavlova sp.*	*Thalassiosira pseudonana*
Days	Egg	2	9	18	18	18	18
	%	%	%	%	%	%	%
14:0	2.5	4.2	13.2	10.2 *a*	6.9 *b*	11.2 *a*	17.5 *c*
16:0	19.9	22.6	23.6	13.3 *a*	12.5 *a*	18.0 *b*	14.1 *a*
18:0	4.1	2.0	12.1	1.9	2.9	1.9	2.0
Total SAT	27.3	30.5	50.9	26.1	23.9 *a*	32.7 *b*	34.1 *b*
16:1n-7	6.8	11.2	6.2	5.5 *a*	8.4 *a*	14.2 *b*	17.1 *b*
16:1n-5	0.3	1.3	8.7	1.4 *a*	4.3 *b*	8.2 *c*	3.0 *b*
18:1n-9	3.7	3.6	1.7	13.2 *a*	3.5 *b*	1.7 *c*	0.8 *c*
18:1n-7	9.6	9.8	3.5	3.1 *a*	3.7 *a*	3.8 *a*	6.3 *b*
20:1n-9	1.4	0.9	2.9	1.3 *a*	3.0 *b*	1.7 *a*	tr
20:1n-7	0.9	0.4	0.6	0.5 *a*	3.8 *b*	0.1 *a*	tr
Total MONO	23.6	25.9	23.6	25.0 *a*	26.8 *a*	29.7 *b*	27.2
16:3n-4	0.3	-	-	2.0 *a*	7.6 *b*	2.2 *a*	8.8 *b*
18:2n-6	1.7	1.2	1.3	5.3 *a*	0.8 *b*	3.5 *c*	1.3 *b*
18:3n-6	0.3	0.7	0.1	1.6	2.5	1.1	1.1
18:3n-3	1.6	0.9	1.2	3.8 *a*	0.9 *b*	1.8 *c*	0.5 *b*
18:4n-3	6.1	3.9	4.7	10.3 *a*	7.5 *b*	3.1 *c*	2.1 *c*
20:2n-6	0.8	0.5	0.6	0.6	0.5	1.6	0.1
20:3n-6	0.3	1.0	-	1.7 *a*	3.4 *b*	2.0 *a*	0.4 *c*
20:4n-6	1.0	0.5	0.7	0.4 *a*	0.4 *a*	3.2 *b*	0.5 *a*
20:5n-3	22.7	18.5	3.4	1.2 *a*	3.5 *b*	6.6 *c*	14.1 *d*
22:5n-6	0.3	0.1	2.2	1.4 *a*	0.5 *b*	4.0 *c*	0.2 *b*
22:6n-3	9.5	3.9	3.5	8.6 *a*	2.6 *b*	2.5 *b*	1.5 *c*
Total PUFA	45.9	33.4	19.0	37.8 *a*	33.7 *b*	32.0 *b*	31.5 *b*
Total FA *(pg/larva)*	4330.6	1855.8	1463.6	2271.8	777.0	1236.6	481.5
C_{20}- C_{22} PUFA	34.4	26.2	10.4	14.7 *a*	14.0 *a*	20.3 *b*	17.7 *c*
EPA/DHA	3.0	4.7	1.0	0.1 *a*	1.3 *b*	2.6 *c*	9.4 *d*
sum (n-7)	17.2	21.4	12.7	9.2 *a*	15.9 *b*	18.2 *c*	23.4 *d*
sum (n-6)	5.1	5.3	4.8	11.0 *a*	8.0 *b*	15.2 *c*	3.6 *d*
sum (n-3)	39.9	27.6	12.8	14.5 *a*	9.6 *b*	11.5 *b*	17.0 *a*
(n-6)/(n-3)	0.1	0.2	0.4	0.8 *a*	0.8 *a*	1.3 *b*	0.2 *c*
sat/unsat	0.4	0.5	1.2	0.4 *a*	0.4 *a*	0.5 *b*	0.6 *b*

* different letters indicate fatty acid differences among day 18 larvae at $p<0.05$

Table 3.8. Polar lipids: relative composition (wt % of total FA) amounts of selected fatty acids in *Placopecten magellanicus* eggs, day 2, and 9 larvae prior to start of feeding trial with four algal species and of day 18 larvae at the end of the trial (mean ± standard deviation; n=3).*

Diet				*Isochrysis sp.*	*Pavlova lutheri*	*Pavlova sp.*	*Thalassiosira pseudonana***
Days	Egg	2	9	18	18	18	18
	%	%	%	%	%	%	%
14:0	0.4	1.1	5.9	3.0	4.7	2.9	4.1
16:0	7.0	7.4	11.5	19.6 *a*	14.4 *b*	14.2 *b*	18.1 *a*
18:0	7.1	8.0	5.5	9.8 *a*	7.4 *b*	7.2 *b*	11.3 *a*
20:0	tr	1.0	5.0	-	-	-	-
Total SAT	15.2	17.6	30.4	34.9 *a*	28.5 *b*	25.2 *b*	36.3 *a*
16:1n-7	2.2	1.1	4.9	1.7 *a*	3.2 *b*	0.9	1.3 *a*
16:1n-5	1.0	1.1	6.0	-	-	-	-
18:1n-9	3.9	2.1	8.1	10.9 *a*	3.5 *b*	3.0 *b*	8.1 *a*
18:1n-7	2.9	6.7	2.4	5.0 *a*	5.3 *a*	3.6 *b*	3.5 *b*
20:1n-11	-	-	0.2	2.2	2.1	2.3	3.2
20:1n-9	5.1	7.3	0.2	4.5	5.3	3.6	4.5
Total MONO	17.0	17.2	21.8	24.4 *a*	19.7 *b*	13.7 *c*	21.8 *b*
18:2n-6	3.8	1.1	7.1	1.9	1.4	2.4	2.5
18:3n-3	1.1	0.8	1.4	2.1	1.2	1.6	1.3
18:4n-3	1.8	2.4	4.0	2.4	1.5	1.6	2.0
20:2n-6	0.4	1.1	1.6	0.9	0.3	0.6	0.2
20:4n-6	3.0	2.4	1.4	2.1 *a*	3.2 *a*	8.1 *b*	3.0 *a*
20:5n-3	28.5	31.8	7.2	3.3 *a*	7.3 *b*	8.5 *b*	6.9 *b*
22:NMID?	0.7	-	5.5	0.7	2.5	1.8	1.5
22:5n-6	0.7	3.2	2.2	3.4 *a*	3.2 *a*	14.6 *b*	2.0 *a*
22:6n-3	16.3	17.3	6.2	19.3 *a*	19.6 *a*	13.3 *b*	14.7 *b*
Total PUFA	58.3	64.1	41.7	38.6	44.0	54.9	38.5
Total FA *(pg/larv)*	1757.9	783.3	1779.2	2707.3	2865.2	2420.2	2174.6
C_{20}- C_{22} PUFA	50.2	57.6	28.3	31.7 *a*	38.8 *b*	48.2 *c*	31.3 *a*
EPA/DHA	1.6	1.8	1.2	0.2 *a*	0.4 *b*	0.6 *c*	0.5 *d*
sum (n-7)	4.9	7.8	8.4	6.9 *a*	8.8 *a*	4.7 *b*	5.9 *b*
sum (n-6)	8.6	10.7	12.3	9.9 *a*	9.1 *a*	26.8 *b*	9.2 *a*
sum (n-3)	51.9	52.5	23.4	29.5 *a*	31.8 *a*	25.1 *b*	26.8 *b*
(n-6)/(n-3)	0.17	0.20	0.53	0.34 *a*	0.29 *a*	1.07 *b*	0.34 *a*
sat/unsat	0.20	0.22	0.48	0.54 *a*	0.43 *b*	0.35 *b*	0.58 *a*

* different letters indicate fatty acid differences among day 18 larvae at $p<0.05$.
** n=2, one sample lost

proportions of MONO (13.7% to 24.5% versus 25.0% to 29.7%, respectively). The (n-3) series fatty acids were also higher in polar lipids (25.0% – 31.7%) than in neutral lipids (9.6% – 17.0%; Tables 3.7, 3.8). A broader array of fatty acids - 18:1n-9, 18:1n-7, 20:1n-11, and 20:1n-9 dominated larval polar lipid monounsaturates despite high levels of 16:1n-7 in the diets. The fatty acid 14:0 was found in lower amounts in the polar lipids

proportions of MONO (13.7% to 24.5% versus 25.0% to 29.7%, respectively). The (n-3) series fatty acids were also higher in polar lipids (25.0% – 31.7%) than in neutral lipids (9.6% – 17.0%; Tables 3.7, 3.8). A broader array of fatty acids - 18:1n-9, 18:1n-7, 20:1n-11, and 20:1n-9 dominated larval polar lipid monounsaturates despite high levels of 16:1n-7 in the diets. The fatty acid 14:0 was found in lower amounts in the polar lipids and was unrelated to dietary levels (Figure 3.2). Significantly higher proportions of 18:0 were found in polar lipid fatty acids but occurred in only trace levels (<1%) in the diet.

Figure 3.4 compares the levels of specific PUFA (essential fatty acids EPA, DHA, ARA, in addition to 22:5n-6) between the neutral and polar lipids of algae and larvae. Long chain C_{20}-C_{22} PUFA were selectively accumulated over shorter chain PUFA in the polar lipids, especially in larvae fed *Isochrysis* sp.. Conservation of DHA in the polar lipids was observed despite low levels in the *T. pseudonana* treatment, although total levels of fatty acids per larva (Table 3.8) were lower in this treatment so that DHA expressed as pg/larvae was less than in other treatments. The relative level of DHA was slightly lower in the *Pavlova* sp. treatment, but there was a notable accumulation of another C_{22} PUFA, 22:5n-6, which was present at high levels in the diet. Selective

Figure 3.2. Proportions of selected fatty acids of the diet treatments (a) compared to neutral lipids (b) and polar lipids (c) of day 18 *Placopecten magellanicus* larvae. Error bars are + standard deviations.

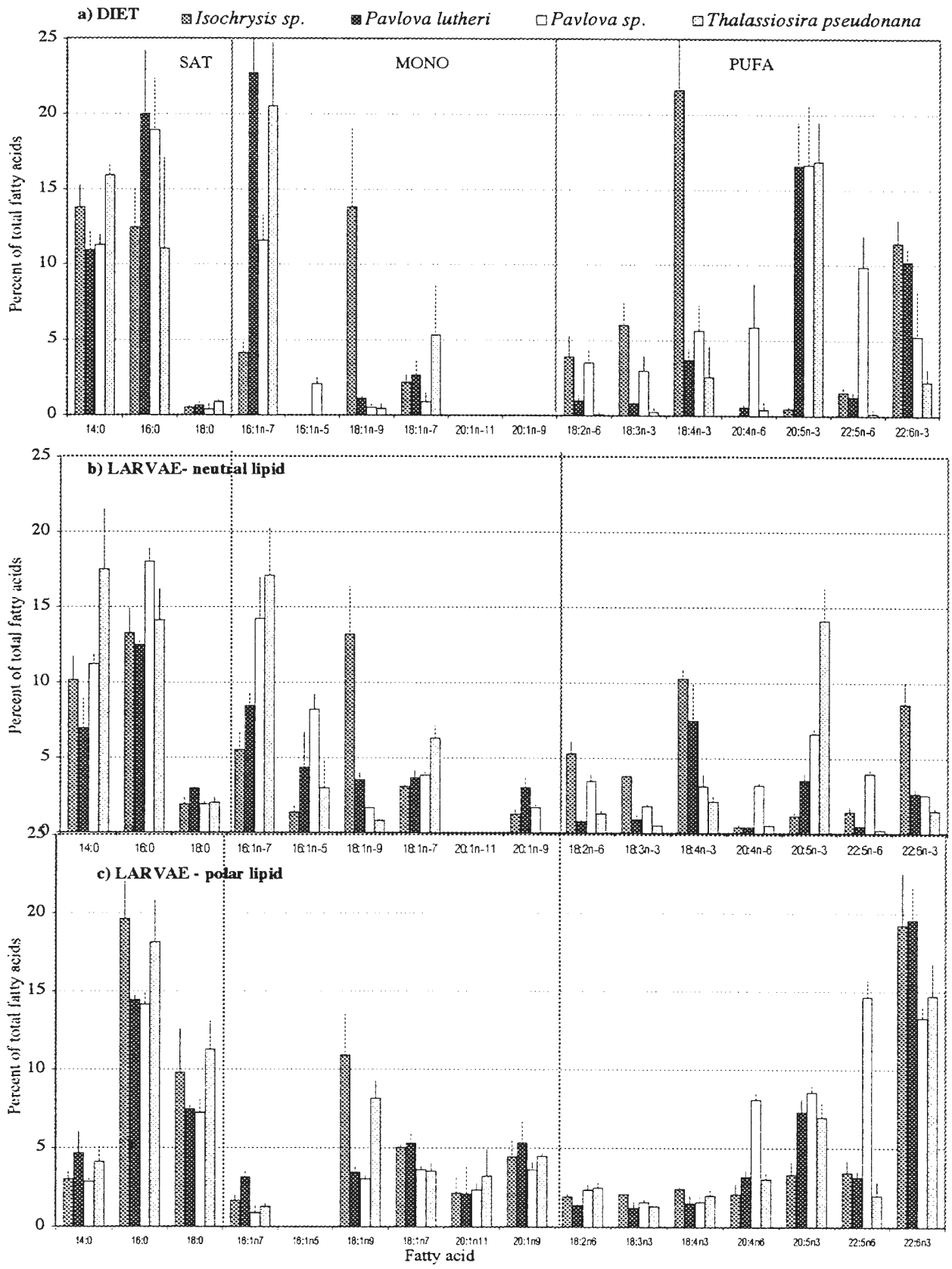

a) DIET
Isochrysis sp.
Pavlova lutheri
Pavlova sp.
Thalassiosira pseudonana
SAT
MONO
PUFA
Percent of total fatty acids
14:0
16:0
18:0
16:1n-7
16:1n-5
18:1n-9
18:1n-7
20:1n-11
20:1n-9
18:2n-6
18:3n-3
18:4n-3
20:4n-6
20:5n-3
22:5n-6
22:6n-3
b) LARVAE- neutral lipid
c) LARVAE - polar lipid
Fatty acid

Figure 3.3. Proportions of important fatty acid groupings and ratios of the diet treatments (a) compared to neutral lipids (b) and polar lipids (c) of day 18 *Placopecten magellanicus* larvae. Error bars are +standard deviations.

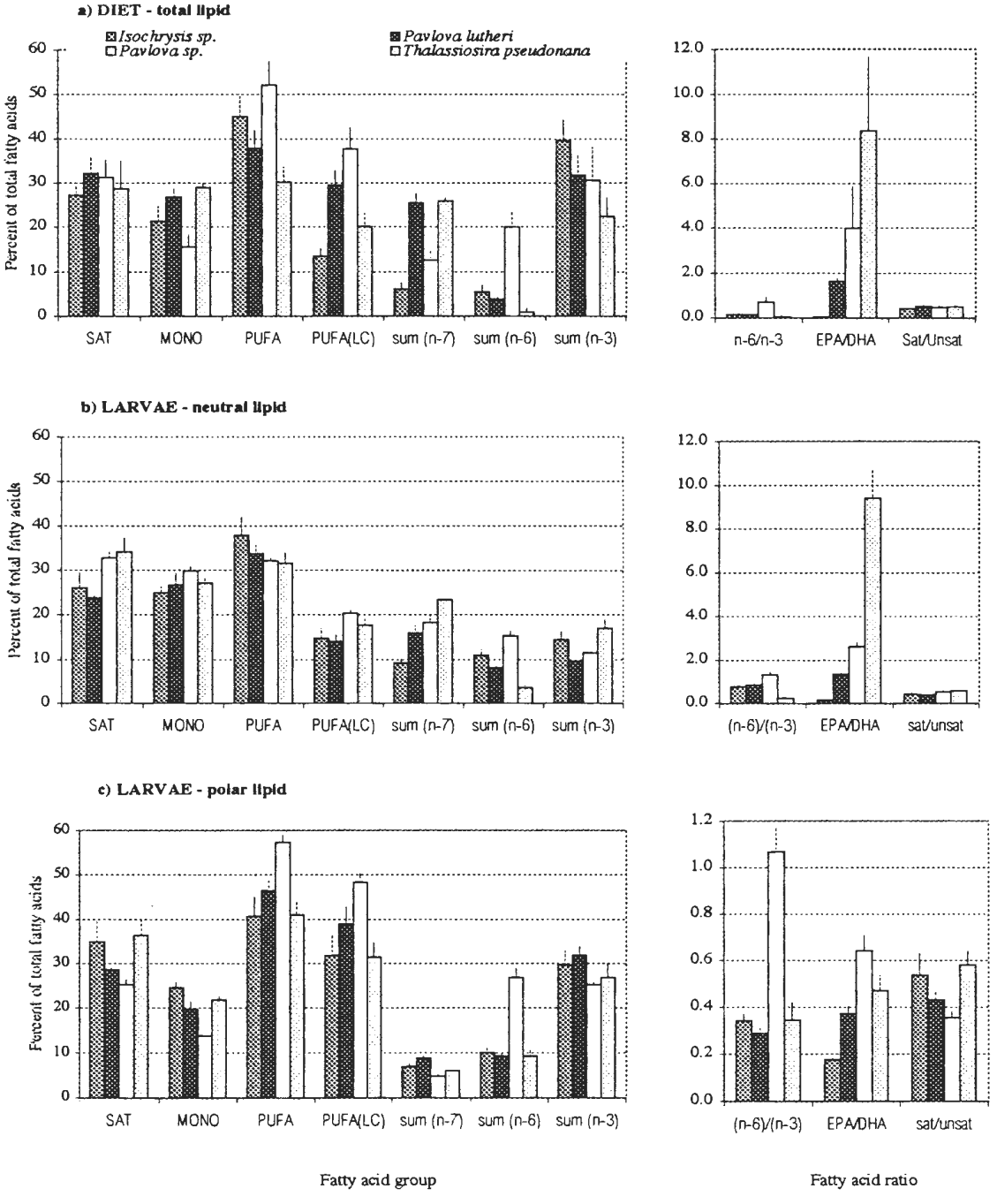
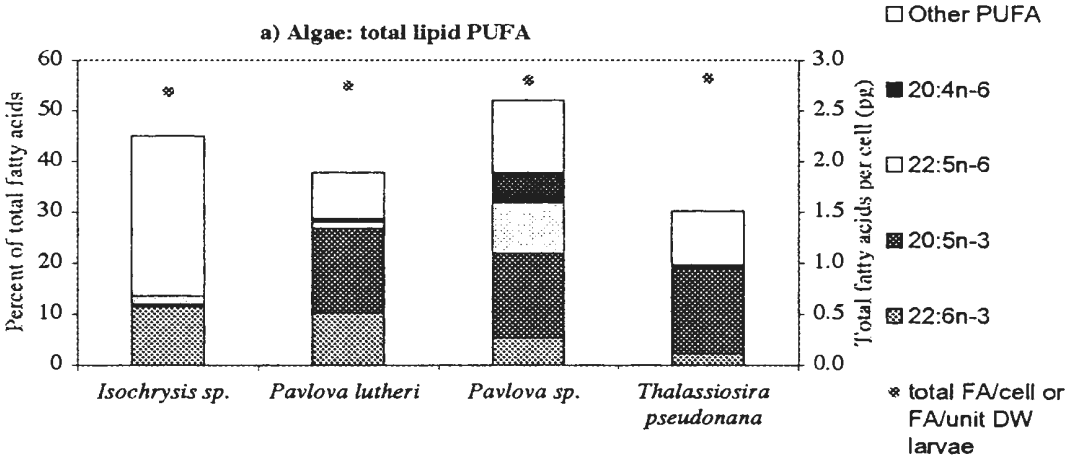
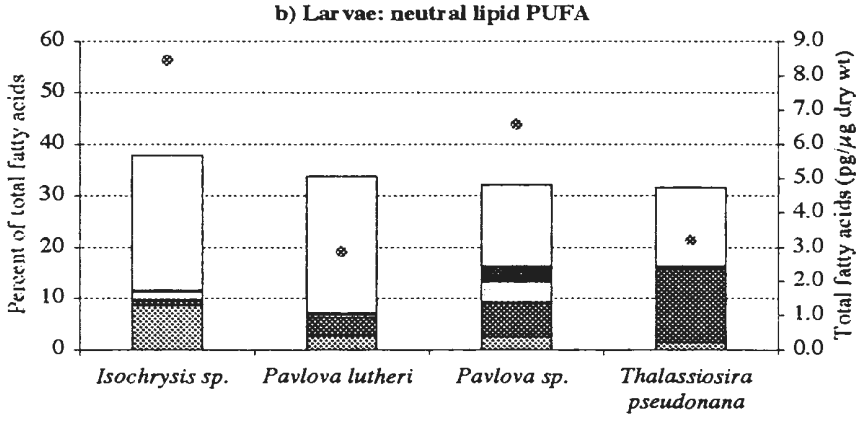
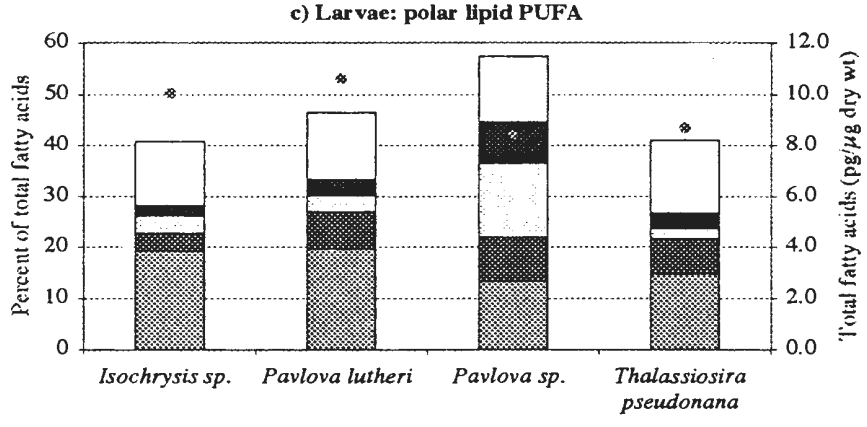

a) DIET - total lipid
Isochrysis sp.
Pavlova sp.
Pavlova lutheri
Thalassiosira pseudonana
Percent of total fatty acids
SAT
MONO
PUFA
PUFA(LC)
sum (n-7)
sum (n-6)
sum (n-3)
n-6/n-3
EPA/DHA
Sat/Unsat
b) LARVAE - neutral lipid
(n-6)/(n-3)
sat/unsat
c) LARVAE - polar lipid
Fatty acid group
Fatty acid ratio

Figure 3.4. Proportions of essential fatty acids and total fatty acid concentration of the diet treatments (a) compared to neutral lipids (b) and polar lipids (c) of day 18 *P. magellanicus* larvae.


a) Algae: total lipid PUFA
Other PUFA
20:4n-6
22:5n-6
20:5n-3
22:6n-3
Percent of total fatty acids
Total fatty acids per cell (pg)
60
50
40
30
20
10
0
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Isochrysis sp.
Pavlova lutheri
Pavlova sp.
Thalassiosira pseudonana
* total FA/cell or FA/unit DW larvae


b) Larvae: neutral lipid PUFA
Percent of total fatty acids
Total fatty acids (pg/μg dry wt)
60
50
40
30
20
10
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Isochrysis sp.
Pavlova lutheri
Pavlova sp.
Thalassiosira pseudonana


c) Larvae: polar lipid PUFA
Percent of total fatty acids
Total fatty acids (pg/μg dry wt)
60
50
40
30
20
10
0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Isochrysis sp.
Pavlova lutheri
Pavlova sp.
Thalassiosira pseudonana

Algal diets

accumulation of EPA was evident in the polar lipids of the low EPA diet (*Isochrysis* sp.), but occurred at lower relative amounts than that provided in the diet for the high EPA treatments (*P. lutheri*, *Pavlova* sp, and *T. pseudonana*). Arachidonic acid was also selectively accumulated in polar lipids. Unlike for EPA, the high levels of ARA found in *Pavlova* sp. were maintained in the polar lipids of the larvae. The total amount of shorter chain PUFA in larval polar lipids was constrained to a small range despite varied amounts in the diets.

3.3.5 Fatty acids associated with growth performance

Growth was lowest in the low DHA/high EPA treatment (*T. pseudonana*), but differences were not significant between the high DHA/ high EPA treatment (*Pavlova lutheri*) and the high DHA/ low EPA treatment (*Isochrysis* sp.). This suggests that DHA may be a more important essential fatty acid than EPA at this stage in larval sea scallop development. The faster growth rate of larvae fed *Pavlova* sp. and dietary transfer of the high ARA and 22:5n-6 fatty acid components in the polar lipids of these larvae suggests that one or both of these long chain (n-6) series fatty acids had a positive impact on larval performance.

Two principal component analyses (PCA) were carried out with the data set as an exploratory test for associations of fatty acids in the diet with larval growth. Individual dietary fatty acids (pg/cell) comprising greater than 2% of the total fatty acids and growth rates over the feeding trial were grouped in the first PCA (Figure 3.5). Axes one and two explained 87.5% of the total variation (50.1% and 37.4% for axes 1 and 2, respectively).

Growth was positively correlated along the first and second axes (component loadings - 0.696 and 0.718, respectively). ARA and 22:5n-6 loaded closest to growth. A number of C_{18} PUFA, 18:2n-6, 18:3n-3, and 18:4n-3, together with the monounsaturate 18:1n-9, were associated with growth on axis 1, but not on axis 2. Likewise DHA correlated with growth on axis 1 (the axis explaining most of the variance), but was neither positively or negatively associated with axis 2. EPA negatively correlated with growth on the primary axis but was associated with axis 2 in the same direction as growth. Similarly, 16:0 was a neutral on the primary axis but positively correlated with growth on the secondary axis. The monounsaturates 16:1n-7 and 18:1n-7, in addition to the saturated FA 14:0, appeared to be negatively associated with growth. In summary, (n-6) long chain PUFA were associated most strongly with growth, and DHA and C_{18} PUFA were positively correlated with growth only on the primary axis. EPA showed a mixed trend, being negatively associated with growth along the primary axis and, together with 16:0, positively associated with growth along the secondary axis (Figure 3.5).

In the second PCA, groupings were considered separately from the individual fatty acids in order to minimize autocorrelation. Axis one accounted for 51.4% of the total variation and axis two 38.4% (89.8% total). Growth was weighted strongly on axis one together with PUFA, the sum of (n-6) series fatty acids, the (n-6)/(n-3) ratio, and the sum of (n-3) fatty acids. The EPA:DHA ratio weighted negatively on axis one, as did total monounsaturates and the sum of (n-7) series fatty acids. Total fatty acids, total saturates, and the sat/unsat ratio in the diet appeared unrelated to growth. Total C_{20} and C_{22} PUFA correlated only weakly with growth (Figure 3.6).

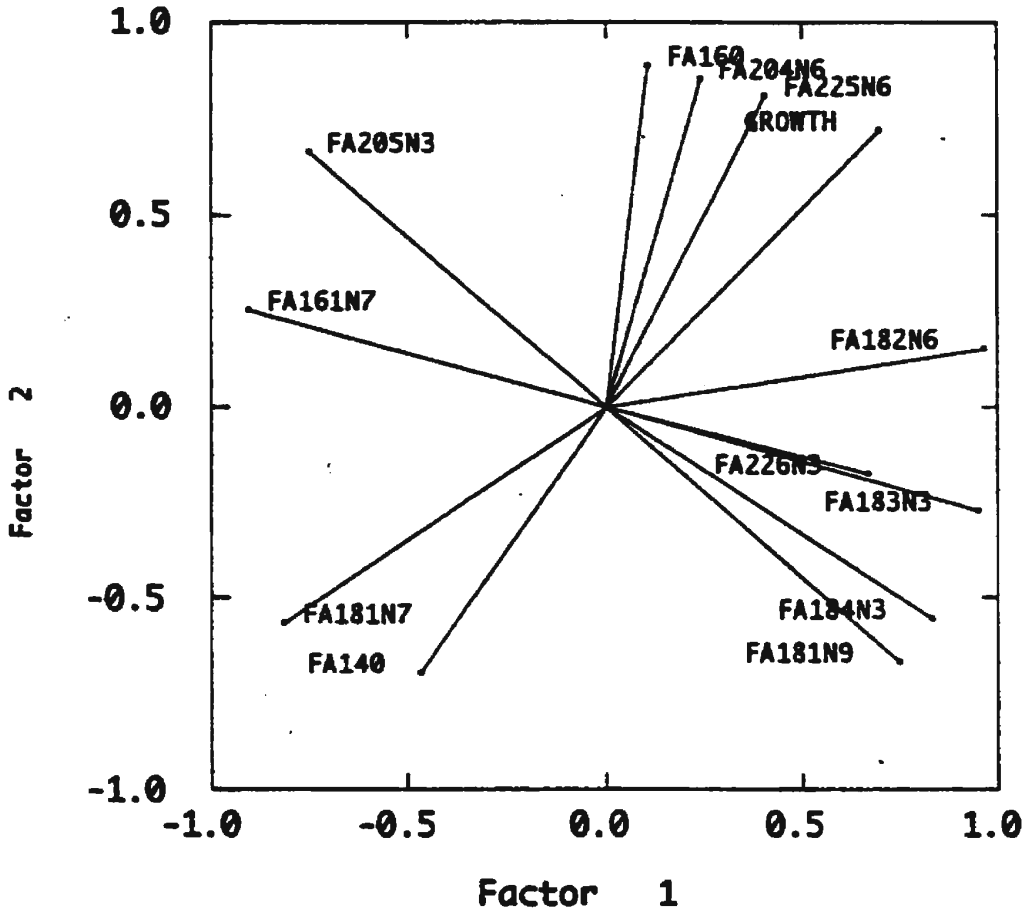


Figure 3.5. PCA results for selected major dietary fatty acids (as FA pg/cell) and larval growth rates. Factor 1 axis explains 50.1% of the total variance and factor 2 axis explains 37.4%.

Legend:
FA161N7 =16:1n-7
FA205N3 = 20:5n-3 (EPA)
FA160 = 16:0
FA204N6 = 20:4n-6 (ARA)
FA225N6 = 22:5n-6
FA225N6 = 22:5n-6
GROWTH = larval growth rates
FA226N3 = 22:6n-3 (DHA)
FA183N3 = 18:3n-3
FA184N3 = 18:4n-3
FA181N9 = 18:1n-9
FA140 = 14:0
FA181N7 = 18:1n-7

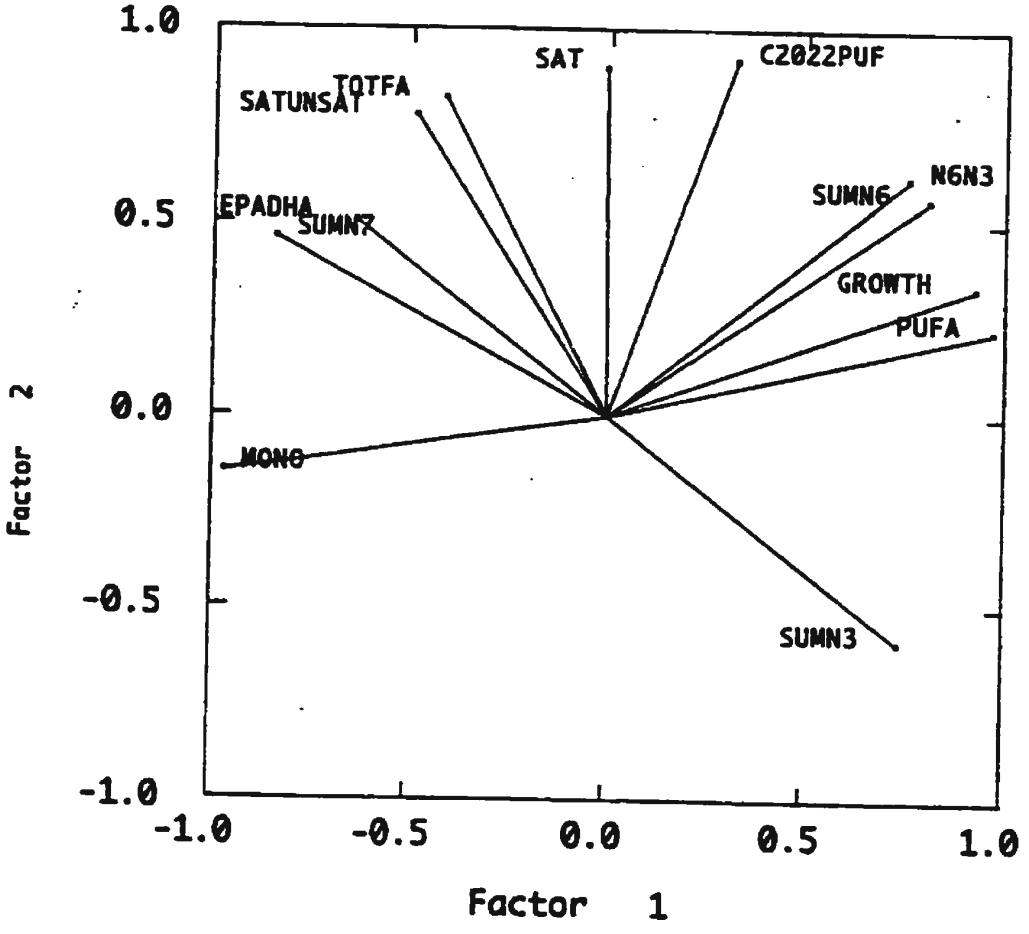


Figure 3.6. PCA results for diet fatty acid groups and larval growth rates. Factor 1 axis explains 51.4% of the total variance and factor 2 axis explains 38.4%.

Legend:
SUMN7 = sum (n-7) fatty acids
EPADHA = EPA/DHA ratio
SATUNSAT = SAT/UNSAT ratio
TOTFA = Total fatty acids
SAT = saturated fatty acids
C2022PUF = C_{20} and C_{22} PUFA
SUMN6 = sum (n-6) fatty acids
N6N3 = (n-6)/(n-3) fatty acid ratio
GROWTH = larval growth rates
PUFA = Polyunsaturates
SUMN3 = sum (n-3) fatty acids

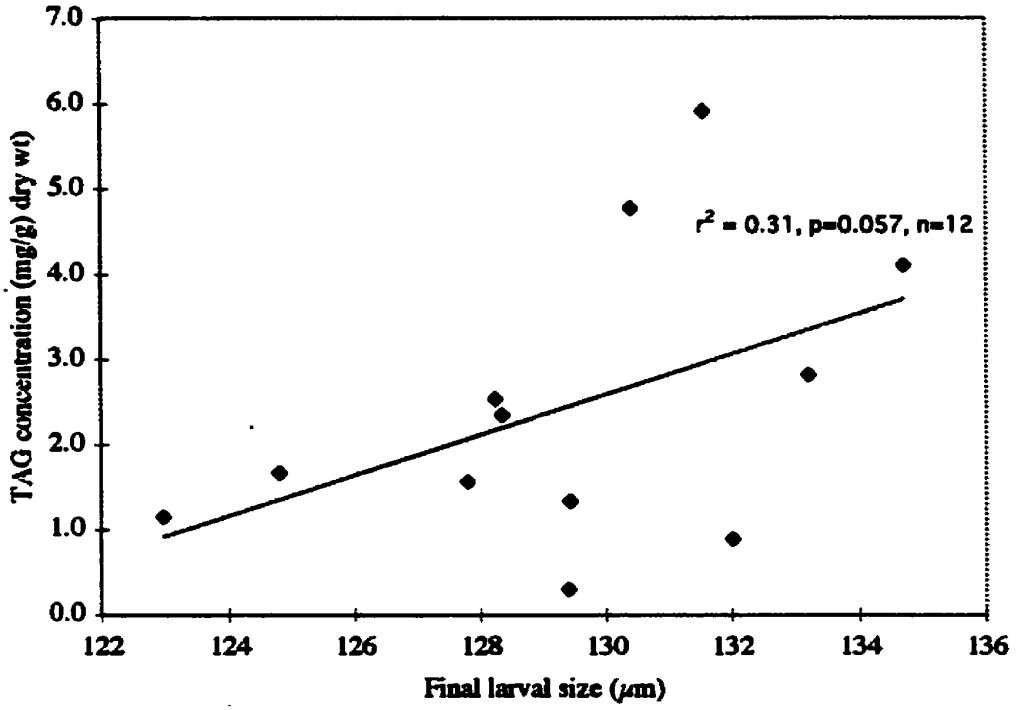


Figure 3.7. Relationship between final TAG concentration and final larval size (day 18 *Placopecten magellanicus* larvae).

TAG in the larvae was a poor predictor of larval growth despite a weak positive trend, larger larvae generally containing greater amounts of TAG per unit estimated dry weight (Figure 3.7; $p=0.057$, $R^2=0.31$, $n=12$).

3.4 Discussion

3.4.1 Algal diets

Algal fatty acid profiles displayed species-specific characteristics consistent with those reported in the literature (Volkman et al., 1989; Viso and Marty, 1993). Some variability in algal fatty acid composition was evident in batch cultures, despite efforts to harvest in a consistent growth phase. Turbidostat or chemostat culture would have provided more precise control over the biochemistry of the algae. Nonetheless, differences in essential fatty acid content were maintained and were useful as an experimental tool. The similarity between total fatty acids per cell and gross composition of the four treatments suggests that growth responses were largely due to dietary fatty acid differences rather than other nutritional factors, although protein and carbohydrate were not directly measured in this study.

Absolute measures of fatty acids in the diet (pg/cell) were used in the PCA analyses, but an examination of relative fatty acid composition of algae is sufficient due to similar levels of total fatty acids per cell across treatments. Feeding on an equivalent cell basis was justified due to the similarity in gross composition, organic weight, cell size, and total fatty acid content of the algal species selected. Furthermore, this protocol is closer to that encountered in commercial hatcheries, where cell density rather than

equivalent dry weights or packed cell volumes is more often used as a basis for determining ration, due to ease of calculation. Other authors (*e.g.*, Parsons et al., 1961; Enright et al.,1986a) have also found little interspecific difference in gross composition in algae grown under similar culture conditions.

The choice of algal species commonly cultured in hatcheries (with the exception of *Pavlova* sp.) is more commercially relevant than fatty acid studies employing algae known to be a poor food for bivalves, such as *Dunaliella* sp., a 'starved' control, or studies with lipid microcapsules (Coutteau and Sorgeloos, 1992). The *Pavlova* sp. treatment served as a useful comparison to *Pavlova lutheri* in that it contained significant quantitities of both (n-3) essential fatty acids (DHA and EPA) but higher levels of ARA than *P. lutheri*. This is the first detailed comparison of these specific diets fed to a bivalve and the chosen treatments provide a greater range of (n-6)/(n-3) series ratios than found in other studies.

Ingestion (clearance rate) was not directly measured in our study, but food availability was equal across treatments since there were no differences in settling rate of cells. Although it is possible that selective ingestion may have occurred (Chretiennot-Dinet et al. 1991), it is unlikely that ingestion differences could have been solely responsible for variability in growth among diet treatments. Additionally, Fritz et al. (1989) concluded that bivalve larvae select smaller sized cells in wild phytoplankton asemblages, but little selection occurs among cell sizes in the range of this study (3-6 μm).

3.4.2 Changes in fatty acid composition of larvae with development

The similarities between the fatty acid profile of prodissoconch I stage veligers and the eggs they were hatched from is not surprising and underlies the importance of egg composition to newly hatched larvae (Millar and Scott, 1967; Berntsson et al., 1997). The large decrease in lipid level from egg to D-stage veligers is consistent with the view that larval development is fueled by lipid (Holland, 1978; Manning, 1986). The decrease in neutral lipid DHA in day 2 larvae compared with the eggs and the maintenance of DHA in polar lipids of the larvae may indicate that PUFA in TAG serve not only as an energy store for catabolism, but also as a pool of structurally important fatty acids for growth and development (Napolitano et al., 1988). The differences in day 9 compared with day 2 larvae reflects an increased dietary influence as well as an endogenous contribution of fatty acid biosynthesis *de-novo*. The fatty acid 18:0 was a minor component in the diet and contributed only 2% of the total fatty acids in day 2 larvae, but slightly over 12% in day 9 larvae. The gradual shift in larval fatty acid profiles from EPA as the dominant fatty acid in early veligers to DHA was also noted by Delaunay et al. (1992) for *Pecten maximus*. It has been suggested that the large EPA content of eggs and early veligers serves mainly an energetic rather than structural role (Pazos et al., 1997).

Although TAG was an important lipid class of *Placopecten magellanicus* larvae in this study, there was no marked accumulation of TAG in older larvae as reported in some studies with other bivalves (*e.g.*, Chu and Webb, 1984, for *Crassostrea virginica*). This may indicate a degree of stress in the larvae due to culture in small containers

(Dupuy, 1975), but it is more probable that development had not progressed far enough for the accumulation of this lipid class to be noted. Some studies have found TAG to be indicative of larval growth (Waldock and Nascimento, 1979; Gallager et al., 1986). Only a general positive trend was found in the data presented here (Figure 3.7), although this trend may have been stronger if we had extended the diet trial. TAG was believed to provide energy for bivalves during metamorphosis, when the animals are unable to feed for a number of days. Because neutral lipids of *P. magellanicus* larvae contain higher amounts of fatty acids such as 14:0, 16:1n-7, and 16:1n-5 (Tables 3.7 and 3.8), fatty acid analysis of larvae may be an indicator of TAG reserves and hence larval vigor (Thompson and Harrison, 1992).

3.4.3 Diet effects on larval fatty acid composition

Both neutral and polar lipid fatty acid composition of sea scallop larvae were influenced by diet. This contrasts with the results of Watanabe and Ackman (1974), who found that adult *C. virginica* and *Ostrea edulis* rapidly converted dietary fatty acids into a species-oriented profile. However, it is well established and not unexpected that it is comparatively easy to alter the fatty acid profile of bivalve larvae although difficult to alter the composition of adult organisms due to the abundant storage pools in larger individuals. Other marine invertebrates such as krill, tunicates, and copepods have also been shown to reflect their diet in terms of their fatty acid composition (Holland, 1978).

As in research with other larval bivalves (*e.g.*, Waldock and Nascimento, 1979), in this study neutral lipid fatty acids reflected diet more than did polar lipids, presumably

because of biochemical constraints placed on polar lipid composition due to their structural role in cell membranes (Delaunay et al., 1992). The conservation or preferential accumulation of important PUFA in polar lipids in the face of dietary deficiency (DHA in the *Thalassiosira pseudonana* treatment and to a lesser extent EPA in the *Isochrysis* sp. diet) underscores the physiological importance of these (n-3) PUFA. The fact that 18:4n-3 prevalent in the *Isochrysis* sp. diet (Figure 3.2) was not apparently converted to 20:5n-3 (EPA) is consistent with the view that the enzymes required to mediate such PUFA modifications (Δ-5 desaturases and/or elongases) are lacking or display low activity rates. Although DHA was largely maintained as a percent of total fatty acids in polar lipids of larvae fed *T. pseodonana*, the smaller size of these larvae and their lower lipid levels also indicate that, as in other bivalves, *P. magellanicus* cannot easily modify these long chain PUFA. Compositional control of polar lipids is probably accomplished through selective assimilation and retention of important PUFA.

Even in neutral lipids, a degree of metabolic control is evident. The presence of 20:1n-9 in larval neutral lipids and lack of correlation between 16:0 and 16:1n-7 in the diet and larval TAG shows an endogenous component to TAG composition. High amounts of 18:4n-3 in the *Isochrysis* sp. diet were apparently catabolized for energy, only appearing in slightly higher amounts in the neutral lipids and as a minor component (<3%) of polar lipids of larvae. The (n-6) and (n-3) series, PUFA, and C_{20} and C_{22} PUFA groups were also less variable in the neutral lipids than in the diet (Figure 3.3). Delaunay et al. (1993) suggested that acyltransferases may be mainly responsible for this partial metabolic control, since elongation and desaturase activity for C_{18} and longer chain

PUFA are low in most bivalves (Waldock and Holland, 1984; Zhukova, 1991). Despite increased MONO in neutral lipids over polar lipids, there is still a significant pool of PUFA. Since PUFA have similar caloric values to MONO and SAT fatty acids, they may serve as a pool for later incorporation into membranes as opposed to being purely an energy store (Napolitano et al., 1988).

The selective incorporation of PUFA may come about through the specificity of lipases in marine invertebrates (Holland, 1978). Brockerhoff et al. (1967) showed that fatty acyl groups in the 1 and 3 position of triacylglycerols were rapidly hydrolyzed in the lobster, *Homarus americanus*, while removal of the acyl group on the number 2 carbon occurred only slowly. The products yielded by this process were free fatty acids and 2-monoacylglycerols. In mammals, these products are re-converted into TAG in the intestinal epithelia, but in marine invertebrates, this TAG would presumably be deposited in the hepatopancreas or intestinal lipid store. Brockerhoff's group (1967) further showed that long chain PUFA in the diet are usually located on the second carbon of the glycerol molecule. Thus these dietary fatty acids may persist in the TAG of marine invertebrates.

3.4.4 Dietary lipid quality and larval performance

The importance of an alteration of animal composition by changes in diet to bivalve hatcheries and to recruitment in the natural environment is their effect on larval performance as opposed to simply dietary labeling. Significant effects on performance in terms of changes in growth rate, although not mortality, were detected in this study.

Growth rates were related to differences in essential fatty acids of both the (n-3) and (n-6) series in the treatments.

Mortality was slightly higher in the *T. pseudonana* treatment (Table 3.1), but in no treatment or replicate was mortality significant. Previous work has shown that culture of bivalve larvae in small containers can be problematic (Dupuy, 1975). The trials should be run again in larger tanks to confirm these results and determine settlement success, but the issue was alleviated here by growing larvae in larger containers for the critical first week before initiation of the diet treatments and by terminating the experiment before 20 days. After 20 days, larval cultures in small containers often exhibit high mortality (Couturier, personal communication). Overall growth of larvae in this study (1.9 μm/ day) was slower than in commercial hatcheries (c.a. 6 μm/day, Dabinett, personal communication), but comparable to another study that was performed in 150-L tanks (Couturier, 1986).

The PCA analysis conducted on diet fatty acids and growth rates should be regarded as exploratory as the number of variables included in each analysis approached the number of cases used. Also, the assumption that growth responds linearly to fatty acid content of the diet may not hold true. Nonetheless, the range of values for many of the dietary fatty acids was significant and broader than that of other studies of a similar type (*e.g.*, Delaunay et al., 1993), and the PCA provides a useful means of visualizing fatty acids and groups that correlate with performance. The results of the PCA provided no clear picture of an optimal fatty acid ration and demonstrate the problems inherent in this type of study where no fixed diet control is available. However, the results do

support the conclusions inferred from the choice of treatments, mainly the importance of dietary PUFA (with chain lengths of C_{18} or greater) over SAT and MONO for optimal larval growth. More specifically, ARA and possibly 22:5n-6 may be more important than previously thought, and DHA is more limiting than EPA.

3.4.4.1 PUFA

As previously mentioned (Chapter 1), the importance of PUFA in the diet is due to the inability of animals to synthesize these compounds without suitable precursors in the diet. Bivalves further need adequate levels of C_{20} and C_{22} PUFA in the diet because they are unable to adequately elongate and desaturate C_{18} PUFA to form the longer and more unsaturated fatty acids that predominate in bivalve phospholipids (especially DHA and EPA). This has been demonstrated by radiotracer experiments (DeMoreno et al., 1976; Waldock and Holland, 1984; Chu and Greaves,1991) and in feeding trials with algae lacking these PUFA (*Dunaliella tertiolecta*, Langdon and Waldock, 1981).

Thompson and Harrison (1992) correlated high levels of saturated fatty acids (14:0 and 16:0) with larval growth of some oyster species. Other studies with bivalves also suggest that when DHA and/or EPA are present at some threshold level, saturated fatty acids (or carbohydrates) become more of a determining factor in performance, presumably because they are simpler to oxidize and provide a better 'fuel' (Whyte et al., 1989; Thompson and Harrison, 1992). Although extra enzymes are required to degrade PUFA, the difference in net energy yield between PUFA and SAT is very little (Napolitano et al., 1988, Parrish, personal communication). It is difficult to draw strict

comparisons between feeding trials conducted differently, but our results indicated PUFA to be more important than SAT or MONO in *P. magellanicus* diets. Whether discrepancies among feeding trials are due to species-specific effects or an artifact of experimental design (*i.e.*, diet choice) is unclear. It is possible though that certain PUFAs, because of their role in maintaining membrane fluidity, may be of greater importance in cold water bivalves compared with oysters or temperate/tropical pectinids. It is also likely that the nutritional requirements for PUFA as a proportion of diet vary with stage of development, and saturates or other nutritional factors unrelated to PUFAs may indeed be important to post set animals where growth is extremely rapid.

3.4.4.2 EPA and DHA

Both EPA and DHA were preferentially incorporated into polar lipids of larvae fed diets deficient in these fatty acids (*Isochrysis* sp. and *T. pseudonana*, respectively). Growth in the *Isochrysis* sp. treatment was significantly better than in the *T. pseudonana* one, but not significantly different from the high EPA/ high DHA *P. lutheri* diet. This indicates DHA to be of greater importance than EPA as an essential fatty acid at this stage of larval development (as previously stated), and inclusion of DHA in the diet should probably be greater than 0.063 pg/cell (the level in *T. pseudonana*; Appendix 3.1) to support adequate larval growth. Thompson and Harrison (1992) fed *Crassostrea gigas* larvae with *T. pseudonana* in which biochemical composition was controlled by manipulating culture conditions. As in this study, they demonstrated that *T. pseudonana* was deficient in DHA with the best larval growth and survival rates obtained with

modified cultures containing the highest proportions of DHA. Delaunay et al. (1993) also found a substantial preferential incorporation of DHA over other prominent (n-3) PUFA (18:4n-3 and 20:5n-3) in *Isochrysis* sp. and *P. lutheri* diets. Growth of *Pecten maximus* larvae in their study, however, was greatest when fed a diet of *Chaetoceros calcitrans*, an alga with fatty acid proportions similar to *T. pseudonana*, contradicting the growth trends in this study. However, *C. calcitrans* in their study contained nearly three times the total fatty acid content of *P. lutheri* and *Isochrysis* sp., such that the DHA deficiency was less than in this study. Furthermore, setting success was low for the *C. calcitrans* treatment indicating that the DHA deficiency may have resulted in deficient reserves rather than slower growth.

Delaunay et al. (1993) also cite a concomitant increase in other (n-3) PUFA (20:5n-3 in the *C. calcitrans* diet and 18:3n-3 in the *D. tertiolecta* diet), with depletion of DHA in these deficient diets. However, levels of 20:5n-3 and 18:3n-3 in absolute rather than relative terms were extremely high in these respective diets. In this study, a greater accumulation of 20:5n-3 in the *T. pseudonana* diet in response to a lower DHA level in polar lipids was not apparent (Figure 3.2c). The accumulation of other (n-3) PUFA as a presumptive replacement of DHA in deficient diets may have been an artifact of the high lipid content of the *C. calcitrans* diet used in Delaunay et al.'s (1993) experiment, rather than indicative of an endogenous metabolic control on the part of the larvae. Another possibility that might explain why other PUFA seem to accumulate in response to a essential fatty acid deficiency is that characteristic dietary fatty acids may accumulate simultaneously as the animal "strives" to incorporate the EFA that is lacking (Soudant,

personal communication). The high degree of conservation of DHA in polar lipids of the *T. pseudonana* treatment in our study seem to support the notion that the physiological role of DHA is highly specific and not easily substituted for by EPA, but this hypothesis should be tested by longer diet trials.

The greater importance of DHA relative to EPA as a nutritional requirement may be specific for *P. magellanicus* or pectinid larvae in general. Helm and Laing (1987) reported that *Isochrysis* sp. was an inadequate diet for larvae of *Crassostrea gigas* (Pacific oyster) and *Crassostrea rhizophorae* (mangrove oyster), although it did support suitable growth in larvae of two clam species. *C. calcitrans* or a mixed diet of *Isochrysis* sp. and *C. calcitrans* supported significantly better growth than *Isochrysis* sp. alone in the oyster diets. They suggest that *Crassostrea* spp. larvae have a high specific requirement for EPA not met by the *Isochrysis* diet. Napolitano et al. (1990) compared *O. edulis* larvae and *P. magellanicus* larvae fed a mixed diet of *C. gracilis*, *C. calcitrans*, and *I. galbana*. Phospholipids of scallop larvae contained a smaller proportion of EPA (7% vs. 13% in oysters) and larger proportion of DHA (20% vs. 17%) in polar lipids. The *P. magellanicus* larvae also exhibited selective accumulation of DHA as evidenced by an increased proportion in polar lipids as opposed to total lipids, whereas the oyster larvae did not, indicating that the DHA content of the diet exceeded the requirements of the oyster larvae but not the scallop larvae. Cary et al. (1981) also concluded that *Isochrysis* sp. was the best of six algal diets tested for rock scallop larvae (*Crassadoma gigantea*). In addition to indicating a high nutritional requirement for DHA in pectinid larvae, these

results point to the importance of evaluating nutritional requirements of bivalve larvae on a species by species basis.

3.4.4.3 (n-6) series fatty acids as an essential nutritional requirement

This study and others (Joseph, 1982; Napolitano et al., 1988; Delaunay et al., 1993) have shown a selective accumulation of ARA in bivalve polar lipids. Thus there is evidence that (n-6) PUFA also play an important role in membrane lipids. There is little consensus in the literature on whether (n-6) fatty acids should also be termed "essential" since they occur in lesser proportions than EPA and DHA in tissues of marine animals, but there is growing recognition that there is a need for these (n-6) PUFA in addition to the (n-3) PUFA. Arachidonic acid is a precursor to prostaglandins and eicosanoids (Henderson et al., 1985), a key signal molecule (Berridge, 1994), and the major component of phosphoinositides (a polar lipid subclass; Bell and Sargent (1985)). Prostaglandins are short-lived local hormones; their synthesis and degradation is irreversible and occurs rapidly in response to physiological or biochemical cues (Stryer, 1993). Delaunay et al. (1993) found a delay in the accumulation of 20:4n-6 in *P. maximus* larvae and proposed that this fatty acid may have a high turnover rate due to its specific metabolic role. Thus ARA may constitute a greater nutritional requirement than is indicated by an examination of fatty acid composition alone.

The fastest growth rate in this study was in larvae of the *Pavlova* sp. treatment. This species was similar to *P. lutheri* in its DHA and EPA levels, but had significantly more ARA and 22:5n-6, indicating that these (n-6) PUFA had a positive effect on larval

performance. Simple feeding trials with *Pavlova* sp. and *Argopecten irradians* have produced better growth performance and earlier settlement in larvae fed this algae (Alix et al., 1996). The most characteristic feature of this diet is its high (n-6) PUFA content together with ample amounts of EPA and DHA, which may explain the improved growth performance reported. Various species of *Chaetoceros* have often been cited as supporting better growth than *Isochrysis* sp. or *Pavlova lutheri* (Enright et al, 1986a,b; Parrish et al., 1999). In the study by Delaunay et al. (1993), *C. calcitrans* had a higher absolute content of ARA (0.06 pg/cell vs. 0.01 for *P. lutheri* and 0.00 for *Isochrysis* sp.) and supported the highest larval growth rate. Napolitano et al. (1990) also found a high proportion of ARA in *Chaetoceros gracilis*-fed *P. magellanicus* larvae (3.50%). Nell and O'Connor (1991) found an unexplained synergistic growth response when larval rock scallops were fed *P. lutheri* in conjunction with *C. calcitrans*. It is possible that ARA could have accounted for this difference.

It is difficult to state whether it was the high ARA level or 22:5n-6 or both in the *Pavlova* sp. diet that accounted for growth differences in the larvae. Feeding trials with *C. gracilis* (which has higher than average amounts of ARA and only trace amounts of 22:5n-6) with *Pavlova* sp. may help to ascertain the nutritional importance of 22:5n-6. Although it seems probable that ARA is the key nutritional requirement due to its metabolic roles described previously, 22:5n-6 was also preferentially accumulated in polar lipids. This was also the case with *P. maximus* larvae (Delaunay et al., 1993) and indicates a specific role for this fatty acid in membranes. The larvae fed *Pavlova* sp. revealed a remarkable accumulation of this fatty acid (14.63%; Figure 3.2c) and a lower

level of DHA than even the larvae from the low DHA treatment (*T. pseudonana*). While the (n-6)/(n-3) ratio in larval polar lipids of the other treatments was maintained at a consistent level (0.29-0.34) regardless of the diet, the (n-6)/(n-3) ratio in the *Pavlova* sp. treatment was over 1. Literature dealing with fatty acids in mammalian tissue (rats) suggests a reciprocal relationship between 22:5n-6 and 22:6n-3 in polar lipids (Stubbs and Smith, 1984). Rats fed diets high in (n-6) fatty acids readily replace 22:6n-3 in polar lipids of tissues containing high amounts of this fatty acid with 22:5n-6 in proportional amounts. Since membrane function and metabolic pathways in lower and higher animals are similar, it is possible that 22:5n-6 may serve a homologous functional role to DHA in phospholipids. From the perspective of membrane fluidity, 22:5n-6 may be as suitable in phospholipids as DHA, since the extra double bond in DHA would have relatively little effect on the melting point of a C_{22} chain PUFA (Farkas et al., 1994). DHA and 22:5n-6 may be competitive substrates in some enzyme pathways. An acceptable purified diet for bivalves, together with fatty acid studies taken to the level of polar lipid subclasses and fatty acid molecular species, would be necessary to further an understanding of the control of fatty acid metabolism.

3.4.5 Other nutritional factors

Although dietary lipid quality had an impact on larval composition and was associated with performance in this experiment, not all the variability in growth rate could be explained by fatty acid differences. Bacteria present in larval and algal cultures, as evidenced by a small percentage of branched and odd-carbon numbered fatty acids

(Appendix 3; Table 3.4) and algal exudates and dissolved seawater components may have had a slight effect on growth (Langdon, 1983). Growth may not respond linearly to changes in diet, so it is difficult to test definitively for nutritional requirements.

An appropriate balance in fatty acid composition is often cited as a determining factor in superior bivalve diets (Webb and Chu, 1982). Large amounts of 18:4n-3 in the *Isochrysis* treatment were apparently catabolized for energy and, judging by a lack of assimilation into polar lipids, were relatively unimportant physiologically. Consistent with the insufficient Δ-5 desaturase activity reported for many bivalves, this fatty acid was not elongated and desaturated in substantial amounts to compensate for the low EPA level in the *Isochrysis* sp. diet. Thus not all PUFA are important and no clear correlation between total PUFA content of an alga and growth response of the larva should be expected. However, our data did support the general importance of C_{20}- C_{22} PUFA (DHA and ARA in particular) in the diet of *P. magellanicus* larvae.

Sterols are also a nutritional component of significance for bivalves (Wikfors et al., 1991), which are unable to synthesize them (Holden and Patterson, 1991). While sterol quality of the diets was not assessed here, the species characteristic sterol composition of *Pavlova lutheri* and *Pavlova* sp. is similar (Ghosh et al., 1998), again indicating that it is differences in the fatty acid profile of these two species that account for larval performance. Minor differences in sterol between *Pavlova* sp. and *P. lutheri* were reported by Ghosh et al. (1988), however, such as a higher cholesterol content in CCMP459. Other algae containing high cholesterol amounts have been linked to that superior growth rates of *C. virginica* juveniles (Wikfors et al., 1991), and it is possible

variability in even this quantitatively small component could affect growth.

Other factors besides fatty acid and sterol composition may influence lipid quality. *Isochrysis* sp. contains very long chain ketones and hydrocarbons. There is debate as to whether these compounds can be assimilated in most marine invertebrates (Volkman et al., 1980), but large amounts may be indigestible, and behave essentially as a laxative (Volkman et al., 1992).

Despite the importance of lipids to larval metabolism, dietary carbohydrate has also been shown to impact larval nutrition (Whyte et al., 1989). Lipids can be synthesized from, but not converted to, carbohydrate (Stryer, 1993). Catabolizing dietary carbohydrate rather than lipid may conserve important PUFA that would otherwise have to be used as a source of energy (Whyte et al., 1989).

3.5 Conclusions

1) Dietary lipid quality (fatty acid composition) had a significant impact on the biochemical composition and growth performance of *Placopecten magellanicus* larvae. This underscores the relative mutability of fatty acid composition of marine invertebrates in their early developmental stages.

2) Neutral lipid composition reflected diet to a greater extent than did polar lipids. This is consistent with the ability of larval bivalves to exercise some control over fatty acid metabolism in order to maintain the physiological integrity of structural components such as membrane lipids, despite a poor ability to synthesize certain essential fatty acids *de*

novo. Dietary influences on the neutral lipid composition of larvae suggests that a degree of control is also exerted on storage components. Neutral lipids may serve as a pool of physiologically important fatty acids in addition to their known function as an energy store (Napolitano et al., 1990).

3) DHA appears to be more important than EPA as an essential fatty acid requirement at this stage of larval development. Larvae exhibited strong selective accumulation of DHA in polar lipids. Growth was lowest in larvae fed DHA deficient *Thalassiosira pseudonana*, but similar between the high EPA/high DHA *Pavlova lutheri* and high DHA/ low EPA *Isochrysis* sp. diet.

4) The selective accumulation of (n-6) series fatty acids 20:4n-6 (ARA) and 22:5n-6 in larval polar lipids and the significantly better growth obtained on the *Pavlova* sp. diet indicated a more important role of one or both of these fatty acids than has been suggested in previous work. A high turnover rate of ARA may account for its importance as a nutritional requirement despite its occurrence at only modest (though consistent) levels in polar lipids. The fatty acid 22:5n-6 may act as a competitive substrate with DHA, apparently without deleterious effects on the larvae.

5) Further studies are needed to compare growth responses of larvae fed *Pavlova* sp., with standard mixed diets and to culture larvae through to metamorphosis. The fatty acid profile of this algal species appears to be very uncommon as it is characterized by significant quantities of both essential fatty acids of the (n-3) series (DHA and EPA)

together with high levels of (n-6) PUFA as ARA and 22:5n-6. This unique profile in conjunction with the superior growth of larvae compared with the *Isochrysis* sp. control indicates that incorporation of *Pavlova* sp. into standard hatchery diets would be beneficial.

CHAPTER 4 – LIPID CLASS AND FATTY ACID COMPOSITION OF *PAVLOVA* SP. (STRAIN CCMP459): CHANGES WITH GROWTH PHASE IN BATCH CULTURES

4.1 Introduction

Previous experiments (Chapter 3) indicated that the CCMP459 strain of *Pavlova* sp. (class Prymnesiophyta = Haptophyta, order Pavlovales) promoted superior growth of sea scallop (*Placopecten magellanicus)* larvae compared with three other algal species commonly used in bivalve hatcheries. Excellent growth and earlier metamorphosis in larvae of another pectinid, *Argopecten irradians*, have also been reported in both unialgal and mixed diet feeding trials with this strain (Alix et al., 1996). The suitability of CCMP459 as a diet for larval bivalves may be related to its fatty acid content — mainly to an enhanced level of (n-6) series fatty acids, specifically 20:4n-6 and 22:5n-6, as well as to significant amounts of the essential fatty acids, 20:5n-3 and 22:6n-3 (Chapter 3). Alix et al.'s study (1996) did not include an analysis of fatty acids, but the authors stated that the increased larval growth found with CCMP459 was likely due to its presumed high PUFA content, typical of *Pavlova* spp.

Members of the genus *Pavlova* are cosmopolitan and found in both oceanic and neritic brackish environments (Green, 1975). Prymnesiophytes as a class and Pavlovales in particular contain a relatively wide array of lipid and fatty acid compositions that reflect a heterogeneous taxonomic class (Volkman et al. 1990). This diversity is evident in ongoing debates concerning the taxonomy of prymesiophytes, the renaming of some species such as *Pavlova lutheri* (previously *Monochrysis lutheri* and considered a

chrysophyte), and the recognition, by some authors, of the Pavlovales as a distinct subclass (Green and Jordan, 1994).

Proximate composition and lipid class and fatty acid content of microalgae are also influenced by culture conditions (*i.e.*, light intensity; Thompson et al. (1990), light/dark cycle, nutrient limitation, pH, growth phase, salinity, and temperature; reviewed by Brown et al. (1989)). Manipulation of growth conditions has been used in attempts to optimize the quality of algae by maximizing PUFA and essential fatty acid content (*e.g.*, Dunstan et al., 1993). Also, it has been used as an experimental tool in feeding trials to vary algal biochemical composition intraspecifically, presumably providing a control for variables such as cell digestibility (Enright et al., 1986b; Ryan, 1999). When fatty acid analysis by gas chromotography (GC) is combined with lipid class composition data obtained by thin layer chromatography with flame ionization detection (TLC-FID), compositional modifications with changes in culture environment may be followed, facilitating a general understanding and localization of the cellular mechanisms by which fatty acid biosynthesis occurs in algal cells (Parrish, 1987; Cohen et al., 1988).

Despite many studies, few generalizations can be made regarding environmental influences on algal fatty acid composition, as the effects are species-specific (Volkman et al., 1992). Thus it is necessary to examine both the species' characteristic fatty acid profile and the effect of culture conditions for each important microalga, especially in groups as diverse as the Pavlovales. In this study, changes in proximate, lipid class, and fatty acid composition of *Pavlova* sp. were examined in relation to growth phase and

nutrient limitation in batch cultures typical of many aquaculture facilities. Harvest strategies appropriate from a nutritional standpoint are discussed and trends compared with other algal species commonly used in hatcheries. While many studies have dealt with lipids of prymnesiophytes and the fatty acid composition of Pavlovales commonly used as aquaculture feeds, such as *Pavlova lutheri* (Volkman et al., 1991), this is apparently the first report of the fatty acid profile of the CCMP459 strain.

4.2 Methods

4.2.1 Algal culture

Pavlova sp. strain CCMP459 was obtained from Provasoli-Guillard Center for the Culture of Marine Phytoplankton, West Boothbay Harbor, Maine (Table 4.1). This species was originally isolated by L. Pravasoli in 1983 from a sample taken in the North Atlantic (38°42.2' N, 72°22.4' W). Cultures were maintained at the Ocean Sciences Centre, Memorial University, St. John's, Newfoundland. Four 2-L Erlenmeyer flasks containing 1500 mL of seawater (31 ppt salinity) filtered nominally at 0.45 μm, enriched with F/2 medium without silica, and autoclaved, were inoculated with 40 mL axenic stock culture under sterile conditions. Cultures were held in a temperature controlled water bath at a temperature of 22- 24°C, lit from behind with white fluorescent light (at an estimated 50 $\mu E \cdot m^{-2}s^{-1}$ at the culture surface) on a continuous 24 hour cycle. Flasks were provided with air filtered to 0.45 μm and enriched with approximately 1% CO_2.

4.2.2 Harvest and sampling

Cell concentrations were determined daily with a Coulter Multisizer™ to obtain a growth curve over the study period of 26 days. Mean growth rate in log and late log phase (K) was calculated according to Guillard (1973):

$$K \text{ (divisions/day)} = 3.322 \times (\log N_1/N_0)/(t_1-t_0)$$

where: K= mean growth rate in divisions/day, N_0 = initial cell density, N_1 = final cell density, t_1 = culture age (days) at time of sampling, t_0 = initial culture age

Samples were occasionally observed microscopically to monitor the condition of the culture and counts cross-checked with a hemocytometer. Approximately 40 mL of each culture was harvested aseptically on days 4, 7, 11, 13, and 17. After determining the cell density and average size on the Coulter Multisizer™, three subsamples of 5-15 mL were collected on precombusted glass fiber filters (Whatman GF/C) under gentle vacuum for subsequent determination of dry weight, CN, and total lipids and for analysis of fatty acids. A set of 2-3 blanks was prepared with each sample series and ran for each analysis.

Filters for dry weight determination were pre-weighed and rinsed after sample collection with 10 mL of 3% ammonium formate to remove excess salt. After drying to constant weight (approximately 3 days) at 45-50°C, filters with samples were stored in a desiccator containing activated silica gel and weighed on a Mettler™ microbalance (± 0.1

μg). CN samples were rinsed with 0.45 μm filtered sea water, similarly dried and stored in a desiccator before analysis on a Perkin Elmer 2400 CHN analyzer using acetanilide as a standard.

Samples for lipid and fatty acid analysis were immediately placed in a test tube containing 2 mL chloroform ($CHCl_3$), sealed under nitrogen, and stored for a short period (less than two weeks) at –20°C before lipid extraction. After subsampling, the remaining 10-20 mL of the original culture sample was filtered (Whatman GF/C) and the filtrate frozen at –20°C for later nutrient analysis.

4.2.3 Lipid extraction

Lipids were extracted using a modified Folch procedure (Folch et al., 1957) as described by Parrish (1999). Prior to extraction, 0.202 mg of a ketone standard, hexdecan-3-one, was added to each sample. This amount was estimated to comprise 10-20% of the total lipids in most samples. Previous analysis indicated that ketones are not found in *Pavlova* sp.. One mL of ice cold methanol was then added to the test tubes containing the sample with 2 mL chloroform for a 2:1 chloroform:methanol ratio. The sample was ground with a stainless steel rod and an additional 1 mL of 2:1 chloroform:methanol solution and 0.5 mL chloroform extracted water added.

The sample mixture was vortexed, sonicated for 4 minutes to further break-up lipid membranes and centrifuged at 4000-5000 rpm for two minutes. The lower chloroform layer was then removed by double pipetting. The sample was washed with 3 mL chloroform and the procedure repeated three more times. The pooled chloroform

layer containing the lipid extract was concentrated under a stream of nitrogen and stored under nitrogen at approximately –20°C.

4.2.4 Total lipid and lipid class quantification

Total lipid and lipid classes were quantified using TLC/FID with a MARK V Iatroscan (Parrish 1987). Between 0.5 and 20 μL of each sample was spotted on each of two silica gel-coated Chromorods with a Hamilton syringe. The mean of these two analytical replicates was used in data analyses. The rods were developed using four different solvent systems and scanned sequentially to resolve lipid class peaks. First, rods were developed twice for 25 and 20 minutes respectively in a 99:1:0.05 (by volume) hexane/diethyl ether/formic acid solution and partially scanned to resolve aliphatic hydrocarbons (HC), wax esters (WE) and steryl esters (SE) and ketones (KET). The other neutral lipids, in order of polarity: triacylglycerols (TAG), free fatty acids (FFA), free aliphatic alcohols (ALC), sterols (ST), and diacylglycerols (DAG), were separated with a 40 minute development in 80:20:0.1 hexane/diethyl ether/formic acid and scanned to a point immediately after the last peak. Lastly, two 15 minute developments in acetone and two 10 minute developments in a 5:4:1 chloroform:methanol:water solution, followed by a total scan of the rods, resolved the acetone mobile polar lipids (AMPL — consisting of monoacylglycerols (MAG), glycolipids (GLY), and pigments) and phospholipids (PL).

The three chromatograms were combined using T-scan software (RSS Inc., California, U.S.A). Lipid class mass on each rod was determined by regression equations

obtained by calibration of the rod set with known amounts of lipid standards (Sigma Chemical Co., St. Louis, MO) consisting of: n-nonadecane for HC, cholesteryl palmitate for SE and WE, hexdecan-3-one for KET, tripalmitin for TAG, palmitic acid for FFA, hexdecan-1-ol for ALC, cholesterol for ST, glyceryl-1-monohexadecanoate for AMPL, and DL-α-phosphatidylcholine for PL.

The lipid class mass in the original sample was calculated as:

$$LC_{sample} = LC_{rod} \times (0.202 \text{ mg} / LC_{standard})$$

where: LC_{sample} = lipid class mass (pg), LC_{rod} = lipid class mass calculated to be on the rod, 0.202 mg = amount of ketone added to the sample as a standard, and $LC_{standard}$ = the ketone amount calculated to have been spotted on the rod

Total lipid was taken as the sum of the masses of each lipid class. Lipid values reported are expressed in relative (percent composition) and absolute terms (cell content and dry weight specific content).

4.2.5 Fractionation of lipid extract

A portion of each algal lipid sample was separated into neutral and polar components by a simple column chromatography method based on Yang (1995). Silica gel, activated by heating at 110°C for 1 hour, was packed into a pasteur pipette and held in place with a small piece of pre-combusted glass wool. The column was washed with 2

mL methanol followed by 4 mL chloroform. A small amount of lipid extract was spotted at the top of the column and neutral lipids eluted with 3 mL of a 9.9:0.1:0.05 (by volume) chloroform:methanol:formic acid solution. By using a solvent amount approximating only one bed volume instead of two, the collection of AMPL in the neutral lipid fraction was minimized (Budge, 1999). Polar lipids were then collected by running 6 mL of methanol through the column.

4.2.6 Fatty acid methyl ester (FAME) derivation

Prior to lipid fractionation and FAME derivation, 0.0625 mg of 23:0 was added to each lipid extract as a fatty acid standard (Parrish, 1999). Lipid extracts were transesterified using 14% BF_3 in methanol (Morrison and Smith, 1964). After evaporating the extract to near dryness, 0.5 mL hexane and 1 mL of BF_3/methanol reagent were added and the vial sealed under nitrogen, shaken, and heated at 80°C for 1 hour. After cooling, 0.05 mL of $CHCl_3$-extracted water and 2 mL hexane were added to the samples and the mixture vortexed and centrifuged at 125 g for 4 minutes. The upper hexane portion containing FAMEs was recovered, concentrated under a stream of nitrogen, and stored at –20°C.

4.2.7 FAME analysis by GC-FID

A Varian 3400 GC with a temperature-programmable injector and a Varian 8100 autosampler was used to analyze FAMEs. Separation was carried out on a flexible fused silica column (30 m x 0.32 mm ID) coated with Omegawax (Supelco, Mississauga, ON)

specifically designed for PUFA separation. Hydrogen served as the carrier gas (2 mL/min flow rate). Oven temperature was programmed as follows: 0.5 minutes at 65°C, then warming at 40°C/min to 195°C and holding for 15 minutes, then warming at 2°C/min until reaching a final oven temperature of 220°C and holding for 0.75 minutes. Flow rates for makeup gas (helium) and combustion gas (air) were 30 mL/min and 300 mL/min, respectively. The injector temperature was programmed to hold for 0.5 minutes at 150°C, then rise at 200°C/min and hold at 250°C for 10 minutes. The FID was held at 260°C.

Fatty acid methyl esters were identified from their retention times compared with known standards (PUFA1, PUFA2, and 37 Component FAME Mix; Supelco, Mississauga, ON). Peaks were integrated with Varian Star Chromatography Software (V 4.02). Peak area reject values were adjusted in each chromatogram to yield a minimum peak area equal to 0.1% of the sum of all peak areas. The amount of fatty acid methyl ester found in each original sample (FA_{sample}) was calculated as:

$$FA_{sample} = (0.0625 \times AREA_{FA}) / AREA_{standard}$$

where: 0.0625 mg = the amount of 23:0 added to each sample as a standard, $AREA_{FA}$ = the peak area of the fatty acid, and $AREA_{standard}$ = the peak area of the standard

The value for FA_{sample} was successfully confirmed by performing the same calculation substituting the peak area and known mass of the ketone standard, which also appeared in the chromatogram, for the 23:0 standard values.

The relative weight percentage of each fatty acid ($FA_{\%}$) was calculated as:

$$FA_{\%} = AREA_{FA} / [AREA_{TOT} - (AREA_{23:0} + AREA_{KET})] \times 100$$

where: $AREA_{FA}$ = the fatty acid peak area, $AREA_{TOT}$ = the sum total peak area, $AREA_{23:0} + AREA_{KET}$ = the peak areas of the two standards

4.2.8 GC-MS analysis of selected FAMEs

The identity of two fatty acids that were found in significant amounts in *Pavlova* sp. was confirmed by GC-MS. A Varian 3400/Saturn GC-MS was used with the same column and temperature program described above. The ionization potential of the mass analyzer was 70 eV, and it scanned over a mass range of 50-500 m/z.

4.2.9 Nutrient analyses

Nitrate and nitrite nitrogen and phosphate in the culture media were determined using an Enviroflow 3500 nutrient analyzer.

4.2.10 Statistical analyses

Data for fatty acid composition were analyzed by ANOVA. When significant differences ($p<0.05$) were found, the ANOVA was followed by Tukey's multiple comparison test. It was deemed unnecessary to use the arcsin transformation for percent data as the range of compared means within each fatty acid class was generally small and

the data satisfied the assumptions of parametric tests. Tests were performed using STATVIEW™ or SYSTAT™ software for Macintosh™ computers.

4.3 Results

4.3.1 Growth rate and batch culture conditions

Pavlova sp. performed well in culture, showing a sigmoidal growth curve characteristic of batch culture (Figure 4.1) with a specific growth rate of 0.50 divisions per day during log phase (day 4 to 6). The cultures reached a peak density of over 24 million cells/mL after 13 days. Growth rate was reduced to 0.27 divisions/day during late log phase (day 6-13). Logarithmic growth occurred after a short acclimatization period, gradually slowing as nutrients (and presumably light) became limiting until a stationary phase was reached where cell concentration remained stable.

Nitrate and nitrite nitrogen in the culture medium dropped rapidly from 1102 $\mu m \bullet L^{-1}$ in the starter culture to 4.39 $\mu m \bullet L^{-1}$ as growth progressed over the first 7 days of culture (Table 4.1). Phosphate levels in the medium fell after day 4. The culture became severely N-limited sometime after day 7 as evidenced by the drop in the Redfield ratio (N/P) from 56.70 in the original culture medium to 0.54 at the termination of the experiment (Table 4.1, Figure 4.1), and compared with the filtered seawater control (1.73).

Table 4.1. Nutrient data for *Pavlova* sp. batch cultures over time (mean ± standard deviation, n=3).

Culture age *(days)*	Nitrite+Nitrate *($\mu m \cdot L^{-1}$)*	Phosphate *($\mu m \cdot L^{-1}$)*	N/P *(molar ratio)*
0	1102.50 ±15.02	22.02 ±9.27	56.70 ±22.36
4	459.11 ±42.25	26.04 ±7.99	18.76 ±5.17
7	4.39 ±1.13	0.40 ±0.04	10.92 ±2.79
11	1.01 ±0.27	1.67 ±0.36	0.62 ±0.19
13	1.35 ±0.51	2.30 ±0.29	0.60 ±0.26
17	1.54 ±0.47	2.92 ±0.98	0.54 ±0.10
seawater	1.01	0.58	1.73

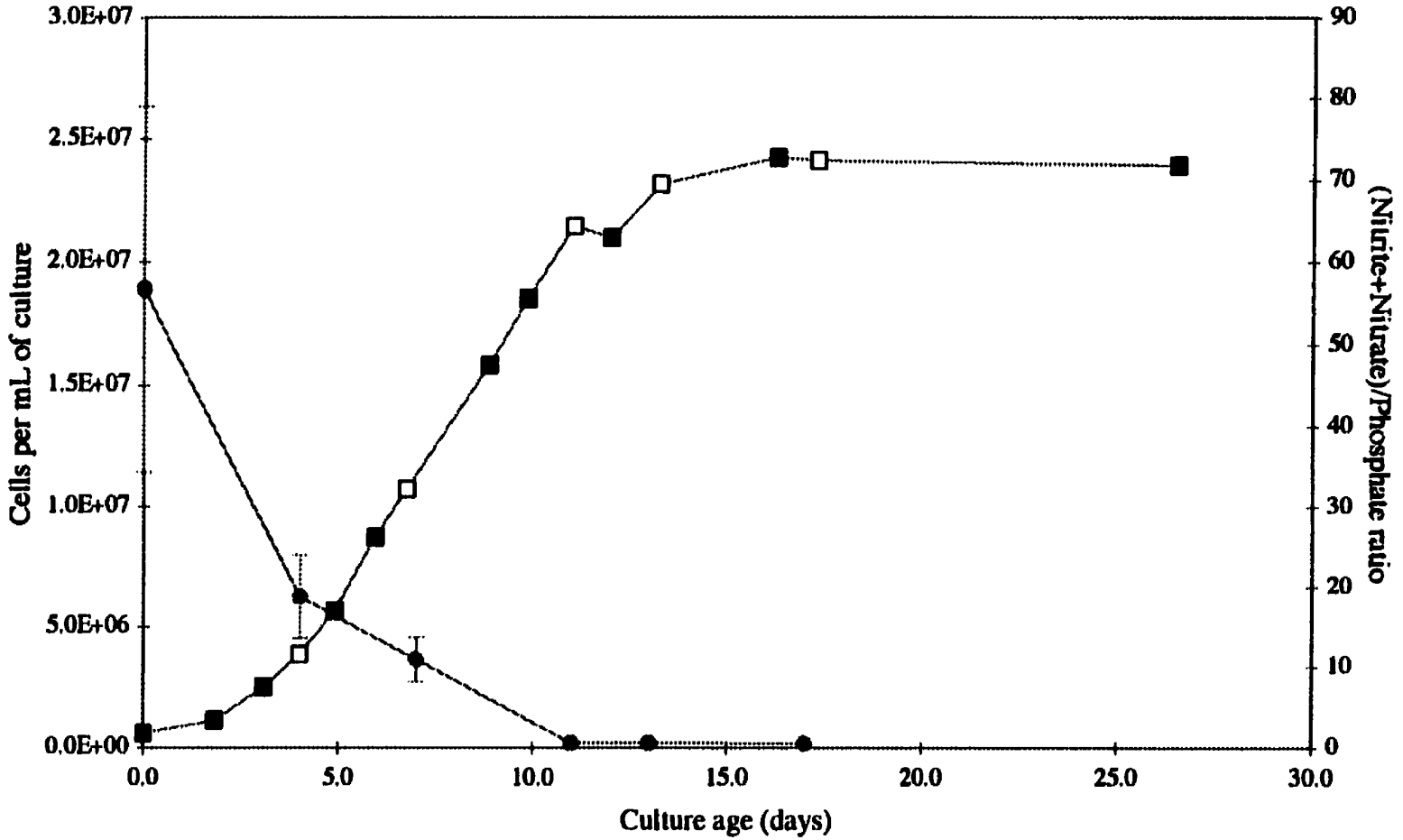


Figure 4.1. Growth (cells/ml) of *Pavlova* sp. (■ / □) and nitrite+nitrate:phosphate molar ratio (●) of culture media over 26 days in batch culture (open squares = sample dates for biochemical analyses) (error bars are ±standard deviation).

4.3.2 Variation in proximate biochemical composition, dry weight, and cell size

Cell dry weight decreased markedly from 18.7 pg/cell (day 4) to 7.5 pg/cell (day 17) as cell density increased. This was accompanied by a decrease in mean cell diameter from 3.7 μm to 3.2 μm over the same time period (Table 4.2.1). Microscopic observation also revealed that the cells appeared to become more oblong in the stationary growth phase, although this trend was not quantified. Total lipid per cell declined from 4.22 pg/cell to 3.17 pg/cell (Table 4.2.1). In contrast, weight-specific lipid content greatly increased, from 24.1% to 42.7% (Table 4.2.2).

The increase in relative lipid content is reflected in the CHN data (Table 4.3). The carbon:nitrogen ratio (by mass) nearly doubled over the culture period rising from 6.16 on day 4 to 11.67 by day 17, attributable to an increase in the relative carbon content from 39.32% to 67.31% accompanied by a modest decrease in relative nitrogen content from 6.40% to 5.77%.

4.3.3 Variation in lipid class composition

The major lipid classes in *Pavlova* sp were phospholipids (48.1-55.4% total lipids), AMPL (14.6%-29.8%), TAG (3.8%-24.7%), and sterols (7.3%-7.6%) (Table 4.2.2). Trace components included HC (0.7-3.1%), FFA (0-1.3%), ALC (0.3-0.7%), and DAG (0.0-0.8%). The low level of FFA indicates that little lipid breakdown occurred prior to sample analysis (Parrish, 1987). Previous analyses (Chapter 3) found none of the ketones that occur in some other species of prymnesiophytes (Volkman et al., 1981).

Table 4.2a. Cell size, total lipid content, and cellular composition of major lipid classes of *Pavlova* sp. (CCMP459) determined by TLC/FID (mean ± standard deviation, n=3).

Culture age *(days)*	4	7	11	13	17
Cell diameter *(μm)*	3.7 ±0.1	3.5 ±0.1	3.4 ±0.1	3.3 ±0.1	3.2 ±0.1
Total lipids *(pg/cell)*	4.22 ±0.84	3.93 ±0.92	3.92 ±0.99	3.71 ±0.71	3.16 ±0.30
TAG *(pg/cell)*	0.16 ±0.04	0.28 ±0.14	0.62 ±0.16	0.76 ±0.03	0.77 ±0.02
PL *(pg/cell)*	2.40 ±0.77	2.08 ±0.93	2.14 ±0.99	1.98 ±0.66	1.54 ±0.30
AMPL *(pg/cell)*	1.15 ±0.07	1.11 ±0.22	0.79 ±0.20	0.54 ±0.01	0.46 ±0.02
ST *(pg/cell)*	0.33 ±0.10	0.31 ±0.10	0.29 ±0.08	0.28 ±0.03	0.24 ±0.02

Table 4.2b. Cell dry weight, weight specific total lipid, and percent composition of major lipid classes of *Pavlova* sp. (CCMP459) determined by TLC-FID (mean ± standard deviation, n=3).

Culture age *(days)*	4	7	11	13	17
Dry Weight *(pg/cell)*	18.7 ±6.7	14.6 ±1.6	10.5 ±0.4	10.2 ±1.6	7.5 ±1.1
Total lipids *(% dry wt)*	24.1 ±14.6	26.6 ±3.5	37.4 ±9.9	36.2 ±11.9	42.7 ±8.8
TAG *(% total lipid)*	3.8 ±0.4	7.0 ±1.9	16.0 ±2.4	21.3 ±4.4	24.7 ±1.7
PL *(% total lipid)*	55.4 ±3.9	51.3 ±10.5	52.1 ±12.2	51.5 ±10.1	48.1 ±4.7
AMPL *(% total lipid)*	28.0 ±3.4	29.8 ±10.9	21.9 ±10.8	15.1 ±4.0	14.6 ±1.7
ST *(% total lipid)*	7.6 ±1.1	7.7 ±0.9	7.3 ±1.2	7.8 ±1.3	7.6 ±1.1

Table 4.3. Proximate biochemical composition of *Pavlova* sp. (CCMP459) as content of carbon and nitrogen (pg/cell and percent dry weight) determined by CHN analysis (mean ± standard deviation, n=4).

	Culture age *(days)*				
	4	**7**	**11**	**13**	**17**
Carbon *(pg/cell)*	7.62 ±0.70	7.55 ±0.29	5.71 ±0.15	5.48 ±0.30	5.25 ±0.31
Nitrogen *(pg/cell)*	1.24 ±0.10	1.06 ±0.06	0.53 ±0.01	0.47 ±0.03	0.45 ±0.03
C:N *(mass ratio)*	6.16 ±0.13	7.14 ±0.15	10.86 ±0.15	11.54 ±0.19	11.67 ±0.08
Cell dry weight *(pg/cell)*	19.99 ±4.67	15.40 ±2.01	10.58 ±0.34	9.88 ±1.48	8.00 ±1.26
Carbon *(% dry wt)*	39.32 ±7.56	49.83 ±8.14	53.97 ±1.36	56.04 ±5.74	67.31 ±14.37
Nitrogen *(% dry wt)*	6.40 ±1.37	6.99 ±1.24	4.97 ±0.06	4.85 ±0.45	5.77 ±1.25

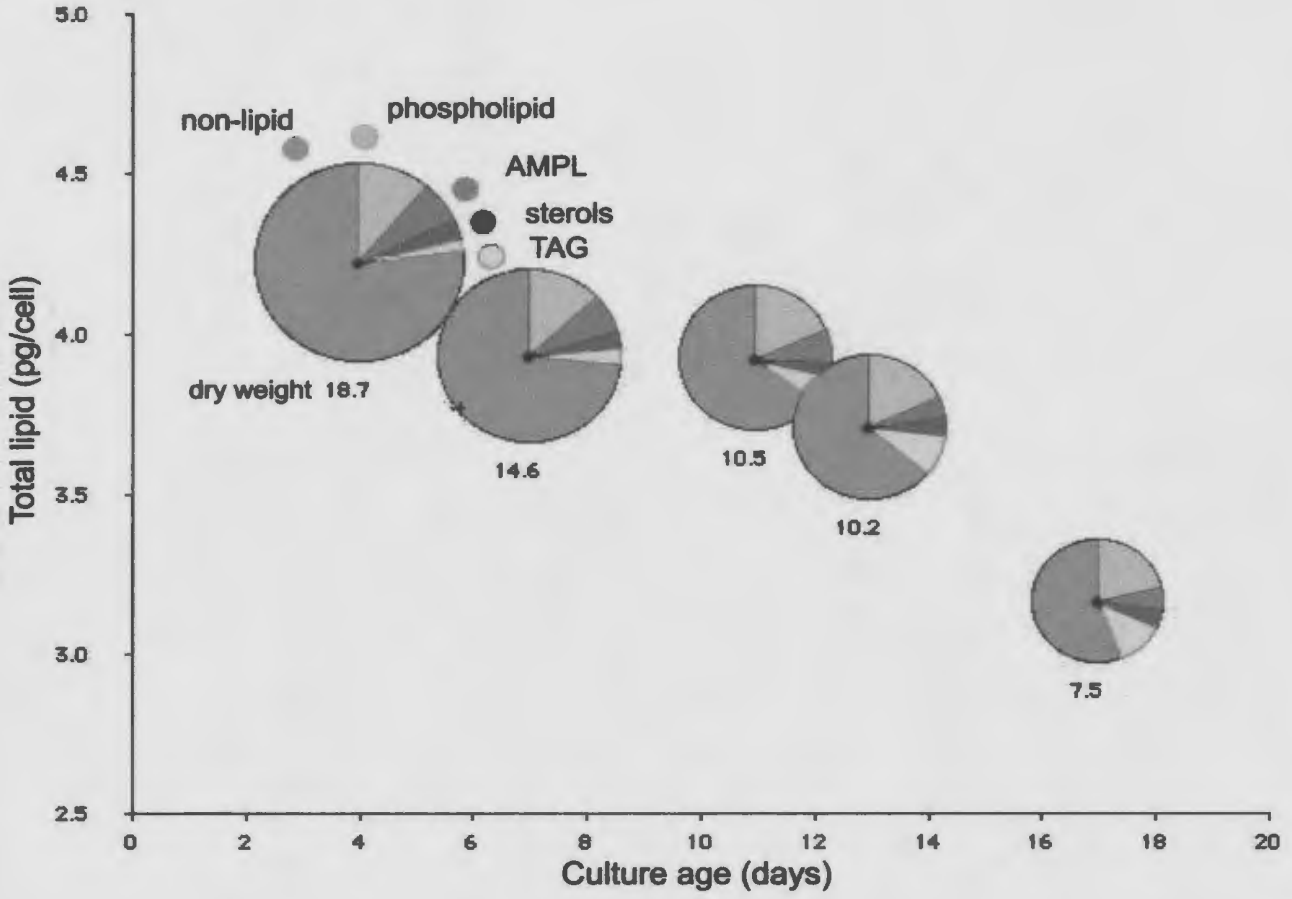


Figure 4.2. Changes in total lipid, lipid class composition, and cell dry weight with culture age in CCMP459 batch cultures. Circle area is proportional to cell dry weight (labeled underneath as pg/cell). The sum area of the four lipid class pie sections represents total lipid as a proportion of dry weight. Subdivisions indicate changing lipid class contributions as marked.

Values for sterol and phospholipid (percent composition) remained relatively stable as the culture aged, while TAG increased considerably from 3.8% in the log phase sample (day 4) to 24.7% in the stationary phase (day 17). AMPL decreased from 28.0% to 14.6% during the culture period (Table 4.2a). In terms of cellular content, PL and sterol decreased due to the declining cell weight. Only TAG increased from 0.16 pg/cell to 0.77 pg/cell, despite the decline in dry weight (Table 4.2b). These trends in lipid class composition and cell dry weight with culture age are summarized in Figure 4.2.

4.3.4 Characteristic fatty acid profile

Total fatty acid concentration ranged from 1.68 to 2.11 pg/cell. The most prevalent fatty acids were 14:0, 16:0, 16:1n-7, 18:4n-3, 20:4n-6, 20:5n-3, 22:5n-6, and 22:6n-3 (83%-87% of the total fatty acids). Other fatty acids comprising greater than 1% of the total included: 16:1n-5, 16:2n-4, 18:2n-6 and 18:3n-3. Present in trace amounts were 18:1n-9, 18:1n-7, 16:3n-4, 18:3n-6, and 18:0 (Tables 4.4 - 4.6). Polyunsaturated FAs comprised approximately half (46-53%) of all fatty acids regardless of culture age. Saturates (mainly 14:0 and 16:0) and monounsaturates (principally 16:1n-7) made up 30-34% and 14-19%, respectively (Table 4.3).

For comparison and an assessment of culture variability, the results of analyses of previous samples of *Pavlova* sp. from a number of different 2-L flask cultures (same culture methods as listed in section 4.2.1) taken over three months is presented in Table 4.7. The results were similar to the data presented above but more variability in 22:6n-3 (2.42 – 9.34%) and 16:0 (14.49 – 22.59%) was evident.

4.3.5 Confirmation of 20:4n-6 and 22:5n-6 with GC-MS

Selected samples were subjected to GC-MS analysis to confirm the presence of 20:4n-6 (ARA) and 22:5n-6 methyl esters. In the mass spectrum for ARA, the protonated molecular ion appeared at a mass/charge (m/z) of 319 – the expected result given its monoisotopic molecular weight of 318 (Figure 4.3). Likewise, the 345 m/z and mass spectrum obtained for the presumed 22:5n-6 peak confirmed the presence of a 22:5 fatty acid compound structure (Figure 4.4)

4.3.6 Variation in fatty acid composition with culture age

The fatty acid composition of *Pavlova* sp. depended on environmental conditions and growth phase in the batch cultures. Trends differed depending on the expression of the data as a weight percent of total fatty acids, or as cellular content (pg/cell), or weight-specific content (pg FA/μg dry weight).

4.3.6.1 FAME composition changes — relative percent

The proportion of 14:0, 18:4n-3, and 20:5n-3 fatty acids declined with culture age while the percentage of 16:1n-7, 16:1n-5, 20:4n-6, and 22:5n-6 increased (Table 4.4, Figure 4.5a). DHA declined slightly compared with samples from the exponential growth phase. The greatest changes occurred between cells in early (day 3-7) and late log phase (day 9-13).

The resultant effect was an increase in the proportion of monunsaturates from 13.6% to 19.2% concomitant with a decrease in saturates and a small decrease in PUFA.

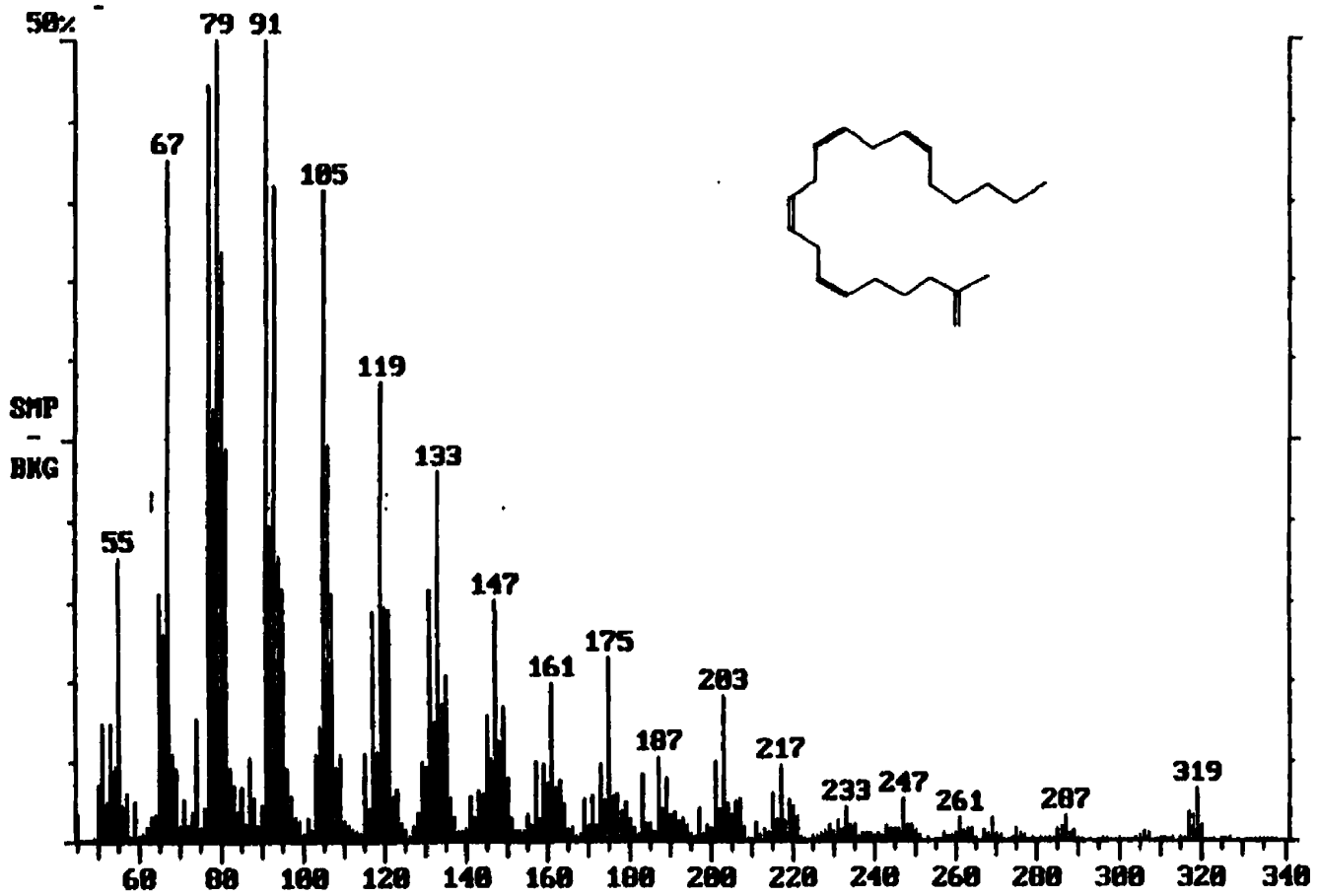


Figure 4.3. Mass spectrum of arachidonic acid, 20:4n-6 (schematic representation, top right), from selected *Pavlova* sp. samples.

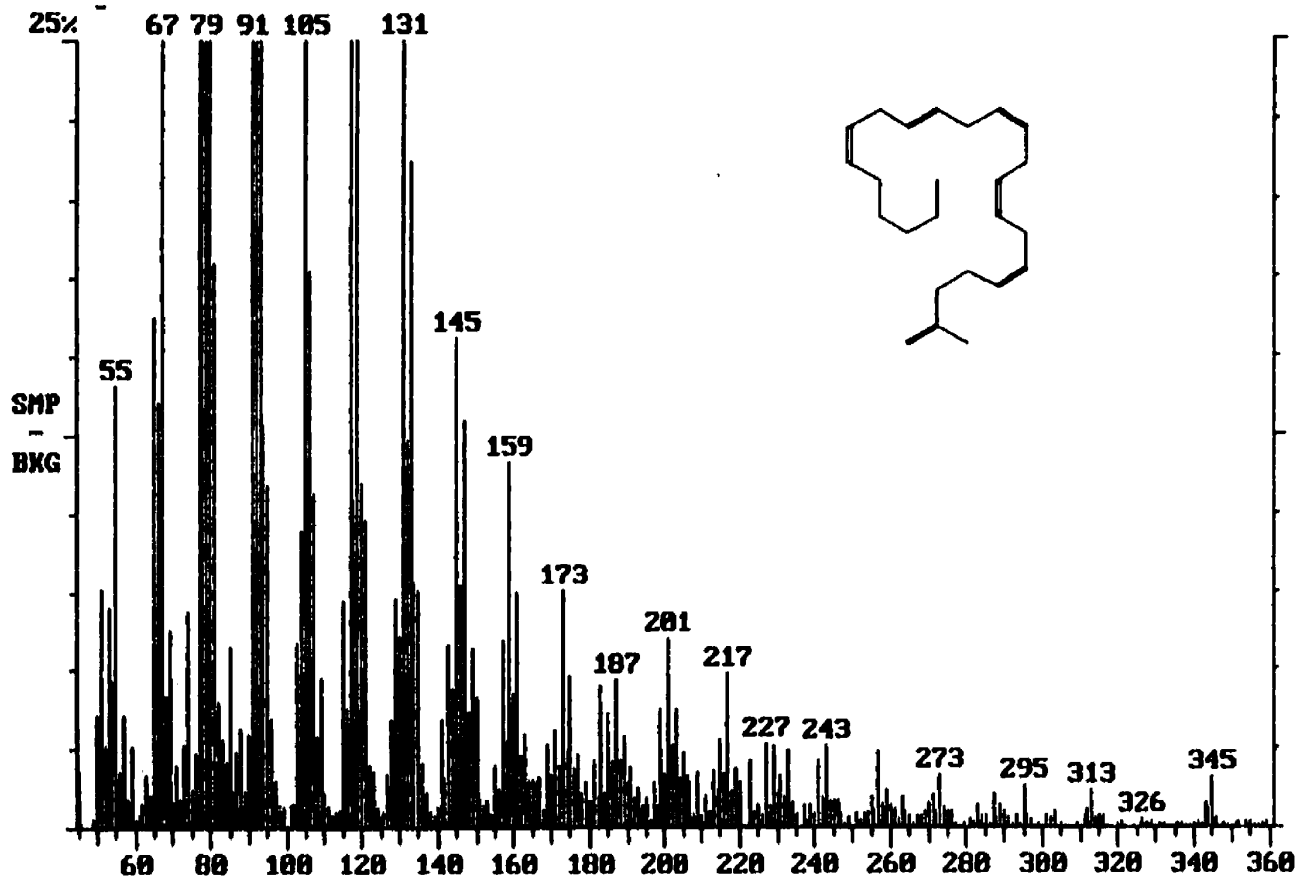


Figure 4.4. Mass spectrum of docosapentaenoic acid, 22:5n-6 (schematic representation, top right), from selected *Pavlova* sp. samples.

The sum of (n-7) and the sum of (n-6) series fatty acids increased and (n-3) fatty acids declined in relative proportion (Table 4.4). The ratio of saturated to unsaturated fatty acids and the DHA to EPA ratio remained relatively stable. The greatest difference was a three-fold increase in the (n-6)/(n-3) series ratio as cultures progressed from the exponential to the stationary phase (Figure 4.6).

4.3.6.2 FAME composition changes – cell content

Changes in fatty acids per cell were similar to changes in relative composition with only minor differences in magnitude (Figure 4.5b). Specifically, the decrease of 14:0 and 20:5n-3 with culture age was even greater, and 16:0 also declined. These data are summarized in Table 4.4. The total fatty acid content per cell peaked in the day 7 samples at 2.113 pg/cell declining to 1.686 pg/cell in the day 17 stationary cultures.

4.3.6.3 FAME composition changes – weight-specific

In contrast to relative and cellular fatty acid profiles, weight-specific fatty acid content increased for almost all fatty acids as the culture aged. The fatty acids shown to be declining in terms of relative percent with culture age did not change in terms of weight-specific content (18:4n-3, 20:5n-3; Figure 4.5c). Total fatty acid content per μg dry weight more than doubled from the log phase to the stationary phase (Table 4.5).

There was an overall decline in PUFA content per cell between days 4 and 11 together with a significant decrease in EPA and an increase in 20:4n-6 and 22:5n-6 (Figure 4.7a). On a specific weight basis, PUFA content increased, mainly due to an

Table 4.4. Variation in the relative fatty acid composition (wt % of total FA) of *Pavlova* sp. with culture age (mean ±standard deviation, n= 3, tr = trace, < 0.1% of total fatty acids)*.).

	Culture age *(days)*									
Fatty acid	**4**		**7**		**11**		**13**		**17**	
14:0	15.55 ±0.06	*a*	15.69 ±0.90	*a*	14.41 ±0.81	*b*	15.29 ±1.16	*ab*	13.87 ±0.28	*b*
15:0	tr		tr		tr		tr		tr	
16:0	16.82 ±0.52		16.77 ±1.54		17.43 ±0.17		17.19 ±0.35		15.47 ±1.02	
18:0	1.33 ±0.01		0.42 ±0.07		0.27 ±0.01		0.32 ±0.01		0.30 ±0.01	
Total SAT	33.70 ±0.57		32.89 ±1.81		32.10 ±0.99		32.80 ±0.81		29.64 ±1.08	
16:1n-7	10.19 ±0.14	*a*	11.86 ±0.71	*b*	13.23 ±0.13	*c*	13.10 ±0.99	*c*	13.71 ±0.32	*c*
16:1n-5	2.35 ±0.42	*a*	3.21 ±0.70	*a*	3.69 ±0.12	*b*	3.88 ±0.32	*b*	4.21 ±0.17	*b*
18:1n-9	0.38 ±0.12	*a*	0.40 ±0.07	*a*	0.71 ±0.07	*a*	1.02 ±0.09	*ab*	1.32 ±0.47	*b*
18:1n-7	0.64 ±0.14		0.93 ±0.38		0.46 ±0.03		tr ±0.01		tr ±0.37	
Total MONO	13.56 ±0.45	*a*	16.40 ±1.40	*b*	18.08 ±0.00	*c*	18.00 ±1.31	*c*	19.24 ±0.27	*c*
16:2n-4	2.46 ±0.15		2.17 ±0.36		1.88 ±0.13		1.99 ±0.17		1.86 ±0.10	
16:3n-4	0.77 ±0.20		0.58 ±0.02		0.43 ±0.01		0.31 ±0.05		0.68 ±0.01	
18:2n-6	2.58 ±0.23		2.88 ±0.69		2.72 ±0.23		2.23 ±0.26		1.94 ±0.09	
18:3n-6	0.52 ±0.02	*a*	0.64 ±0.04	*a*	0.50 ±0.01	*a*	0.46 ±0.03	*ab*	0.36 ±0.04	*b*
18:3n-3	1.92 ±0.12	*a*	2.24 ±0.08	*b*	2.28 ±0.06	*b*	2.53 ±0.03	*ab*	2.15 ±0.00	*b*
18:4n-3	8.92 ±0.34	*a*	6.67 ±0.13	*b*	4.63 ±0.09	*c*	4.79 ±0.12	*c*	4.80 ±0.31	*c*
20:4n-6	4.13 ±0.04	*a*	7.71 ±0.07	*b*	9.11 ±0.29	*c*	10.60 ±0.17	*c*	9.16 ±0.21	*c*
20:5n-3	20.06 ±1.10	*a*	14.58 ±0.06	*b*	11.43 ±1.14	*c*	10.99 ±0.37	*c*	11.08 ±0.51	*c*
22:5n-6	6.79 ±0.44	*a*	8.26 ±0.35	*b*	10.14 ±1.42	*c*	10.19 ±0.29	*c*	11.96 ±0.19	*d*
22:6n-3	4.55 ±0.20	*a*	3.21 ±0.05	*b*	3.01 ±0.67	*b*	2.71 ±0.16	*b*	3.58 ±0.24	*b*
Total PUFA	52.70 ±1.59	*a*	48.93 ±0.50	*b*	46.14 ±3.60	*ab*	46.79 ±0.61	*ab*	47.57 ±1.27	*b*
C_m and C_n PUFA	35.53 ±0.45		33.76 ±0.13		33.69 ±0.88		34.48 ±0.25		35.78 ±0.29	
EPA/DHA	4.41 ±0.06	*a*	4.55 ±0.06	*a*	3.84 ±0.48	*b*	4.05 ±1.14	*b*	3.17 ±0.19	*c*
sum (n-7)	10.19 ±0.14	*a*	11.86 ±0.71	*b*	13.23 ±0.13	*c*	13.10 ±0.99	*c*	13.40 ±0.32	*c*
sum (n-6)	14.02 ±0.41	*a*	19.49 ±0.98	*b*	22.47 ±1.94	*c*	23.47 ±0.70	*c*	22.95 ±0.44	*c*
sum (n-3)	35.45 ±1.48	*a*	26.70 ±0.16	*b*	21.36 ±1.78	*c*	21.02 ±0.13	*c*	20.57 ±0.95	*c*
(n-6)/(n-3)	0.40 ±0.02	*a*	0.73 ±0.04	*b*	1.05 ±0.00	*c*	1.12 ±0.05	*c*	1.12 ±0.04	*c*
sat/unsat	0.50 ±0.02		0.51 ±0.04		0.51 ±0.04		0.51 ±0.03		0.48 ±0.03	

*Means with different designations (*a,b,c,d*) denote significant differences ($p<0.05$)

Table 4.5. Variation in cell content of fatty acids (pg/cell) in *Pavlova* sp. with culture age (mean ±standard deviation, n= 3, tr = trace, <0.1% of total fatty acids).*

	Culture age (*days*)				
Fatty acid	**4**	**7**	**11**	**13**	**17**
14:0	0.308 ±0.063	0.334 ±0.077	0.259 ±0.068	0.270 ±0.032	0.233 ±0.047
15:0	tr	tr	tr	tr	tr
16:0	0.333 ±0.063	0.357 ±0.096	0.312 ±0.068	0.304 ±0.034	0.261 ±0.060
18:0	0.029 ±0.017	0.009 ±0.006	0.000 ±0.003	0.005 ±0.000	0.006 ±0.004
Total SAT	0.670 ±0.125	0.700 ±0.169	0.571 ±0.136	0.578 ±0.065	0.500 ±0.106
16:1n-7	0.203 ±0.044	0.249 ±0.033	0.236 ±0.047	0.231 ±0.041	0.231 ±0.054
16:1n-5	0.047 ±0.015	0.066 ±0.004	0.066 ±0.016	0.068 ±0.008	0.071 ±0.013
18:1n-9	0.008 ±0.003	0.009 ±0.003	0.013 ±0.001	0.018 ±0.000	0.023 ±0.010
18:1n-7	tr	tr	tr	0.006 ±0.000	0.012 ±0.007
Total MONO	0.257 ±0.057	0.323 ±0.031	0.315 ±0.063	0.324 ±0.048	0.336 ±0.067
16:2n-4	0.049 ±0.013	0.046 ±0.011	0.034 ±0.009	0.035 ±0.005	0.031 ±0.006
16:3n-4	0.015 ±0.005	0.012 ±0.002	0.008 ±0.001	0.005 ±0.001	0.011 ±0.002
18:2n-6	0.051 ±0.010	0.060 ±0.011	0.048 ±0.006	0.039 ±0.000	0.033 ±0.009
18:3n-6	0.010 ±0.002	0.014 ±0.003	0.009 ±0.002	0.008 ±0.001	0.006 ±0.004
18:3n-3	0.038 ±0.008	0.047 ±0.009	0.041 ±0.007	0.045 ±0.002	0.036 ±0.008
18:4n-3	0.177 ±0.040	0.141 ±0.027	0.083 ±0.019	0.085 ±0.007	0.080 ±0.016
20:4n-6	0.082 ±0.016	0.163 ±0.032	0.162 ±0.029	0.187 ±0.005	0.155 ±0.037
20:5n-3	0.398 ±0.083	0.308 ±0.057	0.202 ±0.022	0.194 ±0.003	0.186 ±0.036
22:5n-6	0.134 ±0.024	0.175 ±0.036	0.179 ±0.013	0.180 ±0.001	0.202 ±0.045
22:6n-3	0.090 ±0.019	0.068 ±0.013	0.053 ±0.001	0.048 ±0.003	0.061 ±0.016
Total PUFA	1.045 ±0.212	1.033 ±0.190	0.818 ±0.108	0.826 ±0.027	0.799 ±0.176
Total FA	**1.985** ±0.410	**2.113** ±0.399	**1.788** ±0.373	**1.766** ±0.149	**1.686** ±0.371
C_{20}- C_{22} PUFA	0.704 ±0.036	0.714 ±0.035	0.596 ±0.016	0.609 ±0.003	0.603 ±0.033
EPA/DHA	5.462 ±0.072	5.636 ±0.079	4.767 ±0.597	4.051 ±0.142	3.845 ±0.234
sum (n-7)	0.203 ±0.044	0.249 ±0.033	0.236 ±0.047	0.237 ±0.041	0.243 ±0.054
sum (n-6)	0.277 ±0.051	0.411 ±0.076	0.398 ±0.049	0.414 ±0.007	0.395 ±0.092
sum (n-3)	0.703 ±0.148	0.564 ±0.106	0.378 ±0.048	0.371 ±0.015	0.363 ±0.076
(n-6)/(n-3)	0.491 ±0.019	0.905 ±0.050	1.304 ±0.004	1.116 ±0.007	1.338 ±0.054
sat/unsat	0.515 ±0.019	0.516 ±0.051	0.504 ±0.055	0.503 ±0.004	0.441 ±0.033

* Significant differences omitted for clarity. Trends are similar to Table 4.4.

Table 4.6. Variation in weight-specific fatty acid composition (pg/μg dry weight) of *Pavlova* sp. with culture age (mean ±standard deviation, n= 3, tr = trace, < 0.1% of total fatty acids).

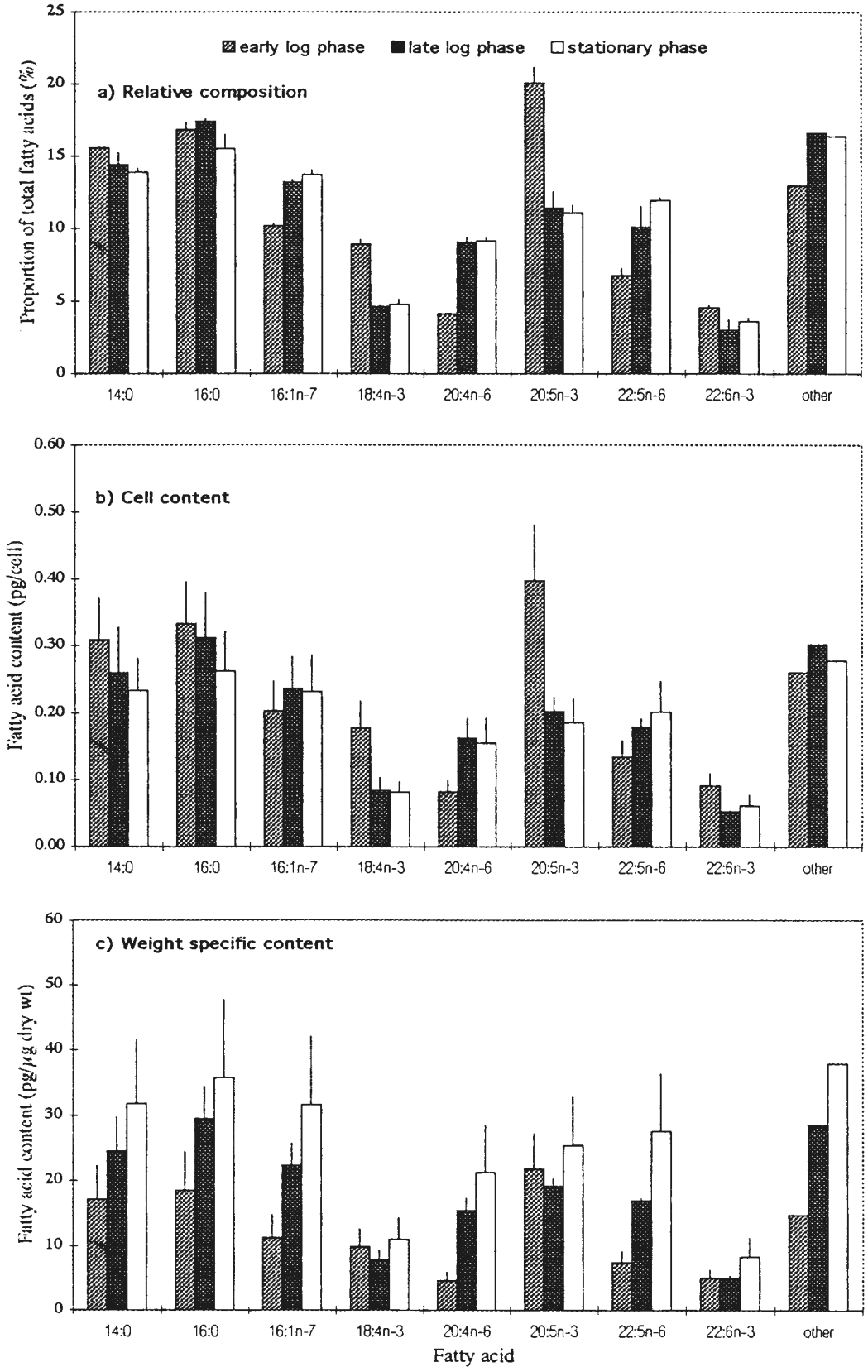
	Culture age (*days*)				
Fatty acid	**4**	**7**	**11**	**13**	**17**
14:0	17.06 ±5.21	23.16 ±5.30	24.43 ±5.16	25.71 ±6.18	31.77 ±9.70
15:0	tr	tr	tr	0.00 ±0.00	tr
16:0	18.50 ±5.88	24.64 ±5.70	29.45 ±4.88	28.92 ±5.98	35.70 ±11.92
18:0	1.98 ±1.14	0.61 ±0.35	0.41 ±0.29	0.45 ±0.26	0.86 ±0.50
Total SAT	35.56 ±11.08	47.80 ±10.78	53.88 ±10.04	55.08 ±5.19	67.47 ±21.53
16:1n-7	11.19 ±3.45	17.23 ±2.80	22.32 ±3.27	22.04 ±4.72	31.57 ±10.42
16:1n-5	2.67 ±1.33	4.55 ±0.59	6.24 ±1.18	6.52 ±1.31	9.61 ±2.78
18:1n-9	0.44 ±0.28	0.60 ±0.18	1.19 ±0.06	1.72 ±0.32	3.17 ±1.70
18:1n-7	0.58 ±0.34	1.42 ±0.69	0.76 ±0.07	0.54 ±0.00	1.59 ±1.06
Total MONO	14.88 ±4.77	23.80 ±3.20	30.51 ±4.45	30.82 ±5.52	45.94 ±13.18
16:2n-4	2.69 ±0.81	3.21 ±0.86	3.20 ±0.72	3.34 ±0.78	4.24 ±1.23
16:3n-4	0.88 ±0.47	0.84 ±0.15	0.72 ±0.10	0.52 ±0.08	1.56 ±0.49
18:2n-6	2.87 ±1.09	4.08 ±0.47	4.57 ±0.33	3.75 ±0.72	4.51 ±1.61
18:3n-6	0.56 ±0.15	0.93 ±0.16	0.84 ±0.11	0.77 ±0.18	0.80 ±0.56
18:3n-3	2.13 ±0.78	3.26 ±0.59	3.84 ±0.50	4.26 ±1.06	4.95 ±1.59
18:4n-3	9.74 ±2.71	9.78 ±1.96	7.84 ±1.38	8.05 ±1.67	10.92 ±3.22
20:4n-6	4.52 ±1.35	11.29 ±2.24	15.34 ±1.91	17.82 ±5.90	21.17 ±7.18
20:5n-3	21.79 ±5.38	21.33 ±4.08	19.14 ±1.08	18.49 ±6.20	25.29 ±7.48
22:5n-6	7.36 ±1.73	12.06 ±2.30	16.94 ±0.28	17.13 ±6.57	27.49 ±8.81
22:6n-3	4.96 ±1.29	4.70 ±0.93	5.00 ±0.35	4.56 ±1.62	8.26 ±2.86
Total PUFA	57.50 ±15.70	71.48 ±13.30	77.44 ±6.07	78.71 ±24.11	108.92 ±34.61
Total FA	**109.77** ±33.60	**146.29** ±28.07	**168.85** ±26.32	**168.23** ±50.73	**230.03** ±73.89
C_{20}- C_{22} PUFA	38.63 ±2.44	49.39 ±2.39	56.42 ±0.90	58.01 ±5.07	82.20 ±6.58
EPA/DHA	5.46 ±0.07	5.64 ±0.06	4.77 ±0.60	4.05 ±1.11	3.84 ±0.23
sum (n-7)	11.77 ±3.45	18.65 ±2.80	23.08 ±3.27	22.58 ±1.72	33.17 ±10.42
sum (n-6)	15.31 ±4.33	28.36 ±4.94	37.69 ±2.63	39.48 ±13.32	53.69 ±17.77
sum (n-3)	38.61 ±10.16	39.07 ±7.54	35.83 ±2.62	35.36 ±10.53	49.42 ±15.13
(n-6)/(n-3)	0.40 ±0.02	0.91 ±0.04	1.30 ±0.00	1.12 ±0.18	1.34 ±0.05
sat/unsat	0.49 ±0.02	0.50 ±0.04	0.50 ±0.06	0.50 ±0.41	0.44 ±0.03

Table 4.7. Variation in relative fatty acid composition (wt % of total FA) in five *different** cultures of *Pavlova* sp. (tr = trace, <0.1% of total FAs).

	Culture				
	a	b	c	d	e
14:0	10.35	11.37	12.11	11.07	11.66
15:0	tr	0.72	1.82	tr	0.51
16:0	14.49	20.48	16.38	22.59	20.94
18:0	0.71	0.35	tr	0.81	0.68
Total SAT	25.55	32.92	30.31	34.47	33.79
16:1n-7	9.08	12.96	13.36	11.6	10.93
16:1n-5	1.97	2.31	2.62	1.77	1.64
18:1n-9	tr	0.64	0.85	0.63	0.5
18:1n-7	tr	0.56	1.13	1.47	1.33
Total MONO	11.05	16.47	17.96	15.47	14.4
16:2n-4	1.2	1.19	1.7	1.48	1.49
16:3n-4	1.05	tr	tr	tr	tr
18:2n-6	3.38	4.76	2.49	3.7	3.18
18:3n-6	0.56	0.44	0.6	0.79	0.75
18:3n-3	1.78	1.99	3.32	3.81	3.88
18:4n-3	8.49	5.74	5.06	4.41	4.47
20:4n-6	3.49	3.24	10.37	6.1	6.21
20:5n-3	23.46	16.8	13.57	14.63	14.85
22:5n-6	9.58	6.75	9.62	11.93	11.52
22:6n-3	9.34	7.64	4.35	2.42	2.47
Total PUFA	62.33	48.55	51.08	49.27	48.82
C_{mn} PUFA	45.87	34.43	37.91	35.08	35.05
EPA/DHA	2.51	2.20	3.12	6.05	6.01
Sum (n-7)	9.08	13.52	14.49	13.07	12.26
Sum (n-6)	17.01	15.19	23.08	22.52	21.66
Sum (n-3)	43.07	32.17	26.30	25.27	25.67
(n-6)/(n-3)	0.39	0.47	0.88	0.89	0.84
Sat/Unsat	0.35	0.51	0.44	0.53	0.53

* this author, unpublished data; same culture methods as section 4.2.1

Figure 4.5. Trends in changes in selected major fatty acids (>2%) in batch cultures of CCMP459, expressed as: a percentage of total fatty acids (a), fatty acid content per cell (b), and fatty acid content standardized per unit dry weight (c). Data shown were condensed to values for early log phase (day 4 sample), late log phase (day 11 sample), and stationary phase (day 17 sample) cultures. Error bars are +standard deviation.


early log phase
late log phase
stationary phase
a) Relative composition
Proportion of total fatty acids (%)
25
20
15
10
5
0
14:0
16:0
16:1n-7
18:4n-3
20:4n-6
20:5n-3
22:5n-6
22:6n-3
other
b) Cell content
Fatty acid content (pg/cell)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
c) Weight specific content
Fatty acid content (pg/μg dry wt)
60
50
40
30
20
10
0
Fatty acid

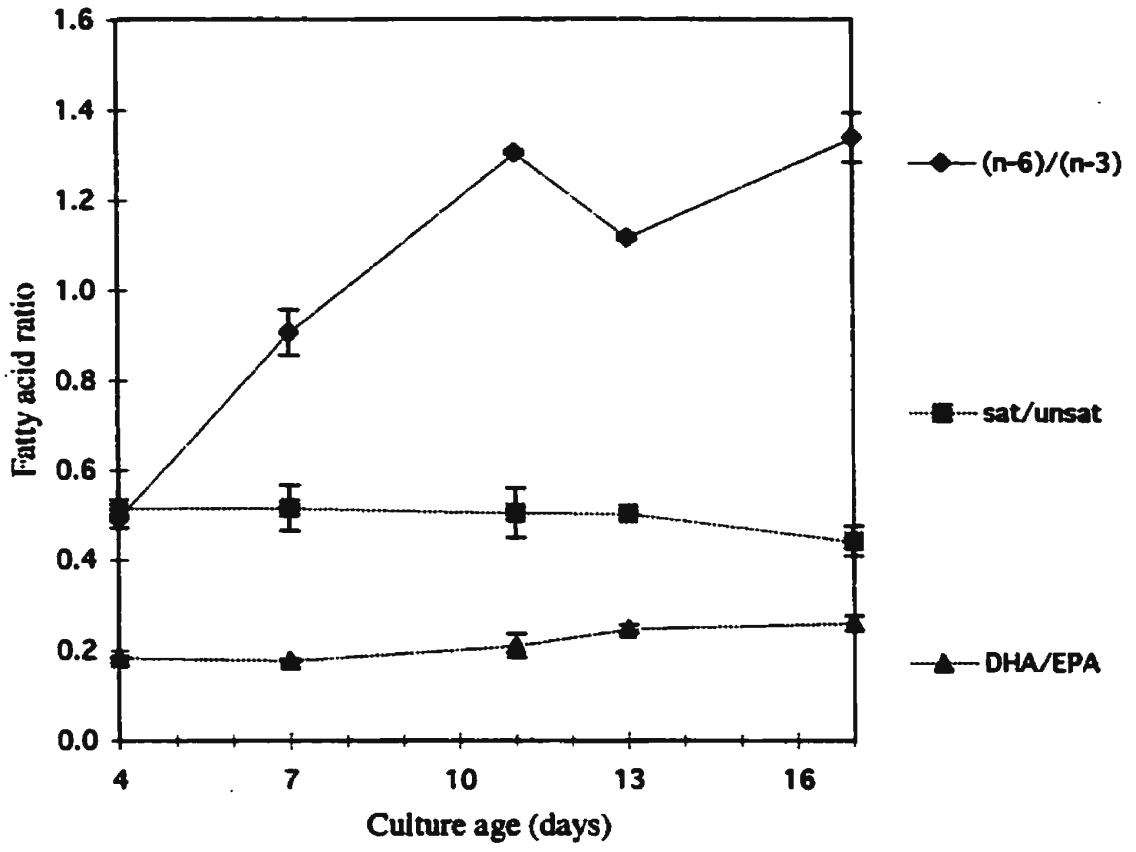


Figure 4.6. Change in (n-6)/(n-3) series, saturated:unsaturated, and DHA:EPA fatty acid ratios of *Pavlova* sp. with culture age. Error bars are ± standard deviations.

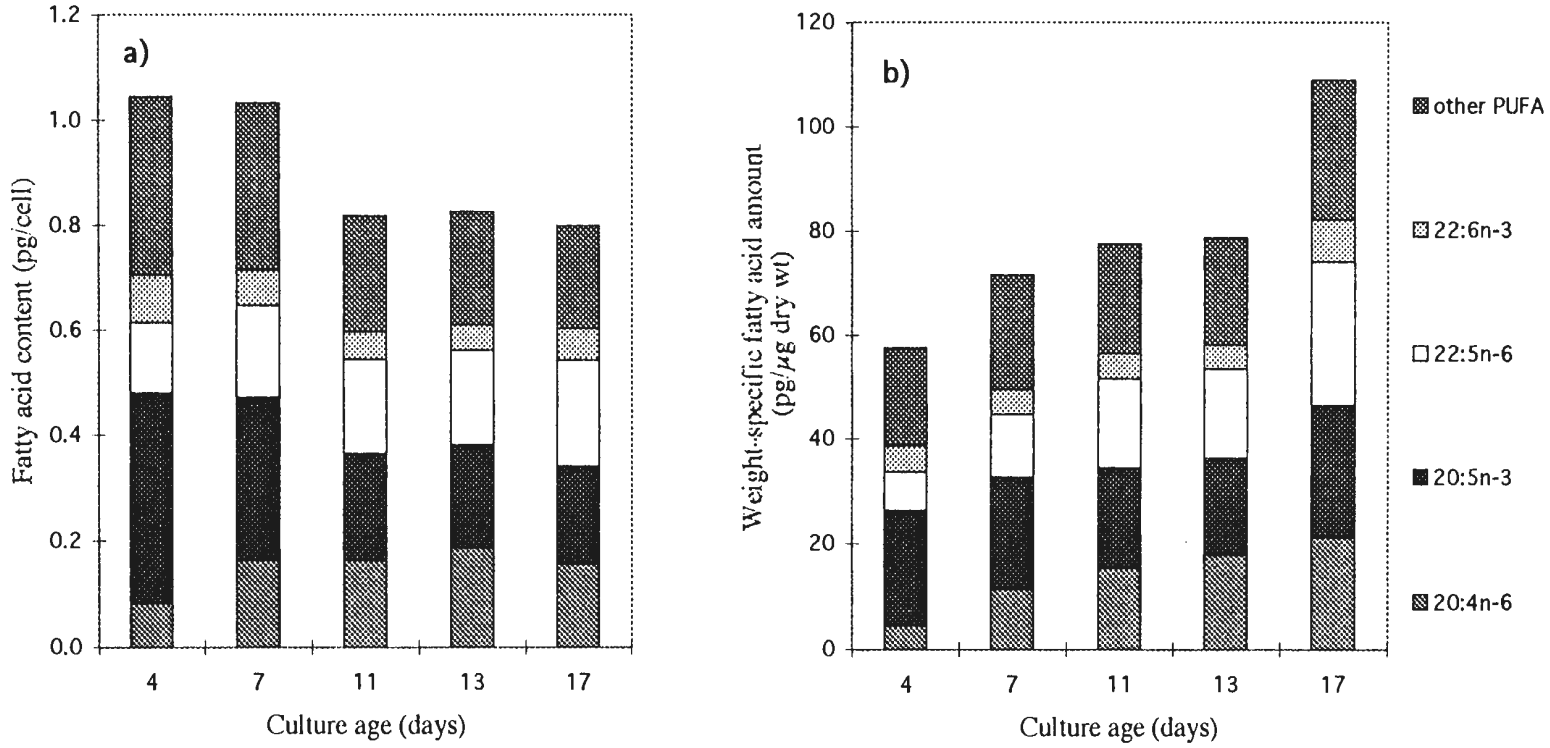


Figure 4.7. Changes in the concentration of selected C_{20} and C_{22} PUFA in *Pavlova* sp. with culture age, expressed as: a) pg per cell and b) pg per μg dry weight.

increase in 20:4n-6 and 22:5n-6 while EPA and DHA changed little (Figure 4.7b).

4.3.7 Fatty acid composition of neutral and polar lipids

Neutral lipids were comparatively enriched in 16:0, 16:1n-7, and 16:1n-5 with slightly more 18:1n-9, 18:2n-6, 20:4n-6, 22:5n-6, and 22:6n-3 (Figure 4.8). They contained less 14:0, 18:4n-3, and much less 20:5n-3 (EPA) than the polar fraction. The DHA to EPA ratio was higher in the neutral lipid fraction, due to the reduced 20:5n-3 level. The (n-6)/(n-3) ratio was also twice as high in the neutral than in the polar fraction. PUFA content was lower in the neutral lipids (38.17% vs. 49.68%, Table 4.8). However, the lower relative content of EPA in the neutral lipids is offset by the increased content of ARA, 22:5n-6 and DHA such that there was no significant difference between C_{20} - C_{22} PUFA as a group between neutral and polar fractions (Table 4.8).

4.4 Discussion

4.4.1 Growth rate and culture variables

The maximum growth rate achieved in the CCMP459 cultures (0.50 divisions/day) was comparable to *Isochrysis galbana* (another haptophyte algae commonly grown in hatcheries) under similar culture conditions (k= 0.55 at 25°C, 2500 lux, 24 hrs light; Hur (1991). With a slight enrichment of CO_2, high cell densities (24 $x10^6$ cells/mL) were obtained in late log phase cultures. Thus, mass culture of this strain should be economical. Temperature and salinity tolerance of this species should also be

Table 4.8. Mean relative fatty acid composition (wt % of total FA) of neutral vs. polar lipids in cultures of *Pavlova* sp. (CCMP459) (mean % ± standard deviation, n= 6).

Fatty acid	Neutral Lipid	Polar Lipid	
14:0	10.35 ±1.03	19.14 ±4.63	**
15:0	0.99 ±1.53	1.47 ±1.22	
16:0	18.59 ±2.80	14.75 ±4.31	*
18:0	0.70 ±0.99	0.68 ±0.44	
Total SAT	29.53 ±3.37	33.89 ±8.44	
16:1n-7	18.61 ±2.74	8.21 ±0.82	**
16:1n-5	6.27 ±4.15	3.56 ±1.80	
18:1n-9	2.28 ±2.08	0.89 ±1.16	
18:1n-7	1.87 ±3.75	0.85 ±0.54	
Total MONO	27.57 ±4.00	13.37 ±1.37	**
16:2n-4	1.10 ±0.68	2.04 ±0.30	**
16:3n-4	0.82 ±0.84	tr ±0.00	
18:2n-6	3.93 ±1.26	3.25 ±2.39	
18:3n-6	tr ±0.00	tr ±0.00	
18:3n-3	1.24 ±0.48	3.31 ±0.63	**
18:4n-3	4.07 ±0.96	8.61 ±2.84	**
20:4n-6	7.74 ±1.86	6.27 ±2.89	
20:5n-3	7.49 ±1.66	13.53 ±5.16	*
22:5n-6	9.04 ±3.77	7.02 ±3.97	
22:6n-3	3.88 ±2.21	1.72 ±1.11	
Total PUFA	38.17 ±5.66	49.68 ±6.82	*
C_{20} and C_{22} PUFA	30.64 ±5.40	31.74 ±5.18	
EPA/DHA	2.21 ±0.65	10.76 ±6.27	*
Sum (n-7)	19.85 ±1.53	9.06 ±0.68	**
Sum (n-6)	22.26 ±2.88	18.28 ±4.50	
Sum (n-3)	16.68 ±3.42	29.41 ±6.85	**
(n-6)/(n-3)	1.33 ±0.24	0.62 ±0.26	**
Sat/Unsat	0.45 ±0.07	0.57 ±0.06	

* = significantly different ($p<0.05$)

** = significantly different ($p<0.01$)

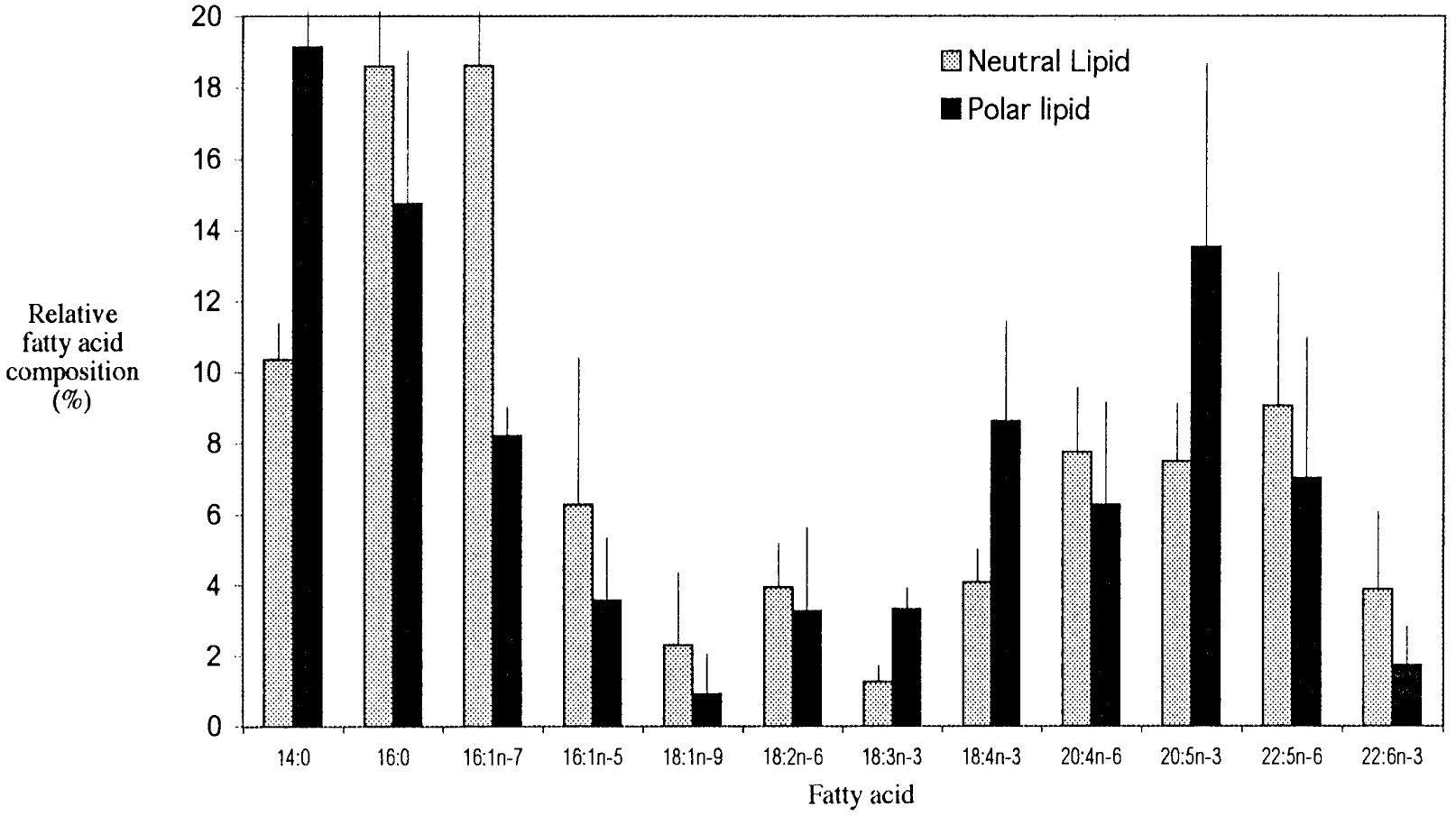


Figure 4.8. Comparison of mean proportions (wt % of total FA) of selected fatty acids of neutral lipids versus polar lipids in *Pavlova* sp. Error bars are +standard deviations.

investigated to determine the range of culture conditions where near optimal growth can be maintained.

The nutrient data indicated that the culture became nitrate limited as it aged (Table 4.1; Figure 4.1). Light may also have been sub-optimal during late log/stationary phase due to self-shading at high cell densities, although irradiance levels were not recorded. In batch algal cultures, endogenous and environmental variables are interdependent. Thus, it is important to note that the terms "culture age" and "growth phase" in this discussion do not necessarily imply direct causal effects, and they are used interchangeably here. Future studies employing turbidostats or chemostats to control growth rate independently of environmental variables are required (Wangersky et al., 1989).

4.4.2 Variability in cell size and dry weight

The combination of the decline in cell size, deviation from spherical shape, and weight-specific increase in total lipid, which is less dense than protein, explains the decrease in cell dry weight with culture age. Cell division and accumulation of storage products (lipids or starch) are regulated at least partially by culture conditions affecting the size and density of microalgae (Norsker and Støttrup, 1991). Brown et al. (1993) found a reduction in cell dry weight of *Isochrysis* sp. at low light levels. Similarly in the present study's results, the apparent variation in algae fatty acid content differed depending on whether the data were expressed in relative (percent) or absolute (cell content and weight specific content) units. Thompson et al. (1994) also found that cell

size of *P. lutheri* grown under high light conditions was larger than for cultures grown under low light.

Fatty acid composition is often presented in the literature in relative proportions due to the ease of generating percent data with GC-FID techniques. However, in experimental studies such as nutritional comparisons in feeding trials, the absolute amount of each fatty acid is a more appropriate measure. In commercial bivalve hatcheries feed is often rationed on a per cell basis, but managers seeking to optimize hatchery protocols should also consider cell dry weight – as considerable intraspecific variation exists. To present results in ways that are both consistent with the literature and experimentally and commercially relevant, the data were reported using all three measures here (Tables 3.3, 3.4, 3.5).

4.4.3 Proximate biochemical composition and changes with growth phase

4.4.3.1 Comparison with other species

Proximate biochemical composition was in the range reported for other species. Commonly, microalgae are comprised of 20-25% protein, 6-12% carbohydrate, and 10-20% lipid as a percent of dry weight, although there is some variation among species (Brown and Jeffrey, 1992). Parsons et al. (1961) found only a small amount of interspecific difference in composition when cells were grown under similar environmental conditions. In previous experiments (Chapter 3) gross composition of four species of algae was also similar when harvested during the same growth phase.

Total lipid per cell was only slightly lower (4.22-3.10 pg/cell) compared with other species in the genus *Pavlova* (4.5-5.7 pg/cell) reported by Volkman et al. (1991), but this discrepancy could be due to differences in analytical or culture techniques. Parrish (1987) reported that Iatroscan lipid values are commonly only 85% of those obtained gravimetrically. As a percent of dry weight, CCMP459 total lipid was as high as (24.1%) or higher (up to 44.1%) than in *Isochrysis* sp. , which is considered a lipid rich microalga (Brown and Jeffrey, 1992).

4.4.3.2 Changes with culture age

Growth phase had a great effect on gross composition. Lipids (primarily TAG) accumulated within the cell with 1.77 times the proportion of lipid in stationary phase cells as log phase cells (Table 4.1b, Figure 4.2). This was reflected in a near doubling of the C:N ratio, which also indicated a loss of protein (Table 4.2). This trend of an increase in lipid with nutrient limitation has been demonstrated for many algae species. Reitan et al. (1994) reported lipid accumulation in response to phosphate limitation for two diatoms, *Phaeodactylum tricornutum* and *Chaetoceros* sp., and two prymnesiophytes, *Isochrysis galbana*, and *P. lutheri*, but not in the green flagellates *Nannochloris atomus* or *Tetraselmis* sp.. Under optimal nutrient conditions, most of the carbon fixed by photosynthesis is incorporated into protein to support rapid cell division, but, when limiting, carbon is shunted into biosynthesis of storage products (mainly lipid) as growth slows (Borowitzka, 1988). Ben-Amotz et al. (1985) also found a significant increase in lipids accompanied by low protein content in nitrate limited cultures of five

taxonomically diverse algae (*Botryococcus braunii*, *Ankistrodesmus* sp., *Nanochloris* sp., *Isochrysis* sp., and *Nitzschia* sp.). In *Dunaliella* spp. however, carbohydrates rather than lipids were accumulated. Carbohydrate accumulation also accompanied lipid storage in *Isochrysis* sp.. Because some authors point to carbohydrate as a possible limiting factor in some bivalve diets (Whyte et al., 1989), more attention should be paid to this nutrient in the future.

4.4.4 Species specific fatty acid profile

4.4.4.1 Total fatty acids

Total fatty acids per cell were slightly lower in *Pavlova* sp. (CCMP459; this study) compared to three other *Pavlova* species (Volkman et al., 1991; 2.6-3.2 pg/cell versus 1.7-2.1 pg/cell for CCMP459). Total fatty acids per cell were comparable to *Isochrysis* sp. (clone T-Iso) – a standard lipid-rich hatchery food (Brown et al., 1993). When expressed as a percent of dry weight, the fatty acid content of CCMP459 was much higher than *Isochrysis* sp. (10.6 - 22.4% for CCMP459 vs. 3.12 - 7.4 %: Brown et al. (1993). However, as previously mentioned, comparisons with values in the literature can be difficult as they are influenced by algal culture conditions and analytical techniques.

4.4.4.2 Fatty acid profile

As in other Pavlovacales, the species-specific fatty acid profile of CCMP459 is diverse compared with other algae used in mariculture (Volkman et al., 1992). Volkman et al. (1991) conducted fatty acid analyses of *P. lutheri* and three other eurythermal

Pavlova species for possible use in tropical hatcheries. The fatty acid profile of CCMP459 (Table 4.3, Figure 4.5) was similar to those reported for other species in Volkman et al.'s (1991) study, with the exception of a much higher level of 20:4n-6 (ARA) in the CCMP459 strain, which is further distinguished from *P. lutheri* by a high level of 22:5n-6 (6.8% - 12.0% vs. < 1-3%, Chapter 3). Significant amounts of 22:5n-6 (3.8-7.4%) were found in *Pavlova salina* and two other unnamed species of *Pavlova* (Volkman et al., 1991) but in lesser amounts than in CCMP459.

Since the content of these two fatty acids in CCMP459 seemed to deviate from other *Pavlova* species, their presence was confirmed using GC-MS (Figure 4.3, 4.4). While MS analysis is not specific for bond position, comparison of the peaks in question with GC retention times for PUFA with related structures (20:4n-3 and 22:5n-3) provides satisfactory confirmation of the 20:4n-6 and 22:5n-6 in our samples (Ackman, 1987).

Despite some differences, the similarities in fatty acid profiles and sterols (Ghosh et al., 1998) support CCMP459's taxonomic designation as a species of *Pavlova*.

4.4.4.3 Fatty acid basis for nutritional value of CCMP459

Pavlova lutheri is known to be a "good" food for mariculture and is commonly incorporated into standard bivalve diets because of the high levels of the essential fatty acids DHA and EPA (Volkman et al., 1992). However, it does not always provide superior growth compared with other species (Enright et al., 1986; Chapter 3). The CCMP459 strain appears to be more nutritious, at least for larval stages of *Placopecten magellanicus* and *Argopecten irradians*, due to the ARA content and possibly the 22:5n-6

fatty acid content (see Chapter 3). ARA is uncharacteristic of prymnesiophytes and normally only a minor component of most marine phytoplankton except some rhodophytes (*i.e.*, *Porphridium*) and a few Bacillariophycaea such as *Astrionella* sp. (Ackman et al., 1968; Viso and Marty, 1993). The combined presence of all essential fatty acids (EPA, DHA, ARA) along with 22:5n-6 in one alga is, as yet, unique to this strain.

4.4.5 Changes in fatty acid composition with culture age

4.4.5.1 Relationship to lipid class composition

The changes in total fatty acid composition with culture age were related to the accumulation of neutral lipids (mainly TAG). Monounsaturated and saturated fatty acids are often associated with lipid storage products such as TAG. Neutral lipids of CCMP459 contained proportionally more 16:1n-7 and 16:0 than polar lipids and hence TAG-rich stationary phase cultures also exhibited a higher proportion of 16:1n-7 and monounsaturates in general. However, the small increase in 16:0 in neutral lipids was offset by a decrease in 14:0 so that total saturates were not significantly different between log and stationary phase cells. The comparatively smaller amount of EPA and 18:4n-3 in neutral lipids versus polar lipids and similar or enhanced levels of 20:4n-6, 22:5n-6, and 22:6n-3 in neutral lipids account for the other trends in total fatty acids.

The AMPL lipid class as a percent of total lipid also decreased with culture age. This may have been due to the decreased effective light intensity in dense cultures. Glycolipids, a principal component of AMPL, have been shown to decline in some

species in response to decreased illumination (Cohen et al., 1988). The fatty acids 18:4n-3 and 20:5n-3 are common in glycolipids of some algae (*e.g.*, T-Iso; Sukenik and Wahnon, 1991), and the reduction of AMPL in late log and stationary phase cultures of CCMP459 may also contribute to the relative reduction of these fatty acids. It would have been useful to separate the AMPL fraction from the total lipid extract to provide a further localization of fatty acid biosynthesis in CCMP459. The marked accumulation of lipid on a weight basis explains the nearly uniform increase in weight-specific fatty acid content.

4.4.5.2 General mechanisms affecting fatty acid trends- comparisons with other algae

Our data support the general conclusion that the fatty acid composition of microalgae is species-specific and related to culture conditions (Ben-Amotz et al., 1985). Many others have studied environmental effects on the biochemical composition of microalgae from nutritional, ecological, and biotechnological (e.g. production of bioactive compounds) perspectives (Borowitzka, 1986). There are underlying, though not immutable, trends in the research.

Lipid accumulation as TAG is one primary mechanism affecting total fatty acids (Webb and Chu, 1983), and it often results in a relative increase in 16:0 and monounsaturates (either 18:1 or 16:1, depending on which is the dominant fatty acid). Like CCMP459, lower levels of 18:4n-3, 20:5n-3, and sometimes 22:6n-3 are often found in studies of fatty acid changes in response to culture environment, due to the association of these fatty acids in declining levels of phospholipid or glycolipid classes (Reitan et al.,

1994). Other culture conditions such as silicate limitation in diatom cultures, decreased light intensity, or stressful temperature conditions can produce a similar effect (Mortensen et al., 1988). Light-dark cycles also affect lipid class composition and hence the total fatty acid profile because TAG is catabolized in the dark phase (Sukenik and Carmeli, 1989).

A reduction in the synthesis of (n-3) fatty acids under suboptimal growth conditions can cause a decrease in total PUFA despite an increase in total lipid (Mortensen et al., 1988). Because CCMP459 contains unusually high levels of (n-6) series long chain fatty acids which appear as prominently in neutral lipids as polar lipids, the reduction in (n-3) fatty acids (20:5n-3 and 18:4n-3) in late log and stationary phase cultures is offset by C_{20} and C_{22} (n-6) fatty acids, and there is comparatively little decrease in total PUFA. For example, saturated and monounsaturated fatty acids comprise approximately 75% of the total fatty acids in neutral lipids of *Isochrysis* sp. (Saoudi-Helis et al., 1994), but only 57% in neutral lipids of CCMP459.

In the rhodophyte *Porphridium*, which also contains relatively high amounts of (n-6) fatty acids, Cohen et al. (1988) found a fatty acid trend similar to CCMP459. The fatty acid 20:4n-6 (ARA) replaced 20:5n-3 (EPA) as the dominant fatty acid in response to decreased light, increased cell density, increased salinity, or suboptimal temperatures or pH. ARA was a main component of neutral lipids and phosphatidylcholine, while EPA was a main component of the glycolipids in *Porphridium*.

Schneider and Roessler (1994) deduced a biosynthetic pathway for PUFA synthesis where EPA is produced through a series of (n-6) fatty acid intermediates.

Figure 4.9a shows two possible pathways for synthesis of EPA. Through [^{14}C]- acetate labeling of *Nannochloropsis* sp. *in-vivo*, Schneider and Roessler (1994) showed that the lower pathway, whereby 20:5n-3 is formed by desaturation of 20:4n-6 at the (n-3) position, predominates. Sukenik et al. (1998) also report the isolation of a mutant strain of *Nannochloropsis* which contains no 20:5n-3 but proportionally more 20:4n-6. A decrease in activity of this proposed desaturase brought about by changes in culture conditions could also explain the trend of decreasing EPA with increasing ARA found for CCMP459. This pathway has also been inferred for *Monodus subterraneus* (Cohen et al., 1992). Furthermore, it could be hypothesized that this shift towards the accumulation of 20:4n-6 could also be related to the increase observed in 22:5n-6 by one of the pathways shown in Figure 4.9b.

4.4.5.3 Within-class differences in fatty acid composition

Saoudi-Helis et al. (1994) found small fatty acid differences within lipid classes (neutral lipids, glycolipids, and phospholipids) related to algal growth rate. For example, DHA levels increased in the neutral lipids of fast-growing chemostat cultures of *Isochrysis* sp. No such differences were found in this study, but the sample size was small (n=3), and the batch culture method used is a less controlled method for manipulating physiological parameters. While changes in the overall fatty acid composition of CCMP459 seem to be most affected by shifts in the proportion of individual lipid classes and the fatty acids associated with them, it is also possible that

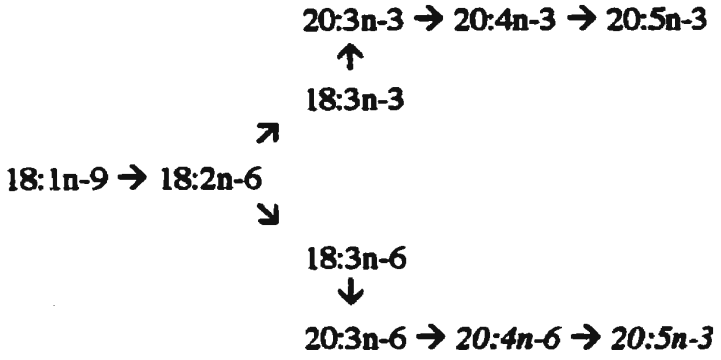


Figure 4.9a. Possible routes of biosynthesis of polyunsaturated fatty acids in algae (adapted from Schneider and Roessler, 1994) showing the linkage between ARA and EPA (in italics).

20:4n-6 → 22:4n-6 → 22:5n-6*

or

20:4n-6 → 20:5n-6 → 22:5n-6

Figure 4.9b. Possible routes of biosynthesis of 22:5n-6 from 20:4n-6 (ARA). *= preferred path in animals.

fatty acid proportions within these individual lipid classes may also change in response to culture environmental or growth phase.

4.4.6 Optimum harvest strategies

Without feeding trials, it is difficult to evaluate optimum harvest periods from a nutritional standpoint. There is still on-going debate over nutritionally desirable characteristics of algae, and the nutritional requirements of bivalves are species specific and life stage dependent (Chapter 1). Nonetheless, considerations include strategies for maximizing PUFA content of cells (mainly EPA, DHA, and ARA), manipulating EPA/DHA and/or (n-6)/(n-3) series ratios, and manipulating gross composition.

4.4.6.1 Maximizing PUFA

Samples taken on day 7 displayed the highest cellular content of total fatty acids and only very slightly reduced total PUFA compared to day 4 samples. EPA was lower but still high (14.5%) and ARA was nearly twice as high (7.7% vs 4.1%). Growth rate also had not slowed by day 7. On a weight basis, PUFA was greatest in late log and stationary phase cultures, although in mass cultures it is common practice to harvest cultures before day 10 because of bacterial concerns (Cornforth, 1990). Dunstan et al. (1993) found PUFA to be highest in stationary cultures of *P. lutheri* due to an increase in total lipid per cell and in the proportion of DHA. PUFA content per cell varied little in *Isochrysis* sp. cultures regardless of growth phase.

PUFA have also been shown to increase in response to decreasing temperatures, presumably in order to maintain cell membrane fluidity, although the pattern of responses reported varies (James et al., 1989). Thompson et al. (1992) reported a "modest but significant" inverse relationship between relative composition of PUFA and temperature for eight species of microalgae commonly grown in hatcheries (including *P. lutheri*). However, except in *Thallassiosira pseudonana*, this trend did not hold true for the nutritionally important essential fatty acid PUFA. Also, because growth slows at lower temperatures, this approach may not be an economical way to enhance PUFA in algae used by commercial hatcheries.

4.4.6.2 (n-6)/(n-3) series ratio

Further studies need to be done to determine optimal (n-6)/(n-3) ratios in microalgal foods, but past work indicates that "good" foods have a relatively high (n-6)/(n-3) ratio (0.5-0.33), whereas "moderate" foods have a lower one (0.25) (Webb and Chu, 1983). CCMP459 has a (n-6)/(n-3) ratio equal to or higher than those of foods listed as "good" in all phases of culture. As pointed out in Chapter 3, few if any feeding trials have included species as rich in (n-6) fatty acids as CCMP459, so it is possible that ratios even higher than 0.5 may be nutritionally superior. It is the levels of arachiodonic acid and possibly 22:5n-6 that account for the high nutritional value of *Pavlova* sp. rather than the ratio per se. For example, *Dunaliella* sp. has a high (n-6)/(n-3) ratio but contains little or no C_{20} - C_{22} chain fatty acids, and is considered a poor food for bivalves. Because this ratio changes dramatically in CCMP459 (from 0.49 in exponential phase cells to 1.34

in stationary phase) this species could be used as an experimental tool to examine appropriate (n-6)/(n-3) fatty acid ratios.

4.4.6.3 Proximate biochemical composition

Few correlations of the gross biochemical composition of diets with growth performance of bivalves have been identified (Brown and Jeffrey, 1992). It is generally assumed that beyond a threshold level, lipid quality impacts on nutrition to a greater extent than total lipid (Gallager and Mann, 1986), and recent emphasis has been on fatty acid and sterol composition (Wikfors et al., 1992). The metabolism of many bivalve larvae is primarily lipid-based (Holland, 1978; Manning, 1986), and the quality of lipids in microalgae is more variable than other components. Chu et al. (1982) analyzed the polysaccharide composition of five algal species used in mariculture and found no major interspecific differences in quality or quantity. Minor differences are found in the composition of essential amino acids, and this may have a small impact on growth performance, but, these differences are less than those found for fatty acids (Webb and Chu, 1983; Enright et al., 1986).

Despite the emphasis on lipid quality in the literature, gross composition is an important consideration in terms of harvest strategies, due to the profound effect of culture conditions. The degree of biochemical variability in CCMP459 was similar to that found by Fabregas et al. (1986) in *Isochrysis* sp. cultures in log and stationary phases. They reported a 177% difference in protein and 136% in carbohydrate content between samples and surmised that this degree of variability must have an important

effect on the nutritive value of this microalga. Some studies have indeed linked an increase in bivalve growth performance with increased carbohydrate in some diets (Whyte et al., 1989; Enright et. al., 1986a) and with dietary protein for juvenile mussels (Kreeger and Langdon, 1993). Ryan (1999) found that log phase algal cultures were generally better for larval nutrition in *P. magellanicus*, and stationary phase cultures were better for post-set animals, and she correlated this with gross compositional characteristics. Utting (1986) found settlement in *Crassostrea gigas* larvae to increase with increasing dietary protein in nitrogen manipulated cultures of *Tetraselmis suecica* and *Chaetoceros calcitrans*, and also found better post set growth on the low-protein *T. suecica* diet. While lipid quality may be the most essential element for adequate nutrition, it seems intuitive that optimization of nutritive value requires an appropriate balance of gross nutrients. These reports also underscore that nutritional requirements of bivalves differ according to developmental stage.

While it has been well established that essential fatty acids (DHA, EPA, and probably ARA) are important for nutrition, attempts to maximize these PUFA in the diet may be misguided. As for total lipid, further inclusion of these EFAs in the diet may not be beneficial beyond a threshold level. Thompson et al. (1992; 1994) reported better growth rates of larval Japanese scallops (*Patinopecten yessoensis*) and Pacific oysters (*Crassostrea gigas*) with diets higher in saturated fatty acids (14:0 and 16:0). The literature suggests that good bivalve diets are high in lipid, have balanced ratios of "essential" fatty acids (Webb and Chu, 1983), and contain an appropriate balance of protein, lipid, and carbohydrate (Whyte et al., 1989). However, due to algal variability,

determining the requirements for lipid quality and gross nutrient balance is difficult without a suitable artificial diet (Langdon, 1983).

4.4.6.4 CCMP459 as food for bivalves — summary

From the perspective of lipid quality, CCMP459 may be a superior food in any culture phase as it contains significant amounts of all essential fatty acids. It also contains Δ-5 sterols (24-ethylcholesta-5,22 dienol) which have been linked to increased juvenile oyster growth (Wikfors et al., 1991). It maintains its characteristically high PUFA profile in stationary cultures due to the high levels of ARA, DHA, and 22:5n-6 in the neutral lipids. Cellular content of EPA is reduced in later growth stages, but remains relatively stable on a specific weight basis and persists in significant quantities in all stages. The result may be that considerable flexibility in the stage of harvest exists from a perspective of lipid quality.

However, a slight increase in the proportion of DHA in CCMP459, as measured in this experiment, may be nutritionally desirable (DHA remained at 3-6% of total fatty acids). Although the possibility that 22:5n-6 could be a suitable substitute for DHA in membranes may mitigate this lower DHA level, incorporating CCMP459 into a mixed diet with a DHA rich algae such as *Isochrysis* sp. may be appropriate. A mixture of *Pavlova* spp. and *Isochrysis* sp. provides a better balance of essential amino acids, and current thinking is that a mixed diet protects against a potential deficiency in some trace nutrients (Webb and Chu, 1983). Nevertheless, there is potential for CCMP459 to replace a multi-species diet and simplify hatchery operations. Fatty acid analysis of other

CCMP459 samples containing DHA up to 9.3% (Table 4.7) shows that DHA can reach higher levels in this strain. It is possible that DHA enrichment may be attained through culture manipulation or genetic selection (Alonso et al., 1992; 1994).

Due to dramatic variation in gross composition and (n-6)/(n-3) ratios, and the potential for fatty acid optimization, use of either specific harvest strategies or a turbidostat or chemostat to control algal biochemical composition is desirable though probably not essential. More research must be carried out to identify the specific nutritional requirements of the farmed animal.

4.5 Conclusions

1) *Pavlova* sp. (CCMP459) performed well in batch culture with growth rates and peak cell densities similar to those for other species of microalgae commonly cultured for mariculture.

2) *Pavlova* sp. is characterized by a comparatively broad fatty acid profile. It is similar in this regard to other *Pavlova* species, but with the important distinction of containing significantly more 20:4n-6 (ARA) and higher 22:5n-6 levels.

3) CCMP459 may be nutritionally superior because of this diverse fatty acid profile and the presence of all the essential fatty acids in significant quantities. It may have the potential to simplify hatchery operations by replacing or reducing the need for a

multi-species diet. At the very least, it could serve as a rich source of ARA and 22:5n-6 when incorporated into mixed diets.

4) Lipid in CCMP459 more than doubled as a percent of dry weight between exponential and stationary growth phases, due mainly to an increase in TAG. This was reflected in a rise in the C:N ratio from 6.16 to 11.67 as the culture aged. AMPL decreased and sterol and PL remained stable as a percent of total lipid.

5) Due to a decline in cell dry weight with culture age, FA trends differed depending on whether data were expressed in terms of weight-specific units or as cell content. As lipid accumulated as a proportion of dry weight with culture age, there was a concurrent increase in weight specific FA content, despite a slight decline in total FA per cell.

6) Fatty acid changes were related to lipid class compositional changes. The (n-3) series fatty acids (especially 18:4n-3 and EPA) were reduced and (n-6) series fatty acids (20:4n-6 and 22:5n-6) increased proportionally. Because of inclusion of long chain (n-6) FA and DHA in neutral lipids, saturates were not substantially elevated in stationary phase cells as sometimes occurs. PUFA remained at relatively high levels regardless of growth stage, with only monounsaturated FA increasing (5-6%).

7) Due to significant changes in gross composition and fatty acids, a chemostat or turbidostat system is recommended for hatchery algal culture systems in order to

optimize the nutritional profile of CCMP459. Nevertheless, significantly more research still needs to be carried out to determine the nutritional requirements of individual bivalve species and different life-history stages of importance to mariculture.

8) Further research with CCMP459 should include an evaluation of culture tolerances (mainly salinity and temperature), and the effect of temperature, irradiance, and light/dark cycles on the fatty acid profile. Experiments should be continued with a turbidostat or chemostat for more refined control of culture variables. Fatty acids should be further resolved to more specific lipid classes (glycolipids, specific polar lipid sub-classes) to further elucidate the mechanisms of fatty acid biosynthesis in this species.

9) It may be worthwhile to continue examining different species and strains of algae that could be nutritionally important, rather than relying on the comparatively small collection of (mostly temperate and subtropical) species and strains that currently make up standard hatchery foods. Only about 50 species out of the several thousand existing in nature have been tested as food for bivalves (De Pauw, 1981). This may be especially important in relation to the farming of cold water bivalves, where lipid quality requirements may be different from that of more temperate/ estuarine species, due to the impact of PL PUFA and/or sterol on maintenance of membrane fluidity (for example) in response to reduced temperatures.

10) **Examining algae species over a range of culture conditions yields better information about their nutritive properties than do isolated reports of species-specific fatty acid profiles.**

CHAPTER 5 - SUMMARY

5.1 Review of findings

The results of this study confirm some of the findings of other pectinid nutrition studies (*e.g.*, Delaunay et al., 1992; Soudant et al., 1996). In terms of the controversy surrounding the importance of EFAs, this study indicated that dietary fatty acids were important in attempts to optimize scallop culture. Diet clearly has an impact on both egg and larval composition. However, the impact of diet lipid quality varies depending on the life history stage of the animal.

The effect of diet on gonad composition varies with the length of the conditioning period and probably with the state of existing endogenous reserves. It appears that the fatty acid composition of eggs is determined, in large part, relatively early in the gametogenic cycle (Soudant et al., 1996). This, in conjunction with the contribution of stored nutrients (Robinson, 1992a,b; Paon and Kenchington, 1996) and the ability of adult animals to partially control lipid metabolism (probably through selective incorporation of important PUFA rather than through modification of assimilated PUFA) serves to constrain the impact of diet. This may explain the failure of some studies to detect differences in egg composition among broodstock fed different diets (e.g. Berntsson et al., 1997). Gonad composition is affected substantially when broodstock are presented with "extreme" diets that are mostly lacking in DHA, EPA, or both, and egg production or quality under these conditions may be decreased (Waldock and Holland, 1984; Chapter 2). DHA and ARA appeared to be conserved to a greater extent

than EPA, especially in polar lipids. This indicates that these compounds were of greater physiological importance, whereas the high EPA content characteristic of eggs may be due to corresponding high levels in many phytoplankton species. Even when the fatty acid profiles of gonads/eggs were altered through manipulation of the diet, proportions of total SAT, MONO, and PUFA, and C_{20} - C_{22} PUFA in polar lipids were maintained. Stress, temperature, genetic, or other as yet unexplained factors may also influence gonad or mean egg fatty acid composition and account for differences between wild stock and artificially conditioned animals. While diminished amounts of ARA and/or DHA in eggs may explain poor hatching rates (Le Pennec et al., 1993), fatty acids cannot explain all of the variability in egg performance exhibited in hatcheries, and egg batches exhibiting a relatively wide range in mean biochemical composition may all produce viable broods (Dorange et al., 1989). It is known that fatty acids from the digestive gland contribute to gametogenesis, but the dynamics of this interaction are poorly understood. Adult tissues not actively involved in lipid metabolism, such as the adductor muscle, are relatively stable in their composition despite changes in diet.

In contrast to the eggs of *Placopecten magellanicus*, larvae are more susceptible to biochemical modification through diet. Neutral lipids in larvae are more sensitive than polar lipids to changes in diet. DHA again appeared to be more important than EPA for larval vigor. A recent study (Jonsson et al., 1999) with larval *O. edulis* also correlated growth with DHA, but not EPA, in the diet. Perhaps the most significant finding is the evidence for enhanced performance of larvae fed diets high in 20:4n-6 (ARA) and 22:5n-6. While ARA as a limiting factor has been suggested in the literature (Napolitano et al.,

1990), it has generally been overshadowed by research concerned with (n-3) PUFA. Although (n-3) fatty acids are predominant in scallop tissue, ARA requirements may be greater than previously thought due to a high turnover rate. A speculative hypothesis for the potential benefits of the more obscure 22:5n-6 fatty acid is that it may exhibit a DHA-sparing effect in membrane lipids. While DHA has a specific, yet unknown, function in pectinids, 22:5n-6 may serve equally well as a structural component in terms of maintaining membrane fluidity, and was readily accumulated in larval polar lipids when present in high amounts in the diet. Dietary optimization in terms of lipid quality is probably more critical for larval rearing than for broodstock management. However, larvae do demonstrate some metabolic control over assimilation of dietary fatty acids.

Chapter 4 presented a more complete picture of the biochemical variability encountered in batch cultures of *Pavlova* sp. with its unique fatty acid profile characterized by high amounts of C_{20} and C_{22} PUFA. Changing batch culture conditions, including available nitrate and phosphate in the culture media and a progressively slower growth rate, produced changes in gross composition and in lipid classes that were in turn linked to varying proportions and content of individual fatty acids. Large changes in both lipid content and quality in batch cultures suggest that time of harvest could have important nutritional ramifications. Thus time of harvest needs to be considered as well as choice of algal species in scallop culture feeding regimes. Alternatively, turbidostat culture could provide a way of controlling algal chemistry in order to standardize feeding. This is important in any attempt to optimize larval culture.

5.2 Significance of findings

The emphasis in this study was on optimized hatchery production of *Placopecten magellanicus*, with an attempt to link and contrast the results with previous studies on bivalve nutrition. However, the results are also important ecologically. Fatty acids have been used in the past as biomarkers of the feeding history of many marine organisms. The effects of lipid quality, especially on larvae, suggest that the fatty acid profile of the standing phytoplankton crop could impact both growth and settlement success in the wild. Algal blooms and plankton dynamics can significantly alter the food quality available to zooplankton (Nelson and Sidall, 1988). Slower larval growth rates will prolong the time to settlement and hence the period that veligers are subject to predation while in the plankton, thus influencing total recruitment. For juvenile *P. magellanicus* as well, Parrish et al. (1995) reported that scallop growth in the field was associated more with particular fatty acids (DHA) present in seston lipids than with total lipid inputs.

5.3 Problems and future research

The study of bivalve nutrition is lagging behind that of fish nutrition because of a lack of suitable defined diets. Although fatty acid profiles of microalgae show species-specific characteristics, variability in their fatty acid profiles is substantial in response to both culture environment and genetic differences among strains. Since there is no direct control over the dietary fatty acid ration but only a choice of algal species or harvest phase in diet treatments, the range of rations is constrained. Data generated may not always conform to the assumptions necessary for statistical comparisons using the

general linear model. Additionally, analyses of lipid classes and fatty acids involve complex multi-step techniques. Errors, in any of these steps, may confound interpretation of results.

These facts may partly explain the divergent views on nutritional requirements of bivalves in the literature. Comparatively little information is gained in simple feeding trials with various microalgal species without an examination of the proximate and micronutrient composition of the diet. While significant advances in the study of bivalve nutrition have been propelled by new analytical and culture techniques, the current approach has been piecemeal and largely descriptive. In lieu of acceptable purified diets for bivalves with nutrient composition manipulated so as to be independent of treatment assignment, a comprehensive battery of standardized feeding trials in conjunction with standardized biochemical analysis and meaningful multivariate analysis is required. Furthermore, because shell growth is at best only an indicator of culture health and vigor, other variables should also be measured in conjunction with shell size. Whyte et al. (1989) found that shell growth of *Patinopecten yessoensis* larvae did not differ among various mixed diet treatments, although the nutritional condition expressed as total energy content of the larvae did. Although some authors contend that a short diet trial is adequate to assess differences in diets, it is important to follow feeding trials past metamorphosis in order to assess the impact of long term dietary deficiencies. Such a comprehensive approach would be a large undertaking, but a necessary one to continue to unify the varied findings. Studies of diet trials taken to more complex biochemical levels, such as molecular species and polar lipid subclass fatty acid composition, are

beginning to appear in the literature (Soudant et al., 1996) and should further our understanding of fatty acid metabolic pathways, dynamics, and specific functions.

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Appendix 2.1. Complete (>0.1%) fatty acid profile (as wt % of total FA) of eggs from *Placopecten magellanicus* fed four different diets (broodstock conditioning experiment #1) (mean ± standard deviation, replicates, n, as indicated).

Diet	*Isochrysis sp.* (n=7)	*T-Iso/Tetraselmis chui mix* (n=6)	*Tetraselmis chui* (n=8)	*Dunaliella tertiolecta* (n=8)	Wild (n=9)
14:0	2.01 ±0.56	1.86 ±0.42	1.56 ±0.45	1.85 ±0.52	2.20 ±0.34
15:0	0.49 ±0.04	0.50 ±0.04	0.55 ±0.05	0.50 ±0.05	0.31 ±0.23
16:0	16.72 ±1.38	16.97 ±1.57	17.04 ±1.31	17.18 ±0.94	18.68 ±2.42
17:0	0.99 ±0.07	0.70 ±0.11	0.71 ±0.10	0.58 ±0.09	0.55 ±0.31
18:0	3.47 ±0.72	3.44 ±0.90	3.46 ±0.51	3.42 ±0.63	4.13 ±0.86
Total SAT	23.27 ±1.68	23.46 ±2.34	23.31 ±1.54	23.53 ±1.01	25.86 ±3.47
16:1n-7	5.08 ±0.77	4.97 ±0.62	4.75 ±0.53	5.26 ±0.94	5.89 ±0.79
16:1n-5	0.29 ±0.20	0.18 ±0.19	0.31 ±0.04	0.38 ±0.23	0.23 ±0.26
18:1n-11	0.14 ±0.18	0.18 ±0.16	0.20 ±0.15	0.11 ±0.14	0.29 ±0.71
18:1n-9	4.94 ±1.54	4.64 ±0.58	4.14 ±0.61	4.05 ±0.80	4.27 ±0.78
18:1n-7	7.67 ±1.48	7.79 ±0.77	7.88 ±0.96	7.22 ±0.92	7.69 ±2.50
18:1n-5	0.42 ±0.09	0.42 ±0.04	0.38 ±0.14	0.37 ±0.14	0.31 ±0.24
20:1n-11	2.43 ±0.47	2.32 ±0.35	2.73 ±0.41	2.44 ±0.68	2.46 ±0.54
20:1n-9	0.75 ±0.19	0.83 ±0.12	0.66 ±0.22	0.64 ±0.11	0.52 ±0.34
20:1n-7	0.46 ±0.21	0.54 ±0.01	0.45 ±0.19	0.47 ±0.11	0.24 ±0.22
Total MONO	22.17 ±2.37	21.86 ±1.39	21.43 ±1.64	20.94 ±0.87	21.90 ±1.86
16:3n-4	0.19 ±0.19	0.15 ±0.17	0.30 ±0.17	0.31 ±0.18	0.11 ±0.16
16:4n-1	1.00 ±0.79	1.44 ±0.43	1.58 ±1.13	1.45 ±0.76	0.21 ±0.63
18:2n-6	2.33 ±0.64	2.53 ±0.60	2.83 ±0.69	2.09 ±0.58	1.66 ±0.30
18:2n-4	0.55 ±0.11	0.52 ±0.10	0.52 ±0.11	0.55 ±0.09	0.56 ±0.28
18:3n-6	0.37 ±0.36	0.48 ±0.36	0.63 ±0.24	0.56 ±0.34	0.06 ±0.11
18:3n-4	0.02 ±0.05	0.06 ±0.09	0.03 ±0.06	0.03 ±0.06	0.01
18:3n-3	3.09 ±1.56	3.49 ±1.40	3.19 ±0.69	3.40 ±1.53	1.73 ±0.75
18:4n-3	6.19 ±2.00	6.30 ±1.28	5.52 ±0.81	5.12 ±0.97	5.63 ±1.12
20:2 NMID	0.25 ±0.18	0.36 ±0.03	0.21 ±0.18	0.18 ±0.14	0.07 ±0.16
20:2n-6	0.98 ±0.23	1.04 ±0.17	1.17 ±0.19	0.92 ±0.23	0.65 ±0.23
20:4n-6	1.38 ±0.23	1.52 ±0.41	2.07 ±0.42	1.58 ±0.39	1.13 ±0.28
20:3n-3	0.55 ±0.40	0.75 ±0.33	0.69 ±0.15	0.76 ±0.38	0.30 ±0.24
20:4n-3	0.36 ±0.06	0.40 ±0.06	0.39 ±0.15	0.38 ±0.16	0.38 ±0.25
20:5n-3	19.42 ±4.85	19.47 ±1.46	20.36 ±1.34	20.83 ±2.90	23.22 ±3.97
21:5n-3	0.72 ±0.32	0.58 ±0.39	0.75 ±0.04	0.73 ±0.29	0.78 ±0.28
22:4n-6	0.14 ±0.19	0.10 ±0.24	0.12 ±0.21	0.20 ±0.19	0.07 ±0.17
22:5n-6	0.72 ±0.54	0.53 ±0.32	0.41 ±0.19	0.56 ±0.36	0.23 ±0.25
22:5n-3	0.67 ±0.23	0.58 ±0.09	0.63 ±0.12	0.73 ±0.14	0.66 ±0.29
22:6n-3	10.46 ±2.08	9.37 ±1.58	8.42 ±1.66	10.07 ±2.15	9.70 ±2.27
Total PUFA	47.55 ±2.47	47.54 ±3.22	47.33 ±1.59	48.09 ±1.45	46.09 ±4.84
20:1 DMA?	1.35 ±0.54	1.31 ±0.46	1.75 ±1.35	1.31 ±0.41	1.18 ±1.04
branched	1.96 ±0.41	2.30 ±0.40	2.25 ±0.58	2.10 ±0.35	1.75 ±1.02
unidentified	1.88 ±0.93	1.41 ±0.82	1.51 ±0.51	1.74 ±0.55	2.13 ±1.37
C_{20}-C_{22} PUFA	35.00 ±5.00	34.20 ±2.03	34.60 ±2.40	36.31 ±2.20	36.50 ±5.31
EPA/DHA	1.92 ±0.61	2.15 ±0.56	2.50 ±0.49	2.17 ±0.55	2.48 ±0.57
sum (n-9)	5.70 ±1.60	5.47 ±0.56	4.79 ±0.59	4.69 ±0.84	4.79 ±0.84
sum (n-7)	13.21 ±2.15	13.30 ±1.30	13.08 ±1.39	12.95 ±1.33	13.82 ±3.03
sum (n-6)	5.79 ±1.23	6.10 ±1.38	7.10 ±1.22	5.71 ±1.28	3.73 ±0.56
sum (n-3)	40.74 ±3.17	40.37 ±2.12	39.21 ±1.45	41.30 ±2.11	41.61 ±4.76
(n-6)/(n-3)	0.14 ±0.04	0.15 ±0.03	0.18 ±0.03	0.14 ±0.04	0.09 ±0.02
sat/unsat	0.33 ±0.03	0.34 ±0.04	0.34 ±0.02	0.34 ±0.02	0.38 ±0.08

Appendix 2.2 Fatty acid composition (as a wt % of total FA) of total lipids of adductor muscles of *Placopecten magellanicus* fed four different diets (broodstock conditioning experiment #1) (mean ± standard deviation).

Diet Fatty acid	*Isochrysis sp.*	*T-Iso/Tetraselmis chui mix*	*Tetraselmis chui*	*Dunaliella tertiolecta*
14:0	1.85 ±0.42	2.86 ±2.21	1.76 ±0.44	2.66 ±1.48
15:0	0.64 ±0.05	0.66 ±0.04	0.44 ±0.39	0.45 ±0.42
16:0	20.00 ±1.30	19.83 ±0.45	20.24 ±2.23	19.33 ±3.13
17:0	0.54 ±0.08	0.55 ±0.04	0.36 ±0.32	0.50 ±0.47
18:0	5.68 ±0.43	6.42 ±0.71	6.35 ±1.86	5.71 ±1.07
Total SAT	28.71 ±0.83	30.31 ±2.45	29.15 ±3.00	28.66 ±4.33
16:1n-7	2.04 ±0.19	2.08 ±0.11	2.40 ±0.12	2.55 ±1.04
16:1n-5	0.58 ±0.10	0.59 ±0.04	0.48 ±0.42	0.34 ±0.29
18:1n-9	2.38 ±0.16	1.89 ±0.70	1.55 ±1.35	1.54 ±0.70
18:1n-7	6.89 ±0.50	7.33 ±0.24	7.57 ±1.33	6.34 ±1.62
18:1n-5	0.34 ±0.08	0.20 ±0.18	0.13 ±0.15	0.00 ±0.00
20:1n-11	0.49 ±0.17	0.59 ±0.02	0.36 ±0.31	0.70 ±0.26
20:1n-9	0.90 ±0.27	1.25 ±0.42	1.54 ±0.53	1.48 ±0.62
20:1n-7	0.85 ±0.33	0.45 ±0.05	0.34 ±0.30	0.26 ±0.24
20:1n-5	0.47 ±0.23	0.00 ±0.00	0.09 ±0.10	0.00 ±0.00
Total MONO	14.94 ±0.54	14.38 ±1.13	14.44 ±0.78	13.21 ±2.83
16:3n-4	0.26 ±0.01	0.35 ±0.07	0.36 ±0.31	0.18 ±0.31
16:4n-1	0.34 ±0.09	0.35 ±0.17	0.09 ±0.10	0.00 ±0.00
18:2n-7?	0.08 ±0.14	0.00 ±0.00	0.20 ±0.35	0.15 ±0.26
18:2n-6	0.78 ±0.11	0.78 ±0.16	0.35 ±0.40	0.67 ±0.33
18:2n-4	0.34 ±0.04	0.21 ±0.19	0.12 ±0.14	0.16 ±0.27
18:3n-3	0.89 ±0.25	0.73 ±0.02	0.54 ±0.49	0.40 ±0.36
18:4n-3	2.47 ±0.42	2.91 ±1.02	2.72 ±0.10	2.32 ±0.10
20:2NMID	0.15 ±0.26	0.00 ±0.00	0.55 ±0.63	0.00 ±0.00
20:2NMID	0.26 ±0.06	0.00 ±0.00	0.00 ±0.00	0.38 ±0.65
20:2n-6	0.99 ±0.29	0.84 ±0.23	0.53 ±0.49	1.18 ±0.59
20:3n-6	0.89 ±0.60	1.19 ±1.00	0.16 ±0.18	1.97 ±1.91
20:4n-6	2.44 ±0.13	2.48 ±0.24	2.35 ±0.29	2.14 ±0.17
20:3n-3	0.29 ±0.02	0.30 ±0.01	0.23 ±0.22	0.14 ±0.24
20:4n-3	0.36 ±0.03	0.54 ±0.20	0.25 ±0.22	0.28 ±0.30
20:5n-3	21.09 ±0.75	21.48 ±2.37	23.41 ±2.01	20.96 ±1.93
21:5n-3	1.11 ±0.13	1.05 ±0.05	1.27 ±0.16	0.91 ±0.43
22:5n-6	0.00 ±0.00	0.00 ±0.00	0.59 ±0.70	0.53 ±0.44
22:5n-3	1.20 ±0.06	1.42 ±0.20	1.76 ±0.84	1.42 ±0.26
22:6n-3	19.55 ±0.67	18.31 ±0.61	19.50 ±0.89	18.97 ±0.91
Total PUFA	53.48 ±1.05	52.95 ±0.55	54.97 ±1.86	52.76 ±2.07
unidentified	1.84 ±0.99	1.32 ±0.27	1.15 ±0.26	3.22 ±3.60
branched	0.40 ±0.28	0.37 ±0.33	0.38 ±0.44	1.89 ±2.52
C_{20}-C_{22}PUFA	48.33 ±1.45	47.62 ±1.56	50.59 ±1.57	48.88 ±2.58
EPA/DHA	1.08 ±0.32	1.17 ±2.65	1.20 ±6.81	1.10 ±2.30
sum (n-9)	3.28 ±0.33	3.14 ±1.13	3.09 ±0.89	3.02 ±0.46
sum (n-7)	9.87 ±0.43	9.86 ±0.28	10.51 ±0.94	9.31 ±2.30
sum (n-6)	5.09 ±0.66	5.29 ±1.28	3.97 ±0.33	6.50 ±2.43
sum (n-3)	46.96 ±0.47	46.75 ±1.48	49.67 ±1.68	45.40 ±1.78
(n-6)/(n-3)	0.11 ±0.02	0.11 ±0.06	0.08 ±0.01	0.14 ±0.09
sat/unsat	0.42 ±0.03	0.45 ±0.07	0.42 ±0.09	0.43 ±0.10

Appendix 3.1. Fatty acid content (pg/cell) of algal diet treatments used in the larval feeding trial (Chapter 2) (mean ± standard deviation, n=4).

Diet	*Isochrysis sp.* (pg/cell)	*Pavlov lutheri* (pg/cell)	*Pavlova sp. (CCMP459)* (pg/cell)	*Thalassiosira pseudonana* (pg/cell)
Branched	0.035 ±0.018	0.047 ±0.024	0.005 ±0.008	0.174 ±0.082
14:0	0.371 ±0.040	0.302 ±0.033	0.317 ±0.018	0.449 ±0.022
15:0	0.015 ±0.006	0.013 ±0.004	0.017 ±0.021	0.022 ±0.004
16:0	0.336 ±0.071	0.550 ±0.116	0.531 ±0.095	0.311 ±0.172
18:0	0.014 ±0.002	0.018 ±0.005	0.010 ±0.011	0.025 ±0.005
Total SAT	0.732 ±0.064	0.883 ±0.104	0.875 ±0.110	0.808 ±0.179
16:1n-9	0.033 ±0.016	0.015 ±0.004	0.013 ±0.014	0.058 ±0.037
16:1n-7	0.111 ±0.018	0.625 ±0.072	0.324 ±0.048	0.579 ±0.119
16:1n-5			0.058 ±0.011	0.000 ±0.000
18:1n-9	0.372 ±0.141	0.031 ±0.004	0.015 ±0.009	0.012 ±0.008
18:1n-7	0.059 ±0.012	0.073 ±0.026	0.025 ±0.017	0.151 ±0.095
18:1n-5	- -	- -	- -	0.008 ±0.016
20:1n-11	0.017 ±0.013	0.005 ±0.000	- -	- -
Total MONO	0.572 ±0.094	0.736 ±0.053	0.435 ±0.079	0.816 ±0.026
16:2n-7	- -	- -	- -	0.095 ±0.045
16:2n-4	0.030 ±0.010	0.027 ±0.005	0.040 ±0.006	- -
16:3n-4	- -	- -	0.006 ±0.013	0.089 ±0.021
16:4n-1	- -	- -	- -	0.016 ±0.015
18:2n-6	0.105 ±0.036	0.027 ±0.009	0.098 ±0.023	0.002 ±0.004
18:2n-4	- -	0.034 ±0.026	- -	- -
18:3n-6	0.034 ±0.000	0.018 ±0.005	0.018 ±0.004	0.006 ±0.008
18:3n-4	- -	- -	- -	0.003 ±0.006
18:3n-3	0.162 ±0.039	0.023 ±0.002	0.083 ±0.028	0.007 ±0.008
18:4n-3	0.582 ±0.104	0.102 ±0.019	0.158 ±0.047	0.072 ±0.058
20:2n-6	- -	0.012 ±0.002	- -	- -
20:4n-6	- -	0.016 ±0.004	0.165 ±0.080	0.011 ±0.017
20:3n-3	- -	0.007 ±0.000	- -	- -
20:4n-3	- -	0.006 ±0.000	- -	- -
20:5n-3	0.012 ±0.003	0.457 ±0.078	0.467 ±0.111	0.476 ±0.074
22:5n-6	0.042 ±0.008	0.035 ±0.007	0.277 ±0.057	0.004 ±0.007
22:5n-3	0.009 ±0.000	0.014 ±0.004	- -	0.013 ±0.017
22:6n-3	0.308 ±0.042	0.281 ±0.026	0.147 ±0.087	0.063 ±0.023
Total PUFA	1.210 ±0.125	1.038 ±0.115	1.457 ±0.163	0.850 ±0.100
Unidentified	0.131 ±0.000	0.100 ±0.000	0.012 ±0.000	0.080 ±0.000
Total FA	2.69 ±0.580	2.75 ±0.477	2.80 ±0.710	2.82 ±0.864
C_{20}-C_{22} PUFA	0.372 ±0.044	0.827 ±0.094	1.055 ±0.133	0.567 ±0.085
EPA/DHA	0.040 ±0.020	1.620 ±0.150	3.980 ±1.900	8.360 ±3.330
sum (n-7)	0.162 ±0.043	0.698 ±0.062	0.350 ±0.058	0.730 ±0.025
sum (n-6)	0.146 ±0.043	0.105 ±0.012	0.558 ±0.098	0.023 ±0.027
sum (n-3)	1.063 ±0.127	0.872 ±0.124	0.854 ±0.212	0.631 ±0.120
(n-6)/(n-3)	0.140 ±0.050	0.120 ±0.020	0.700 ±0.240	0.040 ±0.040
sat/unsat	0.410 ±0.050	0.500 ±0.080	0.470 ±0.080	0.480 ±0.100

Appendix 3.2.1. Complete (>0.1%) fatty acid profile (as a wt % of total FA) of total lipids from eggs, day 2, and day 9 *Placopecten magellanicus* veliger larvae prior to the start of the feeding trial (mean % ± standard deviation, n=3).

Days	Egg (%)	2 (%)	9 (%)
Branched	1.02 ±0.59	1.90 ±0.23	3.77 ±1.11
14:0	2.17 ±0.33	2.17 ±0.22	5.00 ±0.36
15:0	0.51 ±0.29	- -	0.71 ±0.12
16:0	18.80 ±0.97	18.54 ±0.98	28.54 ±1.53
17:0	0.50 ±0.29	0.69 ±0.23	0.57 ±0.50
18:0	3.54 ±0.22	4.29 ±0.20	13.33 ±1.54
20:0	- -	1.06 ±0.32	1.10 ±1.59
Total SAT	24.84 ±1.12	26.75 ±1.95	49.25 ±5.64
16:1n-9	tr -	0.73 ±0.09	1.60 ±0.37
16:1n-7	7.53 ±0.79	6.49 ±0.49	3.00 ±0.56
16:1n-5	- -	- -	- -
18:1n-9	3.81 ±0.22	3.26 ±0.23	3.36 ±0.27
18:1n-7	9.42 ±1.01	9.29 ±0.81	2.26 ±0.11
18:1n-5	- -	- -	- -
20:1n-11	tr -	- -	0.36 ±0.62
20:1n-9	2.97 ±0.21	3.28 ±0.75	2.15 ±0.54
20:1n-7	0.58 ±0.33	- -	0.22 ±0.39
Total MONO	23.93 ±1.95	22.31 ±2.28	12.95 ±2.86
16:2n-4	- -	0.28 ±0.38	0.55 ±0.51
16:3n-4	tr -	- -	1.47 ±1.21
16:4n-3	tr -	- -	- -
18:2n-6	1.36 ±0.07	1.20 ±0.04	1.50 ±0.37
18:2n-4	0.74 ±0.43	0.99 ±0.75	2.61 ±1.04
18:3n-6	- -	- -	- -
18:3n-3	0.90 ±0.52	0.91 ±0.10	1.28 ±0.12
18:4n-3	4.50 ±0.37	3.63 ±0.60	2.58 ±0.29
20:2n-6	0.68 ±0.39	- -	2.00 ±0.33
20:3n-6	tr -	- -	tr -
20:4n-6	1.11 ±0.14	1.19 ±0.16	3.73 ±1.75
20:4n-3	tr -	1.14 ±1.43	tr -
20:5n-3	29.59 ±0.73	24.44 ±0.49	3.96 ±1.59
22:4n-6	tr -	1.09 ±0.35	- -
22:2 NMID?	1.45 ±1.03	- -	0.20 ±0.35
21:5n-3	1.21 ±0.07	- -	- -
22:5n-6	tr -	1.24 ±0.52	1.39 ±0.10
22:5n-3	0.92 ±0.54	- -	0.60 ±0.53
22:6n-3	9.05 ±1.00	8.96 ±0.75	7.41 ±0.50
Total PUFA	48.98 ±1.85	45.05 ±5.58	29.29 ±8.68
Total DMA	tr -	tr -	2.03 ±1.51
Unidentified	0.80 ±0.30	3.20 ±0.78	2.54 ±0.45
Total Fatty acids (pg/larv)	6088.48	2639.09	3242.83
C_{20}- C_{22} PUFA	42.28 ±2.85	38.05 ±3.71	19.30 ±5.14
EPA/DHA	3.29 ±0.27	2.73 ±0.34	0.53 ±0.03
sum (n-7)	16.95 ±1.80	15.77 ±1.30	5.48 ±1.06
sum (n-6)	2.70 ±0.31	4.71 ±1.07	8.61 ±2.54
sum (n-3)	45.56 ±1.15	40.26 ±3.54	15.84 ±3.02
(n-6)/(n-3)	0.06 ±0.01	0.12 ±0.03	0.54 ±0.03
sat/unsat	0.34 ±0.02	0.40 ±0.06	1.17 ±0.20

Appendix 3.2.2. Complete (>0.1%) fatty acid profile (as a wt % of total FA) of the neutral lipid fraction of eggs, day 2, and day 9 *Placopecten magellanicus* larvae prior to the start of the feeding trial (mean % ± standard deviation, n=3).

Days	Egg (%)	2 (%)	9 (%)
Branched	0.78 ±0.62	0.69 ±0.30	3.01 ±0.45
14:0	2.45 ±0.42	4.17 ±0.43	13.17 ±0.96
15:0	0.41 ±0.09	0.79 ±0.10	0.58 ±0.10
16:0	19.90 ±3.64	22.57 ±1.19	23.58 ±1.26
17:0	0.50 ±0.49	±0.00	1.55 ±1.36
18:0	4.07 ±1.36	2.01 ±0.10	12.05 ±1.39
20:0	- -	1.00 ±0.30	- -
Total SAT	27.33 ±3.16	30.54 ±2.22	50.93 ±0.00
16:1n-9	- -	- -	tr -
16:1n-7	6.77 ±2.89	11.17 ±0.84	6.22 ±1.44
16:1n-5	0.34 ±0.24	1.29 ±0.16	8.68 ±0.00
18:1n-9	3.65 ±1.66	3.57 ±0.26	1.69 ±0.14
18:1n-7	9.55 ±3.74	9.80 ±0.86	3.45 ±0.17
18:1n-5	0.39 ±0.28	- -	- -
20:1n-11	0.31 ±0.69	- -	- -
20:1n-9	1.36 ±0.52	0.92 ±0.21	2.93 ±0.74
20:1n-7	0.85 ±0.32	0.43 -	0.58 ±1.00
Total MONO	23.62 ±4.41	25.89 ±2.65	23.55 ±0.00
16:2n-4	- -	- -	0.48 ±0.45
16:3n-4	0.13 ±0.09	0.49 ±0.10	0.23 ±0.19
16:4n-3	0.33 ±0.14	- -	- -
18:2n-6	1.71 ±0.68	1.16 ±0.03	1.3 ±0.32
18:2n-4	0.64 ±0.28	- -	- -
18:3n-6	0.25 ±0.18	0.70 ±0.10	0.1 -
18:3n-3	1.63 ±0.88	0.87 ±0.09	1.23 ±0.12
18:4n-3	6.10 ±2.29	3.94 ±0.66	4.74 ±2.22
20:2n-6	0.78 ±0.32	0.50 ±0.20	0.55 ±0.00
20:3n-6	0.28 ±0.20	1.02 ±0.05	- -
20:4n-6	1.00 ±0.41	0.49 ±0.07	0.67 ±0.08
20:4n-3	0.44 ±0.20	0.28 ±0.35	- -
20:5n-3	22.72 ±2.22	18.50 ±0.37	3.39 ±1.36
22:4n-6?	0.26 ±0.18	1.31 ±0.42	- -
22:2NMID?	- -	- -	0.14 ±0.24
21:5n-3	0.80 ±0.37	- -	- -
22:5n-6	0.29 ±0.21	0.10 ±0.04	2.15 ±0.19
22:5n-3	0.51 ±0.25	0.10 ±0.01	- -
22:6n-3	9.54 ±1.26	3.92 ±0.33	3.45 ±0.23
Total PUFA	45.92 ±5.06	33.38 ±4.13	19.03 -
Unidentified	1.30 ±1.32	6.76 ±2.43	2.36 ±1.24
Tot. fatty acids *(pg/larv)*	4330.58	1855.75	1463.64
C_{20}- C_{22} PUFA	34.38 ±4.42	26.22 ±2.56	10.35 ±2.10
EPA/DHA	3.01 ±0.21	4.72 ±0.00	0.98 ±0.02
sum (n-7)	17.17 ±1.15	21.40 ±1.76	12.71 ±1.18
sum (n-6)	5.11 ±1.35	5.28 ±1.20	4.77 ±0.59
sum (n-3)	39.91 ±2.24	27.61 ±2.43	12.81 ±3.93
(n-6)/(n-3)	0.13 ±0.02	0.19 ±0.00	0.37 ±0.04
sat/unsat	0.39 ±1.53	0.52 ±0.00	1.20 ±0.30

Appendix 3.2.3. Complete (>0.1%) fatty acid profile (as a wt % of total FA) of the polar lipid fraction of eggs, day 2 and day 9 *Placopecten magellanicus* larvae prior to the start of the feeding trial (mean % ± standard deviation, n=3).

Days	Egg (%)	2 (%)	9 (%)
Branched	1.15 ±0.07	tr -	2.36 ±0.12
14:0	0.39 ±0.05	1.05 ±0.11	5.92 ±0.43
15:0	0.18 ±0.01	0.11 ±0.01	0.96 ±0.17
16:0	7.03 ±1.03	7.44 ±0.39	11.48 ±0.61
17:0	0.43 ±0.01	- -	1.51 ±1.32
18:0	7.14 ±0.16	8.01 ±0.38	5.49 ±0.63
20:0		0.99 ±0.30	0.64 ±2.45
Total SAT	15.16 ±1.26	17.60 ±1.28	30.36 ±5.62
16:1n-9	1.36 ±0.03	1.05 ±0.13	4.89 ±1.13
16:1n-7	2.2 ±0.36	1.05 ±0.08	5.99 ±0.00
16:1n-5	1.01 ±0.20	- -	- -
18:1n-9	3.89 ±0.33	2.09 ±0.15	8.12 ±0.66
18:1n-7	2.91 ±0.08	6.66 ±0.58	2.42 ±0.12
18:1n-5	- -	- -	- -
20:1n-11	- -	- -	0.22 ±0.38
20:1n-9	5.07 ±0.05	7.31 ±1.67	0.17 ±0.04
20:1n-7	0.60 ±0.01	0.11 ±0.01	0.00 ±0.00
Total MONO	17.02 ±0.91	17.23 ±1.76	21.82 ±2.33
16:2n-4	- -	0.77 ±1.05	- -
16:3n-4	1.09 ±0.22	0.11 ±0.01	- -
16:4n-3	- -	- -	0.97 ±0.24
18:2n-6	3.76 ±1.00	1.07 ±0.03	7.10 ±2.83
18:2n-4	- -	- -	- -
18:3n-6	0.08 ±0.11	1.39 ±0.02	- -
18:3n-3	1.07 ±0.06	0.84 ±0.09	1.42 ±0.13
18:4n-3	1.78 ±0.19	2.38 ±0.40	3.95 ±0.44
20:2n-6	0.36 ±0.13	1.05 ±0.01	1.60 ±0.40
20:3n-6	- -	1.16 ±0.04	tr -
20:4n-6	2.99 ±0.10	2.35 ±0.32	1.36 ±0.64
20:4n-3	tr -	0.11 ±0.13	tr -
20:5n-3	28.52 ±0.18	31.82 ±0.64	7.18 ±2.87
22:4n-6?	0.49 ±0.06	0.46 ±0.15	- -
22:NMID?	0.69 ±0.02	- -	5.50 -
21:5n-3	0.49 ±0.06	- -	1.04 -
22:5n-6	0.66 ±0.06	3.24 ±1.36	2.25 ±0.16
22:5n-3	0.93 ±0.10	0.11 ±0.01	3.73 ±1.25
22:6n-3	16.29 ±0.74	17.26 ±1.45	6.16 ±0.42
Total PUFA	61.62 ±1.65	64.12 ±7.94	41.73 ±5.99
DMA	5.32 ±0.55	0.80 ±1.20	4.20 ±2.30
Unidentified	2.66 ±0.95	1.20 ±0.22	3.47 ±0.36
Tot. fatty acids(pg/larva)	1757.9	783.3	1779.2
C_{20}-C_{22} PUFA	50.22 ±1.39	57.56 ±5.61	28.30 ±2.35
EPA/DHA	1.57 ±0.06	1.84 ±0.50	1.17 ±0.08
sum (n-7)	4.87 ±0.06	7.82 ±0.65	8.42 ±0.12
sum (n-6)	8.63 ±0.28	10.74 ±2.44	12.31 ±4.03
sum (n-3)	51.90 ±1.71	52.50 ±4.62	23.40 ±5.36
(n-6)/(n-3)	0.17 ±0.01	0.20 ±0.04	0.53 ±0.09
sat/unsat	0.19 ±0.02	0.22 ±0.08	0.48 ±0.06

Appendix 3.3.1. Complete (>0.1%) fatty acid profile (as a wt % of total FA) of total lipids from 18 day old *Placopecten magellanicus* larvae at the end of the feeding trial (mean % ± standard deviation, n=3).

Diet	*Isochrysis sp.* (%)	*Pavlova lutheri* (%)	*Pavlova sp.* (%)	*Thalassiosira pseudonana* (%)
Branched	0.45 ±0.77	1.89 ±1.50	0.75 ±0.98	2.29 ±1.11
14:0	6.44 ±0.99	5.33 ±1.54	5.83 ±0.39	6.03 ±1.39
15:0	0.96 ±0.08	1.16 ±0.36	0.65 ±0.11	1.26 ±0.35
16:0	17.75 ±2.16	14.58 ±0.27	16.13 ±0.80	17.38 ±2.56
17:0	0.81 ±0.05	0.75 ±0.13	0.54 ±0.47	1.10 ±0.41
18:0	6.70 ±1.90	6.77 ±0.31	5.77 ±0.64	9.68 ±1.58
20:0	- -	- -	- -	- -
Total SAT	34.43 ±4.54	31.62 ±0.62	31.77 ±1.46	39.01 ±3.62
16:1n-9	- -	- -	- -	- -
16:1n-7	3.52 ±0.71	4.43 ±0.43	4.12 ±0.81	3.43 ±0.65
16:1n-5	- -	- -	0.76 ±0.10	- -
18:1n-9	12.52 ±3.00	3.61 ±0.47	2.71 ±0.20	6.87 ±0.94
18:1n-7	4.42 ±0.18	5.14 ±0.65	3.88 ±0.19	4.07 ±0.54
20:1n-9+11	3.23 ±0.78	5.05 ±1.22	3.14 ±0.38	3.69 ±0.25
20:1n-7	0.34 ±0.59	1.06 ±0.07	0.79 ±0.16	0.91 ±0.15
Total MONO	24.03 ±1.37	19.28 ±1.83	14.63 ±0.55	18.98 ±0.76
16:3n-4	0.31 ±0.54	0.37 ±0.65	- -	0.38 ±0.66
18:2n-6	3.56 ±0.53	1.31 ±0.12	2.85 ±0.36	2.30 ±0.31
18:2n-4	0.25 ±0.44	0.49 ±0.43	0.81 ±0.34	0.84 ±0.91
18:3n-6	0.19 ±0.32	- -	- -	- -
18:3n-3	2.97 ±0.07	1.19 ±0.36	1.73 ±0.17	1.18 ±0.03
18:4n-3	6.14 ±0.34	2.83 ±0.95	2.16 ±0.54	2.03 ±0.39
20:2n-6	0.77 ±0.08	0.33 ±0.57	0.93 ±0.23	0.16 ±0.28
20:3n-6	0.26 ±0.46	0.55 ±0.95	- -	1.07 ±1.85
20:4n-6	1.43 ±0.43	2.74 ±0.29	6.80 ±0.36	2.60 ±0.37
20:4n-3	- -	0.85 ±0.25	- -	- -
20:5n-3	2.51 ±0.55	6.76 ±0.78	8.28 ±0.40	6.41 ±0.98
21:5n-3	0.93 ±0.07	0.91 ±0.26	0.81 ±0.40	1.25 ±0.84
22NMID?	0.47 ±0.44	2.32 ±1.10	1.29 ±0.78	1.23 ±1.14
22:4n-6	- -	- -	- -	- -
22:5n-6	2.71 ±0.61	2.71 ±0.31	11.72 ±0.84	1.68 ±0.73
22:5n-3	- -	- -	0.20 ±0.35	0.16 ±0.28
22:6n-3	15.38 ±2.62	16.72 ±1.83	10.29 ±0.61	12.81 ±1.83
Total PUFA	36.88 ±4.04	37.10 ±2.22	46.07 ±1.37	30.91 ±2.43
Tot DMA	2.58 ±0.94	3.77 ±2.66	3.39 ±0.78	4.66 ±1.92
Unidentified	1.97 ±2.09	5.97 ±5.20	3.25 ±1.48	2.88 ±0.53
Tot. fatty acids* (pg/larv)	4984.05	3642.13	3656.79	2651.06
dry wt est (pg/larv)	269.73	269.95	287.87	249.96
Tot. fatty acids* (pg/ug)	18.48	13.50	12.70	10.63
C_{20}-C_{22} PUFA	22.04 ±3.28	28.93 ±2.94	37.09 ±1.63	23.51 ±2.63
EPA/DHA	6.17 ±0.35	2.48 ±0.22	1.25 ±0.13	2.02 ±0.28
sum (n-7)	8.28 ±0.86	10.62 ±1.09	8.78 ±0.99	8.42 ±0.03
sum (n-6)	8.48 ±0.90	7.08 ±0.40	22.30 ±1.76	6.74 ±1.01
sum (n-3)	27.94 ±3.44	28.41 ±1.74	23.28 ±0.58	23.68 ±2.77
(n-6)/(n-3)	3.30 ±0.30	4.02 ±0.35	1.05 ±0.10	3.59 ±0.80
sat/unsat	0.57 ±0.10	0.56 ±0.04	0.52 ±0.04	0.78 ±0.09

Appendix 3.3.2. Complete (>0.1%) fatty acid profile (as a wt % of total FA) of the neutral lipid fraction of 18 day old *Placopecten magellanicus* larvae at the end of the feeding trial (mean weight % ± standard deviation, n=3).

Diet	*Isochrysis sp.* (%)	*Pavlova lutheri* (%)	*Pavlova sp.* (%)	*Thalassiosira pseudonana* (%)
Branched	3.03 ±1.25	5.50 ±2.36	2.27 ±1.96	3.00 ±1.46
14:0	10.17 ±1.56	6.95 ±2.00	11.24 ±0.75	17.50 ±4.03
15:0	0.71 ±0.06	1.48 ±0.46	1.55 ±0.26	0.50 ±0.14
16:0	13.28 ±1.61	12.50 ±0.23	18.01 ±0.90	14.10 ±2.07
17:0	tr -	tr -	tr -	tr -
18:0	1.89 ±0.54	2.95 ±0.13	1.90 ±0.21	2.00 ±0.33
20:0	- -	- -	- -	- -
Total SAT	26.05 ±3.43	23.87 ±0.47	32.70 ±1.50	34.10 ±3.17
16:1n-9	- -	- -	- -	- -
16:1n-7	5.53 ±1.12	8.44 ±0.83	14.20 ±2.77	17.10 ±3.25
16:1n-5	1.35 ±0.42	4.33 ±2.34	8.20 ±1.03	3.00 ±1.76
18:1n-9	13.19 ±3.16	3.51 ±0.46	1.66 ±0.12	0.80 ±0.11
18:1n-7	3.10 ±0.13	3.67 ±0.46	3.84 ±0.19	6.30 ±0.84
18:1n-5	- -	- -	- -	- -
20:1n-9+11	1.27 ±0.30	3.03 ±0.73	1.68 ±0.20	- -
20:1n-7	0.52 ±0.90	3.83 ±0.24	0.14 ±0.03	- -
Total MONO	24.96 ±1.42	26.80 ±2.55	29.72 ±1.12	27.20 ±1.08
16:2n-4	0.40 ±0.03	1.43 ±0.26	- -	
16:3n-4	1.95 ±0.38	7.58 ±3.12	2.24 ±1.20	8.80 ±1.24
16:4n-3	0.15	0.45		
18:2n-6	5.28 ±0.79	0.78 ±0.07	3.47 ±0.44	1.30 ±0.18
18:2n-4	- -	2.30 -	- -	- -
18:3n-6	1.58 ±2.74	2.47 ±0.34	1.06 ±0.31	1.10 ±0.55
18:3n-3	3.80 ±0.09	0.91 ±0.28	1.81 ±0.17	0.50 ±0.01
18:4n-3	10.31 ±0.57	7.49 ±2.50	3.12 ±0.78	2.10 ±0.41
20:2n-6	0.55 ±0.06	0.50 ±0.87	1.55 ±0.38	0.10 ±0.17
20:3n-6	1.70 ±1.94	3.42 ±1.93	1.95 ±0.32	0.35 ±0.61
20:4n-6	0.41 ±0.12	0.40 ±0.04	3.18 ±0.17	0.50 ±0.07
20:4n-3	0.53 ±0.20	1.17 ±0.34	0.28 ±0.02	0.80 ±0.01
20:5n-3	1.15 ±0.25	3.54 ±0.41	6.62 ±0.32	14.10 ±2.16
22:4n-6?	- -	- -	- -	- -
22:NMID?	0.11 ±0.10	1.03 ±0.49	- -	- -
21:5n-3	- -	- -	- -	- -
22:5n-6	1.44 ±0.33	0.47 ±0.05	3.96 ±0.28	0.20 ±0.09
22:5n-3	0.25 ±0.03	0.83 ±0.05	0.20 ±0.35	0.10 ±0.17
22:6n-3	8.57 ±1.46	2.64 ±0.29	2.54 ±0.15	1.50 ±0.21
Total PUFA	37.78 ±4.14	33.69 ±2.02	31.98 ±0.95	31.45 ±2.47
Unidentified	8.06 ±8.54	10.14 ±8.83	1.58 ±0.72	4.35 ±0.80
Total FA*(pg/larvae)	2271.77	776.97	1236.57	481.51
C_{20} - C_{22} PUFA	14.71 ±2.19	14.01 ±1.42	20.28 ±0.89	17.65 ±1.97
EPA/DHA	0.13 ±0.01	1.34 ±0.12	2.61 ±0.28	9.40 ±1.31
sum (n-7)	9.15 ±0.95	15.94 ±1.64	18.18 ±2.04	23.40 ±0.08
sum (n-6)	10.96 ±1.16	8.05 ±0.45	15.17 ±1.20	3.55 ±0.53
sum (n-3)	14.45 ±1.78	9.55 ±0.59	11.45 ±0.29	17.00 ±1.99
(n-6)/(n-3)	0.76 ±0.07	0.84 ±0.07	1.32 ±0.13	0.21 ±0.05
sat/unsat	0.42 ±0.07	0.39 ±0.03	0.53 ±0.04	0.58 ±0.06

Appendix 3.3.3. Complete (>0.1%) fatty acid profile (as a wt % of total FA) of the polar lipid fraction of 18 day old *Placopecten magellanicus* larvae at the end of the feeding trial (mean % ± standard deviation, n = 3).

Diet	*Isochrysis sp.*	*Pavlova lutheri*	*Pavlova sp.*	*Thalassiosira pseudonana*
Days	18 (%)	18 (%)	18 (%)	18 (%)
Branched	tr -	0.87 ±0.69	0.09 ±0.03	1.11 ±0.54
14:0	3.04 ±0.46	4.67 ±1.35	2.87 ±0.19	4.11 ±0.94
15:0	1.06 ±0.08	1.03 ±0.32	0.17 ±0.03	1.41 ±0.39
16:0	19.63 ±2.39	14.44 ±0.27	14.15 ±0.70	18.15 ±2.67
17:0	1.36 ±0.09	0.91 ±0.16	0.76 ±0.67	1.33 ±0.50
18:0	9.81 ±2.78	7.44 ±0.34	7.23 ±0.80	11.27 ±1.84
20:0				
Total SAT	34.90 ±4.60	28.49 ±0.56	25.19 ±1.15	36.26 ±3.37
16:1n-9	- -	- -	- -	- -
16:1n-7	1.67 ±0.34	3.18 ±0.31	0.87 ±0.50	1.27 ±0.24
16:1n-5	- -	- -	- -	- -
18:1n-9	10.92 ±2.62	3.47 ±0.45	3.02 ±0.22	8.14 ±1.12
18:1n-7	5.05 ±0.21	5.28 ±0.67	3.63 ±0.18	3.54 ±0.47
18:1n-5				
20:1n-11	2.17 ±1.10	2.10 ±1.82	2.34 ±0.42	3.24 ±1.68
20:1n-9	4.46 ±1.07	5.33 ±1.29	3.63 ±0.44	4.47 ±0.30
20:1n-7	0.18 ±0.31	0.29 ±0.02	1.04 ±0.21	1.10 ±0.18
Total MONO	24.45 ±1.39	19.65 ±1.87	13.67 ±0.51	21.76 ±0.87
16:2n-4	- -	- -	- -	- -
16:3n-4	- -	0.52 ±0.30	- -	0.36 ±0.10
16:4n-3	- -	- -	- -	- -
18:2n-6	1.94 ±0.29	1.38 ±0.12	2.37 ±0.30	2.49 ±0.34
18:2n-4	0.43 ±0.74	0.60 ±0.52	1.15 ±0.47	1.02 ±1.10
18:3n-6	- -	- -	- -	- -
18:3n-3	2.08 ±0.05	1.20 ±0.36	1.58 ±0.15	1.31 ±0.03
18:4n-3	2.42 ±0.13	1.50 ±0.50	1.56 ±0.39	2.00 ±0.39
20:2n-6	0.88 ±0.10	0.27 ±0.46	0.58 ±0.14	0.18 ±0.30
20:3n-6	tr -	tr -	tr -	tr -
20:4n-6	2.09 ±0.62	3.21 ±0.34	8.07 ±0.42	3.04 ±0.43
20:4n-3	0.00 -	1.02 ±0.30	- -	- -
20:5n-3	3.34 ±0.73	7.28 ±0.84	8.52 ±0.41	6.92 ±1.06
22:4n-6?	0.44 ±0.03	0.66 ±0.19	0.00 ±0.00	1.29 ±0.87
22NMID?	0.70 ±0.66	2.54 ±1.21	1.82 ±1.10	1.49 ±1.37
21:5n-3	1.57 ±0.73	1.10 ±0.54	1.15 ±0.19	1.51 ±0.78
22:5n-6	3.45 ±0.78	3.16 ±0.36	14.63 ±1.05	1.99 ±0.86
22:5n-3	- -	- -	0.19 ±0.32	0.18 ±0.30
22:6n-3	19.26 ±3.29	19.58 ±2.14	13.30 ±0.79	14.70 ±2.10
Total PUFA	40.65 ±4.45	46.38 ±2.77	57.33 ±1.71	40.86 ±3.21
DMA	2.06 ±1.24	2.36 ±1.02	2.43 ±0.67	2.40 ±0.78
Unidentified	0.50 ±0.08	4.61 ±1.50	3.83 ±2.02	1.53 ±1.32
Total FA (*pg/larv*) *	2707.3	2865.2	2420.2	2174.6
C_{20} - C_{22} PUFA	31.74 ±4.73	38.82 ±3.94	48.24 ±2.12	31.29 ±3.50
EPA/DHA	0.17 ±0.01	0.37 ±0.08	0.64 ±0.07	0.47 ±0.07
sum (n-7)	6.90 ±0.72	8.75 ±0.90	4.68 ±0.52	5.92 ±0.02
sum (n-6)	9.92 ±1.05	9.12 ±0.51	26.79 ±2.12	9.20 ±1.38
sum (n-3)	29.54 ±3.40	31.76 ±1.95	25.14 ±0.63	26.76 ±3.13
(n-6)/(n-3)	0.34 ±0.08	0.29 ±0.02	1.07 ±0.11	0.34 ±0.08
sat/unsat	0.54 ±0.10	0.43 ±0.03	0.35 ±0.03	0.58 ±0.06