Optimisation of Essential Lipids in Artificial Feeds for Australian Abalone

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FRDC Project 94/85

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1. PROJECT

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Project Number: 94/85

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2. NON-TECHNICAL SUMMARY

94/85 **Optimisation of Essential Lipids in Artificial Feeds for Australian Abalone**

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OBJECTIVE:

To determine the amounts and types of lipids, which should be added to formulated diets to maximise abalone growth rate

NON-TECHNICAL SUMMARY

Lipids and in particular polyunsaturated fatty acids (PUFA) are important for a number of physiological functions in animals. PUFA are also major components of cellular membranes. Many marine animals cannot synthesise "essential" PUFA *de novo* and therefore serious deficiency signs such as low growth rates, reduced survival, low fecundity and lowered disease resistance can occur if sufficient amounts of these fatty acids are not supplied in the diet.

The main focus of our research was to determine the requirement of abalone for PUFA for optimal muscle growth and the appropriateness of alternative lipids as sources of essential PUFA. None of this work had been performed for abalone previously.

1. Current diets and how they are inappropriately formulated to meet the lipid requirements of abalone

Abalone culture in Australia initially relied on the feeding of fresh seaweeds collected from the wild in states where this was permitted. Seaweeds identified by farmers as producing good growth rates in abalone were analysed and found to have a very low lipid content (< 2% dry weight). A trial conducted showed that of two seaweeds, abalone growth rates on the arachidonic acid (ARA; 20:4ω6) rich *Gracilaria ramulosa* cf *cliftonii* was inferior to a basal formulated feed based on fish oils, whereas growth using eicosapentaenoic acid (EPA; 20:5ω3) rich *Polysiphonia perriniae* were comparable to the formulated feed. Due to the variability in abalone growth with different seaweed species, high cost of collection and transport, lack of availability of large quantities of suitable seaweed species year-round and environmental concerns with seaweed collection, alternative feeds to fresh seaweed were required if abalone culture was to expand and be sustainable in the long term.

Australian and international diets available to Australian abalone farmers were analysed and showed a wide range of lipid inclusion levels (ranging from 1 to 11% wet weight). Also they had very different fatty acid compositions indicating the use of oils varying from purely vegetable sources to those substantially of fish origin. It was not known whether the types and amounts of oils used in these diets had been found to be successful for the local abalone for which the food had been designed, or were just the cheapest or most convenient ingredients to use. At the time that the project commenced, Australian abalone farmers were not satisfied that the available feeds were producing the desired abalone growth rates.

Our trials showed that feeds formulated with lipid levels higher than 4% dry weight would suppressed growth and increased the fat content of the abalone's flesh. Such feeds not only reduce productivity for the farmer but possibly affect marketability and value of the product. Feeds without any or enough of the long chain ω3 PUFA (from fish oils) were found to produce low growth rates. Therefore many of the available commercial abalone feeds were inappropriate for these reasons.

2. How this project contributes to our current knowledge of the lipid requirements of abalone

Prior to this project, the lipid class, fatty acid, and sterol compositions of the local species of abalone, and that of the natural and formulated feeds were not known. Such baseline data are useful to indicate which nutrients are important to the animal and the nutritional adequacy of the formulated feeds. Abalone are different to most other cultured species in that they are strict herbivores. Abalone meat was low in lipid and the major fatty acids in the foot muscle were 16:0, 18:0, 18:1ω9, 18:1ω7, 20:4ω6, 20:5ω3 and 22:5ω3. The fatty acid composition of Australian abalone was very similar to other species of abalone, but quite different from other marine animals which typically have 20:5ω3 and 22:6ω3 as the main tissue PUFA, implying that abalone's lipid requirements would also be different. The major sterol in all samples of abalone muscle, like other animals, was cholesterol, irrespective of diet.

Two feeding trials identified that poor growth occurred with a diet containing only the C_{18} PUFA and none of the long-chain PUFA. Enrichment with 20:4ω6 was less effective than with 22:6ω3 whereas diets with both 20:5ω3 and 22:6ω3 produced the highest growth rates. The results of a "winter trial" showed a distinct growth advantage in using oils with elevated levels of 20:5ω3 (relative to other PUFA) in the colder months. The data also showed that 20:5ω3 (high in diatoms, seaweeds and fish oils) cannot be substituted with the same amount of 20:4ω6 (high in most seaweeds) to yield the same growth rates. The results indicated that abalone growth rate was more responsive to the type, rather than the amount of C_{20} PUFA present in the diet. Therefore increased growth rates in abalone can be achieved by using diets with a high ω3/ω6 ratio (up to 2.0) (ie. those that include fish products, and reduced levels of vegetable oils), and which have elevated levels of 20:5ω3 for cooler temperature culture. There was no growth advantage of using a phospholipid instead of a triacylglycerol (oil), even though the former is the major lipid class in seaweeds and also has been shown to be essential for prawns.

Two feeding trials established that growth rate decreased with increasing dietary lipid content. Because the higher lipid levels in the diets were achieved by the addition of fish oil, the ω3/ω6 ratio of the diets increased with lipid level. Unlike the previous PUFA trial, where elevated ω 3/ω6 ratios (up to 2.0) of the diets at constant lipid levels increased growth rates in abalone, even higher ω3/ω6 ratios (up to 4.5) of the diets at elevated lipid levels (>4% dw) did not. There was also a significant growth advantage in adding the kelp powder to the diet.

3. How this project contributes to our current knowledge of digestibility of lipids by abalone

Prior to the current project, the digestibility of total lipid, lipid classes, fatty acids or sterols by abalone had not been examined. This is probably the first single study determining the apparent digestibility of all these lipids by a single organism.

Our findings were that the digestibility coefficient for total lipid, total fatty acids and total sterols was 84.7, 86.6 and 52.5% respectively. The apparent digestibility of the triacylglycerols was much higher (97.9%) than that of the polar lipids (76.2%) present in the formulated diet, indicating that fish oil (triacylglycerols) was highly digestible by abalone. Of the fatty acids, apparent digestibility increased with increasing level of fatty acid unsaturation (SFA 80.3%, MUFA 87.7%, PUFA 91.3% digestible) and ω3 PUFA were generally more

digestible than ω6 PUFA (94.8% cf 89.1%, respectively). Therefore the PUFA in fish oils (long chain and highly unsaturated ω3 PUFA) were more digestible than those derived from terrestrial plant oils (shorter chain, less unsaturated PUFA which are usually ω6 PUFA), when both are present in the diet. Apparent digestibility values for the sterols showed that cholesterol, the main sterol found in animal tissues (and in this case from the fish oil) is much less digestible than the phytosterols derived from plant ingredients (25.7% cf 60-80%). This suggests that adequate cholesterol is obtained from dietary fish oils to satisfy the nutritional requirement of abalone for this sterol and the excess cholesterol is excreted.

4. Recommendations to diet formulators to improve diet formulation in terms of lipid content

Our trials showed that feeds formulated with lipid levels less than 4% dry weight would improve growth and maintain the lipid content of abalone flesh similar to that of wild caught abalone. The project has demonstrated the importance of maintaining adequate dietary concentrations of the long chain ω3 PUFA (from appropriate fish oils) to ensure high growth rates in abalone. Recommendations for abalone diets are:

• Amount of total lipid in the diet	≤ 4% dry weight
• Amount of total PUFA in the diet	no correlation between total PUFA and growth rate in the high PUFA diets tested (type of PUFA more important)
• Total ω3 PUFA	maximise with long chain ω3 PUFA (from fish oils) not short chain ω3 PUFA (from vegetable oils)*
• Total ω6 PUFA	minimise by not using vegetable oils (adequate ω6 PUFA were gained from other plant ingredients used)
• Ratio of ω3/ω6	>1.2*
• Total 20:5ω3	>0.3 % dry weight (especially in colder water temperatures)*
• Total 22:6ω3	>0.3 % dry weight (but 20% better growth achieved when 20:5ω3 added as well)
• Total 20:4ω6	addition better than no long chain PUFA but not as good as the ω3 PUFA listed above
• Sterols	enough obtained from low levels of fish oils in diet

*Maximise within constraints of total lipid level by the addition of appropriate fish oils at the expense of vegetable lipid/oil containing ingredients.

5. Recommended ingredient sources to supply sterols, ω3, ω6 cholesterol, etc in abalone diets

The major PUFA in commercial vegetable oils (eg. sunflower, canola, soya, evening primrose) and other terrestrial-plant products (eg. soya flour and semolina) was usually 18:2ω6; although some also had high levels of 18:3ω3 (eg. linseed oil). Casein on the other hand contained high proportions of saturated fats, and contributes very little PUFA to a formulated feed. In comparison, the main PUFA in most fish oils and other fish products (e.g. fish meal) were long-chain 20:5ω3 and 22:6ω3 fatty acids - the so-called "essential fatty acids" for marine animals. Wax ester rich fish oils (eg. from orange roughy, escolar, and many deep-sea fishes) should be avoided due to the relatively low levels of ω3 PUFA present. Most of the fish oils tested (mackerel, MaxEPA, cod liver oil) contained the recommended levels of ω3 PUFA and produced favourable growth rates. The novel oils developed for the project (worm oil and abalone oil) produced comparable growth rates in abalone.

6. Potential opportunities for marketing abalone as a healthy food

Due to its high proportion of ω3 PUFA and especially 20:5ω3, and it being a particularly unique and good source of high levels of 20:4ω6 and 22:5ω3, abalone could readily be promoted as a healthy low-fat seafood and one that is rich in essential omega 3 fatty acids. Abalone has great potential to supply a niche market for health conscious consumers.

KEYWORDS: Abalone, Diet, Formulated diets, Lipids, Nutritional requirements, Polyunsaturated fatty acids, Sterols

3. BACKGROUND

At the commencement of this project the Australian abalone fishery was worth in excess of $91M p.a. (FRDC Research and Development Plan 1993-94 to 1997-98). With value adding, abalone exports of $168M were recorded for 1992-93 (NFIC newsletter, November 1993). There continues to be a high demand both from Asia and Australian markets for the local species of abalone, but stocks are heavily regulated and less abalone can be taken each year. This has led to the expansion of abalone culture, necessitating a major research effort to identify the specific nutritional requirements of abalone so that an appropriate formulated diet can be formulated and produced.

A review by Dr J. L. McKoy commissioned by the FRDC into the needs of abalone research in Australia identified abalone nutrition to be a high priority area for research. While research overseas has identified particular aspects of the nutritional requirements of their local species of abalone, this information may not be applicable to the Australian abalone which prefer to eat different species of macroalgae with very different biochemical compositions. Harvesting of macroalgae from the natural environment for abalone diets is not considered viable in the longer term due to environmental concerns, and the culture of macroalgae for abalone diets is presently uneconomical. Formulated diets are the preferred option, but increased productivity and profits for the abalone industry will only be realised when these relatively expensive formulated diets have been optimised nutritionally for the local species of abalone.

The potential of abalone aquaculture was recognised by the Cooperative Research Centre for Aquaculture. This CRC has funded studies of the energy, protein and amino acid requirements of abalone by the South Australian Research and Development Institute (SARDI), South Australian Abalone Development (SAABDEV) and the University of Tasmania (UTAS). Research carried out within the FRDC Abalone Aquaculture Sub program has been organised to complement that being done by the CRC. Ultimately, the main objective of both the FRDC sub program and CRC is to develop a cost-effective formulated diet that supports a high growth rate of abalone. The formulated diet developed for the industry will be a modification of the diet identified as producing the best growth rates, but which can also be produced at a competitive price.

Prior to the research outlined in this report, the nutritional requirements of abalone for particular essential lipids had not been examined. While total lipid has been used successfully by pig and poultry researchers to indicate the nutritional lipid status of diets, for practically every marine animal so far tested, it is the presence and amounts of particular lipid components, which are nutritionally important rather than total lipid *per se*. Most important are the long-chain polyunsaturated fatty acids (PUFA or essential fatty acids), although for some organisms particular lipid classes and sterols are also essential. The importance of these dietary lipids is generally peculiar to marine animals and therefore it is not usually possible to extrapolate from nutritional studies on freshwater or terrestrial species.

Our research objectives were to determine the particular lipid components essential for rapid growth and development in the local commercial species of abalone. The research was designed to integrate the work being performed by colleagues on energy and protein requirements of abalone within the FRDC Abalone Aquaculture Subprogram and CRC. The findings of our research will be used to optimise the lipid components in the cost-effective formulated diets being developed by the FRDC Abalone Aquaculture Subprogram and CRC.

This should lead to enhanced growth rates and result in a cheaper diet of high nutritional value, which is presently lacking for the Australian species of abalone being cultured.

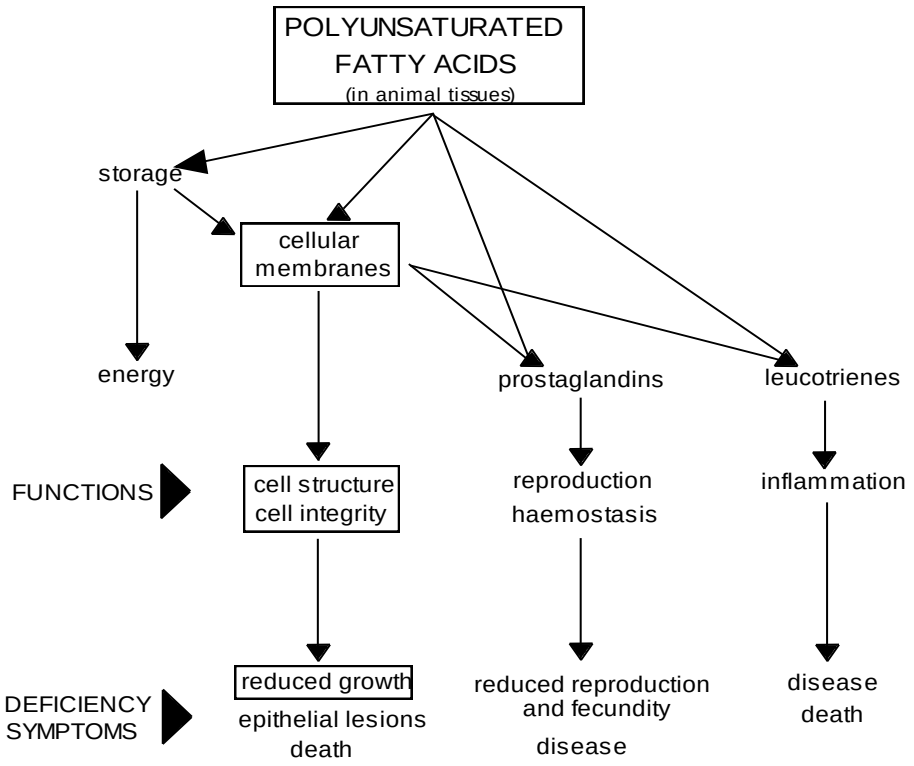
4. NEED

The main problems with the formulated diets available for abalone culture in Australia are that they:

1. produce lower growth rates than the desired industry commercial rate of 25 to 35 mm shell length increase *per annum*;
2. are expensive to produce and costly to use for the extended growth periods required for these slow growing molluscs;
3. do not last long enough in water and result in tank fouling with increased potential of stock loss through infection; and
4. are generally unsuitable for newly settled larvae.

The critical issue of enhancing growth rate through identifying specific lipid requirements was addressed by our research proposal.

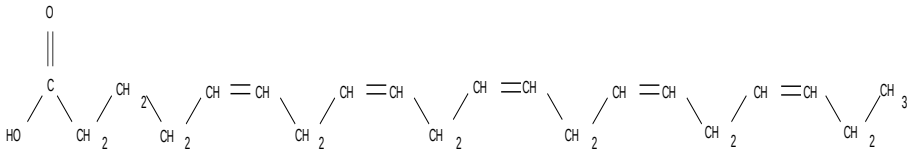
Figure 4.1: Physiological functions and deficiency symptoms due to a lack of essential dietary PUFA in animals



Insufficient quantities of particular dietary lipids, especially the long-chain polyunsaturated fatty acids (PUFA), have been shown to be responsible for low growth rates, reduced survival, low fecundity and poor disease resistance in many marine animals (Figure 4.1).

The naming of fatty acids has been simplified by the use of the following system: As an example, eicosapentaenoic acid (EPA, represented in Figure 4.2) is denoted by 20:5ω3 where the first number (20) refers to the number of carbon atoms in the molecule ("C" in Figure 4.2), the second number (5) refers to the number of double bonds ("=" in Figure 4.2) between carbon atoms, and the third number indicates the position of the first double bond from the methyl end (the "CH_3" in Figure 4.2) of the molecule which nutritionists call ω3 (omega 3) and the chemists call n-3 (n minus 3).

Figure 4.2: Structure of one of the main essential polyunsaturated fatty acids, eicosapentaenoic acid (20:5ω3)



20:5ω3

"20" is the number of Carbon Atoms (C in the figure)

"5" is the number of double bonds (= in the figure)

"3" is the number of carbon atoms to the first double bond from the methyl (CH_3) end of the molecule (omega 3 or ω3)

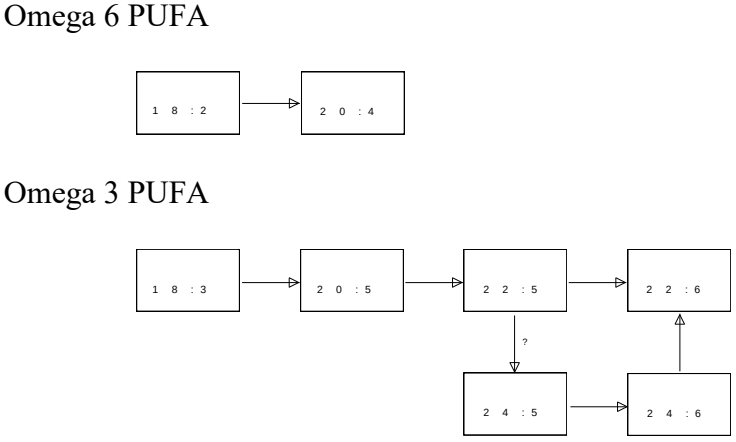
The nutritionally important PUFA are the ω6 and ω3 series (Figure 4.3). Generally these PUFA cannot be synthesised *de novo* by animals and thus must be obtained from the food eaten by the animal. Also ω6 and ω3 PUFA are poorly or unable to be interconverted by animals. That is, ω3 PUFA cannot be formed from ω6 PUFA and visa versa. However animals can produce long-chain (20 and 22 carbon) PUFA from shorter chain (18 carbon) PUFA (Figure 4.3). The efficiency differs between species, and is typically very low in most marine animals. Therefore these animals have a specific dietary requirement for long-chain PUFA, especially 20:5ω3 and 22:6ω3 which are the main PUFA in their tissues. Those PUFA required for normal growth and reproduction, which must be obtained from the diet, are referred to as the "essential fatty acids". The supply of sufficient essential lipids is important in the formulation of a successful formulated food for abalone.

Edible oils contain differing amounts of short-chain and long-chain, omega 3 and omega 6 PUFA. Selection of the correct type or types of oil for inclusion in formulated diets is very important for ensuring adequate amounts of essential PUFA are present in the diet. Just which of these PUFA (Figure 4.3), that were needed to be included in formulated diets for farmed abalone so as to maximise their growth rate, was unknown until this study.

Preliminary experiments indicated that particular PUFA were preferentially assimilated from the natural diet by blacklip abalone implying that they are essential nutrients. Apart from this work, these aspects of abalone nutrition had not been studied for commercial species of Australian abalone. In northern hemisphere species of abalone, particular lipids have not only been identified as essential components of the diet significantly affecting growth rates (Uki *et*

al., 1986), but also shown to induce larval settlement and act as feed attractants (reviewed by Hahn, 1989). It is not possible to extrapolate from nutritional studies of Northern Hemisphere species of abalone due to significant differences in their preferred diets. Similarly, due to the unusual lipid composition of abalone tissues (Uki *et al*., 1986; Dunstan *et al*., 1996), extrapolations of the nutritional requirement of lipids by other marine species to abalone may not be valid.

Figure 4.3: Chain elongation and desaturation of short chain (18 carbon) PUFA into long-chain (20 and 22 carbon) PUFA by animals (minor PUFA intermediates not shown)



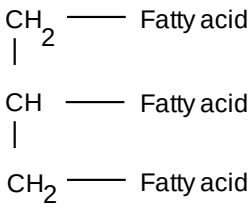
Baseline data on the lipid class, fatty acid and sterol compositions of natural diets of abalone was needed to provide an insight into why these diets produce high growth rates. Also analyses of wild-caught abalone of different ages were lacking. It was expected that such analyses would indicate how closely the lipid in formulated diets matched that of the flesh of abalone. Such differences may also affect consumer preference and therefore the profitability of the product on local and export markets.

The biochemical form (lipid class) of the essential fatty acids in the diet is also important and may affect digestibility and growth rates. Therefore the use of fish oils as a lipid source needed to be examined, since our work had shown that these consist of triacylglycerols while the natural diet of seaweed is rich in structurally dissimilar polar lipids (e.g. phospholipids and galactolipids) (Figure 4.4). Reduced growth rates due to insufficient amounts of polar lipids in the diet would indicate that specific supplements are needed, as is necessary in formulated diets for other marine animals (e.g. penaeid prawns).

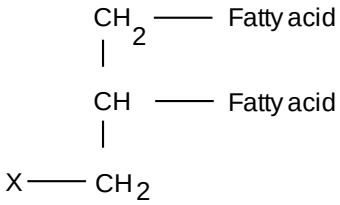
Further work was required to verify the preliminary data and extend our knowledge of the lipids essential to abalone through controlled growth rate experiments under typical industry conditions with appropriate replication for each of the target species. Other expected benefits of our research would be a small reduction in diet formulation costs and reduced tank fouling.

Figure 4.4: The two main forms of dietary lipids are the triacylglycerols and polar lipids

Triacylglycerols (storage lipids, e.g. most commercial oils and fats), contain three fatty acids per molecule.



Polar Lipids (membrane lipids, e.g. lecithin, non-storage muscle lipids) in animals usually contain two fatty acids per molecule.



where X may be a sugar, amino acid or other base

Currently most commercial diets are 3% (or more) lipid, which is primarily fish oil. The Japanese feed "Nihon Nosan Kogyo" contains only 1.5% lipid, but produces significantly increased growth rates in abalone relative to those with 3%. By optimising the diet with respect to lipids we hoped to be able to reduce costs by decreasing the amount of fish oil needed in the diet, and if necessary substitute some of this oil by blending with cheaper oils from different sources. Work will be carried out to establish what level of oil can be assimilated by the abalone and the relationship between this and growth rates.

Further work was urgently required to verify the preliminary data and extend our knowledge of the lipids essential to abalone through controlled growth rate experiments under typical industry conditions with appropriate replication for each of the target species. If the grow-out time for young abalone to reach marketable size can be reduced from the current 24-36 months by just a few months this will represent a substantial saving in costs to the abalone farmer. The savings will be in the form of less formulated diet being used, lower labour costs and increased production rates. This will allow further expansion by individual farmers resulting in increased total production for the Australian industry. Obviously, any reduction of production costs will not only benefit the industry but also open up new markets as the products become more competitively priced relative to other specialty foods and gourmet items.

The proposed research would not only be of direct benefit to the temperate Australian abalone industry, but will improve our understanding of the nutritional requirements of abalone. It may also give strategic insight into future tropical abalone culture and the culture of species with similar habits e.g. trochids and other herbivorous grazing molluscs.

Despite high lipid digestibility, a significant amount of lipid in natural abalone food remains in the faeces (preliminary data). Therefore reducing the amount of non-essential lipid in the formulated diet may also result in less waste and therefore reduced tank fouling.

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5. OBJECTIVES

The objectives of this research proposal were to reduce the production costs for Australian abalone farmers, through optimisation of the diets used at the various stages of growth. We planned to identify the specific lipid requirements of the abalone, as detailed below.

1). Baseline analyses. Determine baseline lipid class, essential fatty acid and sterol composition of the local abalone (from the wild). These baseline data from wild stocks to be compared with data from feeding trials of cultured animals.

2). Essential lipids. Examine the amounts and proportions of nutritionally important lipid components of the abalone diet through growth rate bioassays. Isocalorific diets to be manipulated with respect to fatty acid composition and ω3/ω6 ratio and the effects on abalone muscle composition and growth rates to be monitored. The feasibility of using precursors to be examined as a means of examining uptake and possible bioconversion into biochemically-important compounds.

3). Commercial sources of lipids in formulated diets. Determine supplementation requirements by measuring assimilation rates and apparent digestibility of the lipid components in fish oil based diets (which have compositions different to natural diets) for abalone. Identify the fish oil, oil blend or modified oil, which demonstrates the highest growth rates in abalone within economic constraints.

6. TECHNICAL REPORT – DETAILED RESULTS

6.1 General Materials and Methods

6.1.1 Abalone and management

Greenlip abalone (*H. laevigata*) were provided by Marine Shellfish Hatcheries (MSH). The stocking rates used were designed to mimic that used by the industry. For handling, abalone were anaesthetised in small batches using benzocaine. To ensure similar sized starting populations, five randomly selected abalone were repeatedly allocated to each tank in turn until the desired density was reached. Tagging of abalone was achieved by using Hallmark tags glued to the shell with Superglue Gel. Individual weights were determined (after partial drying with paper towels) using an A&D 3200 balance. Lengths were determined using stainless steel sliding callipers. The long durations of the trials were designed to simulate a true grow-out situation and avoid "change of diet effects" which are possible in trials of short duration. During the trials, diets were fed *ad lib.* every second day. Tanks were cleaned every fourth day by physically swirling the water, allowing waste to collect at the centre of the tank and the tank then drained by removing the central stand pipe.

Hatchery supplied, filtered, continuous flow seawater (1L/min), at ambient temperature, was used so as to maintain commercial relevance. Dissolved oxygen, temperature, pH and salinity levels were monitored periodically to ensure tank hygiene was not compromised. Each tank was aerated with two air-stones and contained PVC half pipe shelters. The tank system was shaded with a 90% shade cloth cover.

6.1.2 Diet manufacture

Diets were made at the hatchery at Bicheno, using a domestic kitchen mixer and pasta extruder, from ingredients sourced from SARDI.

6.1.3 Chemical Analysis

Lipid class, fatty acid and sterol analyses were carried out in the organic chemistry laboratories of the CSIRO Marine Research, where methods have been refined over the last 10 years. The same method for lipid extraction and determination of lipid class, fatty acid and sterol composition was used for diet, animal and algal samples, except where specified in the relevant sections. All analytical methods have been established and particular essential pieces of equipment were dedicated to the project.

6.1.3.1 Lipid extraction and total lipid content

Homogenised subsamples were left to extract overnight in the dark in chloroform/methanol/water (1:2:0.8 by vol; 15 ml) using a modification (Dunstan et al., 1996) of the method of Bligh and Dyer (1959). A further 3 extractions of 10 min (with ultrasonication and centrifugation between each) were performed to ensure maximum extraction. A blank extraction was also performed. The combined extracts from each sample were partitioned against chloroform/water (1:1 vol/vol) (taking sample water content into account) to give a final solvent ratio of chloroform/methanol/water of 1:1:0.9 by vol. NaCl was added to the aqueous phase to aid in phase separation. For each sample, the lower chloroform phase was removed, the upper aqueous phase rinsed with chloroform and both lots of organic solvents containing the lipids were combined and reduced *in vacuo* to recover the lipids. Total lipid was determined gravimetrically on duplicate aliquots of each lipid extract. Lipids were stored under nitrogen at -20°C until analysis.

6.1.3.2 Fatty acid composition

An aliquot of total lipid was transesterified to form fatty acid methyl esters (FAME) using methanol/chloroform/HCl (10:1:1 by vol; 3 ml) at 80°C for 2 h under high purity nitrogen. After cooling, 1 ml of water was added and FAME were extracted with hexane/chloroform (4:1 vol/vol; 3 x 3 ml). FAME samples were analysed with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a flame ionisation detector (FID) at 250°C. FAME samples were injected using an air cooled on-column injector onto a polar BPX-70 fused-silica column (50 m x 0.32 mm i.d.). The carrier gas was high purity hydrogen. The GC oven temperature was initially held at 45°C for 2 min after injection and then increased at 30°C/min to 120°C and at 3°C/min to 240°C, then held isothermal for 10 min. Fatty acids were identified from retention index data on both polar and non-polar columns and confirmed by comparison with known standards and previous data. Fatty acid identifications were verified with a Hewlett Packard 5970B GC/MS system. This type of analysis is only accurate for fatty acids of chain-length greater than or equal to C_{14}, as typically found in abalone. Volatile, very short-chain fatty acids such as found in milk products and some vegetable oils, can be detected, but not accurately quantified, even though they can make up a large proportion of the total fatty acids in such samples. Therefore the percentage fatty acid compositions presented in this study, comprise only the relative amounts of short to long chain fatty acids commonly encountered in the types of samples analysed.

6.1.3.3 Sterol composition

Aliquots of the total lipid were saponified with 5% KOH (wt/vol) in methanol/water (4:1 vol/vol) at 80°C for 2 h under high purity nitrogen. The non-saponifiable lipid fractions were extracted with hexane/chloroform (4:1 vol/vol). This fraction containing sterols was derivatized with N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) for 30 min at 80°C to form the trimethylsilyl ether derivatives of sterols. The sterol and selected FAME samples were analysed using a Shimadzu GC-9A GC fitted with an air-cooled on-column injector, and a non-polar methylsilicone (HP-1) fused-silica capillary column (50 m x 0.32 mm i.d.). GC conditions were similar to that of the polar column GC except that the FID temperature was 330°C, the second oven ramp was 4°C/min, and the final oven temperature was held isothermal at 310°C for 20 min. Sterol identifications were verified with a Hewlett Packard 5970B GC/MS system.

6.1.4 Statistical Analysis

An Analysis of Variance was performed using GENSTAT software.

6.1.5 References

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6.2 Natural abalone diets

6.2.1 Introduction

Adult abalone are unusual as they are one of the few species of marine animal being cultured that feed primarily on macroalgae. Very little work has been performed on the nutritional requirements of these herbivorous marine animals. The main C_{20} PUFA in both red and

brown macroalgae are 20:4ω6 and/or 20:5ω3, brown macroalgae contain significant levels of C_{18} ω3 PUFA as well (Jamieson and Reid, 1972; Johns *et al*., 1979; Virtue and Nichols, 1994). Abalone species from the northern hemisphere and South Africa prefer to feed on brown seaweeds (e.g. Sakai, 1962; Leighton, 1966; Barkai and Griffiths, 1986). Because abalone from Australasian waters have a marked food preference for red macroalgae (Shepherd, 1973; Poore, 1972; Foale and Day, 1992; Fleming, 1995), brown macroalgae were not analysed for the current study.

6.2.2 Methods

As very few data were available on the biochemical composition of the seaweeds the abalone farmers feed to older abalone, these were analysed for lipid composition. Seaweeds and colonial diatoms were collected from the abalone farms involved in the project [Marine Shellfish Hatcheries (MSH), Aquatas and Tasmanian Tiger Abalone Company (TTAC)]. The seaweeds were rinsed in seawater to remove animals, epifauna and epiflora, and finally rinsed with ammonium formate to remove salt. The samples were freeze dried, homogenised and subsamples taken for analysis of total lipid and fatty acid composition. Cultures of microalgae, and particularly diatoms (which form the major food items of very young abalone) had already been analysed for previous FRDC projects (Volkman *et al*., 1995).

6.2.3 Results/Discussion

The type of macroalgae consumed can significantly affect abalone growth rates (e.g. Uki *et al*., 1986b). Analyses of the Australian seaweeds identified by abalone farmers as providing high growth rates in abalone offer different proportions of the nutritionally important PUFA (Table 6.2.1). Most of the macroalgae contained high proportions of PUFA (24-56% of the total fatty acids) and saturated fatty acids (SFA: 27-50%), but low proportions of monounsaturated fatty acids (MUFA: 8-22%) (Table 6.2.1). Typical of macroalgae, C_{22} PUFA were not present in high levels, and of these 22:5ω3 was usually the most abundant (e.g. Jamieson and Reid, 1972, Aknin *et al*., 1992, Khotimchenko, 1993).

Various species of the green macroalga *Ulva* are used as a weaning diet for juvenile abalone between a diatom film diet and formulated diets (M. Cropp pers. com.). *Ulva australis* contained high proportions of short-chain (C_{16} and C_{18}) ω3 PUFA (Table 6.2.1), but low proportions of the C_{20} PUFA abundant in other macroalgae (Table 6.2.1). This composition is similar to that of *Ulva lactuca* from Australian waters (Johns *et al*., 1979) and is typical of other green macroalgae (Aknin *et al*., 1992; Khotimchenko, 1993).

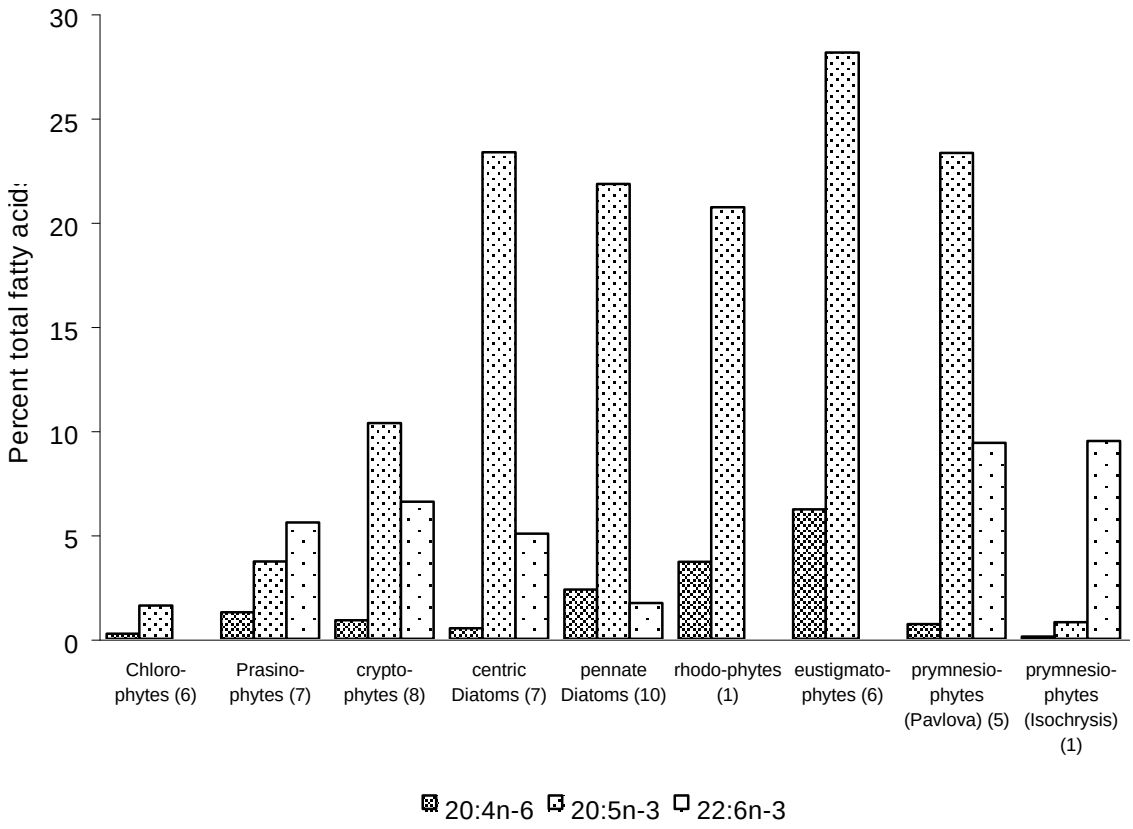
With the exception of *Gracilaria*, the red macroalgae contained high proportions of ω3 PUFA (primarily 20:5ω3, 17-32%), similar to diatoms (Figure 6.2.1, Table 6.2.1). Species of *Gracilaria* contained elevated proportions of ω6 PUFA (primarily 20:4ω6, 37-40%) and negligible levels of ω3 PUFA, resulting in these species having a very low ω3 to ω6 ratio (0.02) relative to the other red macroalgae examined (1.81-3.23). This composition has been shown to be typical for members of this genus (Dawes et al., 1993), and may explain the poor growth rates obtained with *G. ramulosa* cf *cliftonii* compared to other red algae (see section 6.5).

Table 6.2.1: Fatty acid composition of the turf algae and seaweeds identified by abalone farmers as providing high growth rates in abalone

	Colonial diatom	Green seaweed	Red seaweeds					
	Licmophora sp.	*Ulva australis*	*Gracilaria ramulosa* cf *cliftonii*	*Gracilaria* sp.	*Jeannerettia lobata*	*Polysiphonia* sp.	*Polysiphonia perriniae*	*Rhabdonia coccinea*
Saturated fatty acids (SFA)								
14:0	22	1	8	7	9	6	8	5
16:0	18	25	38	40	39	41	35	42
18:0	1	1	1	1	1	1	2	2
total	41	27	48	48	50	49	46	48
Monounsaturated fatty acids (MUFA)								
16:1ω7	17	1	1	tr	5	5	3	2
18:1ω9	1	2	7	5	6	3	6	2
18:1ω7	1	11	1	2	6	4	4	1
total	20	17	10	8	22	13	15	7
Polyunsaturated fatty acids (PUFA)								
16:3ω3	-	**3**	-	-	-	-	-	-
16:4ω3	-	**9**	-	-	-	tr	-	-
18:2ω6	1	**8**	1	-	1	2	3	7
18:3ω3	tr	**16**	tr	-	tr	2	1	-
18:4ω3	1	**9**	tr	-	tr	2	tr	1
20:3ω6	tr	tr	2	1	tr	4	1	1
20:4ω6	1	1	**37**	**40**	**6**	**5**	**5**	**2**
20:5ω3	**22**	2	tr	-	**17**	**18**	**25**	**32**
22:5ω3	tr	3	-	-	-	tr	1	-
22:6ω3	1	1	-	-	-	tr	1	-
total	38	56	42	43	24	38	38	43
total ω3	25	44	1	1	17	24	28	33
total ω6	7	12	41	43	7	13	10	10
PUFA/ SFA ratio	0.91	2.08	0.87	0.90	0.49	0.77	0.82	0.90
ω3/ω6 ratio	3.82	3.65	0.02	0.02	2.50	1.81	2.71	3.23

Newly settled and young juvenile abalone feed on benthic diatoms, turf and crustose coralline algae. Diatoms, in common with most seaweeds, have high proportions of 20:5ω3, and low proportions of 20:4ω6 and 22:6ω3, compared to other groups of microalgae (Figure 6.2.1, Table 6.2.1, Dunstan *et al*., 1994).

Figure 6.2.1: Proportions of nutritionally important polyunsaturated fatty acids in the microalgal species that are commonly used as diets for mariculture (FRDC final report 91/59 and the current study)



Therefore the different natural feeds of juvenile and mature abalone offer lipids with a range of fatty acid compositions. A mixed diet of seaweeds provides the essential lipids.

6.2.4 References

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6.3 Formulated abalone diets and ingredients

6.3.1 Introduction

The time and costs involved in the collection or culture of large amounts of macroalgae for abalone culture is often high. Supply of macroalgae can be unreliable and the environmental consequences of removing seaweeds are also a concern. Thus, the focus of research has moved to the development of convenient and nutritious formulated diets that produce high growth rates in abalone (reviewed by Hahn, 1989 and Fleming *et al*., 1996).

To enable diet formulation, it is essential to analyse ingredients that might be included in the diets. It should be noted that the chemical composition of batches of an ingredient could differ according to supplier, season, and preparatory treatment.

6.3.2 Methods

Formulated abalone diets from around the world were sourced from interested abalone farmers. An early version of the FRDC/CRC diet (ABCHOW) and some ingredients known to be used in other abalone diets were provided by SARDI. Diets were ground to a powder. Powdered diets and ingredients were hydrated and then analysed for total lipid and fatty acid composition.

6.3.3 Results/Discussion

The lipid content and fatty acid composition of a variety of ingredients that could be used in formulated diets were determined (Table 6.3.1). The main differences in lipid composition between ingredients were the levels of individual PUFA, especially 18:2ω6, 20:5ω3 and 22:6 ω3 (Table 6.3.1). The major PUFA in commercial vegetable oils and other terrestrial-plant products (eg. soya flour and semolina) is usually 18:2ω6; although some also have high levels of 18:3ω3. In comparison, the main PUFA in fish oils and other fish products (e.g. fish meal) are long-chain 20:5ω3 and 22:6ω3 fatty acids - the so-called "essential fatty acids" for marine

animals. The very high proportion of saturated fatty acid in the vitamin mix is indicative of synthetic vitamins being used. Milk products contain a large proportion of very short-chain fatty acids which due to their high volatility cannot be quantified accurately using the methods employed (see Section 6.1.3.2).

Table 6.3.1: Fatty acid composition of ingredients, which can be used in formulated diets for abalone

	Casein	Soya flour	Semolina	Vitamin mix	Fish meal	Fish oil
Saturated fatty acids (SFA)						
14:0	13	tr	tr	1	8	5
15:0	1	tr	tr	tr	1	1
16:0	35	18	18	12	20	17
18:0	14	4	2	82	4	3
total	66	24	21	97	33	26
Monounsaturated fatty acids (MUFA)						
16:1ω7	1	tr	tr	-	9	6
18:1ω9	20	11	15	1	7	14
18:1ω7	1	1	1	-	3	3
20:1ω9	tr	tr	tr	-	tr	5
22:1ω9	-	-	tr	-	-	tr
total	27	13	17	1	22	38
Polyunsaturated fatty acids (PUFA)						
18:2ω6	**2**	**55**	**58**	**1**	**1**	**2**
18:3ω3	1	8	4	tr	1	1
18:4ω3	tr	tr	tr	tr	2	3
20:4ω6	tr	-	-	-	1	1
20:5ω3	**tr**	**-**	**-**	**-**	**17**	**11**
22:5ω3	tr	-	-	-	2	1
22:6ω3	**-**	**tr**	**-**	**-**	**14**	**13**
total	6	63	62	2	44	36
total ω3	2	8	4	0	37	31
total ω6	3	55	58	1	5	4
PUFA/SFA ratio	0.09	2.64	2.97	0.02	1.34	1.38
ω3/ω6 ratio	0.66	0.15	0.08	0.16	7.56	6.92
% lipid (wet wt)	1	1	1	2	8	100

A selection of formulated abalone diets from around the world and Australia were analysed for lipid content and fatty acid composition (Tables 6.3.2 & 6.3.3 respectively). Because such diets are under constant development, diet samples from successive years were analysed where available.

Table 6.3.2: Lipid content and fatty acid composition of formulated diets for abalone available from different countries

Country of origin, and year collected	South Africa (1994)	South Africa (+ *Spirulina*) (1994)	New Zealand (early 1994)	New Zealand (late 1994)	New Zealand (1995)	Japan (1994)	Japan (1994)	Japan (1995)	China (1995)
Saturated fatty acids (SFA)									
14:0	4	4	3	3	8	3	2	1	2
16:0	19	21	23	23	26	18	20	18	20
18:0	2	2	7	6	9	3	3	3	4
total	27	29	35	34	49	25	26	23	28
Monounsaturated fatty acids (MUFA)									
16:1ω7	4	4	1	1	1	3	3	2	2
18:1ω9	16	15	13	13	16	12	13	13	14
18:1ω7	2	2	1	1	1	2	3	2	2
20:1ω9	1	1	tr	tr	tr	2	1	1	1
22:1ω9	tr	tr	tr	tr	-	tr	tr	-	-
total	28	27	18	17	22	30	27	21	22
Polyunsaturated fatty acids (PUFA)									
18:2ω6	**5**	**7**	**41**	**42**	**22**	**27**	**29**	**38**	**34**
18:3ω3	3	2	5	6	3	3	3	9	6
18:4ω3	2	2	tr	tr	1	1	1	tr	1
20:4ω6	1	1	-	-	tr	1	1	tr	1
20:5ω3	**9**	**8**	**tr**	**tr**	**tr**	**6**	**5**	**3**	**4**
22:5ω3	1	1	tr	tr	tr	1	1	tr	tr
22:6ω3	**22**	**19**	**tr**	**tr**	**tr**	**5**	**5**	**3**	**3**
total	45	44	47	48	27	45	47	55	50
total ω3	38	33	6	6	4	16	16	15	14
total ω6	7	10	41	42	22	28	31	39	35
PUFA to SFA ratio	1.70	1.53	1.35	1.42	0.54	1.82	1.80	2.37	1.80
ω3/ω6 ratio	5.11	3.20	0.15	0.15	0.20	0.58	0.51	0.39	0.39
% lipid (wet wt)	3	3	2	2	11	2	2	9	6
% lipid (dry wt)	-	-	-	-	12	-	-	9	7

The commercial abalone diets from around the world had a wide range of total lipid content (2-11% wet wt), but in most diets the total lipid comprised less than 5% of the diet (Tables 6.3.2 & 6.3.3). Those with the higher lipid levels were the more recently produced diets.

The fatty acid compositions of the various commercial diets were quite different. Formulated diets generally had high levels of 18:2ω6 (from terrestrial plant-derived ingredients) and/or 22:6ω3 (from fish-derived ingredients) (Table 6.3.1). As indicated by the high proportions of 20:5ω3 and 22:6ω3, most diets from Australia (Table 6.3.3) and elsewhere (Table 6.3.2) (apart from the New Zealand diets) contained some fish oil and/or a fish-derived product such

as fish meal. The use of algal meals would result in elevated 20:5ω3, but not 22:6ω3 (Table 6.2.1), and their inclusion in these diets at low levels with fish products cannot be discounted.

The New Zealand diet contained high levels of very short chain fatty acids (data not shown) indicating a high content of a milk product. The high concentration of total lipid in the 1995 diet from this country suggests that milk fat (or an oil-rich ingredient with similar lipid composition) had been added as the main oil source instead of fish oil.

Table 6.3.3: Lipid content and fatty acid composition of formulated diets for abalone available from different Australian companies

	Company A 1994	Company B 1994	Company B 1995	FRDC diet 1994*	Company C 1997*	Company D 1997*
Saturated fatty acids (SFA)						
14:0	1	2	2	4	3	3
16:0	15	15	19	19	18	17
18:0	5	2	3	4	3	4
total	22	20	26	28	25	26
Monounsaturated fatty acids (MUFA)						
16:1ω7	1	2	2	5	2	3
18:1ω9	21	17	13	13	14	11
18:1ω7	2	2	2	2	2	2
20:1ω9	tr	3	1	2	5	5
22:1ω9	tr	1	tr	tr	3	3
total	25	28	19	27	27	26
Polyunsaturated fatty acids (PUFA)						
18:2ω6	**46**	**41**	**33**	**21**	**26**	**22**
18:3ω3	4	3	2	3	3	3
18:4ω3	tr	tr	1	2	2	tr
20:4ω6	tr	tr	1	1	tr	1
20:5ω3	**1**	**2**	**4**	**8**	**6**	**6**
22:5ω3	tr	1	1	1	1	1
22:6ω3	**1**	**3**	**10**	**8**	**8**	**8**
total	53	52	54	45	48	47
total ω3	6	10	19	22	21	21
total ω6	47	42	35	22	27	24
PUFA to SFA ratio	2.43	2.57	2.05	1.59	1.93	1.79
ω3/ω6 ratio	0.14	0.23	0.54	0.99	0.75	0.91
% lipid (wet wt)	2	1	2	2	3	3

***** These diets contain the oils recommended to the companies/organisations by the author

As indicated by the high 18:2ω6 content (Tables 6.3.2 & 6.3.3), most commercial diets contained a high proportion of vegetable oil, or more likely other terrestrial plant products. The exception was the South African diets, which were based mostly on a fish-derived product. The high levels of 20:5ω3 and 22:6ω3, but low 18:2ω6 and total lipid levels in the South African diet, indicates that this diet contained a very high proportion of fish meal at the expense of terrestrial plant products. This diet contained higher levels of 18:2ω6 and 18:3ω3

than a standard fish oil (Table 6.3.1) indicating either that there was a low inclusion of vegetable oil, lecithin or other plant product or even that freshwater fish were the source of fish products in the diet. The major difference between the standard South African diet and that fortified with 10% *Spirulina*, was the higher levels of gamma-linolenic acid (18:3ω6, data not shown) which is the precursor to 20:4ω6. If ω6 PUFA were to be shown to be important to the abalone diets (see sections 6.6 & 6.7), an oil such as evening primrose oil (10% 18:3ω6) could be added to a formulated diet.

Early Australian diets of companies A & B contained very low levels of fish products (oils or meal) relative to terrestrial plant-derived products (as indicated by the high 18:2ω6, and low 20:5ω3 and 22:6ω3) (Table 6.3.3). A later diet from Company B showed increased inclusion of a fish oil, most likely to be a high 22:6ω3 (relative to 20:5ω3) oil such as tuna oil. The early FRDC diet and more recent diets from Australian Companies C and D (adopting recommendations from the present study), have fatty acid compositions similar to the early Japanese diets (Table 6.3.2). The increased level of terrestrial plant-derived ingredients at the expense of fish-derived ingredients in the more recent diets from Japan and China (increased 18:2ω6) should be noted (Table 6.3.2).

Relative to natural diets (Table 6.2.1), many formulated diets for abalone contained high levels of 18:2ω6 and 22:6ω3 and lower levels of 20:5ω3 and 20:4ω6 (Tables 6.3.2 & 6.3.3, Uki *et al*., 1986; Viana *et al*., 1993). The importance of these fatty acids in diets of other marine animals has been identified (e.g. Enright *et al*., 1986; Mourente *et al*., 1993), but up until the present study their importance for abalone is relatively unknown. Since these fatty acids are important nutrients for a variety of organisms, and the amounts in diets change significantly due to the inclusion/exclusion of various key ingredients, effects of varying their dietary inclusion was examined in sections 6.6 and 6.7.

6.3.4 References

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6.4 Abalone flesh

6.4.1 Introduction

The lipid composition of the two main commercial Australian species of abalone (*H. laevigata* and *H. rubra*) and the hybrid of these species, had until this study, not been determined. The changes in the lipid composition of the muscle of juvenile abalone fed a macroalgae compared to those fed a formulated diet provides valuable baseline data of use to nutritionists and diet formulators.

6.4.2 Methods

Due to their high value and favourable growth characteristics, samples of the greenlip abalone (*Haliotis laevigata*), blacklip abalone (*H. rubra*), and the hybrid abalone (*H. rubra* x *H. laevigata*) were analysed. Wild caught adult *H. rubra* were collected by Craig Sanderson. Juvenile hybrid abalone, as well as wild caught adult *H. laevigata* were provided by MSH. Juvenile *H. laevigata* were fed the green seaweed *Ulva australis*, or a formulated diet.

The visceral mass, gills, head and epipodial tissue of the abalone were removed. The foot muscles of juvenile *H. laevigata* were pooled for analysis due to their small size (<12 mm). The individual foot muscle of juvenile hybrid abalone were analysed separately. Muscle samples (ca. 1 cm^3) from the adult abalone were cut from the centre of the foot and analysed separately. Abalone muscle samples were chopped finely while partially frozen, refrozen with liquid nitrogen and homogenised with a mortar and pestle and analysed for total lipid, fatty acid and sterol composition.

6.4.3 Results/Discussion

Total lipid. Abalone meat is generally low in lipid (Table 6.4.1, reviewed by Olley and Thrower, 1977), as is their natural diet (Table 6.2.1). Most lipid is present in the cellular membranes as polar lipids (de Koning, 1966), with the viscera being the main lipid storage organs (Uki *et al*., 1986b).

Fatty acids. The major fatty acids in the foot muscle of abalone examined were 16:0, 18:0, 18:1ω9, 18:1ω7, 20:4ω6, 20:5ω3 and 22:5ω3, independent of species, age and diet (Table 6.4.1). The fatty acid compositions of Australian abalone were very similar to other species of abalone (Table 6.4.2) and may reflect the biochemical and dietary similarities of wild-caught representatives from the Haliotidae. It should be noted that none of the formulated diets analysed (Table 6.3.2 & 6.3.3) were well matched to the high 20:4ω6, 20:5ω3 and 22:5ω3, but low 22:6ω3, content of the abalone muscle (Tables 6.4.1 & 6.4.2).

The composition of abalone is quite different from other marine animals which typically have 20:5ω3 and 22:6ω3 as the main tissue PUFA. These ω3 PUFA are considered to be the "essential fatty acids" for these animals. This suggests that abalone may have different dietary requirements for essential fatty acids. It is of interest that the preferred food of newly settled abalone are diatoms such as *Licmophora* sp. (Table 6.2.1), *Navicula* sp., *Nitzschia closterium* and *Amphora* sp. Diatoms are high in lipid and 20:5ω3, but low in 22:6ω3 (Figure 6.2.1, Table 6.2.1, Dunstan *et al*., 1994). Similarly, the preferred foods of adult abalone are the macroalgae, which also high in 20:5ω3, but low in 22:6ω3 (Table 6.2.1), suggesting that oils rich in 20:5ω3 may be more suitable in formulated diets for abalone (Dunstan *et al*., 1996b).

Table 6.4.1: Lipid content and fatty acid composition of Australian commercial abalone with different diet histories

species	*H. laevigata*	*H. laevigata*	*H. laevigata* X *H. rubra*	*H. laevigata*	*H. rubra*
age	Juveniles	Juveniles	Juveniles	Adults	Adults
diet	*Ulva australis* (composition shown in Table 6.2.1)	Formulated diet of Company A (1994) (composition shown in Table 6.3.3)	Formulated diet of Company A (1994) (composition shown in Table 6.3.3)	Wild (natural diet of seaweeds)	Wild (natural diet of seaweeds)
Saturated fatty acids (SFA)					
14:0	2	2	2	2	2
15:0	1	1	1	2	1
16:0	20	18	18	19	20
18:0	7	8	9	6	6
total	32	31	31	31	31
Monounsaturated fatty acids (MUFA)					
16:1ω7	1	1	1	3	2
18:1ω9	4	5	5	7	7
18:1ω7	9	6	5	7	8
20:1ω9	tr	1	1	1	tr
total	19	20	19	22	22
Polyunsaturated fatty acids (PUFA)					
18:2ω6	2	7	9	1	1
18:3ω3	3	3	2	1	1
18:4ω3	1	-	tr	tr	1
20:2ω6	tr	2	2	tr	tr
20:3ω6	-	1	tr	tr	tr
20:4ω6	6	4	5	11	14
20:5ω3	15	10	7	10	7
22:2 NMI	3	4	5	6	5
22:4ω6	1	1	1	3	3
22:5ω3	15	12	11	14	14
22:6ω3	2	2	2	tr	tr
total	49	48	48	47	47
total ω3	38	32	28	31	27
total ω6	9	14	17	15	19
PUFA to SFA ratio	1.51	1.56	1.52	1.52	1.51
ω3/ω6 ratio	4.28	2.19	1.59	2.06	1.43
% lipid (wet wt)	0.8	1.5	1.3	0.8	0.8

Like many species of macroalgae, the major C_{22} PUFA in abalone muscle was 22:5ω3 (Tables 6.4.1 & 6.4.2). In contrast, the major C_{22} PUFA in omnivorous and carnivorous marine animals is 22:6ω3 (Dunstan *et al*., 1988), which is the desaturation product of 22:5ω3 (Figure 4.3). The elevated 22:5ω3 and reduced 22:6ω3 in abalone tissue was attributed to a

macroalgal diet by Shimma & Taguchi (1964) and Bannatyne & Thomas (1969). Uki *et al*. (1986b) suggested the elevated levels of the precursors 22:4ω6 and 22:5ω3 in the tissues of abalone was due to a low rate of conversion of 22:4ω6 to 22:5ω6 and 22:5ω3 to 22:6ω3. When abalone are fed diets rich in 22:6ω3, the level of this PUFA increased in the tissues, but 22:5ω3 remained the most abundant C_{22} PUFA (Uki *et al*., 1986a). Thus, both an adaptation to a low lipid macroalgal diet, as well as a low rate of conversion may explain the observed C_{22} PUFA distribution. This suggests that abalone are unusual compared to other marine animals in requiring 22:5ω3 in the diet rather than 22:6ω3. Therefore formulated diets containing high concentrations of 22:6ω3 (Tables 6.3.2 & 6.3.3; e.g. Uki *et al*., 1986a and Viana *et al*., 1993) may not be appropriate for abalone.

Table 6.4.2: Major fatty acids (% of total fatty acids) in the foot muscle of abalone (*Haliotis*) from around the world compared to the early FRDC/CRC formulated diet

	H. discus (adult)[1]	*H. japonicus* (adult) [1]	*H. discus* (adult) [2]	*H. iris* (adult) [3]	*H. laevigata* (juvenile) [4]	*H. laevigata* (adult) [4]	*H. rubra* (adult) [4]	*FRDC formulated diet* [5]
16:0	20.9	19.8	21.9	22.8	20.4	18.6	20.1	18.8
18:0	5.1	3.9	4.5	6.7	6.9	5.5	5.5	3.7
18:1	16.4	17.1	12.0	15.7	12.3	13.5	6.9	15.2
18:2ω6	1.2	1.6	1.3	0.5	1.9	0.7	0.9	**20.5**
20:4ω6	**10.7**	**12.3**	**8.7**	**13.4**	**5.5**	**10.7**	**14.3**	0.5
20:5ω3	8.8	10.0	8.8	8.0	14.5	9.9	6.8	7.8
22:5ω3	**7.3**	**8.4**	**9.3**	**10.4**	**14.8**	**14.1**	**13.5**	0.8
22:6ω3	-	-	0.4	-	1.8	0.2	0.3	**7.8**

[1] Shimma and Taguchi, 1964, [2] Kochi, 1975, [3] Bannatyne and Thomas, 1969, [4] Table 6.4.1, [5] Table 6.3.3

For animals, the nutritionally important PUFA are both the ω6 and ω3 PUFA (Figure 4.3). These PUFA cannot be synthesised *de novo* and must therefore be obtained from the diet. However, animals can produce long-chain (C_{20} and C_{22}) PUFA from shorter chain (C_{18}) PUFA (Figure 4.3), but the efficiency of production differs between species, and is typically low in most marine animals. The main C_{18} PUFA in the formulated diet were ω6, while those in *U. australis* were the more highly unsaturated ω3 PUFA. Feeding juvenile abalone an 18:2ω6 rich/ω3 PUFA-poor formulated diet (as opposed to an ω3 PUFA-rich macroalgae), resulted in elevated 18:2ω6 and reduced 20:5ω3 and 22:5ω3 in the muscle (Table 6.4.1). This reflected the composition of the diet and indicates that these lipids accumulated in the muscle. Uki *et al*. (1986a) have shown similar increased proportions of C_{18} PUFA in the muscle and viscera of abalone fed diets rich in these PUFA. The elevated levels of 20:2ω6 in the abalone fed the formulated diet indicate that the abalone can produce C_{20} PUFA from C_{18} PUFA (Dunstan *et al*., 1996a).

Most marine animals contain low levels of ω6 PUFA, but abalone have high proportions of 20:4ω6 (Tables 6.4.1 & 6.4.2) as does their natural diet (Table 6.2.1). The formulated diet and *U. australis* were low in 20:4ω6. Also, the muscle of both groups of juvenile abalone fed these diets had low proportions of this fatty acid and its elongation product 22:4ω6 compared

with wild-caught adults (Table 6.4.1). This was despite the fact that the formulated diet contained high proportions of the precursor 18:2ω6. Like abalone, species of finfish which consume red and brown macroalgae contain elevated proportions of 20:4ω6 and 20:5ω3 relative to carnivorous finfish with the same low lipid content (Dunstan *et al*., 1988). Therefore 20:4ω6 may be accumulated by animals consuming a diet relatively high in this fatty acid. Although high levels of 20:4ω6 may not be essential for muscle growth, it has been suggested that abalone have a requirement for both ω6 and ω3 PUFA (Uki *et al*., 1986a). Whether the high levels of 18:2ω6 (from terrestrial plant products) in formulated diets (Tables 6.3.2 & 6.3.3), can be used by the abalone to form 20:4ω6, or even if 20:4ω6 is nutritionally important to abalone, had until the present study, not been determined (see section 6.6).

Sterols. The major sterol in all samples of abalone muscle was cholesterol, irrespective of diet (Table 6.4.3). The muscle of juvenile *H. laevigata* fed a formulated diet high in 24-methylcholest-5-enol and 24-ethylcholest-5-enol contained elevated proportions of these sterols compared with the muscle of juveniles fed *U. australis* and wild-caught adult abalone. Observed differences in fatty acid and sterol composition were attributed to the accumulation of lipid, including sterols in the foot muscle of abalone fed a formulated diet (Dunstan *et al*., 1996a).

Table 6.4.3: Sterol composition (% of total sterols), cholesterol (mg/100 g wet wt of tissue) and total sterol content (g/100g wet wt) of natural and formulated diets and of *Haliotis laevigata* and *H. rubra* muscle

sample	*Ulva australis* (macroalgae)	Formulated diet of Company A (1994)	*Haliotis laevigata*	*Haliotis laevigata*	*Haliotis laevigata*	*Haliotis rubra*
age			Juveniles	Juveniles	Adults	Adults
diet			*Ulva australis*	Formulated diet of Company A (1994)	Wild (natural diet of seaweeds)	Wild (natural diet of seaweeds)
Percentage of total sterols						
cholesterol	6.2	15.1	90.0	87.0	95.5	88.6
cholesta-5,24-dienol	-[a]	-	5.1	7.6	1.0	1.4
24-methylcholest-5-enol[c]	-	14.8	0.7	2.4	0.5	1.6
24-methyl-5α-cholestanol[d]	-	10.1	-	-	-	-
24-ethylcholest-5-enol[c]	-	45.7	1.0	2.6	0.5	0.1
24-ethyl-5α-cholestanol[d]	-	14.3	-	-	-	-
24-ethylcholesta-5,24(28)-dienol[c]	93.8	-	1.6	0.4	0.2	3.4
Sterol Content						
cholesterol (mg/100g wet wt)	0.4	23	72	120	46	nd[b]
total sterols (mg/100g wet wt)	6.0	150	80	140	50	nd[b]

[a] not detected, [b] not determined, [c] "phytosterol", [d] "stanol"

It is generally held that gastropods can synthesise cholesterol *de novo* from acetate or mevalonate and that unlike crustaceans, gastropods may not have a dietary requirement for sterols (Teshima, 1982). The precursor to cholesterol, desmosterol (cholesta-5,24-dienol), was identified in all abalone muscle samples, but not in any food items examined (Table

6.4.3). This suggests that abalone can convert phytosterols into cholesterol (Teshima, 1982) and/or intermediates such as desmosterol. The higher proportions of C_{28} and C_{29} phytosterols in abalone fed a formulated diet indicates that these sterols accumulate in the muscle when fed a high lipid diet. However, it should be noted that the stanols present in the formulated diet were not detected in the abalone (Table 6.4.3), implying that they were either catabolised, excreted or stored elsewhere in the body.

The sterol composition of muscle from juvenile abalone fed *U. australis* was similar to that of wild-caught adult abalone, but quite different from *U. australis* (Table 6.4.3). Dietary 24-ethylcholesta-5,24(28)-dienol, which was abundant in *U. australis*, did not accumulate in abalone muscle and was probably converted into cholesterol *via* 24-methylcholesta-5,24(28)-dienol (which was detected only in this sample) and desmosterol (Table 6.4.3). Cholesterol, stigmasterol and some steroids have been shown to be important in the regeneration of damaged shell in gastropods (Whitehead, 1977) and may play a role in enhanced pearl production in molluscs such as abalone.

Human health and marketing implications. An important consideration for abalone fed formulated diets in culture is their acceptance by consumers. From a human health viewpoint, while abalone muscle contains high proportions of the health promoting ω3 or "omega-3" PUFA (Tables 6.4.1 & 6.4.2), the elevated lipid and sterol content of the muscle of abalone fed the formulated diet should also be noted (Tables 6.4.1 & 6.4.3). The level of cholesterol in the muscle of abalone fed *U. australis* (46-72 mg/100 g wet wt) could be considered "low" relative to other seafood (Tucker, 1989; Ackman, 1994).

Because the lipid composition of abalone muscle can be changed by diet (Tables 6.4.1 & 6.4.3; Uki *et al*., 1986a) and since lipids can be important in determining the flavour and odour of seafoods (reviewed by Lindsay, 1988), the use of formulated diets may give cultured abalone different flavours from those associated with wild animals. This could provide an opportunity to tailor the flavour of farmed animals to meet market demand. Similarly, the accumulation of lipid in the muscle of abalone fed a formulated diet may lead to subtle changes in muscle texture. Factors such as these need to be considered with respect to marketing a product, which is generally expensive to produce.

6.4.4 References

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6.5 Seaweed/formulated diet comparisons and digestibility of lipids

6.5.1 Introduction

The aim of this trial was to compare abalone growth rates when fed seaweeds (those recommended by local abalone farmers) or a formulated diet, with different lipid compositions and to determine the apparent digestibility of the various components of lipid in the formulated feed. Prior to this trial the apparent digestibility of the various lipids had not been determined, and this information is important for feed manufacturers.

6.5.2 Methods

Diet and growth experiments were carried out at the commercial facilities of MSH at Bicheno, Tasmania. Nutritional and feeding experiments with formulated diets were carried out using young abalone (2-6 cm) where maximum growth rate is required by the industry. Experimental designs were determined in consultation with the CSIRO biometrics unit to ensure adequate replication and appropriate controls.

A four month trial comparing a reference formulated diet with two seaweed diets was conducted. The seaweeds were selected based on the recommendations of abalone farmers and according to their availability. The three diets were:

1. FRDC reference diet including the indigestible marker 5α-cholestane (no high lipid ingredients except the added oils) was fed for the duration of the trial.
2. *Jeannerettia lobata* collected fortnightly by a diver from Aquatas and delivered fresh to MSH for the first period (42 days) of the trial. *Polysiphonia perriniae* collected

fortnightly by a diver from TTAC was delivered fresh to MSH, for the second period (82 days) of the trial.

3. *Gracilaria ramulosa* cf *cliftonii* collected from a shallow lagoon by staff of Aquatas and delivered fresh to MSH, was fed for the duration of the trial (124 days).

The FRDC reference diet was modified slightly from a formulation from Tom Coote (CRC/SARDI) to be used for determination of amino acid requirements (Table 6.5.1). By using this diet, data gained from our trials could be compared with work being carried out in South Australia. A variation from this low-lipid diet was the addition of 5α-cholestane (as a marker) to the oil and vitamin E mix as a digestibility marker.

Table 6.5.1: Formulation and estimated proximate composition (from ingredient analysis and literature values, dry weight basis) of the FRDC reference diet used in the digestibility trial, and diets for the essential fatty acid requirements trials

	Digestibility trial	EFA trials
Component	Percent	
$CaSO_4$	0.20	0.20
Sodium alginate	0.80	1.80
Mineral Mix	2.00	2.00
Vitamin Mix	0.50	0.50
Vitamin E	0.01	0.01
Vitamin C	0.05	0.05
DL Methionine	0.52	0.52
L Threonine	0.59	0.59
L Lysine	0.32	0.32
Semolina	63.64	61.48
Casein	30.02	29.03
fish oil	1.31	3.50
5α-cholestane	0.05	-
total	100.00	100.00
estimated total protein	40.1	38.7
estimated total carbohydrates	53.6	52.8
estimated total lipid	3.2	5.5
estimated total ash	3.1	3.0

Seaweeds were stored for each fortnight in a 2000 L tank supplied with freshly running seawater supplemented with Aquasol as required. Due to increasing rate of deterioration of harvested *Jeanerettia lobata* as the summer progressed, this perennial macroalgae was replaced with *Polysiphonia perriniae* as the second dietary treatment. Samples of the seaweeds and reference diet were taken at regular intervals and analysed for total lipids and fatty acids to establish any changes in composition with time (Table 6.5.2).

The lipid composition of the diets used in this trial is shown in Tables 6.5.2 (variations in seaweed composition with time), 6.5.3 (formulated diet lipid class composition) and 6.5.4 (average seaweed fatty acid composition during the last two weeks when faeces were collected for apparent digestibility determinations and formulated diet fatty acid composition). The major differences in lipid composition between the two seaweed diets were the higher 20:5ω3 content of *Jeannerettia lobata* (fed to day 42) and *Polysiphonia perriniae* (fed from

day 43 to day 124) compared to *Gracilaria ramulosa* cf *cliftoni* where 20:4ω6 was the predominant PUFA. Total lipid content and proportions of saturated fatty acids (SFA) were comparable for the two treatments, but lower levels of monounsaturated fatty acids (MUFA) were present in *Gracilaria ramulosa* cf *cliftoni*. Generally only minor variations in the lipid composition of the seaweeds with time were noted (Table 6.5.2), although samples of *Gracilaria ramulosa* cf *cliftoni* did tend to contain proportionally less 20:4ω6 towards the end of the experiment. All species contained very low levels of 22:6ω3, unlike the formulated diet which was also rich in 20:5ω3 (Table 6.5.4).

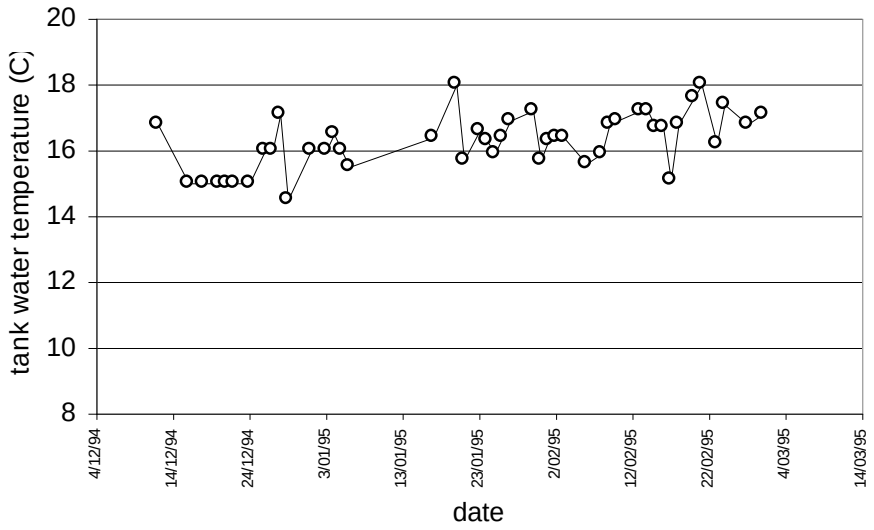
Table 6.5.2: Lipid content and fatty acid composition of the macroalgae used in the seaweed feeding/digestibility trial

	Jeannerettia lobata	*Polysiphonia perriniae*				*Gracilaria ramulosa* cf *cliftonii*			
	3/11/94	27/12/94	25/1/95	17/2/95	24/2/95	3/11/94	19/1/95	17/2/95	24/2/95
Saturated fatty acids (SFA)									
14:0	9	7	9	9	6	8	7	8	9
15:0	1	1	tr	tr	tr	tr	tr	1	tr
16:0	39	32	40	39	30	36	33	42	41
18:0	1	1	1	2	2	1	2	1	2
total	50	42	51	50	41	45	42	52	52
Monounsaturated fatty acids (MUFA)									
16:1ω7	5	3	2	2	3	1	2	tr	1
18:1ω9	6	6	6	7	5	7	7	5	8
18:1ω7	6	3	4	4	4	1	2	1	2
total	22	17	13	15	17	10	11	8	12
Polyunsaturated fatty acids (PUFA)									
18:2ω6	1	3	3	3	3	1	1	1	1
18:3ω3	tr	1	tr	tr	1	1	1	-	1
20:3ω6	tr	1	2	1	1	2	2	1	2
20:4ω6	**6**	**6**	**4**	**4**	**5**	**39**	**40**	**37**	**30**
20:4ω3	-	1	1	1	1	-	tr	-	-
20:5ω3	**17**	**26**	**26**	**22**	**27**	**tr**	**1**	**tr**	**tr**
22:5ω3	-	tr	tr	tr	1	-	tr	-	-
22:6ω3	-	tr	tr	1	1	-	tr	-	-
total	24	40	36	34	41	44	47	40	36
total ω3	17	29	27	24	30	1	2	tr	1
total ω6	7	11	9	10	11	43	45	39	35
PUFA to SFA ratio	0.49	0.94	0.71	0.67	1.02	0.98	1.11	0.76	0.69
ω3/ω6 ratio	2.50	2.53	3.00	2.55	2.82	0.02	0.04	0.01	0.03
% lipid (dry wt)	1	2	2	1	2	1	2	1	2

Nine identical 189 litre commercial tanks were used, enabling triplicate diet treatments. The 120cm diameter round tanks were shallow (15cm deep at perimeter, gently sloping to 20cm deep at centre), centrally drained via a standpipe. Each tank was aerated with two air-stones and contained 6 PVC half pipe shelters, and shaded with a 90% shade cloth cover.

Greenlip abalone (*H. laevigata*) were provided by MSH. The stocking rate was designed to mimic that used by the industry at 500 abalone per tank. 100 abalone from each tank were tagged. Weight and length measurements of tagged animals were made at the beginning of the trial, after 42 days, and at end of trial at 124 days. At the beginning of the trial the average length and weight of measured abalone per tank were (mean ± SD) 18.1 ± 0.1 mm and 0.76 ± 0.01 g respectively. At day 42 a proportion of untagged animals were measured to establish the effect of tagging. From day 42 to day 124 the water temperature in the tanks averaged 16.3°C increasing from a minimum 14.5°C to a maximum of 18.0°C (Figure 6.5.1).

Figure 6.5.1: Experimental tank water temperature fluctuations during the main part of the apparent digestibility trial



For the last two weeks of the trial, faeces samples were collected (for determination of apparent digestibility) according to the following method. The day after each feeding, the tanks were siphoned of faeces and uneaten food, and on the second day faeces were siphoned onto a mesh and the abalone fed again. All faeces for the collection period were combined for each tank and subsamples analysed according to section 6.1.3.

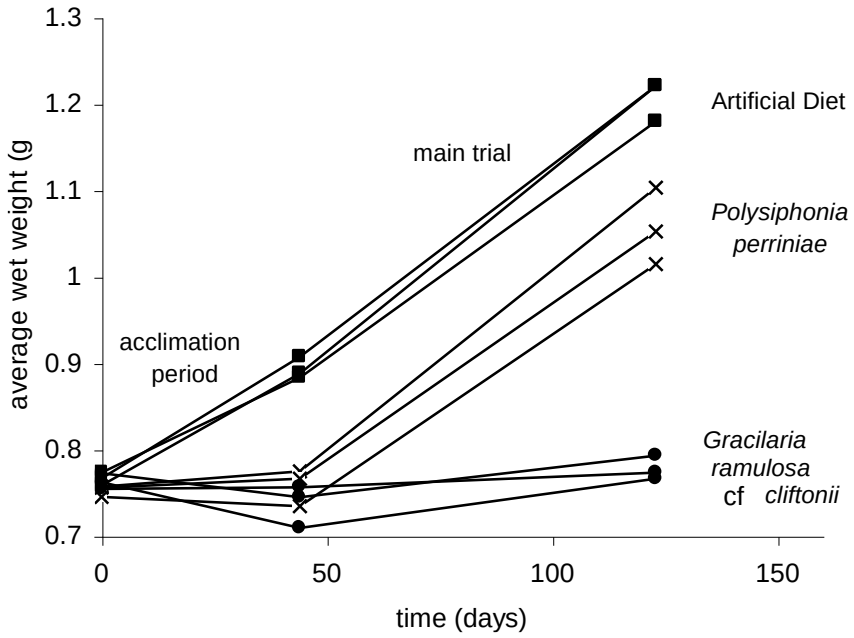
The apparent digestibility coefficients of the different lipids were calculated using the following equation;

$$\text{\% apparent digestibility coefficient} = \frac{\frac{100 \times \text{average g lipid/100g diet}}{\text{g cholestane/100g diet}} - \frac{100 \times \text{average g lipid/100g faeces}}{\text{g cholestane/100g faeces}}}{\frac{\text{average g lipid/100g diet}}{\text{g cholestane/100g diet}}}$$

6.5.3 Results/ Discussion

The average weight of greenlip abalone fed the ω3 PUFA-rich formulated diet increased more rapidly during the first 42 days of the trial compared to 20:5ω3-rich *Jeannerettia lobata* or 20:4ω6-rich *Gracilaria ramulosa* cf *cliftoni* (Figure 6.5.2). In both of these seaweed treatments, growth was negligible (Figure 6.5.3).

Figure 6.5.2: Average weights of triplicate tanks of greenlip abalone fed a 20:4ω6-rich seaweed (*G. ramulosa* cf *cliftoni*), 20:5ω3-rich seaweeds (*J. lobata* to day 42, *P. perriniae* to day 124) or an ω3 PUFA rich formulated diet



Unfortunately *Jeannerettia lobata* deteriorated within a week or two of being harvested, but once this seaweed was replaced with *Polysiphonia perriniae* at day 42, the growth of abalone increased to a rate comparable to those fed the ω3 PUFA-rich formulated diet (Figure 6.5.2). *A*balone fed *Gracilaria ramulosa* cf *cliftoni* actually decreased in length (due to wearing of the shell, which was not being replaced by new growth) and lost weight during this initial period, and over the whole trial gained very little weight or length when fed this seaweed (Figure 6.5.3).

There was very little difference between the average weight of abalone in each triplicate tank and the effect of tagging the abalone using the methods employed was negligible (Figure 6.5.4).

Figure 6.5.3: The average percentage Specific Growth Rates (±SEM) of greenlip abalone fed a 20:4ω6-rich seaweed (*G. ramulosa* cf *cliftoni*), a 20:5ω3-rich seaweed (*J. lobata* to day 42, *P. perriniae* from day 43 to day 124) or an ω3 PUFA-rich formulated diet

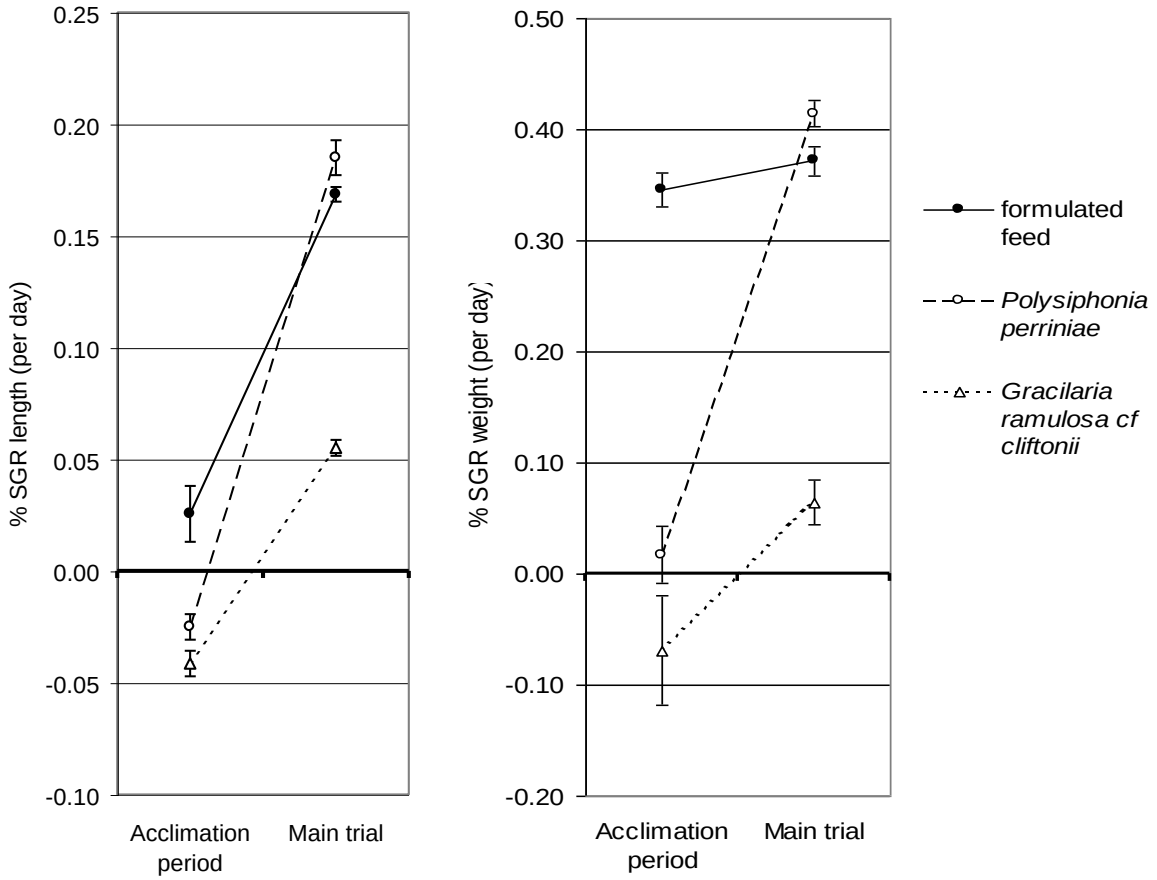
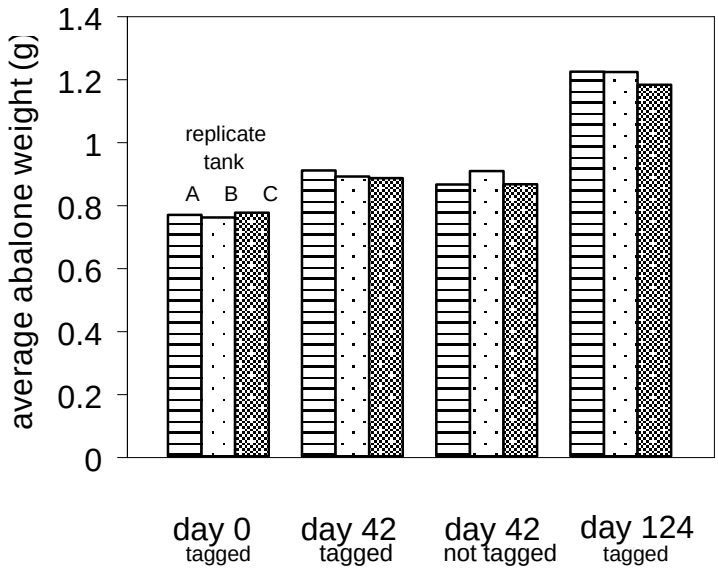


Figure 6.5.4: The effect of tagging on the average abalone weight in each replicate tank for the animals fed the formulated diet containing the digestibility marker



The collection and analysis of faeces during the last two weeks of the trial enabled determination of the digestibility of lipids from the formulated diet by abalone. Total lipid, total fatty acids and total sterols had apparent digestibility coefficients of 84.7, 86.6 and 52.5% respectively (Tables 6.5.3, 6.5.4 and 6.5.6). Also, the apparent digestibility of the individual lipid fractions differed. The main types of lipid in most seaweeds are the polar lipids. Each molecule of the more abundant polar lipids of seaweeds membranes (phospholipids) have two fatty acid molecules (Figure 4.4). In contrast oils such as the fish oils (which are triacylglycerols) typically added to formulated diets are more fatty acid rich and contain three fatty acids per molecule of triacylglycerol (Figure 4.4). The apparent digestibility of the triacylglycerols was much higher (97.9%) than that of the polar lipids (76.2%) present in the formulated diet. Therefore these data shows that fish oil (triacylglycerols) was highly digestible by abalone. It should be noted that the polar lipids in the formulated diet were derived from terrestrial plant ingredients such as the flours etc. added in the making of the diet. Because the composition of the polar lipids of terrestrial plants and seaweeds differ, this lower figure may not accurately represent the apparent digestibility of polar lipids by abalone from seaweeds.

Table 6.5.3: Total lipid content and lipid class composition of a formulated diet (Table 6.5.1) and the faeces from abalone fed this diet, and apparent digestibility values for these lipids

	diet composition	faeces composition	% apparent digestibility coefficient
Lipid class (% of total lipid)			
Polar lipids	31.6	49.1	**76.2**
Free sterols	1.9	4.5	**63.0**
Free fatty acids	3.1	28.3	**-38.6**
Triacylglycerols	60.2	8.4	**97.9**
Total lipid (g/100g dry weight)	3.27	1.07	**84.7**

The negative apparent digestibility of free fatty acids (-38.6) indicates a net excretion of these components by the abalone. Free fatty acids are a product of lipid digestion (coming mostly from the digestion of dietary polar lipids and triacylglycerols), and most are absorbed through the gut wall, and the excess excreted as is evident here.

Of the fatty acids, apparent digestibility increased with increasing level of fatty acid unsaturation (SFA 80.3%, MUFA 87.7%, and PUFA 91.3% digestible). ω3 PUFA were generally more digestible than ω6 PUFA (94.8% cf 89.1% respectively; Table 6.5.4). Therefore the PUFA in fish oils (long chain and highly unsaturated ω3 PUFA) were more digestible than those derived from terrestrial plant oils (shorter chain, less unsaturated PUFA which are usually ω6 PUFA). The branched chain fatty acids were the least digestible (56.9%). These are minor components of the food and are abundant in certain strains of gut bacteria, explaining the higher levels in the faeces and therefore lower apparent digestibility (Table 6.5.4).

Table 6.5.4: Total lipid content and fatty acid composition of the seaweeds and a formulated diet (Table 6.5.1), and of faeces from abalone fed these diets, apparent dry matter digestibility values for these lipids for the formulated diet and expected faeces composition from seaweed-fed animals

Diet	Formulated diet			*Polysiphonia perriniae*			*G. ramulosa* cf *cliftonii*		
Sample	food composition	faeces composition	% apparent digestibility coefficient	food composition	faeces composition	expected* faeces composition	food composition	faeces composition	expected* faeces composition
Saturated fatty acids (SFA)									
14:0	4.2	3.3	**89.5**	6.4	4.8	5.2	8.2	4.7	5.6
15:0	0.5	0.6	**82.8**	0.6	2.0	0.7	0.5	1.2	0.5
16:0	20.3	28.2	**81.3**	31.2	32.1	44.8	41.0	35.1	49.8
17:0	0.2	0.3	**82.4**	0.1	0.5	0.2	tr	0.9	tr
18:0	3.9	9.6	**67.0**	1.6	2.6	4.2	1.3	4.5	2.9
20:0	0.2	0.4	**73.3**	1.4	0.4	2.8	-	0.4	-
22:0	0.2	0.6	**66.7**	0.1	0.8	0.3	0.1	0.7	0.3
24:0	0.2	0.5	**65.9**	-	-	-	-	-	-
Total	29.9	43.6	**80.3**	41.4	43.1	58.1	51.1	47.4	59.2
Branched chain fatty acids									
i15:0	0.2	0.8	**54.6**	0.1	0.8	0.3	0.1	0.6	0.3
a15:0	0.1	0.4	**60.4**	0.2	1.1	0.6	0.4	0.8	0.9
Total	0.4	1.2	**56.9**	0.3	1.8	0.9	0.5	1.4	1.2
Monounsaturated fatty acids (MUFA)									
16:1ω9	0.2	0.3	**78.7**	0.6	0.4	0.9	0.3	0.5	0.5
16:1ω7	3.4	5.3	**78.9**	2.9	6.0	4.8	0.7	6.6	1.0
16:1ω5	0.4	0.5	**81.4**	0.2	0.8	0.3	tr	0.9	tr
18:1ω11	0.4	0.5	**82.1**	-	-	-	-	-	-
18:1ω9	15.5	10.6	**90.8**	5.4	5.5	3.8	6.4	3.5	3.9
18:1ω7	1.8	2.7	**80.1**	3.6	9.6	5.5	1.5	6.7	1.9
20:1ω11	0.4	0.5	**81.3**	0.2	0.5	0.3	0.1	1.2	0.1
20:1ω9	2.6	2.1	**88.8**	0.4	0.9	0.3	-	0.5	-
22:1ω9	3.6	2.6	**90.4**	0.1	0.5	0.1	0.1	0.6	tr
24:1ω9	0.4	0.5	**85.4**	0.2	0.7	0.2	0.6	1.1	0.5
Total	29.5	26.8	**87.7**	16.9	29.6	16.2	10.3	24.3	8.3
Polyunsaturated fatty acids (PUFA)									
18:2ω6	23.7	18.6	**89.4**	2.7	2.8	2.2	0.9	0.9	0.6
20:2ω6	0.2	0.2	**84.2**	0.4	0.6	0.5	0.3	0.5	0.3
20:4ω6	0.3	0.2	**87.3**	5.9	4.4	5.7	32.9	14.7	27.1
18:3ω3	2.3	1.4	**92.0**	1.0	0.4	0.6	0.4	0.5	0.2
18:4ω3	1.6	0.4	**96.5**	0.2	0.3	tr	0.1	0.7	tr
20:4ω3	0.4	0.1	**96.5**	0.6	0.4	0.2	tr	0.1	tr
20:5ω3	4.8	1.9	**94.8**	26.1	10.4	10.4	0.2	1.5	0.1
22:5ω3	0.4	0.3	**90.1**	0.4	0.5	0.3	-	0.4	-
22:6ω3	5.1	1.6	**95.7**	0.7	0.6	0.2	tr	0.5	tr
Total	39.8	25.7	**91.3**	40.7	23.4	24.0	38.0	24.0	31.3
total ω6	24.7	19.8	**89.1**	11.0	10.3	11.1	37.0	20.2	30.9
total ω3	15.0	5.7	**94.8**	29.3	13.0	11.8	1.0	3.8	0.3

*expected faeces composition was calculated using the apparent dry matter digestibility coefficients on the fatty acid concentrations (dry matter basis) of the seaweeds

The high apparent digestibility of C_{20} PUFA is expected as they are major components in the abalone's flesh, and are precursors to compounds essential for a variety of physiological, health and maintenance functions (Figure 4.1). Interestingly, 22:5ω3 was slightly less digestible than 22:6ω3 (90.1 cf 95.7 respectively), even though the proportion of 22:5ω3 in abalone flesh is much higher than 22:6ω3 (Tables 6.4.1 & 6.4.2). Similarly, 22:5ω3 was also slightly less digestible than its precursor; 20:5ω3 (90.1 cf 94.8 respectively), suggesting that due to poor uptake of 22:5ω3, 20:5ω3, may be more important to the abalone's diet (see next section). Also, the apparent digestibility data of fatty acids such as 22:5ω3 which are only present in small amounts, may not be sufficiently accurate to draw such conclusions and need to be determined from diets rich in such fatty acids.

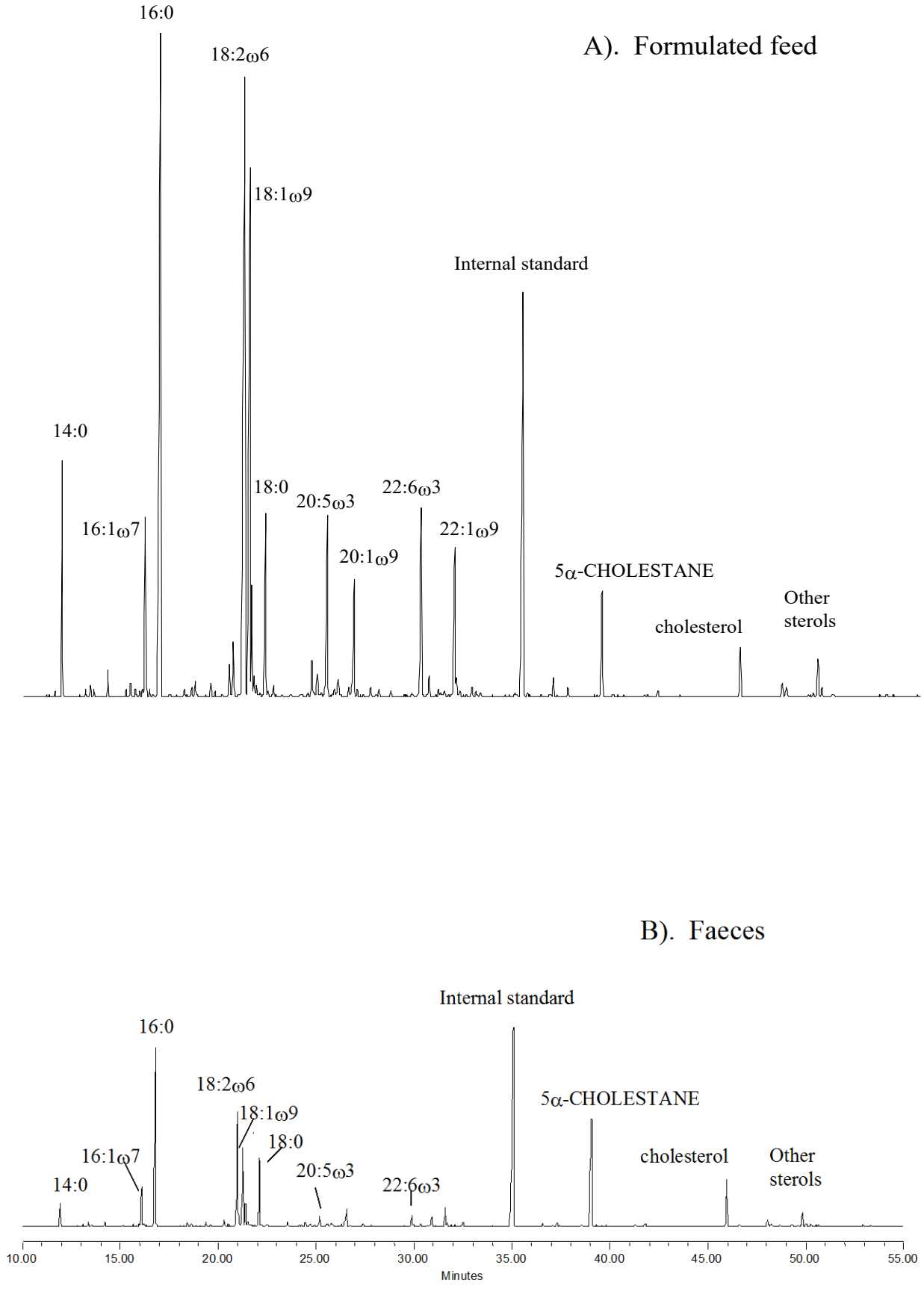
Using the apparent digestibility data of the lipids from the formulated diet to calculate the expected faeces composition from abalone fed a seaweed, and comparing this to the actual faeces composition, yields some interesting results (Table 6.5.4). The average faeces composition from abalone fed ω3 PUFA rich *Polysiphonia perriniae* was similar to that estimated, based on the apparent digestibility values for an ω3 PUFA-rich formulated diet. Conversely the average faeces composition from abalone fed ω6 PUFA-rich/ω3 PUFA-poor *Gracilaria ramulosa* cf *cliftoni*, had significantly lower levels of the main ω6 PUFA, 20:4ω6. This suggests in diets where ω3 PUFA are lacking, the ω6 PUFA uptake increases to compensate. The proportion of ω3 PUFA in the faeces of these animals was higher than in the diet and estimated to be in the faeces, suggesting that in abalone very small amounts of ω3 PUFA are poorly digested. The low growth rate of abalone fed this ω3 PUFA-poor seaweed (Figure 6.5.3), suggests that the lack of ω3 PUFA, and possibly other factors make this species unsuitable for use in farming greenlip abalone.

Overall the saturated fatty acids (0 double bonds) with 16 and 18 carbon atoms which are found in all animal and plant lipids to varying degrees tended to have lower digestibility coefficients (Table 6.5.5). With increasing chain length and number of double bonds (unsaturation), digestibility increased, indicating that fish oils should be recommended over vegetable oils as additives to abalone diets (Table 6.5.5).

Table 6.5.5: Apparent digestibility of fatty acids of differing chain length (number of carbon atoms) and degree of unsaturation (number of double bonds) from the formulated diet (Table 6.5.1)

number of double bonds	0	1	2-3	4	5-6	Legend for shading, showing the typical oils which are rich in the particular fatty acids
Chain length						
14	89.5					animal & plant oils
16	**81.3**	78.9	70.7			olive, canola oils
18	**67.0**	**89.5**	**89.6**	96.5		most vegetable oils
20	73.3	**88.1**	85.9	**92.8**	**94.8**	most seaweeds
22	66.7	**90.4**		87.4	**95.2**	most fish oils
24	65.9	85.4				pelagic fish oils

Figure 6.5.5: Gas chromatograms of lipids from a formulated diet (A) containing 5α-cholestane digestibility marker (Table 6.5.1) and faeces (B) from abalone fed the diet (standardised to 5α-cholestane peak height)



Apparent digestibility values for the sterols (unsaturated) and stanols (saturated) show that cholesterol, the main sterol found in animal tissues (and in this case from the fish oil) is much less digestible than the phytosterols derived from plant ingredients (25.7% cf 60-80%, Table 6.5.6). Even though a very important metabolic nutrient, many animals excrete cholesterol in excess of requirements. Therefore the plant sterols are more important nutritionally than fish oil sterols, which differs to the findings for fatty acids (Table 6.5.5).

Table 6.5.6: Apparent digestibility values for sterols and stanols from the formulated diet (Table 6.5.1)

Sterol or stanol	Percent of total sterols		% apparent digestibility coefficient
	food composition	faeces composition	
cholesterol	35.0	54.4	25.7
5α-cholestan-3β-ol[a]	1.2	0.5	80.9
24-methylcholest-5-enol[b]	11.0	9.2	60.2
24-methyl-5α-cholestanol [a]	9.5	4.0	80.7
24-ethylcholest-5-enol[b]	27.2	16.6	71.3
24-ethyl-5α-cholestanol [a]	5.9	3.2	74.6
Other minor sterols	9.6	10.6	
Total	100.0	100.0	52.5

[a] "stanol" [b] "phytosterol",

The relative decrease in the various lipid components with digestion can be seen in Figure 6.5.5. The vertical scale has been standardised so that the 5α-cholestane (digestibility marker) is shown as the same area for Figures 6.5.5A (the lipid composition of the formulated diet) and Figure 6.5.5B (the lipid composition of the faeces from abalone fed this diet). The large decrease in most components (except cholesterol) is evident, especially the decrease in PUFA. The internal standard, added immediately prior to analysis in known amounts depending on sample lipid content, is used for calculating the concentration of the various lipid components to enable calculation of the apparent digestibility coefficients, and has no effect on the results.

6.6 Dietary PUFA and growth rate - Individual PUFA

6.6.1 Introduction

Dietary lipids have been shown to be important nutrients for maximising growth rates and health of many marine animals. Attention to the specific dietary lipid requirements is especially important for species being grown commercially for aquaculture. Particular lipids are essential for growth of tissues, as an energy source and a number of physiological functions in animals (Figure 4.1). Because of their predominance in the natural diet (Tables 6.2.1 & 6.5.2), formulated diets (Tables 6.3.2 & 6.3.3) and tissues (Tables 6.4.1 & 6.4.2) of abalone, and their highly digestible nature (Tables 6.5.4 & 6.5.5), the identification of the nutritional significance of 20:4ω6, 20:5ω3 and 22:6ω3 in the diet was objective 2 of the current project. The information gained from the baseline data enabled us to formulate diets to determine which types and how much of these lipids should be incorporated into formulated diets to maximise the growth rates in abalone.

6.6.2 Methods

Seven diets containing different amounts of long chain ω3 and ω6 PUFA (Table 6.6.1) were assessed for effect on the growth rate of greenlip abalone (*Haliotis laevigata*). To control the lipid composition of each treatment diet, a reference diet containing none of the high lipid ingredients was used (Table 6.5.1). All ingredients used in the treatment diets were identical except for the added oils. The isocalorific diets all contained the same amount of lipid (3%). A single batch of mixed ingredients was divided into 7 weighed portions, prior to oil addition and binding. Different oils were blended at CSIRO to produce characteristic lipid compositions as determined by ingredient analysis. The oils blended in varying proportions were MaxEPA, RBD-DHASCO, RBD-ARASCO and olive oil. The main differences between the diets were the amounts of the long chain PUFA 20:4ω6 (high in seaweeds), 20:5 ω3 (high in diatoms, most seaweeds and fish) and 22:6ω3 (high in fish). Diets were formulated to maintain short-chain (C_{18}) PUFA (high in vegetable oils and plant derived ingredients) at a constant level, but due to ingredient batch differences this did not occur. Samples of each diet were analysed in triplicate according to section 5.2.2.

The main comparisons to be made were between:

- zero, low (4.6%) and high (12%) levels of 20:4ω6 (Diets CS-1, CS-4, CS-5 respectively),
- zero, mid (8%) and high (14.7%) levels of 22:6ω3 (Diets CS-1, CS-6, CS-7 respectively),
- zero, mid (9.7%) and high (13%) levels of 20:5ω3, with mid (8%) levels of 22:6ω3 (Diets CS-6, CS-2, CS-3 respectively).

Because the diets were formulated to be isocalorific, as the percentage of the PUFA of interest changes, so does the percentage of other fatty acids. Generally the diets contained similar proportions of saturated fatty acids (SFA), with the proportions of monounsaturated fatty acids (MUFA) changing inversely with the levels of PUFA (Table 6.6.1).

Twenty-one identical 53 litre experimental tanks made for the current study were used, enabling triplicate diet treatments. The 70cm diameter round tanks were shallow (13cm deep at perimeter, gently sloping to 15cm deep at centre), centrally drained via a standpipe. Each tank was aerated with one air-stone and contained 4 PVC half-pipe shelters, and the entire system shaded.

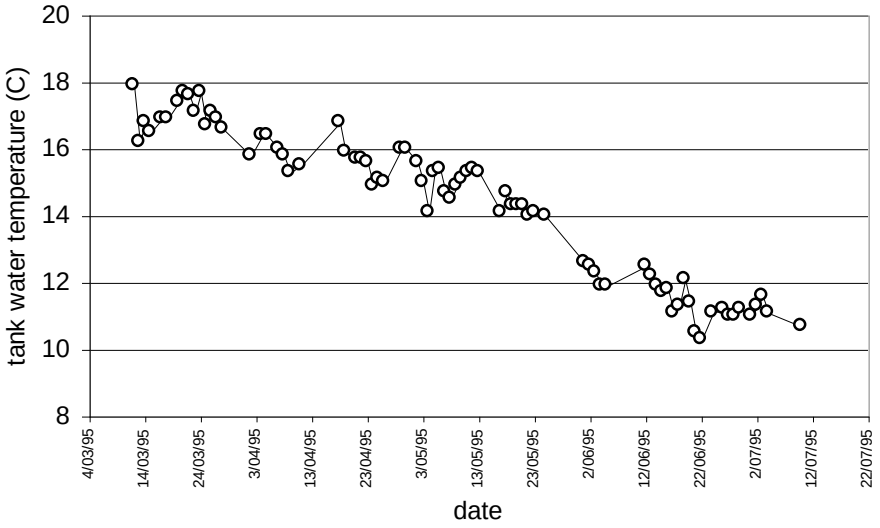
Triplicate tanks of tagged greenlip abalone were fed one of seven diets assigned in a randomised block design to the tanks. To ensure the same number of abalone with identical diet histories and similar sized animals were present in each tank at the commencement of each trial, five randomly selected abalone from each prior treatment were allocated to each of the 21 tanks in turn, until the desired density was reached.

For this trial, the triplicate tanks each contained 117 abalone. Shell length and animal weight measurements of all animals were taken at the commencement of the experiment, at one time during the trial and at the conclusion of the experiment. At the beginning of the trial the average length and weight of measured abalone per tank were (mean ± SD) 20.8 ± 0.2 mm and 1.13 ± 0.03 g respectively. This trial evaluating diets CS-1 to CS-7 was conducted during the autumn to mid winter with temperature decreasing from the annual March maxima of 17.9°C to a minimum of 10.3°C in July, and averaged 14.3°C over the whole trial (Figure 6.6.1).

Table 6.6.1: Lipid content and fatty acid composition of Diets CS-1 to CS-7, formulated to contain different levels of long chain ω6 and/or ω3 PUFA

Diet code	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
Diet features	no long chain ω6 or ω3 PUFA	mid 20:5ω3 mid 22:6ω3	high 20:5ω3 mid 22:6ω3	low 20:4ω6, no long chain ω3 PUFA	high 20:4ω6, no long chain ω3 PUFA	mid 22:6ω3	high 22:6ω3
Saturated fatty acids (SFA)							
14:0	3.8	5.1	6.3	0.6	1.0	3.8	6.5
16:0	16.2	15.4	17.7	11.2	15.7	14.7	14.5
18:0	3.1	2.8	3.6	3.4	5.9	2.9	2.5
total	23.9	24.1	29.0	16.0	23.9	22.1	24.2
Monounsaturated fatty acids (MUFA)							
16:1ω7	0.9	4.1	6.4	0.6	0.7	0.9	1.0
18:1ω9	46.6	24.0	11.0	56.6	37.2	47.5	40.9
18:1ω7	2.7	1.7	2.5	1.7	2.0	1.7	0.9
20:1ω9	0.4	0.7	0.9	0.3	0.4	0.3	0.3
22:1ω9	-	2.7	0.4	-	-	-	-
total	50.9	33.9	24.3	59.2	40.5	50.5	43.3
Polyunsaturated fatty acids (PUFA)							
18:2ω6	23.5	15.4	11.2	18.3	20.0	16.4	13.5
18:3ω6	0.0	0.2	0.2	0.3	0.7	0.2	0.2
18:3ω3	1.5	1.1	1.2	0.9	1.1	1.0	0.9
18:4ω3	0.6	1.3	2.0	0.5	0.8	0.5	0.4
20:4ω6	**-**	**1.0**	**0.8**	**4.6**	**12.2**	**1.3**	**2.3**
20:5ω3	**-**	**9.7**	**13.1**	**tr**	**tr**	**tr**	**0.1**
22:5ω3	-	1.0	1.7	-	tr	tr	0.2
22:6ω3	**-**	**8.0**	**8.6**	**tr**	**-**	**8.0**	**14.7**
total	25.6	39.8	42.7	24.8	35.6	27.3	32.4
total ω3	2.1	21.6	27.8	1.4	1.9	9.4	16.2
total ω6	23.5	17.3	13.4	23.4	33.7	17.9	16.1
PUFA/ SFA ratio	1.1	1.6	1.5	1.6	1.5	1.2	1.3
ω3/ω6 ratio	0.09	1.25	2.08	0.06	0.06	0.53	1.01

Figure 6.6.1: Experimental tank water temperature fluctuations during the individual PUFA growth rate trial



6.6.3 Results/Discussion

The poorest growth resulted from the diet containing only the C_{18} PUFA and none of the long-chain PUFA (Figure 6.6.2). The addition of 20:4ω6 was less effective than the addition of 22:6ω3 whereas diets with both 20:5ω3 and 22:6ω3 produced the highest growth rates.

In view of the importance of long-chain PUFA, these results suggest that

- the conversion of C_{18} PUFA into the C_{20} and C_{22} PUFA (Figure 3), is inefficient in the abalone,
- provision of the long-chain ω6 PUFA, 20:4ω6 does not compensate for a lack of the long-chain ω3 PUFA, 20:5ω3 and 22:6ω3.
- having both 20:5ω3 and 22:6ω3 in the diet was better than having 22:6ω3 alone.

Many marine animals have only a limited capacity to chain-elongate and desaturate C_{18} ω3 PUFA to the corresponding long-chain C_{20} and C_{22} PUFA (Kanazawa *et al*., 1979), as appears to be the case with abalone (Figure 6.6.2), suggesting a specific dietary requirement for long-chain C_{20} and C_{22} PUFA. Dietary lipid quantity and quality (in particular 20:5ω3 and 22:6ω3) has been linked to growth rates in *H. discus hannai* (Uki *et al*., 1985, 1986). Similarly, the current work suggests that C_{20} and C_{22} ω3 PUFA from fish oils cannot be substituted with C_{18} ω6 PUFA-rich vegetable oils.

When growth rates for all 2274 animals in the experiment were compiled, 86% of the top 100 and 84% of the top 200 were fed diets containing 20:5ω3 (Figure 6.6.3), showing the importance of this PUFA to abalone growth rates.

Because the abalone were tagged it is possible to examine the effect of the previous seaweed and formulated diet diets on the growth of abalone in the current trial. A radar plot of average growth rate by weight (mg/day) for each diet (CS-1 to CS-7) and for the previous diet shows the growth response on each diet to be similar and independent of the previous diet (Figure 6.6.4). Highest growth rates were evident in abalone fed the 20:5ω3 diets, and reduced through 22:6ω3, 20:4ω6 diets to the lowest growth on the no long-chain PUFA diet. The

growth of the abalone which had been fed *Gracilaria* (20:4ω6-rich) in the previous trial showed consistently lower growth rates than those fed *Polysiphonia* (20:5ω3-rich) and formulated diet (20:5ω3 and 22:6ω3-rich).

Figure 6.6.2: "Summer trial"- Average change in length to change in weight relationship in each tank with dietary PUFA

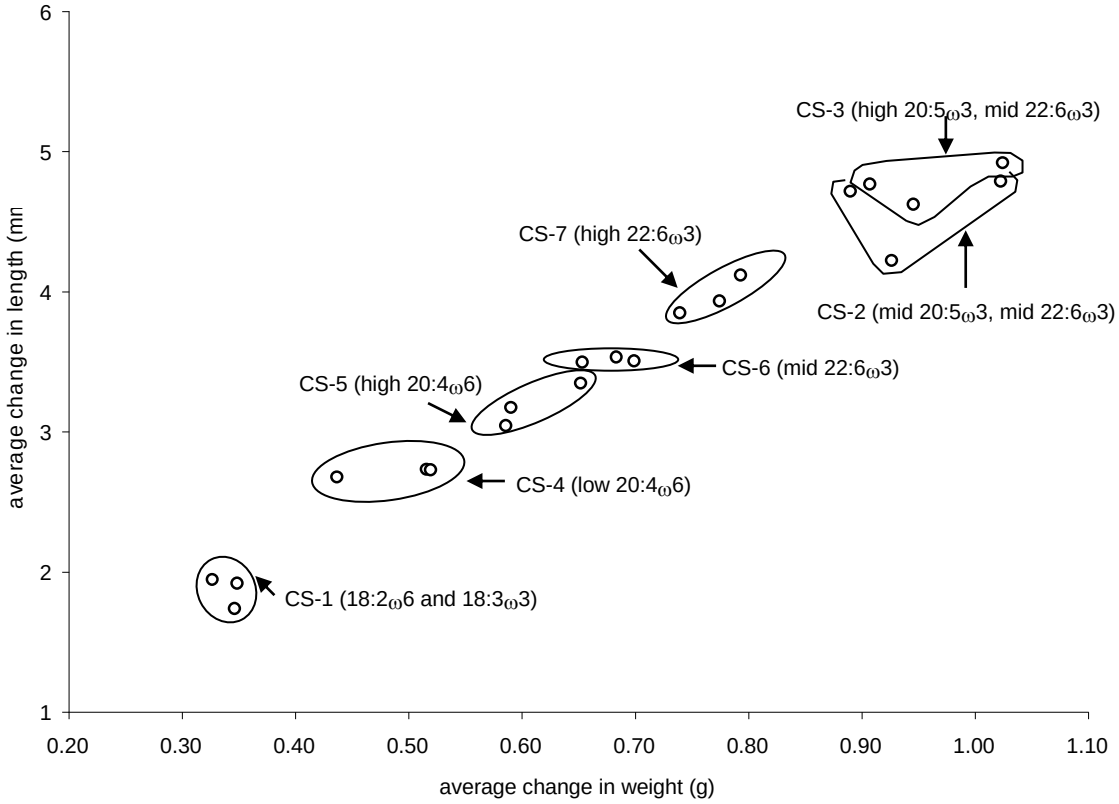


Figure 6.6.3: Histogram showing the number of abalone in the top 200 fastest growers by length with diet

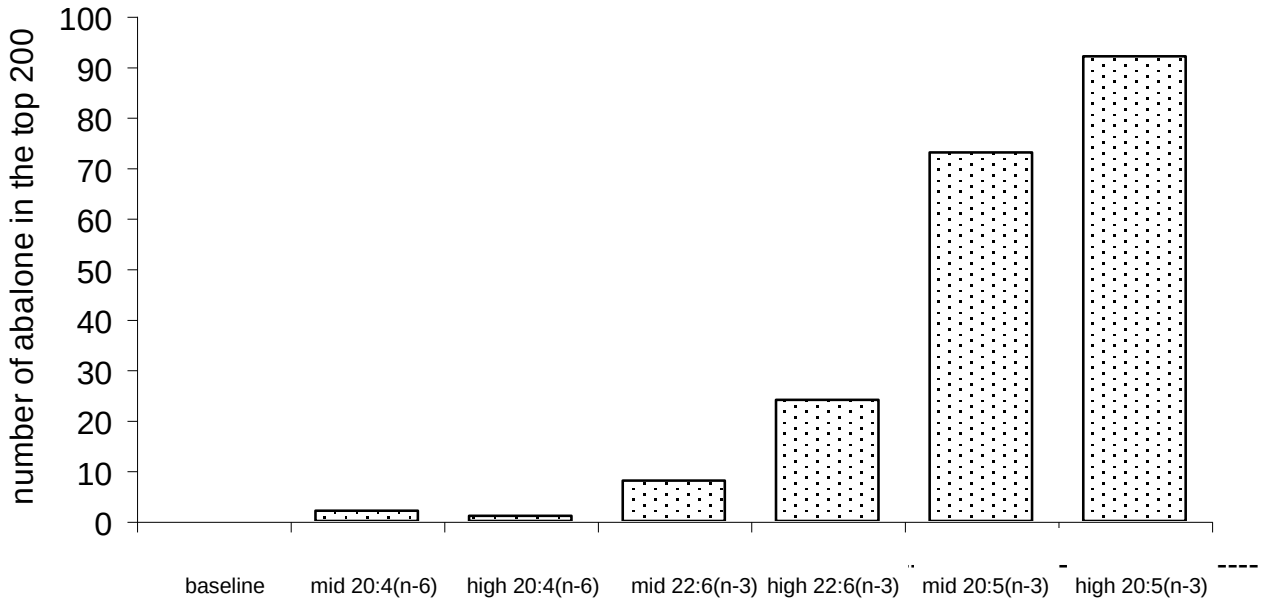
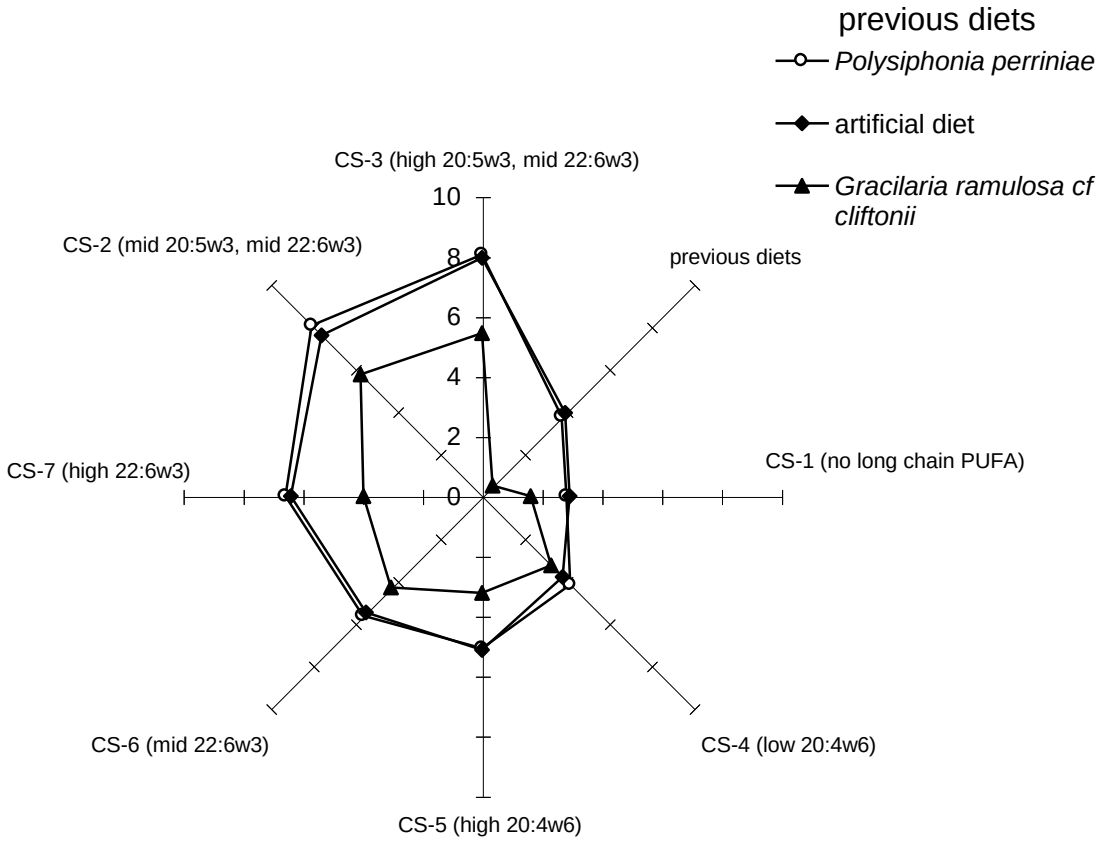


Figure 6.6.4: Average growth rate by weight (mg/day) for each of diets CS-1 to 7 of abalone with three different pre-diet histories



6.6.4 References

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6.7 Dietary PUFA and growth rate - Combinations of Different PUFA

6.7.1 Introduction

Results from the previous PUFA feeding trial showed that diets containing only the C_{18} PUFA produced the lowest growth rates compared with those with C_{20} and C_{22} PUFA, suggesting that the abalone is unable to convert C_{18} PUFA into the C_{20} and C_{22} PUFA efficiently (Figure 4.3). Due to the importance of the long-chain PUFA to abalone growth rates, the growth response of different combinations of the C_{20} and C_{22} PUFA in formulated diets was examined for *H. laevigata*.

6.7.2 Methods

Seven diets containing different amounts of long chain ω3 and ω6 PUFA (Table 6.7.1) were assessed for effect on the growth rate of greenlip abalone (*Haliotis laevigata*).

Table 6.7.1: Lipid content and fatty acid composition of Diets CS-8 to CS-13, formulated to contain different levels of long chain ω6 and/or ω3 PUFA

Diet code	CS-8	CS-9	CS-10	CS-11	CS-12	CS-13
Similar Diet trialed in summer	CS-2	-	CS-3	-	-	-
Diet features	mid 20:5ω3 mid 22:6ω3	mid 20:4ω6, mid 20:5ω3, mid 22:6ω3	high 20:5ω3, mid 22:6ω3	+lecithin mid 20:5ω3, mid 22:6ω3	-lecithin mid 20:5ω3, mid 22:6ω3	mid 20:4ω6, mid 22:6ω3
Saturated fatty acids (SFA)						
14:0	4.3	4.5	6.1	3.8	4.5	3.7
16:0	16.2	16.8	17.4	15.1	16.8	12.9
18:0	4.4	4.9	2.9	3.8	4.1	3.5
total	25.8	27.2	27.2	23.0	26.0	20.8
Monounsaturated fatty acids (MUFA)						
16:1ω7	4.3	3.9	6.1	3.4	3.3	0.8
18:1ω9	15.0	12.9	14.0	14.3	17.9	47.1
18:1ω7	2.1	1.7	3.0	2.1	1.8	1.2
20:1ω9	4.6	5.2	0.7	3.8	4.1	0.3
22:1ω9	4.8	5.4	-	3.2	3.3	-
total	33.2	31.5	24.7	28.3	32.3	49.4
Polyunsaturated fatty acids (PUFA)						
18:2ω6	19.1	11.8	14.5	27.6	22.3	14.1
18:3ω6	0.1	0.4	0.2	0.0	0.0	0.4
18:3ω3	2.2	1.3	1.2	2.6	2.5	0.9
18:4ω3	1.5	1.7	1.9	1.2	1.3	0.5
20:4ω6	0.5	5.4	0.8	0.4	0.4	6.0
20:5ω3	6.4	7.4	13.3	7.1	4.7	0.1
22:5ω3	2.5	2.8	1.6	1.9	1.8	0.1
22:6ω3	7.1	6.2	8.3	5.5	5.4	7.0
total	42.0	40.0	44.8	47.8	40.5	29.6
total ω3	21.2	21.1	27.4	19.4	16.8	8.6
total ω6	20.5	18.7	16.3	28.4	23.3	21.0
PUFA/SFA ratio	1.6	1.5	1.7	2.1	1.6	1.4
ω3/ω6 ratio	1.0	1.1	1.7	0.7	0.7	0.4

To control the lipid composition of each treatment diet, a reference diet containing none of the high lipid ingredients was used (Table 6.5.1). All ingredients used in the treatment diets were identical except for the added oils. The isocalorific diets all contained the same amount of lipid (3%). A single batch of mixed ingredients was divided into 7 weighed portions, prior to oil addition and binding. Different oils were blended at CSIRO to produce characteristic lipid compositions as determined by ingredient analysis. The oils blended in varying proportions were MaxEPA, RBD-DHASCO, RBD-ARASCO, cod liver oil, soya bean oil and olive oil. The treatment diets included the two best-performing diets from the Individual PUFA trial (section 6.6), which were either high or low in 20:5ω3 (with constant 22:6ω3). Other

treatments were a low 20:4ω6 & 22:6ω3, a low 20:4ω6, 20:5ω3 & 22:6ω3, a high phospholipid and a high triglyceride diet. Samples of each diet were analysed in triplicate according to section 6.1.3.2.

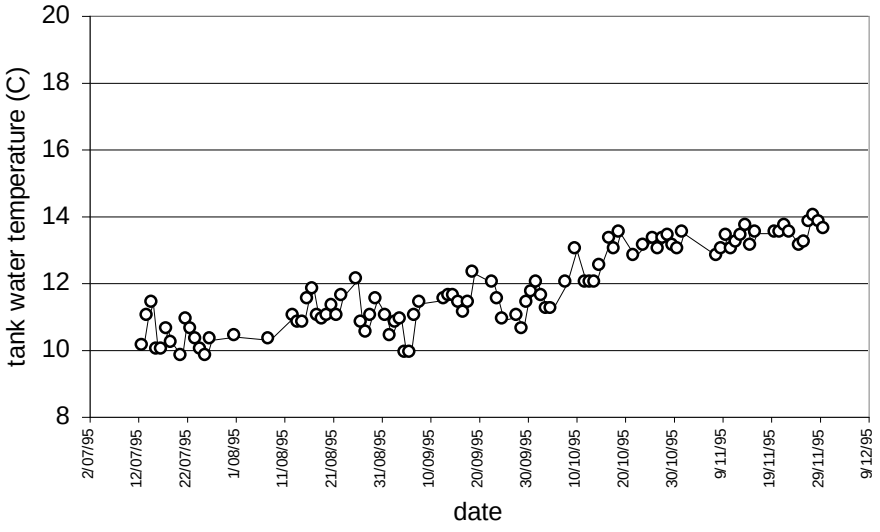
The fatty acid compositions of the diets tested (CS-8 to CS-13) are shown in Table 6.7.1. The main comparisons to be made were:

- mid (6.4%) and high (13.3%) levels of 20:5ω3, both with mid (7-8%) levels of 22:6ω3 (Diets CS-8, CS-10 respectively).
- mid (6.4%) levels of 20:5ω3, compared with mid levels of 20:4ω6 (6%), both with mid (7%) levels of 22:6ω3 (Diets CS-8, CS-13 respectively).
- with mid (7-8%) levels of 22:6ω3 (Diets CS-8, CS-10 respectively).
- Comparable levels of C_{20} and C_{22} PUFA, but with almost half the C_{20} PUFA being 20:4 ω6 (Diet CS-9) compared with all the C_{20} PUFA being 20:5ω3 (Diet CS-10).
- High 18:2ω6 diets with and without lecithin added (Diets CS-11, CS-12 respectively)

The twenty-one identical 53 litre experimental tanks made for the current study were used, enabling triplicate diet treatments. The 70cm diameter round tanks were shallow (13cm deep at perimeter, gently sloping to 15cm deep at centre), centrally drained via a standpipe. Each tank was aerated with one air-stone and contained 4 PVC half-pipe shelters, and the entire system shaded.

Triplicate tanks of tagged greenlip abalone were fed one of seven diets assigned in a randomised block design to the tanks. Each tank contained 100 abalone at the beginning of the trial. The average initial length and weight of measured abalone per tank were 24.8 ± 0.2 mm (mean ± SD) and 1.83 ± 0.06 g respectively. These were fed the treatment diets from July to November. The water temperature in the tanks averaged 11.8°C increasing from a minimum 9.8°C to a maximum of 14.0°C (Figure 6.7.1).

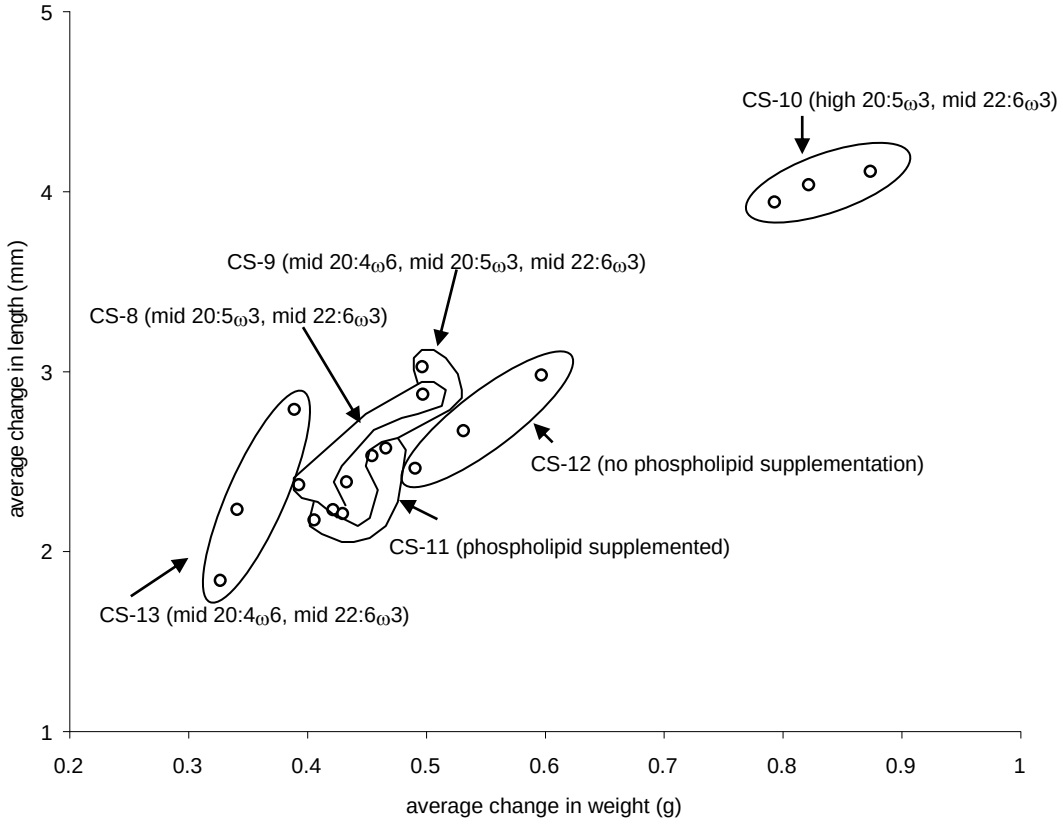
Figure 6.7.1: Experimental tank water temperature fluctuations during the combinations of PUFA growth rate trial



6.7.3 Results/Discussion

The results for this "winter trial" show a distinct growth advantage in using oils with elevated levels of 20:5ω3 (relative to other PUFA) (i.e. diet CS-10) in the colder months (Figure 6.7.2). Whereas in the "summer trial" of the previous experiment, there was no difference in growth with a similar high 20:5ω3 diet (CS-3) to one with moderate levels of 20:5ω3 (CS-2), both giving the highest growth rates of the seven diets evaluated (Figures 6.6.2).

Figure 6.7.2: "Winter trial"- Average change in length plotted against change in weight in each tank for diets differing in PUFA content



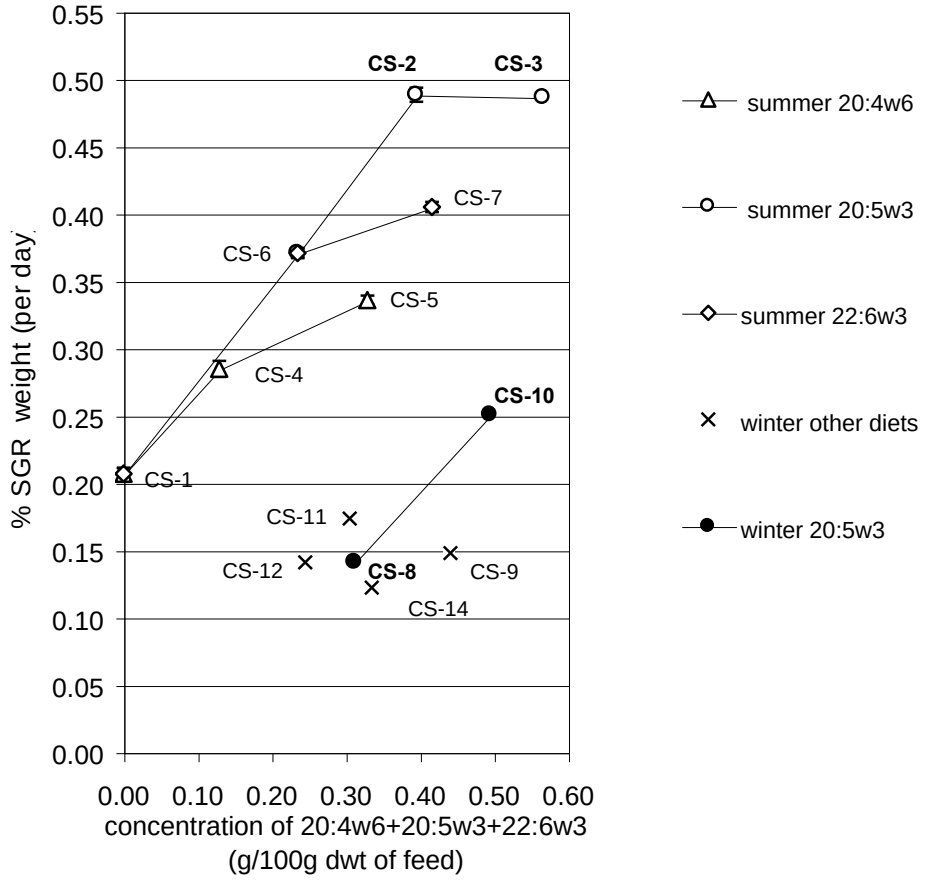
Comparing both PUFA trials there were significant differences in the growth responses between the two seasons when the abalone were fed common diets (i.e. high 20:5ω3/mid 22:6 ω3 and mid 20:5ω3/mid 22:6ω3) (Figure 6.6.2 cf. Figure 6.7.2). Plotting the average growth response during the “summer” and “winter” trials against total amount of major long chain PUFA (g/100g dry wt. of diet), shows the advantage of using the high 20:5ω3 diets (CS-3 and 10), especially in the cooler months (Figure 6.7.3).

The data show that 20:5ω3 (high in diatoms) cannot be substituted with the same amount of 20:4ω6 (high in seaweeds) (diets CS-2 cf. CS-7, Figure 6.7.3) to yield the same growth rates. Similar results were obtained for the mid 20:4ω6,mid 20:5ω3,mid 22:6ω3 and the high 20:5ω 3, mid 22:6ω3 diets (diets CS-9 cf. CS-10, Figure 6.7.3). That is, both had similar amounts, but different types of long chain PUFA and produced different growth rate responses, showing that it was not the amount of C_{20} PUFA, but the type of C_{20} PUFA which is important to growth. There was no growth advantage by using a phospholipid (Figure 4.4) instead of a

triacylglycerol (oil) (Figure 6.7.2), even though the former is the major lipid class in seaweeds and also has been shown to be essential for prawns.

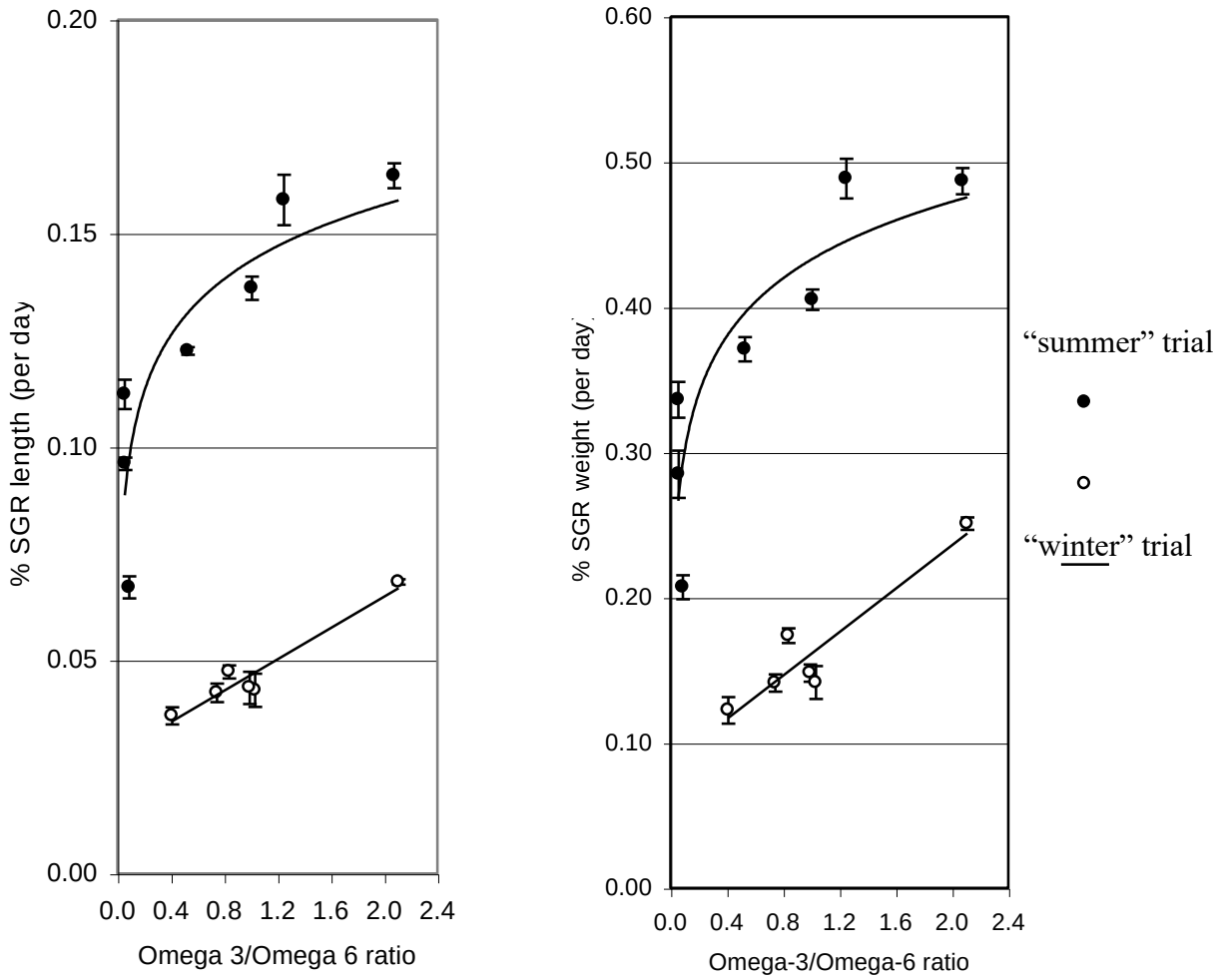
It should be noted that the overall low growth rates in this experiment were likely to have been due to a combination of the cold water temperatures, the fact that the animals used were the slowest growing animals from the cohort (genetic) and the particular low lipid-ingredient (no fish meal) reference diet used. It was necessary to use a diet with low lipid ingredients to isolate the effect of the added oils, but this may have resulted in other essential trace nutrients being removed.

Figure 6.7.3: Average specific growth rate by weight (±SEM) during the "summer" (individual PUFA) and "winter" (combinations of PUFA) trials against total amount of major long-chain PUFA (g/100g dry wt. of diet). Numbers refer to diet numbers appearing in Tables 6.6.1 and 6.7.1



Specific growth rate measured by length and weight increased with increasing ω3 to ω6 ratio (up to 2.0) in both the "summer" (individual PUFA trial) and the "autumn" (combination of PUFA trial) (Figure 6.7.4). High proportions of the ω6 PUFA occur in terrestrial plant ingredients such as oils and flours (Table 6.3.1) and some seaweeds (Tables 6.2.1 & 6.5.2), whereas high proportions of ω3 PUFA occur in fish products such as oils and meals (Table 6.3.1). Therefore increased growth rates in abalone can be achieved by using diets with a high ω3/ω6 ratio (i.e. those that include fish products, but have reduced levels of vegetable oils, Figure 6.7.4), and which have elevated levels of 20:5ω3 for cooler temperature culture.

Figure 6.7.4: Average specific growth rate by length and weight (±SEM) during the "summer" and "winter" trials against omega-3 PUFA to omega-6 PUFA ratio



6.8 Quantity of dietary lipid

6.8.1 Introduction

Not only are the types and amounts of fatty acids present in a diet important to growth rates, but also the total amount of lipid. The total lipid level in the commercial formulated diets analysed ranged from 2 to 11% wet weight (Tables 6.3.2 & 6.3.3). This section of our research investigated the amount of lipid required in formulated diets to maximise abalone growth rates. Two feeding trials were performed to determine the amount of total lipid required for elevated growth rates in greenlip abalone (*Haliotis laevigata*).

6.8.2 Methods

Two feeding trials were performed to determine the amount of total lipid required for elevated growth rates in greenlip abalone. The same system and same methods for abalone measurement were used as for the fatty acid requirements trials above (Sections 6.6 and 6.7). Unlike the previous trials, which used reference diets, both total lipid requirement trials were performed with diets using full-lipid ingredients according to the ABCHOW formulation. In formulating the diet with different lipid levels, only one ingredient of similar digestibility to the fish oil was substituted for the oil. For both trials, diets were fed *ad lib.* every two days.

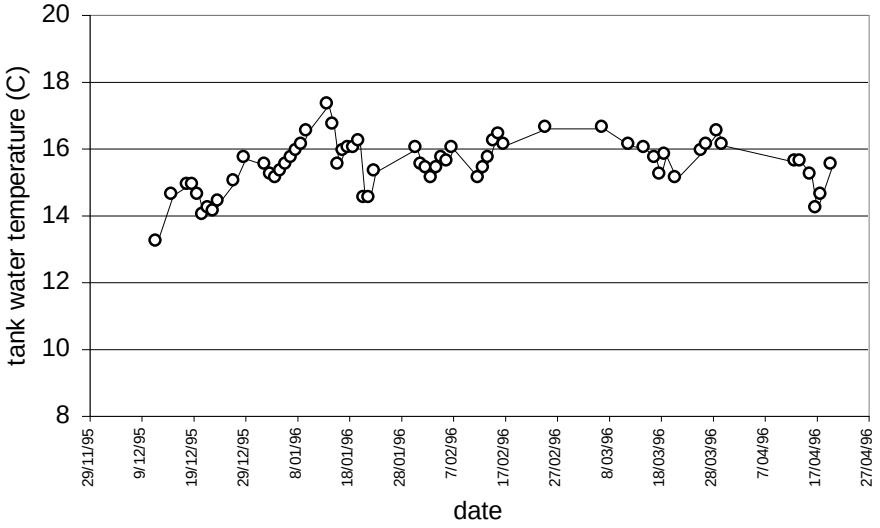
Table 6.8.1: Lipid content and fatty acid composition of Diets CS-15 to CS-21, formulated to contain different levels of oil and to compare abalone growth rates in the dark and light

	Evaluated in light					Evaluated in dark	
	No algal meal						Algal meal
Diet Code	CS-15	CS-16	CS-17	CS-18	CS-19	CS-20	CS-21
oil added (% of diet)	1.5%	4.0%	6.5%	9.0%	11.5%	4.0%	4.0%
Saturated fatty acids (SFA)							
14:0	4.4	4.9	5.2	5.1	5.1	4.9	5.9
16:0	17.3	14.8	14.2	13.6	13.4	15.0	17.1
18:0	4.3	3.5	3.2	3.1	3.0	3.6	3.8
total	27.1	24.6	23.9	23.2	22.8	24.7	28.0
Monounsaturated fatty acids (MUFA)							
16:1ω7	4.9	5.7	6.2	6.3	6.4	5.8	6.1
18:1ω9	11.9	12.2	12.6	12.9	13.2	12.4	13.5
18:1ω7	2.8	3.1	3.2	3.2	3.2	3.1	3.2
20:1ω9	3.8	5.5	6.4	6.7	6.8	5.7	5.8
22:1ω9	5.0	7.5	8.6	9.0	9.2	7.7	8.0
total	32.2	38.8	42.2	42.9	43.5	39.4	41.7
Polyunsaturated fatty acids (PUFA)							
18:2ω6	16.7	9.9	7.1	5.7	4.9	9.7	9.6
18:3ω6	0.0	0.1	0.1	0.0	0.0	0.0	0.0
18:3ω3	2.3	1.7	1.4	1.3	1.3	1.7	1.6
18:4ω3	1.7	2.3	2.4	2.6	2.7	2.3	1.7
20:4ω6	0.5	0.5	0.4	0.5	0.5	0.5	0.6
20:5ω3	7.3	8.3	8.3	8.6	8.7	8.1	6.1
22:5ω3	1.4	1.7	1.7	2.2	2.2	1.6	1.2
22:6ω3	7.7	8.4	8.3	8.7	9.0	8.2	6.0
total	39.6	35.3	32.5	32.4	32.2	34.5	29.1
total ω3	21.5	23.7	23.7	25.0	25.6	23.3	17.8
total ω6	17.8	11.1	8.4	6.9	6.2	10.8	10.9
PUFA to SFA ratio	1.5	1.4	1.4	1.4	1.4	1.4	1.0
ω3/ω6 ratio	1.2	2.1	2.8	3.6	4.1	2.1	1.6
% lipid (wet wt)	3.5	5.7	8.4	10.8	13.4	5.9	5.7
% lipid (dry wt)	3.8	6.3	9.3	12.1	14.3	6.4	6.5

The first trial evaluated 7 different diets (Table 6.8.1). Five diets with different amounts of oil added (1.5, 4, 6.5, 9, 11.5% of ingredients) supplemented with diatoms and other periphyton which settled in naturally lit (10-100 μE) tanks within a hatchery shed (corrugated polymer roof). As a part of this trial, the 4% added oil diet trialed in triplicate lit tanks was also evaluated in triplicate heavily shaded tanks, as was a 4% added oil diet which also contained powdered kelp ("Bloom's Giant Pacific Kelp") at 5% inclusion level. These two latter treatments were to establish if low levels of periphyton were beneficial, and whether this benefit could be simulated with a commercially available seaweed powder in heavily shaded

tanks. At the beginning of the trial the average length and weight of measured abalone per tank were (mean ± SD) 26.3 ± 0.4 mm and 2.39 ± 0.11 g respectively. This first trial was a "summer trial", the abalone were fed the treatment diets from December to April. The water temperature in the tanks averaged 15.5°C increasing from a minimum 13.2°C to a maximum of 17.3°C (Figure 6.8.1).

Figure 6.8.1: Experimental tank water temperature fluctuations during the quantity of dietary lipid-light/dark growth rate trial.



The second total lipid requirements trial evaluated seven feeds in triplicate, with different amounts of oil added (1, 2.5, 4, 5, 6.5, 9, 11.5% of ingredients). All treatments were evaluated in the dark with the entire system heavily shaded with two layers of 90% shade cloth. At the beginning of the trial, average length and weight of measured abalone per tank were 34.4 ± 0.4 mm (mean ± SD) and 5.25 ± 0.17 g respectively. This was a "winter trial", the abalone were fed the treatment feeds from April to August. Water temperature in the tanks averaged 12.8°C, with a maximum of 15.1°C and a minimum of 11.6°C (Figure 6.8.2).

Figure 6.8.2. Experimental tank water temperature fluctuations during the quantity of dietary lipid-dark growth rate trial.

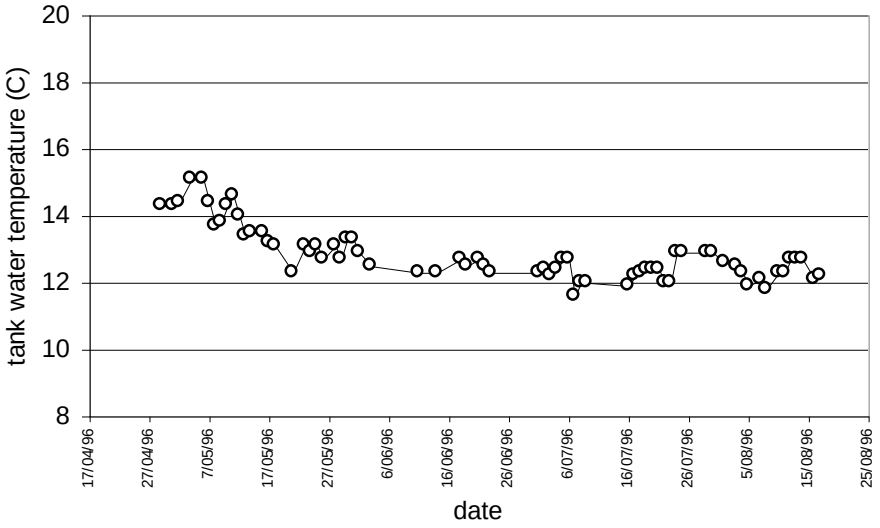


Table 6.8.2: Lipid content and fatty acid composition of Diets CS-22 to CS-28, formulated to contain different levels of oil and to compare abalone growth rates in the dark

Diet Code	CS-22	CS-23	CS-24	CS-25	CS-26	CS-27	CS-28
amount of oil added (% of diet)	1.0%	2.5%	4.0%	5.0%	6.5%	9.0%	11.5%
Saturated fatty acids (SFA)							
14:0	4.4	4.8	4.9	5.0	4.9	5.1	4.9
16:0	18.7	15.6	14.3	13.8	13.4	13.0	12.7
18:0	5.3	4.2	3.8	3.6	3.5	3.3	3.2
total	29.4	25.9	24.4	23.7	23.1	22.7	22.0
Monounsaturated fatty acids (MUFA)							
16:1ω7	4.9	5.8	6.2	6.3	6.5	6.7	6.7
18:1ω9	12.1	12.6	12.9	13.4	14.0	13.9	14.1
18:1ω7	3.0	3.2	3.3	3.3	3.3	3.3	3.4
20:1ω9	2.9	4.7	5.5	5.8	6.2	6.6	6.7
22:1ω9	3.6	6.1	7.3	7.6	8.0	8.6	8.8
total	30.0	37.1	40.5	41.9	43.1	44.4	44.9
Polyunsaturated fatty acids (PUFA)							
18:2ω6	16.2	10.2	7.9	7.0	5.9	4.9	4.5
18:3ω6	0.1	0.1	0.1	0.1	0.0	0.0	0.1
18:3ω3	2.5	1.8	1.6	1.5	1.4	1.3	1.3
18:4ω3	1.3	1.8	2.1	2.2	2.3	2.3	2.4
20:4ω6	0.6	0.5	0.5	0.5	0.5	0.5	0.5
20:5ω3	7.9	8.4	8.6	8.7	8.5	8.7	8.9
22:5ω3	1.5	1.9	2.1	2.1	2.6	2.7	2.8
22:6ω3	7.9	8.4	8.5	8.6	8.6	8.6	8.6
total	39.6	35.7	33.9	33.2	32.5	31.7	31.7
total ω3	22.2	23.8	24.4	24.7	25.1	25.3	25.7
total ω6	17.3	11.5	9.1	8.1	7.1	6.1	5.7
PUFA/SFA ratio	1.3	1.4	1.4	1.4	1.4	1.4	1.4
ω3/ω6 ratio	1.3	2.1	2.7	3.0	3.6	4.2	4.5
% lipid (wet wt)	2.30	3.75	5.08	6.02	7.38	9.90	12.11
% lipid (dry wt)	2.59	4.22	5.72	6.79	8.31	11.16	13.65

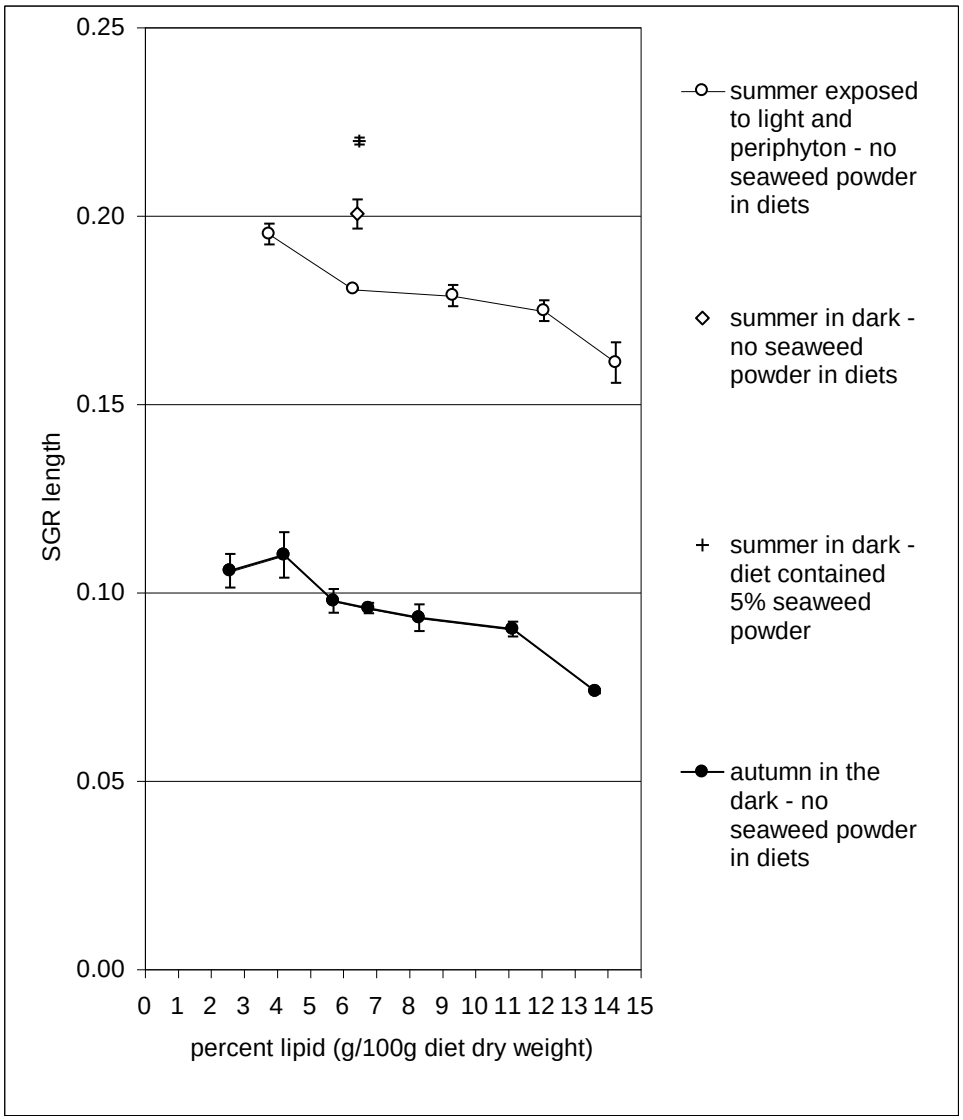
6.8.3 Results/Discussion

The first total lipid requirement trial was run as a "summer trial". Five diets were evaluated, each having different levels of total lipid (3.8-14.3% dry wt., Table 6.8.2) supplemented with diatoms and other periphyton which settled in the naturally lit tanks. The lower the lipid level of the diet, the higher the growth rate (Figure 6.8.3). Because the higher lipid levels in the diets were achieved by the addition of fish oil, the ω3/ω6 ratio of the diets increased with lipid level (Table 6.8.1). Therefore unlike the previous PUFA trial, where elevated ω3/ω6 ratios (up to 2.0) of the diets at constant lipid levels increased growth rates in abalone, elevated ω3/ ω6 ratio (up to 4.1) of the diets (at elevated lipid levels) does not.

As a part of this trial, diet CS-16 was also evaluated in triplicate heavily shaded tanks (labelled CS-20), as was a diet which also contained 5% powdered kelp (CS-21) (Table 6.8.1). These treatments were to establish if low levels of periphyton were beneficial, and whether this benefit could be simulated with a commercially available seaweed powder in heavily shaded tanks. We found that there was no growth advantage from the presence of periphyton (Figure 6.8.3) suggesting that the potentially increased feeding time (darkness) afforded by shading was more important to growth rate. Shelters were provided for the duration of the trial but the results differ to those found previously, where shading of greenlip abalone was not influential if shelters were provided (Maguire et al., 1996b). There was a significant growth advantage in adding the kelp powder to the diet (Figure 6.8.3), as noted when powdered seaweeds were included in previous barrel trials (Dunstan *et al*., 1996a).

The second total lipid requirement trial was run as an "autumn trial". As in the previous light/dark trial the highest growth rates were evident in abalone fed low lipid diets (≤ 4% oil added to the diet) (Figure 6.8.3), which had an ω3/ω6 ratio of 2.7 or less (Table 6.8.2). Lower growth rates were again observed with high lipid (greater than 5% lipid dry wt.), with elevated ω3/ω6 ratio (up to 4.5) diets.

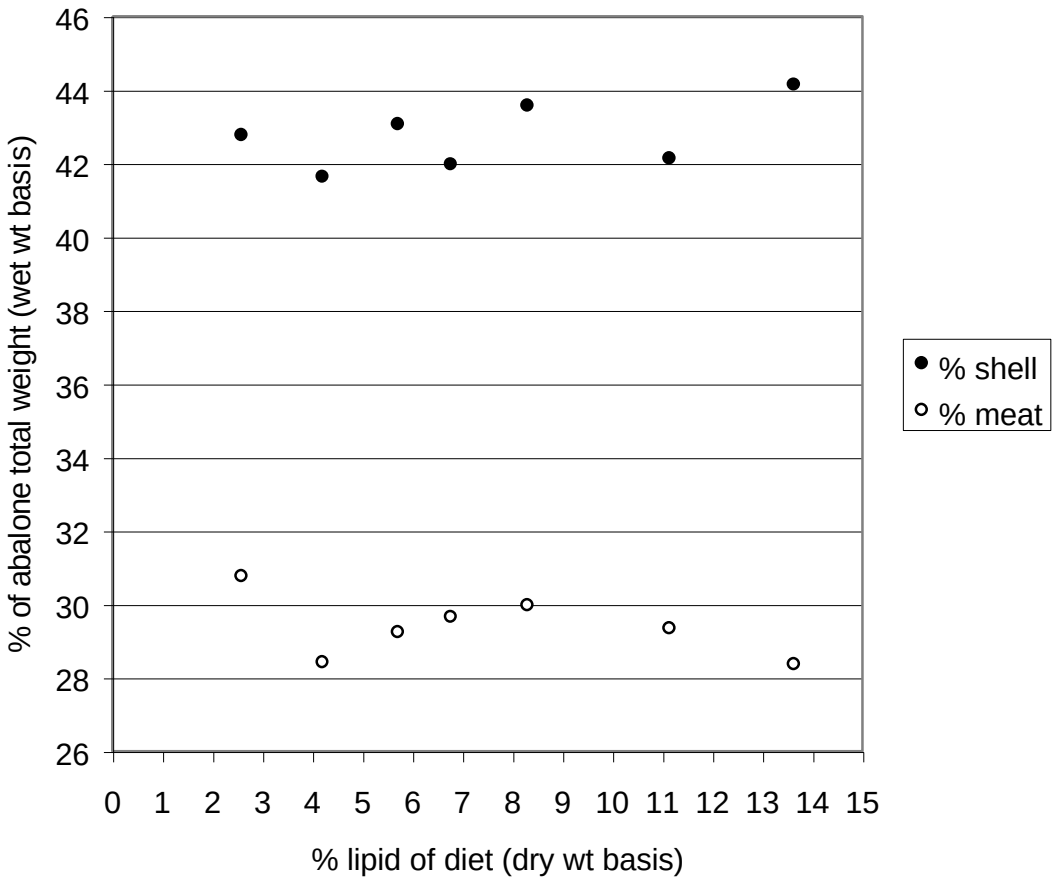
Figure 6.8.3: Average specific growth rate by length (±SEM) during the "summer" and "autumn" trials against the lipid content of the diet



Comparing data from both trials show that lower lipid levels produce higher growth rates in abalone at both warmer and cooler water temperatures (Figure 6.8.3). The highest growth rates were achieved at total lipid levels of between 2 and 4% dry weight (1 and 2.5% oil added at diet formulation respectively). For the lipid levels evaluated, maximum growth rates occurred when lipid was 3.8% in summer and 4.2% in winter. These data suggest that formulated diets with lipid levels between 2 and 5% are optimal for greenlip abalone diets when the abalone are cultured under the conditions of the experiments. Current trends are to increase the lipid levels in formulated diets of some cultured species, for example lipid is added to make up more than 30% in Atlantic salmon diets. However, the present data (Figure 6.8.3), and work on other species of abalone suggest that growth rates in abalone are best when they are fed low-lipid formulated diets (see discussion in Dunstan *et al*., 1996b and Fleming *et al*., 1996).

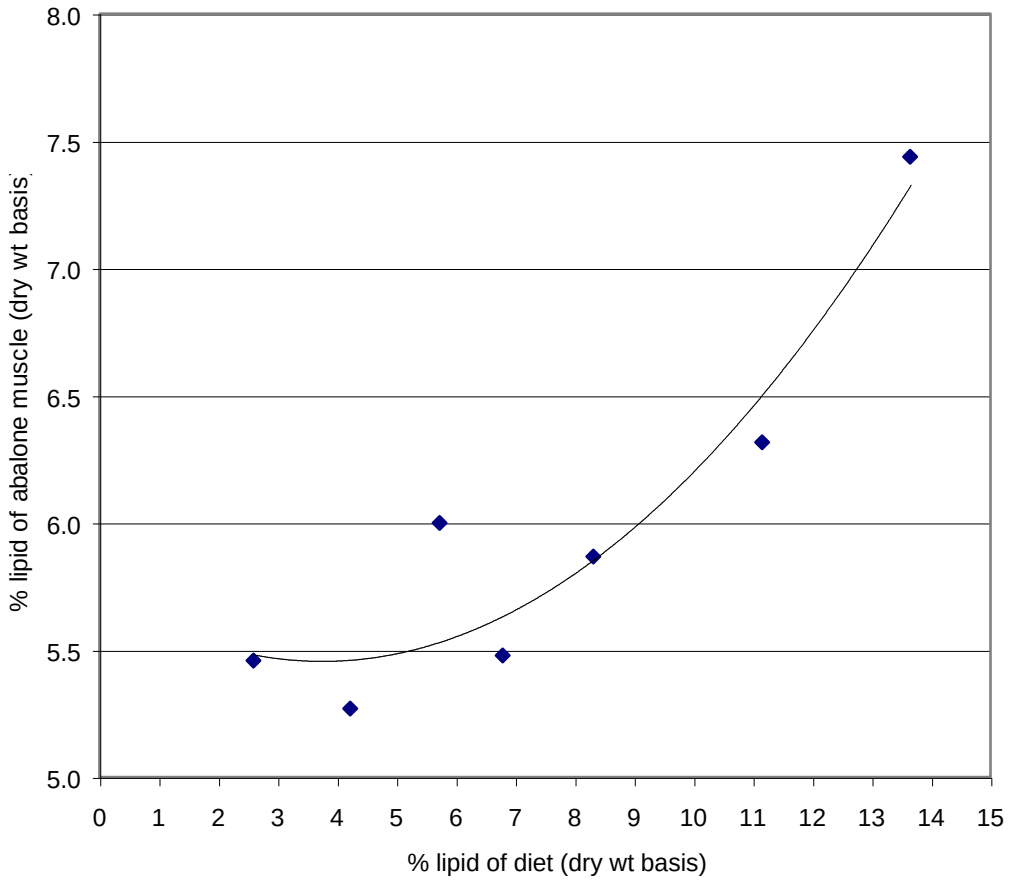
To promote high growth rates of abalone, macroalgal diets with 3-5% lipid (Mercer *et al*., 1993) and formulated diets with 5% lipid have been recommended (Uki *et al*., 1985). Viana *et al*. (1993) have shown accelerated growth rates in *H. fulgens* when fed formulated diets high in lipid (> 9%) compared with those fed a macroalga low in lipid (1%). Similarly, macroalgae with the lowest lipid level produced the lowest growth rates in *H. tuberculata* and *H. discus hannai* (Mercer *et al*., 1993). However, it should be noted that these growth differences may involve nutritional factors other than lipids, as the current trials show that elevated lipid levels from formulated diets decrease abalone growth rates.

Figure 6.8.4: Average percentage shell weight and meat weight of abalone fed diets with different total lipid contents



Changes in the composition of farmed abalone induced by formulated diets is a concern. While changes in the percent meat and percent shell weight were not evident with increasing dietary lipid levels (Figure 6.8.4), significant changes in the flesh lipid content were observed (Figure 6.8.5). Even though glycogen is the main storage compound in abalone muscle (Webber, 1970), it is evident that lipid can accumulate when abalone are grown on a diet high in lipid, and this could affect product quality.

Figure 6.8.5: Average percentage lipid content of muscle of abalone fed diets with different total lipid contents



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6.9 Commercial sources of lipids in formulated diets

6.9.1 Introduction

A feeding trial was performed to determine which particular oil should be included in formulated diets for greenlip abalone. Based on the data for lipid digestibility and the quantity and quality of PUFA compositions shown to increase abalone growth rates during the previous trials, selected oils were incorporated into formulated diets to determine effects on growth of abalone.

6.9.2 Methods

The same system and same methods for abalone measurement were used as for the fatty acid requirements trial above (Section 6.8). The evaluated diets were formulated using a modified ABCHOW formulation. For six of the diets, various oils were added at the levels identified as being optimal from our previous work. Oils were selected on the basis of their fatty acid composition identified as, or thought to be, potentially advantageous as based on trials performed during this study. The investigators have compiled an extensive database on the oil compositions of most Australian fish of commercial value; oils from such species are presently often wasted. The compositions of most commercial vegetable oils have also been analysed. This information was used to determine suitable fish oils from local sources for use in the abalone diet. The oils selected were MaxEPA (fed to the smaller abalone as one treatment, and two treatments for the larger abalone, at two different inclusion levels; 3% and 6% lipid), cod liver oil, mackerel oil, abalone oil and earth worm oil (included in treatment diets for the larger abalone).

At the commencement of the trial (Time 0) all abalone were measured for length and weight. A mid-term (at day 67) length measurement of 25% of each tanks population was made (Time 1), and a measurement of all abalone weights and lengths were again made at Time 2 (at day 105). Because of the extended warm water temperatures during this year and higher feed consumption, it was decided that the experiment should be extended, to enable growth rate determinations during cooler water temperatures. More of the diets were made and some diets changed to enable the continuation of the experiment, and all abalone weights and lengths were again measured at the end of the trial (Time 3, at day 162). Abalone were not sorted between each period. The total duration of the trial was 162 days.

Diets CS-29 to CS-35 (Table 6.9.1) were evaluated during “summer” (between Times 0 and 2). Diets CS-29 and CS-36 to CS-40 (Table 6.9.2) were evaluated during “autumn” (between Times 2 and 3). Diets CS-29 and 30 were from the same batch of an identical diet, the former being a crumbed version (0.5-2 mm cylinders); the latter being pelleted, and there was no

difference in lipid composition with processing (Table 6.9.1). The crumbed CS-29 was fed to smaller animals (average abalone length 22.9mm) whereas the other six diets were fed to larger abalone (average abalone length 40.9 mm). For the batch of diets used in the first part of the trial (Time 0 to 2), the abalone and worm oils were extracted in the laboratory using non-toxic solvents. For the batch of diets used for the second part of the trial (Time 2 to 3), a semi-commercial source of abalone oil was used, but worm oil was not available for further evaluation.

Table 6.9.1: Lipid content and fatty acid composition of Diets CS-29 to CS-35, formulated to contain various types of oil evaluated from Time 0 to Time 2

Diet code	CS-29	CS-30	CS-31	CS-32	CS-33	CS-34	CS-35
amount of oil added (% of diet)	2.5% MaxEPA	2.5% MaxEPA	4% MaxEPA	2.5% cod liver oil	2.5% mackerel oil	2.5% abalone oil	2.5% worm oil
Meal used	fish	fish	fish	fish	fish	fish	fish
Form	crumb	pellet	pellet	pellet	pellet	pellet	pellet
Saturated fatty acids (SFA)							
14:0	5.7	5.6	6.1	4.9	4.7	6.0	4.2
16:0	19.4	19.3	18.6	18.1	20.7	21.5	18.7
18:0	3.9	3.8	3.7	3.3	3.5	3.2	4.6
total	30.6	30.2	29.9	27.5	30.2	31.9	29.3
Monounsaturated fatty acids (MUFA)							
16:1ω7	5.0	5.0	5.9	4.3	4.2	3.9	3.1
18:1ω9	10.7	10.7	10.6	12.3	12.5	10.5	10.6
18:1ω7	2.4	2.4	2.5	2.4	2.0	8.7	2.9
20:1ω9	1.0	1.0	1.0	5.0	2.4	1.4	1.8
22:1ω9	0.5	0.5	0.5	4.2	2.8	0.2	2.0
total	21.1	21.0	22.1	30.6	25.6	27.9	23.0
Polyunsaturated fatty acids (PUFA)							
18:2ω6	13.9	14.1	10.7	15.9	14.9	13.9	20.2
18:3ω6	0.2	0.2	0.2	0.0	0.0	0.1	0.0
18:3ω3	2.1	2.1	1.8	2.5	2.6	2.7	2.8
18:4ω3	1.8	1.8	2.0	2.2	2.4	1.1	1.5
20:4ω6	1.2	1.3	1.2	0.9	1.0	4.0	2.6
20:5ω3	10.6	10.8	12.3	6.4	7.4	5.7	6.7
22:5ω3	1.5	1.5	1.7	1.1	0.9	1.9	1.0
22:6ω3	10.2	10.4	10.4	9.1	11.3	4.9	8.7
total	44.9	45.5	44.0	40.3	42.4	38.3	45.6
total ω3	27.2	27.7	29.2	22.4	25.6	16.8	21.6
total ω6	16.6	16.6	13.5	17.6	16.6	19.2	24.1
PUFA/SFA ratio	1.47	1.51	1.47	1.47	1.41	1.20	1.56
ω3/ω6 ratio	1.64	1.67	2.17	1.27	1.54	0.88	0.90
% lipid (wet wt)	3.0	3.1	4.0	2.9	3.2	3.6	3.3
% lipid (dry wt)	3.3	3.4	4.5	3.2	3.6	4.0	3.7

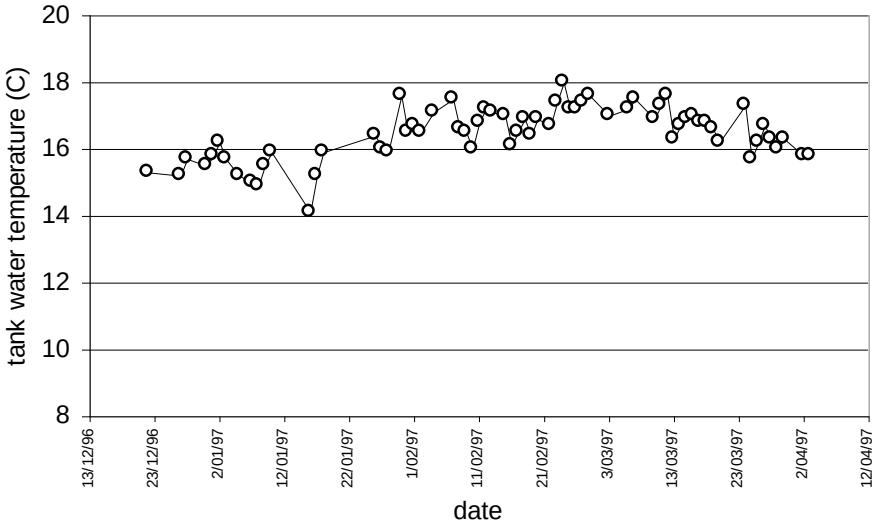
Eighteen of the tanks were stocked with 65 greenlip abalone each, which at the beginning of the trial had an average length and weight of (mean ± SD) 40.9 ± 1.1 mm and 9.49 ± 0.48 g

respectively. The remaining three tanks were stocked with 105 greenlip abalone each, which at the beginning of the trial had an average length and weight of 22.9 ± 0.3 mm (mean ± SD) and 1.62 ± 0.05 g respectively. For this first part of the experiment (between Times 0 and 2), treatment diets were fed from December to April, and the water temperature in the tanks averaged 16.5°C increasing from a minimum of 14.1°C to a maximum of 18.0°C (Figure 6.9.1).

Table 6.9.2: Lipid content and fatty acid composition of Diets CS-29 and CS36 to CS-40, formulated to contain different types of oil, evaluated from Time 2 to Time 3

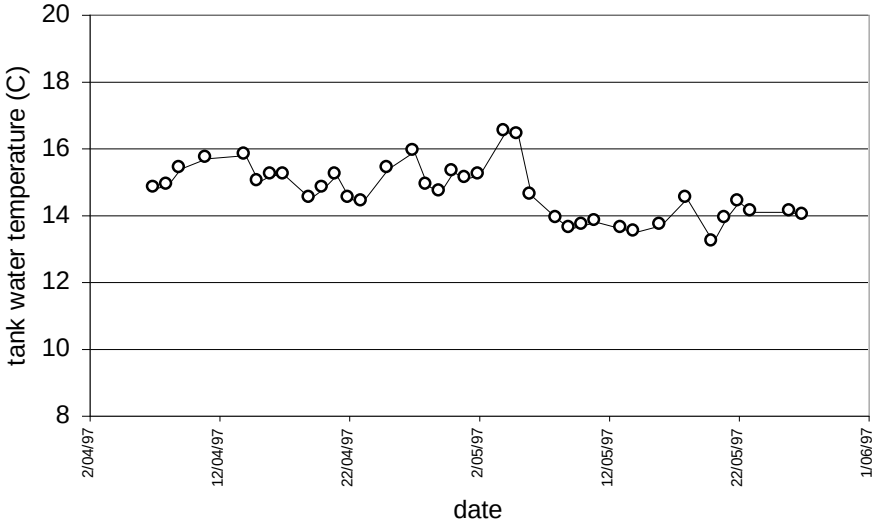
Diet code	CS-29	CS-36	CS-37	CS-38	CS-39	CS-40
oil added (% of diet)	2.5% MaxEPA	2.5% MaxEPA	4% MaxEPA	2.5% Cod liver oil	2.5% mackerel oil	2.5% abalone oil
Meal used	fish	fish	fish	fish	fish	abalone
Form presented	crumb	pellet	pellet	pellet	pellet	pellet
Saturated fatty acids (SFA)						
14:0	5.7	5.1	5.5	4.3	4.5	2.0
16:0	19.3	17.8	17.3	16.0	19.4	13.8
18:0	3.7	3.6	3.4	3.0	3.4	2.8
total	30.1	27.7	27.5	24.3	28.5	19.1
Monounsaturated fatty acids (MUFA)						
16:1ω7	5.4	4.7	5.4	4.0	3.5	1.2
18:1ω9	10.8	11.5	11.1	12.8	12.8	13.6
18:1ω7	2.4	2.2	2.3	2.3	1.8	3.5
20:1ω9	0.8	0.9	1.0	4.6	2.6	0.4
22:1ω9	0.5	0.3	0.5	4.6	2.4	0.0
total	21.2	20.9	21.7	31.1	25.2	19.8
Polyunsaturated fatty acids (PUFA)						
18:2ω6	12.9	18.7	14.9	19.3	19.2	52.3
18:3ω6	0.2	0.1	0.2	0.1	0.1	0.0
18:3ω3	2.0	2.9	2.4	3.1	3.2	2.6
18:4ω3	1.9	1.7	1.9	2.1	2.1	0.5
20:4ω6	1.2	1.2	1.2	0.8	0.9	1.7
20:5ω3	11.4	10.0	12.1	6.3	6.5	1.4
22:5ω3	1.6	1.4	1.6	1.1	0.8	0.1
22:6ω3	10.6	9.4	9.9	8.3	9.8	0.5
total	45.3	48.5	47.5	43.1	44.5	60.6
total ω3	28.5	26.3	28.8	21.8	23.2	5.6
total ω6	15.5	21.1	17.3	21.0	21.0	54.3
PUFA/SFA ratio	1.5	1.8	1.7	1.8	1.6	3.2
ω3/ω6 ratio	1.8	1.3	1.7	1.0	1.1	0.1
% lipid (wet wt)	3.2	3.7	4.5	3.6	3.7	4.2
% lipid (dry wt)	3.6	4.1	5.0	4.0	4.1	4.7

Figure 6.9.1: Experimental tank water temperature fluctuations during the "summer" part of the different oils growth rate trial



At the beginning of the second part of this trial (Times 2 to 3) the greenlip abalone in the eighteen tanks had an average length and weight of 49.3 ± 1.5 mm (mean ± SD) and 15.21 ± 0.82 g respectively. The remaining three tanks with greenlip abalone had an average length and weight of 30.3 ± 0.4 mm (mean ± SD) and 3.59 ± 0.11 g respectively at Time 2. For this second part of the experiment, tanks were fed the treatment diets from April to May, and the water temperature in the tanks averaged 14.7°C decreasing from a maximum of 16.5°C to a minimum of 13.2°C (Figure 6.9.2).

Figure 6.9.2: Experimental tank water temperature fluctuations during the "autumn" part of the different oils growth rate trial

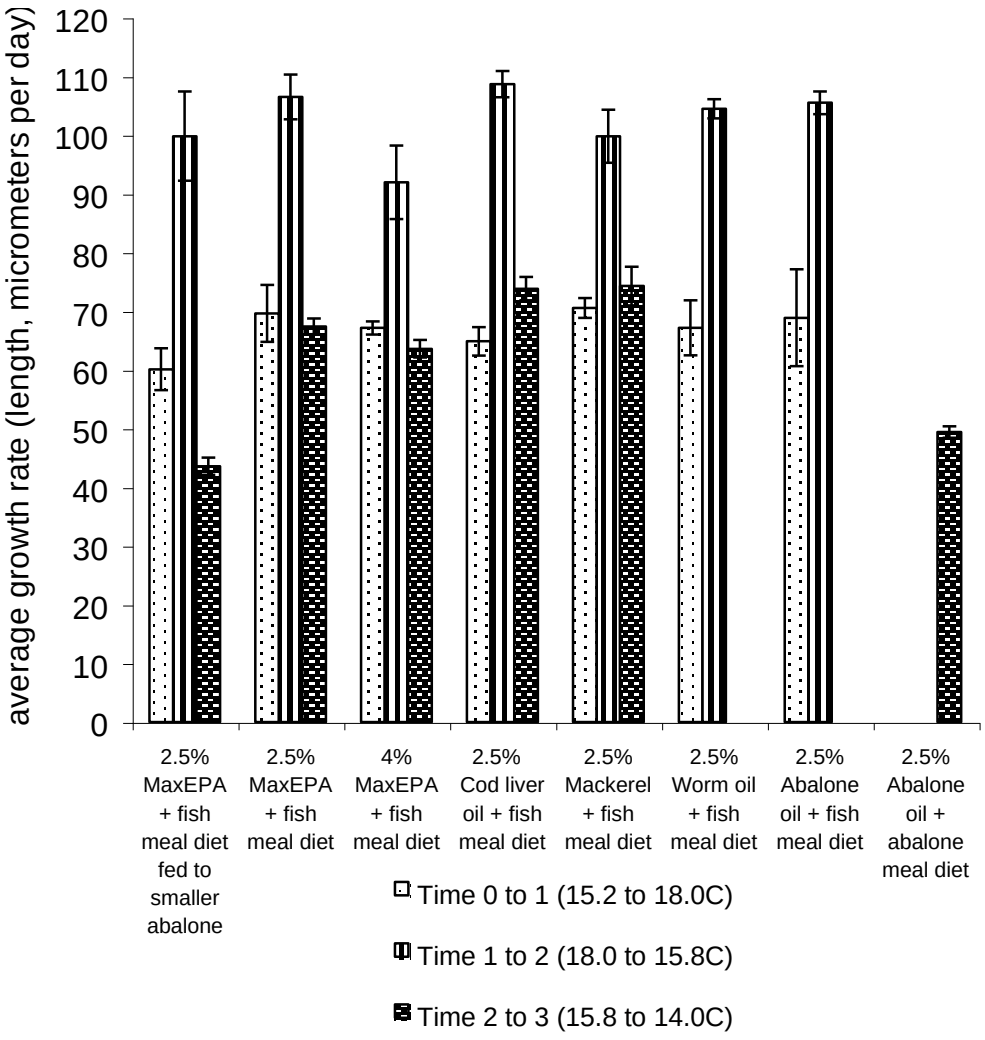


6.9.3 Results/Discussion

All diets with oils at 2.5% inclusion level produced comparable growth rates in larger abalone during the trial (generally >100 μm/day, between Times 1 and 2, and >65 μm/day, between Times 0 and 1, as well as between Times 2 and 3) (Figure 6.9.3). The MaxEPA oil and cod

liver oil, at 2.5% inclusion level, produced the highest average growth rates in the larger abalone between Times 1 and 2. The worm oil diet produced the same high growth rates but was only evaluated between Times 0 and 2 due to its high cost of production in the laboratory. Between Times 1 and 3, growth rates appeared to be higher in large abalone fed 2.5% MaxEPA diets containing less lipid, than those fed diets containing 4% MaxEPA, which is consistent with the data from the previous section on high lipid diets. The smaller abalone grew at lower absolute growth rates (μm/day) than the larger abalone when both treatments were fed the MaxEPA diet. This would be expected for smaller animals, and calculation of specific growth rates show that on the same diet, the SGRw was higher for smaller abalone compared to larger abalone (0.85 and 0.4 respectively).

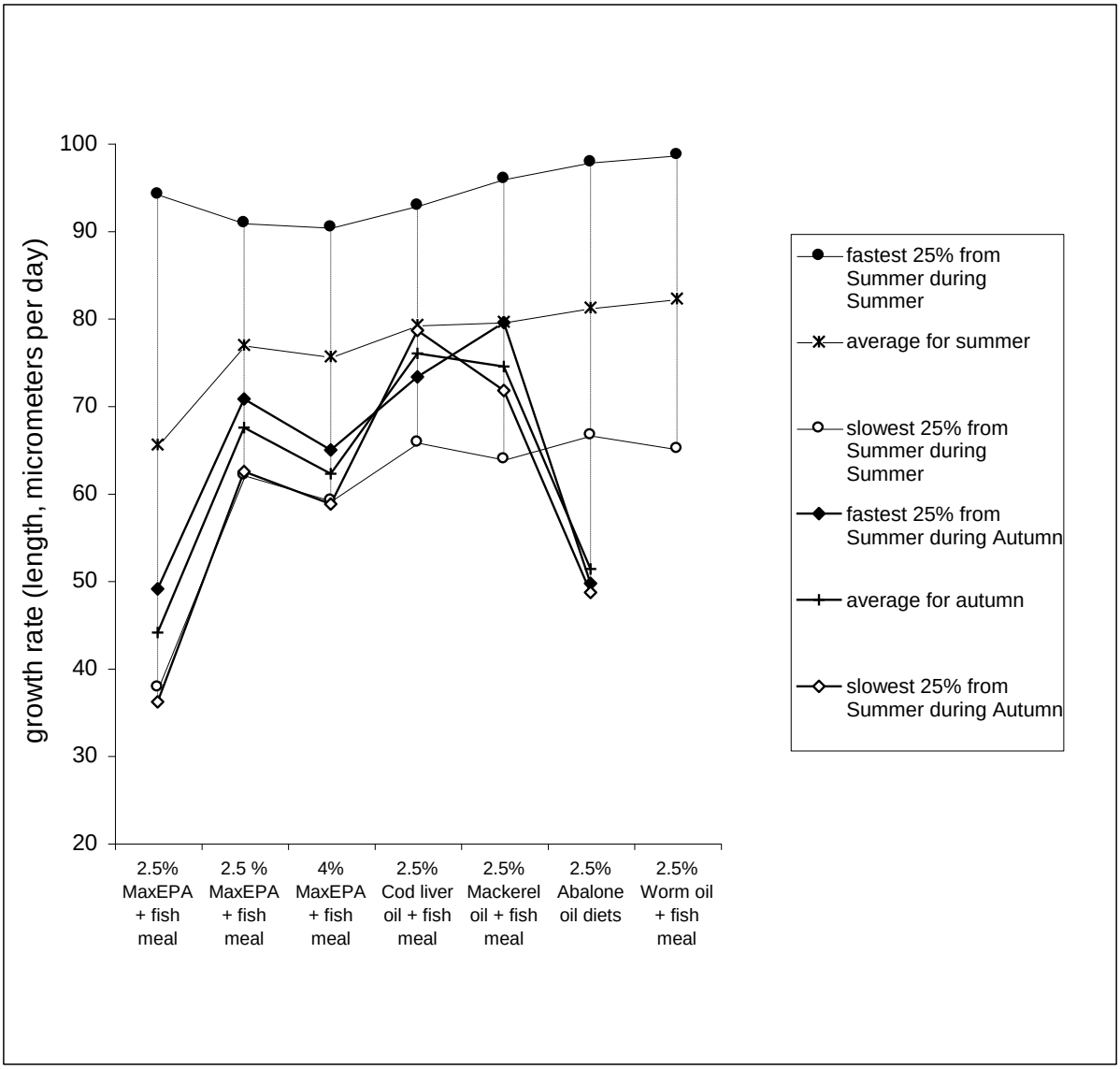
Figure 6.9.3: Average growth rates in micrometers per day (±SEM) for greenlip abalone fed diets containing different types of oil for the three growth periods



When abalone were fed the laboratory prepared abalone oil (between Times 0 and 2), growth rates were as high as those fed the other oils. The diet containing the semi-commercial source of abalone oil used for the second series of diets depressed growth relative to the abalone oil prepared in the laboratory. Fatty acid analysis revealed that the semi-commercial abalone oil

resembled a vegetable oil, with high ω6 PUFA and a low (approx. 0.1) ω3/ω6 ratio (Table 6.9.2 CS-40 cf Table 6.6.1 CS-1). Vegetable oils similar in composition to this oil, when present in high amounts also depress growth (CS-1 Figure 6.6.2, Dunstan et al., 1996b).

Figure 6.9.4: Growth rates during both summer and autumn of the fastest growing 25% and slowest growing 25% greenlip abalone during summer, when fed diets containing different oils



During cooler water temperatures, abalone grew faster using high levels of 20:5ω3 with moderate levels of 22:6ω3 compared to using typical fish oils which contain moderate levels of both these fatty acids (Figure 6.7.2). The natural diets of abalone are diatoms which contain high levels and proportions of 20:5ω3, and the red and brown seaweeds which contain high proportions of this acid (Table 6.2.1, Dunstan and Volkman, 1996). It is for this reason that MaxEPA, abalone, and earthworm oils were selected, since they all contain elevated levels of this fatty acid. Diatom oil as another high 20:5ω3 oil, has already been evaluated, but was found to offer no growth advantage over using fish oils (Maguire et al., 1996).

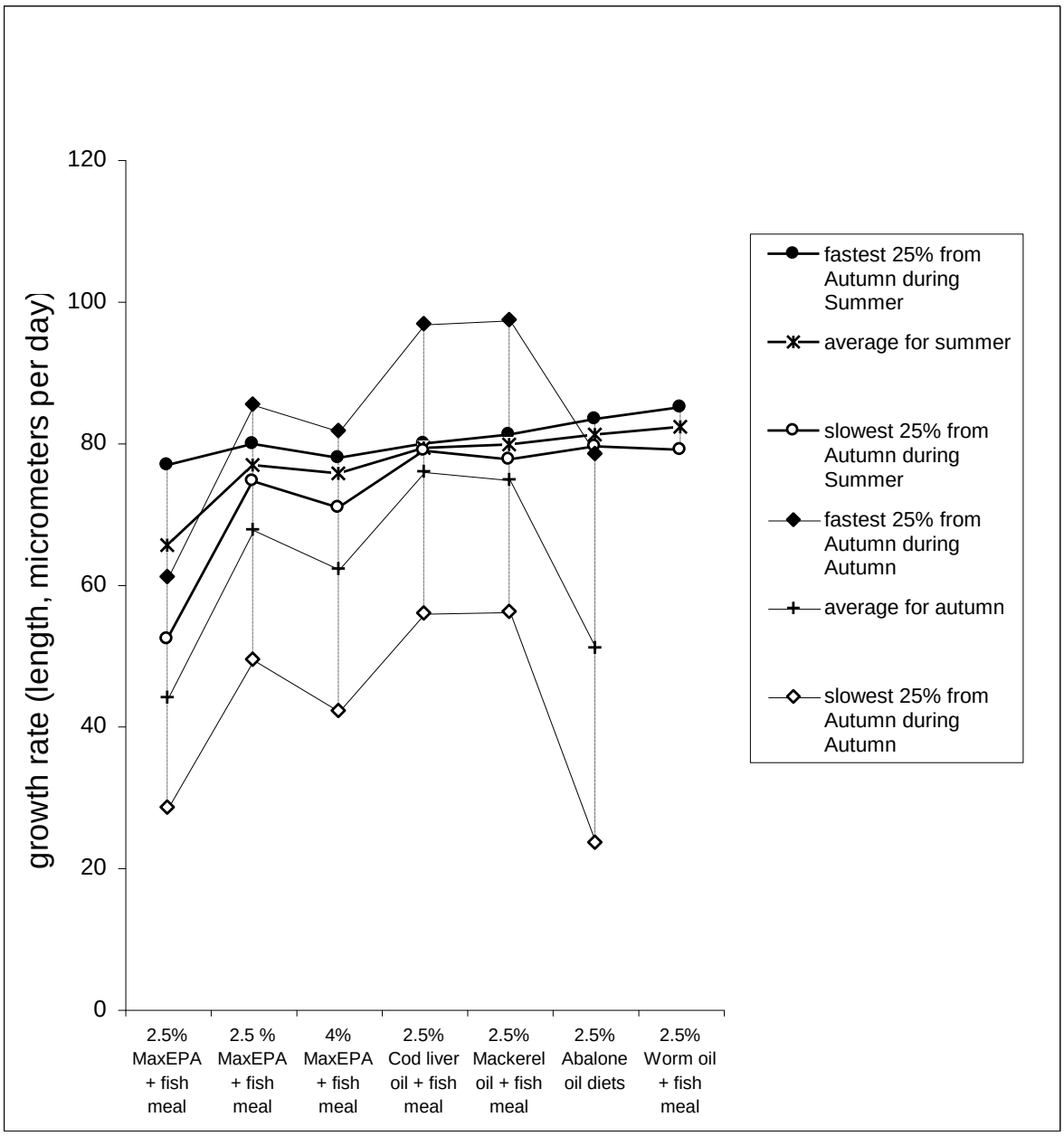
The reference diets from our previous work (Sections 6.5 lipid digestibility and 6.6 & 6.7 dietary PUFA and growth rate) contained the added oil as the sole major lipid source, and therefore any major growth advantage could be attributed to the added oil. In the current trial, the full ABCHOW formulation was used and therefore the lipid from relatively high lipid ingredients such as fishmeal contributes to, and affects the total lipid and fatty acid composition of the diets. Although fishmeal contains 20:5ω3 as a major ω3 polyunsaturated fatty acid, the other essential fatty acid for many marine species, 22:6ω3, is usually the major ω3 acid. Therefore because so little of the high 20:5ω3 MaxEPA oil is added, the beneficial effects of using these oils may be diminished, possibly explaining the lack of growth advantage using high 20:5ω3 oils in the cooler water temperatures in this (MaxEPA – Figure 6.9.3), and other studies (Diatom oil - Maguire et al., 1996a). The lack of a growth advantage in the cooler weather by using the high-20:5ω3-oil containing diets, may also have been due to the higher water temperatures experienced in the current experiment compared to the previous trial (Autumn cf Winter).

Previous work (Figure 6.6.2) showed that during the warmer water temperature months, growth in abalone was similar when fed high 20:5ω3 diets compared to when fed typical commercially available fish oils (high 20:5ω3 and high 22:6ω3), of which cod liver oil and mackerel oils are examples. The same trends were evident in the current study (Figure 6.9.3). Therefore "typical" fish oils are recommended for inclusion in formulated diets for abalone during the warmer seasons, but at low inclusion rates. Diets with <8% fish meal and only vegetable oils added would therefore not provide enough ω3 PUFA to maximise growth rates in greenlip abalone. We must stress that not all fish oils are "typical" in composition, and the oils trialed in the present study were carefully selected based on preliminary research maximising growth rates.

Because each abalone was tagged for the trial, identification of individual growth rates was possible. As is evident from Figure 6.9.4 the fastest growing 25% of the abalone from each treatment were averaging over 90 micrometers/day during the “summer” (i.e. between Times 0 and 2). The slowest growing 25% of the abalone from most treatments were averaging between 60 to 66 micrometers/day, the exception being the smaller abalone fed the powdered 2.5% MaxEPA diet. Particularly noteworthy is that the fastest growing 25% and slowest growing 25% in summer were, during the autumn (i.e. between Times 2 and 3), growing on average at rates similar to the average growth rates for the whole population during autumn.

Similarly Figure 6.9.5 shows that the fastest growing 25% and slowest growing 25% in autumn had, during the summer, grown on average at rates similar to the average growth rates for the whole population during summer. Therefore the fastest and slowest growing abalone during one season, are not necessarily the fastest and slowest growing abalone, respectively, during another season.

Figure 6.9.5: Growth rates during both summer and autumn of the fastest growing 25% and slowest growing 25% greenlip abalone during autumn, when fed diets containing different oils



6.9.4 References

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Maguire G.B., Dunstan G.A., Mansour P., Hindrum S.M., Johns D.R. and Cropp M., 1996. Effects of microalgal or macroalgal supplementation of an abalone diet on growth of juvenile greenlip abalone *Haliotis laevigata*. *Proc. 3rd Ann. FRDC/CRC Workshop on Abalone Aquaculture*, 16-18 August 1996, Port Lincoln. Hone P.W. and Fleming A.E. (Eds). South Australian Research and Development Institute, Adelaide, South Australia.

7. POINT SUMMARY OF FINDINGS

Baseline data. (Objective 1)

6.2 Natural abalone diets

- The natural diets of juvenile abalone (diatoms) and adult abalone (seaweeds) typically contain high proportions of the essential fatty acid 20:5ω3 and low proportions of 22:6ω3.
- Red seaweeds also contain high proportions of 20:4ω6.

6.3 Formulated abalone diets and ingredients

- Formulated diets used for abalone culture contained very little to high proportions of 20:5ω3 and 22:6ω3 (dependent on the amount of fish oils added) and low proportions of 20:4ω6.

6.4 Abalone flesh

- Abalone flesh is low in total lipid.
- Abalone flesh has quite a different fatty acid composition to other seafoods, with high proportions of 20:5ω3, 20:4ω6 and 22:5ω3.
- Abalone flesh contains small amounts of sterols, the main sterol being cholesterol.

Essential lipids (Objective 2)

6.5 Seaweed/formulated diet comparisons and apparent digestibility of lipids

- Abalone growth rates were higher when fed the reference formulated diet containing high proportions of 20:5ω3 and 22:6ω3 or a seaweed containing high proportions of 20:5ω3, than when fed a seaweed containing high proportions of 20:4ω6.
- Abalone growth rates were not affected by tagging.
- Apparent dry matter digestibility of lipid by abalone was high (84.7).
- Apparent dry matter digestibilities by abalone of fatty acid rich lipid classes (triacylglycerols e.g. fish oils and polar lipids that are lecithin like) were higher than for the other lipid classes.
- Apparent dry matter digestibilities by abalone of ω3 and longer chain polyunsaturated fatty acids (as found in fish oils) were higher than for ω6 and shorter chain less unsaturated fatty acids (as found in vegetable oils).

- Apparent dry matter digestibility of phytosterols (plant derived) was higher than for cholesterol (animal derived).

6.6 Dietary PUFA and growth rate - Individual PUFA

- Abalone growth rates were higher when fed the reference formulated diets containing long chain ω3 polyunsaturated fatty acids (as found in fish oils) than when fed those containing long chain ω6 PUFA (as found in red seaweeds). The lowest growth rates were evident when abalone were fed diets containing mostly shorter chain ω6 PUFA (as found in vegetable oils).

6.7 Dietary PUFA and growth rate - Combinations of different PUFA

- Abalone growth rates were higher during colder water temperatures when fed the reference formulated diet containing the highest proportion of 20:5ω3.

- Abalone growth rates were higher when fed reference formulated diets with an ω3/ω6 PUFA ratio of 1.2 to 2 (i.e. more fish oil derived PUFA than vegetable oil derived PUFA).

6.8 Quantity of dietary lipid

- Abalone growth rates were higher when grown in the dark and higher still when a kelp powder was added to the formulated diet.

- Abalone growth rates were higher when fed formulated diets with low lipid levels (only 1.5% added fish oil, total lipid content of 3.8% during summer.

- Abalone growth rates were higher when fed formulated diets with low lipid levels (only 1 and 2.5% added fish oil, total lipid contents of 2.6 and 4.2% respectively) during winter.

- Total lipid content of formulated diets did not affect the % shell or % meat weight of abalone.

- Total lipid content of formulated diets did affect the total lipid content of abalone flesh.

Commercial sources of lipids in formulated diets (Objective 3)

6.9 Commercial sources of lipids in formulated diets

- Abalone growth rates were higher when formulated diets contained MaxEPA or cod liver oils at optimum dietary lipid levels.

- Abalone growth rates were lowest when fed a formulated diet containing an oil resembling a vegetable oil in composition.

8. ARTICLES ARISING FROM THE PROJECT

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9. INTELLECTUAL PROPERTY

The focus of the work was to conduct public domain research so all stakeholders can benefit.

Results will be published and disseminated widely. It is not anticipated that any patents or commercial intellectual property will arise from this project.

10. PROJECT STAFF

Provide the names, positions, qualifications and skills of all staff to be engaged on the project. Indicate, as a percentage of time, the level of contribution of each staff member to the project. A substantial commitment of time is expected by the Principal Investigator throughout the life of the project.

Mr. G.A. Dunstan	CSIRO Marine Research	BSc	60%
Dr J.K. Volkman	CSIRO Marine Research	BSc (Hons) PhD	10%
Dr G.B. Maguire	Department of Aquaculture, UTAS	BSc (Hons) PhD	10%
Mr. M.J. Cropp	Marine Shellfish Hatcheries	Dip. Agriculture	3%
Mr D.R. Johns	Department of Aquaculture, UTAS	Dip. Aquaculture	50%
Mr. D.A. Cropp	Aquatech Australia Pty Ltd	BSc(Hons) AIB	3%

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