AN INVESTIGATION OF DIET MANAGEMENT STRATEGIES FOR THE CULTURE OF THE SOUTH AFRICAN ABALONE, *HALIOTIS MIDAE.*

BRYNN JAMES ALRIC SIMPSON

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A rotation diet feeding scheme was devised to utilize less abundant algae, as well as, the abundant kelp, *Ecklonia maxima*. Rotation diets entailed feeding abalone on *Ecklonia* for 80% of the two week rotation cycle, and on one of the less abundant algae species, or a mixture of the less abundant algae species, for the remaining 20% of the time. Six rotation diets and six single-species control diets were tested simultaneously.

Shell length and body weight growth were ranked highest for the single-species diet *Porphyra*, but rotation diets were placed in the subsequent four ranking positions. Shell length growth rates varied between 27 and 38μm.day^{-1} for abalone fed rotation diets, and between 15 and 53μm.day^{-1} for those fed on single-species diets. Body weight growth rates varied between 34 and 55mg.day^{-1} for abalone fed rotation diets, and between 9 and 74mg.day^{-1} for those fed the control diets. The best rotation diet consisted of *Ecklonia* rotated with *Porphyra*; the next best diets were *Ecklonia* rotated with a mixture of three algae, and *Ecklonia* rotated with *Ulva*.

Alginate bound artificial diets were produced, using powdered algae as a large component (40%) of the ingredients. Each of five artificial diets had a different algae added. Each algae was tested simultaneously as a control diet. Loss of dry matter over 16 hours varied from 12.44% to 21.51%, depending on the algae used in the diet.

Shell length and body weight growth rates for abalone fed on the artificial diets varied between 51 and 87μm.day^{-1} and between -0.18 and 6.79mg.day^{-1} respectively. Shell length growth rates of control diets were higher than the growth rates of abalone fed on the corresponding artificial diet, but significant differences were not apparent ($P>0.05$). Body weight growth rates on artificial diets were substantially less than those of abalone fed on the corresponding control diets.

The six single-species rotation trial control diets were analyzed for protein, energy and ash content, as well as, amino acid composition. Protein content of the six algae varied between 8.13% and 27.74%, energy content varied between 11.77 and 15.81KJ.g^{-1},

while ash content varied between 18.15% and 33.8%. Neither protein content, energy content or ash content was correlated to the growth rates experienced on the diets ($P>0.05$). However, intake of protein and energy was positively correlated to both shell length and body weight growth rates ($P<0.05$). Shell length and body weight growth rates were positively correlated to intake of the essential amino acids histidine and lysine, and the non-essential amino acids alanine and proline. Comparison of essential amino acid patterns and essential amino acid ratios (relative to lysine) of algae with that of *H. midae* tissue did not satisfactorily describe the growth rates achieved on the six control diets.

CHAPTER 1

INTRODUCTION

The Far East provides an almost insatiable market for a wide variety of seafood products. Abalone are one of the more sought after delicacies of the East due to the subtly flavoured meat. Abalone are imported from around the world and command high prices.

Throughout their global distribution, many of the 100 or so species of abalone are commercially and recreationally exploited. The continuing demand for abalone has resulted in the overexploitation of wild stocks throughout the world. Most commercial abalone fisheries are characterised by high yields at the beginning of the fishery, followed by a subsequent decline in yields to present levels (Ebert, 1992; Tarr, 1992).

Abalone culture was pioneered in Japan, partly as a consequence of overexploitation, through the development of reseeding programmes. Hatchery-reared abalone were reseeded into depleted wild abalone populations, and are now harvested sustainably by local fishermen (Shaw, 1982). Similar ventures have been investigated for *Haliotis tuberculata* by Cochard and Flassch (1981), *H. rufescens* by Ebert and Ebert (1988) and Tegner and Butler (1989) , *H. kamtschatkana* by Emmet and Jamieson (1988), and *H. iris* by Schiel (1993).

In addition to reseeding programmes, abalone culture has provided new products for the Far East seafood markets. Abalone are exported live from several countries, as 'cocktail abalone' and command prices in excess of those paid for canned or frozen abalone (Rudd, 1994). *H. midae* is highly sought after in the Far East (Rudd, 1994).

In South Africa, wild populations of abalone have been commercially exploited since 1949 (Tarr, 1992). The only abalone of commercial value is *Haliotis midae*, which is distributed between St Helena Bay (32° 45'S; 18° 10'E) on the West coast and just

north of Port St Johns (31° 40'S; 29° 35'E) on the East coast of South Africa (Wood, 1993). *H. midae* is most abundant between St Helena Bay and Cape Agulhas (Barkai and Griffiths, 1986) and supports a commercial fishery between Cape Columbine and Quoin Point (Tarr, 1992). A small commercial fishery exists at Hamburg on the East coast of South Africa (Wood, 1993).

Research into culture of the South African abalone began in the last decade, encouraged by the decline in wild abalone populations and the development of a market for cocktail sized abalone. Initially abalone culture was not considered to be viable in South Africa, largely due to a lack of knowledge. The initial breakthrough in the culture of *H. midae* followed the demonstration that it was possible to spawn wild abalone through chemical cues (Genade *et al.*, 1988). The realization that the spawning and culture of *H. midae* was possible prompted further research into abalone culture. At present there are approximately 10 commercial companies involved in the preliminary stages of abalone culture in South Africa.

Globally, research has covered many aspects of abalone culture. In the present study, various aspects of growth and nutrition of cultured *H. midae* were researched.

The effects of temperature on growth have been investigated in *H. discus hannai* (Kan-no and Kikuchi, 1962), *H. fulgens* (Leighton *et al.*, 1981), and *H. kamtschatkana* (Paul and Paul, 1981). Between four and five temperatures were tested in each study. These covered normal ambient sea water temperatures and above ambient temperatures, in an attempt to determine optimum temperatures for growth.

Temperatures vary along the coast of South Africa because of the effects of ocean currents and winds. Hecht (1992, 1994) observed the growth rates on *H. midae* at three temperatures to determine the optimum temperature for growth. This study did not relate the effects of temperature to diet. It is important to know the diets fed as optimum temperatures for growth are expected to vary with diet. This is tested in the present study.

In the present study, growth rates of *H. midae* were tested at four temperatures characteristic of sea water temperatures along the West to South coast of South Africa. Three single-species algal diets were fed at each temperature to determine the effects of temperature on abalone growth on a particular diet. The results achieved in this study could indicate the optimum position for abalone culture along the coast of South Africa.

Previous studies have concentrated on single-species algal diets as potential diets for cultured abalone (e.g. Uki *et al.*, 1986). More recently, studies have examined the effects of mixed diets (Owen *et al.*,1984; Fleming, 1994a,b). Day and Fleming (1992) suggested that mixed algal diets provided a broader base for obtaining the nutrients required for growth. This theory is substantiated by the analysis of gut contents of abalone. Gut contents seldom contain less than two different algae species (e.g. Barkai and Griffiths, 1986), which suggests that abalone naturally consume a mixed diet. This study examined the feasibility of a rotated diet scheme which mimics a mixed diet, with the exception that each algae is fed separately. Rotation diets were compared to six single-species diets.

Previous dietary research has considered the use of artificial diets to enhance growth rates of cultured abalone. Artificial diets remove the uncertainty associated with harvesting natural algae diets, but could increase the cost of production. A balance between increased diet cost and increased growth rates is required to make the use of artificial diets in abalone mariculture viable. The addition of powdered algae to artificial diets was investigated. Algae, which constituted 40% of alginate bound artificial diets, replaced more expensive ingredients like dextrin and cellulose.

Proximate analysis was carried out on each of the six single-species algae diets tested to determine protein, energy and ash content and amino acid profile. Regression analysis was used in an attempt to relate various biochemical factors to growth. The factors studied included: protein content and intake, energy content and intake, ash content, amino acid intake, consumption rates and feed conversion efficiencies. Essential amino acid pattern (Knauer, 1994) and lysine related essential amino acid

ratios (Fleming and van Barneveld, 1994) were used to compare the amino acid profile of *H. midae* with the profile of each algae. An attempt is made to explain why some diets produce better growth rates than others.

Ultimately, this study aimed to identify the best diet management options for culture of the South African abalone, *Haliotis midae*.

CHAPTER 2

THE INFLUENCE OF TEMPERATURE AND DIET ON GROWTH OF THE SOUTH AFRICAN ABALONE, *HALIOTIS MIDAE*.

INTRODUCTION

The South African abalone, *Haliotis midae* occurs between St. Helena Bay on the West coast and Transkei on the East coast of South Africa (Newman, 1968: Barkai & Griffiths, 1986). The resource is steadily being depleted due to commercial and sport fishing and poaching (Tarr, 1989). In addition the high price being paid for abalone in the Far East has spurred interest in the land-based culture of abalone in South Africa. Numerous studies on the growth of various abalone species have been undertaken: *H. cracherdoii* (Leighton and Boolootian, 1963), *H. discus hannai* (Uki *et al.*, 1986), *H. fulgens* (Leighton, 1974; Leighton *et al.*, 1981), *H. kamtschatkana* (Paul *et al.*, 1977; Paul and Paul,1981), *H. laevigata* (Shepherd, 1988; Shepherd and Steinberg, 1992), *H. midae* (Newman,1968; Barkai and Griffiths, 1986,1987; Hecht, 1992; Simpson, 1992; Stepto, 1993), *H. roei* and *rubra* (Shepherd and Steinberg, 1992), *H. rufescens* (Greenier and Takekawa, 1992), *H. tuberculata* (Peck, 1983; Peck *et al.*, 1987) and a general overview (Day and Fleming, 1992). Few have measured the interactive effects of temperature and diet on growth.

South Africa's long coastline is influenced by the warm Agulhas Current on the East to South-west coast and the cold Benguella Current on the West coast.

Temperature variation along the coast affects the distribution of *Haliotis midae* (Tarr, 1989) and the range of natural algal diet available to it (Simons, 1990).

This chapter investigates the effect of temperature on growth rates of *Haliotis midae* fed on three single-species algal diets.

METHODS

Growth response trials were carried out at four temperatures using three single-species algal diets. Four temperature control units were used to provide temperatures of 11, 14, 17 and 20°C. These approximated temperatures experienced along the West and South-west coast of South Africa; characterised by temperatures experienced at Yzerfontein on the West coast, 11.3 to 14.5°C (range: 9.5 to 16.7°C; year 1992), and Struisbaai on the South coast, 12.8 to 21.1°C (range: 12 to 23°C; year 1990).

The kelp species *Ecklonia maxima* and *Laminaria pallida* as well as the red algae *Porphyra capensis* were used as single-species algal diets. All three species are accepted but *Porphyra* is not naturally accessible to abalone in the wild as it is found on the highest rocks in the *Littorina* zone. These algal species are found in abundance along the West to South coast of South Africa (Simons, 1990).

Seven hundred and twenty hatchery reared juvenile *H. midae*, approximately 15 months old and 28mm (sd. 3.87mm) shell length, were used to test growth rates. Each diet was tested in duplicate at each temperature. Groups of 30 abalone were placed in two litre transparent plastic jars, supplied with fresh sand-filtered sea water at a rate of 15 litres per hour, with vigorous aeration by airstone. Jars remained clean as a result of the high aeration but were further swirled and emptied twice per week to remove faeces caught between the abalone. Fresh algae was supplied twice weekly to ensure feeding to satiation.

Randomly selected juveniles, initially fed *Ecklonia maxima*, were placed in the jars and were fed on one of the three algae species. Animals were acclimatised to the diets for a period of one month before the initial measurement.

Weight and length measurements were taken every two weeks for six weeks, using a Sartorius balance and vernier callipers.

Body weight (BW) and shell length (SL) growth rates (GR) were calculated in the following way:

$$BWGR = (W_1 - W_0)/t$$

$$SLGR = (L_1 - L_0)/t$$

Where W_0 = mean initial weight, W_1 = mean final weight, L_0 = mean initial length, L_1 = mean final length and t = time in days.

Feed conversion efficiency (FCE) was calculated for trials at 17 and 20°C using the following formula (Uki, 1981):

$$FCE = (growth/ration)*100$$

where growth is the wet body weight gain per day and ration is wet feed intake per day, calculated from consumption of diets over 2 to 3 night feeding periods.

Statistical analysis

A two-way ANOVA was applied to mean shell length for each diet/temperature treatment, but due to variation in initial size it was not possible to tell which treatments were significantly different.

Thereafter, an ANCOVA was applied to growth rate data for each replicate, with initial length as the covariate (Sokal and Rohlf, 1969). It was assumed that the effects of initial size on growth were the same for all treatments. There is no biological reason

to suspect that initial length (within the range used) would effect treatments differently (Rob Day, pers. comm.), although there is not sufficient data to test this assumption.

RESULTS

Growth rates

Shell length and body weight measurements were taken every two weeks for six weeks. Length and weight growth curves at the four experimental temperatures are shown for each single-species algal diet in Figure 1. and 2.. Length and weight growth curves are shown for all diets at each temperature in Figures 3. and 4..

Daily growth rates were calculated from the growth increment over the six week period (Table 1, Figure 5,6). Shell length growth rates ranged from 6.5µm.day^{-1} on *Laminaria* at 11°C to 64.1µm.day^{-1} on *Porphyra* at 20°C. These equate to 0.2 and 1.9mm shell length increase per month respectively.

Table 1. Shell length (µm.day^{-1}) and body weight (mg.day^{-1}) growth rates (n=2) for three diets at four temperatures.

LENGTH				
Temperature	11°C	14°C	17°C	20°C
Ecklonia	7.7	19.0	23.6	40.9
Laminaria	6.5	17.0	32.9	30.8
Porphyra	19.4	28.9	50.6	64.1
WEIGHT				
Temperature	11°C	14°C	17°C	20°C
Ecklonia	5.7	8.8	16.4	15.5
Laminaria	0.6	11.2	17.6	16.6
Porphyra	13.8	23.9	30.7	28.5

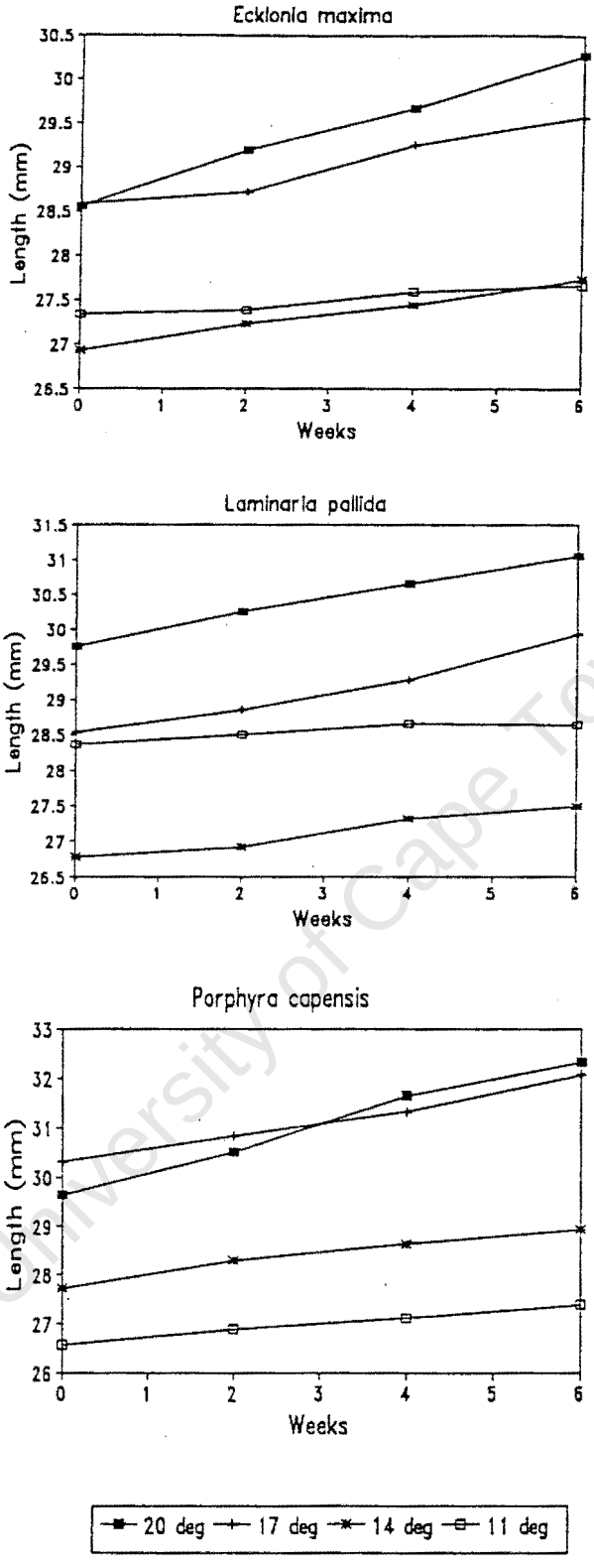


Figure 1. Length growth curves on single-species algal diets of *Ecklonia maxima*, *Laminaria pallida* and *Porphyra capensis* at the four experimental temperatures.

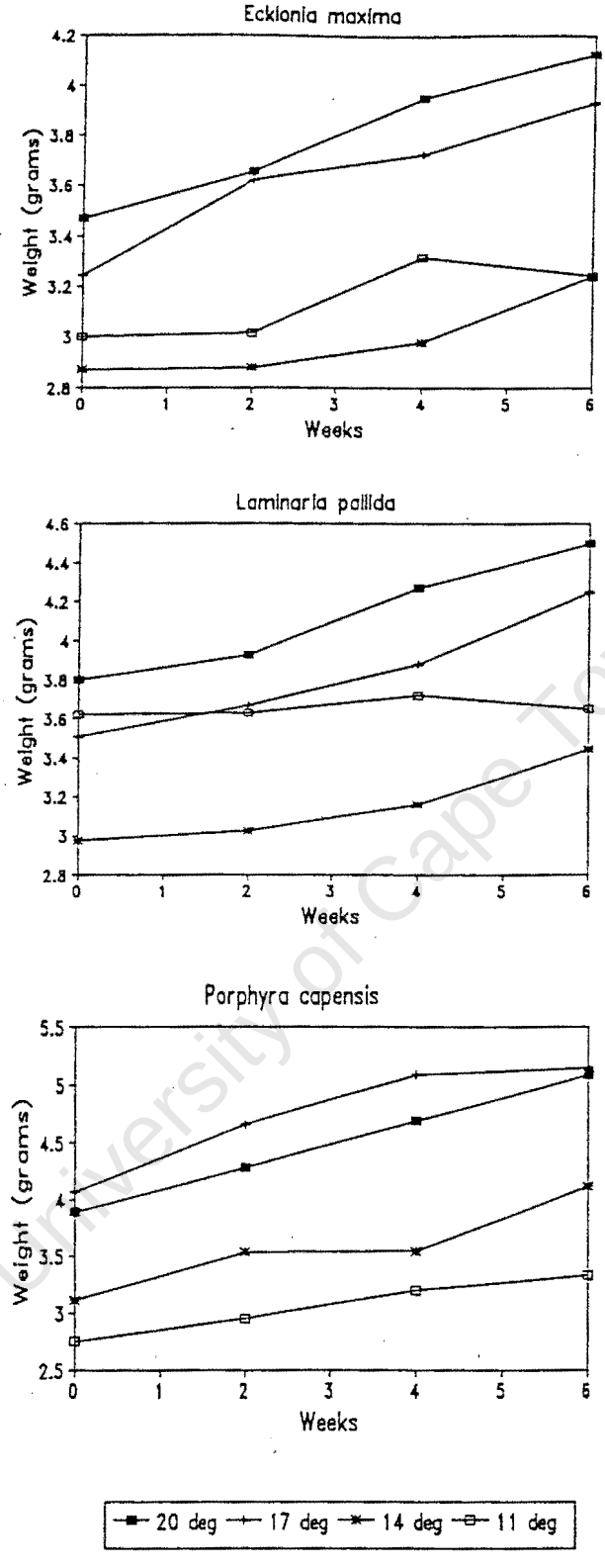


Figure 2. Body weight growth curves on single-species algal diets of *Ecklonia maxima*, *Laminaria pallida* and *Porphyra capensis* at the four experimental temperatures.

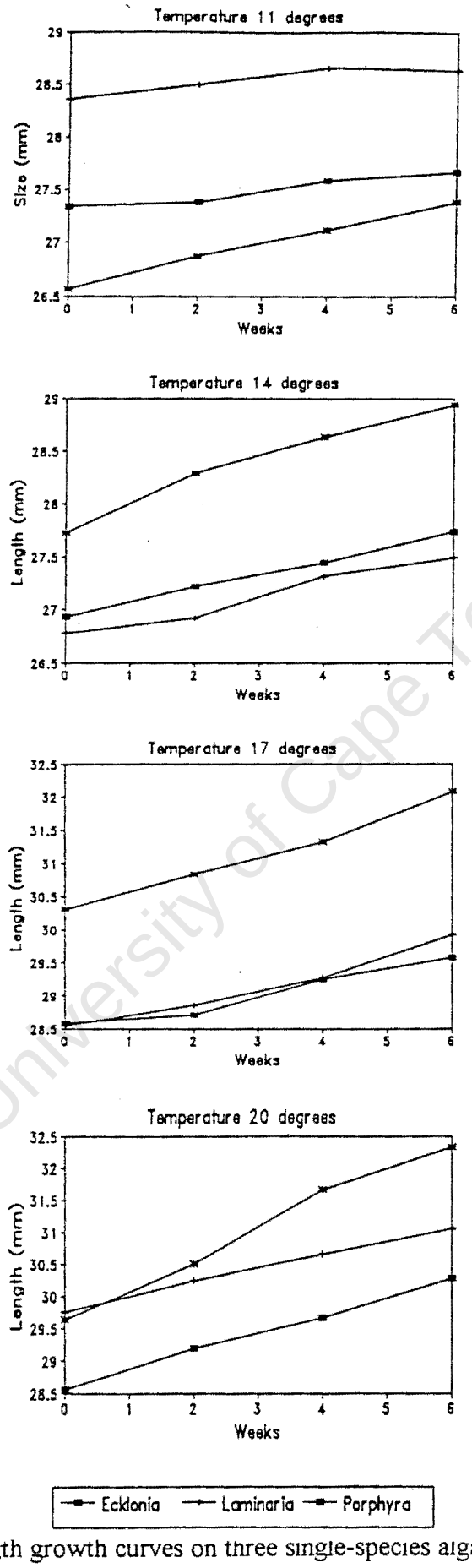


Figure 3. Shell length growth curves on three single-species algal diets at temperatures 11, 14, 17 and 20°C.

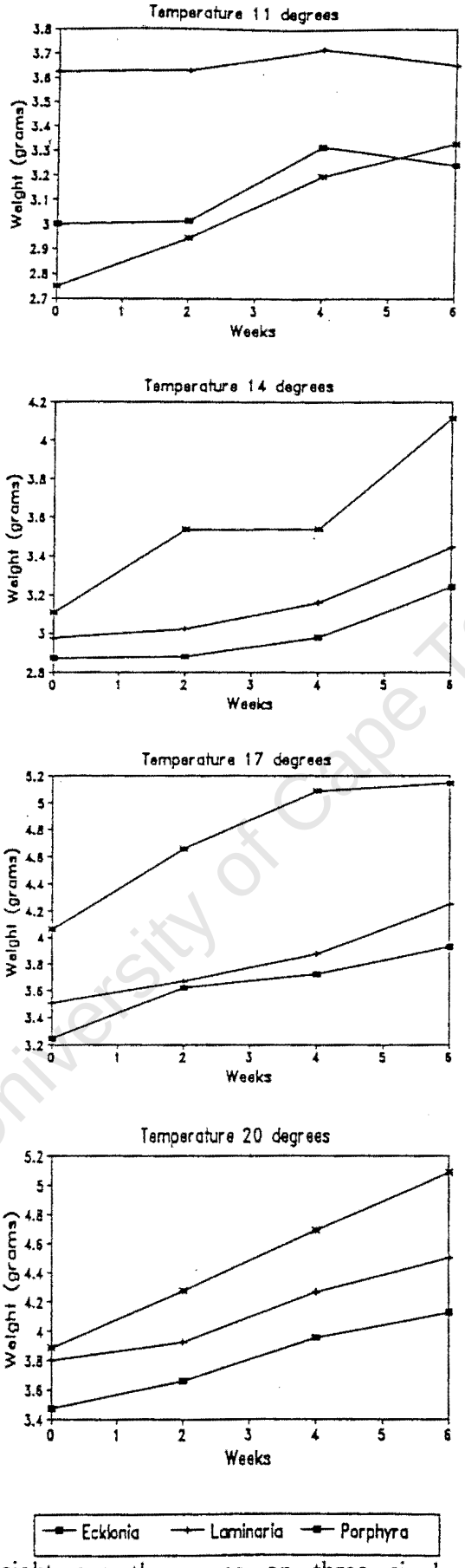


Figure 4. Body weight growth curves on three single-species algal diets at temperatures 11, 14, 17 and 20°C.

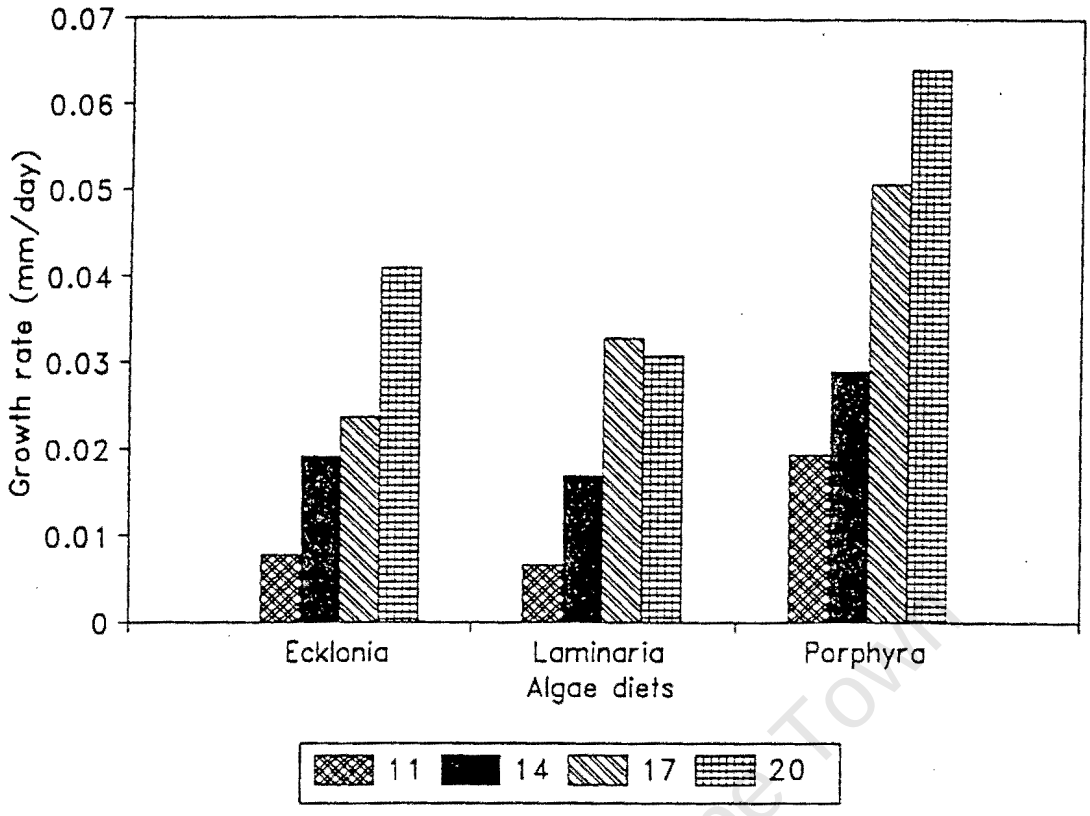


Figure 5. Daily shell length growth rates for each diet at each of the four temperatures.

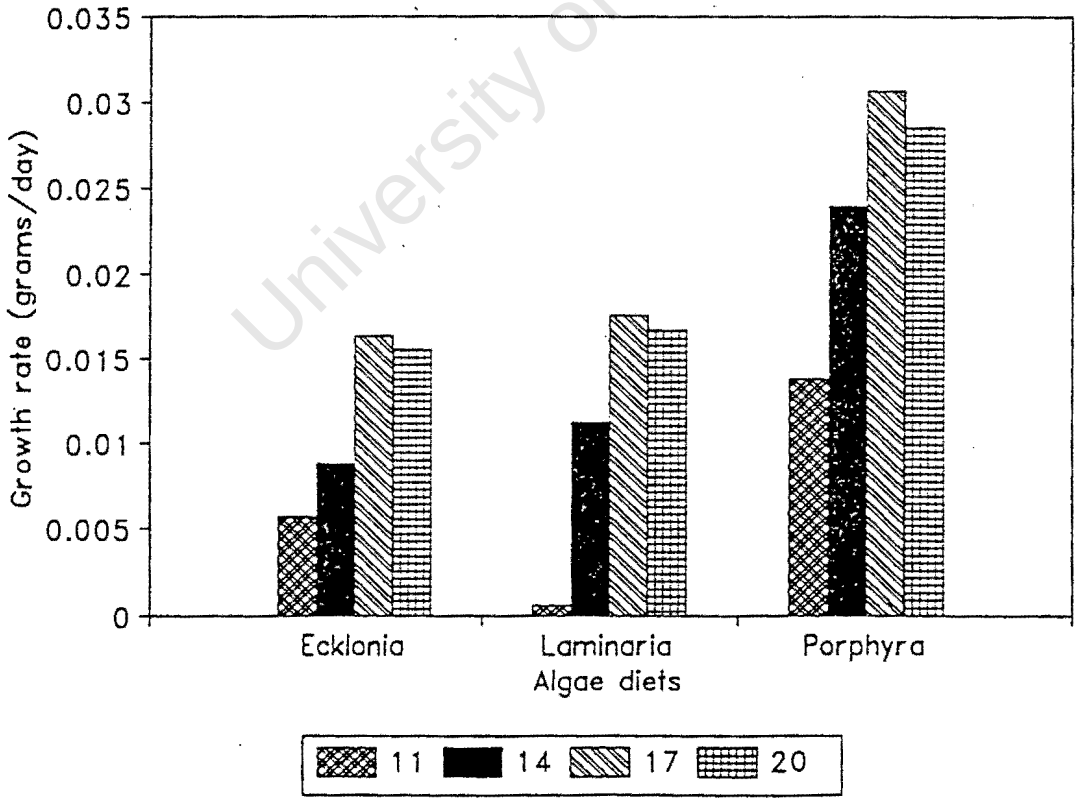


Figure 6. Daily body weight growth rates for each diet at each of the four temperatures.

No significant differences in shell length growth rates were apparent between diets at 11°C and *Ecklonia* and *Laminaria* at 14°C. Shell length growth rates on *Porphyra* at 20°C were significantly higher than all other treatments except *Porphyra* at 17°C.

Body weight growth rates ranged from 0.6 mg/day for *Laminaria* at 11°C to 30.7 mg/day for *Porphyra* at 17°C. This represents a 51 fold difference in body weight growth rates over the range of temperatures and diets tested. Figure 4. shows erratic body weight growth at lower temperatures, while at higher temperatures less variability was apparent.

Shell length and body weight growth rates, for all treatments, were ranked from 1 (highest) to 12 (lowest) (Table 2.). Shell length growth rates increased with temperature for both *Ecklonia* and *Porphyra*, but peaked at 17°C on *Laminaria*. Body weight growth rates peaked at 17°C for all three diets. *Porphyra* took the top two ranks for shell length growth rates and the top three for body weight growth.

Table 2. Diet treatments ranked according to shell length (SL) and body weight (BW) growth rates (GR) from figures 5. and 6. Treatments are ranked from highest growth rate (1) to lowest growth rate (12). Treatment given as T (temperature) 11 to 20°C and D (diet) where 1 = *Ecklonia*, 2 = *Laminaria* and 3 = *Porphyra*. Also shown are statistical differences between treatments for shell length growth rates.

SLGR Rank	Treatment	Tukey Hsd test	BWGR Rank	Treatment
1	T20 D3	a	1	T17 D3
2	T17 D3	ab	2	T20 D3
3	T20 D1	bc	3	T14 D3
4	T17 D2	bc	4	T17 D2
5	T20 D2	cd	5	T20 D2
6	T14 D3	cd	6	T17 D1
7	T17 D1	de	7	T20 D1
8	T11 D3	de	8	T11 D3
9	T14 D1	de	9	T14 D2
10	T14 D2	de	10	T14 D1
11	T11 D1	e	11	T11 D1
12	T11 D2	e	12	T11 D2

Consumption rates and feed conversion efficiencies

Feed conversion efficiencies were measured for 17 and 20°C (Table 3.). These were highest for *Porphyra capensis*, followed by *Ecklonia maxima* and then *Laminaria pallida*. *Porphyra* was eaten in small amounts (Table 3) but gave high growth rates. *Laminaria* on the other hand was eaten in large amounts (Ttable 3) and produced small growth rates.

The lower temperature produced an improvement in FCE for *Ecklonia* and *Laminaria* but a decreased in FCE for *Porphyra*.

Table 3. Consumption rates and feed conversion efficiencies for *H.midae* at 17 and 20°C.

Diet	Consumption (% BW.day^{-1})	FCE
20°C		
Ecklonia	8.69	0.045
Laminaria	11.81	0.037
Porphyra	4.40	0.182
17°C		
Ecklonia	7.82	0.056
Laminaria	9.4	0.051
Porphyra	3.98	0.159

DISCUSSION

Water temperatures vary along the coast of South Africa. Temperatures are lower on the West coast (Atlantic Ocean) where they are influenced by the cold Benguela Current, while on the South-west to East coast they are influenced by the warm Agulhas Current (Indian Ocean). Where *H. midae* and kelp are found together, mean monthly temperatures vary between approximately 9.5 and 16.7°C (Yzerfontein) on the West coast and approximately 14 and 23°C (Cape Agulhas) on the South coast. Temperatures on the West coast are also affected by strong off-shore winds which

blow throughout summer (Southern hemisphere - September to March). These off-shore winds cause upwelling, resulting in decreased inshore temperatures so that a 10 or 11°C difference is sometimes found between inshore and off-shore surface waters (Andrews and Hutchings, 1980). Surface temperatures below 8°C have been reported but are rare (Andrews and Hutchings, 1980).

Upwelling may be reversed by rapid wind change causing warmer off-shore water to be transported inshore (Andrews and Hutchings, 1980). The result of short-term wind changes is a wide variation in inshore water temperatures over a relatively short period of time. This variation may be greater than 5°C in 3 days (Andrews and Hutchings, 1980).

Data from this study and others (Kan-no and Koike, 1962; Leighton, 1974; Leighton *et al.*, 1981; Paul and Paul, 1981; Uki, 1981) show that low temperatures result in low growth rates. The effects of temperature fluctuations on abalone has not been reported extensively. It is reasonable to assume that large fluctuations could stress animals, and result in reduced growth rates (Day and Fleming, 1992). Fluctuations in temperature of more than 5°C are experienced along the West coast of South Africa and could be expected to have a detrimental effect on growth rates of wild *H. midae*. This may in part explain the low growth rates reported for abalone along this coast (Newman, 1968; Tarr, 1989).

Higher temperatures along the South-west to East coast should lead to higher growth rates than those experienced on the colder West coast. Where low growth rates are experienced on the South and East coast, this could be attributed to the low abundance of food and consequent inability of abalone to consume food in excess of the normal metabolic requirements (Tarr, 1989). It is possible that this could result in growth rate being higher when sea temperatures are cooler and metabolic rates are consequently lower.

Leighton *et al.* (1981) measured growth rates for *Haliotis fulgens* at four different temperatures (16, 20, 24 and 28°C) of effluent water. Three of the four temperature levels were above ambient temperatures (range 11.6 to 19.8°C) normally experienced. They found the highest growth rates for both length and weight to be at 28°, the highest temperature tested. Paul and Paul (1981) measured growth rates of *H. kamtschatkana* at four temperatures (5.5, 8.5, 11.5 and 13.5°C) and Kan-no and Kikuchi (1962) tested growth rates of *H. discus hannai* at five temperatures ranging from 5 to 25°C. Both studies found that the highest growth rates occurred at the highest temperature tested. On the other hand, Leighton (1974) measured growth rates of three North American abalone species (*H. rufescens, H. fulgens* and *H. corrugata*), over temperatures ranging from 10 to 30°C, and identified optimum temperatures for maximum shell length growth rates which were below the maximum temperature tested.

In the present study, peak growth rates occurred at a temperature below the maximum experienced by wild abalone; approximately 26°C on the East coast of South Africa (temperature data from Sea Fisheries Research Institute). Growth rates increased as the temperatures increase up to 17°C irrespective of the diet. Above 17°C, the effect of increasing temperature, on shell length growth rates was dependent on the algal diet fed. Shell length growth rates increased for *Porphyra* and *Ecklonia* above 17°C but decreased for *Laminaria*. Body weight growth rates decreased for all diets as temperature was increased above 17°C. This suggests that the optimum temperature for the culture of *H. midae* lies between 17 and 20°C for the three diets tested. Hecht (1992), also working on *H. midae*, found that of the three temperatures tested (15, 18, 22°C), the highest growth rates occurred at 22°C. However, no mention is made of the diet used or whether these are shell length or body weight growth rates. Hecht (1994) showed that the temperature of preference of *H. midae* was 24°C; although growth rates were not reported in this study. It is apparent from the present study that

diet and temperature should be considered together in studies wishing to determine optimum temperatures for abalone culture.

Hecht (1992, 1994) gives the critical thermal maximum (the temperature at which *H. midae* loses its suction ability) as 26°C. Paul and Paul (1981) found peak growth rates for *H. kamtschatkana* to be approximately 4.5°C below the critical thermal maximum for that species. Using similar assumptions for *H. midae*, an optimum temperature of approximately 21°C could be expected.

Shell length and body weight growth rates varied considerably over the temperature range tested. At 11°C shell length growth rates were almost negligible for *H. midae* fed on *Ecklonia* and *Laminaria* (7.7 and 6.5 $\mu m.day^{-1}$) respectively. No significant differences in shell length growth rates were apparent between diets at 11 and 14°C ($P>0.05$) with the exception of *Porphyra* at 14°C, where higher growth rates were found ($p<0.05$). Shell length growth rate on *Porphyra* at 20°C was significantly higher ($P<0.05$) than all other treatments except *Porphyra* at 17°C.

Porphyra, a red algae, was eaten in far smaller quantities than *Ecklonia* and *Laminaria* (both brown kelp) at 17 and 20°C. The best feed conversion efficiencies were exhibited by abalone fed on *Porphyra*. Fleming (1994a) fed a number of single-species algal diets to *H. rubra* and found best growth rates and feed conversion efficiencies on a red alga, *Jeannerettia lobata*. *Porphyra* has the highest protein content of 27% (Stepto, 1993), calorific content of $15.81 KJ.g^{-1}$, as well as the lowest ash (Chapter 5, Table 4.) and water content of the three test alga. Body weight growth rates were lower on *Ecklonia* than *Laminaria* which may be explained by the lower calorific value (contrary to that found by Field *et al*, 1980) and nitrogen content (von Holdt *et al.*, 1955) of *Ecklonia*.

Consumption rates increased with an increase in temperature. More energy is required at higher temperatures to sustain growth and a higher metabolic rate. The decrease in FCE with increased temperature for *Ecklonia* and *Laminaria* is probably due to the higher energy requirement for metabolic activity at higher temperatures and the

inability of the abalone to eat sufficient quantity of these algae to satisfy energy requirements.

In light of the above discussion, the siting of abalone farms utilising natural diets, would be best restricted to the South-west coast. Here sea temperatures are approximately equal to those producing the best growth rates on the three algal diets tested. On the west coast, growth rates at ambient temperature will be slow and the growout period could be too long to sustain a viable enterprise. Abalone culture would only be viable along the West coast if water could be heated to between 17^{o} and 20^{o}C at a low cost.

Results also indicate that growth may be slow on the East coast where temperatures are high; although, the diets tested in the present study are not available on the East coast. It may be necessary to use an artificial diet on the East coast as naturally occurring algae are not sufficiently abundant for use as a food source for commercially farmed abalone.

CHAPTER 3

ROTATION DIETS: A METHOD OF IMPROVING GROWTH OF ABALONE USING NATURAL ALGAL DIETS.

INTRODUCTION

Dietary research on abalone has focused on the natural diet of wild abalone, single-species diets in culture and more recently the production of an artificial diet.

Research into the natural diet of the South African abalone, for example (Barkai and Griffiths, 1986), has shown that abalone feed on a broad selection of algae, normally with at least two species being found in the gut content at any time. Preferences certainly do exist, with red algae usually being favoured by a number of different abalone species (Tutschulte and Connell, 1988; Shepherd and Steinberg, 1992; Stepto, 1993; Fleming, 1994).

Originally research focused on single-species diets in an attempt to isolate algae species producing the highest growth rates and feed conversion efficiencies. Sakai (1962) fed abalone on twelve single-species algal diets. Kikuchi *et al.* (1967) fed twenty different algae to abalone. Uki *et al.* (1986) carried out the largest algal dietary study, testing the dietary value of 57 species of algae, and grouped the algae into four ranks based on the results of their study and previous studies.

Other studies, testing the feasibility of abalone culture, have also focused on single-species diets. A few have added a mixed diet to compare with single-species diets (Owen *et al*, 1984; Cook and Claydon, 1991; Day and Fleming, 1992; Fleming, 1994). The general trend from these studies indicates that mixed diets produce better growth rates than single-species diets. Day and Fleming (1992) state that although an alga may not support sustained growth when fed singly, it may be of great value when part of a mixed diet, providing essential nutrients to the diet. Mixed diets are expected to sustain

growth rates, while growth rates on single-species diets tend to decrease over time (Day and Fleming, 1992).

The present study was designed to determine whether it was possible to increase the growth rate of cultured *H. midae* by utilising less abundant algae species, alternated with an abundant algae species.

MATERIALS AND METHODS

Abalone were fed for six months using a diet rotation scheme. The rotation scheme entailed feeding a primary algae (the most abundant kelp species) for approximately 80 percent of the time, and one of five secondary algae (less abundant species) for the remainder of each two week rotation period. A rotation diet with a mixture of secondary algae was also tested. Control diets were used to determine the effect of secondary algal diets when fed as single-species diets.

Test animals

Four hundred and eighty hatchery reared animals of approximately two years age and 43.68 (± 0.59) mm shell length were used to test the growth response of abalone fed on six diet rotation schemes and the six concurrently run control diets.

Groups of twenty unmarked abalone were housed in 5 litre transparent plastic jars which were supplied with temperature controlled (18.5°C ± 1°C) fresh sand-filtered water (12 $l.h^{-1}$). The water was aerated vigorously. The six rotation diets and six control diets were tested in duplicate (n=2).

The abalone were measured every two months over a period of six months. Shell length was measured with a Sylvac digital verniers calliper and body weight was measured using a Sartorius balance. Growth rates were calculated as in chapter 2.

Diets

Ecklonia maxima was selected as the primary algal species as it is found in abundance along the coast of South Africa (Simons, 1990), making it the most economical feed for commercial abalone culture. The secondary algae species are less abundant but are present in sufficient quantity to make up a small portion of the diet of cultured abalone. The secondary algae were: *Laminaria pallida, Porphyra capensis, Ulva* spp., *Aeodes orbitosa* and *Gracilaria verrucosa.* Fresh algae was supplied twice weekly to ensure feeding to satiation.

The diet rotation scheme cycle was repeated every 14 days. The primary species was fed for 11 days and secondary species was fed for 3 days. Secondary species were fed singly or in a mixture. When a mixture was used it was composed of *Porphyra, Ulva* and *Aeodes* supplied in equal mass amounts. Feeding occurred every three or four days, at which time old algae was removed and the containers cleaned. Each algae species used in the rotation diets was fed as a single species diet in the controls.

Feed conversion efficiency, consumption and mass/length ratio

Feed consumption was measured with rotation diets and the control diets. Feed consumption was measured over three night (Friday to Monday) and four night (Monday to Friday) periods to reduce variation resulting from erratic feeding patterns (personal observations). Daily feed consumption was calculated as a percentage of body weight.

Feed conversion efficiency (FCE) was only calculated for abalone groups fed single-species diets. It is not meaningful to calculate FCE's on rotation diets as at least two different algae were consumed.

Feed consumption and conversion efficiency were calculated as in chapter 2.

The body weight/shell length ratio (BW/SL) was calculated for control diets and rotation diets. For each diet trial the final mean mass was divided by the final mean

length. The BW/SL ratio gives an indication of the flesh volume per unit shell length resulting from feeding on a particular diet treatment.

Statistical analysis

The effect of the interaction between diet and time on mean shell length was analysed for using a two-way ANOVA for the effects of diet and time (Sokal and Rohlf, 1969; Zar, 1984). A Tukey's HSD multiple range test was applied to test for differences between mean abalone size (SL) on different diet treatments at each time. Shell length was chosen as differences between treatments are less apparent than body weight differences.

Statistical analyses were not carried out on growth rates as abalone were not individually marked, thus n is equal to two.

Analysis of variance was performed on consumption rates. Consumption rates for control and rotation diets were analysed separately due to the different method of feeding.

RESULTS

A significant increase in shell length and body weight was detected, over time, for all diets, by the two-way ANOVA. All diets showed positive growth rates (Figures 1,2) with the exception of *Ulva* and *Gracilaria* which yielded a decrease in body weight over the first two month period, after which their growth rates became positive.

Shell length growth curves tended to be linear whereas body weight growth curves tended to increase exponentially. Variation in growth was low amongst rotation diets but high amongst the single-species diets. Shell length growth rates were between 0.027 and 0.038 $mm.day^{-1}$ on rotation diets, and between 0.015 and 0.053 $mm.day^{-1}$ on the single-species diets. Body weight growth rates were between 43 and 55

mg.day^{-1} on rotation diets, and 9 and 74 mg.day^{-1} on the single species algal diets (Table 1.).

Table 1. Mean initial size (mm, g), daily growth rate (mm.day^{-1}, mg.day^{-1}) over the six month growth period, rank and BW/SL ratio for rotation and control diets.

Diet description	Mean initial size (mm) (n+40)	Standard deviation	Mean final size (mm) (n=40)	Standard deviation	Tukey HSD test	Growth rate (mm/day) (n=2)	Tukey HSD test	Growth rate rank
Ecklonia	44.23	4.01	50.33	4.60	bcd	0.033	bcd	6
Laminaria	43.90	4.51	48.87	4.99	abcd	0.027	abcd	8
Porphyra	43.07	3.64	52.80	4.85	d	0.053	e	1
Ulva	42.72	3.69	45.48	4.02	a	0.015	a	12
Aeodes	43.72	3.79	48.14	4.33	abc	0.024	abc	10
Gracilaria	43.67	4.07	47.87	4.26	abc	0.023	ab	11
Ecklonia + Laminaria	42.99	3.58	48.54	4.83	abc	0.030	bcd	7
Ecklonia + Porphyra	43.71	4.39	50.73	5.37	bcd	0.038	d	2
Ecklonia + Ulva	43.61	4.77	50.02	5.54	bcd	0.035	bcd	5
Ecklonia + Aeodes	43.56	6.50	50.26	5.04	bcd	0.036	bcd	4
Ecklonia + Garcilaria	44.89	3.17	49.84	6.14	bcd	0.027	abcd	8
Eck + Por/Ulv/Aeod	44.19	3.06	50.99	4.07	bcd	0.037	cd	3

Diet discription	Mean initial size (mg) (n+40)	Standard deviation	Mean final size (mm) (n=40)	Standard deviation	Growth rate (mg/day) (n=2)	Tukey HSD test	Growth rate rank	BW/SL ratio
Ecklonia	13.70	4.28	20.58	5.37	0.037	cde	8	0.409
Laminaria	13.13	4.12	20.56	6.03	0.040	cde	6	0.421
Porphyra	12.78	3.27	26.33	7.14	0.074	e	1	0.499
Ulva	12.04	3.40	13.69	3.83	0.009	a	12	0.301
Aeodes	13.48	4.47	17.74	5.63	0.023	abc	10	0.368
Gracilaria	13.47	3.97	16.20	4.24	0.015	ab	11	0.338
Ecklonia + Laminaria	12.59	3.28	18.78	4.94	0.034	bcd	9	0.387
Ecklonia + Porphyra	13.36	4.51	23.55	6.83	0.055	ef	2	0.464
Ecklonia + Ulva	13.43	4.45	21.89	7.17	0.046	de	4	0.438
Ecklonia + Aeodes	13.27	4.21	20.62	6.06	0.040	cde	6	0.410
Ecklonia + Garcilaria	14.05	3.41	21.98	4.96	0.043	de	5	0.441
Eck + Por/Ulv/Aeod	13.69	3.39	23.08	6.09	0.051	de	3	0.453

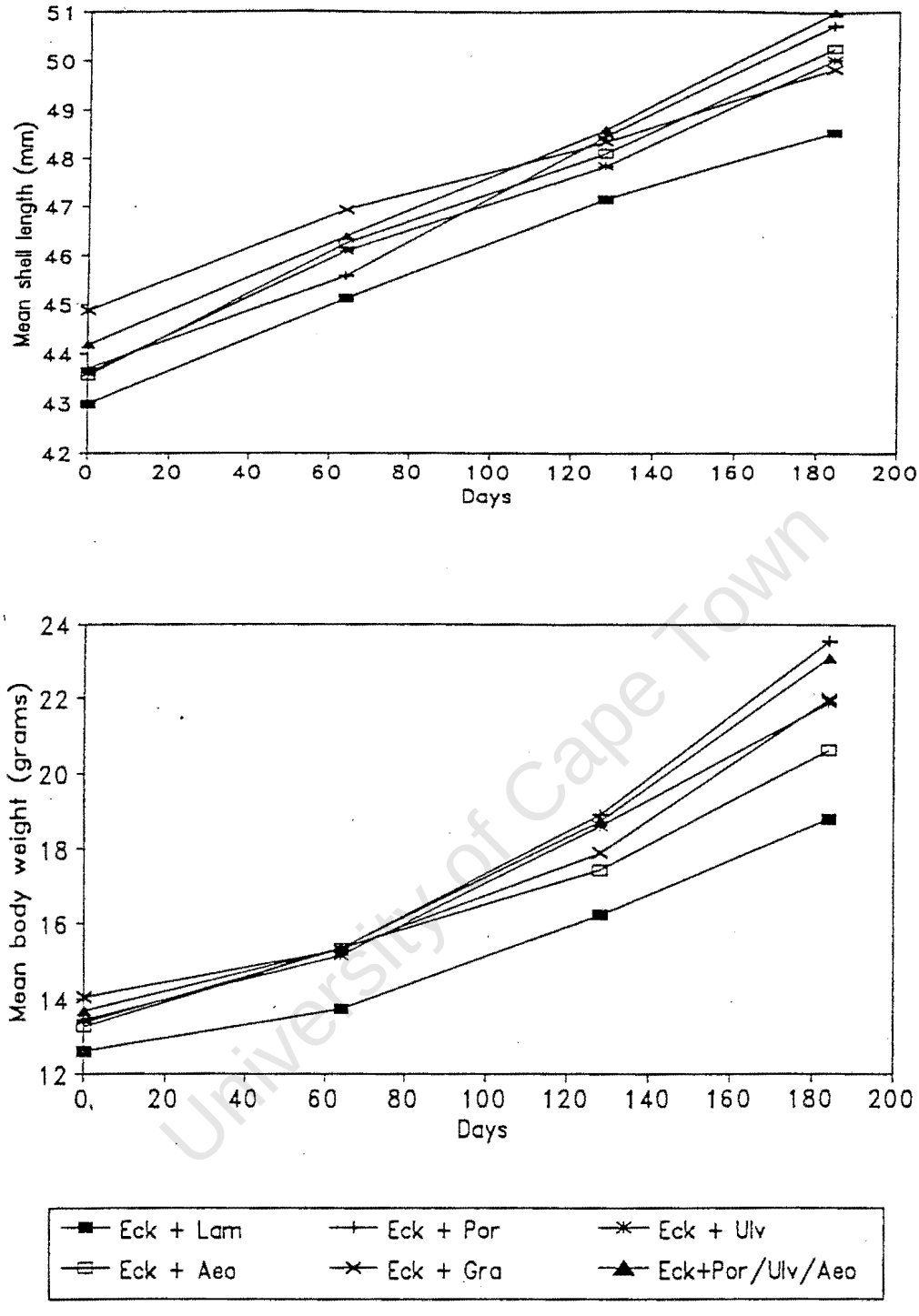


Figure 1. Shell length (top) and body weight (bottom) growth curves for rotation diets.

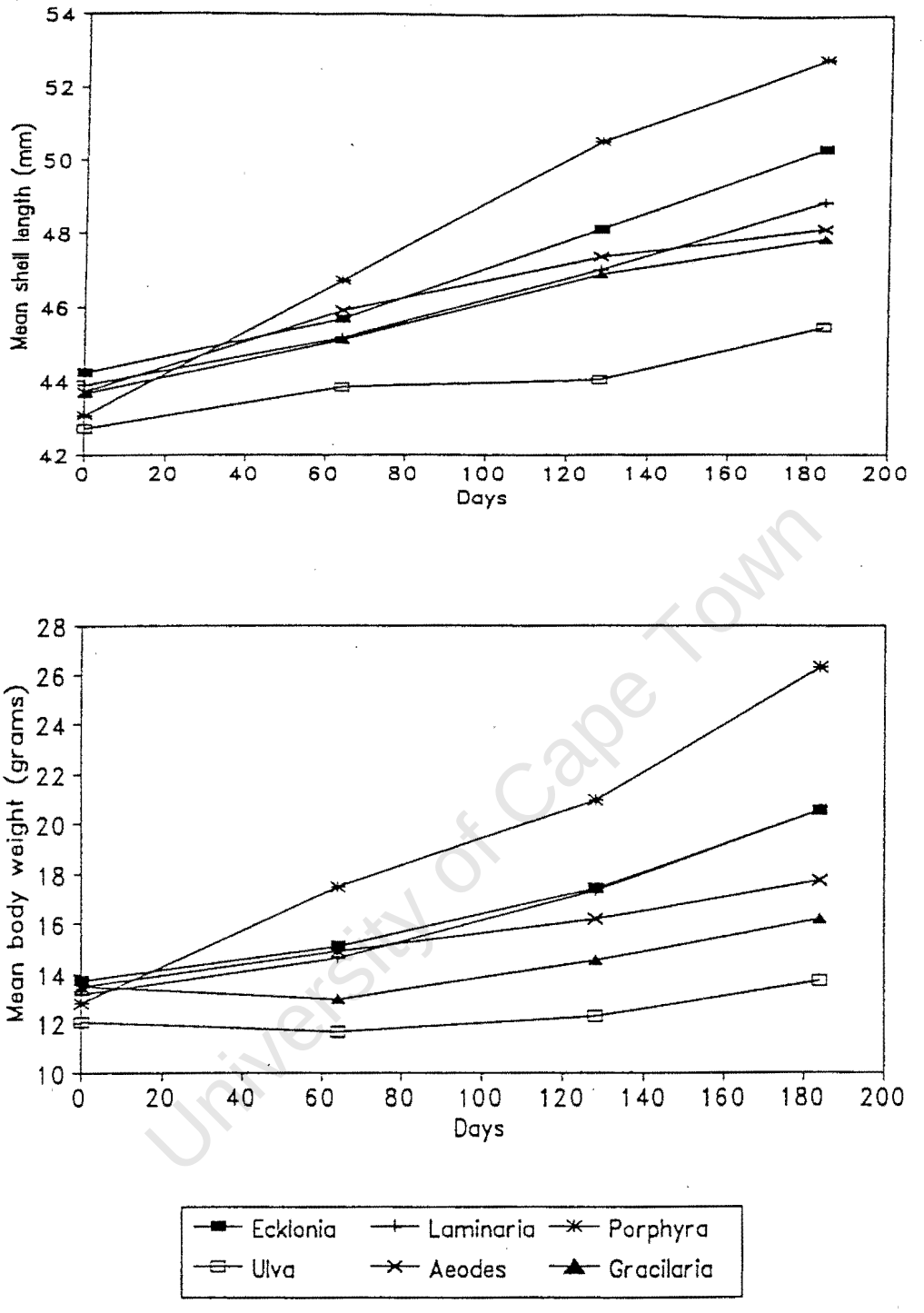
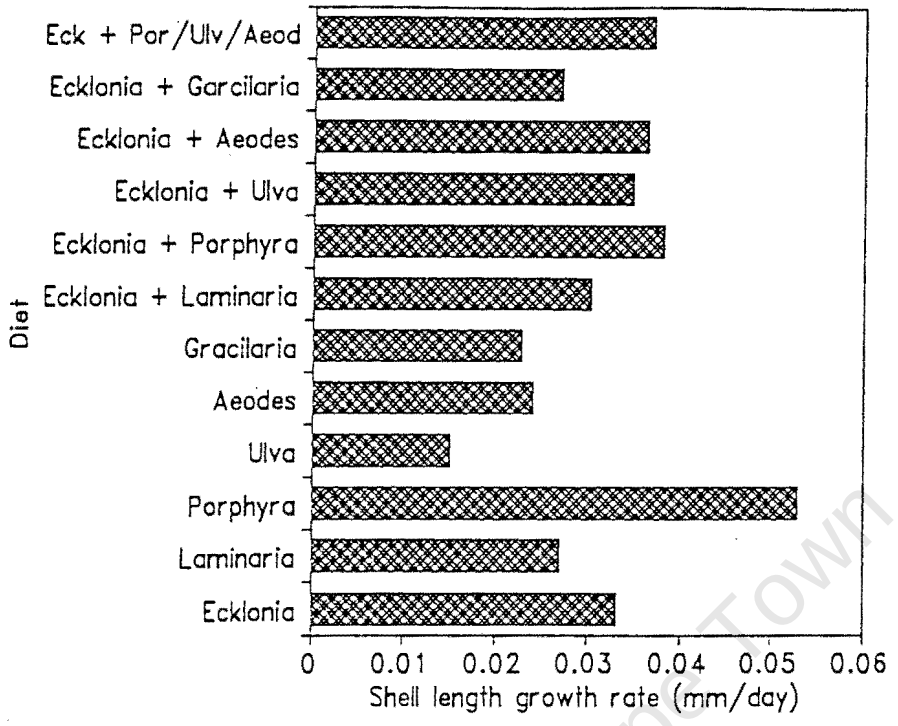


Figure 2. Shell length (top) and body weight (bottom) growth curves for single-species control diets.



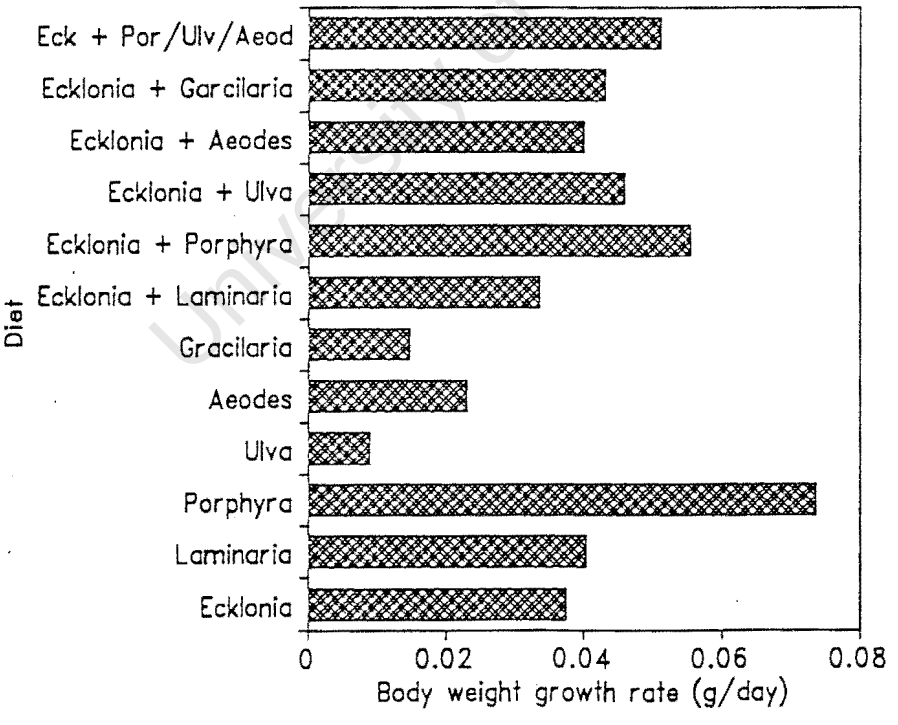


Figure 3. Daily growth rates in Shell length (top) and body weight (bottom) on rotation and control diets.

Statistical analysis of shell length using a two-way ANOVA could only detect differences between treatments ($P<0.05$) after four months. At four months mean abalone shell length for the single-species diet of *Ulva* was significantly less than most rotation diets, with the exception of the rotation diets *Ecklonia* with *Laminaria* and *Ecklonia* with *Ulva*. Mean shell length of abalone fed *Ulva* was also significantly lower than for abalone fed single-species diets of *Porphyra* and *Ecklonia* (Figure 2).

After six months the single-species diet of *Ulva* was significantly different to all diets except the combination of *Ecklonia* and *Laminaria* and the single-species diet of *Laminaria*

Shell length and body weight growth rates, over the six month period, were ranked from highest (1) to lowest growth rate (12) (Table 1). Generally, the rotation diets produced higher growth rates (Table 1, Figure 3) than single-species diets. The exception to this was the single-species algal diet *Porphyra* which produced the highest growth rates for both shell length ($0.053 mm.day^{-1}$) and body weight ($74 mg.day^{-1}$). The best rotation diets were *Ecklonia* with *Porphyra* ($0.038 mm.day^{-1}$, $55 mg.day^{-1}$) and *Ecklonia* with a mixture of *Porphyra, Ulva* and *Aeodes* ($0.037 mm.day^{-1}$, $51 mg.day^{-1}$).

Consumption of the single-species diets ranged from 2.25% $BW.day^{-1}$ for *Porphyra* to 5.44% $BW.day^{-1}$ for *Ecklonia* (Table 2). *Porphyra* was consumed in significantly smaller amounts ($p<0.05$) than *Laminaria* (4.32% $BW.day^{-1}$), *Gracilaria* (4.21% $BW.day^{-1}$) and *Ecklonia*. *Ecklonia* was also consumed in larger amounts than *Aeodes* (2.98% $BW.day^{-1}$) and *Ulva* (2.92% $BW.day^{-1}$).

Consumption of *Ecklonia,* in the rotation diets, was similar for all rotation treatments and the single-species treatment. Consumption rates of secondary algae varied significantly ($P<0.05$). *Laminaria* (5.94% $BW.day^{-1}$) was consumed in larger amounts than all other secondary algae. Consumption of *Ulva* (0.52% $BW.day^{-1}$) was lower than consumption of *Porphyra* (1.53% $BW.day^{-1}$) and *Aeodes* (1.16% $BW.day^{-1}$), in the rotation diet with the mixture of secondary algae.

Feed conversion efficiencies (FCE) were calculated for single-species diet treatments only. FCE ranged from 0.025 for abalone fed on *Gracilaria* to 0.17 for those fed on *Porphyra*. The feeding scheme of rotation diets, with two or more algae being eaten, does not allow for meaningful calculation of FCE ratios.

The BW/SL was calculated for each diet trial using the final mean body weight and shell length. In general BW/ SL ratios were higher for rotation diets than the single-species diets. The diet treatment of *Porphyra* had the highest BW/SL ratio while the diet treatment of *Ulva* had the lowest BW/SL ratio. The range of single-species diet BW/SL ratios (0.301 to 0.499g.mm^{-1}) was greater than the range of rotation diet BW/SL ratios (0.387 to 0.464g.mm^{-1}).

Table 2. Consumption rates (wet weight) in grams per abalone per day and as a percentage of body weight per day, and feed conversion efficiency (FCE) (single-species diets only). Rotation diet consumption rates are given for the primary (*Ecklonia*) and secondary diets in different columns.

Single species algae diets

Diet Description	Consumption (g/abalone/day)	Standard deviation	Consumption %of BW/day	FCE
Ecklonia	0.875	0.224	5.44	0.042
Laminaria	0.701	0.221	4.32	0.057
Porphyra	0.434	0.117	2.55	0.17
Ulva	0.348	0.059	2.92	0.026
Aeodes	0.453	0.129	2.98	0.051
Gracilaria	0.588	0.279	4.21	0.025

Rotation diets

Diet description	*Ecklonia* Consumption (g/abalone/day)	Standard deviation	Consumption %of BW/day	Other algaes Consumption (g/abalone/day)	Standard deviation	Consumption %of BW/day
Ecklonia + *Laminaria*	0.907	0.046	6.6	0.929	0.24	5.94
Ecklonia + *Porphyra*	0.956	0.09	6.23	0.726	0.083	4.19
Ecklonia + *Ulva*	1.048	0.138	6.92	0.36	0.066	2.06
Ecklonia + *Aeodes*	0.818	0.108	5.34	0.298	0.05	1.79
Ecklonia + *Gracilaria*	0.954	0.147	6.3	0.449	0.234	2.56
Ecklonia + mixture	0.785	0.103	5.13			
+ *Ulva*				0.089	0.03	0.52
+ *Porphyra*				0.258	0.021	1.53
+ *Aeodes*				0.197	0.022	1.16

DISCUSSION

Ecklonia maxima is the most abundant algae species found along the South-west and West coast of Southern Africa (Simons, 1990).This alga forms extensive kelp beds extending up to 3 kilometres off-shore, and has a biomass turnover three times per annum (Field *et al*, 1977). *Ecklonia* would be the most likely source of feed for any abalone farms developed along the South-west and West coast of the country as it would be the most easily harvested algae. However, *Ecklonia* does not produce very good growth rates (Cook and Claydon, 1991; Simpson, 1992; and chapter 2). Numerous other species of algae occur along the coast but are not available in sufficient quantity for use as a single-species diet on an abalone farm (Simons, 1990).

Previous studies on other *Haliotis* species, as well as other molluscs, have shown that mixed diets produce better growth rates than single-species diets (Owen *et al.*, 1984; Enright *et al.*, 1986; Day and Fleming, 1992; Fleming 1994a).

The rotation diets tested in this study are similar to mixed algal diets. The difference is that in rotation diet schemes the different algae are provided separately. This technique results in easier regulation of the feeding by abalone on each algae. The single implementation of each algae is important as abalone will only consume preferred algae in a mixed diet. Stepto (1993) found that *Ecklonia maxima* was the least preferred of three algae fed to *H. midae*, and suggested that this may be due to high phenolic levels. In a mixed diet it is likely that *Ecklonia* would be avoided, which would negate the purpose of feeding *Ecklonia* as part of the diet.

Growth rates of abalone fed on rotation diets were generally higher than those of abalone fed single-species diets. The exception is *Porphyra* which produced higher growth rates than any other diet. Subsequent to ranking of all diet treatments, the better rotation diets were placed in positions 2-5 for both shell length and body weight growth rates.

The single-species diets, *Ulva, Aeodes* and *Gracilaria*, produced poor growth rates while *Porphyra* and *Ecklonia* produced good growth rates. The growth rates achieved

on rotation diets were similar between diets and mean shell length could not be shown to differ significantly ($P>0.05$) between diets after six months.

Duncan and Klekowski (1975) stated that essential substances may become limiting when animals are fed one type of food in long-term experiments. This results in decreased growth rates. Growth rates on single-species diets remained constant throughout the 184 day period. Continued single-species feeding could have resulted in decreased growth rates (Day and Fleming, 1992). Feeding trials on *H. rubra* using single-species of dried algae revealed that abalone cease to grow after a period ranging from 50 to 200 days (Day and Fleming, 1992). The rapid effects shown by *H. rubra* may be due to the drying of algae prior to feeding. It is possible that the present study was conducted for insufficient time for dietary deficiencies on single-species diets to materialise.

Shell length growth rates of abalone fed on rotation diets remained constant over time, but the body weight growth rates showed a definite increase over time. The exponential increase in body weight was not as pronounced on single-species diets.

Duncan and Klekowski (1975) stated that it is rare for a any animal to feed on the same food for its entire life. Wild *H. midae* were found with a variety of algae in their guts (Newman, 1968; Barkai and Griffiths, 1986, 1987). This suggests that they obtain the required nutrients for growth by selecting a mixed diet (Day and Fleming, 1992). Fleming (1994a) suggested that preference for certain algae may be due to the presence of essential nutrients not available in other algae.

Although certain algal species (for example *Ulva, Aeodes, Gracilaria*) constitute a poor diet when supplied singly, they may be of great value when supplied as part of a mixed diet, thereby supplying essential nutrients to the diet (Day and Fleming, 1992). This hypothesis was observed for the algae *Ulva* and *Aeodes*, which produced better growth rates when fed as part of a rotation diet. Results demonstrated that combinations of low quality algae often produce good growth rates.

Rotation diets appear to have a greater ability to increase the ratio of body weight to shell length. Body weight/shell length ratios are important in that they indicate the

mass of abalone per unit shell length. Thus, at marketable size (e.g.. 80mm), the value of an abalone, priced by weight, will be dependant on the BW/SL ratio. Certain diets will, therefore, produce more valuable abalone.

Previous studies on other *Haliotis* species, where mixed diets have been tested, have usually consisted of three or more algae species (Owen *et al*, 1984; Fleming, 1994a). One of the rotation diets tested in this study consisted of three secondary algae species which were fed together. Shell length and body weight growth rates on this diet were higher than other rotation diets (except *Ecklonia* with *Porphyra*) but there was no significant difference ($P>0.05$). Abalone fed the rotation diet with a mixture of secondary algae species were able to select preferred algae. This was observed by the difference in consumption rate of *Porphyra, Ulva* and *Aeodes* in the mixed rotation trial. The high growth rate suggests that the abalone may have selectively fed on particular algae to fulfil their nutritional balance requirements. Fleming (1994b) found that *H. rubra* selected foods that maximise the intake of nitrogen and subsequently lead to maximum growth rates.

In rotation diets it is likely that the level of consumption of the secondary algae is related to the nutritional deficiencies present in the primary algae. The secondary algae, *Porphyra*, is eaten at 4.19% BW.day^{-1} when part of a rotation diet, twice that when fed as a single-species diet. It is possible that this makes up for nutritional deficiencies in the primary algae *Ecklonia*. *Ulva, Aeodes* and *Gracilaria* are consumed at lower levels as part of a rotation diet, suggesting that these algae are not nutritionally as good as *Ecklonia*. This is suggested by the low growth rates on *Ulva, Aeodes* and *Gracilaria* when fed as single-species diets and the low feed conversion efficiencies on *Ulva* and *Gracilaria*. Although *Ulva, Aeodes* and *Gracilaria* are inferior diets, they may provide essential nutrients when fed as part of the rotation diet. Thus growth rates of abalone fed on rotation diets with *Ulva, Gracilaria* and *Aeodes* where higher than growth rates of abalone fed either of these, or the primary algae, singly.

Growth rates achieved on the rotation diet of *Ecklonia* and *Laminaria* were low. This is probably due to the similar nutrient content of these alga (Chapter 4.) which are both

Phaeophyta. This suggests that secondary algae in rotation diets should be from a different division to the primary alga. For example, brown algae should be fed in combination with red or green alga and not other brown alga.

Ideally, in a rotation diet, the poorer diet should be fed for a shorter period of time. Poorer algae should be fed for sufficient time for uptake of essential nutrients but not long enough to retard growth rates. *Ulva, Aeodes* and *Gracilaria* should be fed for a short period of time. On the other hand the period of feeding on *Porphyra* should be extended, for as long as supplies allow, to increase growth rates on that particular rotation diet.

This study demonstrates that rotation diets generally produce higher growth rates than single-species diets. Single-species diets are often limiting in one or more nutrients. Feeding on single-species diets for long periods of time often results in decreased growth rates (Day and Fleming,1992). Rotation diets (and mixed diets) provide a wider base for the uptake of essential nutrients. By utilising the different nutrient compositions of different algae, rotation diets ensure that abalone receive a balance of the required nutrients. Growth rate can be improved by regulating the uptake of each algae. This is done by controlling the length of time fed on each algae.

Ecklonia is the most abundant algae along the South to West coast of South Africa and is likely therefore, to form the major part of cultured abalone diets in this area. Growth rates on *Ecklonia* can be improved by addition of either *Porphyra*, *Ulva* or *Aeodes*, or a mixture of all three, in a rotation diet.

CHAPTER 4

THE RELATIONSHIP BETWEEN GROWTH AND PROTEIN, ENERGY AND AMINO ACID INTAKE FOR H. MIDAE FED ON SIX SINGLE-SPECIES ALGAL DIETS.

INTRODUCTION

Research into the culture of abalone has covered all aspects from diet to genetics. In the past decade research has become focused on some of the more complex aspects of culture such as genetic manipulation of inseminated eggs (e.g. Kudo *et al*, 1991), artificial diet binders (Knauer *et al*, 1993, Fleming and van Barneveld, 1994) and control of larval settlement (Matthews and Cook, in press).

Research into optimising the use of both natural and artificial diets has led to faster growth rates which result in shorter grow-out periods. This research is also generating a better understanding of the nutritional requirements of abalone. Research on diets initially concentrated on determining the best single-species algal diet for abalone (e.g. Sakai, 1962; Kikuchi *et al*, 1967). More recently research has been undertaken on optimising growth rates through formulation of artificial foods. Optimum levels of protein, energy and fats have been determined for artificial diets (summarised by Uki and Watanabe, 1986, and Fleming and van Barneveld, 1994)) as well as identifying the best sources of protein (Brits, in press). Amino acid composition has been considered, with researchers striving to match essential amino acid patterns of artificial diets with the essential amino acid pattern of abalone (Fleming and van Barneveld, 1994; Knauer, 1994).

The high costs of artificial feeds prompted the study of single-species and rotation algal diets covered in chapter 3. The present chapter considers the protein, energy and amino acid content of the six single-species diets tested in chapter 3 and how intake of

each factor is related to the varying growth rates experienced between diets. Essential amino acid pattern (Knauer, 1994) and essential amino acid ratios relative to lysine (Fleming and van Barneveld, 1994) are also considered as a method of determining the quality of an algal diet.

MATERIALS AND METHODS

Amino acid content

Hydrolysis of raw samples:

Amino acid analysis of each dry powdered algae species was carried out in duplicate, by the Biochemistry department at the University of Cape Town. The method used was as follows.

Approximately 10mg of each algae sample was weighted into acid cleaned test-tubes. To each test-tube 1 ml of constant boiling HCl (boiling point 110^{o}C) and 10µl of thiodiglycol was added. The tubes were flushed with N_2, to remove O_2, and then evacuated and sealed.

The samples were hydrolysed at 110^{o}C for 20 hours. Thereafter, the samples were removed from the oven and dried in a desiccator.

The hydrolysate was a dark colour and was purified in order to be able to run samples on the analyzer. The purification was carried out as follows:

The hydrolysate was dissolved in 300µl of 625 NLE (citrate buffer, which contains internal standard Norleucine at a concentration of 625nmol.ml^{-1}).

A Sepak cartridge (small disposable C18 column) was primed by passing 0.5ml methanol through it, and subsequently washing the column twice with 1ml water. The column was never allowed to run dry.

The 300µl hydrolysate was inserted into the column and slowly pushed through. The effluent was collected in an Eppendorf tube. Thereafter, the column was washed through twice with 200µl of water. The 200µl fractions were collected each time. At this stage all the amino acids should be eluted from the column, and the remainder of the sample left in the column. The sample should be in 700µl of liquid.

The 700µl of sample was dissolved further so that the final sample used for analysis had a concentration of 50nmol/ml of internal standard (Norleucine).
100µl of each sample was transferred to vials for amino acid analysis.

Amino acid analysis:

Amino acid analysis was carried out on a *Waters* Amino Acid Analyzer equipped with a fluorescence detector. The amino acids were separated using an ion exchange chromatography column which had a strong cation-exchange resin. The column had a length of 25cm and diameter of 0.46cm. The packing material was a styrene-divinyl benzene copolymer, with sulphonic acid functional groups. The amino acids were detected, post column, using o-phthaldialdehyde (OPA) and hypochlorite. The column ran at a temperature of 63^{o}C.
The analysis was performed using a two-buffer system. Buffer A was Tri-sodium Citrate (pH 3.05) and Buffer B was Sodium Nitrate (pH 9.45). The column was equilibrated in Buffer A (low pH with a high concentration of sodium ions). Amino acids entering the column in this acidic buffer, were strongly positively charged, and exchanged with the loosely bound sodium ions. As Buffer B was introduced, increasing the pH over the gradient, each amino acid became less positively charged, and eluted from the column according to it's pK value. The acidic and neutral amino acids eluted first, then the hydrophobic ones, and lastly the basic amino acids.

The gradient program of the two buffers over the 90 minute run, was as follows:

At 0 minutes:	100% A
At 45 minutes	100% B (increase B to 100% from 0 to 45 minutes)
At 80 minutes:	100% B (B maintained at 100%)
At 90 minutes:	100% A (increase A to 100% from 80 to 90 minutes)

Equilibration time between runs: 12 minutes of 100% Buffer A.

The following amino acids were detected in the analysis: threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine, aspartic acid and glutamic acid. Asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, and were thus eluted as aspartic acid and glutamic acid. Tryptophan was not detected as it was destroyed during hydrolysis. Cysteine was detected, but is not very accurate as it is sensitive to hydrolysis. Data for levels of cysteine were therefore disregarded.

Amino acid content was expressed as nmol AA per sample. This was converted to mg $AA.g^{-1}$ of each species of algae (samples = 2). Protein content of diets was calculated by adding the levels of amino acids in each diet. Protein content was then expressed as a percent of the dry algae.

Energy and ash content

Triplicate samples of oven dried algae (80°C for 24 hours) were milled to fine powder and the calorific content measured using a *Digital Data Systems* CP500 bomb calorimeter.

Triplicate samples of dry powdered algae were ashed in an oven at 500°C for five hours.

Growth rates, consumption and feed conversion efficiency

Growth rates, rates of algae consumption and FCE ratios are presented in chapter 3, Tables 1 and 2.

Growth was measured over a six month period and expressed as daily growth in shell length ($\mu m.day^{-1}$) and body weight ($mg.day^{-1}$).

Consumption of single-species algal diets was measured over 3 or 4 day periods. Daily consumption (wet weight) is expressed as the percent of wet body weight consumed per day. For the calculation of protein, energy and amino acid intake in this chapter, daily consumption of algae was expressed as a dry weight. This was done using dry weight/wet weight conversion factors for each algae.

Statistical analysis of data

Linear regression analysis was used to show the relationship between growth rates (SL and BW) and the factors analysed for in each algae. The factors were: protein content and intake, energy content and intake, ash content, amino acid intake, consumption and FCE.

The levels of essential amino acids, expressed as a percent of the total essential amino acid, for *H. midae* were regressed against the corresponding essential amino acid patterns of each algae. The level of correlation between the levels of the essential amino acid patterns of *H. midae* and an algae indicate the similarity of their essential amino acid compositions. In a perfect correlation, the levels of each essential amino acid will be identical in the abalone and the algae.

Regressions were carried out using *Statgraphics* version 6 (Manugistics Inc.).

RESULTS

Protein, energy and ash content

Protein content, energy content and ash content are expressed as percent of dry mass, $KJ.g^{-1}$ and percent dry mass respectively (Table 1).

Protein content of diets ranged from 8.13% for *Ecklonia* to 27.74% for *Porphyra*. The kelp species *Ecklonia* and *Laminaria* had similar protein content which were lower than the other algae tested. *Ulva* had a protein content similar to *Porphyra*. The measured protein content is lower than the true protein content as a consequence of tryptophan and cysteine not being measured.

Calorific values were again highest for *Porphyra* (15.81 $KJ.g^{-1}$) and *Ulva* (15.10 $KJ.g^{-1}$) while calorific values on other algae tested were similar (11.77 to 13.11 $KJ.g^{-1}$).

Ash content was lowest for *Porphyra* (19.31%) and *Ulva* (18.15%). *Ecklonia, Laminaria* and *Aeodes* had similar ash content but *Gracilaria* had a significantly higher ash content.

Table 1. Protein (% dry weight), energy ($KJ.g^{-1}$) and ash content (% dry weight) of the six single-species algal diets. Protein and ash content are given as a percent of the dry weight for each algae. Energy content is given in $KJ.g^{-1}$ of each algae.

Algae	Protein content	Energy content	Ash content
Ecklonia	8.13	11.77	25.44
Laminaria	9.1	13.11	23.13
Porphyra	27.74	15.81	19.31
Ulva	24.82	15.1	18.15
Aeodes	12.94	12.84	24.39
Gracilaria	12.31	12.57	33.8

Neither protein energy or ash content were correlated to shell length or body weight growth rates ($P > 0.05$).

Consumption and feed conversion efficiency

On a wet weight basis, *Ecklonia* was consumed in the greatest quantities and *Porphyra* was consumed in the smallest quantities (Table 2). On a dry weight basis *Ecklonia* was consumed in the greatest quantities but due to the difference in moisture content of the algae, *Ulva* was consumed in the smallest quantities. Consumption was not correlated to growth rate ($P>0.05$).

FCE ratios were highest for *Porphyra* (0.17) and lowest for *Ulva* (0.026) and *Gracilaria* (0.025). A significant correlation ($P<0.05$) was found between FCE and both shell length and body weight growth rates (R^2 = 86%). The best growth rates were produced by abalone fed on *Porphyra* and had a FCE of 0.17.

Protein and energy intake

Daily protein and energy intake were calculated from the daily consumption rates (dry weight)(Table 2). Protein intake is expressed in $mg.day^{-1}$ (per abalone) and energy intake is expressed in $KJ.day^{-1}$ (per abalone).

Table 2. Consumption, feed conversion efficiency, protein and energy intake for abalone fed on six single-species algae diets. Daily consumption shown as a percent of body weight ($\%BW.day^{-1}$) and as dry weight ($dry\ g.ab^{-1}.day^{-1}$). Protein and energy intake given as $mg.day^{-1}$ and $KJ.day^{-1}$ per abalone.

Algae	Consumption $\%BW.day^{-1}$	Consumption $dry\ g.ab^{-1}.day^{-1}$	FCE	Protein intake	Energy intake
Ecklonia	5.44	0.122	0.042	9.9	1.44
Laminaria	4.32	0.098	0.057	8.9	1.29
Porphyra	2.25	0.100	0.170	27.7	1.58
Ulva	2.92	0.070	0.026	17.3	1.05
Aeodes	2.98	0.111	0.051	14.4	1.43
Gracilaria	4.21	0.071	0.025	8.7	0.88

Protein intake was highest for *Porphyra* (27mg.day^{-1}), followed by *Ulva* (17.3mg.day^{-1}) and *Aeodes* (14.4mg.day^{-1}). Protein intake from *Ecklonia*, *Laminaria* and *Gracilaria* were similar, but these were low compared for intake from the other algae. Protein intake was positively correlated to both shell length and body weight growth rates (R^2 = 37.8% and 35.8% respectively; $P<0.05$; Table 3).

Energy intake was also positively correlated to shell length and body weight growth rate (R^2 = 48.3% and 57.7% respectively; $P<0.05$; Table 3). Intake varied from 0.88 KJ.day^{-1} on *Gracilaria* to 1.58KJ.day^{-1} on *Porphyra*.

Table 3. Table showing levels of correlation (R^2 = %) between growth rates (SL and BW) and algae protein content and intake, energy content and intake, ash content and FCE. The level of significance of the correlation is given.

Shell length growth rates:		
Factor	R^2	Significance
Protein content	10.70	0.52
Protein intake	37.75	0.03
Energy content	12.15	0.49
Energy intake	48.33	0.12
Ash content	5.32	0.66
Consumption	20.27	0.14
FCE	86.01	0.007
Body weight growth rates:		
Factor	R^2	Significance
Protein content	8.61	0.57
Protein intake	35.84	0.03
Energy content	14.47	0.45
Energy intake	57.75	0.004
Ash content	12.68	0.48
Consumption	24.44	0.10
FCE	86.76	0.006

Amino acid content of diets

Individual amino acid levels for each algal diet are expressed in mg.g^{-1} of dry algae in Table 4. *Ulva* and *Porphyra* had the highest total amino acid levels. *Ulva* had slightly higher individual amino acid levels for 13 of the 18 amino acids isolated. *Porphyra* has a proline level double that of *Ulva* and at least triple that of *Aeodes* and *Gracilaria*.

Amino acid levels for *Ecklonia* and *Laminaria* were similar, while *Aeodes* and *Gracilaria* also had similar amino acid levels but these were slightly higher.

The levels of amino acid in each diet were expressed as a percentage of the total protein (Table 5, Figure 1). Visual analysis of figure 1 confirms that little variation occurs in the amino acid composition between the six algal diets analysed. The only noticeable differences are the high levels of the amino acid proline (25.71 to 30.15 %) in *Ecklonia, Laminaria* and *Porphyra* and the high level of amino acid alanine in *Porphyra.*

Amino acid intake

Amino acid intake is expressed in grams of amino acid per abalone per day. Intake levels of each individual amino acid for each diet are tabulated in Table 6.

Generally amino acid intake was similar for the following pairs of algae: *Porphyra* and *Ulva, Aeodes* and *Gracilaria, Ecklonia* and *Laminaria.* Intake was always highest for *Porphyra* and *Ulva* with the exception of the amino acid histidine which was highest for the diet *Laminaria.*

The intake of amino acids from the diets *Aeodes* and *Gracilaria* was always higher than the diets *Ecklonia* and *Laminaria*, with the exception of proline.

Regression analysis showed that intake of proline, alanine and lysine were positively correlated to shell length growth rate ($P<0.05$) and threonine and valine were positively correlated to the shell length growth rate at the 10% significance level. Proline, alanine, histidine and lysine were positively correlated to body weight growth rate at the 5% level and threonine was correlated to body weight growth rate at the 10% level.

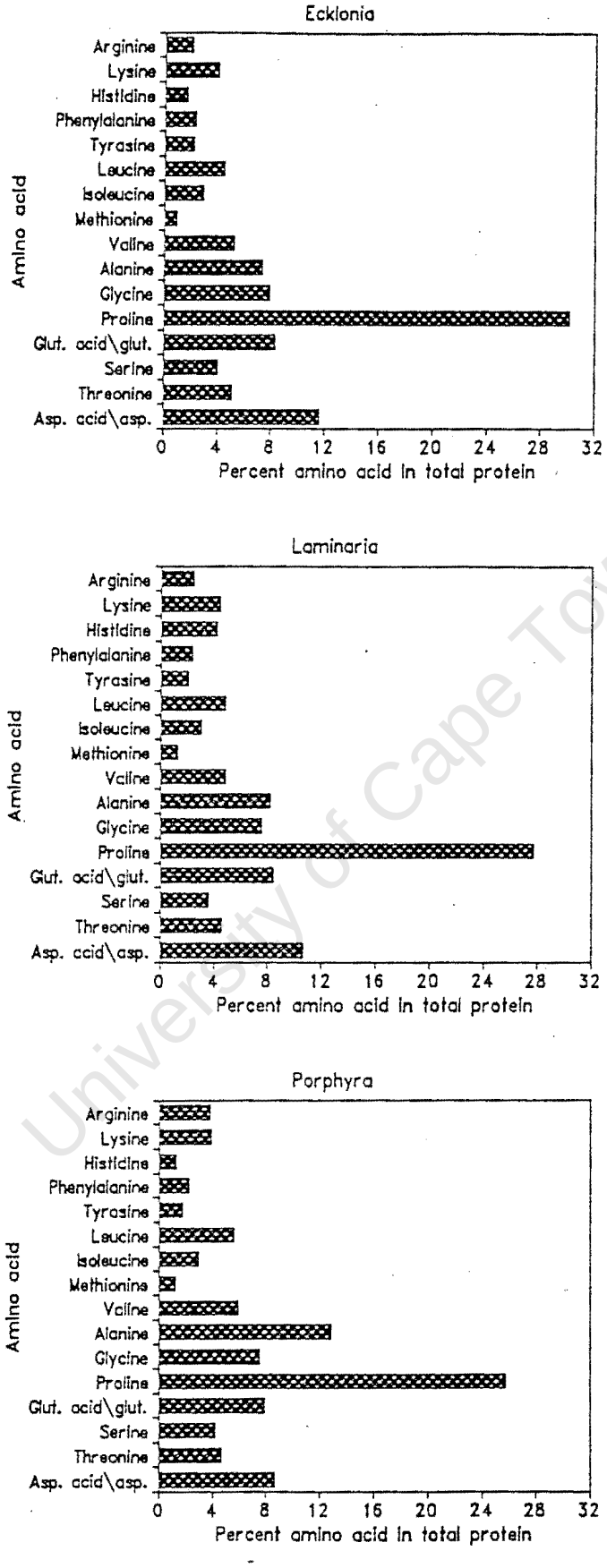


Figure 1. Amino acid composition of protein for the algal diets *Ecklonia maxima*, *Laminaria pallida* and *Porphyra capensis*.

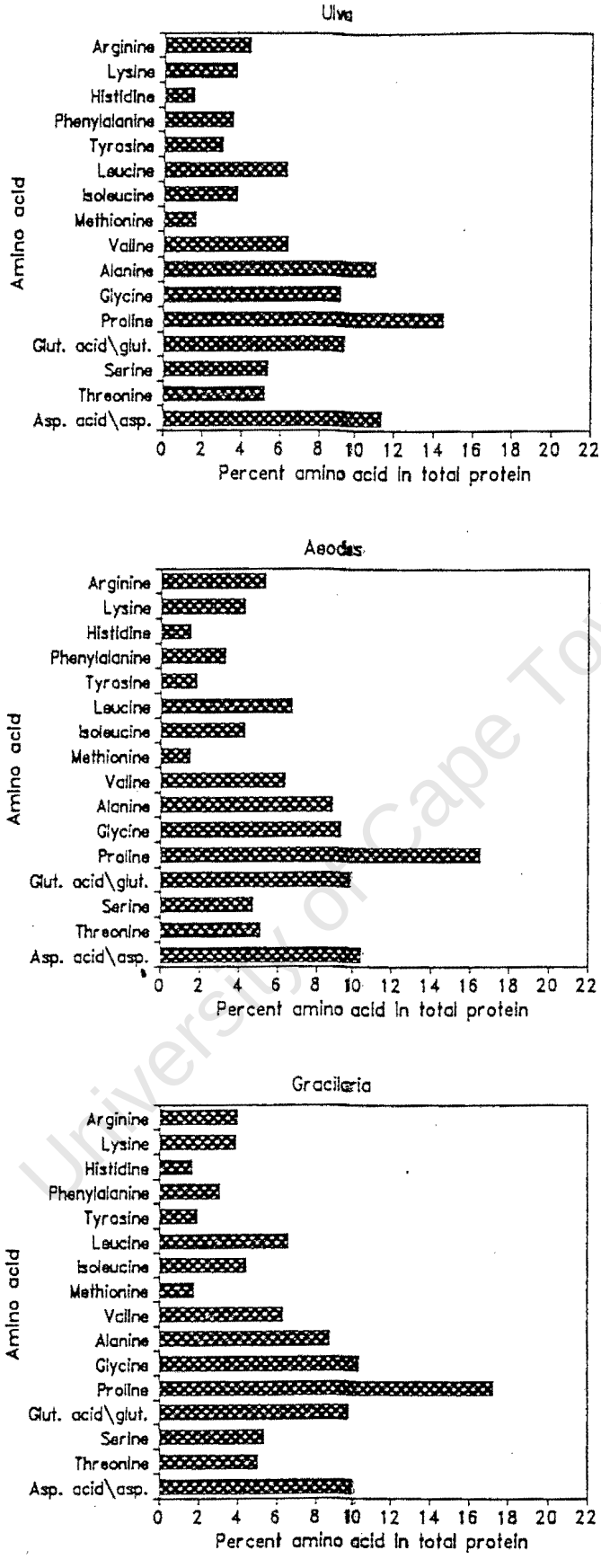


Figure 1. contd. Amino acid composition of protein for the algal diets *Ulva* spp., *Aeodes orbitosa* and *Gracilaria verrucosa*.

Table 4. Amino acid composition of the six single-species algae diets. Amino acid content given in $mg.g^{-1}$ dry algae. Aspartic acid includes asparagine and glutamic acid includes glutamine.

	Ecklonia	*Laminaria*	*Porphyra*	*Ulva*	*Aeodes*	*Gracilaria*
Aspartic acid	9.441	9.705	23.978	28.155	13.530	12.302
Threonine	4.156	4.126	12.991	12.942	6.622	6.163
Serine	3.259	3.257	11.607	13.340	6.135	6.603
Glutamic acid	6.754	7.646	21.867	23.113	12.721	11.919
Proline	24.516	25.249	71.430	35.973	21.453	21.212
Glycine	6.421	6.902	20.798	22.491	11.978	12.724
Alanine	5.960	7.462	35.588	27.418	11.392	10.707
Valine	4.221	4.379	16.325	15.741	8.217	7.768
Methionine	0.777	1.172	3.398	4.049	1.982	2.170
Isoleucine	2.326	2.784	8.209	9.374	5.561	5.463
Leucine	3.629	4.384	15.459	15.636	8.688	8.084
Tyrosine1.782	1.827	4.869	7.413	2.383	2.381	
Phenylalanine	1.851	2.117	6.155	8.756	4.292	3.793
Histidine	1.350	3.774	3.518	3.798	1.973	2.084
Lysine	3.206	3.979	10.675	9.111	5.571	4.791
Arginine	1.625	2.207	10.504	10.939	6.940	4.905

Table 5. Amino acid pattern of six single-species algal diets expressed as a percent of the total protein in each diet. Aspartic acid includes asparagine and glutamic acid includes glutamine.

	Ecklonia	*Laminaria*	*Porphyra*	*Ulva*	*Aeodes*	*Gracilaria*
Aspartic acid	11.62	10.68	8.65	11.34	10.46	10.00
Threonine	5.12	4.54	4.69	5.21	5.11	5.01
Serine	4.01	3.58	4.19	5.37	4.72	5.37
Glutamic acid	8.31	8.41	7.89	9.31	9.83	9.68
Proline	30.15	27.73	25.71	14.49	16.55	17.24
Glycine	7.90	7.59	7.50	9.05	9.25	10.34
Alanine	7.33	8.21	12.84	11.05	8.81	8.70
Valine	5.20	4.82	5.89	6.34	6.35	6.31
Methionine	0.96	1.29	1.22	1.63	1.53	1.76
Isoleucine	2.86	3.06	2.96	3.78	4.30	4.44
Leucine	4.47	4.82	5.58	6.30	6.72	6.57
Tyrosine2.19	2.01	1.75	2.99	1.84	1.93	
Phenylalanine	2.28	2.33	2.22	3.53	3.32	3.08
Histidine	1.66	4.15	1.27	1.53	1.53	1.69
Lysine	3.94	4.38	3.85	3.67	4.31	3.89
Arginine	2.00	2.42	3.79	4.41	5.36	3.99

Comparison of essential amino acid pattern of algae and *H.midae*

Essential AA pattern:

The essential amino acid pattern of an algae or abalone refers to the group of amino acids which were found to be essential to *H. rufescens* by Allen and Kilgore (1975).

Essential amino acid (EAA) composition of the six algae diets and the total animal tissue of *H. midae* (Knauer, 1994) are expressed as a percentage of total EAA (Table 7).

Table 6. Average daily intake of each amino acid for each diet. Amino acid intake in $g.day^{-1}$ per abalone. Aspartic acid includes asparagine and glutamic acid includes glutamine.

	Ecklonia	*Laminaria*	*Porphyra*	*Ulva*	*Aeodes*	*Gracilaria*
Aspartic acid	1.160	0.954	2.397	1.965	1.509	0.869
Threonine	0.509	0.405	1.299	0.903	0.738	0.436
Serine	0.399	0.320	1.160	0.931	0.684	0.467
Glutamic acid	0.827	0.751	2.186	1.613	1.418	0.842
Proline	3.004	2.481	7.141	2.511	2.392	1.499
Glycine	0.787	0.678	2.079	1.570	1.336	0.899
Alanine	0.730	0.733	3.558	1.913	1.270	0.757
Valine	0.517	0.430	1.632	1.099	0.916	0.549
Methionine	0.095	0.115	0.340	0.283	0.221	0.153
Isoleucine	0.285	0.274	0.821	0.654	0.620	0.386
Leucine	0.445	0.431	1.545	1.091	0.969	0.571
Tyrosine0.218	0.180	0.487	0.517	0.266	0.168	
Phenylalanine	0.227	0.208	0.615	0.611	0.479	0.268
Histidine	0.165	0.371	0.352	0.265	0.220	0.147
Lysine	0.393	0.391	1.067	0.636	0.621	0.339
Arginine	0.199	0.217	1.050	0.763	0.774	0.347

There was significant correlation between the EAA patterns of *Porphyra, Ulva, Aeodes* and *Gracilaria* and the EAA pattern of *H. midae* ($P<0.05$). The highest correlation coefficient was between *H. midae* and *Aeodes* ($R^2 = 62.85\%$, $P<0.05$) while the lowest significant correlation was between *H. midae* and *Gracilaria* ($R^2 = 42.39\%$, $P<0.05$). The correlation between EAA pattern of *H. midae* and *Porphyra* and between *H. midae* and *Ulva* were similar. The level of correlation between the EAA levels of *H. midae* and corresponding EAA level patterns of the algal diets was not related to either shell length or body weight growth rates.

Table 7. Essential amino acid pattern expressed as a percent of the total amino acid for the six single-species algal diets tested.

	H. midae		*Ecklonia*	*Laminaria*	*Porphyra*	*Ulva*	*Aeodes*	*Gracilaria*
Threonine	10.83		16.68	13.42	14.10	13.24	12.68	12.95
Valine	9.98		16.93	14.24	17.72	16.10	15.73	16.32
Methionine	4.52		3.12	3.81	3.69	4.14	3.79	4.56
Isoleucine	8.76		9.33	9.05	8.91	9.59	10.65	11.48
Leucine	14.97		14.56	14.26	16.78	15.99	16.63	16.98
Tyrosine8.20		7.15	5.94	5.29	7.58	4.56	5.00	
Phenylalanine	8.38		7.43	6.89	6.68	8.96	8.22	7.97
Histidine	3.95		5.42	12.27	3.82	3.88	3.78	4.38
Lysine	13.37		12.86	12.94	11.59	9.32	10.67	10.07
Arginine	17.04		6.52	7.18	11.40	11.19	13.29	10.30

Table 8. Essential amino acid pattern expressed as a ratio to the amino acid lysine. Limiting amino acids are underlined.

	H. midae	*Ecklonia*	*Laminaria*	*Porphyra*	*Ulva*	*Aeodes*	*Gracilaria*
Threonine	80.99	129.64	103.68	121.69	142.04	118.88	128.63
Valine	74.65	131.64	110.04	152.92	172.76	147.50	162.12
Methionine	33.80	24.24	29.44	31.83	44.43	35.57	45.30
Isoleucine	65.49	72.54	69.96	76.90	102.88	99.83	114.02
Leucine	111.97	113.18	110.18	144.81	171.61	155.96	168.72
Tyrosine	61.34	55.57	45.92	45.61	81.37	42.78	49.69
Phenylalanine	62.68	57.75	53.21	57.66	96.10	77.05	79.17
Histidine	29.58	42.12	94.86	32.95	41.68	35.42	43.51
Lysine	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Arginine	127.46	50.67	55.47	98.40	120.06	124.59	102.36

Ratio of essential amino acids relative to lysine:

After calculation of amino acid ratios (relative to lysine), limiting essential amino acids in the diets are identified as being present at a lower ratio level than that found in *H. midae*, and are underlined (Table 8). Comparison of lysine related EAA ratios of *H. midae* with those of the six diets showed *Ulva* to be the least limiting diet with only one limiting amino acid (arginine). The most limiting diet was *Laminaria* with five limiting amino acids. *Ecklonia* and *Porphyra* had four limiting amino acids, while *Aeodes* and *Gracilaria* had two limiting amino acids. All algae were limiting in arginine and all except *Ulva* were limiting in tyrosine. The number of limiting essential amino acids was not related to growth rates.

DISCUSSION

Proximate analysis of a diet may be used only as a superficial indicator of quality. Regression of protein, energy and ash content with growth rates for the six algal diets tested showed that none of these factors were related to growth. The protein or energy content of a diet has little bearing on its digestibility and therefore the absorption of energy and protein into the body. Fleming (1994b) found nitrogen content of algae to be related to preference but not to growth rate.

Results of the *Ulva* diet trial demonstrate the lack of correlation between either protein or energy content and growth rate. *Ulva* has a high protein (24.82% dry weight) and energy content (15.1 $KJ.g^{-1}$) but produces growth rates inferior to all other diets tested. Similar low growth rates are reported for other species of abalone fed on *Ulva* species (Owen *et al*, 1984; Fleming 1994a,b) as well as sea urchins fed on *Ulva* (Gonzalez *et al*, 1993). This phenomena may be a result of low consumption rates or the presence of antinutritional factors in *Ulva* (Fleming, 1994b).

Antinutritional factors form an important aspect of the optimum foraging (Freeland and Jansen, 1974) and chemical defence theory (Jones, 1979; Anderson and Velimirov, 1982; Tugwell and Branch, 1992; Winter and Estes, 1992). Little is known about the antinutritional factors found in *Ulva*. These reduce growth rates to low levels either by reducing intake or by inhibiting digestion and metabolism (Mercer *et al.*, 1993). Fleming (1994b) suggests that low growth rates on *Ulva* species may also be caused by the mechanical limitation of consuming an alga of two cells thickness. This argument is not supported by this study. Abalone fed a diet of *Porphyra* yielded good growth rates (chapter 2, 3; Uki *et al*, 1986) and *Porphyra* has a similar thickness to *Ulva*.

Intake of protein and energy are better determinants of growth than the protein and energy content of the algal diets. Protein intake is an index of the amount of protein available for absorption into the body. This protein can be use in growth and other

metabolic functions. Energy intake provides an estimate of the amount of energy available for growth and metabolism. Intake, of protein and energy, is only a rough estimate of the protein and energy available for use in the body, as absorption efficiencies may differ on different diets and are dependent on the physiological state of the animal (Lloyd *et al.*, 1959).

Both protein and energy intake were found to be significantly correlated to shell length and body weight growth rates in the present study. *Ulva* produced an 'irregular' point in the protein intake regression with a high protein intake but a low growth rate.

In the gut, protein is broken down into amino acids which are absorbed into the body and used in protein synthesis, synthesis of nitrogen-containing compounds or degradation into nitrogen and carbon skeletons which are used as an energy source (Lloyd *et al*, 1978). Amino acid intake yields an approximation of the amount of AA available for these functions and can give an indication of the more limiting amino acids in the diet.

Allen and Kilgore (1975) found that ten amino acids were essential to *Haliotis rufescens*. These were: threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, lysine, histidine and arginine. These were not produced by the body and must therefore be supplied in the food.

Regression of amino acid intake versus growth rates, showed that the level of intake of proline, alanine, histidine and lysine was significantly correlated to growth rate. This suggests that these are the most limiting amino acids for *H. midae* fed the algal diets tested, and that their supply in significant quantity in an algal diet is essential. It is interesting to note that proline and alanine are non-essential amino acids (Allen and Kilgore, 1975) but appear to be limiting amino acids in this study.

Allen and Kilgore's (1975) work showed that abalone required the same essential amino acids as salmon and growing rats (Meister, 1965), and the shrimp *Paleamon serratus* (Cowey and Forster, 1971), but different essential amino acids to man, dogs and mice (Meister, 1965). It is generally assumed that the same essential amino acid

are required by different species of abalone, although no work has been done on the essential amino acid requirements of species other than *H. rufescens* to support this. Non-essential amino acids, such as proline and alanine, can be produced by abalone (Allen and Kilgore, 1975) but the results of the present study suggest that their supply in an algal diet is important. Production of non-essential amino acids by the body will probably support maintenance of body tissue but may not be sufficient for growth.

Regression of the levels of the essential amino acids of *H. midae* with the levels of the essential amino acids for each diet did not satisfactorily describe growth rates on the algal diets. The level of correlation between the levels of EAA of *H. midae* and the levels in the various algal diets was not related to the growth rates experienced on the diets. Knauer (1994) used this method to select protein sources for the formulation of an artificial diet. This method may have been satisfactory for use in artificial diet formulation but it does not successfully describe the quality of algal diets.

Fleming and van Barneveld (1994) suggested using the ratio of essential amino acids to lysine to compare abalone tissue with various protein sources for artificial diets. Lysine is chosen as the reference amino acid as it is normally the first and major limiting amino acid for agricultural species fed on cereal-based diets (Batterham, 1992). In this study the lysine level in diets were found to be significantly correlated to growth rates. If the requirement for lysine is met then, theoretically, all other essential amino acids should be supplied in sufficient quantity (Fleming and van Barneveld, 1994). This method showed arginine to be the most limiting amino acid, followed by tyrosine, methionine and phenylalanine. Mia *et al* (1994) found high arginine levels in the body of *H. tuberculata* and *H. discus hannai* and suggest that this is likely to be one of the first limiting EAA in most foods..

Comparison of these ratios between *H. midae* and the six algae suggest that growth rates should be highest for *Ulva* followed by *Aeodes* and *Gracilaria* as these diets have the least number of limiting essential amino acids. Growth rates do not correlate with these rankings, which suggests that this is not a satisfactory method for determining

the quality of the algal diets tested in this study. This index may be satisfactory for the appraisal of artificial diets, due to the absence of antinutritional factors which may inhibit consumption, digestion or absorption.

The failure of the essential amino acid pattern and ratio methods for determination of a good quality algal diet may be due to the high variation in digestibility of the algal diets. The high variation in digestibility of algae is caused by differences in morphology and biochemical composition of algae. Artificial diets are generally uniform in morphology and biochemistry and are not impregnated with antinutritional factors. If algal diets were uniformly digestible and the absorption efficiencies were uniform over the range of diets, then the essential amino acid patterns and lysine related ratios may have better described the growth rates experienced on each diet.

In this study protein and energy intake was related to shell length and body weight growth rate. These were linear relationships. It is unlikely that any algae produce levels of protein or energy that will exceed the optimum requirements for abalone. In this study, the optimum daily intake of protein was 27.7 $mg.day^{-1}$ and energy intake was 1.58 $KJ.day^{-1}$, per abalone of approximately 45mm in shell length. Intake of the amino acids lysine, histidine, proline and alanine were also found to be related to growth rates. This shows that non-essential amino acids (proline and alanine) may also be limiting in algal diets. Non-essential amino acids should be considered in selecting protein sources for artificial foods.

Further investigation into the amino acid requirements of *H. midae* needs to focus on the absorption efficiency of each amino acid. This method should be applied to both natural and artificial diet research to determine the required levels of each amino acid.

CHAPTER 5

AN INVESTIGATION INTO THE USE OF ALGAE AS A MAJOR COMPONENT OF ARTIFICIAL FEEDS FOR JUVENILE *HALIOTIS MIDAE*

INTRODUCTION

Until recently, dietary research on cultured abalone dealt with algal diets only (e.g. Sakai, 1962). More emphasis is now being placed on the development of artificial diets. Work on artificial diets has been encouraged by the difficulties associated with supplying sufficient algae for farmed abalone. Problems associated with natural diets are seasonal availability, lack of abundance, the affects of weather on harvesting and tank fouling. Artificial diets are easily manufactured, stored and fed to abalone.

Artificial diets have been shown to produce better growth rates than naturally occurring algae diets (Uki *et al.*, 1985; Viana *et al.*, 1993). Artificial feeds for abalone have been researched in depth, to the extent of improving growth by controlling the levels of each essential amino acid (Knauer, 1994). This complex approach to diet production has resulted in the costs of diets escalating to proportions that threaten the economic viability of abalone mariculture.

Algal diets provide practical difficulties for abalone farmers, especially when dealing with very small juveniles. Thus, an artificial diet would be most useful if produced for just weaned juveniles.

A number of algal species exist which give growth rates comparable to those produced by artificial diets, i.e. over 3mm per month (Uki *et al*, 1986). These diets are usually high in protein, and have a good balance of nutrients, protein and energy (chapter 4).

The aim of this study was to determine whether it would be possible to produce an artificial diet which incorporates powdered algae as its major component. The algae could be supplemented with a rich source of protein (fishmeal), lipid (sunflower oil)

and a vitamin and mineral mixture. This mixture would be bound to form a dry artificial diet. It is possible that the incorporation of dry algae could reduce costs significantly.

MATERIALS AND METHODS

Two artificial diet trials were carried out. In the first trial abalone were fed gelatine bound diets, while in the second, diets were alginate bound.

Test animals were fed a diet of *Ecklonia maxima* prior to the first measurement, after which they were fed on their respective test diets. This ensured that test animals undergoing different diet treatments were of similar size and condition at the commencement of the experiment, and were subject to the effects of the same dietary history.

Test animals

Gelatine bound diet trial:

Four hundred and twenty hatchery reared abalone (*H. midae)* were used to test the growth response to six gelatine bound artificial diets. Animals were not individually numbered. *Ecklonia maxima* was used as the control diet, as it is the most likely algal diet to be used on abalone farms. Abalone were approximately 6 months old, 12.37 mm (±0.18 mm) average length and 255 mg (± 7 mg) average weight.

Groups of 30 abalone were placed in two litre transparent plastic containers supplied with temperature controlled fresh aerated sand-filtered sea water (12 $l.h^{-1}$), at 18.5°C (±1°C). Each diet was tested in duplicate (n=60) over an 88 day period.

Feeding of artificial diets was at two day intervals and feeding of the control diet was every three to four days. The experimental containers were cleaned at every feeding to remove faeces that were not dislodged by the strong aeration.

Shell length and body weight were measured every three weeks. Shell length was measured using manual vernier callipers and body weight was measured using a Sartorius balance.

Alginate bound diet trial:

Four hundred hatchery reared abalone (*H. midae)* were used to test growth response to five alginate bound artificial diets. Abalone were not individually numbered. Five algae were used singly in each artificial diet, as well as being fed as single-species control diets. The five algae species were: *Ecklonia maxima, Porphyra capensis, Ulva* spp., *Aeodes orbitosa* and *Gracilaria verrucosa.* Abalone were 8 months old, 14.49 mm (± 0.33 mm) average length and 520 mg (± 0.45 mg) average weight.

Experimental apparatus was as described for the trial of gelatine bound diets. Growth response was tested in duplicate on groups of 20 abalone (n=40 per diet).

Alginate bound artificial diets were fed daily as they tended to disintegrate in water. Single-species algal diets were fed every three to four days. Plastic containers were washed after every feeding.

Growth response was measured over a 28 day period. Measurement took place at the beginning and end of the 28 day period, using a digital vernier callipers and a Sartorius balance.

Artificial diet preparation

Gelatine bound diets:

Diet formulations are presented in Table 1. Dry powdered algae made up 50 percent of the dry weight of diet ingredients. Algae was mixed with fishmeal and a mineral and vitamin mix as recommended by Hahn (1989). Gelatine constituted 20 or 25 percent of the dry ingredients and was mixed with hot water at a concentration of 1 millilitre water per gram dry diet ingredients. The remaining dry ingredients were added to the gelatine solution and mixed. The resulting mixture was poured into petri dishes, to 5 millimetres depth, and allowed to set in a refrigerator. The resultant gel was cut into strips, 5 cm by 1 cm, and fed to the test animals.

Table 1. Diet formulations for gelatine (top) and alginate (bottom) bound diets. All ingredients given as a percentage of the total dry mass.

Name	Gel-Eck	Gel-Lam	Gel-Por	Gel-Gra	Gel-Bi	Gel-Ulv
Algae	*Ecklonia*		*Laminaria*	*Porphyra*	*Gracilaria*	Bi-product *Ulva*
Algae	50	50	50	50	50	50
Fishmeal	25	25	25	25	20	20
Vit/Min	5	5	5	5	5	5
Gelatine	20	20	20	20	25	25

Name	Algin-Eck	Algin-Por	Algin-Ulv	Algin-Aeo	Algin-Gra
Algae	*Ecklonia*	*Porphyra*	*Ulva*	*Aeodes*	*Gracilaria*
Algae	40	40	40	40	40
Fishmeal	30	30	30	30	30
Semolina	5.8	5.8	5.8	5.8	5.8
Vit/Min	5	5	5	5	5
Oil	5	5	5	5	5
Alginate	11.2	11.2	11.2	11.2	11.2
Catalyst	3	3	3	3	3

Alginate bound diets:

Alginate bound diet formulation is presented in Table 1. Dry powdered algae and fishmeal accounted for 40 and 30 percent of the dry ingredients. The mineral and vitamin mix was added as in the gelatine bound diets. Lipid was supplied in the form of sunflower oil which accounted for 5 percent of the ingredient weight. Semolina, a fine flour, was also added to aid binding.

Initially, sodium alginate was used as the binder as it had been used in previous diets (Uki and Watanabe, 1992; Viana *et al*, 1993). Sodium alginate was later replaced by potassium alginate which is more easily available and shows similar binding capabilities (own findings). Potassium alginate was supplied as an unrefined raw kelp extract with a moisture content of 96 percent (Kelp Products Pty.).

Different concentrations of alginate were tested in preliminary diets. Concentrations ranging from 2 percent to 15 percent dry weight alginate were tested. A concentration of 11.2 percent was found to be acceptable and was used in subsequent experimental diets. Higher binder levels resulted in excessive moisture levels which reduced efficiency of pelet formation.

All ingredients,except the catalyst, were mixed and then added to the alginate. The catalyst, calcium sulphate ($CaSO_4$), was mixed with warm water before addition to the other ingredients. The quantity of warm water added with the catalyst was dependent on the algae used in a particular diet. The final mixture was stirred and then microwaved for 5 to 10 minutes, stirring every 2 minutes.

Subsequently the mixture was extruded using a small hand operated sausage mincer. The sausage mincer die produced ribbons of 1 cm wide, 3 mm deep and of varying lengths. These were dried in a convection oven at 80^{o} C for three hours.The drying procedure caused the ribbons to shrink by approximately 50 percent.

Growth, consumption and feed conversion efficiency

Body weight (BW) and shell length (SL) growth rates (GR) were calculated using the following equations:

$$BWGR = (W_{tf} - W_{ti})/t$$

$$SLGR = (L_{tf} - L_{ti})/t$$

where W_{ti} and L_{ti} are mean initial weight and length, W_{tf} and L_{tf} are mean final weight and length and t = time in days.

Feed consumption (F) and feed conversion efficiency (FCE) were calculated for the alginate bound diet trial only. The gelatine bound diet was only used as a preliminary indicator of growth rates, and therefore consumption was not measured. Approximately 1.5g of alginate bound diet was supplied to each jar (n=20 abalone) at each feeding. Feed consumption was measured over five separate nights. Food was supplied at 5:00 pm each night and uneaten food was removed the next morning at 10:00 am; this time period corresponds with the time period used to test diet stability in water (see below).

Single-species algal diets were supplied to control animals in sufficient quantity to ensure feeding to satiation. Consumption of algae was measured over three or four nights. Daily feed consumption (F) was measured using the following equation (Uki and Watanabe, 1992):

$$F = (GS/100) - R$$

where G is the amount of diet supplied, S is the percentage recovery of the diet (100 - the leaching rate) and R is the diet remaining after the abalone have fed. Feed consumption for artificial diets was measured on a dry weight basis whereas

consumption of control diets was on a wet weight basis. This was converted to daily consumption as a percentage of body weight.

Feed conversion efficiency (FCE) was calculated as follows (Uki, 1981):

$$FCE = W/F$$

where W is the daily weight gain and F is the daily feed consumption.

Diet analysis

Fish meal, supplied by a local fishing company, had a protein content of 67 percent (Concentra Ltd). Protein content of the five algal species used in the artificial diets is given in chapter 4 (table 1).

Protein content of the alginate bound diets was calculated by summing the protein content of the fishmeal and algal portions.

Calorific content of the alginate bound artificial diets was calculated in triplicate using a Digital Data Systems CP500 bomb calorimeter. Triplicate samples of artificial diet were ashed at 500°C for five hours to determine ash content (percent).

Diet stability

Water stability of alginate bound diets were determined over a 17 hour period, under the same conditions as the growth experiments, except that abalone were absent. Similarly, the weight variation of the single-species algal control diets was measured over a 3 night period. These time periods were chosen to correspond to the consumption measurement periods in the diet trials.

Statistical analysis

Mean shell length and mean body weight were analysed using a two-way ANOVA, for the effects of diet and time (diet x time) on growth. A Tukey HSD multiple range test was then applied to test for significant differences in mean shell length and mean body weight between diet treatments. Significant differences were not apparent between diet treatments. Therefore, a one-way ANOVA was used to analyse for differences in growth rates between diet treatments.

RESULTS

Gelatine bound diet trial

Growth rates in the preliminary diet trial, where gelatine was used as the binder, showed that good growth can be achieved using a diet which has algae as the main constituent.

Shell length and body weight growth rates for abalone fed gelatine bound artificial diets were greatest for Gel-Por (0.067mm.day^{-1} and 6.5mg.day^{-1}; Table 2, Figure 2).

Table 2. Shell length and body weight growth rates on gelatine bound diets (n=2).

	Shell Length (mm/day)	Body Weight (g/day)
Ecklonia	0.050	0.0042
Gel-Eck	0.020	0.0020
Gel-Lam	0.040	0.0038
Gel-Por	0.067	0.0065
Gel-Gra	0.054	0.0051
Gel-Bi	0.012	0.0009
Gel-Ulv	0.045	0.0045

Gel-Gra produced the next highest growth rates of 0.054mm.day^{-1} and 5.1mg.day^{-1}. Gel-Bi produced the lowest growth rates at 0.012mm.day^{-1} and 0.9mg.day^{-1}. *Ecklonia*, Gel-Lam and Gel-Ulv produced similar body weight growth rates. Two of the six gelatine bound diets (Gel-Por and Gel-Gra) produced shell length and body weight growth rates higher than the control diet, *Ecklonia*.

Alginate bound diet trial

Leaching rates (over 17 hours) of alginate bound artificial diets varied from 12.44 % (Algin-Eck) to 21.51 % (Algin-Gra). The diets Algin-Eck and Algin-Ulv (13.23 %) were firm after 17 hours, whereas Algin-Por (20.57%) and Algin-Aeo (14.89%) were partially disintegrated and soft after the same time period.

The algae *Ecklonia* and *Gracilaria* had negligible variation in weight over the three night period. The stability of *Porphyra* (-6.25%), *Ulva* (+2.11%) and *Aeodes* (+3.92%) varied over the stability test period. This variation was taken into account in calculations.

Due to the slight variation in initial size, the one month experimental period was insufficient to detect a significant difference in mean shell length and mean body weight between diet treatment groups. Significant differences were apparent between initial and final measurements ($P < 0.01$) for all treatments. A one-way ANOVA which was performed on final measurements revealed significant differences between certain diet treatment groups.

Shell length growth rates (n=2) were highest for the algae *Porphyra* (0.109mm.day^{-1}) followed by the alginate bound diet Algin-Por (0.087mm.day^{-1}) and algae *Ecklonia* (0.076mm.day^{-1}) (Table 3, Figure 3). There was no significant difference between growth rates on these diets.

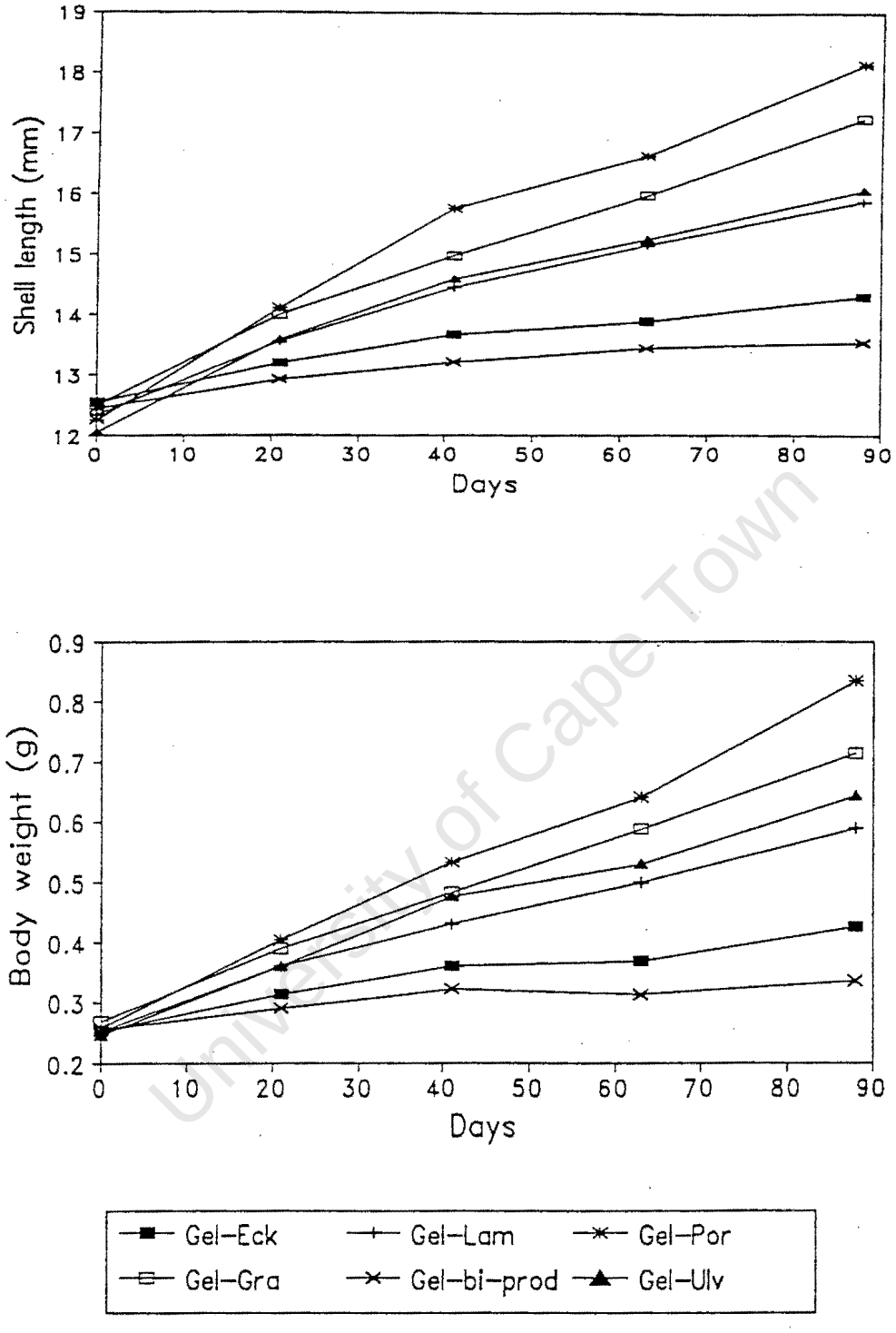


Figure 1. Shell length (top) and body weight (bottom) growth curves for gelatine bound artificial diets.

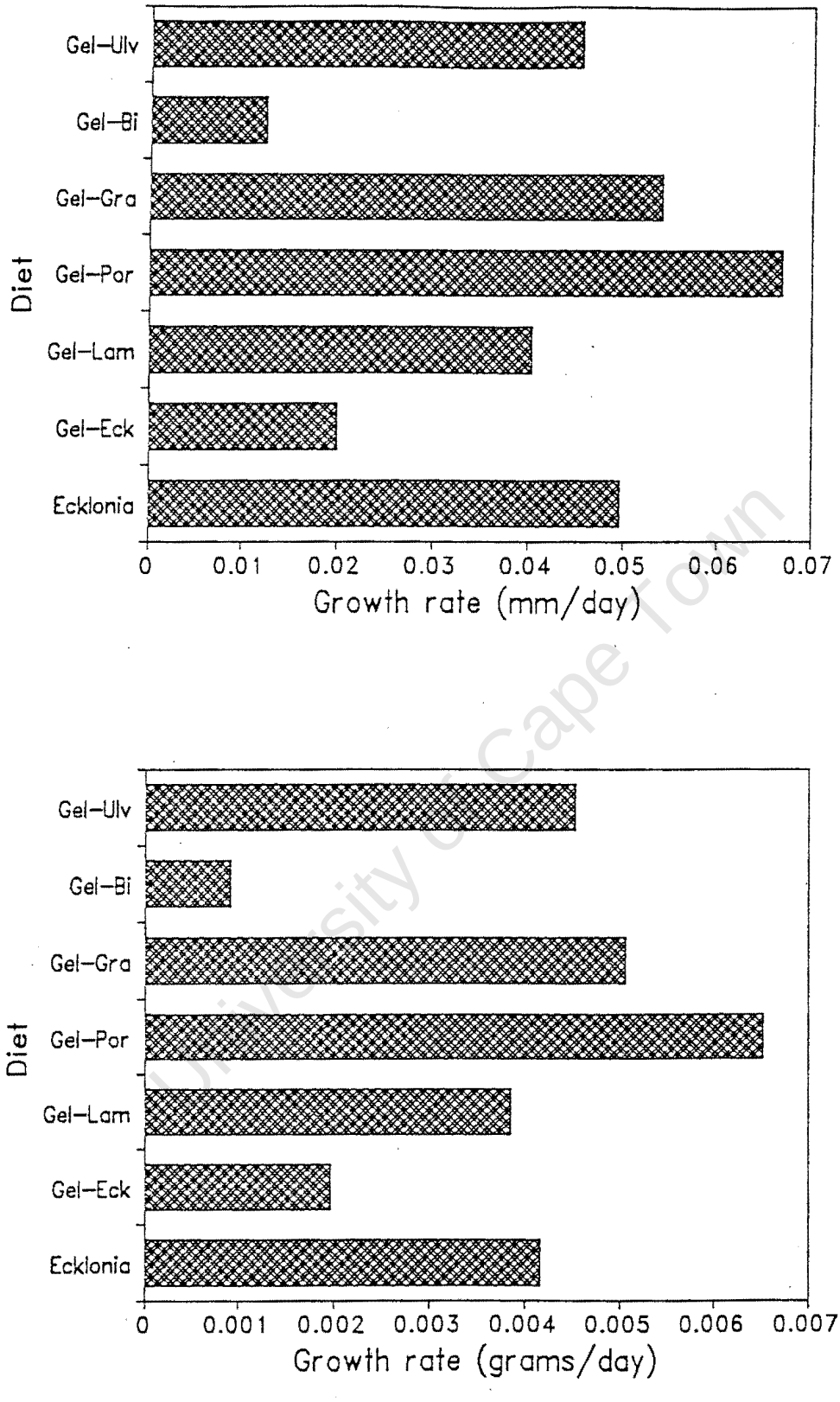


Figure 2. Daily shell length (top) and body weight (bottom) growth rates for gelatine bound artificial diets.

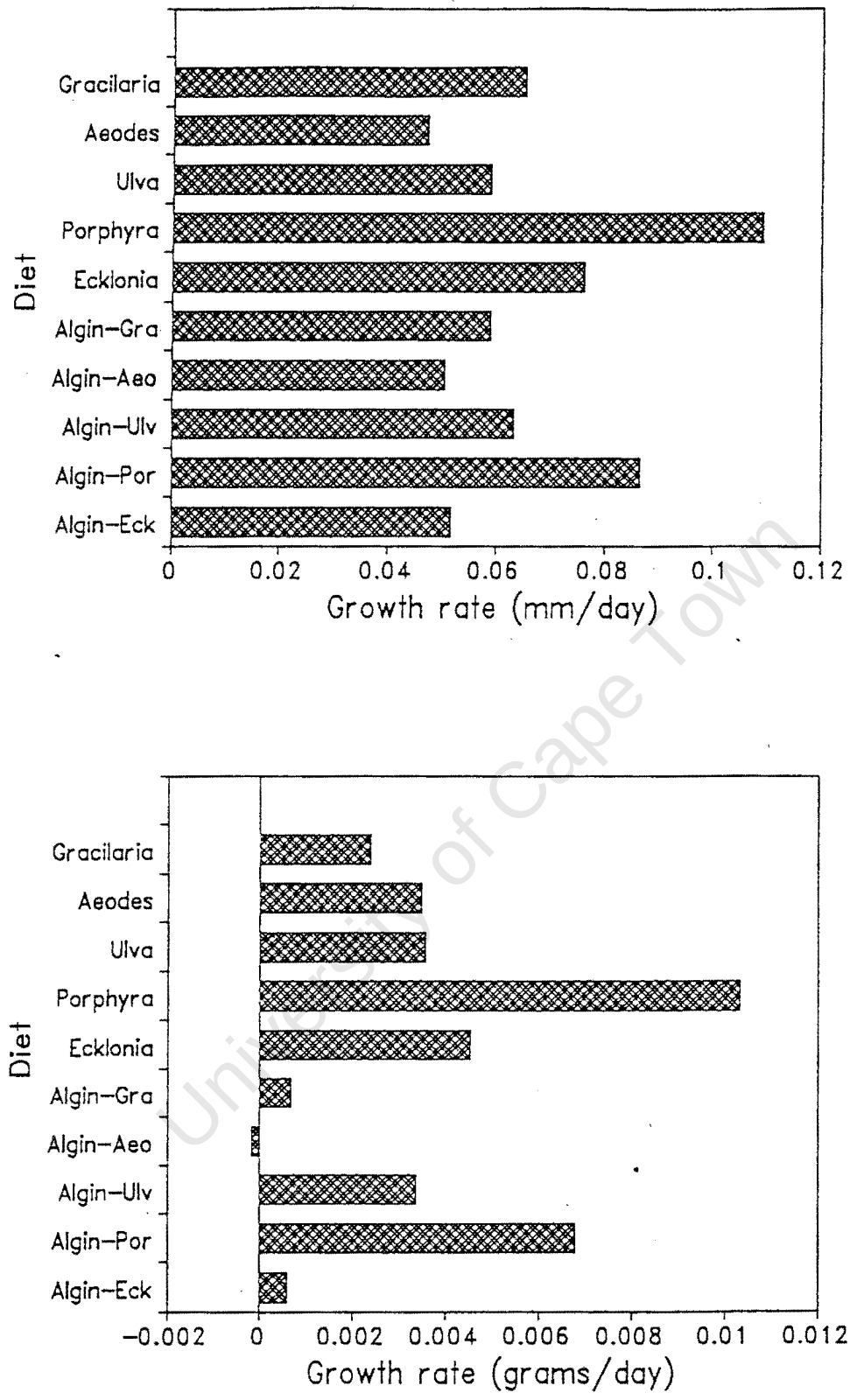


Figure 3. Daily shell length (top) and body weight (bottom) growth rates for alginate bound artificial feeds.

Table 3. Initial and final shell length (mm) and body weights (mg) for alginate bound diet treatment and algal control diets. Also shown are growth rates; shell length ($mm.day^{-1}$), body weight ($mg.day^{-1}$).

Diet description	Initial length (mm)	Standard deviation	Final length (mm)	Standard deviation	Growth rate (mm/day)
Arteck	15.10	3.03	16.55	3.23	0.05
Artpor	14.74	3.00	17.17	3.21	0.09
Artulv	14.17	2.66	15.94	3.02	0.06
Artaeo	14.53	2.04	15.95	2.19	0.05
Artgra	14.43	2.44	16.09	2.64	0.06
Ecklonia	14.35	2.39	16.49	2.56	0.08
Porphyra	13.91	1.89	16.96	2.24	0.11
Ulva	14.65	2.13	16.30	2.25	0.06
Aeodes	14.33	2.21	15.65	2.42	0.05
Gracilaria	14.72	2.00	16.55	2.14	0.07

Diet description	Initial weight (mg)	Standard deviation	Final weight (mg)	Standard deviation	Growth rate (mg/day)
Arteck	599.78	428.43	615.95	406.70	0.58
Artpor	536.53	344.38	726.58	434.22	6.79
Artulv	477.20	321.99	570.49	345.14	3.33
Artaeo	535.25	263.24	531.29	250.63	-0.14
Artgra	510.95	300.96	529.83	298.21	0.67
Ecklonia	499.38	279.00	625.85	321.99	4.52
Porphyra	457.85	217.53	746.48	278.78	10.31
Ulva	536.00	280.94	635.56	256.72	3.56
Aeodes	504.03	314.44	601.43	338.05	3.48
Gracilaria	595.78	474.30	662.80	291.69	2.39

The lowest shell length growth rates were produced by abalone fed on *Aeodes*, Algin-Aeo and Algin-Eck (0.047, 0.051 and $0.052mm.day^{-1}$ respectively). No significant differences ($P > 0.05$) in shell length growth rates were visible between each single-species algae diet and the algae's corresponding artificial diet.

Body weight growth rates were more variable than shell length growth rates (Table 3, Figure 3). A trend was apparent, showing body weight growth rates to be higher on single-species algae diets than on the corresponding artificial diets.

Table 4. Consumption as a percent of BW per day, FCE, protein content (% of dry mass), calorific value (KJ.dry g^{-1}) and percent ash of alginate bound artificial diets and algal control diets. Consumption and FCE ratios for algae are on a wet weight basis.

Diet	Consumption %BW/day n=6	Standard deviation	FCE n=6	Standard deviation	Protein content (%)	Calorific value (Kj/g)	Ash content (%)
Algin-Eck	0.9444	0.6635	0.1575	0.1052	22.9	13.66	28.53
Algin-Por	2.9452	1.4203	0.3601	0.1523	30.74	15.64	24.6
Algin-Ulv	1.4293	3.0049	0.4864	0.1166	29.58	15.59	25.51
Algin-Aeo	3.3617	2.1222	-0.0244	0.0262	24.83	14.51	28.51
Algin-Gra	0.6118	0.5337	0.1082	0.2540	24.57	14.35	29.52
Ecklonia	12.5964	3.5364	0.0568	0.0201	8.13	11.77	25.44
Porphyra	4.6449	1.0643	0.2592	0.0766	27.73	15.81	19.31
Ulva	5.7774	7.4239	0.3606	0.4534	24.82	15.1	18.15
Aeodes	3.2594	1.1334	0.1816	0.0622	12.94	12.85	24.39
Gracilaria	13.0531	3.0790	0.0289	0.0157	12.31	12.57	33.8

With the exception of Algin-Por (6.7mg.day^{-1}), the body weight growth rate on *Porphyra* (10.3mg.day^{-1}) was significantly higher than all other diets. A negative body weight growth rate was produced by abalone fed a diet of Algin-Aeo (-0.178 mg.day^{-1}).

Consumption and FCE

Feed consumption by abalone was measured on a wet weight basis for algae and a dry weight basis for artificial feeds. The consumption of Algin-Aeo was higher than the other artificial diets. Feed conversion efficiencies were highest for the diets Algin-Por and Algin-Ulv. FCE was negative for the diet Algin-Aeo. FCE for algal diets was highest for abalone fed on a diet of *Porphyra*.

DISCUSSION

Artificial diets are a viable approach to solving the feed provision problems associated with commercial abalone mariculture (Uki *et al.*, 1985; Morrison and Whittington, 1991; Mozqueira, 1992; Viana *et al.*, 1993), but the main problem with artificial foods is the cost. The cost of the artificial diets produced in this study was reduced by the addition of low cost fine ground algae which replaces other more expensive ingredients such as dextrin and cellulose, which fulfil the same purpose.

Two gelatine bound diets produced satisfactory growth rates when compared to the control diet *Ecklonia*. The maximum shell length growth rate was achieved by abalone fed on Gel-Por and equated to 2.01mm.month^{-1}. The growth rates (SL and BW) on the gelatine bound diets Gel-Por and Gel-Gra were higher than for the control diet *Ecklonia*. This result supports previous studies (e.g. Viana *et al.*, 1993) which have shown that artificial diets can produce higher growth rates than natural algae diets. It is important to realize that this comparison is dependent on the quality of the algal control diet chosen. This point was considered in the alginate bound artificial diet trial. Each alginate bound artificial diet had a control diet of the same algae. This allowed for comparison between artificial diets and the algae which made up a large part of it's composition. This comparison is necessary as there is no point in producing an artificial diet which produces lower growth rates than the algae that makes up a large portion of its composition.

Statistical analysis of shell length growth rates indicate that, although algal diets always registered higher growth rates than the corresponding alginate bound artificial diets, there was no apparent significant difference between them. There were however, significant differences between BW growth rates on algae and their corresponding alginate bound artificial diets. BW growth rates were significantly lower for juvenile *H. midae* fed artificial diets.

SL and BW growth rates, for abalone fed on alginate bound artificial diets, varied from 0.051 to 0.087 mm.day^{-1} and -0.18 to 6.78 mg.day^{-1} respectively. SL and BW growth rates, for abalone fed on the five algae diets, ranged from 0.047 to 0.109mm.day^{-1} and 2.39 to 10.31mg.day^{-1}.

Besides abalone being pre-adapted to digestion of algae, the algae may also function as a feeding stimulant. According to Harada and Akishima (1985) gastropods are attracted by large amounts of acidic amino acids excreted by algae, as well as carbohydrate levels in algae. This could explain the high ingestion rate of Algin-Por and Algin-Ulv. These diets had high protein (30.74 and 29.58 percent; chapter 4) and carbohydrate content (15.64 and 15.59 KJ.g^{-1}; chapter 4). Algae such as *Porphyra* were found to be preferred by *H. midae* (Stepto, 1993) and in addition, abalone fed on these algae produce good growth rates (chapter 2 and 3).

FCE was highest for the diet Algin-Ulv followed by the diet Algin-Por. The high FCE ratios and high consumption rates equate to the good growth rates (both SL and BW) achieved for abalone fed on these diets. The FCE ratios in this study are low compared to previous studies. Brits (in press) produced FCE ratios between 0.99 and 1.40 for *H. midae* fed on artificial diets. Uki and Watanabe (1992) found ratios to fall between 0.57 and 1.23 for *H. discus hannai* fed on artificial diets. In the previous studies, FCE ratios were low on diets where fishmeal was used as the protein source.

Fishmeal was used as the protein source in this study which may explain the low FCE ratios. Low FCE ratios may result from an inability to digest and assimilate fishmeal. Uki and Watanabe (1992) found that heat treatment of fishmeal and casein used in artificial diets, severely reduced growth rates of *H. discus hannai*. In this study, diet ingredients were cooked in a microwave oven prior to extrusion. Temperatures in the microwave oven would have reached 100^{o}C. Further heat was applied when diets were subsequently dried at 80^{o}C in a convection oven. This heat treatment may have detrimentally affected the fishmeal and algae included in the artificial diets, resulting in decreased digestibility of artificial diets (Uki and Watanabe, 1992). Control diets (algae) were not subjected to heat treatment. This could partially explain why abalone

fed on algae produced better growth rates than those fed on artificial diets. It should be noted though that other peleted foods for fish and abalone are produced using extrusion methods. Extrusion results in higher temperatures thatn experienced here and does not appear to spoil the protein (Maria-Teressa Vianna, personal communication). Ash content of alginate bound artificial diets was negatively correlated with BW ($P < 0.05$) and SL growth rates ($P < 0.1$). High ash content was also related to low FCE ratios.

High levels of leaching (low stability), in artificial diets, result in the loss of important vitamins, minerals and soluble proteins shortly after immersion (Grabner *et al*, 1981). Leaching rates for the present study are lower than the leaching rates of alginate bound artificial diets produced by Knauer *et al.* (1993) (± 53% after 12 hours). Growth rates in the present study did not appear to be related to stability, but leaching rates between 12.44 and 21.51% are thought to be unsatisfactory. This suggests that a greater proportion of the diet should be taken up by the alginate binder, but this will increase costs substantially. In the present study, the potassium alginate binder made up 11.2 percent of the diet ingredients (dry weight) which was higher than that tested by Knauer *et al.* (1993) (2 percent), but lower than the concentrations used by Viana *et al.* (1993) (23.4 and 25.5 percent) and Uki and Watanabe (1992) (20 percent). Even at 11.2 percent, the binder makes up a substantial portion of the feed cost.

Abalone fed on artificial diets, with algae constituting 40% of the dry weight, produced shell length growth rates that were not significantly different to those produced by abalone fed on the corresponding algae diets. This suggests that it will be feasible to use algae as a large component of artificial diets. *Porphyra* and *Ulva* are the best algae to use in artificial diets.

The semi-purified diets used in this study need refinement. Further research should test different levels of algae incorporated into diets, different varieties of fishmeal and different binders. Heat treatment of ingredients should be avoided.

CHAPTER 6

CONCLUSIONS AND SPECULATIONS

Temperature and availability of an algal food source are the most important factors in considering the positioning of an abalone farm. Temperature related growth rate experiments show that abalone grow optimally at different temperatures on different diets. Of the three single-species algal diets tested in the temperature trials *Laminaria* produced optimum growth rates at approximately 17°C while *Ecklonia* and *Porphyra* produced optimum growth rates at a slightly higher temperature. This study suggests that the conclusions drawn by Hecht (1994) are incorrect. His conclusions are based on the incorrect assumption that the preferred temperature corresponds to the temperature for optimum growth.

Temperatures along the West coast are lower than the optimum temperatures for growth on the three algal species tested. This suggests that growth rates on the West coast may be too slow to sustain viable abalone culture operations unless water is heated to a constant temperature of between 17 and 20°C.

South-west coast mean monthly temperatures range between 15.7°C and 20.9°C (for Gansbaai), which are around the optimum temperature for growth on the three test diets, making this the most suitable stretch of coast for abalone farming. Beyond Agulhas, the southern-most point of Africa, the abundance of kelp species decreases. Therefore results from this study for *Ecklonia* and *Laminaria* are not applicable to the coast east of Agulhas.

The length of time abalone spend in a farm is crucial to the economic viability of the farm. As a result, careful diet management will be important in controlling the period of time which abalone spend in culture.

Diet management schemes may involve the use of algae or artificial feeds. Harvested algae provide a cheap and abundant source of food along the West and South-west coast of South Africa. Single-species algal diets are generally accepted to be inferior to mixed diets, because mixed diets provide a greater range of nutrients. In this study rotation diets, which are simple mixed diets, were shown to produce better growth rates than the single-species algal diets. The exception to this was the growth rate of abalone fed the single-species diet *Porphyra*. The growth rates on this diet exceeded those on all other diets tested. Unfortunately, however, the species is not sufficiently abundant in its natural environment to support large abalone farms alone.

Rotation diets produced consistently high shell length and body weight growth rates that varied little between treatments. Growth rates were generally lower and varied more between single-species algal diets than between rotation diets.

Shell length growth rates are more important than body weight growth rates as it is possible to increase the body weight of abalone over a short period of time prior to harvesting. Of the single-species algal diets, *Porphyra* produced the best shell length growth rates followed by *Ecklonia*.

Porphyra is not available in sufficient quantity to be used as a single-species diet in abalone culture; therefore one of the following rotation diet schemes could be considered: *Ecklonia* with a mixture of *Porphyra, Ulva* and *Aeodes*; *Ecklonia* with *Porphyra*; *Ecklonia* with *Aeodes*; or *Ecklonia* with *Ulva*. The choice of secondary algae in a rotation diet scheme may be dictated by the abundance of these species at the site.

The more expensive dietary option is to feed cultured abalone on some form of artificial diet. Artificial diets are generally expensive but produce better growth rates than natural algae diets. Powdered dry algae was successfully used as a large component of semi-purified artificial diets, in an attempt to reduce the costs. Potassium alginate, in a raw form, was found to be a suitable binder. Greater quantities could be included in future diets to reduce high leaching rates. Alternatively, a cheaper and more effective binder needs to be isolated.

Five algae species were used to make five alginate bound artificial diets. Comparison of shell length growth rates showed that there was no significant difference between growth on each artificial diet and growth on the algae used in the artificial diet. However, body weight growth rates on artificial diets tended to be lower than the corresponding algae, with certain artificial diets resulting in loss of body weight over the one month experimental growth period. *Porphyra*, *Ulva* and *Gracilaria* were found to be satisfactory components of both gelatine and alginate bound artificial diets, with *Porphyra* being the best algal additive tested.

It may be possible to enhance the growth rates of abalone fed on these artificial diets by increasing the quantity and quality of fishmeal in the diet. Additionally the stability of the diet should be improved. Heat treatment of ingredients prior to extruding should also be reduced. Heat treatment detrimentally affects the diet ingredients, resulting in decreased digestibility.

Proximate analysis of the six algal species, tested as control diets for the rotation diets, revealed that growth rates of abalone were not correlated to protein, energy or ash content of algal diets. However, intake of both protein and energy was positively correlated to both the shell length and body weight growth rates of abalone.

Analysis of the amino acid composition of each algae revealed that growth rates were best correlated with the intake of histidine, lysine, proline and alanine. This suggested that these amino acids are the most limiting amino acids in algal diets. It is interesting to note that growth is limited by non-essential amino acids. This suggests that non-essential amino acids should also be considered in the formulation of artificial foods. Previously only essential amino acids were considered.

Comparison of essential amino acid pattern and essential amino acid ratios (relative to lysine) between *H. midae* tissue and each species of algae tested, revealed that neither method could satisfactorily predict the growth rates experienced on the different algae. The apparent lack of validity of the essential amino acid pattern and ratio methods for comparison of abalone body tissue with algal tissue, may be a result of variation in digestibility of different algae. Further investigation of this is required.

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