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SEASONAL VARIATION IN NUTRITIONAL CONTENT OF  
THE KELP *ECKLONIA MAXIMA* ON THE WEST AND  
SOUTH WEST COASTS OF SOUTH AFRICA, WITH  
REFERENCE TO ITS USE AS ABALONE FEED.

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## Abstract

Knowledge of the chemical composition of marine macroalgae is important to understand their nutritional value for abalone as well as their potential as a source of protein, carbohydrate and lipid for commercial use. This study investigates the seasonal variations in chemical composition and nutritive value of *Ecklonia maxima* collected from various kelp beds near commercial abalone farms on both the west and south west coasts of South Africa. It has been suggested by numerous abalone farmers that west coast kelp is not as good as the south west coast for abalone feed.

Measurements of tissue moisture in the kelp samples did not reveal differences between location but showed a seasonal pattern in which concentrations increased during spring and summer (82 – 91%) and decreased during autumn and winter (75 – 79%). Similarly seasonal variation in averaged carbon content reflected the seasonal growth pattern of storage carbohydrates, with a higher content in summer and autumn (33 – 37%) and decreasing contents after growth started in winter (31 – 33%). Carbon content was higher ( $33.82 \pm 0.17$ ) for all the months on the south west compared to the west coast ( $31.17 \pm 0.22$ ). Concentrations of fibre were also significantly higher on the south west coast ( $41.34 \pm 1.21\%$ ) compared to the west coast ( $28.64 \pm 1.22\%$ ) but these values did not show a seasonal pattern. On the contrary, ash, phosphorus, sodium and potassium had higher values on the west coast and showed a slight increase in concentration during the late-winter spring months. Protein concentrations were

not significantly different between the two locations and did not reveal a seasonal pattern. Similarly, fat content was low and constant throughout the year.

Results show nutrient composition alone is insufficient for predicting the superiority of *Ecklonia maxima* growing in one location over the other. Kelp growth rate trials as well as abalone feeding trials need to be added to this study to provide conclusive answers to the main question posed.

# CHAPTER 1

## INTRODUCTION

### 1.1 The global wild fisheries catch and aquaculture industry

The world aquaculture industry contributes about 30% to the total commercial fisheries production (36 million tons, net value of US \$ 52 billion, 1998) (AASA 2004). Due to the increased demand and consequent decline in supplies from wild fisheries there has been a more than 40% increase over the past two decades in global aquaculture production (FAO 2004).

Of the total world wild fish catch, Africa contributes 6% (570 000 tons) while South Africa contributes 9% of Africa's contribution amounting to 0.5% of the total world catch (AASA 2004). Aquaculture will play a vital role in the future supply of fish food as most of the world's fishing areas have reached their maximum harvesting potential (Naylor *et al.* 2000; Troell *et al.* 2003). For aquaculture in marine environments (by weight), 44% is seaweed production, 46% molluscs, 8.7% fin-fish farming and 1% is from farming crustaceans (FAO 2004). Africa contributes <1% of the world aquaculture production and South Africa is <1% of that (John Bolton 2007. pers. comm.). As the human population continues to grow and the demand for seafood continues to increase, the need to expand aquaculture production will increase accordingly.

## 1.2 The South African abalone industry

Triggered by a drastic decline in yields from wild fisheries worldwide (from 50% - 95% over the past 25 years) (Fishtech 2003), a rapid development of abalone cultivation took place in the 1990's and is now widespread in many countries including USA, Mexico, South Africa, Australia, New Zealand, Japan, China, Taiwan, Ireland, and Iceland (Hahn 1989a, Gordon & Cook 2001). Over the past decade there has been a marked increase in abalone aquaculture production in South Africa. Total production increased from 3000 tons (worth R51 million) in 1997 to 4030 tons (worth R146 million) in 2000. This reflects an increase of 31% in weight and 35% in value from 1997-2000. (AASA 2004).

The large South African abalone, *Haliotis midae* or "perlemoen" as it is locally known, is the only species of abalone commercially exploited locally. It is found naturally in the rocky coastal waters from Cape Columbine on the west coast to just North of Port St Johns in Transkei on the southeast coast (Newman 1965, Muller 1986, Wood 1993). Harvesting of *H. midae* from natural populations is mainly carried out in the South Western Cape. The abalone live in the area below the low tide mark to approximately 25m depth, but mainly occur at depths of 2-10m and within *Ecklonia maxima* kelp beds (Tarr 1992) Juvenile abalone live in the subtidal zone, usually using small rocks and sea urchins as a refuge from predators. (Tarr 1989, SANCOR 1996, Tarr *et al.* 1996 & Day 1998). Abalone are among the most highly valued seafoods in the world, selling for US\$ 20-40/kg live weight (Rudd 1994). The prime demand is in Asian countries where "cocktail-size" abalone

and abalone products form part of traditional cuisine and ceremony (Rudd 1994). There are about 100 species world wide (Hahn 1989a) but only approximately 22 species are of economic importance, with respect to fisheries and aquaculture (Hahn 1989a). Six species of *Haliotis* have been recorded on the South African coast of which only *H. midae* is harvested commercially.

Since 1986, the South African wild abalone fishery has been regulated by minimum legal size of 138 mm shell length (114 mm shell breadth), a restricted fishing season and a strict quota system. Despite this, natural abalone stocks have steadily depleted resulting in the authorities implementing Total Allowable Catches (TAC) in 1997 (Cook 1998) and since December 2004 the recreational abalone season has been closed till further notice (DEAT 2004). Over-exploitation of wild abalone stocks by poaching and high market prices have been the main drivers for its cultivation. Access to relatively cheap labour, together with favourable coastal water quality and infrastructure has also facilitated the rapid growth of the on-shore abalone farming industry (Troell *et al.* 2006).

Today, there are 22 commercial abalone farms in South Africa with an estimated investment of R346.5 million, with productions of 527 tons in 2003 and 750 in 2004 (Robertson-Andersson 2007. pers. comm.) (Fig. 1B). According to AFASA (Abalone Farmers Association of Southern Africa) the expansion of the industry will continue (Bennett 2002). However, the lack of suitable coastal land and the dependence on wild harvest of kelp for feed may

restrict further development in some areas (Troell *et al.* 2006). The development of nutritionally complete pelleted feeds is seen by a number of analysts (Hahn 1989c, Fallu 1991, Britz *et al.* 1994) as being fundamental for the expansion of abalone farming. Dry pelleted artificial feeds are being used in Japan, China, Australia, New Zealand and South Africa (Abfeed®). Seaweed is not necessarily cheaper than artificial feeds as savings are offset by the cost benefits achieved from low feed ratio and a shorter production cycle offered by pelleted feeds. Despite costs, the major advantage of pelleted feed lies in their reliability and convenience from a farm management point of view (Robertson – Andersson 2003). Harvesting and the use of natural kelp in abalone farming is dependant on sea conditions. This complicates farm management and adds to financial risks. Another advantage in favour of artificial feeds is that there is less restriction on the location of the farms. Because kelp does not grow naturally in the Eastern Cape, the use of pelleted feeds make farming in this area possible (Robertson-Andersson 2003).

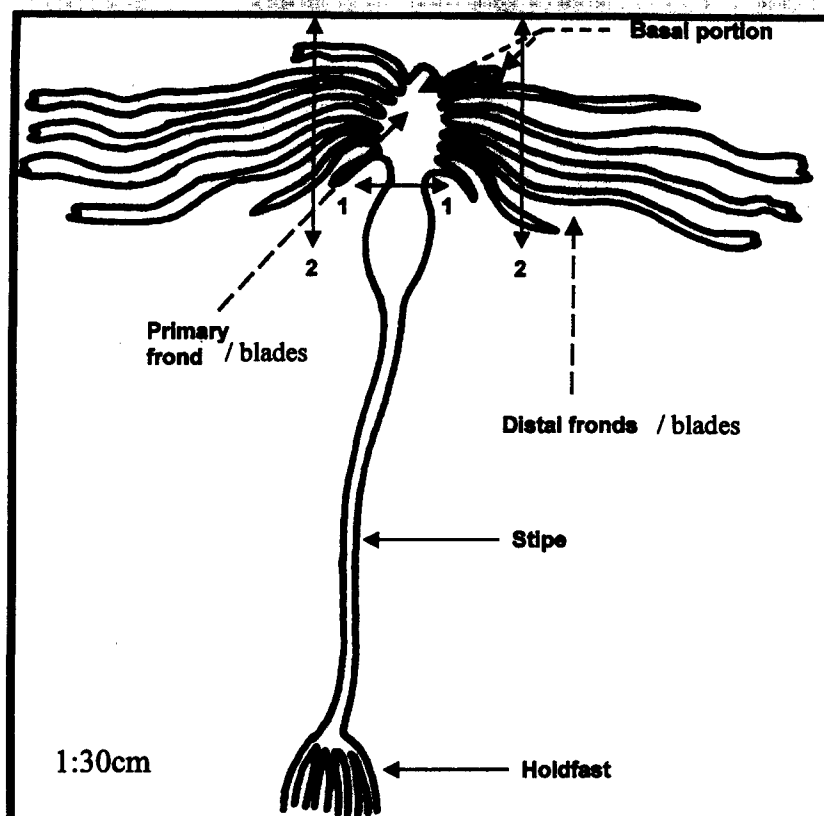
The conflict between the use of kelp or other seaweeds versus artificial feed on an abalone farm is related not only to the price of feed and availability and accessibility of fresh seaweed but also to food conversion ratio of the different feed (FCR), cost of handling and storage and final quality of abalone and culture environment. (Troell *et al.* 2006). Kelp was estimated at R500 per mt cheaper than Abfeed in 2005 but had a higher FCR (between 1:12 and 1:17) compared to Abfeed (1:5 – 1:9) (Hahn 1989a & Britz 1996). This means that feed would cost between R10 800 – R15 300 to produce a tonne of abalone

fed on kelp compared to R7000 – R12 600 to produce a tonne of abalone fed on Abfeed. These figures are very similar and because artificial feed is simpler to use it seems that there must be benefits gained by feeding with kelp. Abalone grow faster on Abfeed until they reach 50mm shell length but are weaned to a combination of Abfeed and kelp for two main reasons. Firstly, Abfeed causes an increase in sabellid infection as they feed on the more nutrient-rich faeces produced; and secondly shell growth rates tend to be higher when fed kelp. A new Abfeed based on dried kelp is being tested (Abfeed K26) which is claimed to show equivalent or better growth compared to fresh kelp in the > 50 mm size classes (P. Britz, pers comm).

Kelp is relatively low in protein and abalone tend to show good shell growth but a low meat weight gain. With Abfeed, meat weight gain is high but shell length tends to decline. The low protein K26 Abfeed formulation was developed in response to the sabellid problem and to improve growth rates (P. Britz, pers comm). Now the “old” high protein Abfeed is used for the young juveniles and the low protein pellet is designed to be used for the >50mm size classes.

### 1.3 The South African kelp industry

Kelps are large brown seaweeds (Class Phaeophyceae) which belong to the Order Laminariales. Kelp beds extend along the rocky coast from Cape Agulhas, the southern most point of Africa, up the west coast and into Namibia (Stegenga *et al.* 1997). From Cape Agulhas to Cape Columbine the dominant inshore species is *Ecklonia maxima* (Stegenga *et al.* 1997).

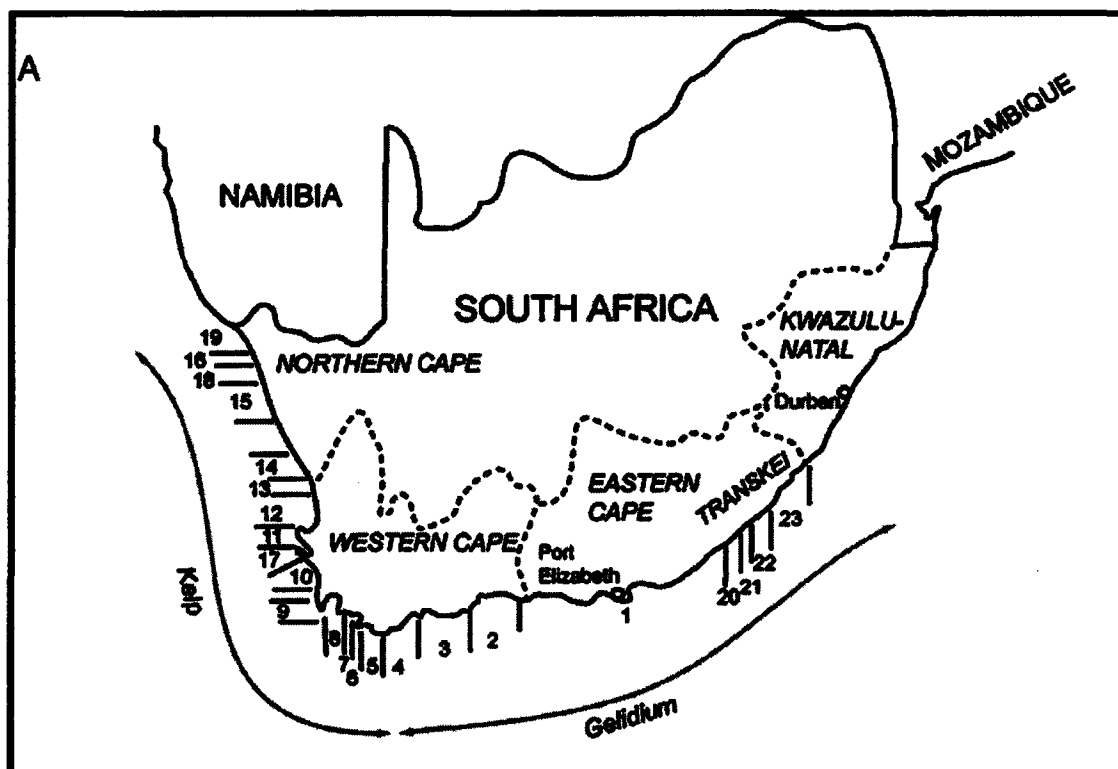


**Figure 1 Diagram of *Ecklonia maxima* sporophyte. Harvesting techniques: 1 = primary and attached secondary fronds/blades; 2 = Distal part of the secondary fronds/blades. (Anderson**

The other kelp occurring in this area is *Laminaria pallida* (Diekmann 1978). It seldom reaches the surface in the south west region and forms extensive beds in deeper waters. It has a 2m long stipe, no hollow bulb and a digitate frond. North of Cape Columbine and in Namibian waters, *L. pallida* develops a hollow stipe and gradually replaces *E. maxima* as the dominant inshore kelp (Troell *et al.* 2006).

Kelp harvested for abalone feed is either done by cutting the entire head (primary frond and attached secondary fronds) or by cutting off the distal parts of the secondary fronds from a boat (Fig 1). *Ecklonia* is presently preferred by abalone farmers as it is reported to have a lower food conversion ratio (dry feed fed/wet weight gain), approximately 10 – 15, compared to *Laminaria*, however this is unsubstantiated research (Britz 1995)

Harvestable kelp grows on the west coast of South Africa and on the south-west coast (between the Cape Peninsula and Cape Agulhas) but not on the south coast (east of Cape Agulhas). Since the early 1950s, the large dominant inshore kelp, *Ecklonia maxima* has been collected as beach-cast, and shipped to Europe, North America and Asia for alginate production (Anderson *et al.* 1989). Until the development of abalone farming, the only kelp directly harvested from beds was used as a liquid growth stimulant for agricultural crops (Anderson *et al.* 2003). Consequently, the growing demand for kelp by the abalone industry has greatly increased harvesting. In 2003, coastal management authorities (Marine and Coastal Management, Department of Environmental Affairs and Tourism, Cape Town) estimated that a maximum sustainable yield (MSY) of kelp was approached in parts of the two main areas of abalone farming; from Quoin Point to Cape Hangklip and in the Cape Columbine area (Fig 2B). Under the Marine Living Resource Act, 1998 seaweed resources are managed on an area basis, with 23 monitored concession areas between Namibian border and the southern border of Kwazulu - Natal (Fig 2A).



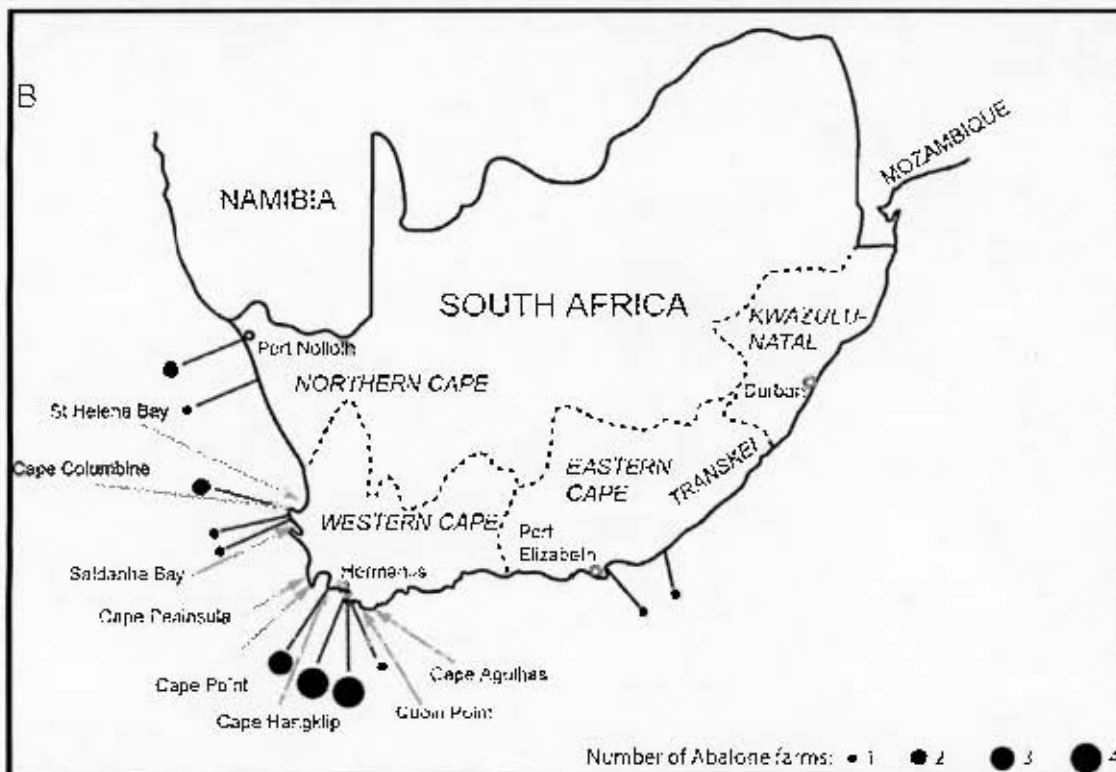


Figure 2 (A) Map of South Africa showing the Seaweed Concession areas (Anderson *et al.* 2003). Areas 1, and 20-23 is where *Gelidium* is currently collected from. Kelp is collected in areas 5-9, 11-16 and 18-19. No seaweed is collected from areas 2-4, 10, 20, 22, and 23. Line separate concession areas. B Map showing the distribution of abalone farms along the coast of South Africa (Troell *et al.* 2006)

Both the seaweed and abalone industries bring important economic benefits to South Africa. They generate export earnings, boost local and regional economies and provide employment among poor coastal communities (Troell *et al.* 2006). If the South African abalone industry continues to grow exponentially (Fig 3), there is a need to identify and analyse the relationships, from both economic and ecological perspectives, between these two industries and to consider effects on South African coastal ecosystems.

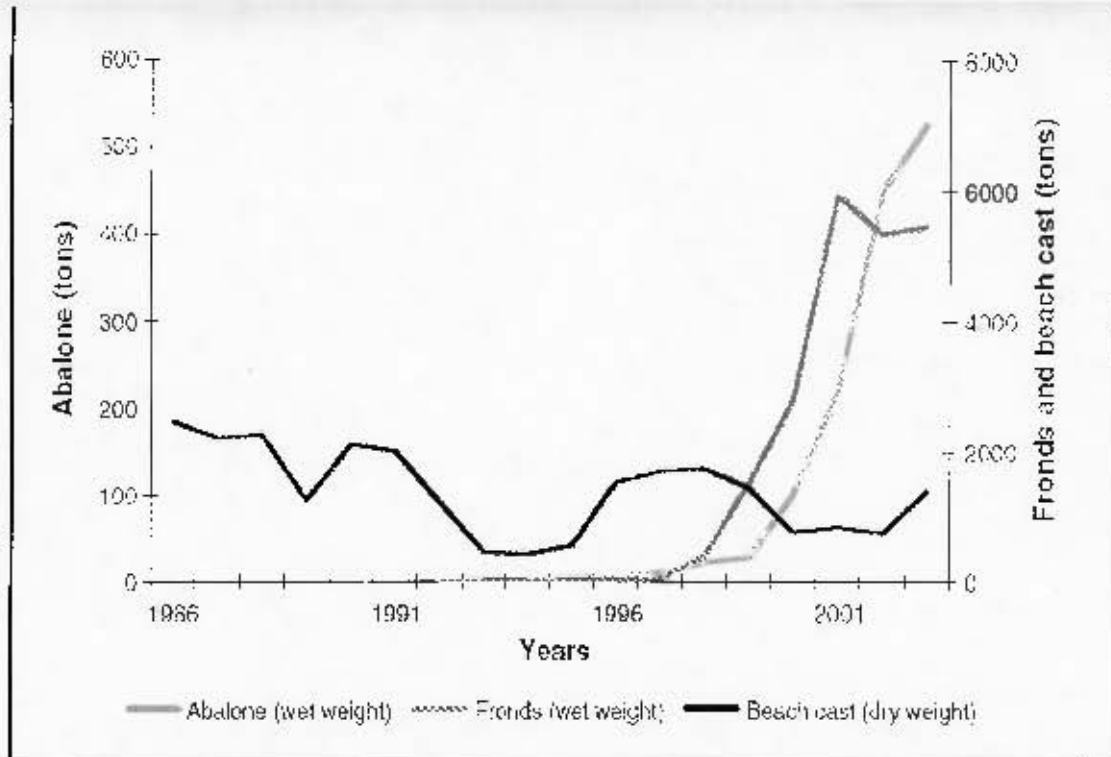


Figure 3. Graph showing the exponential growth of abalone production, the amount of kelp fronds utilised by abalone farming and the total amount of beach-cast kelp (Troell *et al.* 2006).

Large amounts of inappropriate harvesting from these areas may impact negatively on the kelp ecosystem (Anderson *et al.* 2003). For example the recovery of *E. maxima* has been shown to take approximately 2.5-3 years after harvesting the whole sporophyte. Harvesting of the *E. maxima* did not change the understory communities over the 3-year period (Levitt *et al.* 2002) or affect juvenile plants over 1.5 years old. However, populations of three obligate red algal epiphytes that grow on *E. maxima* took at least 2 years longer to recover than the kelp host itself (Anderson *et al.* 2006). Therefore, there is a need for conservation management not only in South Africa but a potential shortage of kelp supply has also emerged in other abalone farming countries such as USA and in countries where the industry is anticipated to develop rapidly such as Chile (McBride 1998). Thus, alternatives to wild kelp as abalone feed need to be made to combat a

potential bottleneck effect for increased abalone production in South Africa (Fig. 3).

Abalone, are herbivorous marine gastropods belonging to the genus *Haliotis*. They feed on diatoms and other microalgae as early juveniles, and then switch to predominantly macroalgal diet as they grow larger (McShane & Smith 1988). The adult abalone eats between 10-30% of its body weight per day (Hahn, 1989b). A farm that produces 250 000 abalone per year requires 0.5-1 tonne of seaweed per week (Fallu 1991), if using an exclusive kelp based diet. The development of a suitable diet and the subsequent growth rate is an important criterion for success in abalone aquaculture (Capinin 1996). Owing to the logistic and supply problems associated with the use of fresh seaweed, intensive abalone culture is becoming increasingly dependant upon formulated artificial feed (Hahn 1989b, Britz *et al.* 1994) although it is important to note that 6000t of kelp were harvested in South Africa in 2006 (Troell *et al.* 2003) and thus artificial feed has up to this date not shown any signs of reducing kelp harvested for feed.

#### 1.4 Morphological description of *Haliotis*

During planktonic development (Newman 1964 1966 1967 1968, Fallu 1991, Branch *et al.* 1994) the larvae undergo changes from swimming trochophore to veliger stage, before metamorphosis and settlement on a firm substrate as a post-larval juvenile. At this stage the larva is commonly termed "spat" (Fig 4).

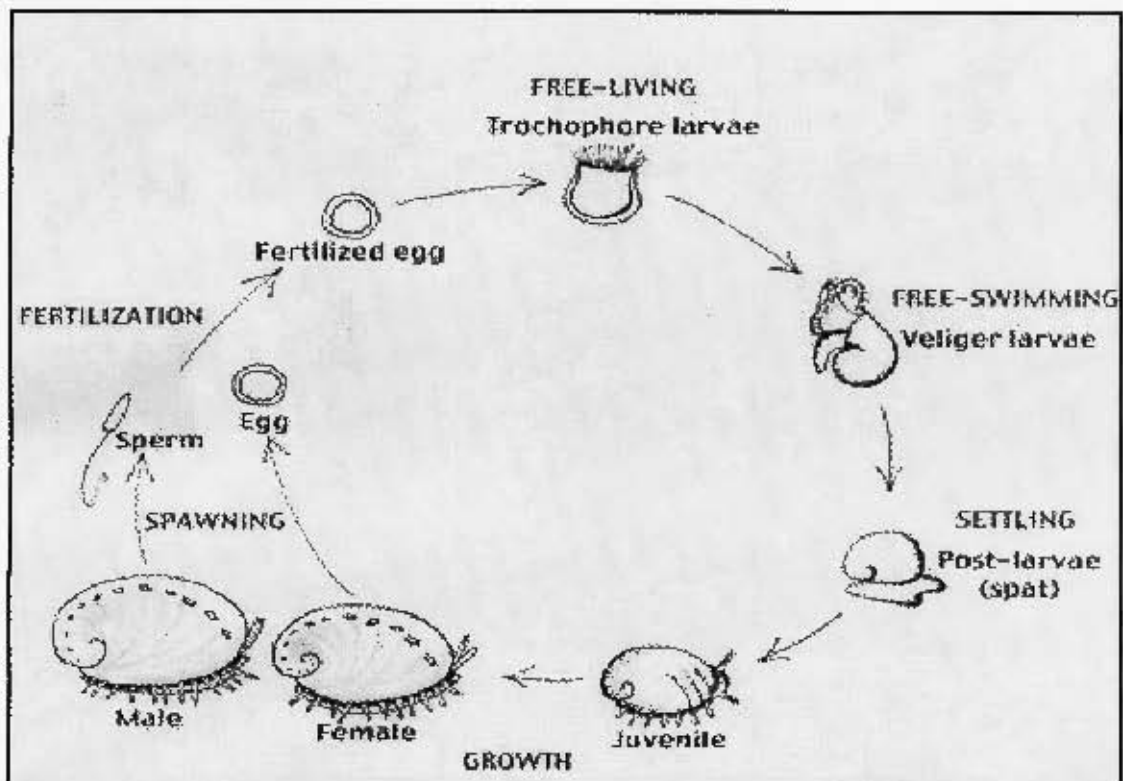


Figure 4 The life cycle of the South African abalone, *Haliotis midae*

(<http://www.hikabalonefarm.co.za>)

Adult abalone have a flattened, spiral shell with rows of small holes along the right side (Day 1974, Fallu 1991). The holes are largest towards the anterior and decrease in size towards the posterior, those at the back being almost blocked (Fig. 4). The holes assist in both respiration and removal of waste; sea water enters the gill cavity anteriorly, passes over the gills, oxygen is taken up, waste gases are given off and the exhaled sea water exits through the holes. During growth periods, new holes are created and old ones are filled with aragonite. The shell covers the large muscular foot, which is used for both locomotion and gripping firmly onto a firm substrate. The part of the foot that is not completely covered by the shell is protected by a tough outer skin. The edge of the foot has sensory tentacles that detect predators and food items. The gut is coiled in a space between the stalk of the foot and the rim of the shell (Fallu 1991). A thin layer of skin grows out of the stalk and

adheres to the surfaces of the shell, sealing off the mantle cavity and gut from the external environment.

### 1.5 Abalone diet and nutritional requirements

Abalone need food for two main purposes, to provide energy and to provide the basic material for growth. If the requirements for these two purposes are not met the abalone will grow very slowly, if at all. The cheaper kind of food to provide energy is carbohydrates but fats and protein do the same at a greater expense (Fallu 1991). For the present study, fibre was measured, which is made up of a number of complex substances which are all types of carbohydrates. For tissue growth, abalone require specific chemical building blocks such as amino acids (the components of protein) and polyunsaturated fatty acids (Fallu 1991).

The development of artificial feed (Abfeed® which has fishmeal as the primary protein source) for abalone has resulted in many of the South African west coast farms feeding a mixture of kelp and artificial feed (Bennett 2002). The drawback of artificial feed is that it gives the meat and shell of the abalone an undesirable light colour thus considerably reducing its market value. A mixed algal diet however, results in a red to brown coloured meat, which is ideal for the export market.

Nutritionists have a theory that if a supplemented diet contains similar chemical components to the animal that feeds on it, then the diet should be correct. *H. midae* is, to a certain extent, similar in chemical composition to its

natural kelp diet of *Ecklonia maxima*, containing very low levels of fat (1.1%), high levels of reserve carbohydrates (37%), and relatively low levels of protein 11.05) (Knauer *et al.* 1994b).

Unlike most vertebrates which store energy in the form of lipids, abalone store energy in the form of glycogen. (Webber 1970 & Goudsmit 1972). This is represented by the high carbohydrate/low fat composition of the *H. midae* tissue and shows that the energy metabolism of abalone is carbohydrate based (Britz 1995). Britz & Hecht (1997) found maximum growth rates in *H. midae* at dietary carbohydrate levels of 33-58%.

Although the natural diet of abalone has a high moisture content (68-83%) (Hahn 1989b, Robertson-Andersson 2003) and relatively low nutrient levels studies with formulated dry feeds have shown that abalone can efficiently digest highly concentrated protein and carbohydrates, but that their ability to utilise high levels of fat is limited (Uki & Watanabe 1992, Britz *et al.* 1994, Mai *et al.* 1995) It has been reported that macroalgal diets containing 3-5% lipid promotes a high growth rate for abalone (Mercer *et al.* 1993)

Abalone require a balanced diet of lipid and essential and non-essential amino acids (Mai *et al.* 1995). Protein is one of the essential components in the diet and although seaweeds consumed by abalone generally contain less than 20% (Nisizawa *et al.* 1987), they have been shown capable of digesting high levels of dietary protein (20-50%) in concentrated form (Knauer *et al.* 1996, Uki *et al.* 1986, Taylor 1992, Viana *et al.* 1993, Mai *et al.* 1995). Casein,

in particular, is said to be the most suitable protein for artificial diets when compared to various other proteins such as soya bean meal, rye grass concentrate, egg albumin, whole egg and fishmeal. (Uki *et al.* 1985). Britz (1996) suggested that a dietary protein level higher than 20-30% might be required to achieve maximum abalone growth rate. Previous studies have also shown that a 30-40% protein level in the abalone diet is suitable for both small and large size classes (Hahn 1989b, Mai *et al.* 1995, Britz & Hecht 1997).

It was suggested that all abalone have similar requirements which are normally obtained from their food and absorbed from the surrounding seawater (Fallu 1991). Additionally, Uki *et al.* (1985) confirmed that dietary mineral supplementation does improve growth rates of captive *Haliotis discus hannai*.

In South Africa fresh seaweeds are being used as an alternative to expensive formulated feed. These include brown algae (kelp), *Ecklonia* and *Laminaria*, green algae, *Ulva* and red algae, *Gracilaria*. Kelp is used as the primary feed for abalone because of its abundance in the south west and west coast of South Africa, however previous research on natural diets of the South African *Haliotis midae* showed that abalone feed on a variety of algae with at least two species found in the gut at any time (Barkai & Griffiths 1986). It would appear that supplementation with other algae and/or artificial feed may be necessary in order to obtain growth rates fast enough to justify intensive shore-based farming of *Haliotis midae*. Results from Naidoo *et al.* (2006)

showed that abalone grew well ( $0.056\text{g day}^{-1}$  body weight) on all fresh seaweed combinations, but grew best on a mixed diet of kelp plus other seaweed ( $0.074\text{g day}^{-1}$  body weight). An exclusively Abfeed ® diet only grew abalone at  $0.046\text{g/day}$  body weight.

## 1.6 Morphology and seasonal growth patterns of kelps

Kelp forests grow predominantly in cool, nutrient-rich waters and are among the most biologically productive habitats in the marine environment. They are found in shallow coastal waters, and the larger forests are restricted to areas of monthly mean seawater temperatures less than  $20^{\circ}\text{C}$ . They also extend into the tropics where the water is cool enough e.g. Namibia. A dependence upon light for photosynthesis restricts them to clear shallow water and in South Africa kelp rarely grows in waters deeper than 15m (Fallu 1991). However, kelps have been known to grow in depths of more than 100m in very clear waters such as in parts of the Mediterranean Sea (Lüning 1993). Not only do they provide various services to the ecosystem such as shelter, shade and a substratum for attachment of shellfish for example, but kelp forests are also a major food source for grazers and filter feeders (Fallu 1991). Kelp can be the single largest source of fixed carbon in these habitats. Furthermore, a significant part of the energy fixed by kelp through photosynthesis enters the ecosystem as dissolved organic matter, via bacteria pathways. Kelps are more than primary producers in these systems; they effectively govern the structure and diversity of the food-web (Graham 2004, Sjøtun *et al.* 1995).

Kelp's life histories have heteromorphic alternation of generations, comprising 2 free-living life phases, a macroscopic sporophyte generation and a microscopic gametophyte generation (Schiel & Foster 2006). The sporophytes are typically structured into a holdfast, stipe and one or more fronds (Fig. 5). Kelp grows by intercalary meristems that are mainly active at the base of the fronds.

It has a hollow stipe up to 9m-long, with a gas-filled bulb at the top, above which the smooth elongated strap-like fronds are suspended near the water surface (Fig 1). The inshore specimens have shorter stipes while the deeper water specimens are longer and more flexible. The conical-shaped holdfast has many entwined branched haptera and can be up to 40cm in diameter.

Several seaweeds have been identified to exhibit distinctive growth and reproductive cycles, which are believed to be correlated with seasonal fluctuations in light intensity, photoperiod, temperature and nutrients, i.e. the primary ecological factors (Lüning & Dieck 1989) as well as an endogenous circannual clock. Dieckmann (1980) measured the growth of South African *Laminaria pallida* fronds and stipes and found that the lowest growth rates were recorded in winter ( $1\text{mm day}^{-1}$ ) and highest in summer ( $13\text{mm day}^{-1}$ ). The growth pattern of *Ecklonia maxima* has not been researched and published, however it assumed that it is likely to be comparable to other temperate kelp species which have been investigated in detail. For example many kelp species start new growth during the winter months and stop or reduce growth during the summer months as demonstrated by the cold-

temperate kelp *L. hyperborea* (Sjøtun *et al.* 1995). This European species sheds the old blade in May while new growth of the blade starts in December and stops in June or July. (Kain 1963 1979, Lüning 1979). In winter, when growth starts, nutrient content of seawater is high whereas temperature, light intensity and day length is low. In summer the reverse occurs when growth stops or slows down.

It is also advantageous to start growth from stored carbon in early winter, well ahead of the spring phytoplankton bloom. This is because there is a wealth of nutrients in the seawater after the autumn storms have resulted in remineralisation of plankton (Lüning, 1993). During summer when the light intensity and day lengths are high seaweeds reduce growth, even if sufficient nutrients are available, and they distribute the assimilated carbon to reserve materials in order start growing again during the low light in winter. Research by Lüning (1993) showed that seasonal growth cycle of *L. hyperborea* is controlled by an endogenous circannual clock, with day length as a synchronizer.

Red seaweeds are high in nutritional quality and tend to be the preferred food of wild abalone. They have a relatively high protein content when compared with other macroalgae (Fallu 1991). The red alga *Palmaria palmata*, which grows along both sides of the North Atlantic Ocean, from the Arctic to cold-temperate regions (Lüning 1990) is used in coastal areas of Northern Europe and North America. A study conducted by Galland-Irmouli, (1999) in France showed that *Palmaria palmata* was significantly different in protein content

according to season. The highest protein content ( $21.9 \pm 3.5\%$ ) was found in the winter–spring period and the lowest ( $11.9 \pm 2.0\%$ ) in the summer–early autumn period. Buchal *et al.* (1998), reported that *Palmaria mollis* (Dulse), used as a settlement substrate and food for red abalone (*Haliotis rufescens*), was found to be superior to kelp (*Nereocystis luetkeana*) in conditioning broodstock, resulting in eggs of higher dry weight, lipid and protein content.

### 1.7 Hydrology of the study areas

The western coast of South Africa is characterised by a temperate climate. During spring and summer strong south-easterly winds are frequent, causing localised upwelling. Together with high light intensities, these conditions are optimal for plankton growth (Andrews & Hutchings 1980). During winter, northerly winds are frequent and are often accompanied by large swells and rain. Most of the Western Cape coast is directly exposed to the pounding of these large westerly swells (Dieckmann 1978)

The west coast has lower annual mean sea water temperatures ( $12 - 16^{\circ}\text{C}$ ) than the south west coast ( $17 - 19^{\circ}\text{C}$ ), a result of strong southeasterly winds generating a semi-permanent upwelling system in which cold nutrient-rich water is brought to the surface in summer. This is then followed by periods of downwelling, ultimately affecting the export and import of organic material and nutrients in the water column surrounding the kelp beds. In winter, the occurrence of upwelling is reduced if not entirely absent (Dieckmann 1980) which is accompanied by a drop in nutrients. The higher nutrient concentrations during summer may reduce the late spring decline in growth

rates shown by kelps elsewhere. No seasonal growth rate figures are available for *Ecklonia maxima* in the literature but evidence for different kelp species around the world show similar annual patterns, including the other major South African west coast kelp *Laminaria pallida* (Diekmann 1978, 1980).

Seasonal patterns have been shown in the nitrogenous uptake rates in which tissue nitrogen increased during upwelling events (Probyn & McQuid 1985). Spore production also varies seasonally with low values in late summer and winter, and high values in spring/early autumn. (Joska & Bolton 1987). Seasonal variations in ash, alginic acid (a naturally occurring hydrophilic colloidal polysaccharide) and mannitol (a carbohydrate in tissues that are increasing by cell division) of *Ecklonia maxima* fronds were demonstrated in a study by Von Holdt *et al.* (1955). Mineral ash values reached a maximum in mid-winter (June), fell to minimum on either side of the month and then rose again towards the summer. The alginic acid curves rose from a minimum in winter to a maximum in summer and in this respect were the converse of those for ash. Mannitol values tended to be higher in winter than in summer (Von Holdt *et al.* 1955).

### 1.8 Aims and objectives of the present study.

In addition to the studies discussed above research has also been carried out on the ecology (Velimirov *et al.* 1977, Field *et al.* 1980), productivity (Mann *et al.* 1979, Newell *et al.* 1980, Jarman & Carter 1981), commercial harvesting (Simons & Jarman 1980) and environmental tolerances of the gametophytes

of the *Ecklonia maxima* species (Branch *et al.* 1974, Bolton & Levitt 1985). However little or no research has been done on the seasonal variations in chemical composition. Even though the food conversion ratio (kelp wet weight/abalone weight gain) for abalone feeding on *Ecklonia maxima* is not scientifically substantiated data, the south west coast has been reported by abalone farmers, to be (1:12-15) lower than the west coast (1:15-19) (pers. comm. Deborah Robertson-Andersson 2007). Consequently, the south west coast abalone farmers (Irvin and Johnson, Avuca and HIK) claim that kelp growing in this area is superior for abalone feed than the kelp growing along the west coast (Jacobs Bay Sea Products) as less kelp is needed. The aim of this study was to investigate and compare the nutritional constituents and seasonal differences between *Ecklonia maxima* collected from the south west and west coasts of South Africa.

## CHAPTER 2

### MATERIALS AND METHODS.

#### 2.1 Sampling and the determination of moisture content

From the 5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005 replicate samples of kelp, *Ecklonia maxima*, were collected once every two weeks or once every month depending on weather and ocean conditions. These samples were collected by the farm workers and not by the author, therefore there tends to be a reduction in the number of samples over the summer holiday period, when farms are short-staffed (December-January). The samples were commercially collected and consequently there are variable numbers of samples in different months in different locations (Appendix 1). Inconsistent kelp harvesting has

caused a variation in sample number per month for each location, however for nutritional concentrations there were on average 7 samples per month for the south west coast and an average of 4 samples per month for the west coast except were months have missing values completely (i.e. February, May and December for the west coast and September for the south west coast). For moisture content an average 16 samples for the south west and 7 samples for the west were collected per month.

This, for the purpose of analysing seasonal variation in the data, the data is presented as calendar months, and therefore April, May and June have combined data from both years. In 15 months a total of 80 samples were collected from JSP (Jacobsbaai Sea Products Abalone farm) on the west coast and 199 from HIK (Haw, Ingels & Krohn), AVA (Avuca Abalone farm) and I&J (Irvin & Johnson) on the south west coast (Fig 5). Replicate samples were averaged for each month, separated by location and graphically represented in Excel with standard error

For moisture content an average 13 samples for the south west and 5 samples for the west were collected per month. After harvesting, by cutting the fronds only, the kelp was then transported by boat to the various farms at the shore where it was washed, weighed into 500g or 1 kg portions, packed into labelled bags and frozen. The reason for the separation of sites was to ensure that we received different samples from farms and who had different kelp contractors.

Each sample was then transported to The University of Cape Town or Marine and Coastal Management where they were defrosted and washed in distilled deionised water, reweighed and placed in a drying oven for 70 hours at 70°C. During the freeze-thaw process there is an unknown amount of water which leaks out however; this insignificant amount should not drastically change the seasonal pattern which is being calculated. Following this the dry samples were weighed again and ground in a 3 stage process to a particular diameter of less than 1mm. The moisture content was determined using the following equation:

$$\% \text{MOISTURE} = \frac{(\text{Wet} - \text{Dry})}{\text{Wet}} \times 100$$

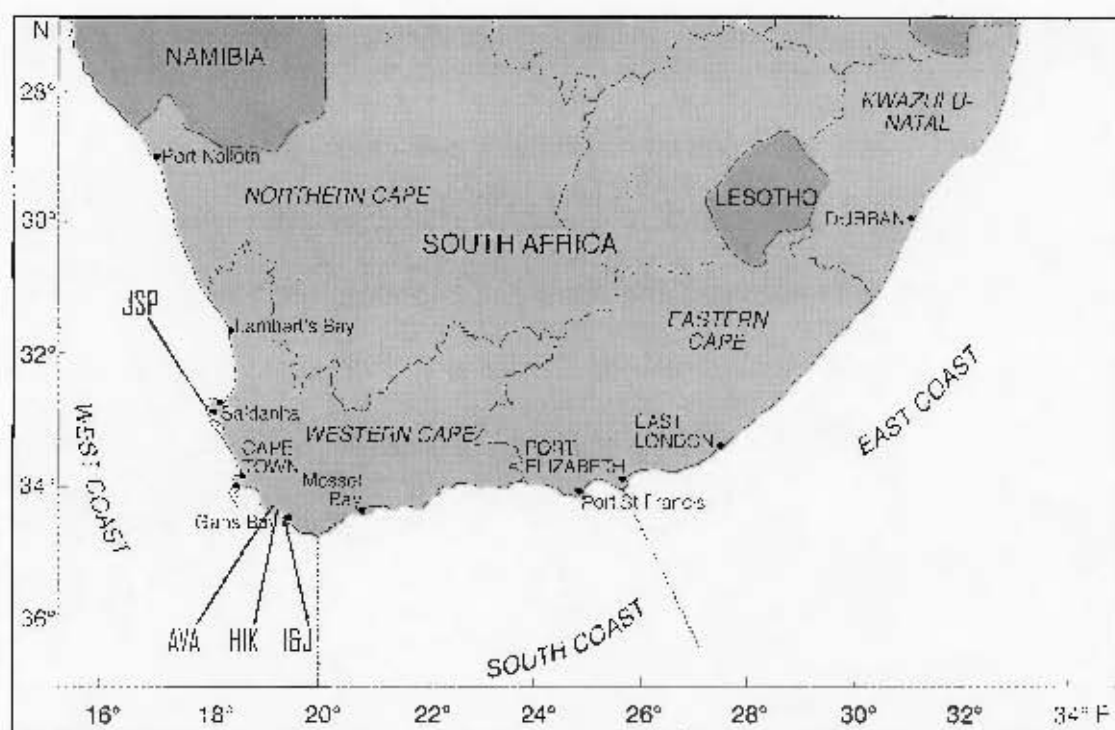


Figure 5. Map showing location of kelp samples used in this study. JSP = Jacobsbaai Sea Products. AVA = Avuca Abalone. HIK = Haw, Ingels & Krohn. I&J = Irvine & Johnson

## 2.2 Carbon and Nitrogen determination

Tissue nitrogen and carbon concentrations were analysed by the author using an elemental analyser (LECO CHNS-932) at the University of Stockholm. The

instrument uses infrared detection to determine the weight percentage of the carbon and nitrogen. An average of 6 samples per month for the south west coast and an average of 3 samples per month for the west coast were weighed (2.6mg), numbered, recorded and placed in the elemental analyser. The samples were heated in a 950°C oxygen-rich environment. The carbon dioxide was measured and then removed and nitrogen was measured by thermal conductivity. The carbon dioxide was measured and then removed and nitrogen was measured by thermal conductivity. A standard was run after every 12<sup>th</sup> sample and a blank after every 6<sup>th</sup> sample. The results were calibrated using a standard (EDTA, acetanilid) and a blank (Watkins *et al.* 1987). The carbon-nitrogen ratio (C:N) was calculated to show whether the kelp was storing nitrogen (low C:N value) or if growth was nitrogen limited (high C:N value) (Sjøtun *et al.* 1995, Hanisak 1983).

### 2.3 Mineral Composition

Protein, fibre, fat, ash, calcium, sodium, magnesium, potassium, phosphorus, zinc, copper, manganese, lead and iron were analysed by a commercial feed analysis company. An average of 6 samples per month for the south west coast and an average of 3 samples per month for the west coast were sent to the Cedara Feed Laboratory in Pietermaritzburg, South Africa, for commercial feed analysis. The resultant graphs state whether the data was analysed by the author i.e. in Stockholm or if it was commercial feed laboratory data. The determination of crude protein was based on the Kjeldahl digestion method and test for nitrogen content (Bradstreet 1984). This gives an indication of the amount of protein present by multiplying nitrogen by a constant of 6.25. The

kelp samples were digested by heating with concentrated H<sub>2</sub>SO<sub>4</sub> using a catalyst and a temperature elevator to convert all nitrogen to ammonia. The ammonia was removed by Gerhardt distillation and end point titration to determine the amount of Nitrogen present (Watkins *et al.* 1987). Fibre (ADF) was determined using an acid detergent solution (amylase and sulphite) on a digested distillate (Van Snoest *et al.* 1991), fat was determined by filtering the dried kelp samples through a Soxhlet distillation system with petroleum ether and ash was measured by heating samples in a muffle furnace set at 600°C for 5 hours.

Mineral analysis on the feed samples for the trace elements; Ca, Na, Mg, K, Zn, Cu, Mn, Fe, P and Pb involves a process whereby samples are digested, diluted and then analysed on an Atomic Absorption Spectrophotometer and UV-VIS spectrophotometer.

#### .2.4 Statistical analysis

Each nutrient concentration was treated statistically by a two-way analysis of variance (ANOVA) for assessing main effects of two variables (seasonality and location) and their interaction. Comparisons after ANOVA were made using the post hoc Tukey test to individualise specific differences. (ANOVA; Zar 1999) Prior to the statistical analysis equality of variance and normality were checked using the Levene's and Kolmogorov Smirnov test respectively. When the assumption of homogeneity of co-variance was not fulfilled, a non-parametric Kruskal Wallis ANOVA test was applied. Each variable is presented as a mean (with a standard error bar) for either the entire data set,

showing difference between location, or means of samples in each month, showing a seasonal pattern. Hierarchical cluster analysis (Euclidean distance, complete linkage) was used to identify natural groupings in the data. A significance test for each cluster group was then performed using one-way ANOVA to analyze designs with a single categorical independent variable.

## CHAPTER 3

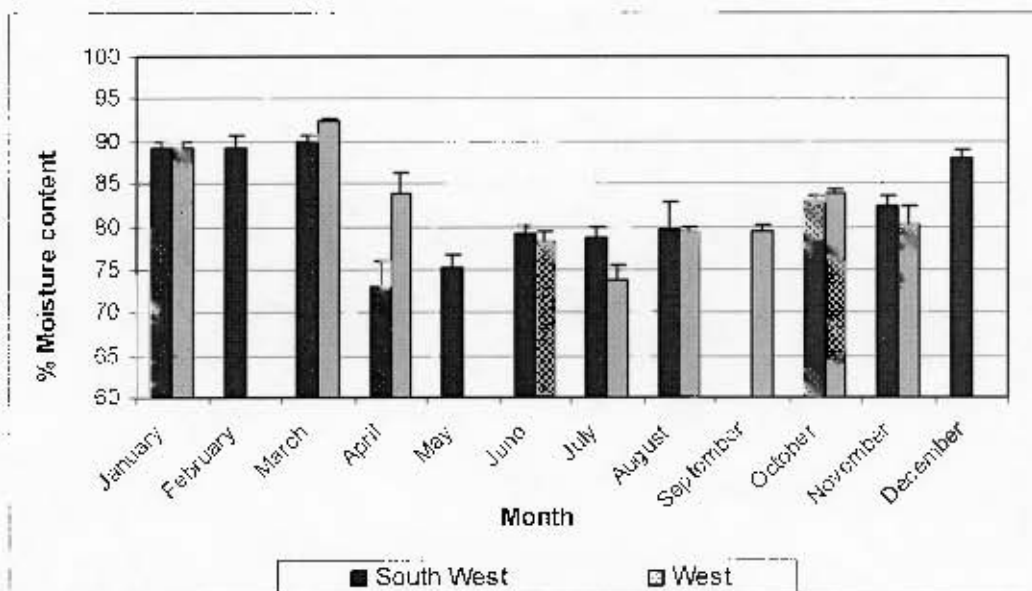
# RESULTS

The average differences for all samples in moisture content (percentage wet weight) and nutritional concentrations (percentage dry weight) of *Ecklonia maxima* between the south west and west coast of South Africa is shown in Table 1. For all p-values less than 0.05 the Null Hypothesis of equal variances is rejected and the alternative hypothesis that variances are significantly different is accepted.

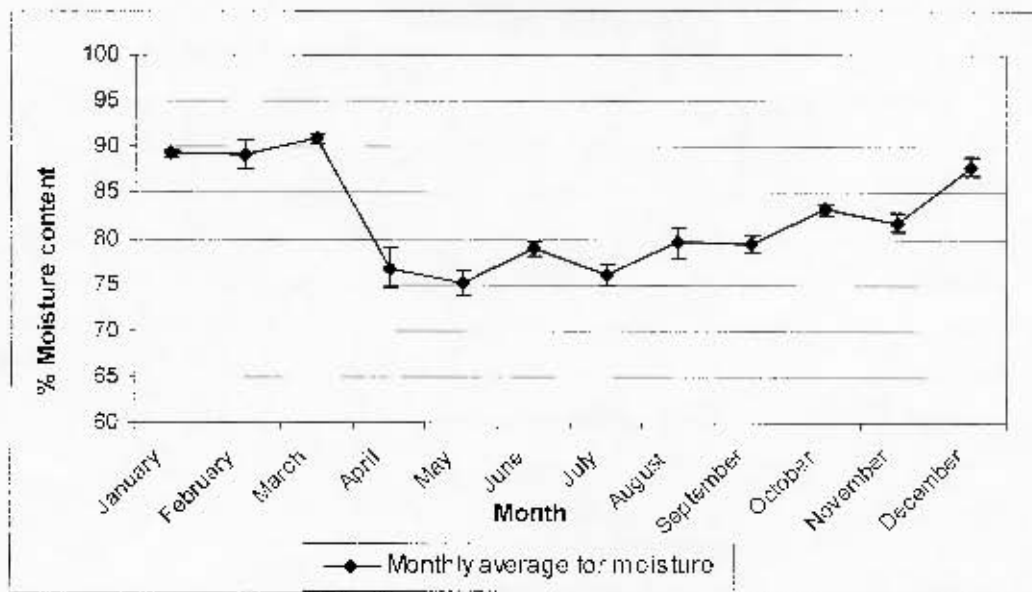
**Table 1 Averages and standard errors for all samples showing moisture content (percentage wet weight) and nutritional concentrations (percentage dry weight) and mineral content (percentage of ppt). A two way ANOVA or a non-parametric Kruskal Wallis ANOVA was applied to test significant differences [\*] in location (west coast or south west coast) and month (seasonality).**

		South West	West	Sig. in location	Sig. in month
Percentage wet weight	Moisture content	79.39 ± 0.75	81.95 ± 0.877.74		*
Percentage dry weight	Carbon	33.82 ± 0.17	31.17 ± 0.22	*	*
	Nitrogen	1.77 ± 0.015	1.70 ± 0.022		
	Crude protein	11.00 ± 0.17	11.13 ± 0.17		*
	Fat	1.16 ± 0.09	1.01 ± 0.06		
	Ash	19.41 ± 0.29	22.70 ± 0.56	*	*
	Fibre(ADF)	41.34 ± 1.21	28.64 ± 1.22	*	
	Calcium	1.17 ± 0.02	1.12 ± 0.02	*	*
	Phosphorus	0.23 ± 0.005	0.3 ± 0.006	*	
	Magnesium	1.46 ± 0.14	0.89 ± 0.011	*	*
	Sodium	2.70 ± 0.06	3.08 ± 0.07	*	*
	Potassium	2.95 ± 0.67	3.94 ± 0.12	*	*
	Copper (ppt)	0.0003 ± 2.01E-05	0.0002 ± 2.75E-05	*	
	Zinc (ppt)	0.0014 ± 6.59E-05	0.0016 ± 0.0001		*
	Manganese (ppt)	0.0006 ± 5.11E-05	0.0004 ± 4.82E-05	*	*
Iron (ppt)	0.01021 ± 0.001	0.006 ± 0.0006	*		

There were no significant differences in tissue moisture content (Kruskal Wallis ANOVA,  $H = 3.07$ ,  $p > 0.05$ ) between the south west and west coast (Table 1), however, moisture content differed significantly from month to month (Kruskal Wallis ANOVA,  $H = 104.12$ ,  $p < 0.05$ ). The monthly average moisture content at both locations was at its lowest during autumn and winter from April to August (Fig 6). The moisture content rose during spring and summer, and reached a maximum in March (Fig 7).



**Figure 6** Average moisture content (% wet weight) (analysed by the author) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.



**Figure 7** Total average moisture content for both locations (% wet weight) (analysed by the author) showing seasonal variation from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

Carbon concentrations were significantly different (Kruskal-Wallis ANOVA,  $H = 55.29$ ,  $p < 0.05$ ) between locations with higher average values on the south west coast compared to the west coast (Table 1). For every month carbon concentrations were on average higher on the south west coast than on the west coast (Fig 8). Carbon also differed significantly (Kruskal Wallis  $H = 44.26$ ,  $p < 0.05$ ) among months (Table 1) implying a seasonal variation in concentration. The south west coast values were high during summer (December – March) and lower for the rest of the year (Fig 9) but winter (June-July) had higher values on the west coast.

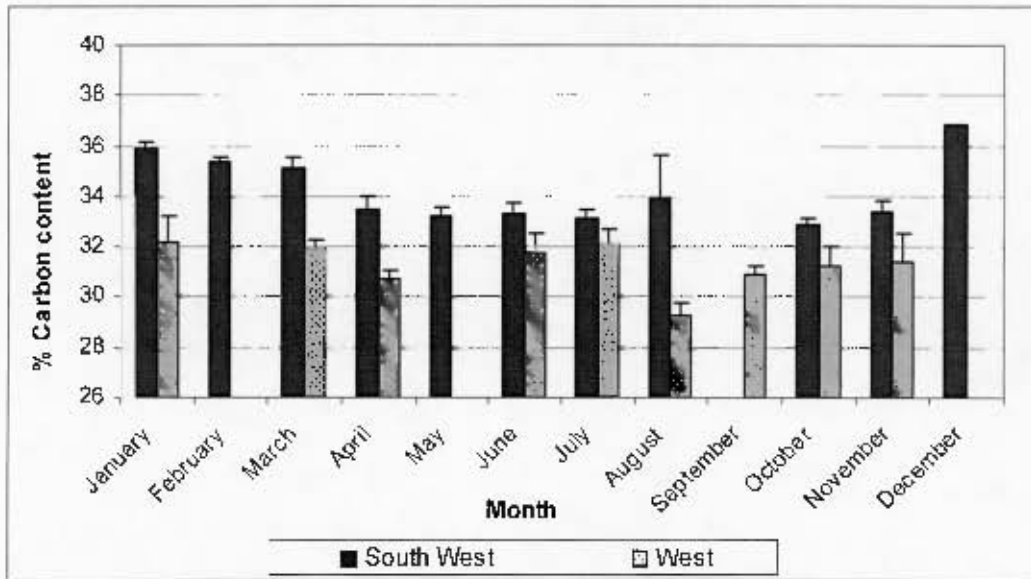


Figure 8 Average carbon content (% dry weight) (analysed by the author) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

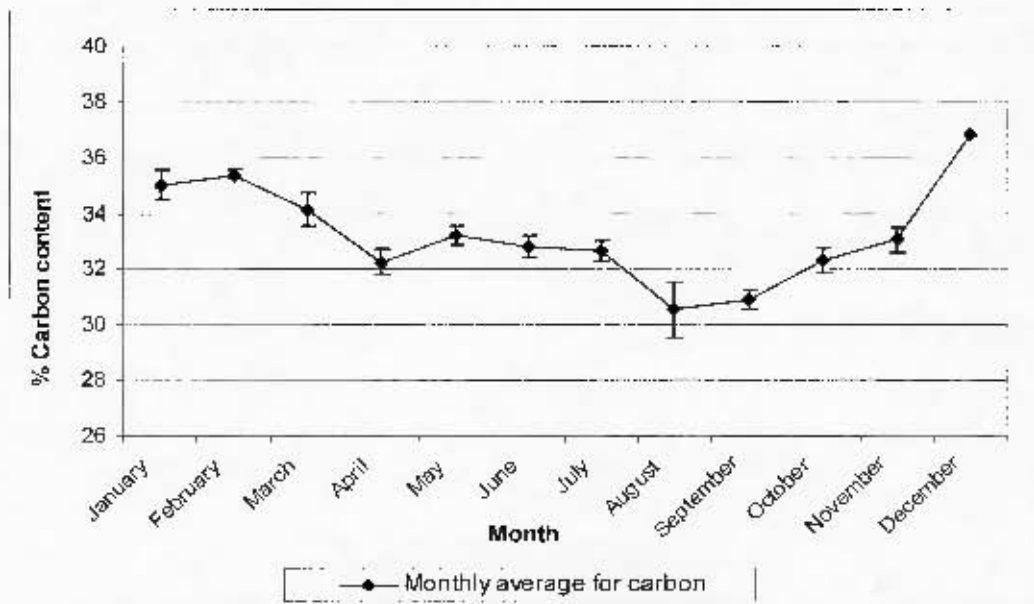
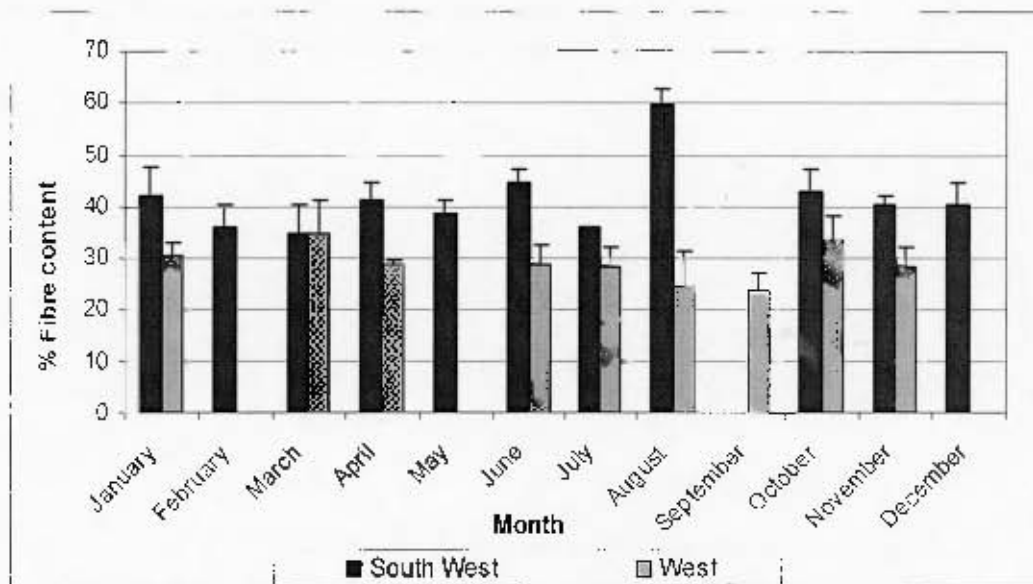


Figure 9 Total average carbon content for both locations (% dry weight) (analysed by the author) showing seasonal variation from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

Fibre differed significantly (ANOVA,  $df = 10$ ,  $F = 31.71$ ,  $p < 0.05$ ) between location with much higher average values for the south west coast compared to the west coast (Table 1). Analogous to the carbon trends (Fig 8), fibre concentrations were also equal or higher for every month on the south west coast (Fig 10). Concentrations were not significantly different (ANOVA,  $df = 11$ ,  $F = 1.071$ ,  $p > 0.05$ ) among the months (Table 1) and did not reveal a seasonal pattern (Fig 11).



**Figure 10. Average fibre content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.**

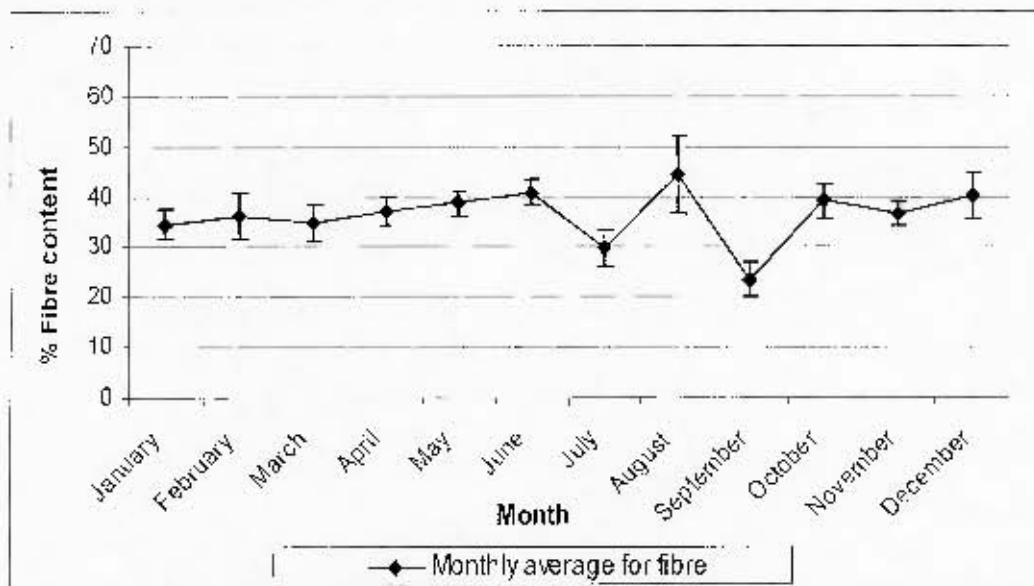
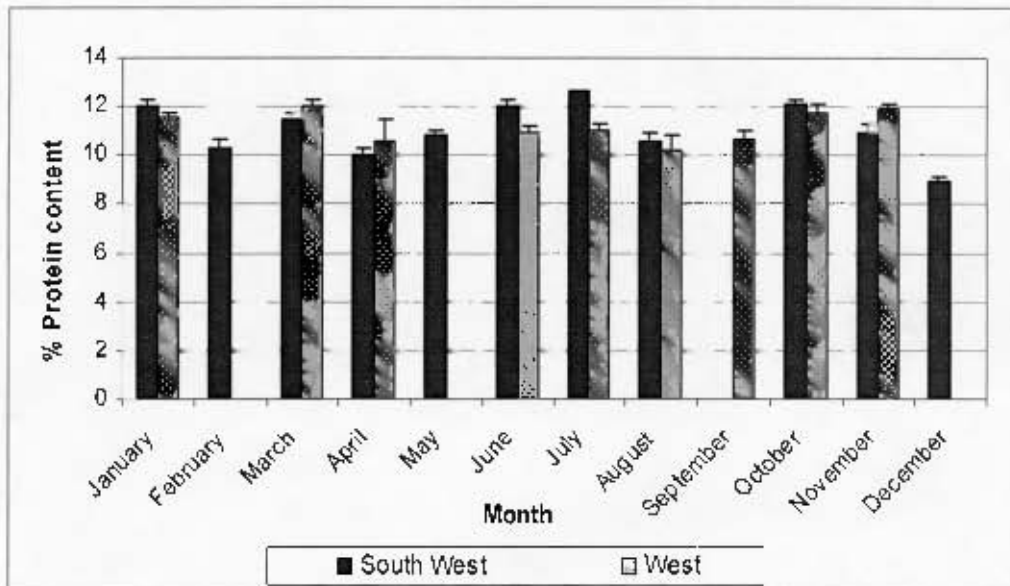


Figure 11. Total average fibre content for both locations (% dry weight) (commercial feed laboratory data) showing seasonal variation from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

Results showed that the protein concentrations on the west coast were not significantly different (ANOVA,  $df = 11$ ,  $F = 5.64$ ,  $p > 0.05$ ) to those on the south west coast (Table 1). In contrast protein concentrations tended to vary throughout the year with significant differences among months (ANOVA,  $df = 11$ ,  $F = 1.07$ ,  $p < 0.05$ ). However, these results did not reveal a seasonal pattern as December and April in particular were significantly lower (Tukey test,  $p < 0.05$ ) than the other months, which all remained relatively constant (Fig 14).

Nitrogen did not differ significantly among month (ANOVA,  $df = 11$ ,  $F = 1.75$ ,  $p > 0.05$ ) or location (ANOVA,  $df = 1$ ,  $F = 3.21$ ,  $p > 0.05$ ) (Table 1). The nitrogen

results were similar to the commercially-determined protein (Fig. 14) in that concentrations also remained relatively constant throughout the year.



**Figure 12 Average protein content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.**

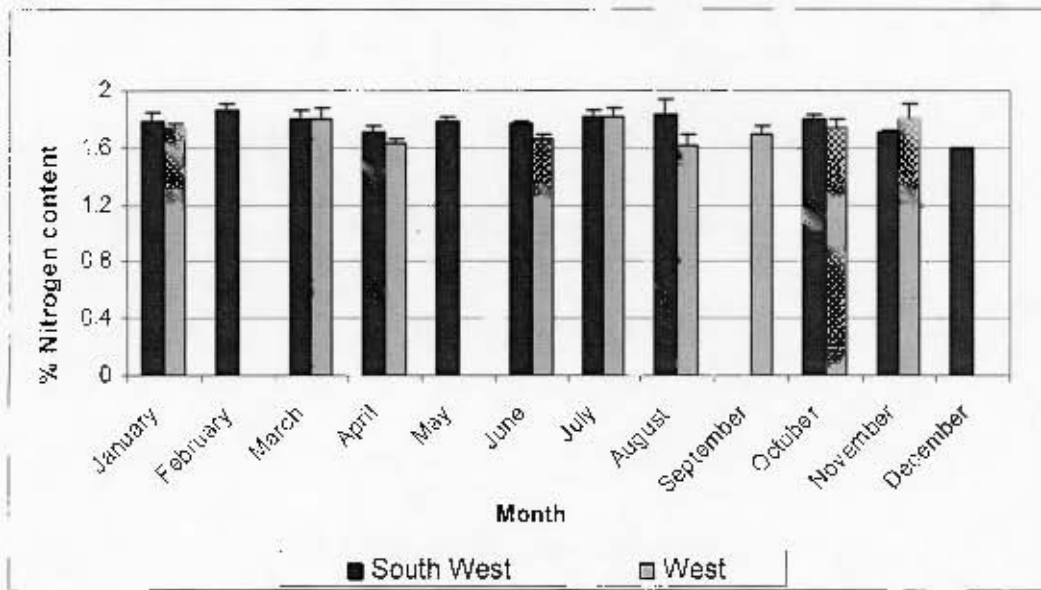


Figure 13. Average nitrogen content (% dry weight) (analysed by the author) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error. (n=278)

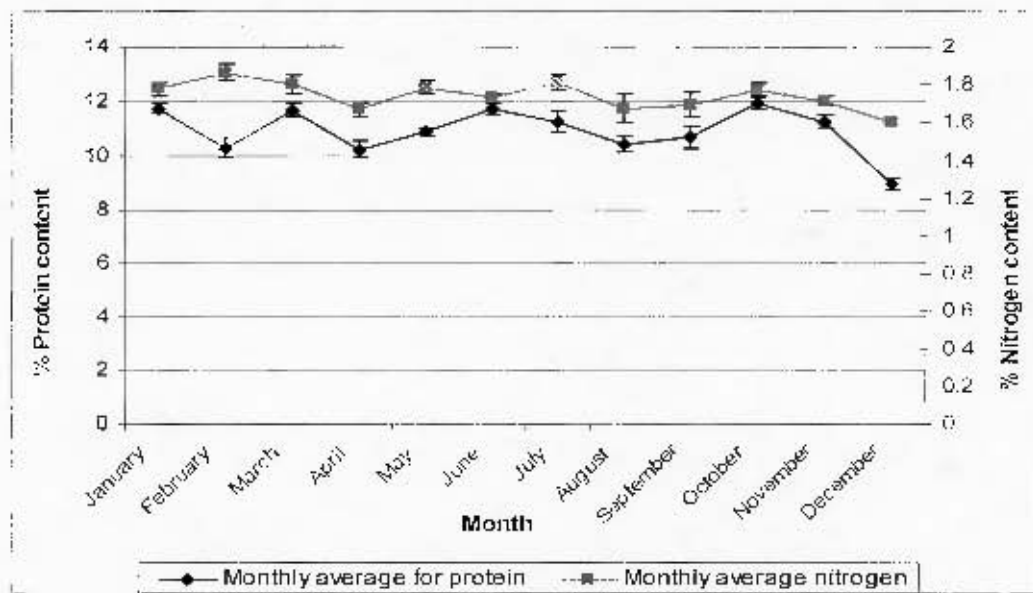
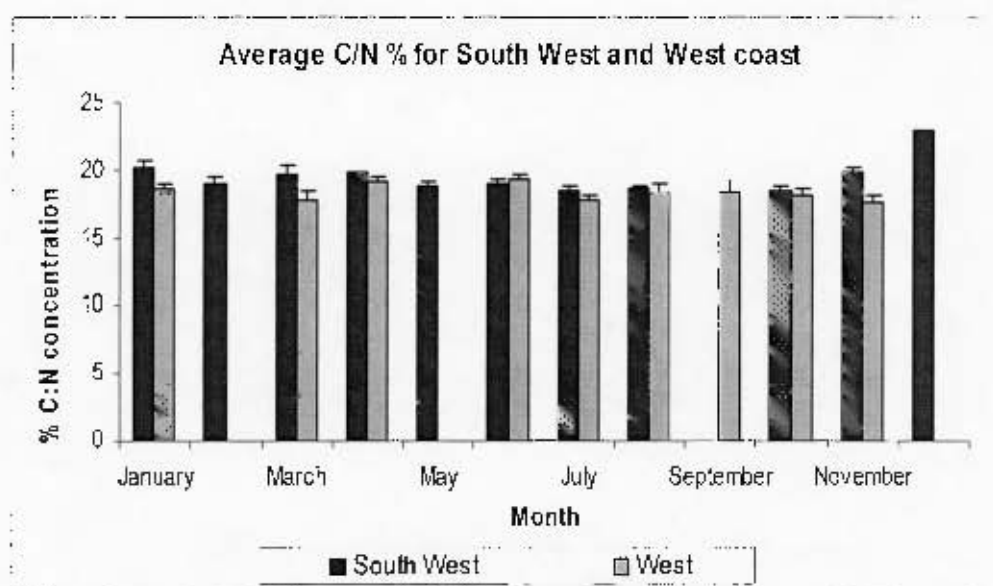
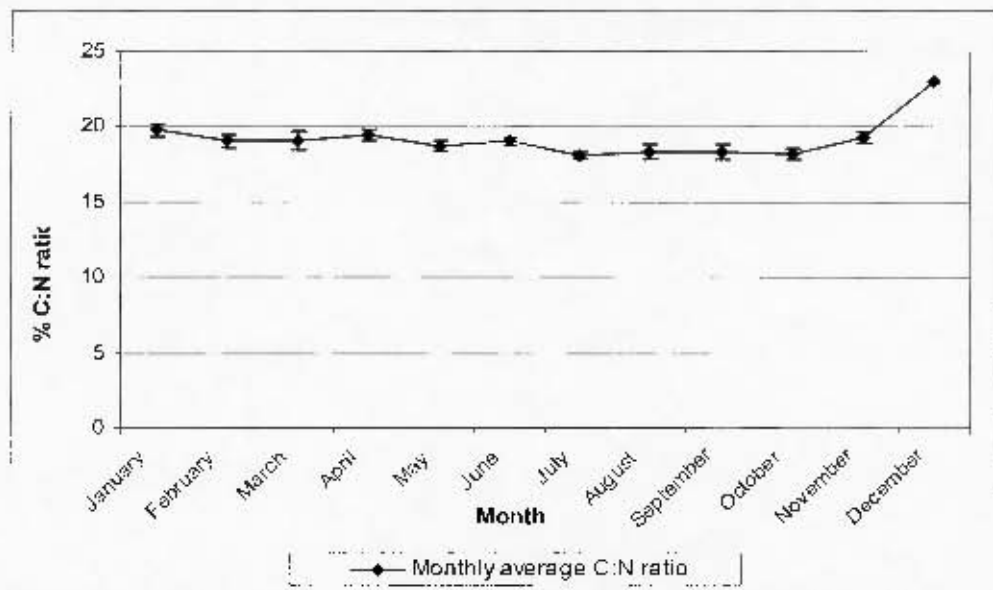


Figure 14. Total average protein for both locations (commercial feed laboratory data) and nitrogen content (analysed by the author) showing seasonal variation from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

The carbon/nitrogen ratio was significantly different (Kruskal-Wallis  $H = 7.56$ ,  $p < 0.05$ ) between the two locations with higher values found on the south west coast compared to the west coast (Fig 15). There were no significant differences between the months (Kruskal-Wallis  $H = 17.12$ ,  $p > 0.05$ ), and the values remained relatively constant throughout the year. There tends to be a slight seasonal pattern in the C:N ratio which is caused by the seasonal pattern of carbon. Concentrations tend to increase during summer (December – March). For the rest of the year the ratio is fairly constant which is caused by the lack of seasonal variation in nitrogen (Fig 16).



**Figure 15** Average C/N content (% dry weight) (analysed by the author) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error. (n=278)



**Figure 16** Total average carbon and nitrogen content for both locations (% dry weight) (both analysed by the author) showing seasonal variation from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show the standard error.

The south west coast showed a higher average fat content compared to the west coast (Table 1). Statistically, there were no significant differences among month (Kruskal-Wallis  $H = 17.56$ ,  $p > 0.05$ ) and between locations (Kruskal-Wallis  $H = 0.15$ ,  $p > 0.05$ ), therefore no seasonal patterns were revealed and values were fairly constant throughout the year (Fig. 18). There was only one measurement taken in July on the south west coast which can be ruled out as an outlier.

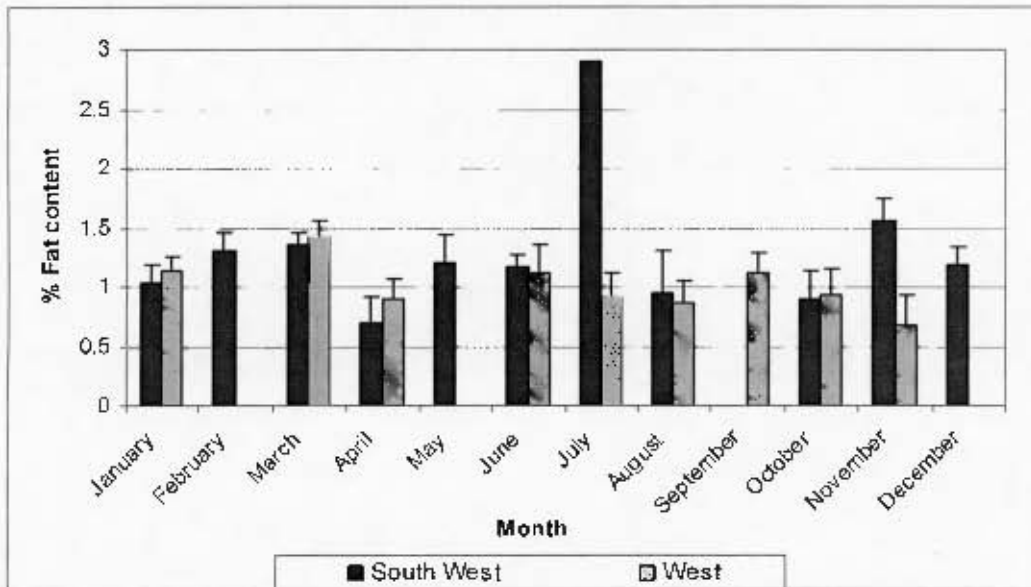


Figure 17. Average fat content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error (n=278)

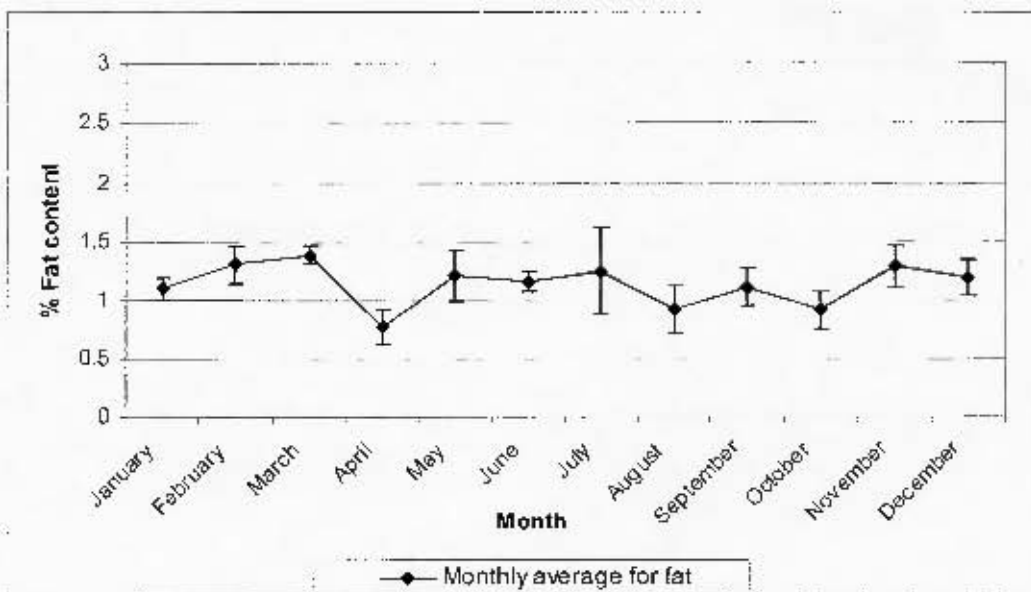


Figure 18. Total average fat content for both locations (% dry weight) (commercial feed laboratory data) over a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show the standard error.

The ash content differed significantly for both location (Kruskal-Wallis ANOVA,  $H = 24.48$ ,  $p < 0.05$ ) and month (Kruskal-Wallis ANOVA,  $H = 27.15$ ,  $p < 0.05$ ). The average for all the samples was higher on the west coast compared to the south west coast (Table 1). Contrary to the fibre and carbon data, ash concentrations were higher for all the months on the west coast compared to the south west coast (Fig 19). There tended to be a seasonal pattern with a distinct increase in concentration during the late-winter spring months, particularly on the west coast, reaching a maximum in September. Concentrations tended to be lower for the rest of the year (Fig 20).

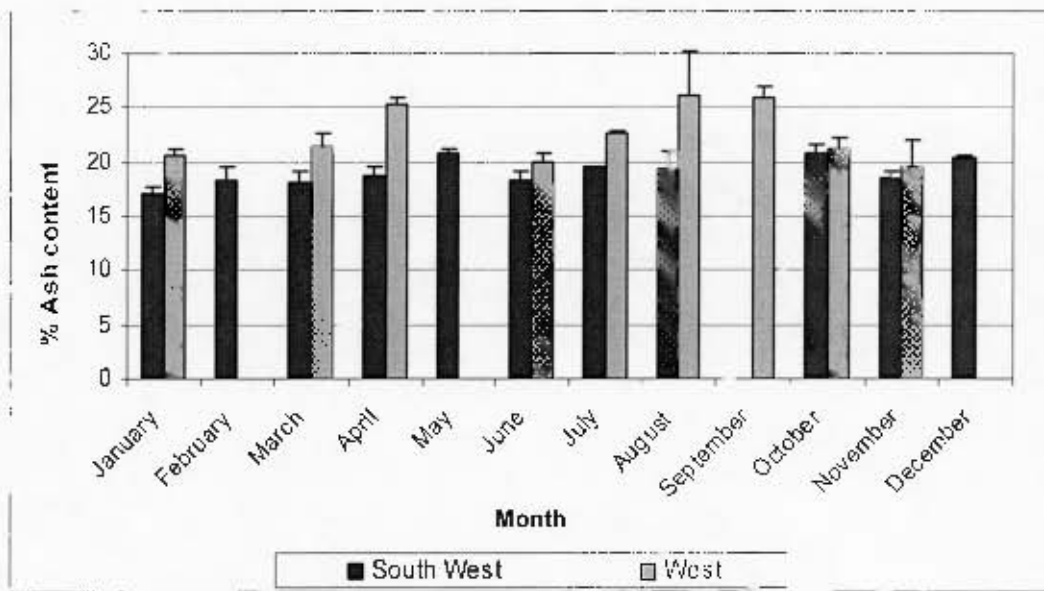


Figure 19. Average ash content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

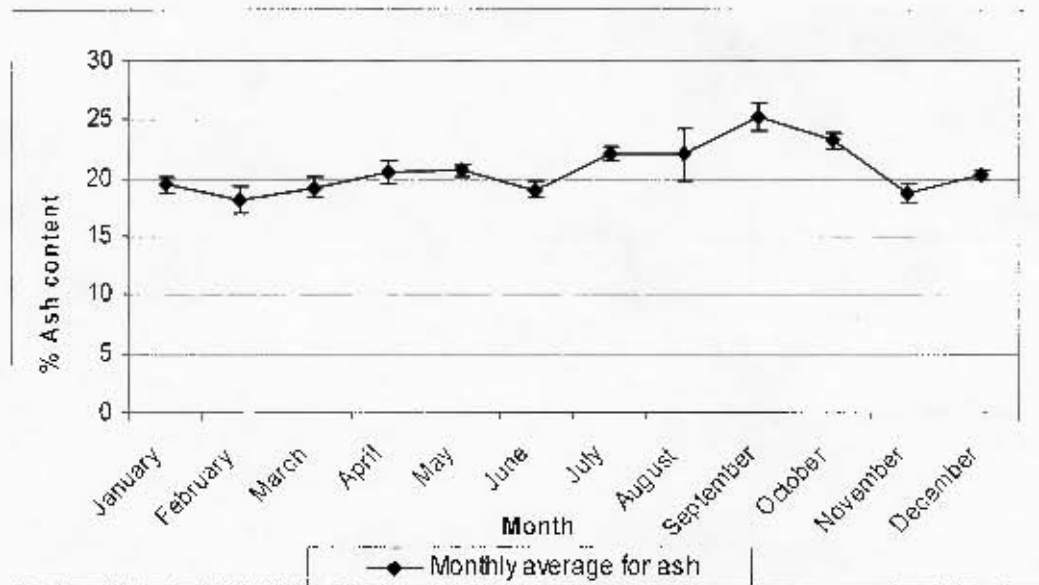


Figure 20. Total average ash content for both locations (% dry weight) (commercial feed laboratory data) showing seasonal variation from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show the standard error.

Concentrations of phosphorus were significantly different between location (ANOVA,  $df = 1$ ,  $F = 66.518$ ,  $p < 0.05$ ), higher average values for the west coast compared to on the south west coast (Table 1). Similar to the ash data, phosphorus concentrations were higher for all the months on the west coast compared to the south west coast (Fig 21). Even though phosphorus did not differ significantly with month (ANOVA,  $df = 11$ ,  $F = 1.819$ ,  $p > 0.05$ ), concentrations tended to decrease during summer reaching a minimum in December (Fig 22). For the rest of the year concentrations remained relatively constant

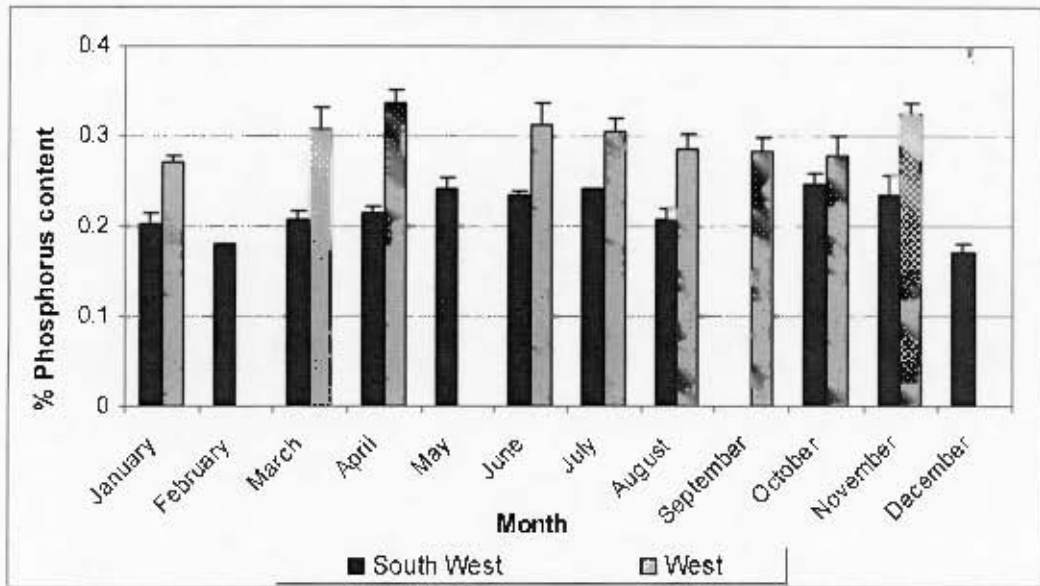


Figure 21. Average phosphorus content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

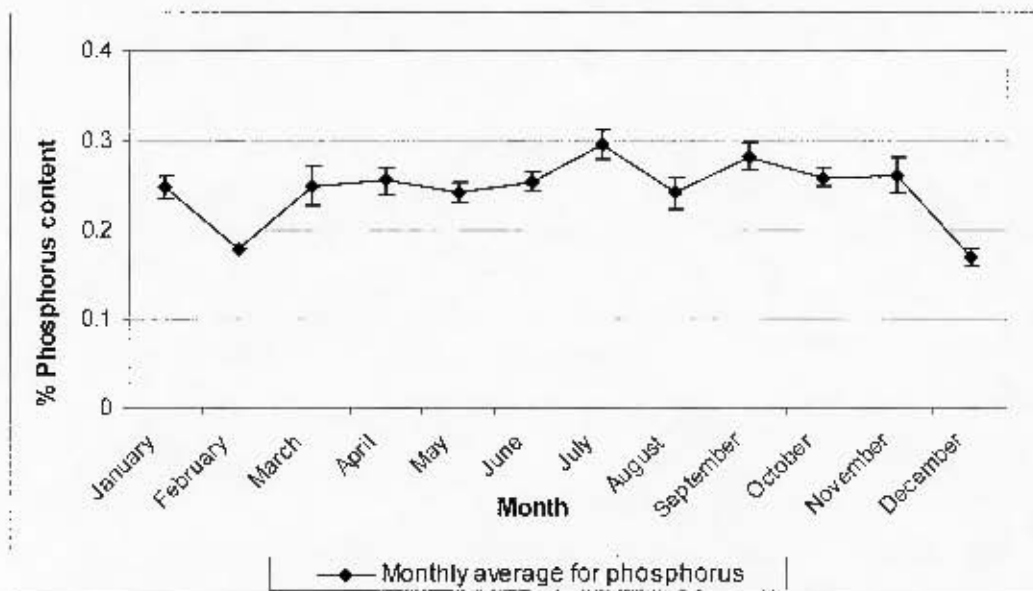


Figure 22 Total average phosphorus content for both locations (% dry weight) (commercial feed laboratory data) over a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

Calcium was significantly different for both month (ANOVA,  $df = 11$ ,  $F = 0.0668$ ,  $p < 0.05$ ) and location (ANOVA,  $df = 1$ ,  $F = 6.481$ ,  $p < 0.05$ ) with, in contrast to the ash data, higher average values for the south west coast than for the west coast (Table 1). Concentrations did not appear to show a seasonal pattern but values tended to drop during autumn reaching a minimum in April. The rest of the year remained relatively constant (Fig. 24).

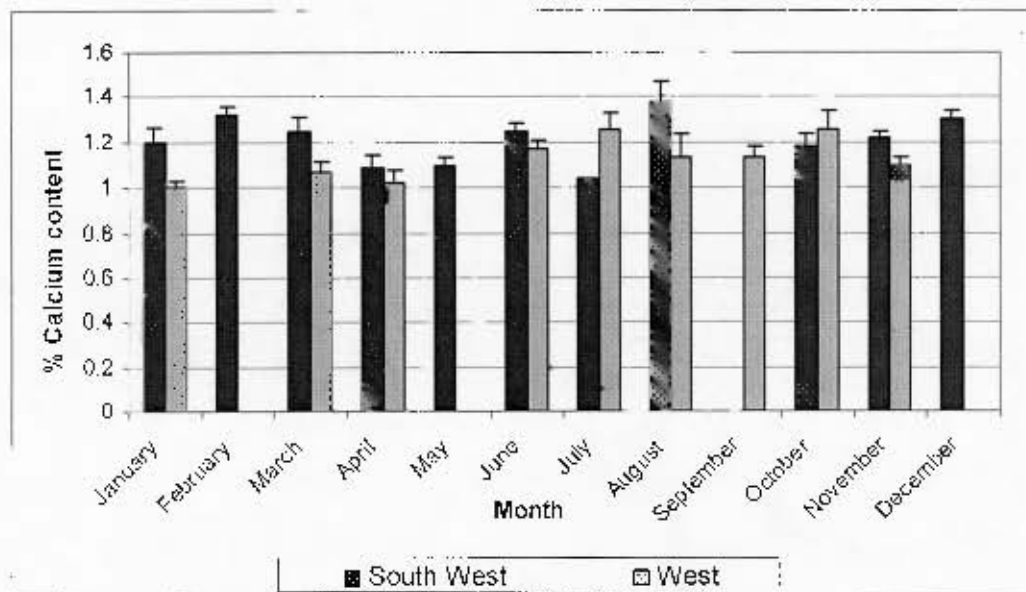


Figure 23. Average calcium content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

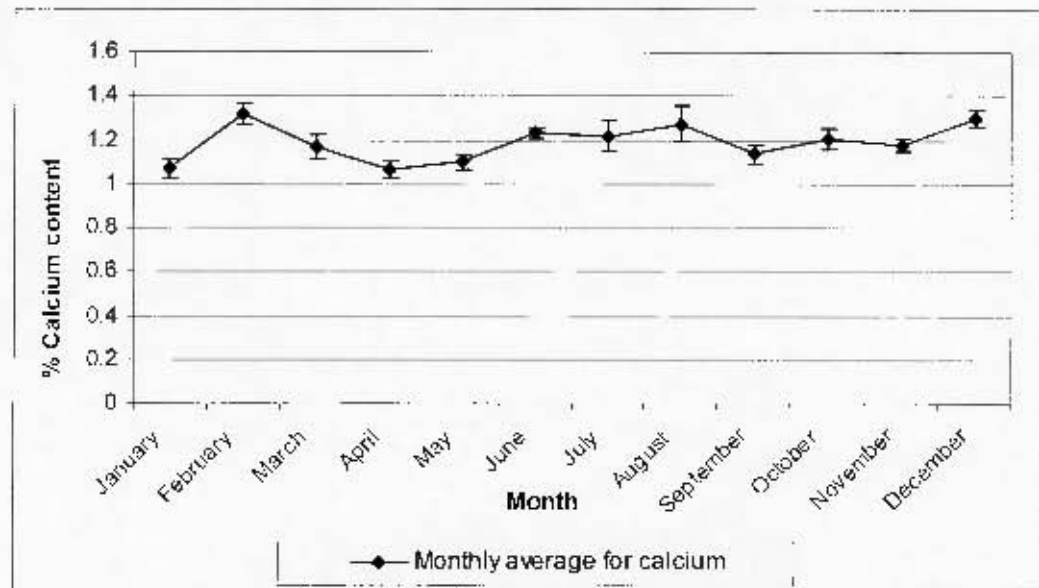


Figure 24. Total average calcium content for both locations (% dry weight) (commercial feed laboratory data) for a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

Magnesium was significantly different for both month (Kruskal-Wallis ANOVA,  $H = 29.489$ ,  $p < 0.05$ ) and location (Kruskal-Wallis ANOVA,  $H = 3.98$ ,  $p < 0.05$ ) with higher average values for the south west coast than for the west coast (Table 1). Concentrations remained constant throughout spring and summer but increased during autumn and winter reaching a maximum in August (Fig 26)

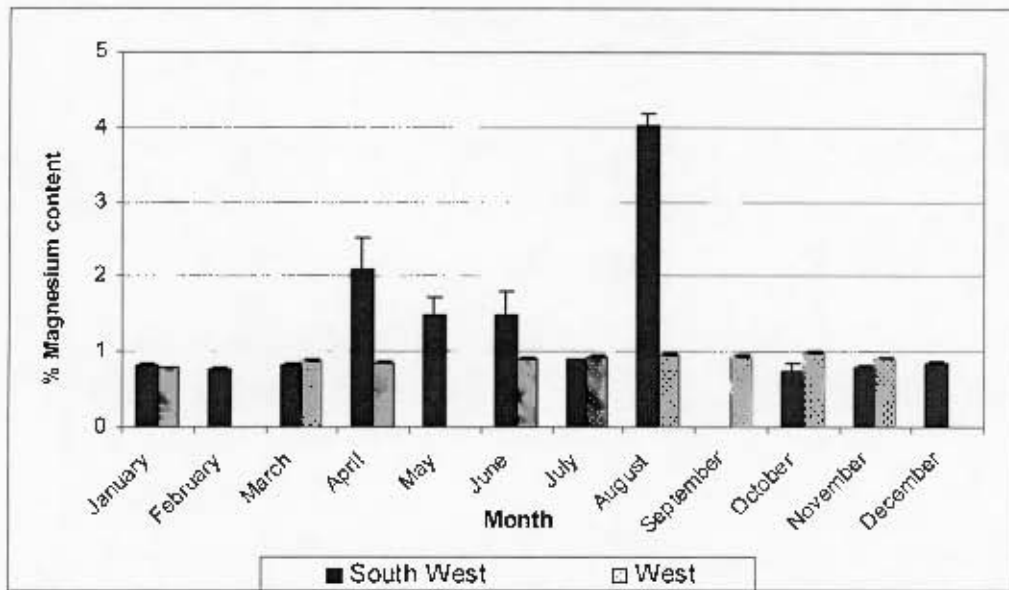


Figure 25. Average magnesium content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

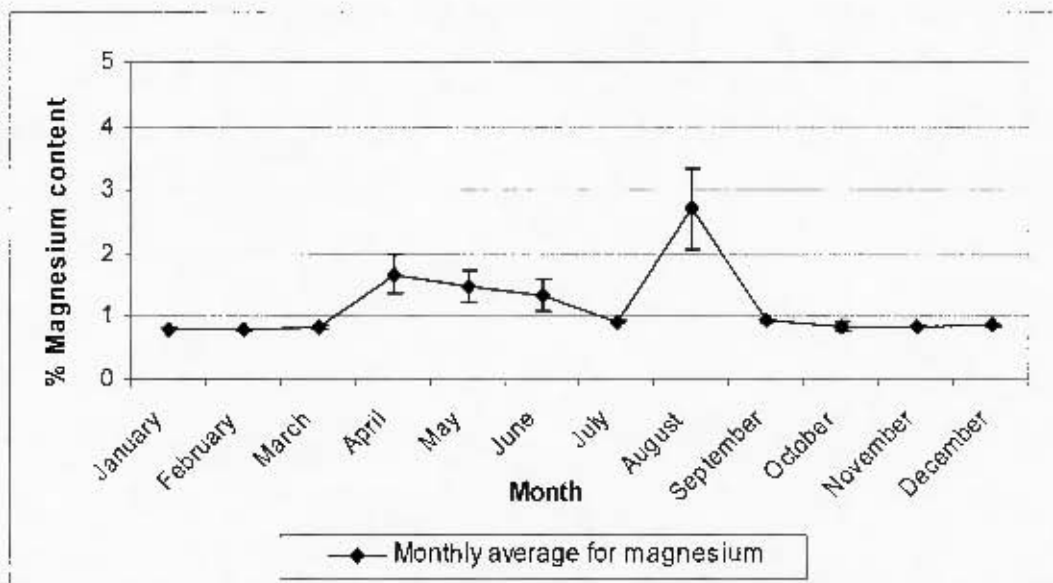


Figure 26. Total average magnesium content for both locations (% dry weight) (commercial feed laboratory data) from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

Sodium was significantly different for both month (Kruskal-Wallis ANOVA,  $H = 35.38$ ,  $p < 0.05$ ) and location (Kruskal-Wallis ANOVA,  $H = 12.79$ ,  $p < 0.05$ ) with higher average values for the west coast than for the south west coast (Table 1). Similar to the ash and phosphorus concentration, the sodium concentration on the west coast had, on average a higher value for all the months (Fig 27). Concentrations tended to be lower in spring and summer reaching a minimum in February, whereas during autumn and winter the concentrations tended to rise reaching a maximum in September (Fig 28).

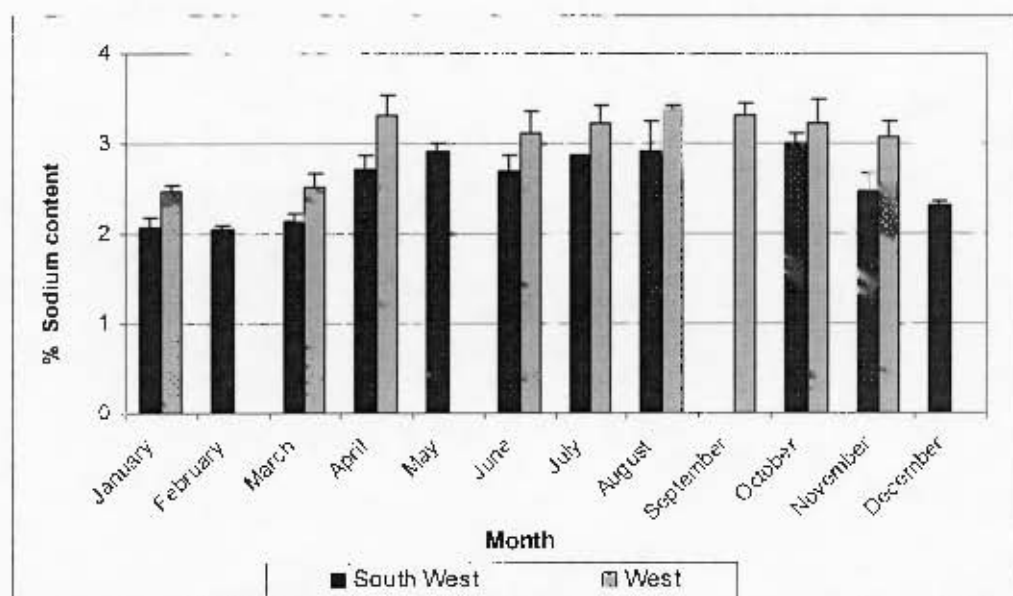
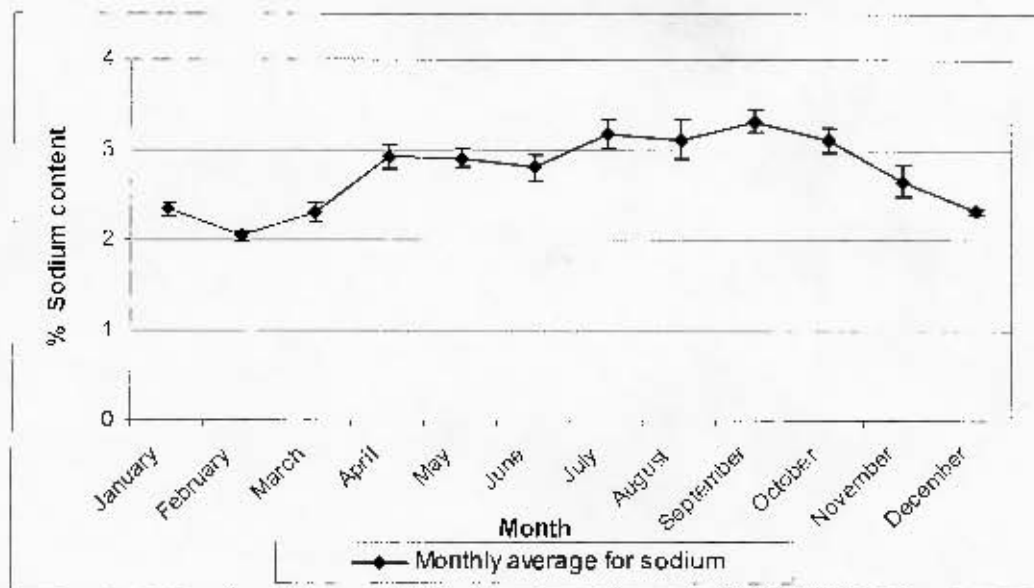


Figure 27. Average sodium content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.



**Figure 28. Total average sodium content for both locations (% dry weight) (commercial feed laboratory data) from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.**

The potassium concentrations were significantly different for both month (Kruskal-Wallis ANOVA,  $H = 21.99$ ,  $p < 0.05$ ) and location (Kruskal-Wallis ANOVA,  $H = 40.35$ ,  $p < 0.05$ ) with higher average values for the west coast than for the south west coast (Table 1). Analogous to the ash, sodium and phosphorus results, the west coast had higher average values for all the months (Fig 29). The concentrations tended to be quite variable throughout the year however, there did seem to be a decrease during summer and autumn and an increase during late winter and spring (Fig 30).

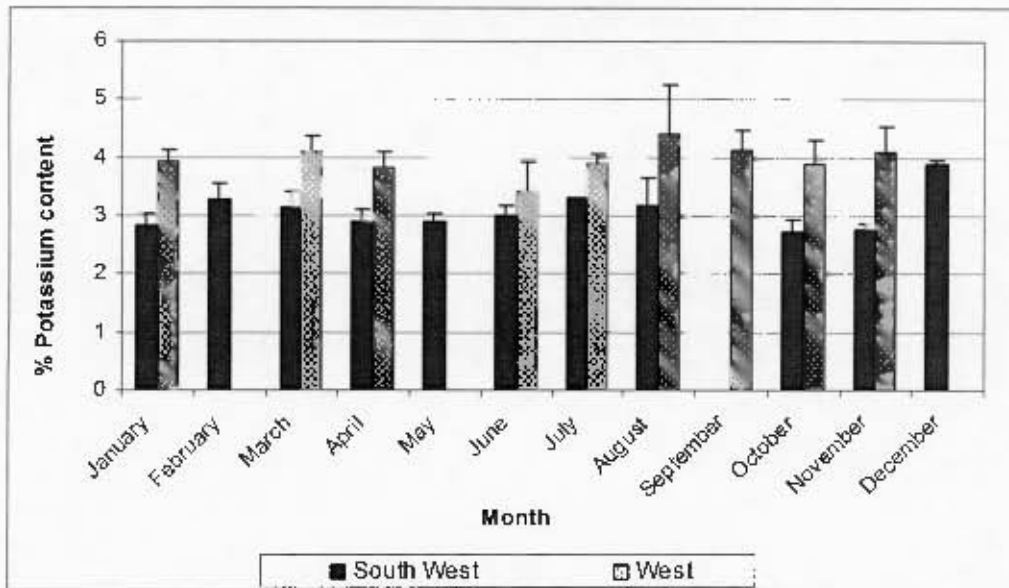


Figure 29. Average potassium content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

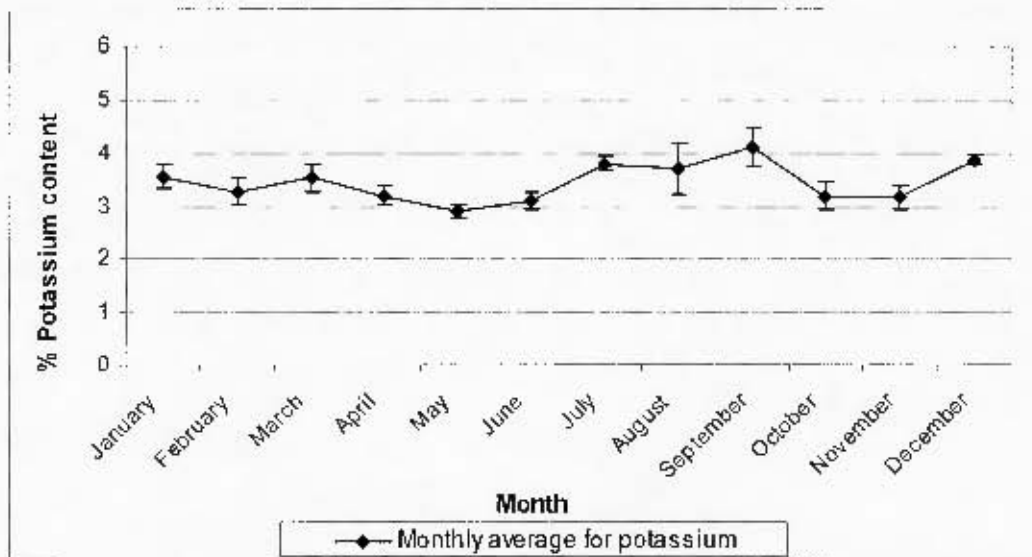


Figure 30. Total average potassium content for both locations (% dry weight) (commercial feed laboratory data) from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

Copper, zinc, manganese and iron were summed and averaged and presented as a total trace metal concentration. These values were low and did not show significant differences among months and between locations, however, there does appear to be higher values for the south west coast compared to the west coast (Fig 31). The concentrations appeared to be variable throughout the year suggesting that seasonal patterns can not be identified from these results (Fig. 32).

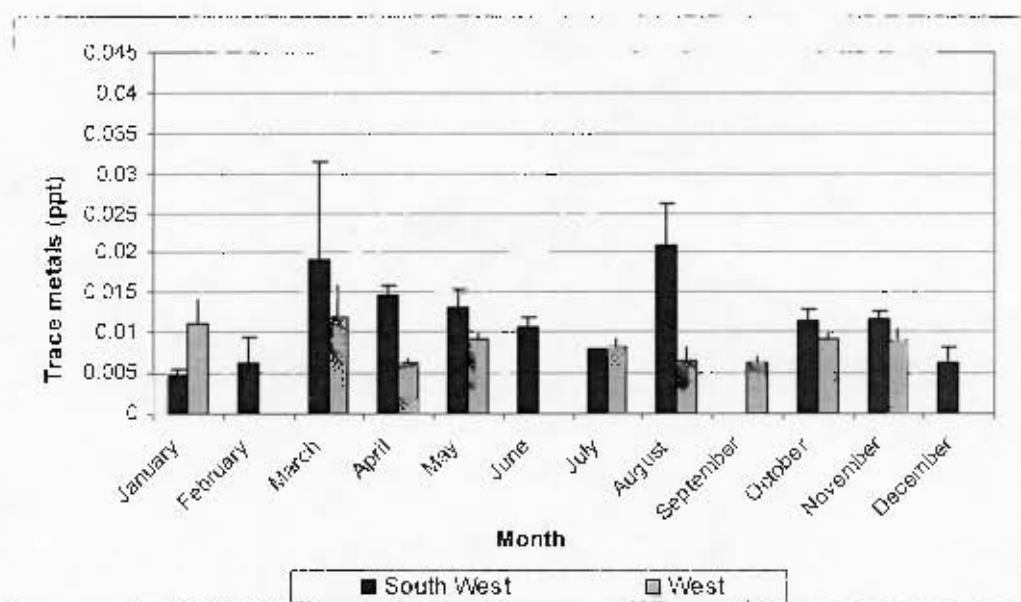


Figure 31. Average trace metal content (% dry weight) (commercial feed laboratory data) for the south west and west coast from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

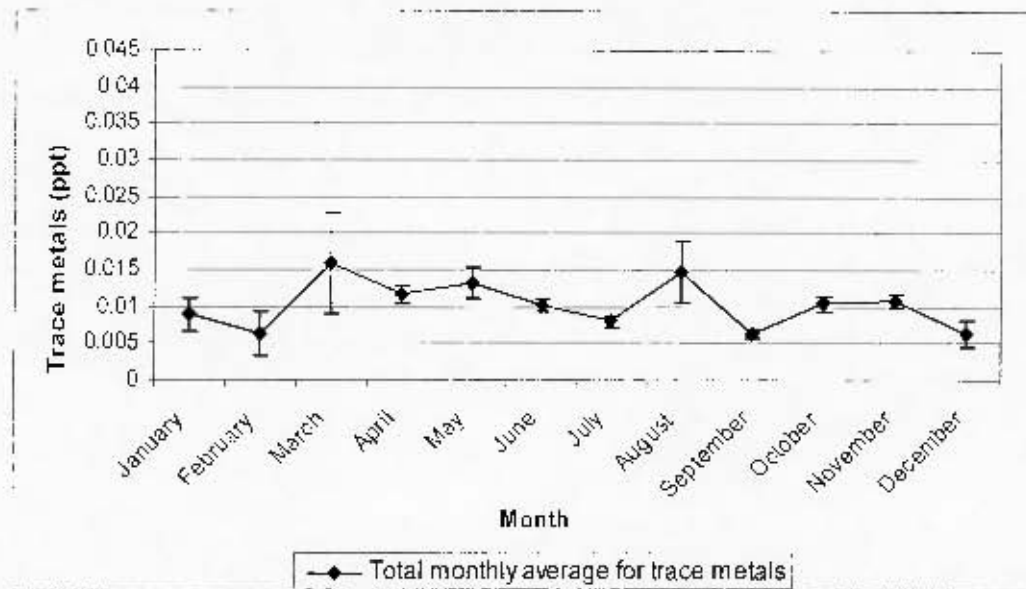


Figure 32. Total average trace metal content for both locations (% dry weight) (commercial feed laboratory data) from a 15 month period (5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005) which has been combined into a year. Error bars show standard error.

The cluster analysis of the nutrient composition from both locations (Fig 33) indicated three main groups, namely Group I, II, and III. Group I was comprised of 41% of South West kelp (58% West coast kelp), group II included 73% South West kelp (26% West Coast kelp) and group III consisted of only South West kelp. A (one-way ANOVA) test revealed that group III was significantly different to group I and II in phosphorus, magnesium, copper and iron concentrations. Group II was significantly different to group I and III in protein and fibre concentrations and group I was significantly different to Group II and III in potassium and calcium concentration. The rest of the nutrient concentrations were not significantly different between each group. The groups did not appear to be clustered in groups of seasonal variation.

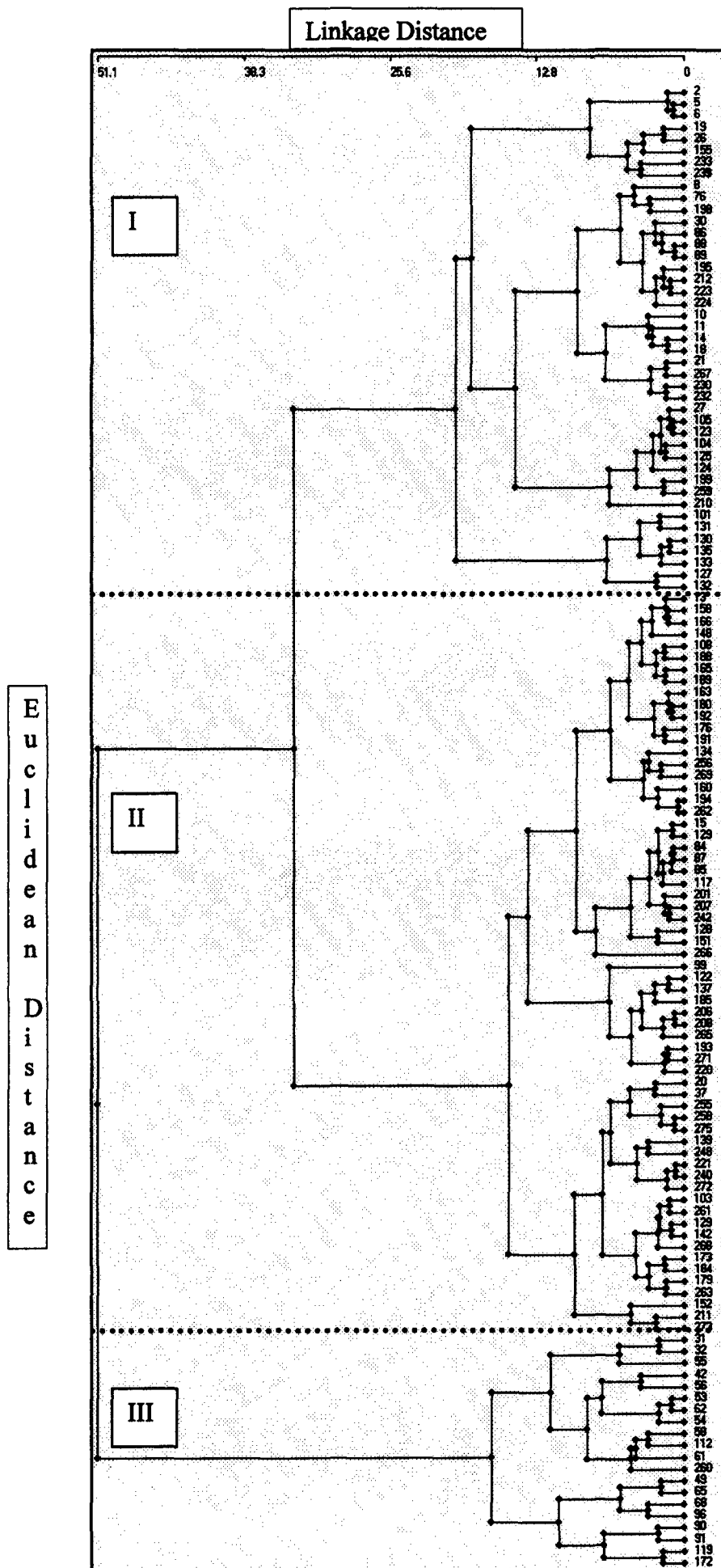


Figure 33. Hierarchical cluster analysis (complete linkage, Euclidean distance measure) of the nutritional composition of *Ecklonia maxima* from the west and south west coast of South Africa, collected from the 5<sup>th</sup> April 2004 to the 20<sup>th</sup> June 2005.

## CHATER 4

# DISCUSSION

### 4.1 Environmental triggers in kelp seasonality

Biological and environmental factors that influence the growth and chemical composition (nutrient uptake) in *Ecklonia maxima* are temperature, light, photoperiod, salinity, turbidity, nutrient and carbon supply, pH, grazer density and epiphyte density (Duke *et al.* 1986, Duke *et al.* 1989, Friedlander *et al.* 1991, Lüning 1990). It is also likely that seasonal growth and phenology patterns may be controlled by an endogenous circannual clock as has been demonstrated, for example, in the Pacific kelp *Pterygophora californica* (Lüning, 1993). These factors operate together affecting growth rate, physiological processes, chemical composition (Lüning, 1993) and, presumably, overall feed quality of the *Ecklonia maxima* species. As a result, if one factor changes then this could alter one or more of the others (Lüning, 1993). For example, the west coast is renowned for its intense seasonal inshore upwelling system which is caused by the offshore southerly winds and introduces cold, nutrient-rich bottom water from the northward flowing Benguela Current into the surface layer. A study conducted by Probyn & McQuaid (1985) showed that nitrogen uptake by *Ecklonia maxima* was linearly related to ambient concentration which resulted in a significantly higher tissue nitrogen content under upwelling conditions during summer. The nitrogen results (Fig 13).in the present study showed no apparent changes in chemical composition due to upwelling events, probably because samples within a month were combined, and upwelling works on a shorter time scale. .

In Southern Africa the west coast and south west coast experience markedly different water temperature regimes, with means of 12 and 19°C respectively, in summer and 13 and 14°C respectively, in winter (Shannon & Stander 1977). Temperature is an important determinant of plant production. Its effect on respiration and photosynthesis is well known. Generally temperature has an inverse relationship with nutrients on these coasts, with cool, upwelled water having higher nutrient levels than warmer, downwelled water. From available data, the cool west coast will have higher overall average nutrient levels, and particularly higher nutrients levels under upwelling conditions in the summer, than on the warmer south west coast (Probyn & McQuaid 1985). Ash, phosphorus, sodium and potassium in the present study, revealed higher concentrations for every month on the west coast compared to the south west coast. However, these nutrients on the south west coast were higher during winter as opposed summer (the upwelling season), which would have been expected. In contrast carbon, fibre, fat, calcium, magnesium and the trace minerals had higher values for the south west coast compared to the west coast and only carbon and fibre was higher during summer.

#### 4.2 Seasonal differences in nutritional composition between the south west coast and west coast.

Seasonal growth rates have not yet been recorded for *E. maxima* but several northern hemisphere kelps such as *Laminaria hyperborea* reflected the seasonal growth pattern of storage carbohydrates, with high content in the

summer and autumn (slow growth period), and decreasing content after growth started in January (Sjøtun *et al.* 1995).

Even though calorific content was not measured in the present study seasonal variations in *M pyrifera* and *U. lactuca* was suggested to have been a function of the change in the ash content, and changes in the chemical composition of the organic portion associated with seasonal growth and uptake of nutrients. (Lamare *et al.* 2001) Previous studies showed that an increase in calorific content could be a result of an increase accumulation of carbohydrates during spring and summer (Vergara *et al.* 1997). Hernandez *et al.* (1997) found the C:N ratio was highest in late spring and summer because of an increase in carbon content. These results could have been a result in an increase in the calorific content during spring.

Carbon content in the present study projected a seasonal pattern in which it increased during summer and decreased during winter which could have been related to calorific content. This is contrary to a study by Von Holdt *et al.* (1955) in which mannitol or carbohydrate concentrations of *E. maxima* had higher values for winter than in summer. The carbon values in the present study were significantly higher on the south west coast compared to the west coast and the average values for all the samples ( $32.93 \pm 2.08\%$ ) were approximately 5% higher to those obtained by Sjøtun (1993) for the species *Laminaria saccharina*.

In the present study tissue moisture content is high when carbon is high (in the summer). If *Ecklonia maxima* has a similar growth pattern to other kelp species such as species of *Laminaria* (Sjøtun 1993) then the high carbon content could be due to the kelp optimally photosynthesizing but at the same time growing slowly (Lüning, 1993). During winter photosynthesis slows down and growth speeds up (hence low carbon) (Sjøtun 1993). The moisture content showed an unexpected result as it should have been higher in winter when the kelp is growing faster. If the kelp is not growing, but it is photosynthesizing, it would have a higher carbon content and a lower moisture content. Nevertheless, a low water content during winter may also suggest a high content of other constituents such as ash, phosphorus, magnesium sodium and potassium, which increase during winter and decrease during summer (John Bolton 2007. pers. comm.).

Protein was estimated by multiplying nitrogen by a constant 6.25. Evidence shows that protein is frequently the principle constraint to the growth, fecundity and survival of abalone and that their diet selection generally maximizes diet protein (Duffy & Hay 1991). Protein would therefore be a good indicator as to whether the south west kelp was superior, as an abalone feed, to the west coast kelp. However, there were no significant differences in protein between the two locations. Previous studies revealed that the protein content and similarly the nitrogen content of *Laminaria hyperborea* was high during the growth period, winter and low for the rest of the year (Sjøtun *et al.* 1995). This did not seem to be the case in the present study as seasonal variation in nitrogen content can not be distinguished from the data. However,

the pattern of high versus low nitrogen content may also indicate storage of nitrogen in a period of high ambient nitrate concentration (Black & Dewar 1949). Studies of *L. longicruris* have shown that this growth pattern can result from a high concentration of nitrate in the sea-water during winter and spring and from nitrate limitation during the summer and autumn (Chapman & Craigie 1977). It was expected that the west coast would have more nitrogen, particularly during summer, due to the input of nutrients during an upwelling event but in the present study nitrogen values were not significantly different between the two locations. Asare & Harlin (1983) found that values exceeding 1% nitrogen content of tissue dry weight represented intracellular stored nitrate in *L. saccharina*. If this also applies to *Ecklonia maxima*, it suggests that nitrate is being stored in the plants during both winter and summer at both locations. On the contrary these hypothesized high levels of nitrate storage may also mean that measurement of nitrogen (and multiplying by 6.25) is not a true reflection of protein content of this kelp.

Nitrogen is one of the resources limiting seaweed growth in the natural environment and seaweed growth rates tend to parallel nitrogen supply (Duke *et al.* 1989). This implies that the nitrate content of the seawater may temporarily control the amplitude of the growth rate, just as other primary ecological factors do (Luning 1993). Additional environmental parameters influencing the water column composition and growth measurements of *Ecklonia maxima* species would have been useful in the present study. but, nevertheless, nitrogen should have been abundant on the west coast during upwelling conditions in summer (Probyn & McQuaid 1985). The south west

coast only has a few months of abundant ambient nitrogen in comparison. In the present study nitrogen did not differ significantly among month or between locations and remained relatively constant throughout the year.

The C:N ratio in macroalgae is important in terms of protein requirements for consumers such as abalone. Russell-Hunter (1970) calculated that most animals need a C:N ratio of 17 or less in their diet. A study conducted by Yoshikawa *et al.* (2001) revealed that the C:N ratio of *U. pinnatifida* ranged from 10.1 to 16.4. Carbon and nitrogen ratios of 10-15 have been found to be critical in several macroalgae, with lower values indicating storage of nitrogen and higher values causing nitrogen-limited growth (Hanisak 1983). However in other kelps, such as *L. longicuris* and *L. digitata*, the ratio is in the range of 13.8–27.2 (Mann, 1972), indicating that these algae are a poor nitrogen source at certain times of the year. In the present study the C:N ratio for both locations was greater than 15 for all the months. Seasonal reductions in calorific content was suggested by McQuaid (1985) to be related to a reduction in carbohydrate content during rapid growth, and an associated increase in carbon: nitrogen ratio (C:N). This is substantiated by the high carbon and fibre values particularly on the south west coast.

The fat concentration corresponds to 0.7 – 1.4% which is lower than the range for other *Laminaria* species (1-3%), however, abalone species show a low fat requirement (3-8%), typical of herbivore molluscs and fish (Mai *et al.*, 1995a). This low fat requirement has been associated by some authors (Durazo-Beltran *et al.*, 2004) to the low use of dietary lipids as an energy source of

abalone based upon its low metabolic rate. Dietary lipid levels that are too high (10%) seem to negatively affect abalone growth (Thongrod *et al.* 2003, Britz *et al.* 1997). However, it was demonstrated that the lowest lipid level produced the lowest growth rates in *H. tuberculata* and *H. discus hannai* (Mercer *et al.* 1993). It should be noted that the differences in growth rates could involve nutritional factors other than lipids, for example high levels of fibre, and hence carbohydrates, enhance abalone growth. Abalone have enzymes capable of hydrolyzing complex carbohydrates (Flemming *et al.* 1996) and a good capacity to synthesize non-essential lipids from carbohydrates. In the present study fibre (ADF) values were exceptionally higher on the south west coast ( $41.34 \pm 1.21\%$ ) but within the range of the optimal abalone growth requirements (33-58%) (Britz & Hecht 1997) and were comparable to other *Laminaria* species (37.3%) (Nisizawa *et al.* 1987). Conversely, the fibre concentrations on the west coast ( $28.64 \pm 1.22\%$ ) were below the abalone requirement range for maximum growth and were not nearly as much as those of other kelp species.

The composition of ash contains mostly sodium, potassium, magnesium, calcium, phosphorus and iron as well as trace minerals in small amounts. The ash content differed significantly for both location and month. The west coast had a higher ( $22.7 \pm 0.56\%$ ) average ash concentration for all the months compared to the south west coast ( $19.41 \pm 0.29\%$ ). This could be correlated with a higher calorific content described by Lamare and Wing (2001) in which *Macrocystis pyrifera* and *Ulva lactuca* showed that calorific content is negatively correlated to ash content. For maximum growth abalone only

require a low percentage of ash (12%) (Knauer *et al* 1994a) as high ash concentrations in algae could limit the presence of other nutrients and reduces nutrient digestibility (Horn 1989, Hay *et al.* 1998). The ash content tended to reveal a seasonal pattern which was inversely related to the carbon and moisture content. Higher values were found during winter while lower values were found for the rest of the year. This is analogous to the results recorded by Von Holdt *et al.* (1955), the ash in the *E. maxima* stipes reached a maximum value in mid-winter (June), it dropped to a minimum on either side of this month and then rose again towards summer. In the present study the average ash content for both locations was 5% lower than that obtained by Knauer *et al.* (1994b) for *E. maxima*

Phosphorus in seawater is available to marine algae at concentrations of 1-3  $\mu\text{m}$  (De Boer 1981). This resource may be exhausted under high seaweed and phytoplankton densities and therefore would be a limiting factor for seaweed growth. Contrary to expectation the phosphorous values, in the present study tended to be slightly higher during the winter months as apposed to summer when upwelling events occur. However a seasonal pattern is difficult to distinguish. The concentrations are low, in fact 3% lower than those calculated by Whyte *et al.* (1974) for the *Nereocystis luetkeana* species. Nevertheless, analogous to the ash concentration, the west coast had higher values for all the months compared to the south west coast. This suggests that the input of nutrients during upwelling events could be an important factor for *Ecklonia maxima* growth and development. The sudden

increase in phosphorus in January (Fig. 21) for example, could have been a direct result of upwelling events.

The calcium concentrations did not reveal a seasonal pattern but values were higher on the south west coast compared to the west coast. Calcium is an important component in the formation and strengthening of the abalone shell. The shell consists of three layers: an outer horny periostracum, (occasionally fibrous), a thick calcareous prismatic layer and an inner pearly nacreous layer (Day 1974). The prismatic layer is composed of calcium crystals and is responsible for linear growth of the shell (Culver *et al.* 1997).

Magnesium concentrations remained low and constant throughout spring and summer but during autumn and winter the south west coast concentrations suddenly increased while the west coast remained at a constant level. The study sites, particularly on the south west coast, are in a winter rainfall area, therefore this increase in concentrations could have been due to a terrestrial environmental influence in which a pollutant was introduced to the system via surface run-off.

Sodium and potassium revealed higher concentrations for all the months on the west coast. Concentrations tended to be lower during spring and autumn but rose during autumn and winter reflecting a potential seasonal pattern.

The trace metals, copper, zinc, manganese and iron were low and did not show a seasonal pattern. Although trace metals are an essential element as a

co-factor of enzymes and key participants in several metabolic pathways (Stauber & Florence, 1987; Gaetke & Chow, 2003), at elevated concentrations they become toxic (Florence & Stauber 1986, Gledhill *et al.* 1997). Nevertheless, a range of macro and micronutrients as well as various trace elements are necessary for algal growth (De Boer 1981).

According to the cluster analysis (Fig 33) the nutritional differences correspond to the difference in location rather than seasonal variation. The south west coast had higher calcium, carbon and fibre (hence higher carbohydrates) concentrations than the west coast which could make it slightly nutritionally richer and therefore better as an abalone feed than the west coast.

## CHAPTER 5

# CONCLUSIONS

Based on a calculated food conversion ratio, the south west coast abalone farmers claim that kelp growing in this area is more beneficial as an abalone feed than the kelp growing around the west coast. The aim of this study was investigate this theory and provide reasons why this may be the case. The following conclusions have been deduced:

- Overall the study found no single trend in change of chemical composition with season and the differences were mainly to do with location.

- Moisture content was lower during winter and autumn (which may be the growth season although no data are available in the literature for seasonal growth of *E. maxima*).
- Carbon content was high during summer and decreased during winter. Other kelp species *Laminaria longicruris* (Gagné, 1982), *Laminaria solidungula* (Lunning 1993) and *Laminaria hyperborea* (Sjøtun et al. 1995) for example, have been known to grow in winter from stored carbon which was assimilated during the summer months (Sjøtun et al. 1996).
- Protein did not show a seasonal pattern and concentrations were no different on the south west compared to the west coast.
- Carbon and fibre contents are markedly greater on the south west coast which contributed to the high C:N ratios.
- Fat concentrations were low and did not reveal a seasonal pattern
- Ash, phosphorus, sodium and potassium had higher values on the west coast particularly in spring which could be explained by the more prevalent inshore upwelling system found in this area.
- *Ecklonia maxima* is a nutritional rich species for abalone farming in terms of carbon and fibre but it is important to note that the nutritional values here are based on feed analysis. Biological analysis using animal feeding trials would be required to establish the complete nutritional value of this kelp. However, the difficulty with doing feeding trials between two coasts is that it is costly and logistically extremely difficult to test fresh kelp in controlled conditions from sites 300 km apart.

If details of the exact nutritional requirements of abalone and the composition of seaweeds are known, mixing and matching appropriate seaweeds could produce an efficient diet. (Fallu 1991). It has generally been accepted that a balanced level of protein (>15), lipid (3-5%) and carbohydrates (20-30%), without any toxic substances in the natural algae, are essential for an optimal growth performance of abalone (Mercer *et al.* 1993). On the south west coast and the west coast *Ecklonia maxima* in this study was low in protein and lipid but high in carbohydrates, particularly on the south west coast. Further feeding trials with live abalone may show carbon ,fibre, ash, phosphorus, sodium and potassium concentrations in kelp are important elements for healthy abalone growth. If these prove to be critical factors then the results of this study will enable the South African abalone farmers to select the optimal kelp forests for abalone feeding. However, based on current knowledge, its not possible to state that one location is superior as an abalone feed over the other.

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<http://www.hikabalonefarm.co.za>. 21 March 2007



42	I & J	I & J	2004-05-03	10:00	80.4					10.42	1.10	16.50	54.40	1.13	0.02	3.64	2.03	2.50	0.0005	0.0011	0.0005	0.01
43	I & J	Buffeljags	2004-05-18	11:00	79.93	34.10	1.84	18.53														
44	I & J	Buffeljags	2004-05-18	11:00	80.6	33.90	1.65	20.55														
45	I & J	Buffeljags	2004-05-18	11:00	80.01	30.20	1.57	19.24														
46	I & J	Buffeljags	2004-05-03	12:00	80.55	35.30	1.8	19.61														
47	AVA	Beach	2004-06-18	12:00	79.07	34.90	1.65	21.15														
48	AVA	Beach	2004-06-01	12:00	77.61	35.40	1.68	21.07														
49	AVA	Beach	2004-04-05	08:00	81.48				11.28	1.30	16.70	64.20	1.24	0.21	3.95	2.54	2.70	0.0004	0.0015	0.001	0.0153	
50	AVA	Beach	2004-06-01	12:00	77.66	34.80	1.72	20.23														
51	AVA	Beach	2004-04-05	12:45	76.44																	
52	AVA	Beach	2004-04-20	12:00	74.7																	
53	AVA	Beach	2004-06-01	12:00	81.23				11.4	1.30	15.20	57.00	1.18	0.23	3.34	1.89	2.16	0.0004	0.0013	0.0006	0.0139	
54	AVA	Beach	2004-06-01	12:00	81.82				10.59	1.40	13.20	57.40	1.23	0.21	3.30	2.04	2.08	0.0004	0.0014	0.0006	0.0125	
55	HIK	Dyer Island	2004-06-19	16:00	72.38				9.57	0.40	23.10	54.20	1.16	0.24	4.22	3.72	4.53	0.0004	0.0016	0.0007	0.0165	
56	HIK	Dyer Island	2004-06-19	16:00	81.82				10.77	0.80	19.90	55.40	1.43	0.21	3.99	3.01	2.95	0.0004	0.0013	0.0007	0.0105	
57	AVA	Beach	2004-04-20	12:00	77.09																	
58	AVA	Beach	2004-04-05	12:45	78.13				10.77	2.90	17.90	51.80	1.10	0.25	3.54	2.87	1.71	0.0004	0.0014	0.0007	0.0183	
59	AVA	Beach	2004-06-18	12:00	83.25																	
60	AVA	Beach	2004-06-18	12:00	76.5																	
61	AVA	Beach	2004-04-05	12:45	76.23				9.1	0.50	15.80	49.80	1.05	0.23	3.36	2.47	2.24	0.0004	0.0011	0.0007	0.0161	
62	AVA	Beach	2004-06-01	12:00	83.6				10.58	1.30	15.20	57.40	1.19	0.25	3.44	2.52	2.34	0.0004	0.0016	0.0007	0.0115	
63	AVA	Beach	2004-04-05	12:45	82.76	34.80	1.95	17.85														
64	AVA	Beach	2004-06-01	12:00	78.04	34.50	1.7	20.29														
65	HIK	Dyer Island	2004-06-19	10:00	83.28				11.14	0.80	15.00	63.80	1.80	0.19	4.29	2.16	2.34	0.0006	0.0017	0.0007	0.0335	
66	HIK	Dyer Island	2004-06-19	10:00	85.5	35.60	1.84	18.35														
67	AVA	Beach	2004-04-20	12:00	0	34.90	2.15	16.23														
68	HIK	Dyer Island	2004-06-19	10:00	79.98				10.79	2.00	19.30	65.60	1.32	0.19	3.62	2.77	2.93	0.0005	0.0019	0.0007	0.0116	
69	HIK	Dyer Island	2004-06-19	10:00	83.31	32.20	1.71	18.83														
70	JSP	Omdraai	2004-06-18	12:00	77.04	30.70	1.53	20.07														
71	HIK	Plankhuis	2004-06-10	11:00	79.53	32.70	1.86	17.58														
72	JSP	Omdraai	2004-07-13	09:00	79.78	32.30	1.77	18.25														
73	JSP	Omdraai	2004-07-29	11:00	75.93	30.40	1.68	17.99														
74	JSP	Omdraai	2004-06-27	11:00	80.39	30.10	1.67	18.02														
75	JSP	Omdraai	2004-06-18	12:00	78.42	34.90	1.69	20.65														
76	JSP	Omdraai	2004-06-27	11:00	74.9				10.88	0.80	20.70	32.20	1.21	0.34	0.86	3.80	3.79	0.0001	0.0027	0.0003	0.0087	
77	JSP	Omdraai	2004-08-12	12:00	77.96	28.50	1.57	18.15														
78	JSP	Omdraai	2004-06-27	11:00	82.59	32.30	1.77	18.25														
79	JSP	Omdraai	2004-06-27	11:00	77.93	29.90	1.61	18.57														
80	JSP	Omdraai	2004-07-29	11:00	73.18	33.20	1.91	17.38														
81	JSP	Omdraai	2004-08-12	12:00	79.07	30.20	1.51	20.00														
82	JSP	Omdraai	2004-07-14		70	32.70	1.93	16.94														
83	JSP	Omdraai	2004-07-14		80.79	34.50	2.12	16.27														
84	JSP	Omdraai	2004-07-29	11:00	69.55				11.06	0.70	22.30	34.60	1.36	0.31	0.94	3.29	3.68	0	0.0017	0.0009	0.0084	

85	JSP	Omdraai	2004-07-29	11:00	72.63				10.25	0.90	22.80		34.60	1.29	0.30	0.95	3.41	4.14	0.0001	0.0021		0.0005	0.0062
86	JSP	Omdraai	2004-06-27	11:00	72.84				10.41	0.70	18.80		30.80	1.26	0.34	0.94	3.45	4.06	0.0002	0.003		0.0011	0.0044
87	JSP	Omdraai	2004-07-29	11:00	78.15				10.93	0.30	22.80		35.00	1.44	0.36	0.99	3.80	4.28	0.0001	0.0021		0.0012	0.0045
88	JSP	Omdraai	2004-11-01	13:45	83.86				11.79	0.50	18.80		30.60	1.09	0.32	0.88	3.26	4.07	0.0002	0.0025		0.0003	0.0085
89	JSP	Omdraai	2004-11-01	13:45	84.33				12.09	0.20	19.00		30.00	1.18	0.36	0.97	3.39	4.07	0.0001	0.0029		0.0008	0.0051
90	HIK	Planhuis	2004-05-31	11:00	80.65				11.06	4.40	22.60		61.20	1.09	0.30	4.23	3.71	2.00	0.0004	0.002		0.0007	0.0133
91	HIK	Planhuis	2004-05-31	11:00	75.56				10.37	4.20	24.50		60.20	1.09	0.25	4.43	3.85	2.42	0.0004	0.0024		0.0007	0.0133
92	HIK	Planhuis	2004-05-31	11:00	63.46	31.80	1.77	17.97															
93	HIK	Planhuis	2004-05-31	11:00	74.61	34.10	1.97	17.31															
94	HIK	Planhuis	2004-05-31	11:00	73.76	33.60	2.06	16.31															
95	JSP	Omdraai	2004-11-01	13:45	77.92	32.50	1.91	17.02															
96	HIK	Planhuis	2004-05-31	11:00	77.56	32.90	1.86	17.50	11.6	4.10	19.20		63.60	1.09	0.23	3.93	2.54	2.12	0.0006	0.0016		0.0008	0.0269
97	HIK	Planhuis	2004-06-10	11:00	73.74	31.70	1.66	18.87															
98	HIK	Planhuis	2004-06-10	11:00	68.72	30.60	1.87	18.36															
99	JSP	Omdraai	2004-11-01	13:45	80.67				12.32	0.60	14.00		35.40	1.14	0.32	0.92	2.53	3.06	0.0002	0.0027		0.0011	0.0063
100	JSP	Omdraai	2004-11-01	13:45	83.77																		
101	JSP	Omdraai	2004-11-01	13:45	70.08	30.30	1.68	18.04	11.63	1.41	26.45		17.24	0.98	0.30	0.87	3.07	5.18	0.0001	0.0008		0.0002	0.003
102	JSP	Omdraai	2004-09-30	11:00	82.96	31.10	1.55	20.06															
103	JSP	Omdraai	2004-10-12	11:00	83.15				12.7	0.60	18.50		40.20	1.39	0.30	0.97	2.79	3.60	0.0002	0.0027		0.0008	0.0034
104	JSP	Omdraai	2004-10-12	11:00	83.76	31.50	1.74	18.10	10.95	1.55	22.94		19.95	1.07	0.21	0.90	2.83	4.11	0	0.0011		0.0002	0.0078
105	JSP	Omdraai	2004-09-30	11:00	78.99	32.60	1.91	17.07	12.19	1.16	22.45		18.71	1.07	0.31	0.91	2.82	3.86	0.0005	0.0012		0.0002	0.0082
106	JSP	Omdraai	2004-09-30	11:00	80.05	31.50	1.81	17.40															
107	JSP	Omdraai	2004-10-12	11:00	84.06	32.40	1.66	19.52															
108	JSP	Omdraai	2004-10-12	11:00	82.49				11.96	0.90	20.30		38.80	1.38	0.30	1.09	3.78	4.89	0.0003	0.0022		0.0008	0.0055
109	HIK	Dyer Island	2004-04-05	10:30	82.91	27.20	1.6	17.00															
110	HIK	Dyer Island	2004-04-05	10:30	81.49	33.80	1.74	19.43															
111	HIK	Dyer Island	2004-04-19	10:00	84.36	35.30	2	17.65															
112	HIK	Dyer Island	2004-04-05	10:30	78.16				9.17	0.60	18.50		51.20	1.06	0.20	3.56	2.50	2.84	0.0005	0.0014		0.0006	0.0149
113	HIK	Dyer Island	2004-04-05	10:30	88.44	35.10	1.74	20.17															
114	HIK	Dyer Island	2004-04-19	10:00	81.87	32.40	1.8	18.00															
115	HIK	Dyer Island	2004-04-19	10:00	83.25	33.20	1.77	18.76															
116	JSP	Omdraai	2004-10-12	11:00	85.59	29.90	1.83	16.34															
117	JSP	Omdraai	2004-10-12	11:00	83.89				11.48	0.50	22.70		34.80	1.19	0.30	0.93	3.55	2.88	0.0002	0.0015		0.001	0.0085
118	HIK	Dyer Island	2004-10-13	10:00	83.11	32.00	1.8	18.84															
119	HIK	Dyer Island	2004-10-13	10:00	85.75				11.03	0.40	21.30		62.40	1.15	0.20	0.11	3.27	2.72	0.0005	0.0014		0.0007	0.0129
120	JSP	Omdraai	2004-08-24	13:00	81.36	28.50	1.67	17.07															
121	HIK	Dyer Island	2004-10-13	10:00	85.1	34.30	1.94	17.68															
122	JSP	Omdraai	2004-06-27	11:00	82.55				11.89	1.20	18.40		34.80	1.12	0.33	0.93	2.67	1.96	0.0003	0.0021		0.0008	0.0046
123	JSP	Omdraai	2004-07-13	09:00	80.51	32.60	1.86	17.53	11.93	1.22	22.13		19.47	1.14	0.27	0.90	2.76	3.50	0.0005	0.0012		0.0002	0.0039
124	JSP	Omdraai	2004-06-18	12:00	77.74	32.70	1.67	19.58	10.39	1.77	21.69		17.70	1.08	0.24	0.87	2.69	3.87	0.0001	0.001		0.0002	0.0061
125	JSP	Omdraai	2004-07-13	09:00	79.01	32.10	1.73	18.55	10.77	1.48	23.13		18.35	1.04	0.29	0.88	2.88	3.89	0.0003	0.0017		0.0002	0.0045
126	JSP	Omdraai	2004-07-29	11:00	72.58	29.30	1.54	19.03															
127	JSP	Omdraai	2004-08-12	12:00	79.53	28.20	1.43	19.72	9.25	1.09	32.36		16.58	1.07	0.29	0.89	3.30	5.61	0.0005	0.0012		0.0002	0.0027





214	HIK	Dyer Island	2005-02-07	04:00	90.89	35.70	1.78	20.06													
215	HIK	Dyer Island	2005-01-24	06:00	90.6	36.10	1.89	21.36													
216	HIK	Dyer Island	2005-01-31	15:55	89.6	35.90	1.89	21.24													
217	HIK	Dyer Island	2005-01-24	06:00	85.43	34.80	1.84	21.22													
218	HIK	Dyer Island	2005-02-07	04:00	80.44	35.60	1.78	20.00													
218	HIK	Dyer Island	2005-01-31	15:55	88.32	36.40	1.85	22.06													
220	HIK	Dyer Island	2005-03-07	15:35	89.74				11.35	1.59	18.50	32.92	1.15	0.19	0.78	1.93	2.33	0.0001	0.001	0.0004	0.0087
221	HIK	Dyer Island	2005-03-30	15:55	92.23				12.29	1.47	19.03	46.56	1.41	0.19	0.91	2.12	3.15	0.0004	0.0008	0.0007	0.054
222	HIK	Dyer Island	2005-03-15	15:45	89.23	36.20	1.85	21.94													
223	HIK	Dyer Island	2005-05-23	15:45	89.5				11.28	1.34	19.23	29.64	1.35	0.20	0.83	2.07	3.42	0.0002	0.0007	0.0005	0.0167
224	HIK	Dyer Island	2005-05-23	15:45	88.86				11.41	1.49	17.10	28.59	1.17	0.20	0.77	2.01	2.74	0.0002	0.0006	0.0007	0.0556
225	HIK	Dyer Island	2005-03-15	15:45	88.92	34.70	1.74	19.94													
226	HIK	Dyer Island	2005-05-23	15:45	91.83	35.30	1.93	18.29													
227	HIK	Dyer Island	2005-03-28	15:45	87.93	35.90	1.87	21.50													
228	HIK	Dyer Island	2005-01-31	15:55	91.19	36.40	1.8	20.22													
229	HIK	Dyer Island	2005-03-28	15:55	91.33	33.10	1.88	19.70													
230	JSP	Omdraai	2005-04-22	11:50	91.08				12.55	1.24	22.05	25.17	1.17	0.34	0.95	2.68	3.94	0.0001	0.0009	0.0004	0.0035
231	JSP	Omdraai	2005-04-28	15:20	90.96	31.60	1.72	18.37													
232	JSP	Omdraai	2005-03-15	15:30	91.62				11.98	1.18	22.09	23.90	1.09	0.32	0.89	2.57	4.47	0.0003	0.0017	0.0009	0.015
233	JSP	Omdraai	2005-04-22	11:50	91.14				13.55	1.36	24.21	32.85	1.18	0.38	0.90	2.90	4.31	0.0002	0.0011	0.0004	0.0042
234	JSP	Omdraai	2005-04-22	11:50	88.14	33.10	1.84	17.99													
235	JSP	Omdraai	2005-03-15	15:30	92.77	32.20	1.94	18.60													
236	JSP	Omdraai	2005-04-28	15:20	91.93	29.70	1.72	17.27													
237	JSP	Omdraai	2005-04-28	15:20	92.23	30.60	1.84	18.66													
238	JSP	Omdraai	2005-04-22	11:50	88.89	32.20	1.8	17.89													
239	JSP	Omdraai	2005-04-22	11:50	92.78				10.64	1.01	25.57	30.91	1.03	0.34	0.85	3.07	4.70	0.0001	0.0009	0.0004	0.0029
240	JSP	Omdraai	2005-03-15	15:30	93.26				12.47	1.44	19.07	46.00	1.13	0.28	0.90	2.21	3.54	0.0001	0.0014	0.0004	0.0113
241	JSP	Omdraai	2005-04-12	12:40	91.37	30.00	1.59	18.87													
242	JSP	Omdraai	2005-03-15	15:30	91.72				11.46	1.64	23.12	34.89	0.97	0.34	0.82	2.78	4.25	0.0001	0.001	0.0004	0.0031
243	JSP	Omdraai	2005-03-15	15:30	92.57	31.20	1.89	18.46													
244	JSP	Omdraai	2005-03-15	15:30	91.88	32.30	1.77	18.25													
245	JSP	Omdraai	2005-04-22	11:50	90.09	29.20	1.59	18.36													
246	JSP	Omdraai	2005-04-12	12:40	91.77	29.00	1.64	17.68													
247	JSP	Omdraai	2005-04-12	12:40	91.27	31.60	1.68	18.81													
248	HIK	Dyer Island	2004-11-11	14:15	82.52				9.46	1.18	18.00	46.39	1.28	0.18	0.77	2.06	3.28	0.0001	0.001	0.0004	0.014
249	HIK	Dyer Island	2005-01-17	14:30	91.2	36.80	2.02	18.12													
250	HIK	Dyer Island	2004-12-06	15:30	88.95	36.80	1.6	23.00													
251	HIK	Dyer Island	2004-11-11	14:15	92.82	35.50	1.72	20.64													
252	HIK	Dyer Island	2005-02-28	15:50	91.67	35.10	1.85	18.97													
253	HIK	Dyer Island	2005-02-28	15:50	92.06	35.70	2.01	17.76													
254	HIK	Dyer Island	2005-02-28	15:50	91.56	34.80	1.91	18.22													
255	HIK	Dyer Island	2004-12-06	15:30	84.74				9.11	1.36	19.99	45.31	1.27	0.18	0.88	2.30	3.86	0.0002	0.0006	0.0004	0.0082
256	HIK	Dyer Island	2004-11-11	14:15	92.39				9.66	1.69	16.65	39.22	1.15	0.20	0.75	1.89	3.01	0.0001	0.0006	0.0004	0.0088

