August 2002

Formulated feeds for newly settled juvenile abalone based on natural feeds (diatoms and crustose coralline algae)

Graeme A. Dunstan
Malcolm R. Brown
John K. Volkman
Greg B. Maguire

UNIVERSITY OF TASMANIA

FRDC Project No. 1996/386

National Library of Australia Cataloguing-in-Publication Entry

Formulated feeds for newly settled juvenile abalone based on natural feeds : diatoms and crustose coralline algae

Bibliography.
ISBN 1 876996 22 6.

1. Abalones - Feeding and feeds - Australia.
I. Dunstan, Graeme A.
II. CSIRO. Division of Marine Research.
III. Title.

639.48320994

Table of Contents

1. NON-TECHNICAL SUMMARY

1996/386 **Formulated feeds for newly settled juvenile abalone based on natural feeds (diatoms and crustose coralline algae)**

PRINCIPAL INVESTIGATOR: Graeme A. Dunstan
ADDRESS: CSIRO Marine Research
GPO Box 1538
Hobart, Tas. 7001
Telephone: 03 6232 5274
Fax: 03 6232 5000

OBJECTIVES:

1. Use information on the nutritional and attractant factors present in natural food items to develop formulated diets for very young juvenile abalone (« 15 mm).
2. Manufacture and evaluate the diets for water stability and palatability for various diet delivery mechanisms including gels, pellets, pastes and adhesion diets.
3. Produce formulated diets of high nutritional value, which produce high growth rate in very young abalone (< 15mm), as verified by growth experiments.
4. Provide information to the FRDC sub-program, so as to improve the existing formulated diet used for the "grow out" phase (>15 mm).
5. Identify the nutrients incorporated into the actively growing tissues of abalone when fed on diatoms by assessing fatty acid metabolism and carbon and nitrogen retention in juvenile abalone using stable isotopes.

NON-TECHNICAL SUMMARY:
Diatoms, crustose coralline algae, turf algae and bacteria are the main natural food of small abalone. Due to high infrastructure and labour costs associated with long-term diatom culture, and the limited time diatom supply can meet demand, development of formulated diets to replace diatoms was seen by industry as a high priority. The overall aim of this research was to develop innovative formulated diets for very young abalone based on the chemical composition of their natural diet, and optimised to maximise the animals' growth.

OUTCOMES ACHIEVED

Formulated diets based on the chemical composition of diatoms, as an alternative to using cultured diatoms, were shown to be quite effective in improving growth rate of small abalone (<15 mm). A series of controlled feeding experiments with a total of 28 formulated diets, were carried out to identify the amounts of certain key nutrients (crude protein and fish oil) and which other ingredients should be included to improve growth rate. The research showed that growth rate of small abalone was maximised by feeding high protein diets. When soya flour (main protein source) was incrementally added to formulated diets with decreasing maize starch (main carbohydrate source), a protein level >36% dry weight (DW) was required for maximum abalone growth rate. Addition of diatomaceous earth did not change growth rate. However, the addition of a sugar mix (6% glucose, 0.5% mannose and 2.5% xylose DW ingredients) or 3% oyster glycogen was shown to produce higher growth rates than adding maize starch or single simple sugars such as glucose and sucrose. A high fishmeal/algal meal diet produced high abalone growth rate comparable to that of the formulated "diatom" diet. Growth rate of abalone fed a diet where the protein and lipid was entirely of plant origin was lower than one where fish products were used. Substitution of maize starch with semolina or wheat starch gave a slight but not significant growth advantage during the cooler months. Small blacklip abalone fed on appropriately formulated diets grew at rates equal, or superior, to those fed on mass cultured, pasted or flocculated diatoms.

A second outcome was to advance knowledge about the dietary assimilation of essential fatty acids and their metabolic fate in small abalone. The apparent digestibility of different classes of fatty acids was determined and their metabolic fate traced using stable isotope mass spectrometry. Saturated and monounsaturated fatty acids were less digestible than polyunsaturated fatty acids (PUFA) but plant PUFA (predominantly ω6 fatty acids) were equally as well digested as fish PUFA (predominantly ω3 fatty acids). Oils rich in 20:4ω6, 20:5ω3 and 22:5ω3 were preferentially assimilated or converted from other dietary PUFA, and therefore diets should contain these long chain PUFAs or their precursors.

Outputs of the research include several dietary formulations that under experimental conditions appear equal or superior to cultured diatoms as food for small abalone. The importance and metabolic fate of dietary fatty acids in small abalone were determined.

Based on the findings from this project, it is recommended that further work be done to address two aspects. Firstly, to validate the experimental findings of this project by carrying out large-scale, industry studies to compare formulated diets against mass cultured diatoms.

Formulations that are recommended for comparative study are detailed below:

Ingredient	Diet code and description			
	MG53 (34% soya)	MG54 (48% soya)	MG56 (diatom)	MG57 (Fish/algal)
Soya flour	34.0	48.0	25.0	0
Fishmeal	9.0	9.0	9.0	20.0
Fish oil	3.0	3.0	3.0	1.9
Glucose	0	0	6.0	0
Mannose	0	0	0.5	0
Xylose	0	0	2.5	0
Semolina	0	0	0	30.0
Diatomaceous earth	0	0	4.3	0
Kelp powder	5.0	5.0	5.0	16.0
Maize starch	29.3	15.3	25.0	12.9
Calcium carbonate	1.0	1.0	1.0	0.5
Fixed ingredients[1]	18.7	18.7	18.7	18.7
Total	100	100	100	100

[1] Included in final diet (%): casein, 10.0; sodium alginate, 3.0; vitamin mix, 2.0; vitamin C, 1.0; vitamin E, 0.01; mineral mix, 1.0; calcium sulphate, 0.8; methionine, 0.3; threonine, 0.3; and arginine, 0.3.

Secondly, further research is needed to understand the role, and to optimise the composition, of the 'sugar mix' and other carbohydrates in abalone formulated diets. The current work showed that a sugar mix of 6% glucose, 0.5% mannose and 2.5% xylose had a significant growth stimulatory effect, particularly when used with high protein diets. Further research is needed to better understand the dietary importance of these simple sugars and other non-structural carbohydrates. The usefulness of a sugar mix in diets for larger (>15 mm) abalone also warrants investigation.

KEYWORDS: Abalone, Carbohydrate, Diatom, Formulated diet, Lipid, Polyunsaturated fatty acids, Protein, Sugar.

2. BACKGROUND

Epiphytic and epilithic diatoms, crustose coralline algae, turf algae and bacteria are significant dietary components of newly settled and small (<15 mm) abalone. Most of Australia's hatcheries rely on natural colonisation of plates by such diatom communities for the first diets of newly settled abalone. The voracious abalone spat fed such diets grow at a high growth rate. However, consumption rate rapidly exceeds diatom production, due to the reliance on natural colonisation of plates, seasonal decline of diatoms with water temperature and the finite resources required to maintain the increasing demands of a large number of abalone. As a result, the hatchery can run out of this important food resource. Limiting of the natural food source results in reduced growth rate and can result in a high mortality rate. Prior to this happening, the abalone may be weaned onto formulated diets, but this generally leads to a reduced growth rate. Similarly at the grow-out phase (>15 mm), a diet of natural diatom communities generally produces higher growth rates than if the abalone are fed on formulated diets (M. Cropp, pers. com.). In the wild, it is not until the abalone are much larger (approx 25 to 30 mm) that they tend to feed less on diatom and turf communities and more on seaweeds. For optimum nutrition, small (<15 mm) abalone may require a diet similar in composition to that of diatom communities for a significantly longer period of time than presently is the case in hatcheries.

It is important to identify the nutritional requirements of the very young abalone in order to improve the formulated diets currently available. Research identifying the specific chemical characteristics of the components of diatom communities and reproducing them in formulated diets for very young abalone may substantially improve productivity. Maximising the growth rate of very young abalone by providing them with better diets should significantly reduce the time it takes them to grow to a commercial harvest size.

Previously we have shown that low levels of oils rich in Omega 3 polyunsaturated fatty acids (ω3 PUFA) should be added to abalone diets for maximum growth of grow-out abalone (Dunstan et al., 2000). The highest growth rate achieved with long chain ω3 PUFA was two and a half times that achieved with a long-chain PUFA deficient diet. Also, 20:4ω6 (arachidonic acid; AA) which is high in most seaweeds, or 22:6ω3 (docosahexaenoic acid; DHA) which is high in most fish oils, could not alone be substituted for, and produce the high growth rate achieved with 20:5ω3 (eicosapentaenoic acid; EPA) which is high in diatoms. But no work had been performed on small abalone. Diatoms contain a large proportion of their dry weight as lipid (Dunstan et al., 1994), but seaweeds contain only a low proportion of lipid (Dunstan et al., 2000). Thus, there was a need to establish whether an ontogenetic shift away from lipid and towards carbohydrate as a preferred energy source occurs as abalone change from one diet source to the other. Once suitable diets have been identified it would be necessary to reduce cost by substituting cheaper, commercially available food ingredients.

3. NEED

There was very little focussed work on why diets of diatoms, turf algae and crustose coralline algae are so beneficial for very young abalone. Unfortunately, it becomes impractical to provide enough diatom-covered plates to satisfy the requirements of the rapidly growing abalone. Thus alternative diet sources such as seaweeds and/or formulated diets must be investigated. Harvesting of seaweeds on a very large scale may prove inappropriate due to environmental concerns. The culture of seaweeds for abalone food is presently uneconomic in Australia. Formulated diets are convenient and the FRDC diet developed at SARDI for grow-out abalone is proving to be more economical than imported diets. Unfortunately, small

abalone fed solely on formulated diets for extended times tend to grow slowly. Thus the factors (which enable small abalone to grow rapidly) in the natural food items seemed to be missing from presently available formulated diets. Work currently under way is determining the digestibility of many nutritional factors for grow-out abalone, but there is very little information on requirements of very young abalone (<15 mm). There is no information as to the precise nutrients that are assimilated by very young abalone when fed on diatoms. Some of these aspects are addressed in this project.

The reliance on natural diatom settlement onto plates for feeding very small abalone involves high infrastructure and labour costs. The development of stable nutritional diets which are growth enhancing for the abalone, should result in less diatom plates being required, thus reducing capital costs. Similarly by moving the abalone off the diatom plates at an earlier age and allowing higher densities of abalone to be grown in existing tanks, labour costs should decrease while productivity could increase. Presently, abalone must be stocked at relatively low densities (especially at lengths >7 mm) to provide enough food in the form of the natural diatom film covering the surface of tanks and plates. Also with such diets, there should be less reliance on natural light levels (affecting diatom growth), allowing for a longer feeding and growing time. Similarly there should be reduced labour costs involved in light regulation through shade cloth manipulation.

Another problem facing the industry is the low growth rate during the transition onto currently available formulated diets. Such diets have been formulated for grow-out abalone, and ontogenetic changes in specific nutritional requirements for smaller abalone have not been taken into account. By providing diets that have been formulated to better satisfy the nutritional requirements of small abalone, a cohort with a narrower size range should result with fewer slower growing individuals. Development of diets specifically formulated for the different culture systems should not only benefit farmers growing the very young abalone on-shore in tanks but also farmers using barrels and cages offshore. If all of these problems are adequately addressed by development of a nutritionally adequate diet for small abalone, it is estimated that current production could expand by 10-fold. For farmers not intending to expand operations, such developments could result in up to 10 to 20% increase in production rate with existing infrastructure. Such increases would greatly accelerate the success of this fledgling industry.

4. OBJECTIVES:

- Use information on the nutritional and attractant factors present in natural food items to develop formulated diets for very young juvenile abalone (« 15 mm).
- Manufacture and evaluate the diets for water stability and palatability for various diet delivery mechanisms including gels, pellets, pastes and adhesion diets.
- Produce formulated diets of high nutritional value, which produce high growth rate in very young abalone (< 15mm), as verified by growth experiments.
- Provide information to the FRDC sub-program, so as to improve the existing formulated diet used for the "grow out" phase (>15 mm).
- Identify the nutrients incorporated into the actively growing tissues of abalone when fed on diatoms by assessing fatty acid metabolism and carbon and nitrogen retention in juvenile abalone using stable isotopes.

5. TECHNICAL REPORT – DETAILED RESULTS

5.1 General Materials and Methods

5.1.1 Abalone and management

Blacklip abalone (*Haliotis rubra*) were provided by Abalone Farms Australia P.L. for all experiments except the isotope experiment, which were purchased from Tasmanian Abalone Farms. Greenlip abalone (*H. laevigata*) and "tiger" (*H. laevigata* X *H. rubra* hybrid) abalone were provided by Tasmanian Tiger Abalone Company (TTAC). All experiments were conducted on "small" abalone, which for this project were defined as <15mm (maximum shell length) in comparison to "grow-out" abalone defined as >15mm long. For handling, small batches of abalone were anaesthetised using benzocaine. To ensure same average-sized starting populations, five randomly selected abalone were repeatedly allocated to each tank in turn until the desired density was reached.

Abalone length measurements were made from photographs. At the beginning and end of experiments all abalone were photographed in batches outside the tanks. When abalone were measured during experiments, to minimise disturbance the animals were photographed *in situ* when each tank was drained for cleaning. Photographs were taken from a constant distance with a framer (object to film distance 34.5cm) incorporating a 10 mm scale, using a 55 mm Nikon Micronikkor lens on a Nikon 401s (f22, 1/60sec) with built-in flash. These lengths were determined from the photographs with stainless steel sliding callipers. Length calibrations on the 10mm scale were preformed using the stainless steel sliding callipers and measured lengths corrected accordingly. For determination of average weight, all abalone in the tank were blotted dry using paper towelling, weighed on an A&D 3200 electronic balance and the average derived by dividing the total weight by the number of animals.

The long duration of the experiments was designed to simulate a true grow-out situation and avoid "change of diet effects", which are possible in experiments of short duration. During the experiments, diets were fed *ad lib* at 1 to 2% body weight (season dependent) on Monday, Wednesday and Friday of each week. That is, all tanks containing the same sized abalone were fed the same ration, approximately twice the amount eaten as recommended by Uki et al. (1985a), and adjusted for changes in feeding rate with temperature and abalone size. Prior to feeding, tanks were cleaned every Monday, Wednesday and Friday by physically swirling the water, allowing waste to collect at the centre of the tank and the tank was then drained by removal of the central standpipe.

Hatchery-supplied, filtered, continuous-flow seawater (1L/min), at ambient temperature, was used 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 four flat PVC shelters. The entire tank system was housed in a steel shed with transparent alcynite roofing and was covered with black PVC sheeting affording maximum shading.

5.1.2 Diet manufacture

Diets were made at CSIRO Marine Research, Hobart, using a domestic kitchen mixer and pasta extruder, formulated using ingredients sourced from commercial suppliers, and as in previous research (Dunstan et al., 2000), all lipid and highly water-soluble ingredients were premixed with the oil. This was to ensure lipid soluble ingredients were dissolved in the lipid and also to reduce leaching of the water-soluble ingredients. As in the previous research, the dried algal powders and binders used were rehydrated prior to mixing in the diet, and a

CaSO4/10% dry ingredient mix added last. The base soya flour/maize starch diet formulation initially used, was based on the then (1996) current version of the SARDI/FRDC experimental diet. As improvements were made, the experimental diets were adjusted accordingly.

5.1.3 Chemical Analysis

Fatty acid and sugar analyses were carried out in the organic chemistry laboratories of CSIRO Marine Research, Hobart as detailed hereunder.

5.1.3.1 Lipid extraction and total lipid analysis

Homogenised sub-samples 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, 2000) of the method of Bligh and Dyer (1959). A blank extraction was also performed. The extracts 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 twice with chloroform and the organic solvent mixtures were combined and reduced *in vacuo* to recover the lipids. Total lipid was determined gravimetrically. Lipids were stored under nitrogen at -20°C until analysis.

5.1.3.2 Fatty acid analysis

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 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.

5.1.3.3 Sugar analysis

Samples were first hydrolysed by heating with 0.5M H_2SO_4 for 4 h at 100ºC according to Brown et al. (1997). The hydrolysates were neutralised by addition of an excess of solid barium carbonate. Samples were clarified by centrifugation (2000 x g for 10 min), and the supernatants were passed through small columns (1 mL) containing AG 1-X8 (acetate form; 100-200 mesh, Bio-Rad Laboratories). The eluants were immediately passed through additional columns (1 mL) of AG 50W-X8 (H^+ form; 100-200 mesh, Bio-Rad Laboratories). These ion-exchange steps removed material such as amino acids and amino sugars that might have interfered with the assay. The eluants from the second column were freeze-dried. The constituent neutral sugars were converted to alditol-acetate derivatives as outlined by Blakeney et al. (1983). These were separated on an aluminium-clad capillary-column (25 m x 0.53 mm i.d.; BP-225 from S.G.E. Pty. Ltd., Melbourne, Australia) fitted to a Hewlett-Packard 5890 gas chromatograph equipped with a flame-ionisation detector. The carrier gas was high purity hydrogen. The GC oven temperature was initially held at 170°C for 2 min after injection and then increased at 6°C/min to 220°C and then held isothermal for 5 min.

5.1.4 Fatty acid Nomenclature

The naming of fatty acids has been simplified by adoption of the convention: X:YωZ (eg 20:5 ω3), where "X" refers to the number of carbon atoms in the molecule (eg 20), "Y" refers to the number of double bonds in the molecule (eg 5), and "Z" indicates the position of the first double bond from the methyl end (CH_3) of the molecule. The latter is generally referred to as Omega Z (eg ω3 or omega 3) or alternatively *n*-Z (eg *n* minus 3).

5.1.5 Statistical Analysis

Experimental designs were chosen in consultation with the CSIRO biometrics unit and discussed with other members of the FRDC sub-program and/or CRC staff to ensure scientific rigour was maintained. All treatments involved three replicates. Statistical comparisons of abalone growth rates with different replicated treatment diets within a trial were made using a single factor Analysis of Variance (ANOVA). Where significant differences were identified between diet treatments with the ANOVA (i.e. $p<0.05$), pair-wise comparisons were made and significance tested using Fisher's protected least significant difference (PLSD) test. For the PLSD test, t for each treatment data pair (a and b) was calculated according to the following equation;

$$t = \left| (Mean_a - Mean_b) \right| / \sqrt{[(\text{within group MS}) \times (1/n_a + 1/n_b)]}$$

Where $Mean_a$ and $Mean_b$ are the averages of replicates from treatments a and b respectively, MS is the mean of squares and from the single factor ANOVA table and n_a and n_b are the number of replicates from treatments a and b respectively. The significance of the difference between the paired means was determined using a two-tailed students t-test and the within group degrees of freedom. Differences between pairs where $p<0.05$ were significant are indicated on Figures using dissimilar letters i.e. a, b, c, etc. (eg Figure 5.7.3).

5.2 Natural abalone diets

5.2.1 Introduction

Diatoms, crustose coralline algae, turf algae and epiphytic bacteria are significant natural foods of newly settled and small abalone. In Australian hatcheries, abalone are settled and raised on plates covered with these natural food items. Abalone fed in this way exhibit a high growth rate. As the abalone increase in size, it becomes increasingly difficult to provide enough of these natural foods on plates, and alternative diet sources such as seaweeds and/or formulated diets must be provided. It was suspected that the nutritional and attractant factors present in the natural food items conducive to high growth rate in small abalone were possibly missing from formulated diets that were available. An examination of the factors present in these natural food items may assist in developing improved formulated diets and particularly for the very small abalone. The development of high performance and economical formulated diets for small (<15mm) abalone is crucial for the expansion of the abalone industry in Australia.

5.2.2 Methods

Previous research by the authors has established baseline data for the lipid, amino acid and sugar composition of diatom lipids (FRDC 1991/59 and FRDC 1990/63), and the lipid composition of abalone tissues and of formulated diets (FRDC 1994/085).

To establish the chemical composition of "natural" diatom communities (specifically those naturally settling in on-shore tanks and on plates) and crustose coralline algae which abalone also prefer to eat, samples were collected at the Tasmanian Tiger Abalone Company hatchery at Dunalley. Dominant diatoms in each sample were tentatively identified by Mike Wing. The samples were freeze dried, homogenised and sub-samples taken for analysis of fatty acid and sugar composition (Section 5.1.3).

5.2.3 Results and Discussion

The major fatty acids of the diatom communities (Table 5.2.1) were 16:0, 16:1ω7, and 20:5ω3, with low levels of 20:4ω6, 22:5ω3 and 22:6ω3. This is in contrast to abalone tissues which contain high proportions of 16:0, 18:1ω9, 18:1ω7, 20:5ω3, 20:4ω6 and 22:5ω3 (Dunstan et al., 2000), and most other marine animals (and the oils from fish) which contain high proportions of 16:0, 18:1ω9, 18:1ω7, 20:5ω3 and 22:6ω3 (Dunstan et al., 1988; Dunstan et al., 1999). The fatty acid and sugar composition of diatom communities were similar to monospecific cultures of marine diatoms (Dunstan et al., 1994; Brown et al., 1997). We believe these are the first analyses of *Cocconeis* sp., although the sample did contain small amounts of other algae. The major fatty acids of the crustose coralline algae were 16:0, 18:1ω9, 20:4ω6, and 20:5ω3, with low levels of 22:5ω3 and 22:6ω3, a composition typical of other rhodophytes (red algae) (Dunstan et al., 2000).

Table 5.2.1: Fatty acid composition of diatom communities and crustose coralline algae collected from abalone culture tanks

Sample and source	Dominant diatom in community				Crustose coralline algae (drain)	Crustose coralline algae (S. Daume Victoria)
	Navicula sp. (standpipe in tank)	*Cocconeis* sp. (round abalone tank)	*Campylo-discus* sp./ *Nitzschia closterium* (plates)	*Nitzschia closterium* (diatom plates)		
Saturated fatty acids (SFA)						
14:0	8.5	5.1	4.9	6.2	4.1	3.5
16:0	14.3	18.4	25.5	25.7	31.1	33.1
18:0	1.5	1.1	1.0	0.9	0.8	2.0
total	26.0	26.2	32.9	33.7	36.6	40.7
Monounsaturated fatty acids (MUFA)						
16:1ω7	20.4	21.2	31.2	32.9	4.7	1.5
18:1ω9	1.5	1.9	2.9	2.8	4.5	9.8
18:1ω7	3.6	4.2	1.4	1.6	1.1	3.9
20:1ω9	0.3	0.3	0.1	0.2	0.2	0.2
total	28.0	29.7	36.5	38.2	11.6	17.1
Polyunsaturated fatty acids (PUFA)						
16:2ω7	1.6	1.5	-	0.4	0.3	0.3
16:3ω4	2.2	2.6	2.3	1.3	0.3	-
16:4ω1	2.5	2.7	1.6	1.0	0.2	-
18:2ω6	1.8	1.5	1.2	1.1	0.8	1.3
18:3ω6	1.3	0.8	1.1	1.3	0.6	1.3
18:3ω3	0.6	0.6	0.6	0.7	0.8	0.3
18:4ω3	3.1	2.1	1.9	2.3	0.4	0.5
20:3ω6	0.4	0.4	0.3	0.3	0.3	0.6
20:4ω6	1.8	1.9	1.3	1.4	36.0	24.6
20:5ω3	13.6	14.4	13.7	12.2	8.7	10.9
22:5ω3	0.4	0.4	0.3	0.3	0.1	0.2
22:6ω3	2.9	2.3	1.8	1.8	0.2	-
total	34.3	32.3	26.6	24.4	48.7	40.6
total ω3	21.0	20.3	18.7	17.6	10.2	12.3
total ω6	10.8	9.3	6.2	5.9	38.3	28.3
PUFA/SFA	1.3	1.2	0.8	0.7	1.3	1.0
ω3/ω6 ratio	1.9	2.2	3.0	3.0	0.3	0.4

Minor fatty acids are not shown.

Table 5.2.2: Sugar composition of diatom communities and crustose coralline algae collected from abalone culture tanks

Sample and source	Dominant diatom in community					
	Navicula sp. (standpipe in tank)	*Cocconeis* sp. (round abalone tank)	*Campylo-discus* sp./ *Nitzschia closterium* (plates)	*Nitzschia closterium* (diatom plates)	Crustose coralline algae (drain)	Crustose coralline algae (S. Daume Victoria)
	Major sugars (% of total sugars)					
Rhamnose	4.1	4.9	2.5	6.8	4.1	18.8
Fucose	6.6	5.7	6.9	14.3	5.8	5.0
Ribose	3.8	2.4	1.7	0.4	-	-
Arabinose	1.9	2.7		0.7	-	-
Xylose	13.3	9.0	10.4	10.2	5.0	24.8
Mannose	2.6	2.5	4.9	5.7	3.7	-
Galactose	6.4	9.5	4.0	6.5	-	-
Glucose	37.9	53.3	43.7	44.4	46.3	11.5
Inositol	0.5	-	-	-	25.8	-
Unknown	10.1	4.5	9.8	7.0	1.8	39.3

The major sugars of the diatom communities were glucose, xylose, fucose and an unidentified sugar (Table 5.2.2). The major sugars of the crustose coralline algae were glucose, rhamnose, and either inositol or xylose and an unidentified sugar. Very little work has been performed on the importance of the various types of carbohydrates in the abalones diet. The composition of the natural diet of small abalone described above, and research already performed by the authors (eg. Dunstan et al., 1988; Dunstan et al., 1994; Brown et al., 1997; Dunstan et al., 1999, Dunstan et al., 2000) provided baseline data to enable development of a formulated diet to mimic the nutritional composition of natural diatoms.

5.3. Commercial abalone diets

5.3.1 Introduction

The high labour and infrastructure costs involved in growing and maintaining the diatom-covered plates widely used by industry were the main reasons for developing an alternative diet. Although such plates have been successfully used for many decades for the settlement and growth of small abalone, the development of a cost effective and nutritious diet was seen as a high priority.

The formulated diets available at the time of the study were analysed to enable comparison with the natural diets of small abalone (Section 5.2) and the grow-out diets used previously (Dunstan et al., 2000). This would assist feed manufacturers to interpret the results of the planned research and accordingly to modify their current formulations to better meet the nutritional needs of small abalone.

5.3.2 Methods

Commercially available abalone diets that were formulated specifically for small abalone were analysed. The powdered and crumbed diets were ground to a very fine powder, hydrated and then analysed for total lipid and fatty acid composition (Section 5.1.3).

5.3.3 Results and Discussion

All the diets analysed contained similar low levels of lipid (between 4 and 5% wet weight). Grow-out greenlip abalone had previously been shown to grow at a faster rate, when fed diets containing low levels of lipid (best growth at lipid levels of ≤ 3.8% WW, 4.2% DW, equivalent to 2.5% added fish oil on a wet weight basis, Dunstan et al., 2000).

Reasonably high levels of 20:5ω3 and 22:6ω3 were evident in all Australian diets, and were comparable in composition to each other and the more recently developed diets used for larger abalone (Dunstan et al., 2000). As indicated by the high 18:2ω6 and elevated 18:3ω3 content (Table 5.3.1), most commercial diets for small abalone contained high proportions of terrestrial plant products. The imported diet contained approximately twice the level of these C_{18} PUFA compared to the Australian diets. This indicates that there were equal amounts of fish and vegetable oils added to the imported diet, whereas the Australian diets contained fish oils as the primary source of lipid. The addition of C_{18} PUFA-rich vegetable oils significantly reduced the relative abundance of long-chain omega 6 PUFA; 20:4ω6, and omega 3 PUFA; 20:5ω3, 22:5ω5 and 22:6ω3 in the diet. These long-chain PUFA have previously been shown to be important to the growth of abalone and/or are major components of their tissues (Uki et al., 1986b, Dunstan et al., 2000). The higher the proportions of the long chain PUFA (and particularly the omega 3 PUFA) in the diet of *H. laevigata*, the higher the growth rate (Dunstan et al., 2000). Relative to natural diets (Table 5.2.1), all the formulated diets for abalone contained high proportions of 18:2ω6 and 22:6ω3 and lower levels of 20:5ω3 and 20:4ω6 (Table 5.3.1).

Table 5.3.1: Lipid content and fatty acid composition of formulated diets for small abalone available from Australian and Asian manufacturers

Fatty acid	Origin and form						
	Australia: Weaning powder	Australia: Juvenile powder	Australia: Juvenile crumbs (large)	Australia: Juvenile crumbs (small)	Australia: Juvenile powder	Australia: Juvenile pellets	Asia: Juvenile powder
Saturated fatty acids (SFA)							
14:0	3.8	3.4	3.3	3.9	3.3	3.3	1.0
16:0	19.0	18.5	18.5	17.5	17.6	17.5	15.3
18:0	3.1	2.7	2.8	2.9	4.1	4.1	3.3
total	27.2	25.5	25.8	25.5	26.2	26.2	20.5
Monounsaturated fatty acids (MUFA)							
16:1ω7	2.7	2.6	2.6	2.9	2.8	2.6	1.0
18:1ω9	13.9	13.0	13.0	12.2	12.3	11.2	16.5
18:1ω7	1.8	1.6	1.8	1.8	2.6	2.3	2.2
20:1ω9	4.7	4.3	4.4	5.0	5.4	5.3	0.7
22:1ω9	2.3	2.6	2.8	3.1	3.3	3.4	0.7
total	26.4	24.8	25.5	26.0	29.1	26.3	22.3
Polyunsaturated fatty acids (PUFA)							
18:2ω6	24.8	26.0	25.0	21.1	22.5	22.4	43.5
18:3ω3	2.7	2.7	2.7	2.4	3.5	3.5	5.6
18:4ω3	1.7	1.8	1.8	2.0	0.2	0.2	0.2
20:4ω6	0.5	0.5	0.5	0.6	0.6	0.7	0.4
20:5ω3	5.3	6.4	6.4	7.5	5.5	6.4	3.0
22:5ω3	1.3	1.4	1.4	1.6	1.2	1.4	0.2
22:6ω3	7.5	8.2	8.4	9.9	6.9	8.4	2.9
total	45.5	48.8	48.0	47.3	42.5	45.0	56.5
total ω3	19.7	21.6	21.9	24.9	18.7	21.4	12.3
total ω6	25.7	27.0	25.9	22.2	23.7	23.6	44.2
PUFA/SFA	1.7	1.9	1.9	1.9	1.6	1.7	2.8
ω3/ω6 ratio	0.8	0.8	0.8	1.1	0.8	0.9	0.3
% lipid (WW)	4.2	4.5	4.6	4.3	3.9	4.2	5.0
% lipid (DW)	nd	nd	nd	nd	4.2	4.6	5.5

Minor fatty acids are not shown; nd denotes not determined.

5.4 Evaluation of stability, attraction and palatability of experimental diets

5.4.1 Introduction

The commercial potential of reducing the reliance on diatoms for the culture of small abalone by the development and use of practical growth promoting formulated diets, resulted in strong industry support for this project. Part of this project was devoted to producing diets for evaluation under controlled experimental conditions, as well as for abalone farmers to evaluate diets with their own systems and species. Thus the stability of diets using different binding techniques and levels of binder needed to be assessed in a variety of systems by the abalone farmers.

Not only is the stability of the diets important but also including nutrients that encourage feeding. A great variety of ingredients have been identified as feed attractants and/or phagostimulants for abalone. These include some lipids (Sakata & Ina, 1985; Harada, 1987; Harada et al., 1987; Ando et al., 1997), amino acids (Harada, 1987; Harada et al., 1987; Harada, 1989), nucleic acid related compounds and purified and crude fractions from algal extracts (eg. Harada et al., 1984, Sakata et al., 1984; Harada & Akishima, 1985; Sakata & Ina, 1985) and terrestrial plant ingredients (Harada et al., 1996; Harada & Miyasaki, 1997). Harada et al. (1993) suggested that sweet substances also act as attractants to abalone. For humans, D-fructose is twice as sweet as sucrose and is metabolised in animals into glycogen (the main form energy is stored in abalone). It is the most readily soluble sugar (Tomasik, 1997) and this may lead to a higher leaching rate than for other sugars. Inclusion of this pentose sugar may result in diets being more attractive to abalone, even though it was not detected in the natural diatom communities previously analysed (Section 5.2).

This research was designed to identify which particular ingredients should be added to formulated diets to increase their palatability and hopefully also the rate at which small abalone grow. A second objective was to enable abalone farmers to assess diets with different attractants and levels of stability, in their own and varied systems. Ten diets were formulated to evaluate different binding techniques and the effectiveness of feed attractants that have been proven for abalone or fish, and/or which have been shown to accelerate diatom settlement (on the chance that abalone will detect this cue and be attracted to new food sources).

5.4.2 Methods

Diet Formulations

Of the diets, only MG1 was microencapsulated (Table 5.4.1). This involved encapsulating the highly water soluble ingredients such as some of the vitamins, minerals and crystalline amino acids in two different lipid coats to reduce leaching of these ingredients. Diets MG8 and MG10 contained elevated levels of the binder sodium alginate so that increased stability of the diets could be evaluated by farmers. The other diets contained a range of ingredients – sugars (mannose, fructose), aniseed oil, dried microalgae (*Dunaliella salina*), or glucodeltalactone (GDL)– all known or suspected of being feed attractants for a variety of marine animals.

Samples for Industry Evaluation

The diets were supplied to industry for evaluation of their suitability; attractiveness and palatability to small abalone reared under the various systems used on Australian abalone farms. The formulation of the diets is shown below (Table 5.4.1).

Table 5.4.1: Formulation of diets MG1 to MG10 used for the industry evaluation of stability, attraction and palatability experiment

Ingredient	Diet code and description									
	Batch 1				Batch 2					
	MG1 Micro-encaps	MG2 Not microencaps	MG3 Mannose	MG4 Fructose	MG5 Fructose	MG6 Aniseed Oil	MG7 *Dunaliella salina*	MG8 High alginate	MG9 GDL	MG10 Mid Alginate
Lecithin	0.9	0.9	0	0	0	0	0	0	0	0
Mannose	0	0	2.7	0	0	0	0	0	0	0
Fructose	0	0	0	2.7	3.0	0	0	0	0	0
Aniseed oil	0	0	0	0	0	1.0	0	0	0	0
D. salina	0	0	0	0	0	0	2.1	0	0	0
Na alginate	1.8	1.8	1.8	1.8	2.0	2.0	2.1	8.4	2.0	5.0
Glucodelta-lactone	0	0	0	0	0	0	0	0	3.0	0
Soya flour	37.2	37.2	36.5	36.5	37.0	37.8	38.1	38.9	37.0	37.0
Maize starch	29.9	29.9	29.4	29.4	28.0	28.6	26.8	21.1	28.0	25.0
Casein	7.4	7.4	7.3	7.3	8.0	8.2	8.2	8.4	8.0	8.0
Fishmeal	8.0	8.0	7.9	7.9	8.5	8.7	8.8	8.9	8.5	8.5
Fish oil	3.7	3.7	3.6	3.6	3.0	3.1	3.1	3.2	3.0	3.0
Minerals	3.7	3.7	3.6	3.6	3.9	4.0	4.0	4.1	3.9	3.9
CaCO3	2.8	2.8	2.7	2.7	1.6	1.6	1.7	1.7	1.6	4.6
Dried kelp	2.8	2.8	2.7	2.7	3.0	3.1	3.1	3.2	3.0	3.0
Fixed Ingredients[1]	1.9	1.9	1.8	1.8	2.0	2.0	2.1	2.1	2.0	2.0
Total %	100	100	100	100	100	100	100	100	100	100

[1]Included in final diet (%): DL-methionine, 0.4; L-threonine, 0.5; CaSO4, 0.5; Vitamin mix, 0.5; Vitamin C, 0.05; Vitamin E, 0.01.

The first batch of four diets was sent to seven farms in August 1996, and a second batch of six diets was sent to nine farms in January 1997. The diets were offered in various forms: powders, crumbs or barrel noodles. Most farmers chose to evaluate each diet in two forms, generally selected according to the size of the animals being fed and the particular system being used. Farmers were requested to visually evaluate diet stability in the water and whether the abalone moved towards or ate the diet, and to fill in a questionnaire for each form of the diet detailing their observations.

Controlled experiments

Concurrently with the industry evaluation of the diets, some of the diets were evaluated for attractiveness under controlled experimental conditions. These experiments were conducted at Abalone Farms Australia P/L using a commercial tank containing over 700 Greenlip abalone (*Haliotis laevigata*). The water inflow system to a 170 litre round tank was modified using an inverted funnel blocked at the narrow end so that the water entered the tank in a symmetrical pattern (round central spray) (see Figure 5.4.1). This design was used because at night many abalone in the tanks were observed to congregate at the water inflow and at air-stones, even though during the day they were evenly distributed around the tank perimeter. After installation of this system, the abalone were more evenly distributed at night.

For the diet evaluation, twelve Petrie dishes were placed equidistant in a circle in the tank (Figure 5.4.1a). Four powdered diets were added to three replicate dishes each, so that no replicates of one diet were adjacent to the same diets in the same order. Air-stones were removed while these diets were in the tank. The number of abalone in each dish (signifying that the abalone were eating the food, i.e. phagostimulant) or touching each dish (signifying that the abalone were attracted to the dish, i.e. feed attractant) were recorded at regular time intervals until feeding activity decreased (usually before midnight). The sums of total number of abalone in and touching each dish were compared statistically using a single factor Analysis of Variance to determine the significance of the difference between treatments at each time interval.

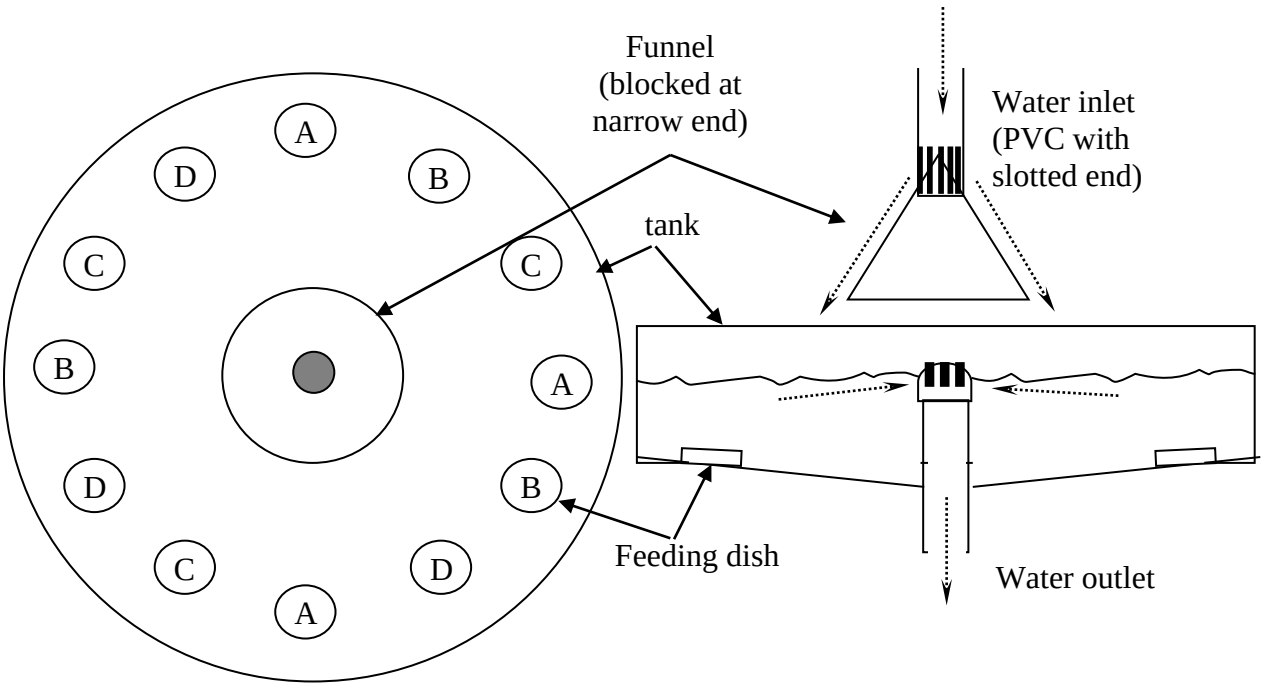


Figure 5.4.1. Diagram showing top (a) and side (b) views of tank used in feed attractant experiments where in (a) feeding dishes containing like diets are labelled the same and in (b) water flows are identified by dashed arrows.

5.4.3 Results and Discussion

Results of Industry Evaluation

There was only one respondent to the evaluation questionnaire for the first batch of four diets sent to the seven farms in August 1996. The respondent evaluated powdered and crumbed diets. Generally there were very few between-diet differences noted, but this respondent considered the powdered diet to produce more growth than the crumbed diet for greenlip abalone. There were two respondents to the evaluation questionnaire for the second batch of six diets sent to nine farms in January 1997. The first respondent, feeding small blacklip abalone crumbed versions of the diets, noted that the fructose diet (MG5) and then the glucodeltalactone (GDL) diet (MG9) exhibited the highest feed attraction and consumption rate. The second respondent, feeding small greenlip abalone crumbed and powdered versions of the diets, noted that the *D. salina* diet (MG7) and then the aniseed oil diet (MG6) exhibited the highest feed attraction and consumption rate, and noted powdered diets were not suitable for use in the high water flow rate tanks used. Both respondents noted that all six diets were stable after 48 hrs in the water. Unfortunately, the low return rate of questionnaires limits any firm conclusions being drawn about the industry relevance of these diets.

Results of Controlled Experiments

Two sets of data showing the average number of abalone in and/or touching feeding dishes containing the different diets are presented for two separate nights (Figures 5.4.2, and 5.4.3). As was evident from experiment 1, the imported diet appeared to consistently be more attractive to the small abalone (more touching the feeding dish), and more phagostimulatory (more in the feeding dish) than the local diet or two of our experimental diets (Figure 5.4.2). Unfortunately the numbers of abalone in and/or touching replicate feeding dishes at any time were highly variable. Abalone rapidly moved between dishes and others remained within a particular dish over successive times resulting in a lack of statistical significance being detected at each time (p=0.2534 to 0.5446). Had an overall significant difference been identified between diet treatments with the ANOVA (ie $p<0.05$), pair-wise comparisons could have been made to test the significance using Fisher's protected least significant difference (PLSD) test to identify differences between each diet pair.

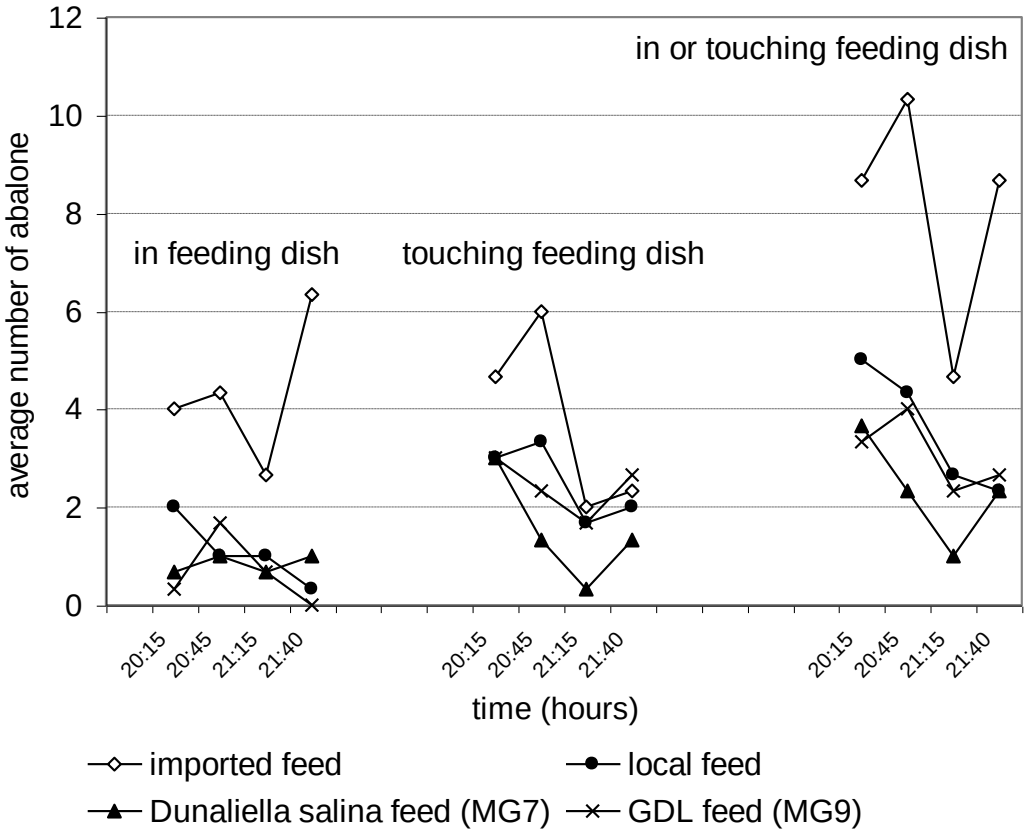


Figure 5.4.2. Temporal feeding responses of greenlip abalone to four different diets in Experiment 1.

Highest feeding activity in Experiment 2 was evident between 2020 and 2120 h with a decrease after this time (Figure 5.4.3). The fructose diet (MG5) and aniseed oil diet (MG6) produced a slightly higher feeding response than the *D. salina* diet (MG7) or the GDL diet (MG9) in greenlip abalone from 2020h to 2100h. But these differences were not significant at each time (p=0.2099 to 0.8895). This again was because the numbers of abalone in and/or touching replicate feeding dishes at any time were highly variable due to the high mobility of the abalone while actively feeding. Having stimuli from four different treatments emanating into the water of a single tank from a total of twelve different dishes may have been confusing during peak feeding activity, resulting in the variable data.

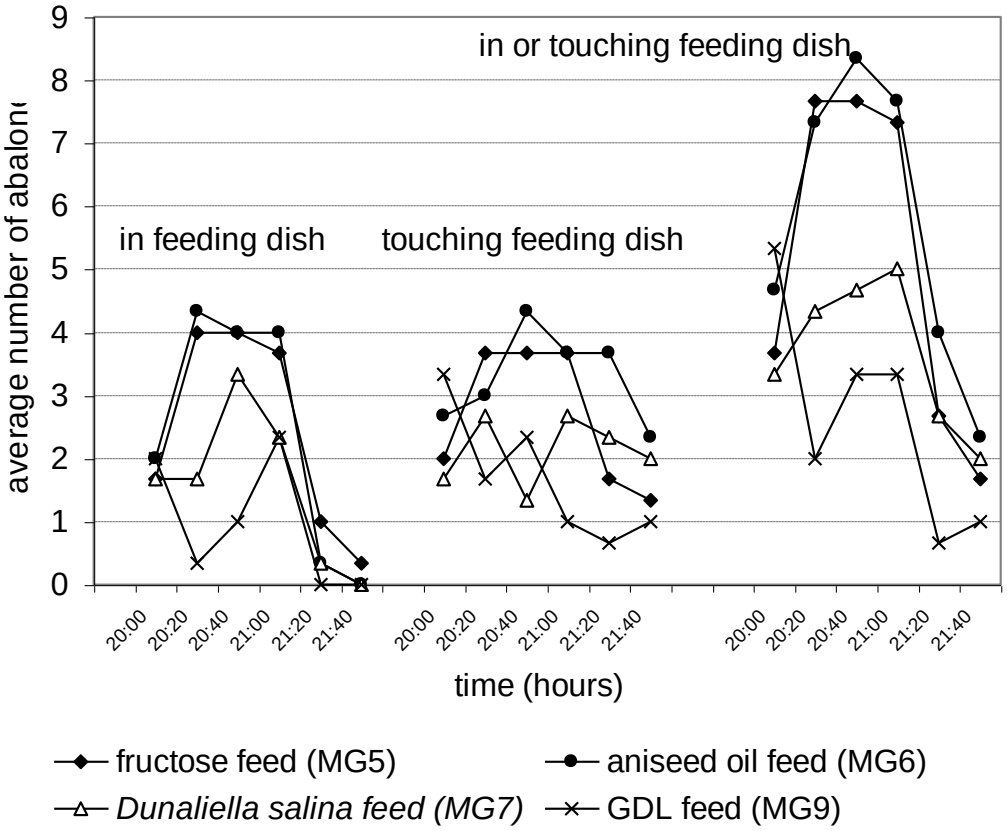


Figure 5.4.3. Temporal feeding responses of greenlip abalone to four different diets in Experiment 2.

D. salina should be nutritious to abalone and should readily leach many of the compounds identified as effective attractants for Japanese abalone (Harada & Akishima, 1985; Sakata & Ina, 1985). This dried microalgae was not as good an attractant as expected when added into the diet (MG7, Figures 5.4.2, and 5.4.3). However, as all the diets evaluated contained powdered kelp (which contains similar compounds), this may have masked any specific attractive effects of *D. salina*. Throughout all experiments GDL was shown to be ineffective as an attractant. It is interesting that the questionnaire respondents identified the fructose diet (MG5) and, to a lesser extent GDL diet (MG9) as eliciting a greater feeding response in blacklip abalone. The *D. salina* diet (MG7) and to a lesser extent the aniseed oil diet (MG6), were reported as eliciting a greater feeding response in greenlip abalone although the controlled experiment did not show them to be more attractive than the other diets.

The lack of significance in the controlled experiments may have been overcome by having more replicates, fewer treatments and/or fewer abalone per tank. In view of the results and as limited data on the effects of different systems used (from questionnaires) was provided, these types of trials were ceased and more effort expended in feeding trials.

5.5 Diet delivery mechanism

5.5.1 Introduction

Due to the problem of abalone consuming all of the diatoms ("natural diet") from abalone culture plates, a preliminary study to evaluate the addition of a formulated diet to diatom coated plates for abalone was undertaken. This was to establish whether it would be useful to develop methods for coating plates with formulated diets (e.g. via sprays and other means) while abalone are *in situ*. If the addition of formulated diets onto the plates (as diatom availability became limiting) improved the growth rates of juvenile abalone, then further work would be warranted. Also as preliminary research, we experimented with diets formulated with different binders, and produced in different forms; to examine how such diets may be applied to the plates, if they were found to be of use.

5.5.2 Methods

5.5.2.1 Binders in pellets and smears

Initially four diets (MG34-37) were formulated and produced to examine the effectiveness of particular diets' binders according to section 5.1.2. The binders selected were feed grade sodium alginate, gum Arabic (ICN Biomedicals Inc), guar gum (ICN Biomedicals Inc), and high viscosity sodium alginate (ICN Biomedicals Inc) (Table 5.5.1).

Table 5.5.1: Formulation of diets MG33 to MG36 used to examine binder effectiveness

Ingredient	Diet code and description			
	MG33 Feed grade sodium alginate	MG34 Gum Arabic	MG35 Guar gum	MG36 High-viscosity sodium alginate
sodium alginate	5.0			
gum Arabic		5.0		
guar gum			5.0	
high viscosity sodium alginate				5.0
Fixed Ingredients[1]	95.0	95.0	95.0	95.0
Total %	100	100	100	100

[1]Included in final diet (%): pre-gelatinised maize starch, 31.4; soya flour, 25.0; fishmeal, 8.5; casein, 8.0; semolina, 5.0; fish oil, 3.0; kelp powder 4.0; dried *Dunaliella salina*, 1.0; DL-methionine, 0.3; L-threonine, 0.3; Arginine, 0.3; minerals, 3.0; calcium carbonate, 2.0; calcium sulphate, 1.2; vitamin C, 1.0; vitamin mix, 1.0; vitamin E, 0.01.

Each diet was prepared (Section 5.1.2) and a portion extruded to form pellets (approx 1.5 mm x 2-3 mm cylinders also called "crumbs") and another portion was applied to a flat PVC tray (T). Diet MG33 was also smeared onto water proof paper (W) and 10 mm oyster mesh (M). The portions were applied to surfaces in a thin layer 0.5-1.5 mm ("smears") with a spatula and allowed to dry overnight, and visually evaluated the next day. The pelleted form of the diets were dried overnight in an oven at 40 C. Pellets and smears which remained attached to the surfaces were visually evaluated in the laboratory after being immersed in 200 ml of filtered seawater after 24 hours.

5.5.2.2 Supplementation of diatoms with formulated diets

To examine supplementation of the diatom plates with a formulated diet, four 300 L tubs each containing one pack of four diatom covered plates were set up at the Tasmanian Tiger Abalone Company hatchery, Dunalley. Each tub was stocked with 200 "tiger" (*H. laevigata*

X *H. rubra* hybrid) abalone and supplied with filtered flow-through heated water and an airstone. Powdered food was used for this preliminary experiment due to its ease of application and the small size of the abalone (6.4 ± 1.52 mm, mean ± SD). For two of the tubs, the powdered food (MG33) was added to each plate twice weekly; the other two tubs were not fed. Tubs were cleaned once weekly by siphoning detritus from the bottom of the tanks. Abalone were photographed from a constant distance for shell length determination at the commencement and conclusion of the experiment, as described previously (Section 5.1.1). At the conclusion of the experiment, sample scrapings were taken and the dominant diatom species on the plates and in the tanks were tentatively identified.

5.5.3 Results and Discussion

5.5.3.1 Binders in pellets and smears

Table 5.5.2: Notes from visual inspection of fresh mixture, dry smears and immersed pellets of diets containing different binders (diets MG33 to MG36). The smears were applied to a flat PVC tray (T), and in the case of MG33 also to water proof paper (W) and oyster mesh (M).

Diet type	Diet code and description			
	MG33 Feed grade sodium alginate	MG34 Gum Arabic	MG35 Guar gum	MG36 High viscosity sodium alginate
Fresh diet when mixed	Very sticky	Not as sticky as MG33	Only a little sticky	Difficult to extrude, not too sticky
Fresh smear when applied	Appeared to attach well to T, W, & M	Did not attach well to T	Did not attach well to T	Did not attach well to T
Dry smear after 24 hours-attachment	No attachment to T, well attached to W & M	No attachment to T	No attachment to T	No attachment to T
Dry smear after 24 hours-appearance	Diet brittle, T & W smears had curled up at edges, M smear just starting to shrink away from mesh	Diet brittle, T had curled up at edges	Diet brittle, T had curled up at edges	Diet brittle, T had curled up at edges
Dried attached smears after 24 hours immersion in seawater -attachment and appearance	smear starting to detach from W, (flattened out and swollen), M smear still held within mesh	-	-	-
Dried pellet after 24 hour immersion in 200ml seawater	Swelled, water yellowish, held together well	Swelled, water discoloured, held together well	Not as swollen or discoloured as MG33 & MG34, signs of falling apart	Not as swollen or discoloured as MG33 & MG34, signs of falling apart

This was only a preliminary laboratory-based experiment to examine the feasibility of whether further investigations of the diets (replicated hatchery based leaching/stability experiments) were warranted. Had any diet been shown to have qualities or features for abalone diets which were more beneficial than the currently used feed grade alginate, then further research along these lines would have been warranted. Examining dietary ingredients which were less effective than the currently used ones was not a part of this work.

A summary of the laboratory notes from visual inspection of fresh mixture, immersed pellets and dry smears of diets containing different binders (diets MG33 to MG36) is shown in Table 5.5.2. Overall none of the diets MG34, MG35 or MG36 appeared to be more stable or more amenable to application as a smear to the surfaces examined than the sodium alginate diet (MG33). Therefore further examination of diets with the other binders were not conducted. As none of the diets were suitable as smears, further work on applying diets to these surfaces was not conducted. However, due to the observations on the stability of the MG33 diet with feed grade sodium alginate (Table 5.5.2) a trial examining a powdered form of diet MG33 was performed, and the results are detailed below.

5.5.3.2 Supplementation of diatoms with formulated diets

Samples from tubs and plates that had not been supplemented with formulated diets contained *Bacillaria* sp., *Nitzschia closterium*, *and Pleurosigma* sp. as the dominant diatom species. Samples from tubs and plates, which had been supplemented with the formulated diet, contained *N. closterium* and *Pleurosigma* sp., or *Thalassionema* sp., *Pleurosigma* sp. and *Cocconeis* sp. as the dominant diatom species. Mortalities were relatively high: diatom plate treatment (9 and 20%); and formulated diet supplemented treatment (19 and 23%). This high mortality was thought to be due to mechanical injury both during cleaning and resulting from aerator vibration of the packs of plates. The growth rate data for this 39-day experiment are shown in Figure 5.5.1.

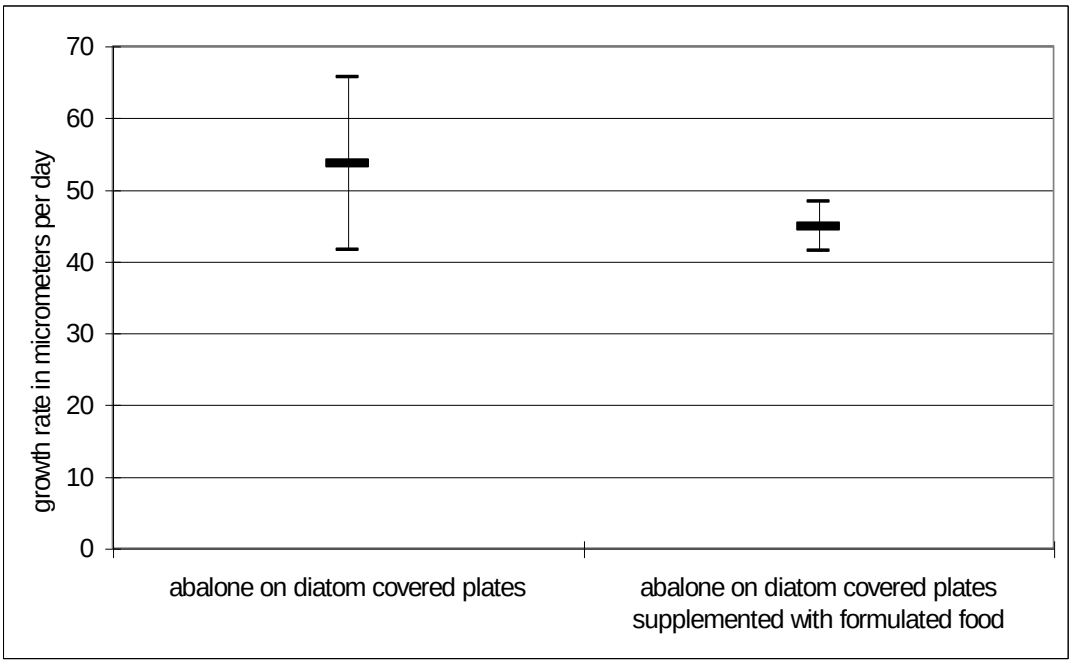


Figure 5.5.1. The growth rate (micrometres per day; ± SEM) of "tiger" abalone grown on diatom-covered plates in duplicate tubs, with and without supplementation of a formulated diet.

There was no significant difference in abalone growth rate between supplemented and non-supplemented diatom covered plates (Figure 5.5.1). One tub, which had not been supplemented with formulated food (and coincidentally, visually contained a lighter epiphyte cover), did exhibit higher abalone growth rate (65.6 μm/day, and hence the higher SEM for this treatment). This was not the tub with the higher mortality (and resultant lower stocking density by the end of the experiment) for this treatment. The other three tubs exhibited comparable and low growth rates (41.4 to 48.3 μm/day). In this preliminary experiment, there was no correlation between growth rate and diatom species dominance or diet treatment. Therefore, supplementing small abalone on diatom-covered plates with a powdered formulated diet did not accelerate growth rate.

Development of a novel and suitable delivery mechanisms (paste, spray, pellet, gel, plate etc) for small abalone could be important if commercially available diets were found to be unsuitable either because pellets were too large for the small abalone, or powdered diets were carried away or dissolved (also causing fouling problems in areas of low current). Preliminary attempts at attaching thin films of diets using different binders to a variety of surfaces resulted in most diets becoming detached while drying. The most successful medium was an oyster mesh where some of the diet was still intact after 24 hrs immersion in seawater. But using such plates coated with diet presents logistical problems for commercial operations. The main one being removal of exhausted diet plates and replacement with new ones, which causes increased handling of abalone and labour costs. Any application of diet to plates where abalone are already present (such as via sprays or other coatings) would be labour intensive and result in the abalone being covered in the food, temporarily reducing access to fresh seawater for oxygen and nutrient supply and waste disposal. Such considerations and the evaluation of the cost versus benefit for the abalone farmer of such feeds really are for the farmer to determine. For these reasons and after discussion with industry collaborators, these diets were not examined further. Free, flocculated and pasted preparations of a diatom commonly used for abalone culture (*Nitzschia closterium*) were evaluated in other sections of this report. These preparations were found to be unsuccessful relative to formulated diets (Sections 5.7 & 5.10). From these investigations, it was concluded that providing formulated diets in either powder or crumb form would remain the best option for small abalone for now.

5.6 Effect of dietary simple carbohydrates, lipid levels and a formulated "diatom" diet on the growth of small abalone

5.6.1 Introduction

At the time this project began, abalone farmers were very keen to have a formulated diet, which produced high abalone growth rates, but without the labour and infrastructure costs associated with a diatom covered plate system. This raises the question "why are diatoms so nutritious to abalone?" Therefore, establishing the biochemical composition of diatoms, formulating a diet based on their composition and substituting expensive with cheap ingredients without compromising growth, was the strategy adopted in the present study. If successful, the developed formulated diatom diet would additionally be useful as a control diet in subsequent experiments to evaluate alternative formulations used to assess the nutritive/attractiveness value of different ingredients and/or nutrient levels.

Complex carbohydrates (polysaccharides) have been shown to affect growth rate in a variety of animals (Uki et al., 1985a; Cruz-Suárez et al., 1994) and affect immune systems (Jeney et al., 1997; Nazarova et al., 1998) in marine animals. Simple sugars (monosaccharides and disaccharides) have also been shown to be important especially in the nutrition of larval marine animals (Diaz et al., 1994), and some have been shown to be growth enhancing in prawns (Cruz-Suárez et al., 1994) and fish (Stickney, 1994). But very little work has been performed on the effect of dietary simple sugars on growth of small abalone. Due to the high water solubility of simple sugars, they are readily leached from formulated aquatic diets. In comparison, complex carbohydrates are relatively water insoluble and are less expensive. While carbohydrates are generally considered to be non-essential nutrients for prawns (Akiyama et al., 1991) and most complex carbohydrates such as starch and cellulose are generally poorly utilized by carnivorous aquaculture species (Stickney, 1994), they are important economically because of their protein sparing effect. For an herbivorous animal like the abalone whose natural diet is relatively rich in carbohydrates and who has a digestion system adapted for such diets, it was suspected that more consideration of carbohydrates was warranted.

Newly settled abalone feed on high carbohydrate-containing diatoms with a gradual transition in food preference to macroalgae (seaweeds) as they increase in size. Abalone of a size greater than about 25 mm would feed almost exclusively on seaweed. The main carbohydrate reserve of diatoms is chryslaminarian (a $\beta 1 \rightarrow 3$ linked polysaccharide of D-glucose, with $\alpha 1 \rightarrow 6$ branch linkages; Percival, 1968). Seaweeds (taxa dependent) can contain a variety of polysaccharides including laminarian (a $\beta 1 \rightarrow 3$ linked glucan of D-glucose and mannitol residues), and/or galactans, mannans and/or xylans, which are comprised predominantly of D-galactose, D-mannose and D-xylose residues respectively, as well as starch-like polysaccharides also comprising a variety of monosaccharides (Percival, 1968). The main carbohydrates in commercial abalone diets are starches from higher plants. The action of amylases on these $\alpha 1 \rightarrow 4$ linked glucans produce D-glucose and D-maltose. The main energy reserves of abalone are carbohydrates, and in particular the complex carbohydrate glycogen (an $\alpha 1 \rightarrow 4$ linked glucan of glucose residues, with $\alpha 1 \rightarrow 6$ branch linkages). It has also been suggested that sulphated complex carbohydrates play an important role in shell formation and hence in growth of gastropods (Marxen et al., 1998). From a knowledge of the composition of the natural diets of small abalone (eg Section 5.2) and published data, evaluation of simple and complex carbohydrates as dietary nutrients was seen to be important in developing formulated diets for small abalone.

Previously we have shown that low levels of oils rich in Omega 3 polyunsaturated fatty acids (ω3 PUFA) should be added to abalone diets for maximum growth of grow-out abalone (Dunstan et al., 2000). But no work had been performed on small abalone. Diatoms contain a large proportion of their dry weight as lipid (Dunstan et al., 1994), but seaweeds contain only a low proportion of lipid (Dunstan et al., 2000). Thus, there was a need to establish whether an ontogenetic shift away from lipid and towards carbohydrate as a preferred energy source occurs as abalone change from one diet source to the other. Therefore this section of the study examined the lipid level and importance of some simple and complex carbohydrates for abalone, and compares growth rate to an experimental formulated "diatom" diet.

5.6.2 Methods

Seven abalone diets were formulated for evaluation. Five of the diets contained only commercially available ingredients (MG27 to MG31), one contained purified oyster glycogen (MG26), selected as an ingredient because glycogen is the major form of energy storage in abalone, and the seventh diet was formulated to broadly mimic the chemical composition of a diatom (MG32) (Table 5.6.1). This "formulated diatom diet" also contained some purified ingredients. The main differences to the other diets being the addition of a "sugar mix" (6% glucose, 0.5% mannose, 2.5% xylose) and 7% diatomaceous earth (pre-combusted swimming pool filter material). The levels of the ingredients in this diet were established by comparing against the dry weight contents of the various nutrients in diatoms.

Table 5.6.1: Formulation of diets MG26 to MG32 used for the simple sugar, oil and formulated "diatom" diet growth rate experiment

Diet number	MG26	MG27	MG28	MG29	MG30	MG31	MG32
Description	3% glycogen	3% glucose	9% glucose	9% sucrose	3% oil added (control)	6% oil added	formulated "diatom" diet
Oyster glycogen	3.0	0	0	0	0	0	0
Glucose*	0	3.0	9.0	0	0	0	6.0
Mannose*	0	0	0	0	0	0	0.5
Xylose*	0	0	0	0	0	0	2.5
Sucrose	0	0	0	9.0	0	0	0
Fish oil	3.0	3.0	3.0	3.0	3.0	6.0	6.0
Diatomaceous earth	0	0	0	0	0	0	7.0
Maize starch	28.4	28.4	22.4	22.4	31.4	28.4	12.4
Fixed ingredients[1]	65.6	65.6	65.6	65.6	65.6	65.6	65.6
Total %	100	100	100	100	100	100	100

[1]Included in final diet (%): soya flour, 25.0; fishmeal, 8.5; casein, 8.0; semolina, 5.0; dried *Dunaliella salina*, 5.0; sodium alginate, 5.0; DL-methionine, 0.3; L-threonine, 0.3; Arginine, 0.3; Minerals, 3.0; calcium carbonate, 2.0; calcium sulphate, 1.2; vitamin C, 1.0; vitamin mix, 1.0; vitamin E, 0.01.

* collectively referred to as the "sugar mix" in the formulated "diatom" diet

The proximate composition of the diets was calculated from ingredient analysis, product specifications and other literature (Figure 5.6.1). Diets MG26 to MG30 contained comparable levels of energy and proximate nutrients, while MG31 and MG32 contained twice the lipid, and MG32 contained twice the ash of the other diets at the expense of carbohydrate (primarily gelatinised maize starch). The total energy (MJ/kg dry weight) was calculated using gross

energy equivalents for protein, carbohydrate and lipid of 20.1, 17.6 and 39.7 J/g, respectively (Whyte, 1987).

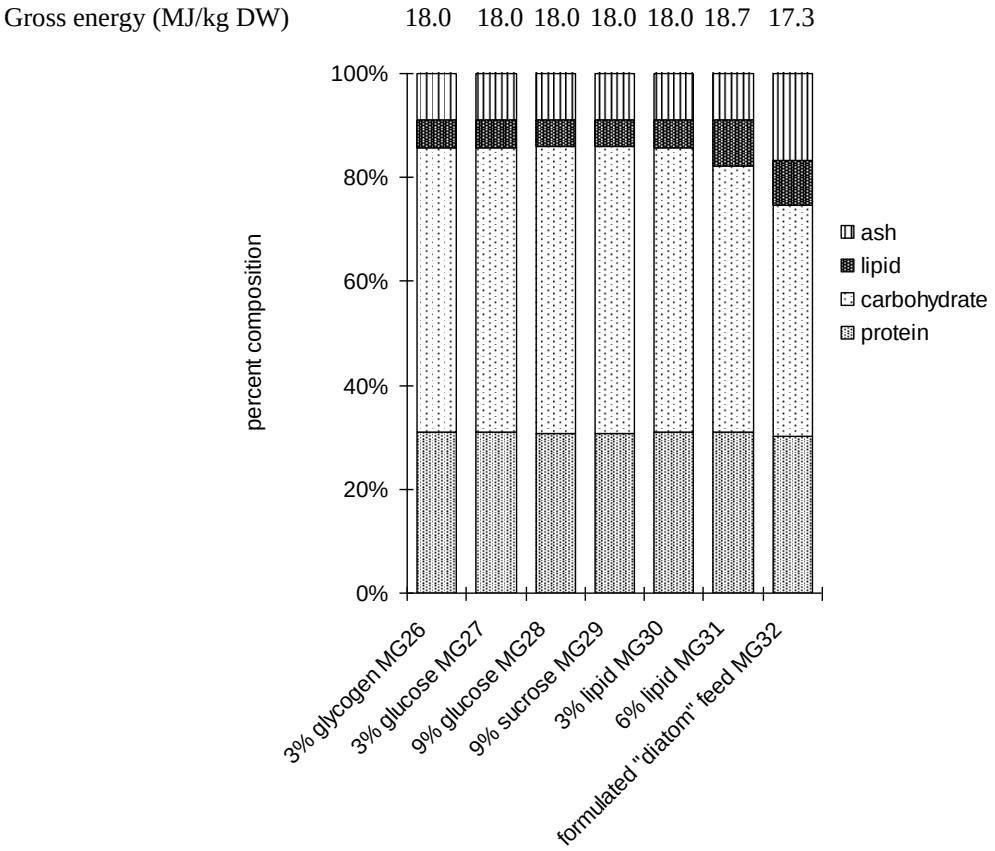


Figure 5.6.1: Proximate composition and gross energy of diets MG26 to MG32

Twenty-one identical 53 L experimental tanks were used for this experiment. The 70 cm diameter round tanks were shallow (13 cm deep at perimeter, gently sloping to 15 cm deep at centre), centrally drained via a standpipe. Each tank was aerated with two air-stones and contained four flat PVC plate shelters, and the entire system was shaded.

The seven dietary treatments were randomly assigned in triplicate. Blacklip abalone (10 mm average shell length) were anaesthetised with benzocaine and randomly allocated at a stocking rate of 120 per tank. Following a 14-day acclimatisation period, abalone were photographed from a constant distance for length determination and this constituted the initial measurement. Abalone were photographed at approximately monthly intervals for determination of shell growth (as detailed in section 5.1.1). The diets were fed *ad lib* (at approximately 1 to 2% body weight per day) in powdered form for the first 80 days and then fed in crumbed form up to day 151.

This experiment evaluating diets MG26 to MG32 was conducted during spring to autumn with temperature increasing from a minimum of 12.5°C to a maximum of 17.8°C in summer, and averaged 15.5°C over the whole experiment (Figure 5.6.2).

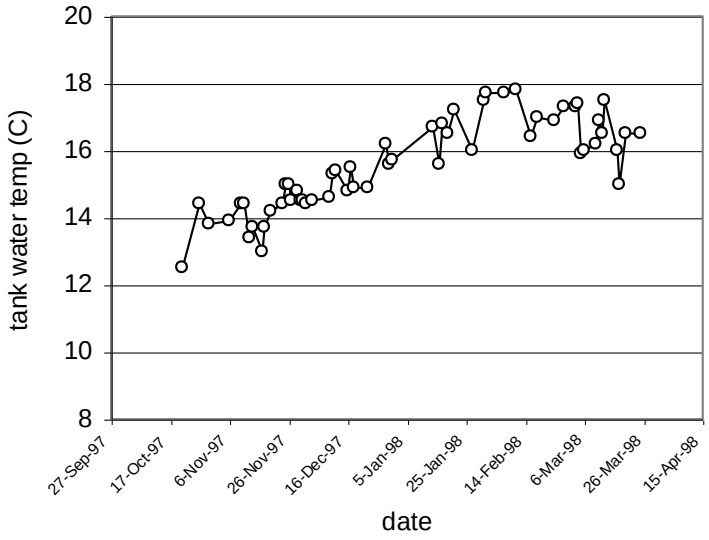


Figure 5.6.2: Experimental tank water temperature fluctuations during the evaluation of diets MG26 to MG32 used for the simple sugar, oil and formulated "diatom" diet growth rate experiment

5.6.3 Results and Discussion

Generally higher growth rates were evident when abalone were fed crumbs as opposed to powders (Figure 5.6.3). This was consistent with reduced leaching of nutrients from crumbed diets relative to the powdered diets with their larger surface area. Single factor Analysis of Variance for pairwise comparisons was performed on the growth rates of abalone fed powdered feeds and on those fed crumbed diets. There was no significant difference between powdered treatment diets (p=0.37), but there was for crumbed treatment diets (p= 0.037). This significance was tested using Fisher's PLSD test.

Powdered diets with 3% of either glycogen (MG26) or glucose (MG27), or 3% extra gelatinised maize starch (MG30), and the diatom diet (MG32) produced slightly lower growth rates than abalone fed powdered diets with 9% glucose (MG28), 9% sucrose (MG29) or 6% oil (MG31). However these differences between the powdered diet treatments were not significantly different (p>0.05).

With crumbed diets, inclusion of the complex carbohydrate oyster glycogen (MG26) at 3% produced a significantly higher growth rate in abalone than when this ingredient was substituted with glucose (MG 27). Glycogen is structurally similar to amylopectin (a starch of higher plants; Kristensen, 1972) and comprised mostly of glucose. Abalone grew slightly faster (but not significantly) when fed a diet containing oyster glycogen (MG26) than when fed one richer in starch (MG30). Kristensen (1972) suggested that the storage carbohydrates (eg in this case is glycogen) of the diet are more nutritionally important to the animal than structural polysaccharide (eg starch, cellulose). The structural polysaccharides of plants such as starch and cellulose are degraded by gut bacteria and their utilisation by the animal is generally poor (Kristensen, 1972).

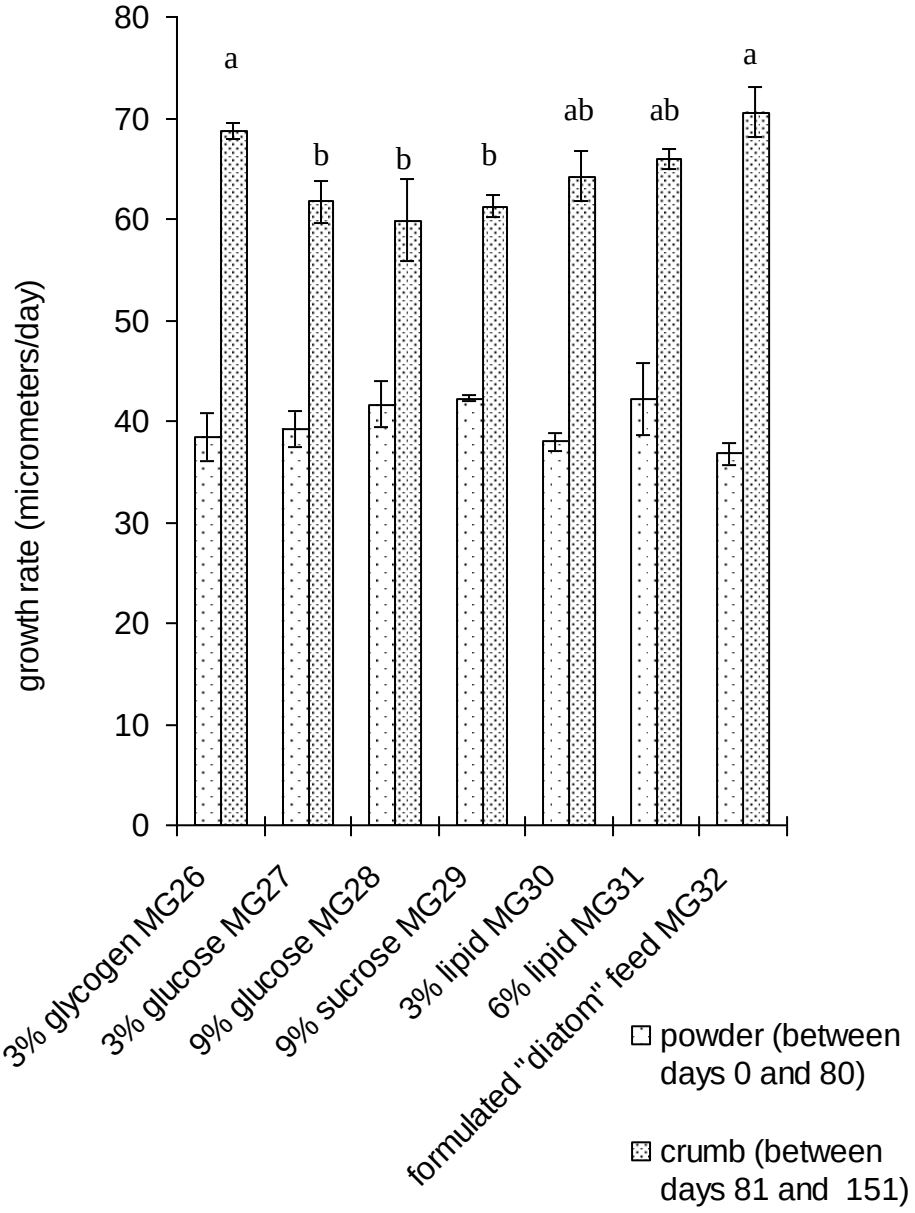


Figure 5.6.3: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed seven different diets initially in powdered form, and later as crumbs.

The inclusion of the simple sugars, glucose (3%, MG27; 9%, MG28) and sucrose (9%, MG29) in crumbed diets resulted in similar but reduced growth rates relative to the complex carbohydrates. Sucrose is a disaccharide comprised of glucose and fructose. Indications were that fructose in the diet was slightly attractive to abalone (Section 5.4), but when included as sucrose, did not provide a growth advantage for small abalone (Figure 5.6.3). It should be noted that strong to weak hepatopancreas carbohydrase activities for amylose (a starch), sucrose, glycogen and laminarin (similar to the chryslaminarin of diatoms) were identified in most of the 22 marine invertebrates (including omnivorous gastropods) examined by Kristensen (1972), suggesting ubiquity. The present data (Figure 5.6.3) suggest that abalone either posses the ability to utilize glycogen (but not glucose or sucrose directly), were more attracted to diets containing glycogen or that the simple sugars had leached from diets MG27 to MG29 before they could be utilized and therefore were of no benefit. Therefore, structural

complex carbohydrates (such as starch which are relatively cheap ingredients and protein sparing), may not be the best carbohydrates to include in an optimised formulated diet for abalone.

Even though diets MG28 and MG29 had the same levels of simple sugars (9% glucose and 9% sucrose respectively) as the formulated "diatom" diet (MG32) (9% of the sugar mix) the latter produced a higher growth rate when fed as a crumb. However, this "diatom" diet differed in the type of the sugars added, ie 6% glucose, 0.5% mannose and 2.5% xylose, so as to mimic that of natural diatoms. The formulated "diatom" diet had intermediate levels of glucose (6%) compared to MG27 (3%) and MG28 (9%), which suggests that it is not the glucose that is responsible for the observed differences in growth of the abalone. The periwinkle *L. littorea* (also an omnivorous marine gastropod) was the only species of 22 marine invertebrates to exhibit strong to weak carbohydrase activity for xylan (predominantly xylose; Kristensen, 1972). Thus it may be the xylose that resulted in the growth advantage from the formulated "diatom" diet. Whether this sugar improves phagostimulation or nutritional qualities of the diet are unknown. Previously, another pentose sugar (fructose) was identified as possibly attracting abalone to feeds (Section 5.4). However the formulated "diatom" diet contained a number of other different ingredients, which have been subsequently examined (see later sections).

Previous work had shown that grow-out greenlip abalone grew faster when fed low lipid diets (Dunstan et al., 2000). For these larger abalone, growth rate was highest when diets had less than 2.5% of added fish oil (4.2% DW of the diet). Larger abalone usually feed on seaweeds, which are low in lipid (Dunstan et al., 2000), whereas newly settled and small juvenile abalone feed on relatively lipid-rich diatoms (Dunstan et al., 1994). Diets MG30 (3% fish oil added) and MG 31 (6% fish oil added) were examined to establish whether the smaller abalone had a higher requirement for lipid than grow-out abalone. The data suggest that there was a slight but not significant growth advantage from adding twice the lipid (MG31, 6% added as oil resulting in a total of 8.8% lipid DW) to a standard grow-out type diet (MG30, 3% added as oil resulting in a total of 5.3% lipid DW) when in powder form. A higher growth rate with higher energy diets (protein level constant) would indicate either that there is a higher requirement for lipid and possibly essential polyunsaturated fatty acids, or that the protein content is not optimal and the increased growth was due to protein sparing effects. While the protein level of the diet can be spared by increasing energy concentration (Jantrarotai et al., 1998), previous studies and the current experiment have shown that increasing the lipid levels of abalone diets (protein level constant) did not significantly increase growth (Figure 5.6.3; MG30 cf MG31), and can actually decrease it in older abalone (Dunstan et al., 2000). Without such an effect, there is no indication of whether the level of protein used in these diets (30 to 31%) was optimal and further work was required on this issue (see Sections 5.8 and 5.9).

The results of this experiment suggest that the chemical composition of the animal's natural food (diatoms) may be a valuable indicator for diet formulation and may be more revealing than using the animal's chemical composition (as suggested by Fleming et al., 1996). Because of the enhanced growth rate in small abalone, these features were examined further in subsequent studies.

5.7 Effects of commercial sources of lipid, carbohydrate and protein and free and flocculated *Nitzschia closterium* on the growth of small abalone

5.7.1 Introduction

Following advice from the FRDC/CRC Abalone Aquaculture steering committee, an experiment was designed to evaluate commercially available ingredients for their efficacy in improving abalone growth rate. Some commercially available ingredients such as fishmeal, casein, and powdered algae were not evaluated, as their growth promoting effects were evident (Uki et al., 1985a; Dunstan et al., 2000). A variety of commercially available complex carbohydrate sources have already been evaluated for abalone. *H. discus hannai* grew faster on a diet containing dextrin (intermediate chain length polysaccharides from the action of amylases on starch) than one containing the same amount of α-starch (Uki et al., 1985a). Similarly increasing the level of dextrin at the expense of cellulose increased *H. discus hannai* growth (Uki et al., 1985a). Interestingly, work on the greenlip abalone (*H. laevigata*) has shown that most isolated gut bacteria had the ability to hydrolyse cellulose, starch and agar (Harris et al., 1998). Structural polysaccharides of plants such as starch and cellulose are degraded by gut bacteria and their utilisation by the animal is generally poor, the storage carbohydrates of the diet are more nutritionally important to animals (Kristensen, 1972). The complex carbohydrate sources gelatinised maize and wheat starch and semolina were examined during this growth assay. Consistent with the aims of the project, the formulated diets containing commercial ingredients were compared to a diet of *Nitzschia closterium*, which was presented as free/mass cultured and as a concentrated culture (by flocculation).

5.7.2 Methods

The experiment was performed at Abalone Farms Australia P/L. Twenty-four identical 53 L experimental tanks were used for this experiment. The 70 cm diameter round tanks were shallow (13 cm deep at perimeter, gently sloping to 15 cm deep at centre) and centrally drained via a standpipe. Each tank was aerated with two air-stones and contained four flat PVC plate shelters; the entire system was shaded.

Table 5.7.1: Formulation of diets MG38 to MG43 used for the commercial ingredients growth rate experiment

Diet number	MG38	MG39[1]	MG40	MG41	MG42	MG43
Description	No oil added	3% oil added (control)	8% oil added	High protein/ soya	Wheat starch	Semolina
Fish oil		3.0	8.0	3.0	3.0	3.0
Soya flour				20.0		
Wheat starch					58.8	
Semolina	5.0	5.0	5.0	5.0	5.0	30.0
Maize starch	61.8	58.8	53.8	38.8		33.8
Fixed ingredients[2]	33.2	33.2	33.2	33.2	33.2	33.2
Total %	100	100	100	100	100	100

[1]fed to greenlip and to blacklip abalone, the remaining diets only fed to blacklip abalone

[2]Included in final diet (%): casein, 10.0; fishmeal, 9.0; kelp powder, 4.0; *Dunaliella salina*, 0.5; sodium alginate, 3.0; vitamin mix, 2.0; vitamin C, 1.0; vitamin E, 0.01; mineral mix, 1.0; calcium carbonate, 1.0; calcium sulphate, 0.8; methionine, 0.3; threonine, 0.3; arginine, 0.3.

Six abalone diets (MG38 to MG 43) were formulated (Table 5.7.1) using only commercial ingredients and evaluated with blacklip abalone. The control diet (MG39) was also evaluated using greenlip abalone as a seventh treatment. The eighth treatment comprised the feeding of the diatom *N. closterium*. This species was selected as it is readily eaten by small abalone, commonly used to inoculate settlement plates by abalone farmers, and easily mass cultured.

The proximate composition of the formulated diets was calculated from ingredient analysis, product specifications and other literature (Figure 5.7.1). Diets MG39 & MG42 to MG43 were formulated to contain comparable levels of all proximates but differed with respect to source of ingredients. MG41 contained elevated protein, MG38 had no additional fish oil and MG40 had 8% fish oil added at the expense of gelatinised maize starch. The total energy (MJ/kg DW) was calculated using gross energy equivalents for protein, carbohydrate and lipid of 20.1, 17.6 and 39.7 J/g, respectively (Whyte, 1987).

Gross energy (MJ/kg DW)	17.3	18.0	19.3	18.4	18.2	18.2

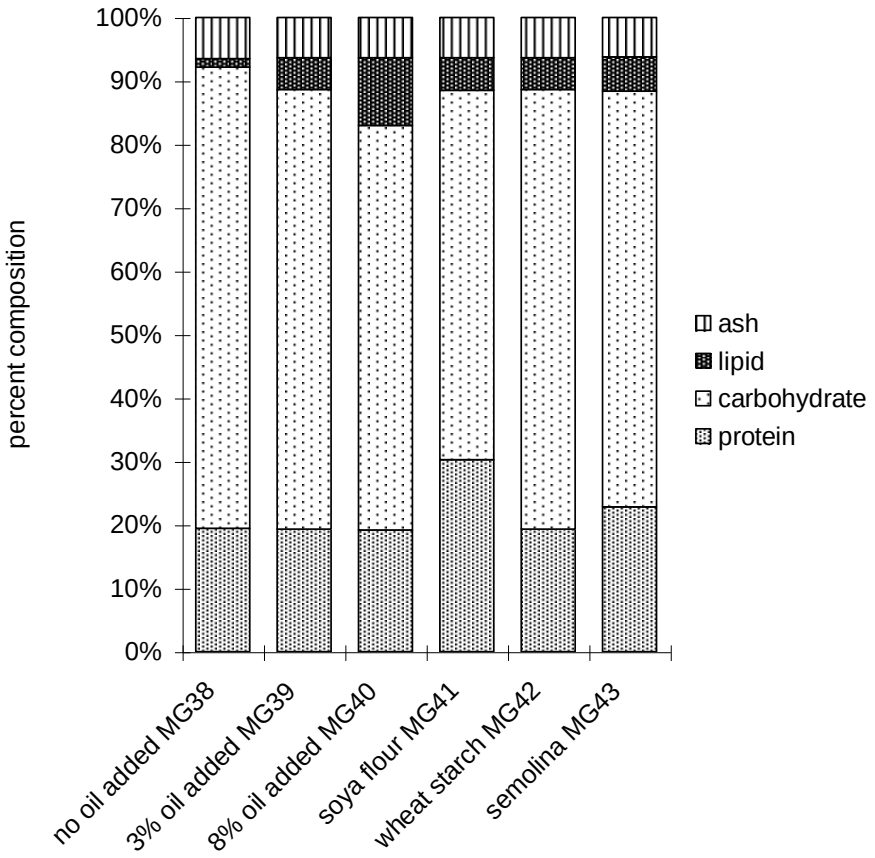


Figure 5.7.1: Proximate composition and gross energy of diets MG38 to MG43

For the first month (days 0 to 28), *N. closterium* was concentrated by settling from bag cultures. The culture was added directly to the tanks and aeration and water flow was stopped for 2 h during feeding. For the second month (days 29 to 53), *N. closterium* was added as a flocculated concentrate (CRC Aquaculture technology), using a syringe. It was not necessary to reduce aeration or water flow for the addition of this diet. For the third and subsequent months (days 54 to 132), a commercially available diet was used in place of the *Nitzschia* treatment. All other diets were fed in a powdered form between days 0 and 53 and then in a crumbed form until the conclusion of the experiment at day 132.

Each of the eight treatments (in triplicate) was assigned randomly to the tanks. The tanks were stocked initially with 120 blacklip abalone of 10.5 mm average length, or in the case of greenlip abalone, with 150 abalone of 6.3 mm average length. At the commencement of the experiment, the abalone were photographed for shell length determination and this was repeated at approximately one-month intervals. The experiment was conducted during the autumn to late winter with water temperature decreasing from a maximum of 14°C to a minimum of 10°C in July, and averaged 11.4°C over the whole experiment (Figure 5.7.2).

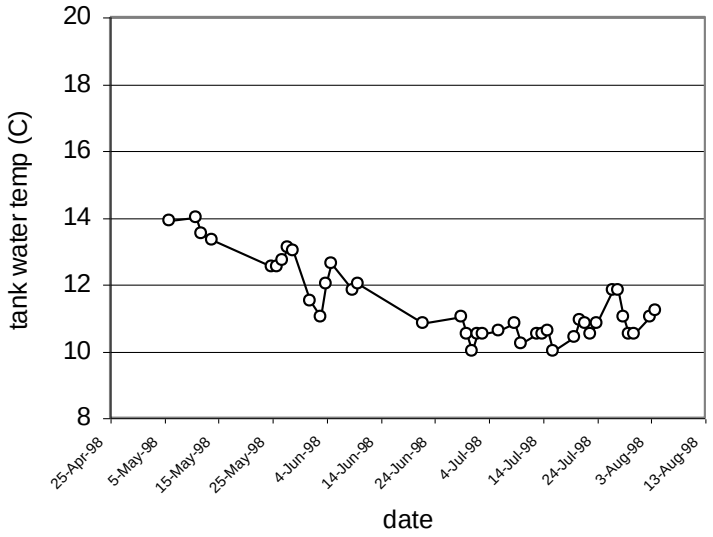


Figure 5.7.2: Experimental tank water temperature fluctuations during the evaluation of diets MG38 to MG43 used for the commercial ingredients growth rate experiment

5.7.3 *Results and Discussion*

Single factor ANOVA for pairwise comparisons was performed on the growth rate of abalone fed powdered feeds and on those fed crumbed diets (Figure 5.7.3). There were significant differences between powdered treatment diets ($p<0.05$), but not for crumbed treatment diets ($p = 0.34$). This significance was tested using Fisher's PLSD test. Overall, the growth rates for this experiment were low compared to other experiments, resulting in reduced significance between treatments. Previous work with similar low protein diets produced higher growth rates in abalone (eg Vandepeer et al., 1999), but it should be noted that the current experiment was run under ambient hatchery conditions during the coldest water temperatures for the year (average 11.4° C) compared to aquaria with constant 18°C water temperature (Vandepeer et al., 1999). Temperatures less than 12°C produce very low growth rates in abalone (Britz et al., 1997; Lopez et al., 1998). This explains the overall reduced growth rates evident here, and the lack of significant differences in growth rate during the latter/colder part of the trial when crumbed diets were evaluated. While these growth rates were disappointing, and resulted in reduced differences between treatments, diets such as these need to be tested at water temperatures experienced under genuine industry conditions.

The treatment diets MG38 to MG40 were included to examine if small abalone require high dietary lipid levels. The natural food of small abalone is high-lipid diatoms, whereas older animals eat low-lipid seaweed. Therefore it may be expected that a higher lipid diet could produce better growth in the smaller animals. These diets were formulated to contain protein levels (Figure 5.7.1) lower than in previous experiments. This was to examine a protein

sparing effect, whereby better growth rates would be expected in high fish oil diets as the oil is used for energy instead of protein thus enabling the protein to be used for growth. For the initial part of the experiment (to day 53), similar growth rates were evident in blacklip abalone fed a powdered formulated diet with 0, 3 or 8% fish oil (Figure 5.7.4). Slightly lower growth rates were evident in blacklip abalone fed the crumbed formulated diet with 8% fish oil compared to crumbed formulated diets with 0 or 3% fish oil but these differences were not significant (Figure 5.7.4). Previous experiments have shown that high levels of lipid (>4% added oil) reduced growth rate in grow-out greenlip abalone in winter and summer (average water temperature 12.8°C and 15.5°C respectively, Dunstan et al., 2000). This is contrary to that expected if a protein sparing effect was occurring. Thus carbohydrate is the main energy source for abalone. During the current winter trial (average water temperature 11.4°C) the growth rate of small blacklip abalone was less affected by high dietary lipid levels than as seen for grow-out sized greenlip abalone.

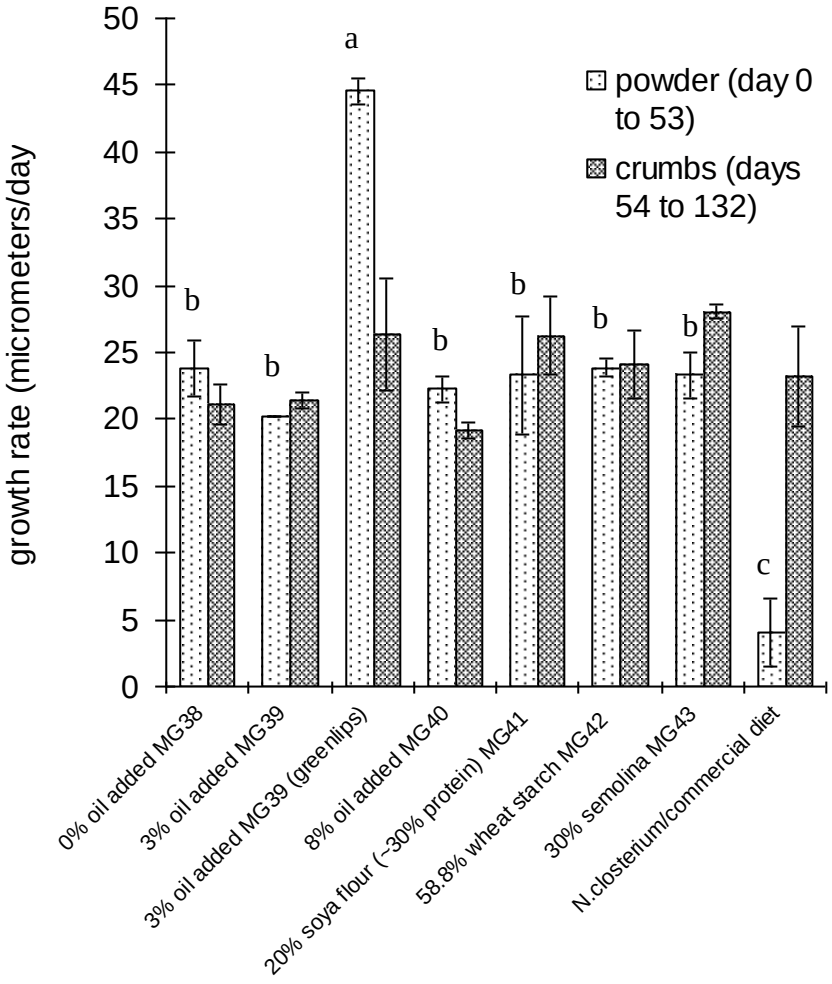


Figure 5.7.3: Average growth rate (micrometres per day ± SEM) of blacklip or greenlip abalone fed formulated diets from this commercial ingredients experiment. Growth rates on crumbed feeds were not significantly different between treatments.

Fishmeal contains lipid and the long chain PUFA, while other ingredients can contribute some lipid hence even though no fish oil was added to MG38, low amounts of essential PUFA and other fatty acids were still present. The longer pelleted abalone diets are in water, the higher their dry weight lipid content becomes due to leaching of water-soluble components (unpublished data). This would be particularly evident with powdered diets (but difficult to

quantitate accurately). Therefore by the time the abalone ingest the diets, not only could some of the important water soluble nutrients have leached out, but also the lipid levels would have increased above those shown in Figure 5.7.1. The results suggest that it may not be necessary to add any fish oil to diets for small blacklip abalone during periods of low growth such as the cooler months, the fish oil in the fishmeal providing adequate amounts of the long-chain ($\geq C_{20}$) PUFA. Also very small abalone can be weaned from a high lipid diet of diatoms to a formulated diet using the macroalga *Ulva* spp (Dunstan et al., 1996). This low lipid macroalga does not contain significant proportions of the essential polyunsaturated fatty acids like most other algae or formulated diets (Dunstan et al., 2000). It appears from this and Figure 5.7.4, that abalone can efficiently assimilate the small amounts of long-chain PUFA, or produce it from the more abundant short-chain (C_{18}) PUFA present in the diet. Caution should be taken though, as previous work has shown that reduced growth rate results if there is no source of fish oil in formulated abalone diets during the warm season when the abalone are actively growing (Dunstan et al., 2000).

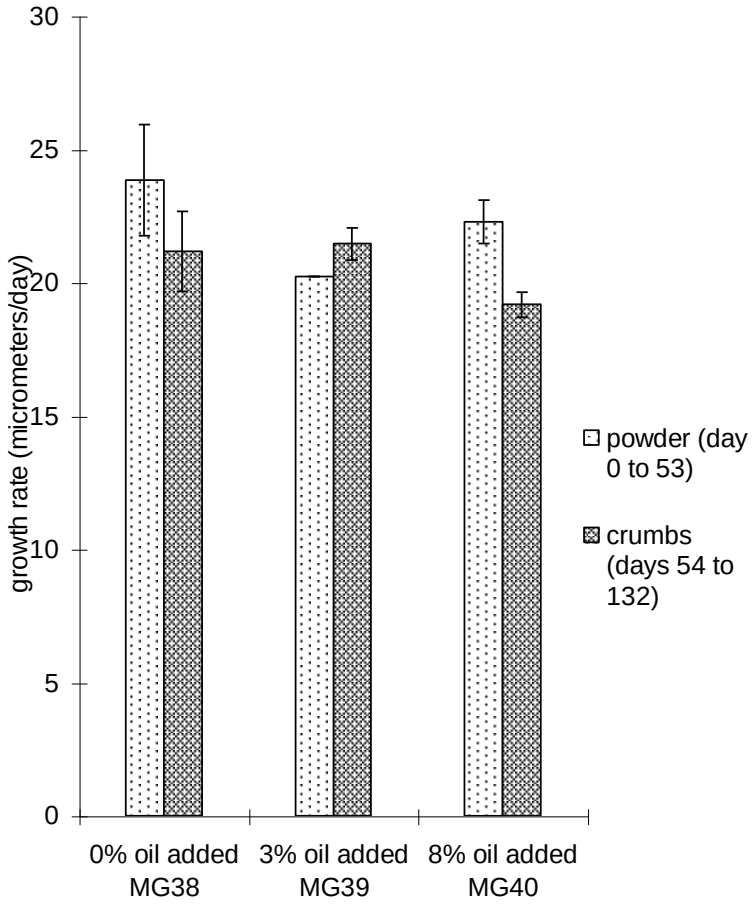


Figure 5.7.4: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed formulated diets with different levels of fish oil.

During both parts of the experiment, slightly higher growth rates were evident in blacklip abalone fed a powdered or crumbed formulated diet with elevated protein levels (30.3% DW protein cf 19.3% DW protein) (Figure 5.7.5). The higher protein content resulted from the addition of soya flour (20%) at the expense of gelatinised maize starch (Table 5.7.1).

Blacklip abalone grew slightly faster when wheat starch (MG42) was used instead of gelatinised maize starch (MG39) as the predominant carbohydrate source in powdered or crumbed formulated diets (Figure 5.7.6). In *Penaeus vannamei* prawn studies (Cousin et al.,

1996), the apparent digestibility of diets based on gelatinised maize starch or wheat starch was similar and high for starch and protein. Interestingly, reduced lipid digestibility was evident when maize starch was used instead of wheat starch. In other *P. vannamei* studies (Akiyama et al. 1991), maize starch had the lowest apparent dry matter digestibility compared to high protein ingredients. Shrimp growth rate was also lower when whole maize flour was substituted for whole wheat flour (Cruz-Suárez et al., 1994). These results lend support to our findings in abalone that wheat starch may be a better source of carbohydrate than maize starch.

Maize starch gelatinises at between 67 and 100°C (Tomasik, 1997). During preparation of the diets for the current experiments, the ingredients never reached such high temperatures to enable gelatinisation and so pre-gelatinised maize starch was used. Most of the binding of the diet was due to the sodium alginate. Had steam pelleting been used to produce the diets, the results may have been different. Therefore, the use of native maize starch in such diets should not necessarily be discounted. Whether gelatinised maize starch interferes with the uptake of essential lipids in the abalone (Cousin et al., 1996), or is less suitable for abalone than other ingredients has yet to be determined for Australian abalone. Our data suggest that maize starch is not the best source of complex carbohydrate to be included in formulated diets to maximise the growth of small abalone during the colder months.

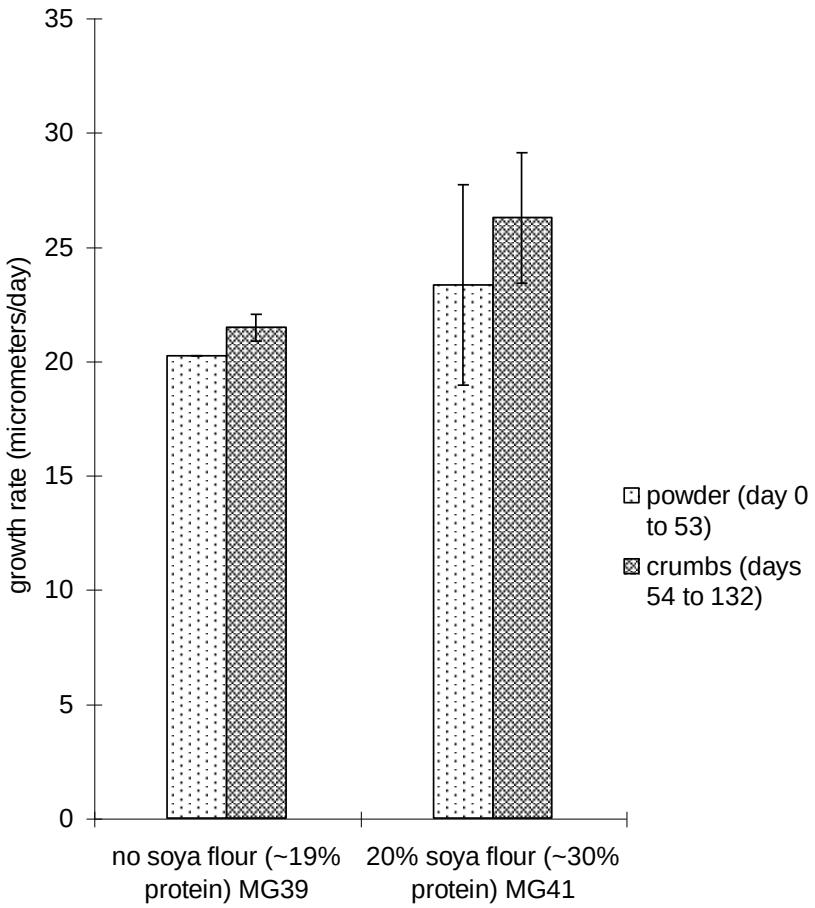


Figure 5.7.5: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed formulated diets with different levels of soya flour.

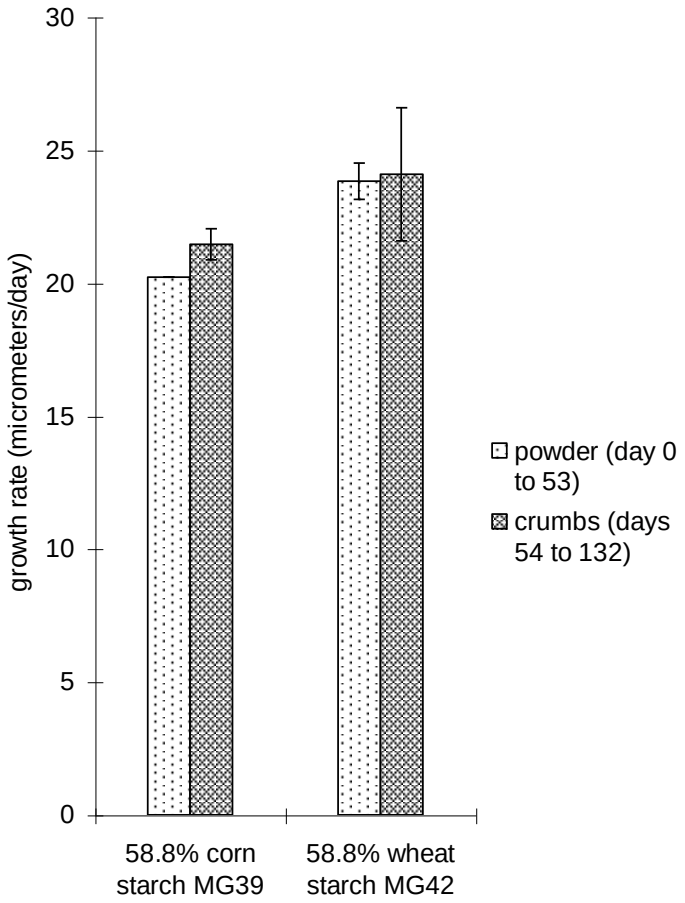


Figure 5.7.6: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed formulated diets with different types of starch.

Slightly higher growth rates were seen with blacklip abalone fed a powdered or crumbed formulated diet with elevated semolina (30% of ingredients added WW) relative to a low semolina diet (5% WW) (Figure 5.7.7). Semolina is a wheat-based product and like the wheat starch (Figure 5.7.6) produced higher growth rates than gelatinised maize starch. This could possibly be due to the slightly higher protein level (22% cf 19%) afforded by the use of semolina at the expense of the maize starch in diet MG43 (Figure 5.7.1). However, the growth rate on the semolina diet was better than for the high protein (soya flour) diet with 30% protein (Figure 5.7.7 cf. Figure 5.7.5).

During the initial part of the experiment (days 0 to28), the smaller (5.7 mm) greenlip abalone grew much faster (53.9 micrometers/day) than the larger (10.8 mm) blacklip abalone fed the same powdered diet MG39 (19.5 micrometers/day) or fed mass cultured *N. closterium* (7.6 micrometers/day) (Figure 5.7.8). Unfortunately, similar sized abalone of the two different species were not available for evaluation as they were sourced from different farms. Therefore such observed differences could be due to the particle size of the diet, as smaller animals may have found the smaller particles of a powder easier to ingest while the larger animals could have difficulty feeding on this form of diet. Alternatively these differences could have been due to differences in the nutritional requirements of the two abalone species.

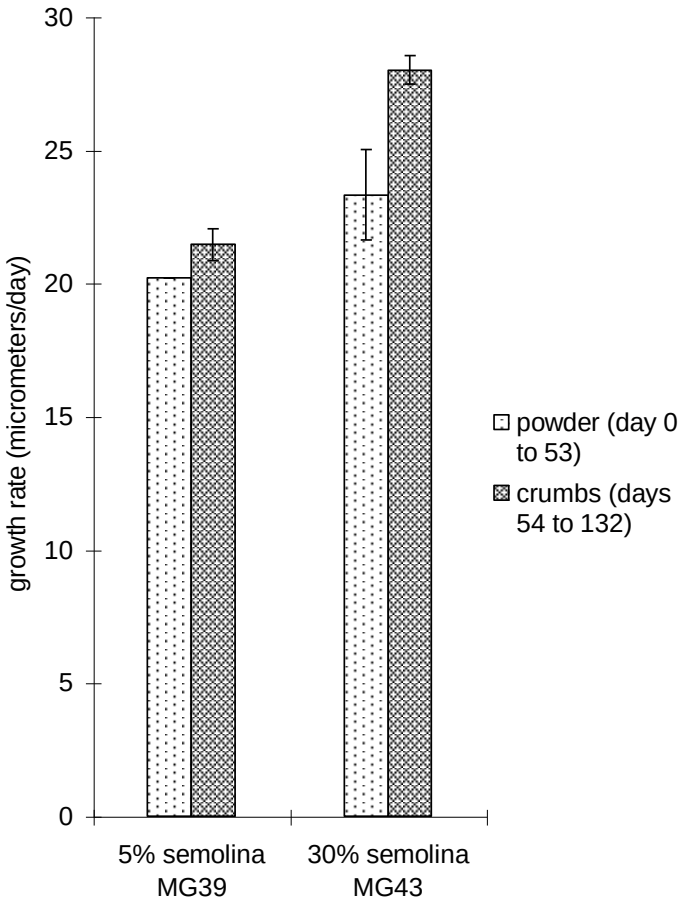


Figure 5.7.7: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed formulated diets with different levels of semolina.

During the second part of the experiment (days 29 to 53), the smaller greenlip abalone grew faster (34.1 micrometers/day) than the larger blacklip abalone fed the same powdered diet MG39 (20.0 micrometers/day) or flocculated *N. closterium* (0.1 micrometers/day) (Figure 5.7.8). From these data it is evident that the control diet (MG39) in a powdered form produced higher growth rates in both blacklip and greenlip abalone than mass cultured or flocculated *N. closterium* (days 0 to 28 and days 29 to 53 respectively) fed to blacklip abalone. While some losses of the mass cultured *N. closterium* could be expected to have occurred once the water flow was reinstated, thus reducing feeding ration, much of the flocculated algae remained on the bottom of the tank for the two days prior to the next cleaning and feeding. This suggests that the process of flocculation adversely affected the nutritional value of the diatom for blacklip abalone.

During the final (and coldest, see Figure 5.7.2) part of the experiment (days 54 to 132), smaller greenlip abalone grew at a comparable growth rate (26.4 micrometers/day) to blacklip abalone fed the same crumbed diet MG39 (21.5 micrometers/day) and blacklip abalone fed a commercial abalone diet (23.2 micrometers/day) (Figure 5.7.8).

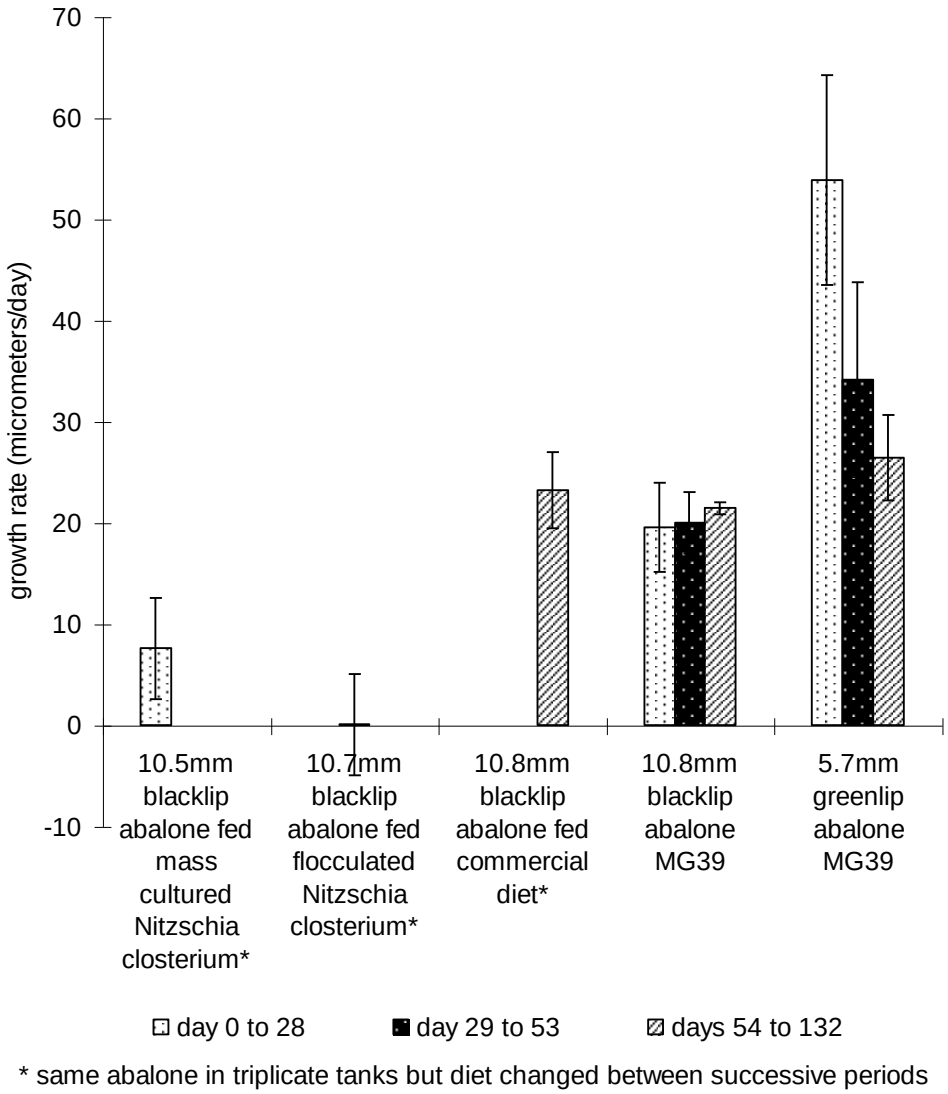


Figure 5.7.8: Average growth rate (micrometres per day ± SEM) of blacklip and greenlip abalone fed formulated (initially in powdered form or a live feed, days 0 to 53, and later as crumbs, days 54 to 132).

In conclusion, high protein, semolina or soya flour based diets, which are low in lipid and maize starch, are recommended during the colder, low growth periods of the year. The preparations of mass cultured and flocculated *N. closterium* produced lower abalone growth rates compared to the formulated diets. However, it is clear from this experiment that new diets based on the composition of natural diets, utilising innovative ingredients were needed.

5.8 Effect of simple and complex carbohydrates and diatomaceous earth in the formulated "diatom" diet on the growth of small abalone

5.8.1 Introduction

The work previously outlined indicated that the addition of fructose could increase the attractiveness of a diet (Section 5.4), and that abalone growth rate could be increased by the addition of oyster glycogen (Section 5.6). The commercial ingredient experiment (Section 5.7) showed that even during the coldest months of the year, small gains could be achieved by manipulating the proportions of key commercial ingredients to optimise the basal diet. Complex carbohydrate sources such as wheat starch and protein/carbohydrate sources such as semolina were shown to be beneficial for small abalone (Section 5.7). But the overall low growth rates evident in small abalone fed such diets and various forms of live diatoms indicated the need for new and innovative formulated diets.

From previous analyses of natural diets (Section 5.2) and experiments with simple and complex carbohydrates (Section 5.6), the formulated "diatom" diet was re examined to establish which components were important in producing the elevated growth rate in abalone. Two of the key differences with this diet were the "sugar mix" and the addition of diatomaceous earth. Thus the aim of this experiment was to evaluate and optimise formulated diets based on the composition of natural diets and identify cheaper substitutes for some components of the sugar.

5.8.2 Methods

In view of the gains observed in previous experiments from manipulating the ingredient source and the amount of protein and carbohydrate in the diet, six diets (MG44 to MG49) were formulated to establish which ingredients were important in the formulated "diatom" diet (Section 5.6) and which could be substituted with cheaper alternatives. A seventh diet (MG50) was formulated as an alternative high protein diet based on fish and algal meal (Table 5.8.1). The proximate composition of the diets was calculated from ingredient analysis, product specifications and other literature (Figure 5.8.1). The total energy (MJ/kg dry weight) was calculated using gross energy equivalents for protein, carbohydrate and lipid of 20.1, 17.6 and 39.7 J/g, respectively (Whyte, 1987). Diets MG44 to MG48 were formulated to contain comparable levels of protein (22-23%DW), while MG49 and MG50 contained a higher amount of protein (32% DW). Diets MG48 & MG49 contained diatomaceous earth (7% WW), and all diets were formulated to contain the same amount of lipid. Additions of ingredients were at the expense of carbohydrate (primarily gelatinised maize starch). Diets were also analysed for fatty acid composition (Section 5.1.3.2) and all diets were shown to be similar in composition (Table 5.8.2).

Table 5.8.1: Formulation of diets MG44 to MG50 used for the carbohydrate and formulated diatom diet growth rate experiment

Diet number	MG44	MG45	MG46	MG47	MG48	MG49	MG50
	0% simple sugar (cf MG43 Section 5.7)	3% sucrose	9% sucrose	Formulated "diatom" diet (no diatomaceous earth)	Formulated "diatom" diet (control)	Formulated "diatom" diet (25% soyaflour)	Fish/algal meal diet
Fish oil	3.0	3.0	3.0	3.0	3.0	3.0	1.9
Sucrose	0	3.0	9.0	0	0	0	0
Glucose*	0	0	0	6.0	6.0	6.0	3.0
Mannose*	0	0	0	0.5	0.5	0.5	0
Xylose*	0	0	0	2.5	2.5	2.5	0
Diatomaceous earth	0	0	0	0	7.0	7.0	0
Soya flour	0	0	0	0	0	25.0	0
Fishmeal	9.0	9.0	9.0	9.0	9.0	9.0	20.0
Kelp powder	4.0	4.0	4.0	4.0	4.0	4.0	15.0
Semolina	30.0	30.0	30.0	30.0	30.0	5.0	30.0
Maize starch	33.3	30.3	24.3	24.3	17.3	17.3	9.9
Calcium carbonate	1.0	1.0	1.0	1.0	1.0	1.0	0.5
Fixed ingredients[1]	19.7	19.7	19.7	19.7	19.7	19.7	19.7
Total %	100	100	100	100	100	100	100

[1]Included in final diet (%): casein, 10.0; dried *Dunaliella salina*, 1.0; sodium alginate, 3.0; vitamin mix, 2.0%; vitamin C, 1.0; vitamin E, 0.01; mineral mix, 1.0; calcium sulphate, 0.8; DL-methionine, 0.3; L-threonine, 0.3; arginine, 0.3.
* collectively referred to as the "sugar mix"

The seven abalone diets were evaluated with blacklip abalone. Twenty-one, identical 53 L experimental tanks were used for this experiment. The 70 cm diameter round tanks were shallow (13 cm deep at perimeter, gently sloping to 15 cm deep at centre) and centrally drained via a standpipe. Each tank was aerated with two air-stones and contained 4 flat PVC plate shelters, and the entire system was shaded. Each of the seven treatments (in triplicate) was assigned randomly to the tanks, and each stocked with 123 abalone. At the commencement of the experiment, all abalone were photographed for shell length determination and this was repeated at approximately one-month intervals. Initial and final weights were also taken. At the beginning of the experiment the average (mean ± SD) length and weight of measured abalone per tank were 13.7 ± 0.95 mm and 0.42 ± 0.02 g, respectively.

The experiment was conducted during the spring to autumn with water temperature increasing from a minimum of 11.5°C in October to a maximum of 17.9°C in early March, and averaged 14.8°C for the whole experiment (Figure 5.8.2).

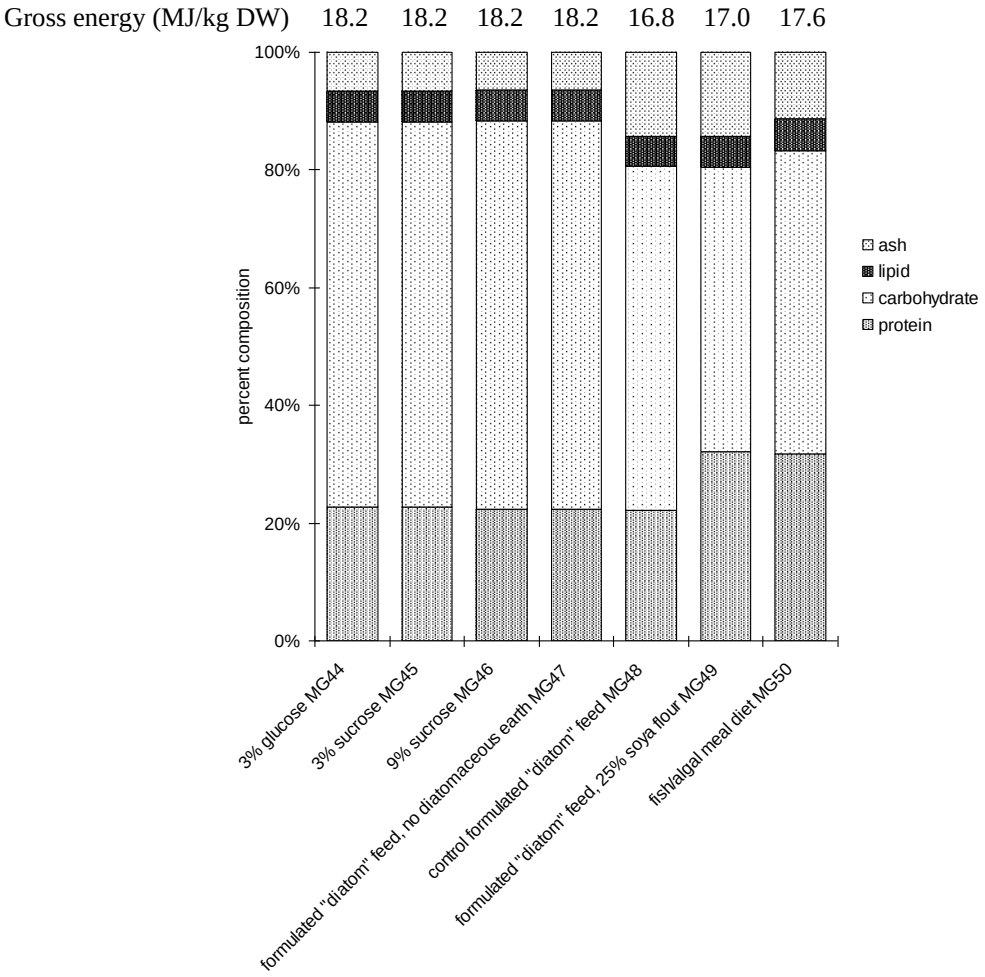


Figure 5.8.1: Proximate composition and gross energy of diets MG44 to MG50 used for the carbohydrate and formulated diatom diet growth rate experiment

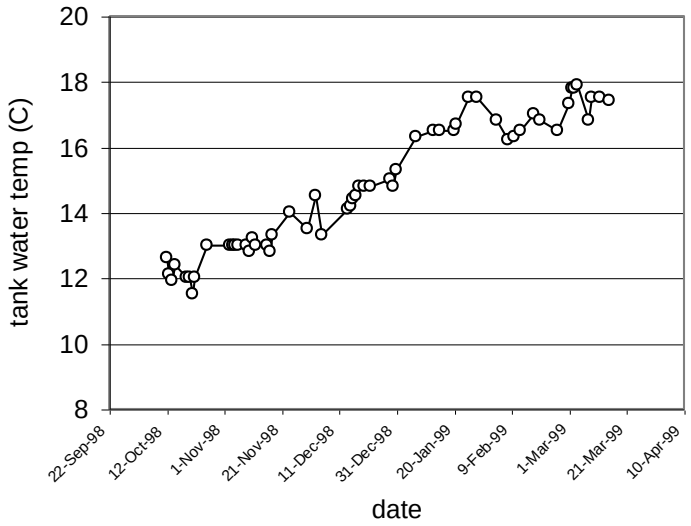


Figure 5.8.2: Tank water temperature during the evaluation of diets MG44 to MG50 for the carbohydrate and formulated diatom diet growth rate experiment

Table 5.8.2: Fatty acid compositions of diets MG44 to MG50 used for the carbohydrate and formulated diatom diet growth rate experiment

Diet number	MG44	MG45	MG46	MG47	MG48	MG49	MG50
Description	0% simple sugar (control)	3% sucrose	9% sucrose	Formulated "diatom" diet (no diatomaceous earth)	Formulated "diatom" diet (control)	Formulated "diatom" diet (25% soyaflour)	Fish/algal meal diet
Saturated fatty acids (SFA)							
14:0	5.8	5.9	6.0	5.9	5.8	5.9	5.5
16:0	18.2	17.6	17.6	17.8	17.8	17.5	17.6
18:0	3.6	3.6	3.5	3.4	3.7	3.9	3.7
total	29.2	28.8	28.7	28.8	28.9	28.8	28.4
Monounsaturated fatty acids (MUFA)							
16:1ω7	6.1	6.1	6.3	6.5	6.4	6.2	5.3
18:1ω9	12.7	12.2	12.3	12.1	12.8	12.8	16.0
18:1ω7	3.2	3.6	3.6	3.7	2.9	3.5	3.3
20:1ω9	1.7	1.7	1.7	1.6	1.7	1.7	1.5
20:1ω7	1.8	1.8	1.8	1.7	1.9	1.8	2.7
22:1ω9	0.7	0.6	0.6	0.6	0.7	0.7	0.5
24:1ω9	0.7	0.7	0.7	0.6	0.7	0.7	0.9
total	31.0	32.2	33.0	32.4	31.4	31.5	34.1
Polyunsaturated fatty acids (PUFA)							
18:2ω6	8.5	7.7	7.3	7.8	7.6	8.2	7.6
18:3ω3	1.1	1.0	1.0	1.1	1.1	1.5	1.2
20:4ω6	1.3	1.3	1.3	1.3	1.4	1.3	2.2
20:5ω3	12.3	12.1	11.9	11.9	12.5	12.2	9.7
22:5ω3	1.7	1.7	1.7	1.7	1.8	1.7	1.5
22:6ω3	10.1	10.1	9.8	9.8	10.3	10.0	11.0
total	37.3	36.1	35.3	35.8	37.0	37.1	35.2
total ω3	25.7	25.3	24.9	24.9	26.2	25.8	23.7
total ω6	11.6	10.8	10.4	10.9	10.8	11.3	11.6
PUFA/SFA	1.3	1.3	1.2	1.2	1.3	1.3	1.2
ω3/ω6 ratio	2.2	2.3	2.4	2.3	2.4	2.3	2.0
% lipid WW	4.4	4.2	4.7	4.4	4.3	4.7	4.7
% lipid DW	4.8	4.7	5.2	4.9	4.9	5.1	5.3

Minor fatty acids are not shown.

5.8.3 Results and Discussion

Figures 5.8.3 (length gain rate) and 5.8.4 (weight gain rate) show the data for the current experiment. Single factor Analysis of Variance for pairwise comparisons was performed on the growth rates of abalone fed the crumbed diets. There was a significant difference between treatment diets ($p<0.05$). This significance was tested using Fisher's PLSD test.

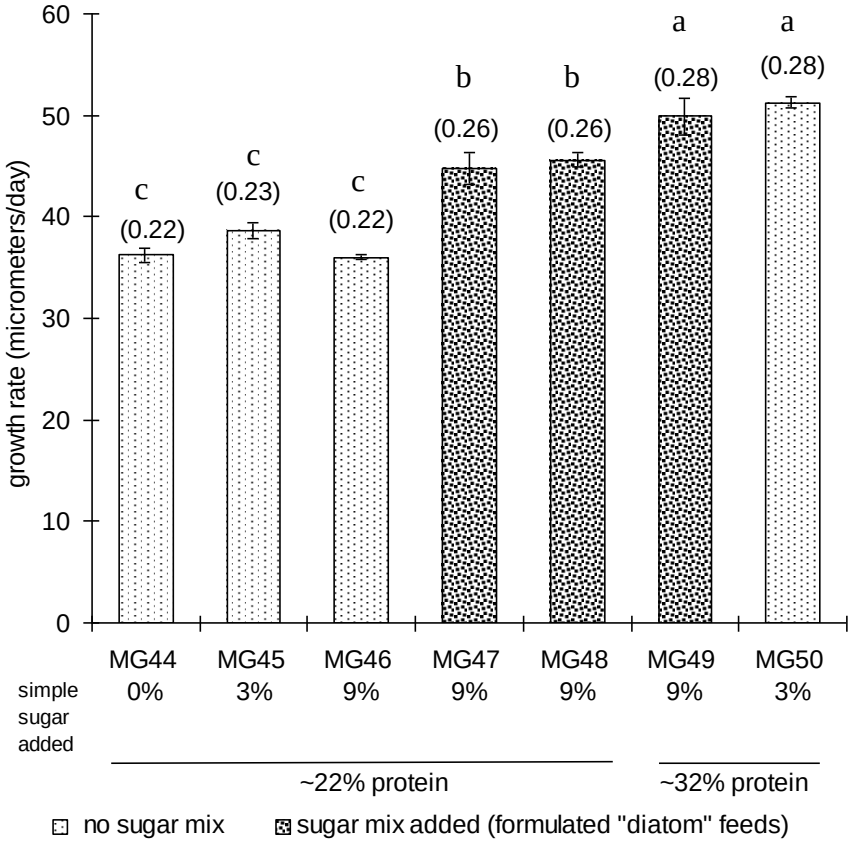


Figure 5.8.3: Average length gain rate (micrometers per day ± SEM) and SGRL (in parenthesis) of blacklip abalone fed diets with different sugars and protein level

The use of the sugar mix (compared to other simple sugars) and high protein levels resulted in elevated growth of small abalone. The greatest weight gain was achieved when the sugar mix was combined with high protein (32%) (MG49), while the greatest length gain was achieved when high protein (32%) diets were used (MG49 and MG50). These two diets were formulated to have similar proximate and gross energy composition (Figure 5.8.1); one being formulated to biochemically resemble a diatom, but modified by increasing protein and reducing ash levels (MG49) while the other was based on fish and algal meals.

There was a slight, but not significant advantage of adding diatomaceous earth to the formulated "diatom" diet (Figure 5.8.5). Even though living diatoms contain a comparable amount of similar silicious material, this was not an unexpected result. Diatomaceous earth is considered to be an inert ingredient during ingredient evaluation studies with other species. Studies with European seabass (*Dicentrarchus labrax*) examining various bulk agents showed that silica reduces protein retention at low inclusion rates (10%), but at higher inclusion rates (20%) reduces growth slightly; zeolite (a silicate) however, was found to have no effect (Dias et al., 1998). These authors cite other studies where clay-based silicates have been beneficial or had no effect on the growth of various species. It is fortuitous that inclusion of diatomaceous earth does not significantly increase growth rates in abalone (Figure 5.8.5), as inclusion of relatively indigestible components in formulated diets would lead to increased faecal waste and suspended solids discharge from farms. Inclusion of highly digestible nutritious ingredients is preferred.

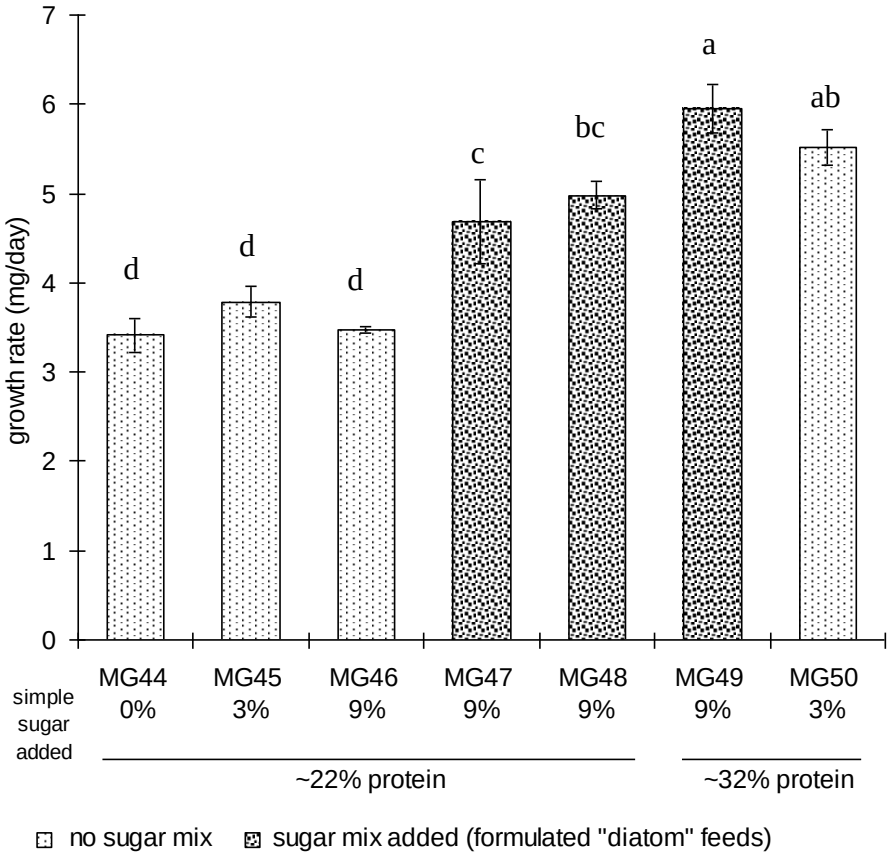


Figure 5.8.4: Average weight gain rate (mg per day ± SEM) of blacklip abalone fed diets with different sugars and protein level

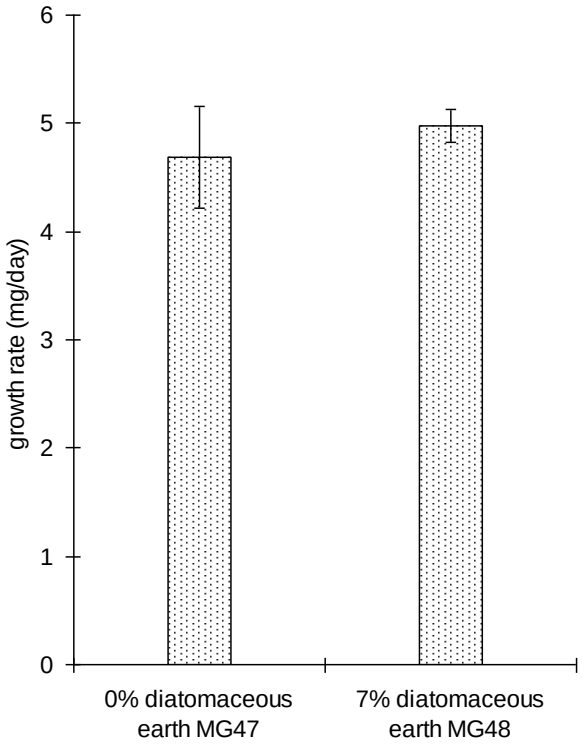


Figure 5.8.5: Average growth rate (mg per day ± SEM) of blacklip abalone fed the formulated "diatom" diet with different levels of diatomaceous earth

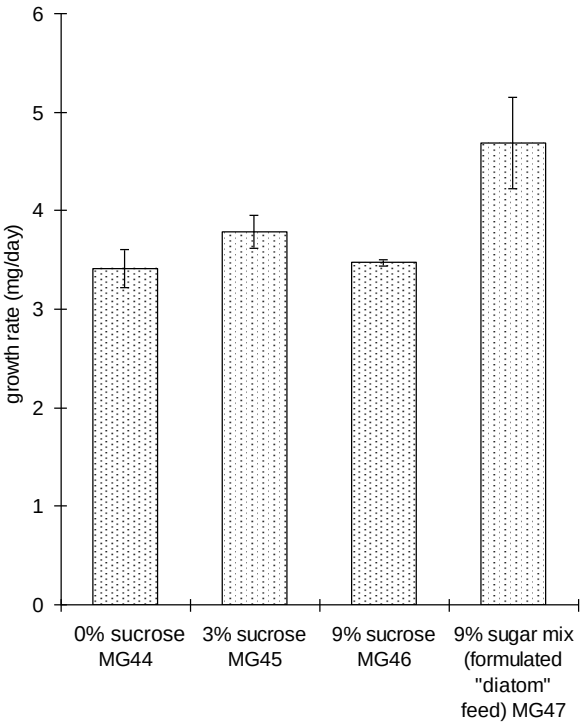


Figure 5.8.6: Average growth rate (mg per day ± SEM) of blacklip abalone fed formulated diets with different levels of sucrose, compared to the "diatom" diet

As in the experiment detailed in Section 5.6, the inclusion of simple sugars (0, 3 or 9% sucrose) did not increase abalone growth rate compared to the "diatom diet" (MG47) with 9% simple sugars (and comparable protein levels) (Figure 5.8.6). Interesting is the markedly different growth rate for diets MG46 and MG47. These two diets contained the same levels of ash, lipid, protein, carbohydrate and simple sugars, and the same gross energy (Figure 5.8.1). The only difference was the inclusion of the 9% simple sugar mix in diet MG47 instead of 9% sucrose in diet MG46 (Table 5.8.1). Likewise for diet MG44, the 9% simple sugar mix was replaced with 9% gelatinised maize starch and produced a similar low growth rate as MG46. It is not known whether this result was because the sugars of the sugar mix were more easily digested than sucrose or gelatinised starch, were essential nutrients for growth, or were more effective feed attractants.

Increased protein level resulted in a growth advantage (Figure 5.8.7). Abalone fed the low protein "diatom" diet (MG48, semolina 30%, soya flour 0%) grew more slowly than those fed the high protein "diatom" diet (MG49, semolina 5% soya flour 25%). The fish/algal meal diet (MG50) which had a protein content and proximate composition comparable to the high protein "diatom" diet (MG49), but with very different ingredients included to achieve this, produced similar abalone growth rates to the high protein "diatom" diet and higher abalone growth rates than the low protein "diatom" diet.

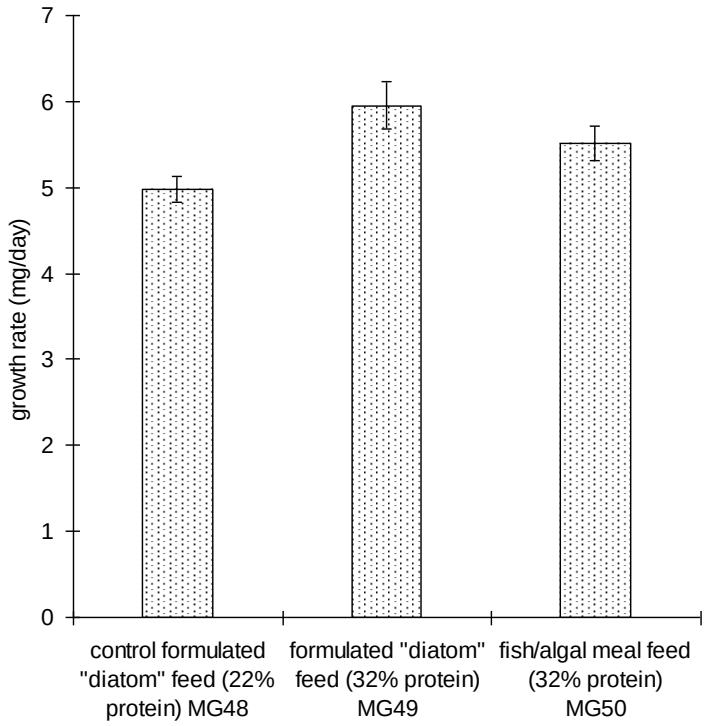


Figure 5.8.7: Average growth rate (mg per day ± SEM) of blacklip abalone fed the formulated "diatom" diet with different levels of soya flour, compared to a fish/algal meal diet.

These findings indicate the need of small abalone to be fed on diets that are high in protein (32% DW) and which contain a sugar mix. This further illustrates the need for research examining the importance of carbohydrates in the diets of herbivorous animals being cultured.

5.9 Effect of protein/carbohydrate level and formulated "diatom" diets on growth of small abalone

5.9.1 Introduction

Previous work (Section 5.8) showed that high protein diets improved abalone growth rate, but the optimum amounts were not known for small blacklip abalone. The main aim of this experiment was to identify the dietary protein level that maximised abalone growth rate. While casein has been shown to be a valuable protein source for abalone (Uki et al., 1985b), it is expensive. Hence soya flour was chosen as the primary protein source. The second aim was to investigate different sources of protein.

5.9.2 Methods

Seven diets were formulated for this feeding experiment (Table 5.9.1). To establish optimum protein level, four diets providing a protein gradient between 26 and 45% DW were formulated (MG51 to MG54) by increasing the level of defatted soya flour at the expense of gelatinised maize starch. Due to the success of the formulated diatom diet and the fish/algal meal diet (Sections 5.6 and 5.8), these diets were refined and evaluated again (MG56 and MG57). The main source of protein in the plant ingredient-only diet (MG55) and the formulated diatom diet (MG56) was soya flour, while in the fish/algal meal diet (MG57) it was fish and algal meal.

Table 5.9.1: Formulation of diets MG51 to MG57 used for the protein growth rate experiment

Diet number	MG51	MG52	MG53	MG54	MG55	MG56	MG57
Description	13% soya flour	25% soya flour (control diet)	34% soya flour	48% soya flour	Plant ingredients only	Formulated "diatom" diet (cf MG49)	Fish/algal meal diet (cf MG50)
Soya flour	13.0	25.0	34.0	48.0	36.0	25.0	0
Fishmeal	9.0	9.0	9.0	9.0	0	9.0	20.0
Fish oil	3.0	3.0	3.0	3.0	0	3.0	1.9
5α-Cholestane	0	0.5	0	0	0	0	0
Vegetable oil	0	0	0	0	4.0	0	0
Glucose	0	0	0	0	0	6.0	0
Mannose	0	0	0	0	0	0.5	0
Xylose	0	0	0	0	0	2.5	0
Diatomaceous earth	0	0	0	0	0	4.3	0
Semolina	0	0	0	0	0	0	30.0
Kelp powder	5.0	5.0	5.0	5.0	5.0	5.0	16.0
Maize starch	50.3	37.8	29.3	15.3	34.3	25.0	12.9
Calcium carbonate	1.0	1.0	1.0	1.0	2.0	1.0	0.5
Fixed ingredients[1]	18.7	18.7	18.7	18.7	18.7	18.7	18.7
Total %	100	100	100	100	100	100	100

[1]Included in final diet (%): casein, 10.0; sodium alginate, 3.0; vitamin mix, 2.0; vitamin C, 1.0; vitamin E, 0.01; mineral mix, 1.0; calcium sulphate, 0.8; methionine, 0.3; threonine, 0.3; arginine, 0.3.

The proximate composition of the diets was calculated from ingredient analysis, product specifications and other literature (Figure 5.9.1). Diets MG52, and MG55 to MG57, all had similar protein levels. Diets MG51 to MG54 had protein contents increasing from 26 to 45%

DW. Some diatomaceous earth was included in the formulated "diatom" diet (MG56) so as to balance the increased ash content from the fishmeal of diet MG57. Therefore MG56 and MG57 had elevated ash contents relative to the other diets. The total energy (MJ/kg dry weight) was calculated using gross energy equivalents for protein, carbohydrate and lipid of 20.1, 17.6 and 39.7 J/g, respectively (Whyte, 1987).

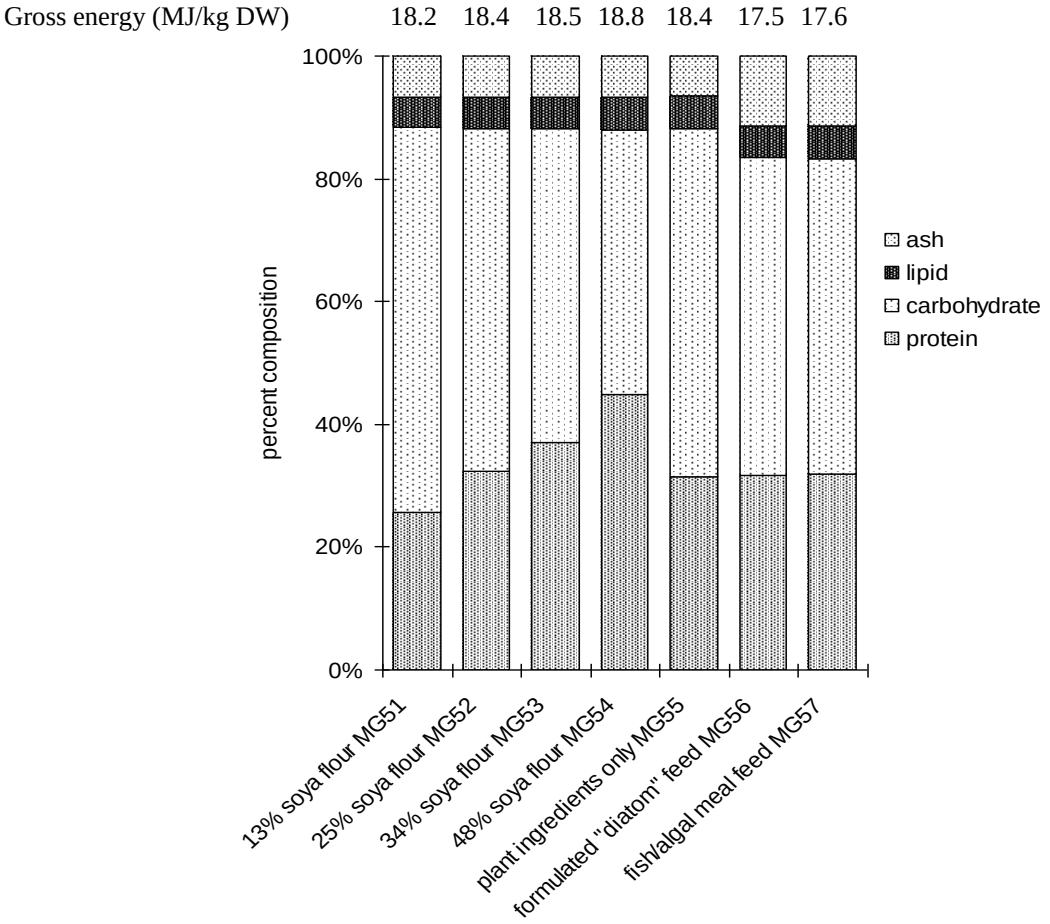


Figure 5.9.1: Proximate composition and gross energy of diets MG51 to MG57 used for the protein growth rate experiment

The protein and lipid contents of the formulated diets for this protein evaluation experiment was determined (Table 5.9.2). There was close agreement between the calculated and determined chemical composition of the diets.

Table 5.9.2: Verification of calculated proximate composition of diets MG51 to MG54 used for the protein growth rate experiment

diet	Calculated protein* (%DW)	Crude protein (by analysis) (%DW)	Calculated lipid* (%DW)	Crude lipid (by analysis) (%DW)
MG51	25.7	25.1	5.0	5.0
MG52	32.4	31.7	5.2	6.0
MG53	37.2	36.2	5.3	5.6
MG54	44.8	43.6	5.5	6.2

*calculated from ingredient analysis and literature values

The fatty acid composition of all diets was similar apart from the plant ingredients-only diet (MG55) which contained higher proportions of 18:2ω6 and 18:1ω9, reduced proportions of 16:0 and 16:1ω7 and lacked the essential long-chain ω3 PUFA (Table 5.9.3).

Table 5.9.3: Total lipid content and fatty acid composition of the formulated diets MG51 to MG57 used for the protein growth experiment

Diet number	MG51	MG52	MG53	MG54	MG55	MG56	MG57
Description	13% soya flour	25% soya flour	34% soya flour	48% soya flour	Plant ingredients only	Formulated "diatom" diet (cf MG49)	Fish/algal meal diet (cf MG50)
Saturated fatty acids (SFA)							
14:0	6.0	5.7	5.5	5.6	0.6	6.0	5.8
16:0	18.2	17.9	17.7	17.5	10.1	17.7	17.5
18:0	4.0	4.1	4.0	4.1	5.4	4.1	4.1
total	29.7	29.4	28.8	28.7	17.8	29.4	29.1
Monounsaturated fatty acids (MUFA)							
16:1ω7	5.8	5.6	5.5	5.4	0.2	5.9	4.8
18:1ω9	11.3	11.3	11.1	11.0	17.7	11.2	12.5
18:1ω7	3.0	2.9	2.8	2.9	0.9	3.0	2.8
20:1ω9	2.0	2.1	1.9	1.9	0.2	2.0	3.3
20:1ω7	0.3	0.3	0.3	0.3	0.1	0.3	0.3
22:1ω9	1.2	1.2	1.1	1.1	-	1.2	1.9
24:1ω9	0.6	0.5	0.5	0.5	-	0.6	0.7
total	27.9	27.6	27.0	26.7	19.3	28.1	29.9
Polyunsaturated fatty acids (PUFA)							
18:2ω6	8.9	10.2	11.5	12.5	58.9	9.1	7.0
18:3ω3	1.7	2.0	2.2	2.5	2.0	1.9	1.4
20:4ω6	1.2	1.1	1.1	1.0	0.2	1.1	1.6
20:5ω3	12.2	11.5	11.4	11.2	0.1	12.0	10.6
22:5ω3	1.8	1.8	1.7	1.7	-	1.8	1.9
22:6ω3	10.2	9.6	9.6	9.4	-	9.9	11.6
total	41.1	41.5	42.6	43.3	61.9	41.0	39.5
total ω3	29.2	28.1	28.2	27.9	2.3	28.9	28.8
total ω6	11.7	13.1	14.3	15.2	59.4	11.8	10.3
PUFA/SFA ratio	1.4	1.4	1.5	1.5	3.5	1.4	1.4
ω3/ω6 ratio	2.5	2.2	2.0	1.8	0.0	2.4	2.8
% lipid (WW)	4.6	5.5	5.2	5.7	5.0	5.0	5.5
% lipid (DW)	5.0	6.0	5.6	6.2	5.4	5.5	6.0

Minor fatty acids are not shown.

The blacklip abalone used in the experiment were collected from diatom and turf algae covered settlement plates. They were distributed to the on-site tanks by randomised allocation as described in Section 5.1.1. Tanks were described in Section 5.8 and each tank was aerated with two air-stones and contained four flat PVC plate shelters; the entire system was shaded. Diets were assigned in triplicate in accordance with the randomised block design of the experiment. Abalone were photographed for shell length measurement at the commencement of the experiment (week 0) and at the conclusion (week 6).

The experiment was conducted during the autumn. In contrast to previous experiments (Sections 5.6 to 5.8), a heat exchanger was incorporated into the system so as to maintain a relatively constant water temperature (17°C±0.7) over the whole experiment (Figure 5.9.2).

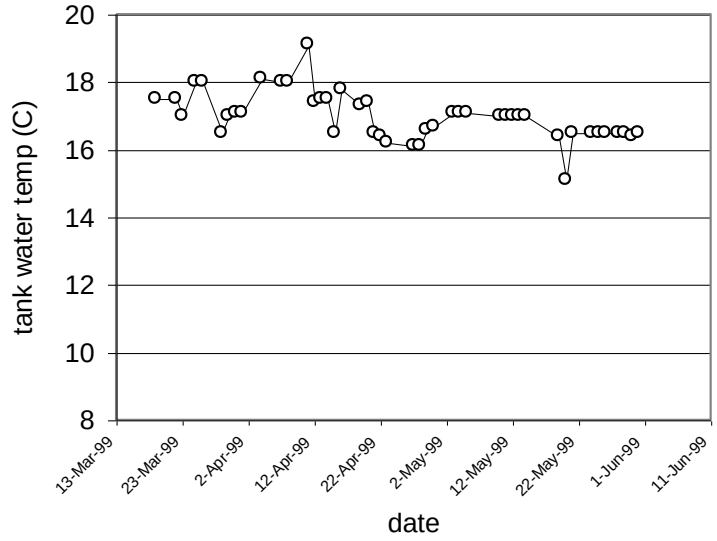


Figure 5.9.2: Experimental tank water temperature fluctuations during the evaluation of diets MG51 to MG57 used for the protein growth rate experiment

5.9.3 Results and Discussion

Figure 5.9.3 shows the results for all treatment diets for the experiment. Single factor Analysis of Variance for pairwise comparisons was performed on the growth rate of abalone fed the crumbed diets. There was a significant difference between treatment diets (p<0.05). This significance was tested using Fisher's PLSD test.

Highest abalone growth rate was observed with diets containing 34% and 48% soya flour which were equivalent to dry matter dietary protein concentrations of 36 and 44% DW, respectively (Table 5.9.2, Figure 5.9.3). Thus in the present study, protein levels greater than 36% produced the best growth rates (Figure 5.9.4).

Soya flour was used in the current study because it is a relatively inexpensive protein source. The growth rate of *Haliotis discus hannai* was maximised at dietary protein concentrations of 45 to 50% and >40% respectively where casein or whitefishmeal had been used as the protein source (Uki et al., 1986a). However feed conversion was optimised at lower dietary protein concentrations of 38% and 20% respectively. Similarly, growth of large and small *H. midae* was maximised with diets of 44 and 34% protein respectively (Britz & Hecht, 1997). In contrast, a dietary protein concentration of 27% was stated to be optimal for the tropical *H. asinina* (Bautista-Teruel & Millamena, 1999) although growth rate was best on the 31.5% protein diet, which was the highest protein concentration investigated. Other work has shown that increasing protein level in natural diets also increases the growth rate of abalone (Shpigel et al., 1999). Thus, most authors report that increased protein levels lead to increased growth rate. However, this is not the only consideration. As the level of protein in the diet increases, the efficiency with which protein is utilised by the abalone decreases. The level of energy in the diet is also important, especially with respect to protein sparing effects. Ideally, protein (expensive ingredient) should be used primarily as a source of amino acids for growth while the animal's energy requirements for maintenance, activity and somatic growth should be sourced from lipid (concentrated energy ingredient) and carbohydrate (cheap ingredient). If

the diet contains insufficient lipid, some protein will be used for energy and thus, reducing the growth benefits of a high protein diet. But as shown previously, diets with more than 3% oil added to pelleted diets (Dunstan et al., 2000) reduced the growth of older greenlip abalone in both the warm and cold seasons. In contrast, we have shown that changing dietary lipid levels (3 and 6% lipid during warm months, see Section 5.6, or 0, 3, 8% lipid during cold months, see Section 5.7) for small blacklip abalone did not affect growth rate. Carbohydrate appears to be the main energy source in abalone (Section 5.7). Increasing dietary protein levels can also increase abalone whole body protein composition (Uki et al., 1986a; Britz & Hecht; 1997), which may affect product quality.

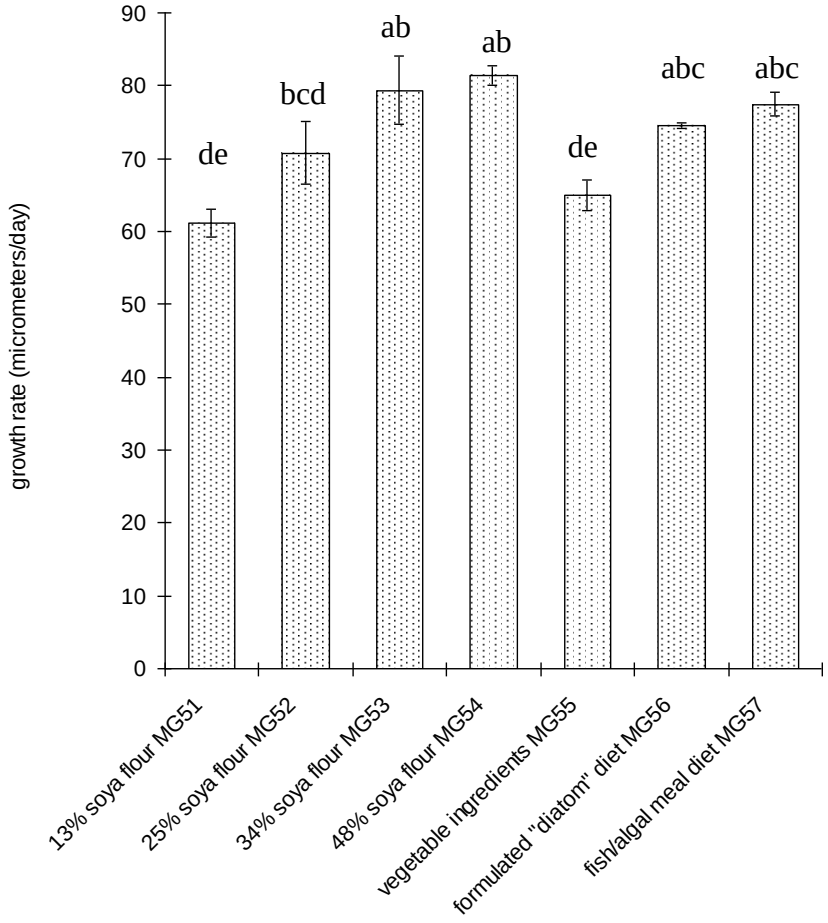


Figure 5.9.3: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed formulated diets with different amounts and sources of protein.

Not only the amount, but also the type of protein is important. In the present experiment, the diet with elevated fish and algal meals as the main protein source (MG57) produced the highest growth rate. This was only slightly but not significantly higher than for the formulated diatom diet (MG56), but higher than for the control diet (MG52). Both of the latter diets used soya flour (with some fishmeal and casein) as the main protein source. It should be noted that all of these aforementioned diets contained the same level of casein and comparable proximate composition. However the ingredients making up these diets needed to be quite different if they were to contain no animal products, but still have the same proximate composition. Therefore the protein source was not the only determinant of its nutritional adequacy for small abalone. Lopez et al., (1998) examined a fishmeal/algal meal diet (similar to MG57) and a casein meal/algal meal diet and also found the former to produce a higher growth rate in *Haliotis tuberculata*. Uki et al., (1985b) have shown that casein is a valuable

protein source, with soybean meal next best while that of white fishmeal being inferior for *H. discus hannai*. The poorest growth was achieved with the diet containing no fish products at all (MG55) even though it contained protein levels that were similar to the other three aforementioned diets. However, the poor growth seen with Diet MG55 could equally have been due to vegetable oil being used as the lipid source instead of fish oil. Markedly reduced growth rates in grow-out greenlip abalone resulted from diets deficient in fish oils (Dunstan et al., 2000). Uki et al., (1985b) found that a protein concentrate from rye grass was better than a white fishmeal based diet, but all of their diets contained the same fish/vegetable oil mix.

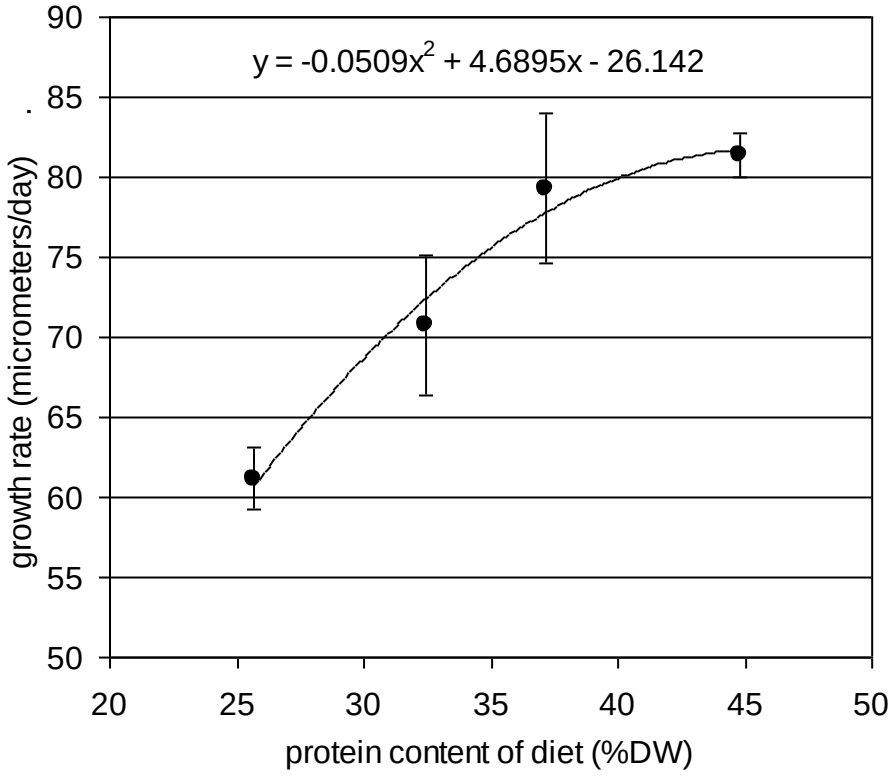


Figure 5.9.4: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed formulated diets MG51 to MG54 with different protein levels.

In conclusion, high protein diets are recommended to improve abalone growth rates. Also both the formulated diatom diet (MG56) and the fish/algal meal diet (MG57) (each with 32% DW protein) produced slightly, but not significantly lower growth rates in abalone than the two high protein diets (with 36% and 44% DW protein, MG53 and MG54 respectively).

5.10 Fatty acid digestibility and assimilation by small abalone fed three different diets

5.10.1 Introduction

Dietary lipids are important nutrients for maximising growth rate and health of the animal. The essential lipid requirements of abalone need to be established. To do this requires knowledge about the digestibility and assimilation of the different classes and sources of lipids. The nutritionally important PUFAs for most animals are both the ω3 and ω6 PUFA. These PUFAs cannot be synthetised *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, but the efficiency of production differs between species, and is typically low in most marine animals. Using isotope mass spectrometry, the sources and fates of fatty acids during metabolism can be estimated.

Previous experimentation (Sections 5.6 to 5.8) examined the nutritional value of feeding abalone on free or flocculated *Nitzschia closterium* diets and compared these to diets formulated to mimic the diatom or to evaluate specific dietary ingredients. The aims of the present work were three fold: to determine which lipids are assimilated by abalone when fed a live diatom; to evaluate a preparation of pasted *N. closterium*; and to measure the fatty acid digestibility and assimilation by abalone fed formulated diets either rich in long-chain PUFA or deficient in these PUFA. The latter aspect was to identify whether varying the source of the fatty acid altered its subsequent assimilation.

5.10.2 Methods

Experiment overview

This experiment formed part of the experiment evaluating diets MG51 to MG57 (protein requirements, Section 5.9). Blacklip abalone were purchased from Tasmanian Abalone Farms P/L, and the feeding experiment was conducted as detailed in Section 5.9.2. The experiment was conducted during the autumn, with a heat exchanger incorporated into the system to maintain a constant temperature of 17°C±0.7.

Diets

Diets MG55 (vegetable ingredient-only diet), MG52 (control diet including fish products) and the pasted *Nitzschia closterium* (supplied fresh every week for 6 weeks, and stored at 4°C) were used in the isotope study of fatty acid assimilation. The formulation of the diets used for the fatty acid digestibility (MG52) and fatty acid assimilation components of the experiment (MG55 and MG52) is detailed in Table 5.10.1. Diet MG52 was used for lipid digestibility determination and contained the digestibility marker, 5α-cholestane. The proximate composition of the formulated diets was calculated from ingredient analysis, product specifications and other literature (see Figure 5.9.1 in Section 5.9). Diets MG55 and MG52 had similar calculated ash, lipid, carbohydrate and protein levels.

Sampling for biochemical analysis (fatty acid assimilation)

Samples of ten abalone per tank were removed at weeks 0, 2, 4, 6 and 10 for biochemical analysis, and photographed for size and growth rate determinations. Gastric emptying time and total food passage time for abalone (fed an alga at 15°C) was found to be 10 h and 44 h respectively (Mai et al., 1998). Therefore, abalone were not fed for two days prior to sampling for biochemical analysis, as gut contents would compromise the analysis. At the time of initial allocation of the 21 groups of abalone to the 21 tanks for the previous (protein) experiment, a 22nd and 23rd group were placed into separate tanks and fed diets MG55 and

MG52 respectively. Abalone from these two groups were tagged and used to replace those removed for fatty acid assimilation determination.

Table 5.10.1: Formulation of diets MG55 and MG52 used for the lipid digestibility and assimilation growth rate experiment

Diet number	MG55	MG52
Description	Plant ingredients only	Fish ingredients included
Soya flour	36.0	25.0
Vegetable oil	4.0	0
Fishmeal	0	9.0
Fish oil	0	3.0
5α-Cholestane	0	0.5
Calcium carbonate	2.0	1.0
Gelatinised maize starch	34.3	37.8
Fixed ingredients[1]	23.7	27.3
Total %	100	100

Included in final diet (%): casein, 10.0; kelp powder, 5.0; sodium alginate, 3.0; vitamin mix, 2.0%; vitamin C, 1.0; vitamin E, 0.01; mineral mix, 1.0; calcium sulphate, 0.8; methionine, 0.3; threonine, 0.3; arginine, 0.3.

Also at the time of initial allocation, the remaining abalone were placed into a 24th tank and fed an imported powdered formulated diet for 4 weeks. This was to change the isotopic signature of the algal-fed abalone (transferred directly from diatom/turf algae plates) to one of a vastly different formulated diet. After the four weeks acclimation on this diet, the abalone were then sorted according to the randomising system described earlier (Section 5.1.1). These were placed into a further three tanks and fed pasted *N. closterium* at the same frequency as the formulated diets. The remaining abalone from the 24th tank were tagged and used to replace those removed from the triplicate tanks fed *N. closterium* for fatty acid assimilation determination. Tagged abalone were not used for growth rate determinations or biochemical analysis.

Growth rate determination

For determination of growth rate during the fatty acid assimilation part of the experiment, all abalone fed MG55 were photographed for shell length measurements at week 0 (commencement of the protein experiment and this treatments' assimilation experiment) and at week 6 (end of protein determination experiment). Abalone fed the pasted *N. closterium* were photographed for shell length measurements at week 4 (commencement of this treatments' assimilation experiment) and at week 10 (its conclusion). Abalone fed the control diet of MG52, were photographed for shell length measurements at weeks 0, 2, 4, 6, 8 and 10. Between weeks 0 and 6, treatments MG55 and MG52 were compared, and between weeks 4 and 10, treatments pasted *N. closterium* and MG52 were compared. Thus all abalone subjected to the treatment diets were grown for a period of 6 weeks.

Lipid and fatty acid apparent digestibility determination

During the last 4 weeks of the experiment (between day 43 and day 71), faecal samples were collected from the tanks of abalone fed diet MG52 (containing the digestibility marker 5α-cholestane), according to the following method. The morning after each feeding, the tanks were siphoned of faeces and uneaten food and solids discarded. On the second morning, faeces were siphoned onto a mesh and the abalone fed again that night. All faeces for the

collection period were combined for each tank and sub-sampled and analysed according to sections 5.1.3.1 & 5.1.3.2. The apparent digestibility coefficients (ADC) of the different lipids were calculated using the following equation:

$$ADC = 100 \times [1-(Ci/Cf) \times (Lf/Li)]$$

where C and L are the respective dry matter concentrations of cholestane and lipid in the ingested diet (i) and faeces (f) (Fleming et al., 1998).

Isotope analysis
GC-IRMS analysis of the FAME of the abalone flesh and diets was performed with a Finnigan MAT delta S mass spectrometer interfaced to an Hewlet packard 5890 Series 2 Gas chromatograph via a cupric oxide/nickel/platinum combustion furnace (940°C) and a copper reduction furnace (640°C). Isotopic calibration used an external CO_2 standard introduced at intervals through the GC run and checked using a standard mixture of deuterated *n*-alkanes. This standard mixture was run after every 5 to 6 analyses. Samples were injected into an on-column injector set at 45°C injecting into a BPX70 SGE polar 50m, 0.33 mm id, and 0.25 um film thickness column using helium for the carrier gas. The oven was ramped to 130°C at 30°C/min and then to 250°C at 2°C/min. The final temperature was held for 15 min. Each sample was injected twice with the analytical error of the $-\delta^{13}C$ values being about 0.2%.

Percentage of the sources contributing to the final fatty acid isotopic $-\delta^{13}C$ ratio was calculated using the equation:

$$A\% = \frac{(-\delta^{13}CX - -\delta^{13}CB) \times 100}{(-\delta^{13}CA - -\delta^{13}CB)}$$

$$B\% = 100-A\%$$

where; "$-\delta^{13}CA$" and "A%" is the isotope ratio and percentage contribution from fatty acid "A" respectively , "$-\delta^{13}CB$" and "B%" is the isotope ratio and percentage contribution from fatty acid "B" in producing fatty acid "X" with an isotopic ratio of "$-\delta^{13}CX$". Where a third possible contribution was identified a "C%" was calculated, and ranges determined by setting each source variable in turn to zero and solving for the other fatty acids contributions.

5.10.3 Results and Discussion

Growth rate
Single factor Analysis of Variance for pairwise comparisons was performed on the growth rate of abalone fed the crumbed diets. This significance was tested using Fisher's PLSD test (Figure 5.10.1). The growth of abalone fed the fish ingredient based diet (MG52) was higher than for the plant ingredient diet (MG55) (Figure 5.10.1), but this difference was not significant (p=0.29). The main reason for including these diets was to examine the assimilation of long-chain PUFA (from the fish ingredient diet), and the assimilation of short-chain PUFA (from the plant ingredient diet). The growth of abalone fed the fish ingredient based diet (MG52) was higher ($p<0.05$) than for the pasted *N. closterium* diet. Formulated diets evaluated against other forms of this microalgae (free and flocculated) produced similar results (Section 5.7). *N. closterium* is one of the main species of microalgae grown for abalone culture. Abalone readily consume and grow well on this species of diatom, but whether presented free, flocculated or pasted, abalone growth rates were inferior to the formulated diets in these trials.

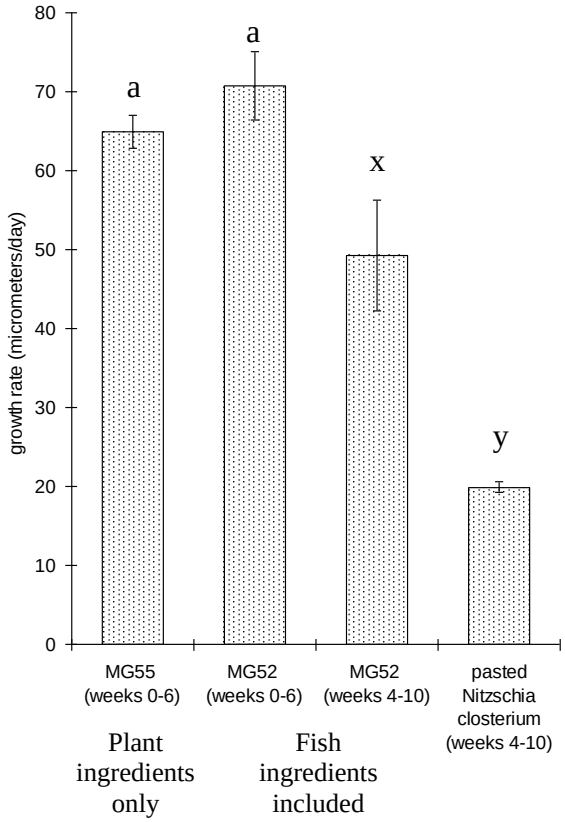


Figure 5.10.1: Average growth rate (micrometres per day ± SEM) of blacklip abalone fed pasted *N. closterium* or formulated diets with different sources of protein and lipid.

Interestingly, the growth rates of abalone fed MG52 during the two six week periods (weeks 0 to 6, and weeks 4 to 10) differed significantly. Generally growth rate on this diet dropped off with time (Figure 5.10.2). Even though a heat exchanger was used, there was a slight decrease in temperature over the period of the experiment (Figure 5.9.2), which may have contributed to this result. Alternatively, after an initial high growth rate on the newly introduced diet (MG52), deficiencies developed due to a lack of appropriate nutrients in the diet.

Fatty acid composition

The fatty acid compositions (as a percentage of total fatty acids) of the pre-diets, diets, and abalone for the three treatments (diets MG52 and MG55 and pasted *N. closterium*) are presented in Tables 5.10.2, 5.10.3, and 5.10.4 respectively. The main points to note are that the pre-diets and diet were intentionally quite different in composition. Also, the fatty acid composition of the abalone generally levelled out to resemble that of the treatment diets after 2 to 6 weeks on each diet.

In the case of the fish ingredient diet (MG52; Table 5.10.2), the high levels of dietary 18:1ω9, 18:2ω6 and 22:6ω3 led to an increase of these fatty acids in the abalone tissue, while the lower levels of 16:0, 20:4ω6 and 22:4ω6, led to a decrease in the proportions of these fatty acids. Interestingly, the proportion of 20:5ω3 remained constant, high and similar to the diet. Also, the proportion of the other main ω3 PUFA in abalone tissue, 22:5ω3, initially decreased at week 2, even though the proportion of this fatty acid in the diet was higher than in the pre-

diet. Thereafter it remained constant. The PUFA to SFA ratio, ω3 to ω6 ratio, proportion of total ω3 PUFA and lipid content of the abalone tissues, all increased on this diet.

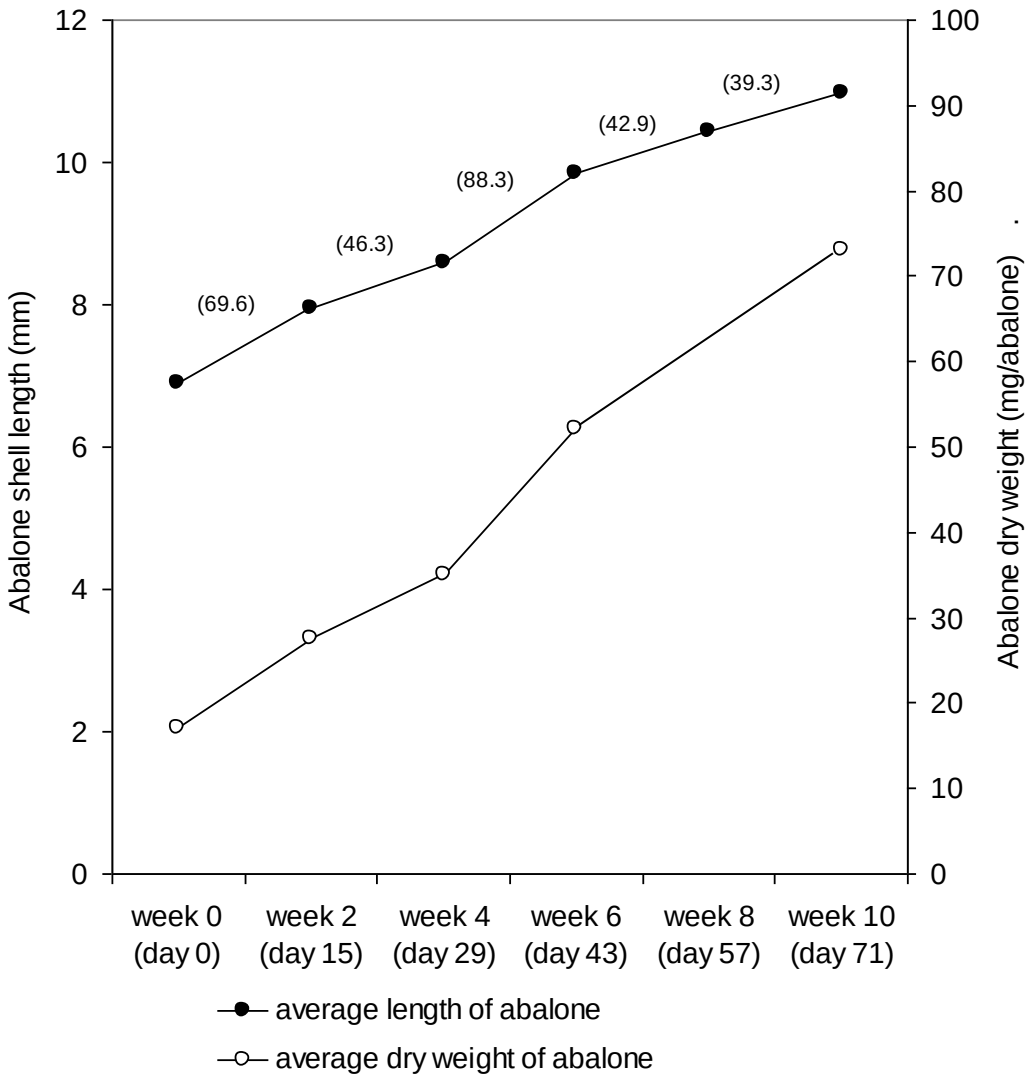


Figure 5.10.2 Average length (mm) and dry weight (mg) of the abalone fed diet MG52 collected for biochemical analysis at each time during the experiment. Numbers in parenthesis indicate calculated growth rate (micrometers per day) between the sampling periods.

In the case of the plant ingredient diet (MG55, Table 5.10.3), the high dietary levels of 18:1ω9 and 18:2ω6 led to an increase in the proportions of these fatty acids in the abalone tissue. The lower level of 16:0 and deficiency of long-chain highly unsaturated PUFA correspondingly led to a decrease in the proportions of these fatty acids in the abalone tissue. The exception was 20:2ω6, which increased, suggesting chain elongation of 18:2ω6, but production of longer chain more highly unsaturated ω6 PUFA was not apparent. The PUFA to SFA ratio, the proportion of total ω6 PUFA and the lipid content of the abalone tissue all increased, while the ω3 to ω6 ratio and the proportion of total ω3 PUFA decreased on this ω6 PUFA-rich diet.

Table 5.10.2: Fatty acid composition and lipid content of pre-diet, diet, whole abalone, and faeces and apparent digestibility coefficients for the long-chain ω3 PUFA-rich fish ingredient diet (MG52)

Sample	Diatoms & algae	Diet MG52	Whole abalone composition					Faeces	Apparent digestibility
Week(s)	Pre-diet	0 to 10	0	2	4	6	10	6 to 10	6 to 10
Saturated fatty acids (SFA)									
14:0	8.1	5.7	1.9	3.5	3.4	3.3	3.1	5.5	**82.3**
16:0	23.9	17.9	19.5	18.3	17.6	17.1	17.1	18.4	**80.7**
18:0	1.9	4.1	7.0	5.4	5.6	4.7	5.7	5.6	**73.8**
20:0	0.8	0.3	0.4	0.1	0.2	0.2	0.1	0.4	**61.4**
total	37.2	29.4	32.0	28.8	28.3	26.8	27.6	32.3	**79.3**
Monounsaturated fatty acids (MUFA)									
16:1ω9	0.9	0.3	1.3	0.9	0.7	0.9	0.8	1.0	**40.0**
16:1ω7	10.6	5.6	1.7	2.8	2.8	2.9	2.4	6.8	**77.5**
16:1ω5	0.8	0.6	1.6	1.2	1.0	1.1	1.1	0.9	**71.1**
18:1ω9	6.6	11.3	2.8	6.1	6.4	6.7	6.2	10.7	**81.5**
18:1ω7	4.0	2.9	8.3	7.4	6.9	6.9	6.6	5.0	**66.7**
20:1ω11	-	0.3	3.8	2.5	2.6	2.8	3.2	1.0	**25.6**
20:1ω9	0.5	2.1	1.2	2.4	2.9	3.5	3.3	2.9	**71.9**
20:1ω7	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	**77.0**
22:1ω11	0.3	0.7	0.5	0.8	0.8	0.5	0.5	1.6	**56.6**
22:1ω9	0.3	1.2	0.9	0.8	0.7	0.6	0.5	0.4	**93.8**
24:1ω9	0.1	0.5	0.2	0.2	0.2	0.2	0.2	0.7	**76.6**
total	26.8	27.6	23.3	26.2	26.1	27.1	25.9	31.5	**78.1**
Polyunsaturated fatty acids (PUFA)									
18:2ω6	3.9	10.2	1.8	5.7	6.2	6.6	5.8	7.0	**86.8**
18:3ω6	1.0	0.3	0.5	0.2	0.2	0.2	0.2	0.5	**63.8**
18:3ω3	6.8	2.0	1.8	1.3	1.3	1.2	1.0	1.4	**86.2**
18:4ω3	4.0	1.8	0.8	0.5	0.5	0.6	0.5	1.2	**87.5**
20:2(5,11)	-	-	0.4	0.6	0.9	0.9	1.0	0.6	*
20:2ω6	0.3	0.2	0.5	0.6	0.7	0.7	0.7	0.5	**54.1**
20:3ω6	0.4	0.2	0.3	0.3	0.2	0.2	0.2	0.3	**69.6**
20:4ω6	3.9	1.1	7.9	3.3	2.4	2.2	2.3	1.4	**76.0**
20:3ω3	0.4	0.2	0.3	0.2	0.2	0.2	0.1	0.3	**60.5**
20:5ω3	6.8	11.5	12.4	12.9	12.7	12.1	12.4	8.6	**85.8**
22:2(7,13)	0.3	-	4.4	3.9	4.2	4.2	5.2	0.7	*
22:4ω6	0.3	0.1	1.6	0.6	0.4	0.4	0.3	0.1	**78.3**
22:5ω6	0.3	0.3	0.7	0.3	0.3	0.3	0.3	0.2	**84.5**
22:5ω3	0.5	1.8	8.3	6.4	6.2	6.3	7.0	1.6	**82.3**
22:6ω3	1.2	9.6	1.5	5.4	6.2	6.7	6.1	7.9	**84.6**
total	32.3	41.5	43.6	43.5	44.1	44.5	44.8	33.3	**84.8**
total ω3	20.8	28.1	25.6	27.6	28.3	28.4	28.5	22.1	**85.2**
total ω6	10.8	13.1	12.9	11.0	10.4	10.6	9.9	9.9	**84.3**
PUFA/SFA	0.9	1.4	1.4	1.5	1.6	1.6	1.6	1.0	
ω3/ω6	1.9	2.2	2.0	2.5	2.7	2.7	2.9	2.2	
% lipid WW	0.6	5.1	1.2	1.0	1.6	1.7	1.4	0.4	
% lipid DW	5.7	5.5	2.9	2.3	3.8	3.7	3.1	2.6	**77.8**

Minor fatty acids are not shown. *fatty acid not found in diet

Table 5.10.3: Fatty acid composition and lipid content of pre-diet, diet and whole abalone for the 18:2ω6-rich plant ingredient diet (MG55)

Sample	Diatom turf algae	Diet MG55	Whole abalone composition			
Week(s)	Pre-diet	0 to 6	0	2	4	6
Saturated fatty acids (SFA)						
14:0	8.1	0.6	1.9	1.5	1.0	1.0
16:0	23.9	10.1	19.5	13.6	10.4	10.6
18:0	1.9	5.4	7.0	5.8	5.9	4.7
20:0	0.8	0.4	0.4	0.2	0.2	0.2
total	37.2	17.8	32.0	22.6	18.7	17.5
Monounsaturated fatty acids (MUFA)						
16:1ω9	0.9	-	1.3	0.7	0.6	0.7
16:1ω7	10.6	0.2	1.7	0.8	0.7	0.8
16:1ω5	0.8	-	1.6	0.8	0.6	0.7
18:1ω9	6.6	17.7	2.8	8.1	10.3	10.2
18:1ω7	4.0	0.9	8.3	5.1	3.1	3.1
20:1ω11	-	-	3.8	1.9	1.9	1.8
20:1ω9	0.5	0.2	1.2	1.6	2.1	2.0
20:1ω7	0.5	0.1	0.3	0.1	0.1	0.1
22:1ω11	0.3	-	0.5	0.3	0.2	0.2
22:1ω9	0.3	-	0.9	0.6	0.3	0.3
24:1ω9	0.1	-	0.2	0.1	-	-
total	26.8	19.3	23.3	20.4	20.3	20.4
Polyunsaturated fatty acids (PUFA)						
18:2ω6	3.9	58.9	1.8	30.2	39.6	40.0
18:3ω6	1.0	0.2	0.5	0.3	0.2	0.2
18:3ω3	6.8	2.0	1.8	2.3	2.1	2.0
18:4ω3	4.0	0.2	0.8	0.2	0.1	0.1
20:2(5,11)	-	-	0.4	0.7	1.0	1.0
20:2ω6	0.3	0.1	0.5	2.2	2.3	2.4
20:3ω6	0.4	-	0.3	0.5	0.4	0.3
20:4ω6	3.9	0.2	7.9	3.9	2.3	2.4
20:3ω3	0.4	-	0.3	0.3	0.2	0.2
20:5ω3	6.8	0.1	12.4	4.9	2.6	2.7
22:2(7,13)	0.3	-	4.4	3.2	3.1	3.3
22:4ω6	0.3	-	1.6	0.5	0.3	0.3
22:5ω6	0.3	-	0.7	0.2	0.1	0.1
22:5ω3	0.5	-	8.3	3.4	2.0	2.0
22:6ω3	1.2	-	1.5	0.5	0.3	0.5
total	32.3	61.9	43.6	55.4	59.2	60.4
total ω3	20.8	2.3	25.6	12.5	8.5	8.7
total ω6	10.8	59.4	12.9	36.7	44.3	45.0
PUFA/SFA ratio	0.9	3.5	1.4	2.4	3.2	3.5
ω3/ω6 ratio	1.9	0.0	2.0	0.3	0.2	0.2
% lipid (WW)	0.6	5.0	1.2	1.7	1.8	1.8
% lipid (DW)	5.7	5.4	2.9	4.0	4.2	3.9

Minor fatty acids are not shown.

Table 5.10.4: Fatty acid composition and lipid content of pre-diet, diet and whole abalone for the pasted *Nitzschia closterium* diet

Sample	Formulated diet	Diet Pasted *Nitzschia closterium*			Whole abalone composition	
Week(s)	Pre-diet	20/4/99	27/4/99	17/5/99	4	10
Saturated fatty acids (SFA)						
14:0	3.9	10.9	10.4	10.2	2.7	4.9
16:0	16.2	11.6	10.4	8.3	16.6	19.3
18:0	3.6	-	0.1	0.1	6.5	5.7
20:0	0.3	0.7	0.5	0.5	0.3	0.2
total	25.2	23.8	22.0	19.7	27.7	32.3
Monounsaturated fatty acids (MUFA)						
16:1ω9	0.2	0.1	-	-	0.8	0.7
16:1ω7	5.4	15.4	17.1	14.4	2.5	4.6
16:1ω5	0.2	1.8	1.2	1.4	0.8	1.6
18:1ω9	12.6	0.2	0.3	0.2	6.2	7.8
18:1ω7	2.9	0.8	0.6	0.4	7.1	0.4
20:1ω11	0.6	-	-	-	3.2	3.0
20:1ω9	0.6	-	-	-	3.1	0.9
22:1ω11	1.7	0.2	0.1	0.2	0.8	0.3
total	29.0	20.0	20.8	18.5	27.0	23.7
Polyunsaturated fatty acids (PUFA)						
16:2ω7	0.4	1.4	1.4	1.4	-	0.2
16:2ω4	0.5	5.6	4.8	5.9	0.1	0.7
16:3ω4	0.4	12.0	13.3	13.7	-	0.3
16:4ω1	-	0.8	0.1	0.1	-	-
18:2ω6	23.7	0.9	1.0	1.1	11.9	2.3
18:3ω6	0.2	1.3	1.1	0.9	0.2	0.3
18:3ω3	3.6	-	-	0.1	2.9	0.5
18:4ω3	1.2	2.8	2.4	2.2	0.5	0.3
20:2(5,11)	-	-	-	-	1.0	0.4
20:2ω6	0.2	-	-	-	1.4	0.4
20:3ω6	0.1	0.1	0.2	0.1	0.6	0.2
20:4ω6	0.6	5.0	7.0	6.1	2.4	7.5
20:3ω3	0.1	-	-	-	0.4	0.1
20:5ω3	6.3	18.7	19.3	23.1	8.3	12.9
22:2(7,13)	-	-	-	-	4.2	4.3
22:4ω6	0.1	0.1	-	-	0.4	0.7
22:5ω6	0.2	1.2	1.3	1.5	0.2	1.3
22:5ω3	1.5	0.1	0.2	0.1	5.2	7.3
22:6ω3	5.8	1.8	1.7	2.0	3.3	2.0
total	44.9	50.6	52.4	56.8	44.2	42.7
total ω3	19.3	35.6	36.9	41.2	21.7	24.3
total ω6	25.4	14.2	15.4	15.5	16.2	13.2
PUFA/SFA ratio	1.8	2.1	2.4	2.9	1.6	1.3
ω3/ω6 ratio	0.8	2.5	2.4	2.6	1.3	1.8
% lipid (WW)	5.0	2.3	1.9	1.9	1.6	1.2
% lipid (DW)	5.3	15.5	13.1	12.9	3.8	2.8

Minor fatty acids are not shown.

In the case of the pasted *N. closterium* (Table 5.10.4), the high dietary levels of 16:1ω7, C_{16} PUFA, 20:4ω6 and 20:5ω3 led to an increase in the proportions of these fatty acids in the abalone tissue. The lower levels of 18:2ω6, 18:3ω3 and 22:6ω3 led to a concomitant decrease in the proportions of these fatty acids. The proportion of 22:5ω3 increased, suggesting chain elongation of dietary 20:5ω3 and/or saturation of stored 22:6ω3 from the pre-diet. The ω3 to ω6 ratio and proportion of total ω3 PUFA increased, while the PUFA to SFA ratio, proportion of total ω6 PUFA and lipid content of the abalone tissues decreased on this diet, commensurate with its composition.

Apparent digestibility

The total lipid apparent digestibility coefficient (for the formulated diet which contained the digestibility marker i.e. MG52) was 77.8 (Table 5.10.2). Of the fatty acids, apparent digestibility was similar for SFA (79.3%) and MUFA (78.1), while PUFA were more digestible (84.8%). The ω3 and ω6 PUFA had comparable digestibility coefficients (85.2% cf 84.3% respectively; Table 5.10.2). The highest apparent digestibility was found in di and triunsaturated C_{16} and C_{18} PUFA and tetraunsaturated C_{18} PUFA (Table 5.10.3). Thus the long-chain fish oil type PUFA (20:5ω3 and 22:6ω3), as well as the shorter chain vegetable oil type PUFA were highly digestible and preferentially digested over saturated and monounsaturated fatty acids in these small blacklip abalone. This was particularly evident in the rapid accumulation of large proportions of 18:2ω6 into the tissues of the abalone fed the formulated diets containing large amounts of this fatty acid (Tables 5.10.2 and 5.10.3). In contrast, previous experiments with grow-out greenlip abalone showed apparent digestibility coefficients to increase with degree of unsaturation (SFA < MUFA < ω6 PUFA < ω3 PUFA; Dunstan et al., 2000). 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 typically ω6 PUFA) in older greenlip abalone but this was not evident in the younger blacklip abalone examined in the present work. Thus, fish oils were recommended over vegetable oils as additives to diets for grow-out abalone (Dunstan et al., 2000), but this may be less important for small abalone.

Table 5.10.5: Apparent digestibility coefficients of fatty acids of differing chain length (number of carbon atoms) and degree of unsaturation (number of double bonds) by small blacklip abalone from diet MG52. Major fatty acids in the formulated diet are shown in bold.

Number of double bonds	0	1	2-3	4	5-6
Chain length					
14	82.3				
16	**80.7**	**75.2**	90.9		
18	**73.8**	**80.9**	**86.2**	87.5	
20	61.4	67.5	42.5	76.0	**85.8**
22	59.4	79.7		78.3	**84.2**
24	48.1	76.6			

Fatty acid assimilation

Data for the stable carbon isotope (-δ13) values for fatty acids from the pre-diets, diets and whole abalone fed the treatment diets for the six week period of this study are shown in Table

5.10.6. Abalone fed diets MG52 (fish ingredient-rich) and MG55 (plant ingredient-rich) had been taken directly from settlement plates upon which they had been feeding on a relatively isotopically heavy (less negative -δ13 value) pre-diet of diatoms and turf algae. They were then fed the isotopically lighter formulated diets for six weeks, during which time their initially "heavy" fatty acids at week 0, became progressively lighter due to assimilation of dietary fatty acids. Abalone fed pasted *N. closterium* as a treatment diet, were initially fed an isotopically light pre-diet for four weeks prior to sampling. Thus they were initially isotopically light at week 4. After feeding with the isotopically heavier diatom, their fatty acids became progressively heavier by week 10 due to assimilation of dietary fatty acids.

Table 5.10.6: Stable carbon isotope (-δ13) values for fatty acids from the pre-diets, diets and whole abalone fed the treatment diets

Treatment diet	MG52 and MG55	MG52			MG55			*Nitzschia closterium*			
Sample	Pre-diet	**Diet**	Whole abalone		**Diet**	Whole abalone		Pre-diet	**Diet**	Whole abalone	
Week(s)	<0	0-6	0	6	0-6	0	6	0-4	4-10	4	10
Saturated, monounsaturated fatty acids and derivatives											
14:0	-21.2	**-24.9**	-21.4	-23.5		-21.4	-21.8	-24.6	**-23.1**	-27.0	-18.6
16:0	-22.6	**-26.4**	-21.7	-24.9	**-29.0**	-21.7	-27.3	-26.9	**-20.8**	-26.2	-21.1
16:1's	-20.7	**-23.6**		-24.1			-22.2	-23.4	**-22.1**	-21.6	-15.8
18:0	-23.4	**-25.0**	-20.7	-24.6	**-27.6**	-20.7	-27.8	-26.2		-26.2	-21.9
18:1ω9	-22.3	**-25.3**	-21.0	-26.0	**-27.6**	-21.0	-28.3	-26.3		-24.4	-20.1
20:1's		**-25.2**	-20.6	-25.0		-20.6	-28.1	-24.2		-27.5	-23.5
20:2(5,11)				-22.3			-27.2			-26.2	
22:2(7,13)			-20.9	-26.7		-20.9	-28.2			-26.0	-24.7
Omega 6 polyunsaturated fatty acids											
18:2ω6	-22.8	**-27.8**	-22.7	-28.8	**-30.0**	-22.7	-29.6	-30.9	**-18.8**	-30.4	-24.3
20:4ω6	-24.9	**-23.3**	-20.5	-25.1		-20.5	-27.1	-23.4	**-25.3**	-24.9	-21.4
Omega 3 polyunsaturated fatty acids											
18:3ω3	-25.6	**-28.7**	-25.1	-27.2	**-26.5**	-25.1	-28.5	-30.8		-30.7	-22.5
18:4ω3	-25.2	**-25.5**						-25.0	**-25.2**		
20:5ω3	-27.2	**-27.2**	-23.8	-27.5		-23.8	-29.5	-25.1	**-26.6**	-26.8	-23.1
22:5ω3		**-26.0**	-22.5	-28.0		-22.5	-25.4	-24.0		-24.9	-26.1
22:6ω3		**-27.7**	-23.1	-28.9		-23.1	-26.4	-24.8	**-23.9**	-24.3	-21.9

Assimilation was determined as a calculation of the percentage of the sources contributing to the final fatty acid isotopic -δ^{13}C ratio. By such calculations, percentage contribution of each fatty acid from the diet could be determined relative to that which already was present in the abalone tissue. This was complicated by the fact that not all of a particular fatty acid will be assimilated intact. Some may be chain elongated and/or desaturated (Figure 5.10.3), resulting in a complex relationship of sources and sinks within the abalone tissue.

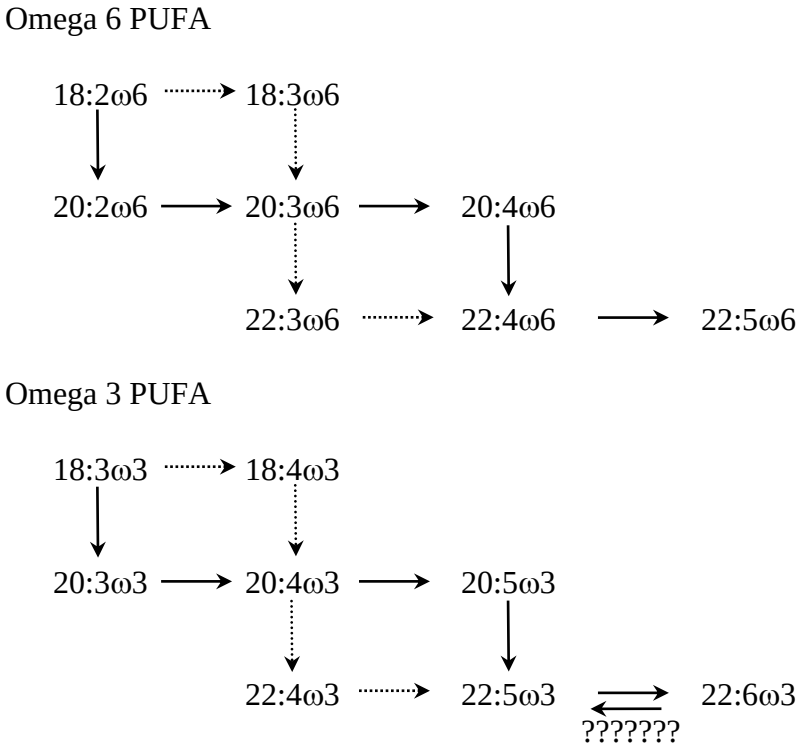


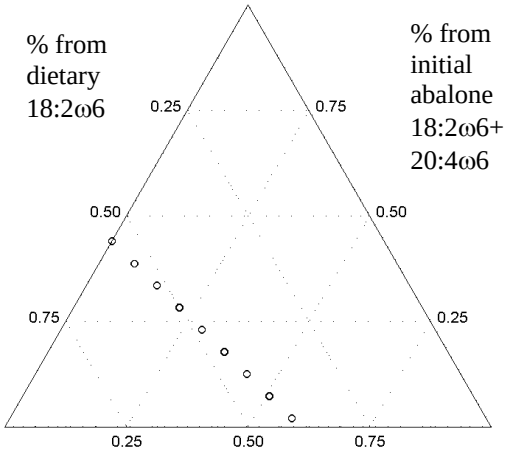
Figure 5.10.3 Most likely paths (solid arrows) for chain elongation (addition of two carbons from acetate units, shown as downward pointing arrows in figure) and desaturation (addition of a methylene interrupted double bond between carbons, shown as right pointing arrows in figure) of short-chain (18 carbon) PUFA into long-chain (20 and 22 carbon) PUFA by abalone. Retroconversion of 22:6ω3 to 22:5ω3 is identified by the arrow with question marks.

Each fatty acid making up the final composition of the abalone can originate from four main sources: a) original component within the abalone tissue; b) directly incorporated from the diet unchanged; c) precursor fatty acids from the original abalone tissue; and/or d) precursor fatty acids from the diet which are chain elongated and/or desaturated. As not all fatty acids within the diet and/or tissues were distinguishable isotopically, $-\delta^{13}C$ ratios for such fatty acids were averaged to gain an estimate of contributions from various pooled sources. Where three possible fatty acid sources existed, a simple method of determining a range of values for the percentage contribution from these sources was used and graphical examples of these are shown in Figure 5.10.4 and 5.10.5. The line of circles on these ternary diagrams, represent the range of possible percentage contributions from the three fatty acids, which make up the axes. All the most likely sources and sinks using these methods are shown in Figures 5.10.6, 5.10.7 and 5.10.8 for the fish ingredients diet (MG52), plant ingredients diet (MG55) and the pasted *N. closterium* diet respectively.

As it is thought that abalone, in common with most other marine animals, can not produce 18:2ω6 *de novo*, the large increase in 18:2ω6 observed with this diet (Table 5.10.2) was due to dietary 18:2ω6. Thus the final tissue 18:2ω6 source was identified as 100% from the diet (Figure 5.10.6). In Figures 5.10.4a and 5.10.6, the three possible origins of the final tissue 20:4ω6 from the diet containing fish ingredients (MG52) were the ω6 PUFA from the abalone at the start of the experiment (18:2ω6 and 20:4ω6), dietary 20:4ω6 and dietary 18:2ω6 which had been elongated and desaturated by the abalone after digestion. Using the ternary diagram, 39 to 56% of the final abalone tissue 20:4ω6 originated from dietary 18:2ω6. Similarly when

the abalone were fed a diet rich in 18:2ω6 but lacking dietary 20:4ω6 (ie the plant based MG55), 66% of the final tissue 20:4ω6 and 95% of the final tissue 18:2ω6 were calculated to have originated from dietary 18:2ω6 (Figure 5.10.7). Abalone fed the live pasted *N. closterium* diet that was low in 18:2ω6 but relatively rich in 20:4ω6, showed that dietary 18:2ω6 still contributed approximately 58% of the final tissue 20:4ω6, the other 42% from initial and dietary 20:4ω6 (Figure 5.10.8). Interestingly with this low 18:2ω6 diet, the amount of tissue 18:2ω6 actually decreased relative to the original amounts present in the abalone. Thus 18:2ω6 was not as important a component of abalone tissue as the 20:4ω6 that was formed from it. The data suggest that the isotopically lighter (more negative) fraction of 18:2ω6 in the original abalone tissue was used for energy and/or to form final tissue 20:4ω6 (Figure 5.10.8). This is because the final tissue 18:2ω6 -δ^{13}C ratio was lower than that of the original tissue 18:2ω6 even though the amount of tissue 18:2ω6 actually decreased.

a); Possible sources of 20:4ω6 in abalone



b); Possible sources of 22:5ω3 in abalone

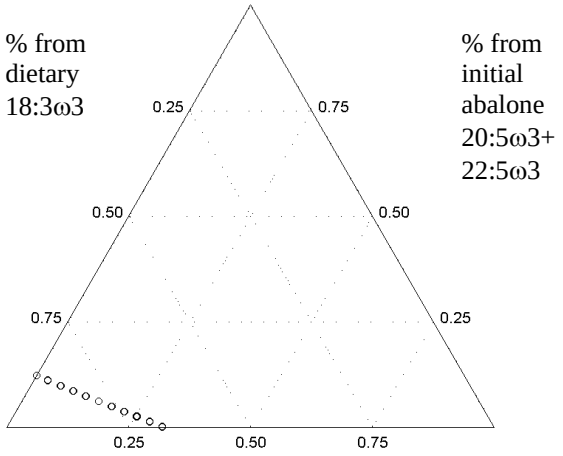


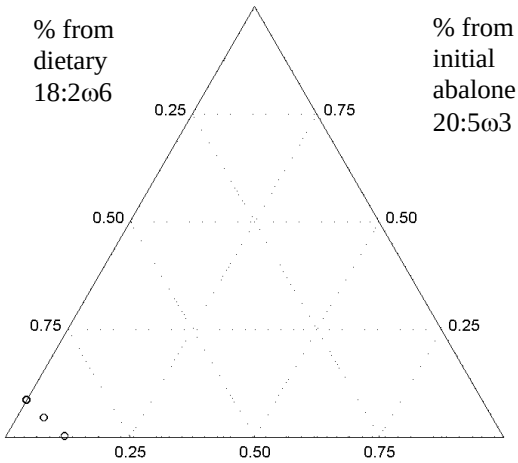
Figure 5.10.4: Ternary diagrams showing the range of percentage contributions to various PUFA in the abalone tissue from three PUFA sources for diet MG 56 (fish ingredients). Where -δ13 values were indistinguishable values for some fatty acid sources were pooled.

Therefore the results of all three dietary treatments indicated that 18:2ω6 was a major and readily convertible precursor of 20:4ω6 in abalone tissue. As 20:4ω6 is one of the main PUFA in abalone tissue and in turn, is a precursor for the prostaglandins and leucotrienes, which control a variety of physiological functions in animals, it is very important that the requirement for this fatty acid be met. Formulated diets used for abalone culture already contain large amounts of 18:2ω6 (Section 5.3), and thus a deficiency of this fatty acid is unlikely.

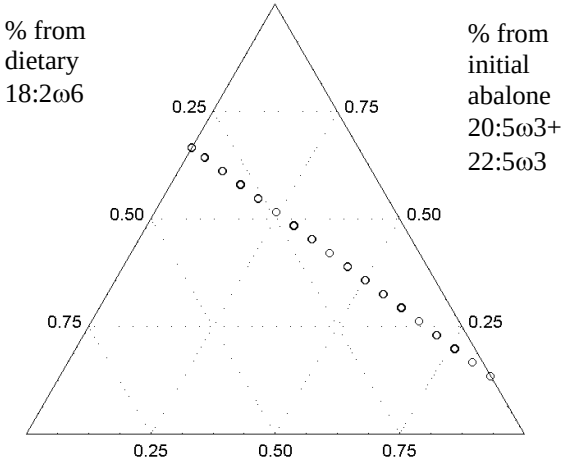
In the abalone fed the diet containing fish products (MG52), most of the 18:3ω3 and 20:5ω3 probably originated from dietary ω3 PUFA (59% 18:3ω3, and 88% 18:3ω3 and 20:5ω3 respectively, Figure 5.10.6); the residual being from the original abalone tissue. Similarly, most of the 22:5ω3 (Figure 5.10.4b) and 22:6ω3 in the abalone probably originated from

dietary ω3 PUFA, the -δ^{13}C ratios suggesting that most was derived from newly acquired ω3 PUFA originating from dietary 18:3ω3 (Figure 5.10.6).

a); Possible sources of 20:5ω3 in abalone



b); Possible sources of 22:5ω3 in abalone



c); Possible sources of 22:6ω3 in abalone

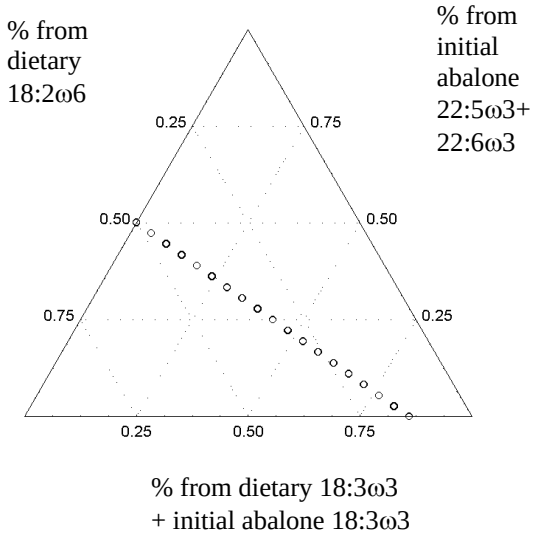


Figure 5.10.5: Ternary diagrams showing the range of percentage contributions to various PUFA in the abalone tissue from three PUFA sources for diet MG 51 (plant ingredients). Where -δ13 values were indistinguishable values for some fatty acid sources were pooled.

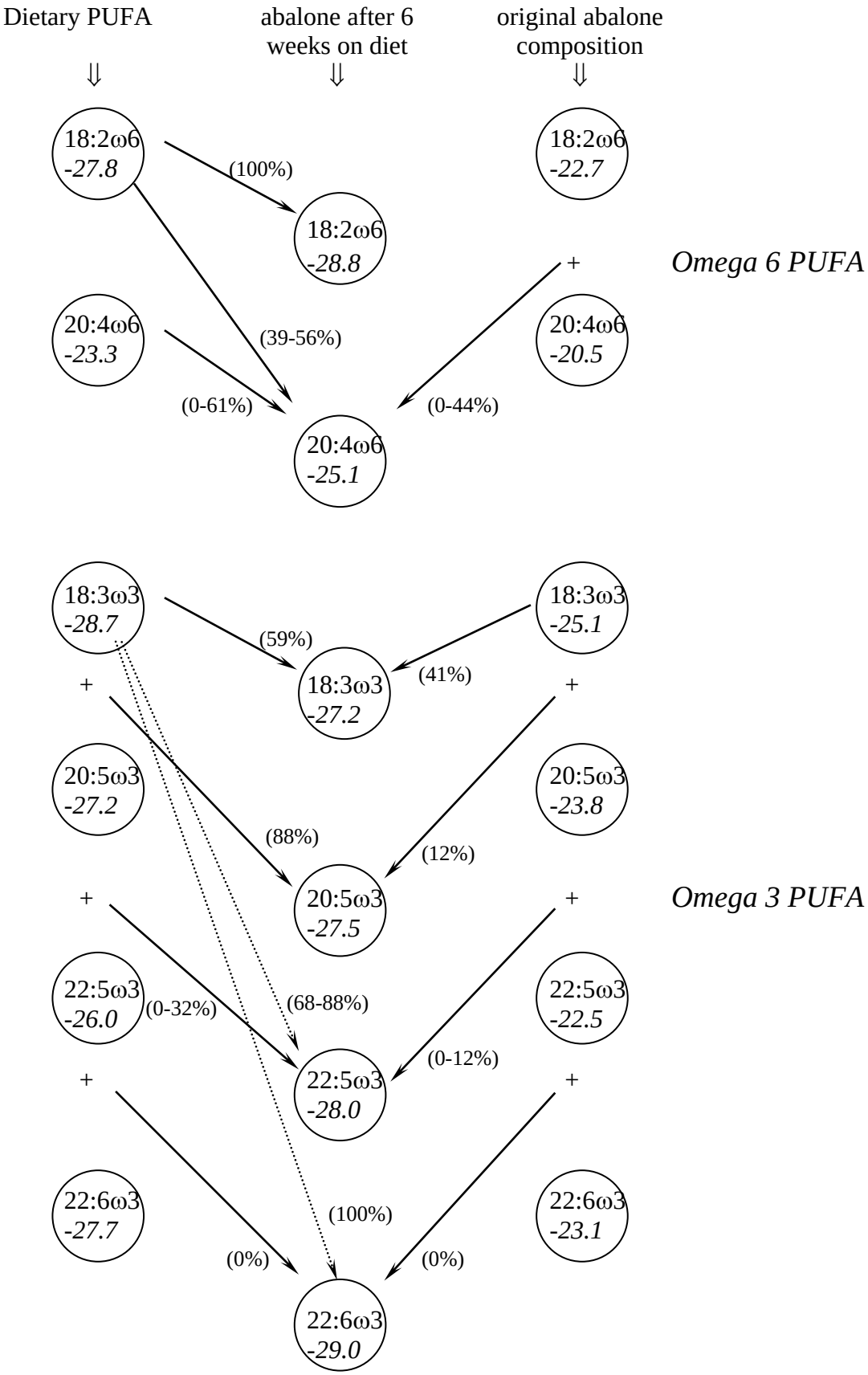


Figure 5.10.6: Flow diagram representing possible PUFA sources (dietary and original composition) and estimates of percentage contribution (calculated from the -$\delta^{13}C$ ratios shown), which resulted in the final abalone composition after being fed diet MG52 (fish ingredients) for six weeks.

The formation of long-chain fatty acids from short-chain fatty acids (Figure 5.10.3) requires the addition of acetyl Co-A, a two-carbon molecule which can originate from the break down of other fatty acids or from other sources within the body. Thus the long-chain ω3 PUFA formed from short-chain ω3 PUFA will include some carbon from another source which is likely to have a different -δ^{13}C ratio. It is generally considered that marine animals cannot produce ω3 PUFA from ω6 PUFA. Photosynthetic plants, a few strains of bacteria and some yeast-like organisms have been shown to do this. But, results for ω3 PUFA formation in abalone fed both the long-chain PUFA-deficient MG55 (plant ingredient) diet (Figure 5.10.7) and the pasted *N. closterium* (Figure 5.10.8) suggest that some of the long-chain ω3 PUFA was derived from 18:2ω6. It is more likely that during chain elongation acetyl Co-A (derived from the breakdown of 18:2ω6 or other carbon source with a light -δ^{13}C ratio) had been added to a pre-existing ω3 PUFA, such as 18:3ω3 (both diets) and 20:5ω3 (pasted *N. closterium* diet) thus affecting the total -δ^{13}C ratio of the longer chain PUFA. However, this conclusion was not supported by the finding that the final 18:3ω3 in the abalone tissue also became isotopically lighter (more negative) when fed the plant ingredient diet than either of the 18:3ω3 sources (Figure 5.10.7). As no carbon is added during the acquisition of 18:3ω3, the -δ^{13}C ratio would be expected to be intermediate between both sources (dietary and inherent 18:3ω3). Therefore it must be concluded that either the 18:3ω3 was being formed from dietary 18:2ω6 (isotopically lighter), which is thought to be unlikely for animals, or the heavier isotope components of 18:3ω3 were used for the elongation and desaturation products ie the long-chain ω3 PUFA.

The final 18:3ω3 in the abalone tissue became isotopically heavier (more positive) when fed the pasted *N. closterium* diet than the main 18:3ω3 source (original 18:3ω3) (Figure 5.10.8) suggesting a similar conclusion as above for the plant ingredient diet. That is, that the 18:3ω3 was being formed from dietary 18:2ω6 (isotopically heavier). But as there was less 18:3ω3 (μg/animal) in the final abalone tissue than originally present, this suggests rather that the opposite was the case for this diet ie the isotopically lighter fraction of 18:3ω3 was used for energy and/or as a precursor for long-chain ω3 PUFA. Likewise there was a loss of 22:6ω3 from the abalone on this diet, the final 22:6ω3 in the abalone tissue became isotopically heavier (more positive) relative to possible sources, also suggesting that the isotopically lighter fraction of 22:6ω3 was used for energy and/or possibly as a precursor of 22:5ω3 (Figure 5.10.3). Because these two ω3 PUFA (18:3ω3 and 22:6ω3) were quantitatively reduced, while 20:5ω3 and 22:5ω3 were quantitatively increased at their expense, this suggests that the former two fatty acids were used to produce the latter two and that the latter two ω3 PUFA are more important for normal abalone functions.

Excluding the case above for 22:6ω3 where there was a loss of this fatty acid from abalone fed the pasted *N. closterium* diet, the isotope ratios of the C_{22} carbon ω3 PUFA (22:5ω3 and 22:6ω3) appear different to the C_{18} (18:3ω3) and C_{20} (20:5ω3) carbon ω3 PUFA in the final abalone compositions (Table 5.10.6). The C_{22} carbon ω3 PUFA of abalone fed the relatively isotopically light fish ingredient diet (MG52) were also slightly isotopically lighter than the C_{18} and C_{20} carbon ω3 PUFA in these abalone. The C_{22} carbon ω3 PUFA of abalone fed the relatively isotopically light plant ingredient diet (MG55) were isotopically heavier than the C_{18} and C_{20} carbon ω3 PUFA in thse abalone. The 22:5ω3 of abalone fed the relatively isotopically heavy pasted *N. closterium* diet was slightly isotopically lighter than the C_{18} and C_{20} carbon ω3 PUFA in these abalone. These trends may possibly be due to the acetyl Co-A from 18:2ω6 or similar as indicated by the dashed lines in Figures 5.10.7 and 5.10.8.

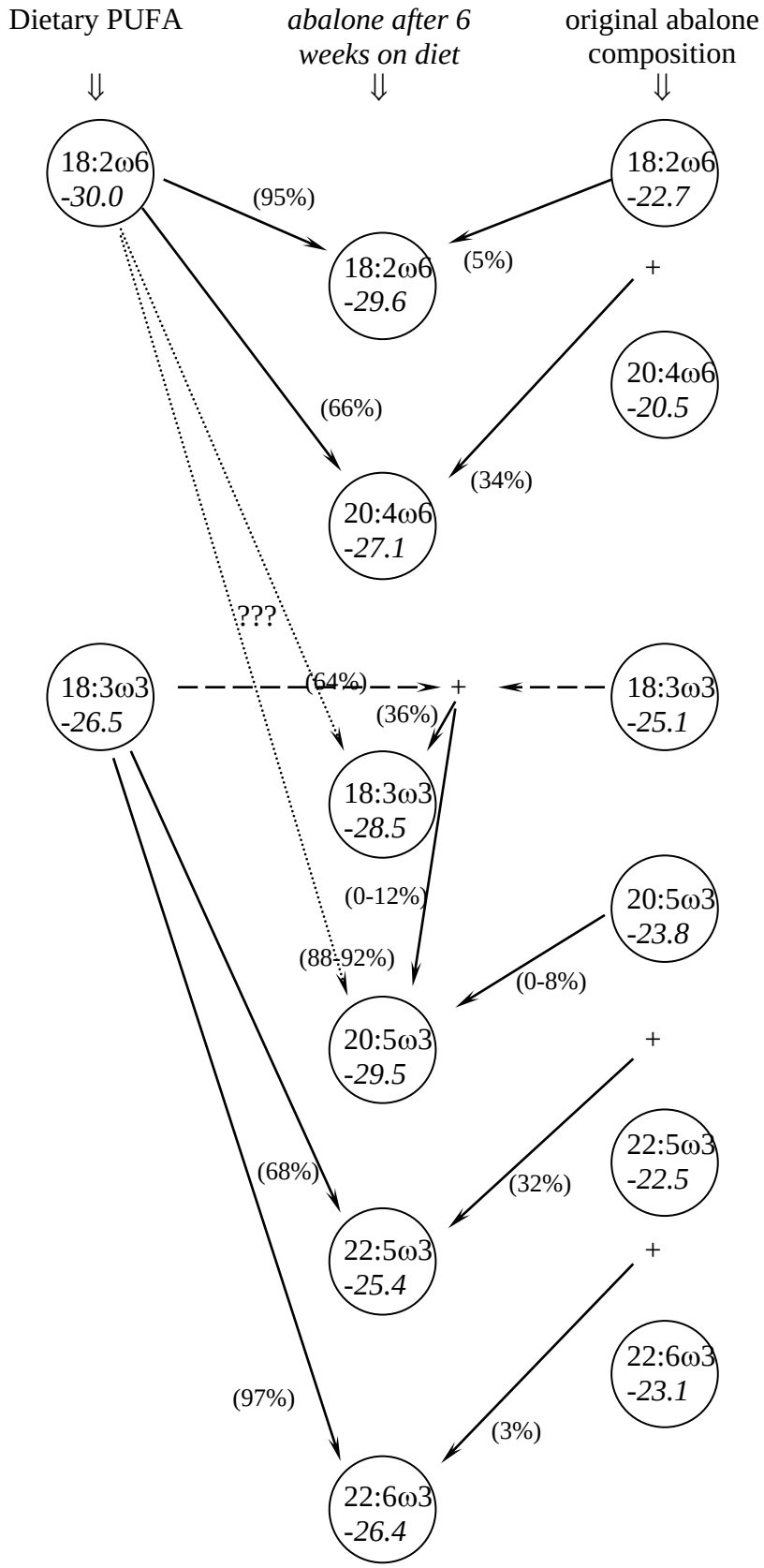


Figure 5.10.7: Flow diagram representing possible PUFA sources (dietary and original composition) and estimates of percentage contribution (calculated from the -δ^{13}C ratios shown), which resulted in the final abalone composition after being fed diet MG55 (plant ingredients only) for six weeks.

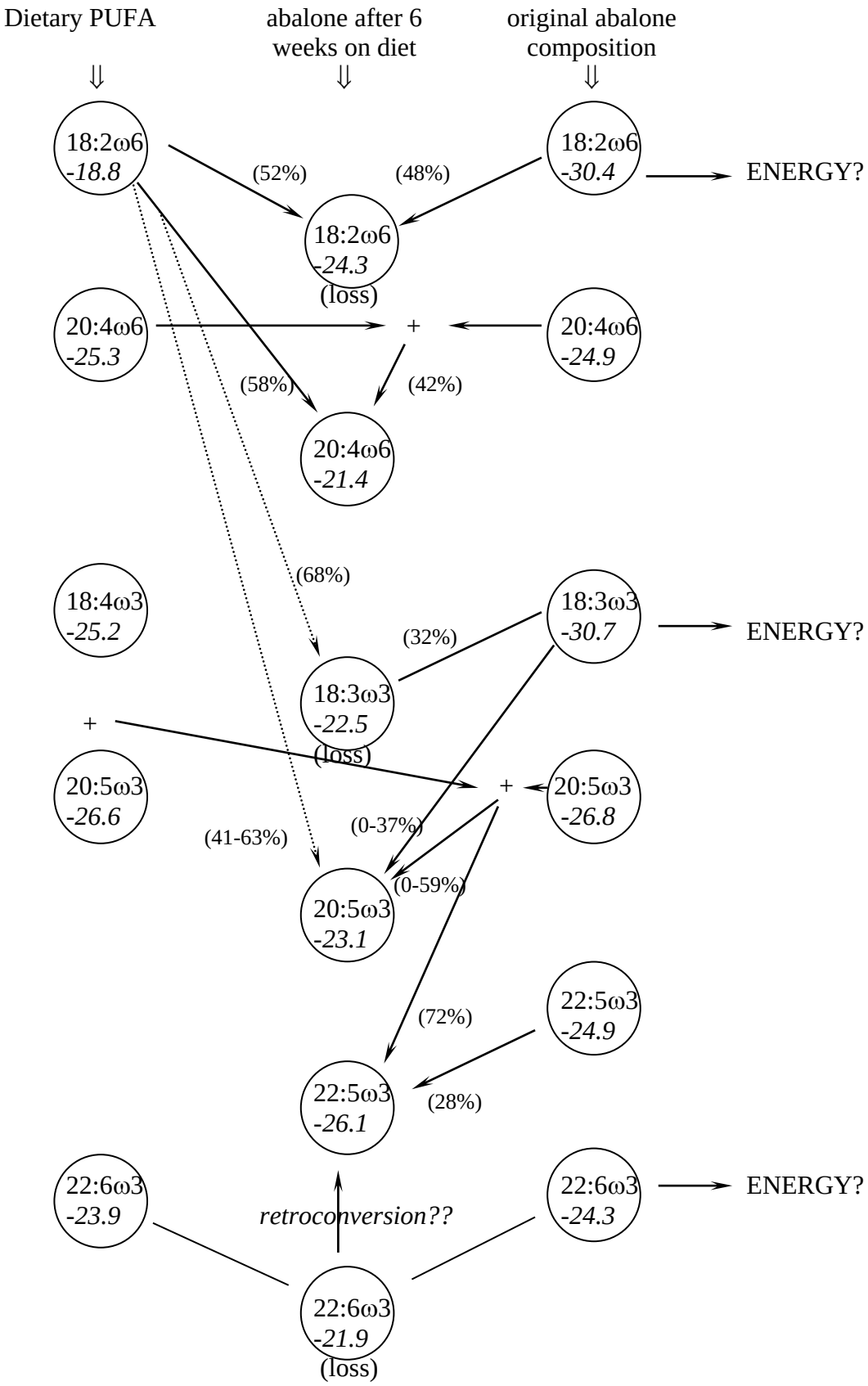


Figure 5.10.8: Flow diagram representing possible PUFA sources (dietary and original composition) and estimates of percentage contribution (calculated from the -δ^{13}C ratios shown), which resulted in the final abalone composition after being fed pasted *Nitzschia closterium* for six weeks.

In conclusion, the primary source of ω6 and ω3 PUFA in the abalone tissue was derived from dietary sources of these PUFA. In abalone, the apparent digestibility of lipid was highest for PUFA, and then monounsaturated and saturated fatty acids. Lipid assimilation studies using stable isotope mass spectrometry demonstrated that abalone were able to synthetise long-chain ω6 and ω3 PUFA by chain elongation and desaturation of other PUFA. However, rates of synthesis did not meet the animal's requirements for maximum growth when abalone were fed on a plant ingredient diet deficient in these PUFA. In the context of dietary essentiality, 20:5ω3 was more important than 20:4ω6. Important also was 22:5ω3, but whether it was being synthetised by retroconversion of 22:6ω3 could not be established.

6. BENEFITS

It was suspected that small abalone (i.e. those <15 mm) require a markedly different dietary specifications to those required by larger, grow-out abalone (i.e. those >15 mm). This may explain why existing formulated feeds for grow-out abalone have proved less than satisfactory alternatives to cultured diatoms for small abalone. The research has developed several formulations that appear under experimental conditions to support growth of small abalone that is equal if not superior to that achieved with cultured diatoms. Studies on the apparent digestibility of different fatty acids, their sources and metabolic fates, have advanced our knowledge on the essentiality of fatty acids for small abalone. These advances offer excellent opportunities for commercial diets to be developed tailored to the needs of small abalone.

Abalone farmers will be the main beneficiaries of the research, but feed manufacturers should also gain considerably from the research. Using formulated diets as the primary food source for small abalone would be a significant benefit to the abalone farmer since it would reduce the bottleneck of diatom culture and associated infrastructure costs. Formulating diets that are more suited to the nutritional requirements of small abalone will provide another feed product for feed manufacturers to market. Whether or not these benefits will be realised will depend on a number of factors. Firstly, it must be acknowledged that the results were generated under controlled experimental conditions. Clearly, the findings need to be validated in large-scale studies under industry conditions. If confirmed, the research would then need to be commercialised for the benefit to be realised. For this to happen, either feed manufacturers must be prepared to initiate such confirmatory studies on their own accord and at their risk or alternatively the abalone farmers themselves must commission feed manufacturers to make these diets for their own commercial evaluation. It is hoped that publication of the findings of this project will provide the necessary impetus for feed manufacturers to embark on work that ultimately will lead to full commercialisation of the outputs of this research.

7. FURTHER DEVELOPMENT

This Project developed and evaluated formulated diets for small (<15 mm) juvenile abalone based on the chemical composition of their natural diatom diet. Growth rate of small abalone was maximized by feeding diets that contained at least 36% DW protein (when soya flour, casein and fishmeal were together used as the main protein sources) and when a sugar mix comprising 6% glucose, 0.5% mannose and 2.5% xylose or 3% oyster glycogen of DW ingredients was included. A diet based predominantly on fishmeal and algal meal produced high growth rates in small abalone that were comparable to a diet that mimicked the natural diatom. Diets based primarily on vegetable products reduced abalone growth rate compared to those based on fish products.

Observations from the current work and recommendations to improve diet formulations for small (<15 mm) blacklip abalone are summarized below:

- Small abalone grew faster with high protein diets (>36% DW) and benefited from the inclusion of a ‘sugar mix’ (6% glucose, 0.5% mannose and 2.5% xylose DW);
- The amount of oil added to the diet did not significantly affect growth rate during colder months, provided fishmeal (supplying some essential PUFA) was present;
- Oils rich in 20:4ω6, 20:5ω3 and 22:5ω3 were preferentially assimilated or converted from dietary PUFA. Diets should contain these PUFAs or their precursors;
- The amount of total carbohydrate in the diet should be <60% DW; and

- Small blacklip abalone fed on appropriately formulated diets grew at rates equal, or superior, to those fed on mass cultured, pasted or flocculated diatoms.

Based on the findings from this project, it is recommended that further work be performed to address two aspects. Firstly, to validate the experimental findings of this project by carrying out larger scale, industry studies to compare formulated diets against mass cultured diatoms. Formulations that are recommended for comparative study are detailed in Table 7.1.

Table 7.1 Formulation (% DW) of diets that produced high growth rates in small abalone in this project

Ingredient	Diet code and description			
	MG53 (34% soya)	MG54 (48% soya)	MG56 (diatom)	MG57 (Fish/algal)
Soya flour	34.0	48.0	25.0	0
Fishmeal	9.0	9.0	9.0	20.0
Fish oil	3.0	3.0	3.0	1.9
Glucose	0	0	6.0	0
Mannose	0	0	0.5	0
Xylose	0	0	2.5	0
Semolina	0	0	0	30.0
Diatomaceous earth	0	0	4.3	0
Kelp powder	5.0	5.0	5.0	16.0
Maize starch	29.3	15.3	25.0	12.9
Calcium carbonate	1.0	1.0	1.0	0.5
Fixed ingredients[1]	18.7	18.7	18.7	18.7
Total	100	100	100	100

[1] Included in final diet (%): casein, 10.0; sodium alginate, 3.0; vitamin mix, 2.0; vitamin C, 1.0; vitamin E, 0.01; mineral mix, 1.0; calcium sulphate, 0.8; methionine, 0.3; threonine, 0.3; and arginine, 0.3.

Secondly, further research is needed to understand the role, and to optimise the composition, of the 'sugar mix'. The current work showed that a sugar mix of 6% glucose, 0.5% mannose and 2.5% xylose had a significant growth stimulatory effect, particularly when used with high protein diets. Substitution of the above sugar mix with an equivalent amount of sucrose or complex carbohydrate (eg pre-gelatinised maize starch) was comparatively ineffective. Clearly, more research is needed to better understand the dietary importance of these simple sugars and other non-structural carbohydrates which until the current study have been relatively ignored for Australian abalone. The usefulness of a sugar mix in diets for larger (>15 mm) abalone also warrants investigation.

Since formulated diets developed in this project were shown to produce superior abalone growth to mass cultured, pasted and flocculated diatom diets, it is suggested that formulated diet research for small (<15 mm) abalone should be a higher priority than research on alternative diatom feed delivery systems.

8. PLANNED OUTCOMES

The planned outcome of the research carried out in this project was to provide hatcheries with an alternative to using cultured diatoms as the only food source for rearing small (<15 mm) abalone.

The approach was to formulate a diet that mimicked the chemical composition of diatoms on which these small abalone naturally feed and then to test this diet and other formulated diets as alternatives to a diet of cultured diatoms. During the course of the project, 57 diets were formulated from food ingredients sourced from provender millers or other commercial sources and 28 of these diets were subsequently evaluated by growth assay as possible alternatives to diatoms for small abalone. Four formulated diets (2 high protein, 1 fish/algal meal and 1 'formulated diatom diet') produced high growth rates (75 to 80 μm per day at 17ºC) in very young abalone (6.7±0.17 mm mean shell length). Crumbed diets (approximately 2 mm cube) were generally better than powder diets (<0.75 mm particle size) for young blacklip abalone but the reverse was the case for greenlip abalone.

In order to understand the importance of fatty acids in diets for small abalone, the apparent digestibility of the different classes of fatty acids was determined and their metabolic fate traced using stable isotope mass spectrometry. Saturated and monounsaturated fatty acids were less digestible than PUFA but plant PUFA (predominantly ω6 fatty acids) were equally as well digested as fish PUFA (predominantly ω3 fatty acids). Most dietary PUFA was found to end up in the abalone tissue as 20:4ω6, 20:5ω3 and 22:5ω3 after chain elongation and desaturation of dietary precursors or preferential assimilation of these fatty acids. However, the amount of oil added to the diet was much less important than that found previously for larger (>15 mm) greenlip abalone.

This research has identified a number of diet formulations that are worthy of large scale, industry studies to validate their efficacy compared to the existing rearing protocol of using mass cultured diatoms. Suggestions for further research to advance diet formulation for small abalone have also been made in Section 7.

9. CONCLUSIONS

Objective 1: Use information on the nutritional and attractant factors present in natural food items to develop formulated diets for very young juvenile abalone (<15 mm).

Findings: Natural abalone food items were characterized (Section 5.2) by:
- Having high amounts of 20:4ω6 and 20:5ω3, and of glucose, xylose and fructose; and
- Having low amounts of 18:2ω6 and 22:6ω3.

Commercial abalone diets were markedly different in composition to diatoms, and were characterized (Section 5.3) by:
- Having high amounts of 18:2ω6 and moderate amounts of 20:5ω3 and 22:5ω3; and
- Having low amounts of 20:4ω6.

Based on the compositional data of natural diatoms, a nutritional specification for a formulated diet that would mimic diatoms was developed for young abalone.

Objective 2: Manufacture and evaluate the diets for water stability and palatability for various diet delivery mechanisms, including gels, pellets, pastes and adhesion diets.

Findings: Feed ingredients of reputed attractiveness to abalone were included in a series of diets, which were manufactured as powders, crumbs or barrel noodles and subjected to appraisal by industry and in a controlled preference experiment (Section 5.4). A poor return of the evaluation questionnaire from industry participants diminished the usefulness of this activity although some preference for diets containing fructose, and to a lesser extent glucodeltalactone and aniseed oil was noted. Controlled preference testing of the diets indicated fructose and aniseed oil diets to be slightly more preferred by abalone. Attempts at attaching diets to existing diatom plates as a thin film using different binders (high and low viscosity alginic acid, gum arabic, guar gum) proved unsuccessful (Section 5.5). Irrespective of the binder used, all diets became unattached within 1 h of immersion.

Objective 3: Produce formulated diets of high nutritional value, which produce high growth rate in very young abalone (<15 mm) as verified by growth experiments.

Findings: Four growth assay experiments were carried out to examine the growth efficacy of formulated diets containing simple (Section 5.6) and complex (Section 5.8) carbohydrates, and alternative lipid sources (Section 5.7) and diets that sought to optimise the dietary protein/carbohydrate content (Section 5.9). Diets were compared against feeding a cultured diatom (commercial ingredients vs *Nitzschia closterium*, section 5.7) or a diet that mimicked the chemical composition of diatoms (Sections 5.6, 5.8, 5.9). Main findings from these experiments were:

- Formulated diatom diet and fish/algal meal diets increased abalone growth rate, relative to a maize starch based diet with comparable proximate composition.
- Abalone grew slower on mass cultured free, flocculated or pasted *N. closterium* compared to the experimental formulated diets
- When fishmeal was present in the diet, addition of fish oil (0, 3,or 8%) did not affect small blacklip abalone growth rate
- Wheat flour and semolina based diets slightly increased abalone growth rate relative to maize starch based diets
- When used in low protein diets a “sugar mix” (6% glucose, 0.5% mannose and 2.5% xylose) produced higher growth rates than when substituted with 9% glucose, 9% sucrose or 9% maize starch
- Optimum growth rates were evident in abalone fed high protein diets (≥ 36% DW)
- Diets containing fish ingredients increased abalone growth rate relative to vegetable-ingredient based-diets
- Diatomaceous earth (7%) in the formulated diatom diet did not affect growth rate

Objective 4: Provide information to the FRDC Abalone sub-program, so as to improve the existing formulated diet used for the “grow out” phase (>15 mm).

Advice: The present work showed that small (<15 mm) abalone require formulated diets to be high in protein (> 36% DW) and that a lipid content of up to 8% was well tolerated. By comparison, previous studies with grow-out (>15 mm) abalone found that the dietary lipid content should not exceed 4%. The marked differences in dietary specifications preferred by small and grow-out size abalone are a likely consequence of the natural feeding transition of the animals from diatoms to macro-algae that occurs at this time. Recognizing the differences between small and grow-out size abalone in their preferred dietary specification is an important outcome of the present work. However, the growth stimulatory effect of including simple carbohydrates in the diet formulation as observed in the current project with small abalone warrants investigation with grow-out size abalone.

Objective 5: Identify the nutrients incorporated into the actively growing tissues of abalone when fed on diatoms by assessing fatty acid metabolism and carbon and nitrogen retention in small abalone using stable isotopes.

Findings: The apparent digestibility and metabolic fate of the different classes of fatty acids (eg. saturated, monounsaturated or PUFA; ω3 or ω6) were investigated. Main findings were:

- The apparent digestibility of saturated and monounsaturated fatty acids was less than for PUFA while ω6 (mainly from vegetable oils) and ω3 (mainly from fish oil) oils were equally well digested;
- The fatty acid composition of the abalone tissue lipid tends to resemble that of the dietary lipid after a feeding period of 2 to 6 weeks;
- Most dietary PUFA was found to end up in the abalone tissue as 20:4ω6, 20:5ω3 and 22:5ω3 after chain elongation and desaturation of dietary precursors or preferential assimilation of these fatty acids from the diet.

10. ACKNOWLEDGMENTS

We acknowledge the funding of this project by the Fisheries Research and Development Corporation. The execution of our feeding experiments would not have been possible without significant industry involvement. In particular, we thank Miles Cropp (Abalone Farms Australia P/L formerly Marine Shellfish Hatcheries) and Mark Heather and Mike Wing (Tasmanian Tiger Abalone Company) for dedication of resources and staff time, and to representatives of the companies who participated in the evaluation of our early diets (Miles Cropp of Abalone farms Australia P/L, Peter Rankin of Ocean Wave Seafoods and Jim Morrison of South Australian Mariculture). Many useful discussions and encouragement from industry representatives such as Dr Dom O'Brien (Huon Aquaculture Company) and Ross Heather (Tasmanian Tiger Abalone Company) are also gratefully acknowledged. Helpful discussions and supply of a regular shipment of diatom pastes from Mike Heasman and John Diemar (Port Stephens Research Centre), and flocculation of diatoms by Richard Knuckey (formerly of UTas) is also gratefully acknowledged. The expertise with the analysis of samples with isotope mass spectrometer of Dr Andy Revill and Dan Holdsworth are also gratefully acknowledged. We also wish to thank Brad Evans for setting up the tank system at Tasmanian Tiger Abalone Company. A significant contribution by Dr Kevin Williams for constructive comments on this manuscript is also gratefully acknowledged.

11. REFERENCES

Akiyama, D.M., Dominy, W.G. & Lawrence, A.L. 1991. Penaeid shrimp nutrition for the commercial feed industry: revisited. In: Akiyama, D.M. & Tan, R.K.H. Proceedings of the aquaculture feed processing and nutrition workshop. September 19-25, 1991. American Soybean Association, Singapore.

Ando, Y., Nakamura, J. & Ota, T., 1997. Phagostimulant activity of phosphatidylcholine molecular species for young abalone, *Haliotis discus hannai*. Fish. Sci., 63: 1048-1049.

Bautista-Teruel, M.N. & Millamena, O.M. 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein/energy levels. Aquaculture 178; 117-126.

Blakeney, A. B., P. J. Harris, R. J. Henry & B. A. Stone, 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohyd. Res.,* 113, 291-299.

Bligh, E.G., & Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Britz, P.J. & Hecht, T. 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. Aquaculture. 156; 195-210.

Britz, P.J., Hecht, T. & Mangold, S. 1997. Effect of temperature on growth, feed consumption and nutritional status of *Haliotis midae* fed a formulated diet. Aquaculture 152; 191-203.

Brown M.R., Jeffrey S.W., Volkman J.K. & Dunstan G.A. 1997. Nutritional properties of microalgae for mariculture. *Aquaculture* 151, 315-331.

Cousin, M., Cuzon, G., Guillaume, J. & AQUACOP. 1996. Digestibility of starch in *Penaeus vannamei*: in vivo and in vitro study on eight samples of various origin. Aquaculture. 140; 361-372.

Cruz-Suárez, L.E., Ricque-Marie, D., Pinal-Mansilla, J.D. & Wesche-Ebelling, P. 1994. Effect of different carbohydrate sources on the growth of *Penaeus vannamei*: economical impact. Aquaculture. 123; 349-360.

Diaz, J.P., Mani-Ponset, L., Guyot, E. & Connes, R. 1994. Assimilation of dissolved glucose from sea water by the sea bass *Dicentrarchus labrax* and the sea bream *Sparus aurata*. Mar. Biol. 120; 181-186.

Dias, J., Huelvan, C., Dinis, M.T. & Métailler, R. 1998. Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass (*Dicentrarchus labrax*) juveniles. Aquat. Living Resour. 11; 219-226.

Dunstan, G.A., Sinclair, A.J., O'Dea, K. & Naughton, J.M. 1988. The lipid content and fatty acid composition of various marine species from southern Australian waters. Comp. Biochem. Physiol. 91B: 165-169.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.-M. & Jeffrey, S.W. 1994. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35: 155-161.

Dunstan, G.A., Baillie, H.J., Barrett, S.M. & Volkman, J.K., 1996. Effect of diet on lipid composition of wild and cultured abalone. *Aquaculture*, 140: 115-127.

Dunstan, G.A., Olley, J. & Ratkowsky D.A. 1999. Major environmental and biological factors influencing the fatty acid composition of seafood from Indo-Pacific to Antarctic waters. Rec.Res. Devel. Lipids Res., 3: 63-86.

Dunstan, G.A., Volkman J.K. & Maguire G.B. 2000. Optimisation of essential lipids in artificial feeds for Australian abalone. FRDC Final Report Project No. 94/85. CSIRO Marine Research, 68pp.

Fleming, A.E., Van Barneveld, R.J. & Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. Aquaculture, 140: 5-53.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., Vandepeer, M.E., & Kruk, J.A., 1998. Complementary additivity of the digestibility coefficients of feed ingredients fed to juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17: 641-647.

Harada, K., Maruyama, S. & Nakano, K., 1984. Feeding attractants in chemical constituents of brown alga for young abalone. Bull. Jap. Soc. Sci. Fish., 50: 1541-1544.

Harada, K. & Akishima, Y., 1985. Feeding attraction activities of amino acids, lipids and nitrogenous bases abalone. Bull. Jap. Soc. Sci. Fish., 51: 2051-2058.

Harada, K., 1987. Relationship between structure and feeding attraction activity of certain L-amino acids and lecithin in aquatic animals. Nippon Suisan Gakkaishi, 53: 2243-2247.

Harada, K., Eguchi, A. & Kurosaki, Y., 1987. Feeding attraction activities in the combinations of amino acids and other compounds for abalone, oriental weatherfish and yellowtail. Nippon Suisan Gakkaishi; 53: 1483-1489.

Harada, K., 1989. Feeding attraction activity of L-dipeptides for stevioside for abalone, oriental weatherfish and yellowtail. Nippon Suisan Gakkaishi, 55: 1629-1634.

Harada, K., Miyasaki, T. & Tamura, Y., 1993. Feeding attraction activity of stevioside for aquatic animals. Nippon Suisan Gakkaishi, 59: 1955.

Harada, K., Miyasaki, T. & Karimata, A, 1996. Attraction activities of terrestrial vegetable extracts for aquatic animals. Fish. Science, 62: 675-682.

Harada, K. & Miyasaki, T., 1997. Attractivity of exotic fruit fleshes for the abalone, the oriental weatherfish and the yellowtail. Fish. Sci., 63: 671-675.

Harris, J.O., Bourke, C.M. & Maguire, G.B. 1998. Characterisation of the digestive tract of greenlip abalone, *Haliotis laevigata* Donovan. II. Microenvironment and bacterial flora. J. Shellfish Res. 17; 9889-994.

Jantrarotai, W., Sitasit, P., Jantrarotai, P., Viputhanumas, T. & Srabua, P. 1998. Protein and energy levels for maximum growth, diet utilization, yield of edible flesh and protein sparing of hybrid *Clarias* catfish (*Clarias macrocephalus* X *Clarias gariepinu*s). J. World Aquaculture Soc. 29: 281-289.

Jeney, G., Galeotti, M., Volpatti, D., Jeney, Z. & Anderson, P. 1997. Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. Aquaculture. 154: 1-15.

Kristensen, H.J. 1972. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar. Biol. 14; 130-142.

Lopez, L.M., Tyler, P.A. & Viana, M.T. 1998. The effect of temperature and artificial diets on growth rates of juvenile *Haliotis tuberculata* (Linnaeus, 1758). J. Shellfish Res. 17; 657-662.

Mai, K., He, G. & Xu, W. 1998. Studies on postprandial changes of digestive status and free amino acids in the viscera of *Haliotis discus hannai* Ino. J. Shellfish Res. 17; 717-722.

Marxen, J.C., Hammer, M., Gehrke, T. & Becker, W. 1998. Carbohydrates of the organic shell matrix and the shell-forming tissue of the snail *Biomphalaria glabrata* (Say). Biol. Bull. 194; 231-240.

Nazarova, I.V., Shevchenko, N.M., Kovalev, B.M. & Khotimchenko, Y.S. 1998. Immunomodulating properties of polysaccharides isolated from red algae: effects on complement system. Russian J. Mar. Biol. 24; 47-50.

Percival, E. 1968. Marine algal carbohydrates. Oceanogr. Mar. Biol. Ann. Rev. 6; 137-161.

Sakata, K., Itoh, T. & Ina, K., 1984. A new bioassay method for phagostimulants for young abalone, *Haliotis discus* Reeve. Agric. Biol. Chem., 48: 425-429.

Sakata, K. & Ina, K., 1985. Digalactosyldiacylglycerols and phosphatidylcholines isolated from a brown alga as effective phagostimulants for a young abalone. Bull. Jap. Soc. Sci. Fish., 51: 659-665.

Shpigel, M., Ragg, N.L., Lupatsch, I. & Neori, A. 1999. Protein content determines the nutritional value of the seaweed *Ulva lactuca* L. for the abalone *Haliotis tuberculata* L. and *H. discus hannai* Ino. J. Shellfish Cult. 18: 227-233.

Stickney R.R., 1994. Principles of aquaculture. John Wiley & Sons, Inc. New York. p298.

Tomasik, P. 1997. Saccharides. In: Sikorski, Z.E., Chemical and functional properties of food components. Technomic Publishing Company, Inc. Pennsylvania, USA. 285pp.

Uki, N., Kemuyama, A. & Watanabe, T. 1985a. Development of semipurified test diets for abalone. Bull. Jap. Soc. Sci. Fisheries. 51; 1825-1833.

Uki, N., Kemuyama, A. & Watanabe, T. 1985b. Nutritional evaluation of several protein sources in diets for abalone *Haliotis discus hanna*. Bull. Jap. Soc. Sci. Fisheries. 51; 1835-1839.

Uki, N., Kemuyama, A. & Watanabe, T. 1986a. Optimum protein level in diets for abalone. Bull. Jap. Soc. Sci. Fisheries. 52; 1005-1012.

Uki, N., Sagiura, M. & Watanabe, T., 1986b. Requirement of essential fatty acids in the abalone *Haliotis discus hannai*. Bull. Jpn. Soc. Sci. Fish, 52: 1013 - 1023. (in Japanese)

van Barneveld, R.J., Fleming, A.E., Vandepeer, M.E., Kruk, J.A. & Hone, P.W. 1998. Influence of dietary oil type and oil inclusion level in manufactured feeds on the digestibility of nutrient by juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res., 17; 649-655.

Vandepeer, M.E., Hone, P.W., van Barneveld, R.J. & Havenhand J.N. 1999. The utility of apparent digestibility coefficients for predicting comparative diet growth performance in juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res., 18; 235-241.

Whyte, J. N. C., 1987. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture 60, 231-241.

12. APPENDIX 1: INTELLECTUAL PROPERTY

The focus of the work was to conduct public domain research so all stakeholders could benefit. Results will be published and disseminated widely. It is not anticipated that any patents or commercial intellectual property will arise from this project.

13. APPENDIX 2: STAFF

Principal Investigator:			
Mr. G.A. Dunstan	CSIRO Marine Research	BSc	33%
Co-Investigators:			
Dr M.R. Brown	CSIRO Marine Research	BSc (Hons) PhD	5%
Dr J.K. Volkman	CSIRO Marine Research	BSc (Hons) PhD	5%
Dr G.B. Maguire	Department of Aquaculture, UTas (currently Fisheries Western Australia)	BSc (Hons) PhD	5%
Research Assistants			
Mr D.R. Johns	Department of Aquaculture, UTas	Dip. Aquaculture	33%
Mr S. Hindrum	Department of Aquaculture, UTas	Dip. Aquaculture	
Ms. M. Augerinos	CSIRO Marine Research	Dip. Appl. Sci.	30%

14. APPENDIX 3: ARTICLES AND PRESENTATIONS ARISING FROM THE PROJECT

Dunstan G.A., Brown M.R., Augerinos M., Johns D.R. & Knuckey R. 1998. Formulated Feeds for Juvenile Abalone. FRDC/CRC Abalone Aquaculture Sub Program Annual Workshop, Hobart, July 1998

Dunstan G.A. 1999. Formulated "Diatom" Diets for Juvenile Abalone. FRDC/CRC Abalone Aquaculture Sub Program Annual Workshop, World Aquaculture Society Meeting, Sydney, April 1999

Dunstan et al., (manuscript in prep). Fatty acid budget for juvenile abalone.