Can the pathway of abalone aquaculture effluent be traced using carbon and nitrogen stable isotope analysis of kelp (*Ecklonia maxima*) and mussels (*Mytilus galloprovincialis*)?

(Photograph by A. Thomas)

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1. Abstract

Abalone farms are becoming more common along the coast of South Africa. The effects these farms are having on the coastal habitats they occupy are becoming of increasing concern due to the possible negative effects of the effluent discharged from the farm. This study was conducted along the coast of Jacobsbaai (32°58′22.09″ S and 17°53′10.56″ E) and Mauritzbaai (32°58'50.75"S and 17°52'59.44"E) near Saldanha on the South African west coast, situated approximately 120 Km from Cape Town (Fig.1). The aim of this study is to determine if the outflow of effluent from the Jacobsbaai Sea Products (Pty) Ltd, abalone farm in Jacobsbaai can be traced using stable carbon and nitrogen isotopes. Kelp (*Ecklonia maxima*) and blue mussels (*Mytilus galloprovincialis*) along the coast near the farm (in Jacobsbaai) were collected from 8 sites and used as tracers of the farms effluent. A ninth site was sample in another bay (Mauritzbaai) which functioned as our control. We hypothesised that if the kelp and mussels are taking up the nitrogen and carbon from the farm, the amount of nitrogen and carbon taken up would decrease with decreasing distance from the effluent outfall. Using this we could map the geographic distribution of the abalone effluent alone the coast to measure the extent of eutrophication due to abalone effluent. Our results suggest that the effluent from the farm is having a localized effect on both *Ecklonia maxima* and *Mytilus galloprovincialis*.

2. Introduction

The South African abalone industry started early in the 1990s and is now the largest producer outside of Asia (Troell *et al.* 2006). With a decrease in natural stocks as the result of over harvesting, poaching and high market prices, there has been a shift to cultivation (Troell *et al.* 2006). Abalone farming is now common in many countries including USA, Mexico, Ireland, South Africa, China, Australia, Taiwan, Iceland, New Zealand and Japan (Troell *et al.* 2006). In 2002 the global production of abalone was approximately 22,600 metric tonnes of this around 8600 metric tonnes were farmed, the total value of which was estimated to be around US$ 0.8 billion (Troell *et al.* 2006). The success of these farms is based on their access to the coast and its resources (fresh sea water and food *i.e.* seaweed, Troell *et al.* 2006).

In South African most of the abalone farms are based on a system where water from the sea is pumped into land based tanks which are run in a flow-through mode, releasing the used water back into the sea; however some of these farms do employ recirculation technology (Troell *et al.* 2006). Both sewage and aquaculture effluent have the potential to supply the coast with nutrient and organic matter enrichment which can lead to eutrophication, which may be a major factor contributing to the alteration of coastal habitats (Chopin *et al.* 2001 and Vizzini & Mazzola 2006). The discharge of nutrient-rich effluent into coastal waters could stimulate algal growth (Gil *et al.* 2005) and or lead to phytoplankton blooms (Cole *et al.* 2004). Eutrophication could result in a decrease in species richness and diversity as well as changes in trophic structures and nutrient cycling (Chopin *et al.* 2001 Cole *et al.* 2004 and Vizzini & Mazzola 2006). The mechanisms through which these changes can occur include anoxia, enhanced sediment metabolism, turbidity, acidification,

high nitrogen and phosphate flux, sulphate reduction and sulphide accumulation among other things (Chopin *et al.* 2001). It is therefore important to be able to trace and therefore monitor the nutrients released from effluent using suitable indicators (Ahn *et al.* 1998, Cole *et al.* 2004, Gil *et al.* 2005 and Rogers 2003).

It is advantageous for the industry to develop systems that minimise habitat destruction, untreated effluents, spreading of pathogens and the use of non-indigenous species (Troell *et al.* 2006). A study by Sankar (2005, unpublished data) looking at the effects of the Jacobsbaai Sea Products abalone farm outflow effluent on the rocky shore community showed that there was no significant impact on the rocky shore environment around the pipe. They did however find a species shift up shore with *Ecklonia maxima* growing 32cm (vertical distance) above the kelp zone on the adjacent rocky coast (Sankar 2005, unpublished data). Compared to fish-cage farming abalone farming, releases only a limited amount of nutrient waste into the open ocean (Troell *et al.* 2006). It is becoming increasingly popular to use integrated farming where the waste water from the abalone is being used to grow seaweed which in turn feeds the abalone before the water is returned to the sea (Ahn 1998, Chopin *et al.* 2001 and Troell *et al.* 2006). The benefits of developing integrated aquaculture include nutrient bioremediation, economic diversification, mutual benefits to integrated organisms and increased profitability per cultivation unit (Chopin *et al.* 2001).

The rate at which macroalgae take up nitrogen depends on environmental factors such as temperature, water currents and irradiance as well as on the concentration of the nitrogen source (Ahn *et al.* 1998). Nitrogen is an important nutrient affecting algal growth in marine environments (Naldi & Wheeler 2002). In general nutrient uptake

rates are affected by biological factors such as age of plant, type of tissue, past nutritional history as well as interplant variability (Ahn *et al.* 1998). Environmental factors that affect both kelp (Ahn *et al.* 1998) and mussels (Dame 1996) include temperature, salinity, tides, sedimentation and wave exposure. Kelp produce carbon through photosynthesis while absorbing all the other nutrients they require for growth, repair and reproduction in the form of dissolved inorganic compounds. Mussels on the other hand, being filter-feeders, take up all the nutrients they need (such as carbon, nitrogen phosphates, iron etc) in the form of particulate organic matter from the water. The tissues of both kelp and mussels provide appropriate material to study the long-term availability of nitrogen in the water column which will be representative of any anthropogenic inputs of nitrogen (Umezawa *et al.* 2007).

Stable carbon and nitrogen isotopes have been used as reliable tracers of nutrient sources through food webs as well as tracers of natural N and C sources (Cole *et al.* 2004, Felsing *et al.* 2006, Fredriksen 2003, Gartner *et al.* 2002, Kaehler *et al.* 2000, Smit 2001). Kaehler *et al.* looked at the trophic structure of the marine food web at the Prince Edwards Islands in the Southern Ocean and found that autochthonous sources of organic material, such as kelp-derived material and micro-phytoplankton blooms, are important components of the diets of all the organisms within the system except zooplankton. In the northern coast of Brittany (France) a study using stable isotopes found that the amphipod *Talitrus saltator* is a key consumer of macroalga detritus and that they preferentially utilize *Fucus serratus* as a food source (Adin & Riera 2003). The stable carbon isotope ratio of a consumer closely reflects (usually within 1‰) the ratio of the dietary carbon while the stable nitrogen value of the consumer is generally enriched (frequently within 3-4‰) in $\delta^{15}N$ relative to their diet (Page & Lastra 2003, Vizzini & Mazzola 2003).

However using laboratory experiments it was found that there was lower trophic enrichment for $\delta^{15}N$ where the mean difference in $\delta^{15}N$ between the diet and the consumer was lower in marine (1.48‰) than in fresh water organisms (2.28‰, Tamelander *et al.* 2006) suggesting that there is variability in trophic enrichment. Stable isotopes (N and C) have also been used to determine if terrestrial and marine plants use nitrogen derived from seabird guano as a source of nutrients (Wainright *et al.* 1998). This study found that there was a trend towards higher $\delta^{15}N$ values in marine (6.5‰ enriched in both $\delta^{15}N$ and $\delta^{13}C$) and terrestrial (22‰ enriched $\delta^{15}N$) plants near bird colonies suggestive of their ornithogenic nitrogen uptake (Wainright *et al.* 1998).

Several studies have shown that seaweeds and mussels take up effluent-derived nitrogen (Ahn *et al.* 1998, Anderson *et al.* 1999, Cole *et al.* 2004, Gartner *et al.* 2002, Gil *et al.* 2005, Rogers 2003 and Vizzini & Mazzola 2006). Gil *et al.* (2005) showed that *Ulva rigida* removed sewage-derived nitrogen from culture media. *Laminaria saccharina* and *Nereocystis luetkeana* were shown to take up ammonium and nitrate released from a nearby salmon cage-farm suggesting that these species might be suitable for integrated cultivation of salmon/kelp (Ahn *et al.* 1998). It was found that the biota (*Ulva*, limpets and mussels) surrounding Moa Point, New Zealand (sewage outfall) had $\delta^{15}N$ isotope values closely linked to contaminated material from the sewage effluent indicating that the organisms took up their nitrogen from the effluent discharged in the bay (Rogers 2003). It has been shown that $\delta^{15}N$ is a better indicator of organic enrichment and uptake of anthropogenic material within marine food webs than $\delta^{13}C$ (Vizzini & Mazzola 2006).

Most of the work done in the marine ecosystem to date has been on trying to tease apart the intricacies of the relationships between marine

organisms using food webs (Cole *et al.* 2004, Felsing *et al.* 2006, Fredriksen 2003, Gartner *et al.* 2002, Kaehler *et al.* 2000, Smit 2001). Table1 shows some of the $\delta^{15}N$ and $\delta^{13}C$ values of components of the food web taken from the literature.

The aim of this study is to determine if the outflow of effluent from the abalone farm in Jacobsbaai can be traced using stable carbon and nitrogen isotopes. The idea is that the kelp and mussels along the coast act as tracers picking up the enriched effluent discharged from the abalone farm. We hypothesised that if they are taking up the nitrogen and carbon from the farm, the amount of nitrogen and carbon taken up would decrease with decreasing distance from the effluent outfall. Using this we could map the geographic distribution of the abalone effluent alone the coast to measure the extent of eutrophication due to abalone effluent.

Table 1: Stable isotope compositions of various components of marine food webs taken from the literature.

	n	Average $\delta^{13}C$ ‰	±SD	Average $\delta^{15}N$ ‰	±SD	Reference
Sea water	-	-20.1	0.1	6.5	0.3	Rogers (2003)
Ulva (non polluted site)	-	-17.1 to -16.7	-	7.8 to 7.6	-	Rogers (2003)
Phytoplankton (POM)	-	-22.03	1.62	6.77	3.2	Wainright *et al.* (1998)
Ice algae	-	-20.7	-	7.5	-	Wainright *et al.* (1998)
Kelp (spp. not specified)	-	-18.0	1.66	8.42	0.96	Wainright *et al.* (1998)
Laminaria spp.	-	-18.0	1.80	8.2	0.14	Wainright *et al.* (1998)
Fucus serratus	3	-15.8 to -14.3	-	5.6 to 7.4	-	Adin & Riera (2003)
Callophyllis laciniata	3	-33.7 to -33.5	-	6.1 to 7.1	-	Adin & Riera (2003
Enteromorpha spp.	3	-15.8 to -13.8	-	8.6 to 8.9	-	Adin & Riera (2003
Palmaria palmata	-	-18.93	2.12	5.31	1.43	Fredriksen (2003)
Helcion pellucidum L.	-	-16.16	0.48	5.33	2.15	Fredriksen (2003)
Aplysia punctata	-	-30.97	1.93	5.56	0.47	Fredriksen (2003)

N: number of samples, -: not given

3. Methods

This study was conducted along the coast of Jacobsbaai (32°58′22.09″ S and 17°53′10.56″ E) and Mauritzbaai (32°58'50.75"S and 17°52'59.44"E) near Saldanha on the South African west coast, situated approximately 120 Km (Sankar. 2005) from Cape Town (Fig.1). Samples were collected during spring tides between 10:00 - 11:30 on the 18th June 2007 and the 4th July 2007.

Kelp (*Ecklonia maxima*) and blue mussels (*Mytilus galloprovincialis*) were collected at sites progressively further from the outflow effluent pipes of the abalone farm (Jacobsbaai Sea Products (Pty) Ltd). The sites started at 5m (site 1) from the pipe which was directly in the flow of the effluent, 15m (site 2, which was directly next to the flow from the pipe) and continued at 20m (site 3), 60m (site 4), 100m (site 5), 200m (site 6), 350m (site 7) and 500m (site 8) from the effluent stream at low tide. The control was collected from Mauritzbaai *ca.* 4km from Jacobsbaai. From each site 6 kelp samples and 6 mussel samples were collected. But were unable to collect mussel samples from site 1 (5m from the pipe) as there were no mussels in this area because site one was directly in the effluent stream above the intertidal zone. Blue mussels do not grow permanently submerged and as the farm continuously pumps effluent from the pipe the mussels are unable to occupy this habitat. To ensure sample site integrity all algal samples were taken from mature specimens attached to rocks rather than free-floating individuals. To control for the age, reproductive and metabolic activity of the kelp we collected the middle section of a middle secondary blade for analysis. Mussels of similar size (*ca.* 5-7 cm in length) were also collected to control for body size. Samples of sediment from the seashore just below the outflow pipe and from the bottom of one of the abalone tanks within the farm, as well as

samples of the artificial feed (Abfeed®) used to feed the abalone were collected. The sludge from the bottom of one of the abalone tanks was sucked up with a pipe and filtered through a 2µm sieve to extract the sludge from the water while the sludge from outside the pipe was scooped up into plastic bags which were sealed for transport. All the samples were bagged and transported back to the UCT Phycology Laboratory for analysis.

The kelp and mussels were gently brushed and rinsed with deionised water to remove any micro-organisms and epiphytes. The abalone sludge was sedimented out of solution using Imhoff sedimentation funnels for 48hrs after which the supernatant was siphoned off leaving the sediment behind. The mussels were opened and the mussel meat was removed. All the samples were dried at 60 °C to constant weight and ground to a fine powder using a Retsch mm 200 ball mill (RETSCH GmbH, Haan, Germany). The samples were weighed into tin capsules to an accuracy of 1 microgram using a Sartorius micro balance for isotope analysis: 2 mg kelp, 0.5 mg mussels, 0.5 mg Abfeed and 1 mg of sludge. The samples were combusted in a flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer, Thermo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). We used Nasturtium as a standard which was calibrated against IAEA (International atomic energy agency) standards. Peedee belemnite was used as a standard for CO_2 while atmospheric nitrogen was used for nitrogen. The isotope composition was expressed as $\delta^{13}C$ and $\delta^{15}N$ values where:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{sample}/R_{standard})-1] \times 1000$$

R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Statistical Analysis

Statistical analysis was preformed in Statistica® version 7.0 (StatSoft Inc. 2004). One-way ANOVAs were run on all the variables ($\delta^{13}C‰$, $\delta^{15}N‰$, % C, % N and CN ratios) to determine if there were significant differences between the sites, followed by Tukey's post hoc tests. Box and whisker graphs were plotted to give a visual representation of the data.

(a)

(b)

Figure 1: (a) Map of the study area showing site 1-8 along the coast (b) Map showing all the sites along the coast (edited from Google Earth™ 2007). The blue circles are a function of Google that can not be removed showing tourist spots.

4. Results

4.1. Seawater and effluent

We were unable to measure isotopic values for the seawater in Jacobsbaai and the effluent both in the farm and from the pipes (Jacobsbaai Sea Products) because we did not have access to the equipment needed to analysis liquid samples. We have therefore taken a value from the literature as a proxy for what sea water should be (table 1). Although this value comes from Moa Point in New Zealand we believe it is a close enough approximation for our purposes. Table 2 depicts all the averaged values for the abalone feed (Abfeed®), Farm sludge and Pipe sludge taken from the outlet which reflects the isotopic values of the effluent coming out of the pipe. Nitrogen and carbon isotope averaged values over the 9 sites are presented in Table 3 (Appendix) for reference.

Table 2: Stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) and nitrogen (%) and carbon (%) for Abfeed, farm sludge and pipe sludge at the farm (± SD= standard deviation).

	% N	**δ^{15} N**	**% C**	**δ^{13}C**	**C:N Ratios**
Abfeed®	6.34	7.78 ± 0.23	42.82	-17.96 ± 0.08	6.76
Farm sludge	3.37	6.64 ± 0.17	20.20	-16.24 ± 0.34	6.00
Pipe sludge	0.92	5.13 ± 1.12	9.45	-8.88 ± 2.32	10.77

4.2. Kelp - *Ecklonia maxima*

4.2.1. $\delta^{15}N$ values

There is a distinct pattern in the $\delta^{15}N$ isotope values for the kelp samples (Fig 2). A significant statistical difference was found between the 9 sites (ANOVA, df= 8, F=3.44, p=0.0036) at a 95% significance level with site 1 and 6 significantly different from site 9 (all comparisons, $p<0.05$). Site 1 which was situated directly in the effluent released from the abalone farm shows low $\delta^{15}N$ values (4.81‰ to 6.89‰). Site 9 on the other hand was the control site showing higher $\delta^{15}N$ values (7.12‰ to 9.99‰).

There appears to be an almost cyclical pattern between the sites with $\delta^{15}N$ isotope values increasing between sites 1-3, decreasing between sites 3-6 and then increasing again between sites 6-8. Site 2 which is 15m from the pipe (2.83‰ to 8.26‰) and site 3 (20m from pipe, 6.30‰ to 9.37‰) seem to be more enriched relative to site 1 (5m from the pipe). Site 4 (60 m from the pipe, 6.54‰ to 8.15‰) and 5 (100m from pipe, 5.72‰ to 7.74‰) on the other hand are more enriched relative to site 2 but less than site 3. Site 6 which is 200m from the pipe is relatively depleted in $\delta^{15}N$ (5.20‰ to 6.66‰) compared to site 2-5 and is in the range of site 1. Site 7 (350m from pipe, 5.82‰ to 6.98‰) and 8 (on the outer edge of the bay, Fig 1(a) and (b), 5.55‰ to 9.13‰) are more enriched relative to site 6, approaching site 9 the control (in another bay Fig 1(b)).

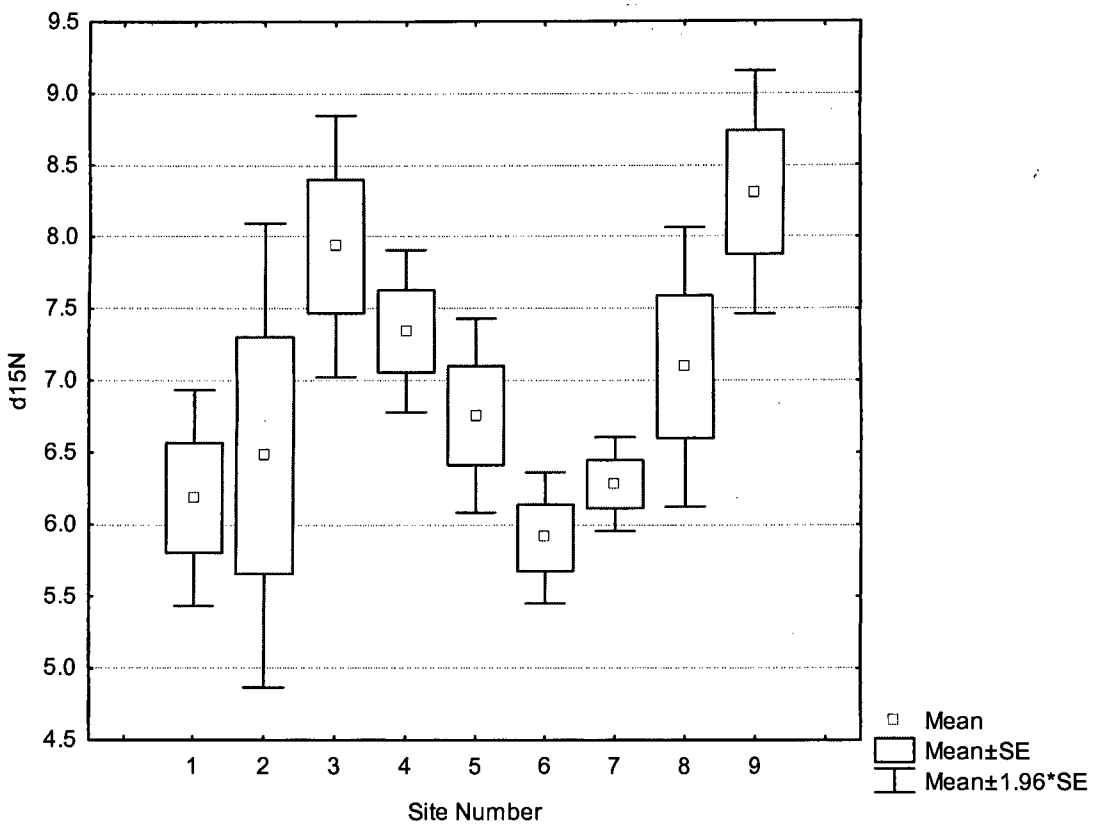

Figure 2: $\delta^{15}N$ values for kelp-*Ecklonia maxima* from Jacobsbaai (sites 1-8) and Mauritzbaai (site 9), South Africa.

4.2.2. $\delta^{13}C$ values

There was a significant difference between the sites (ANOVA, df=8, F=2.24, p=0.0419) indicating variation but there was no significant differences when comparing the individual sites to each other at a 95% significance level (all comparisons, p>0.05), so no two sites were significantly different from each other but there was overall variation in sites.

4.2.3. % N values

Site 1 had a higher % N content than the other 8 sites (Fig 3). Nitrogen decreases with distance from pipe and then starts to increase slightly again after site 3 (20m from the pipe). The effect of the pipe is not evident at site 3 or 20m from the pipe. There was a significant difference between the sites at a 95% significance level (ANOVA, df=8, F=7.748, p= 0.000002) with significant differences between sites 1 and 3-9 (all comparisons, $p<0.05$). Site 1 and 2 were not significantly different from each another. Kelp from immediately below the outfall pipe thus had considerably elevated nitrogen concentration.

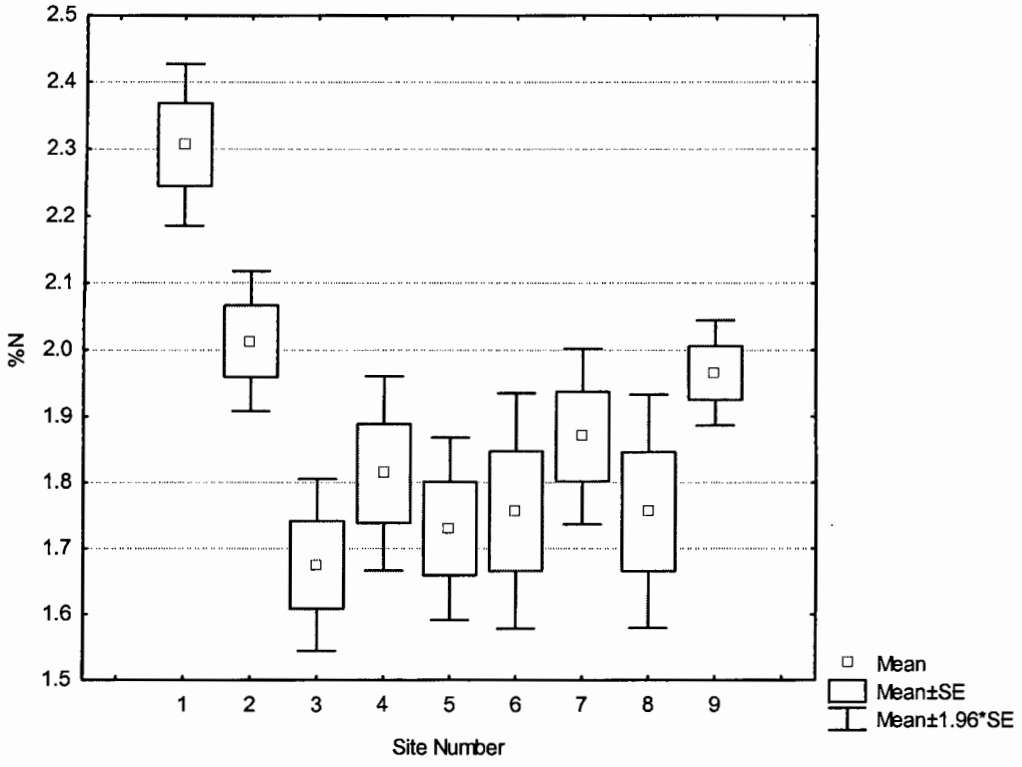

Figure 3: Nitrogen content (%) for kelp-*Ecklonia maxima* from Jacobsbaai (site1-8) and Mauritzbaai (site 9), South Africa.

4.2.4. % C values

Site 1 had a higher % C content than sites 2-8 while site 9 (control) had the highest (Fig 4). The sites were significantly different from each other at a 95% significance level (ANOVA, df=8, F=5.027, p=0.00018). There was a significant difference between sites 1 and 6-8 and between site 9 and 3-8 (all comparisons, $p<0.05$).

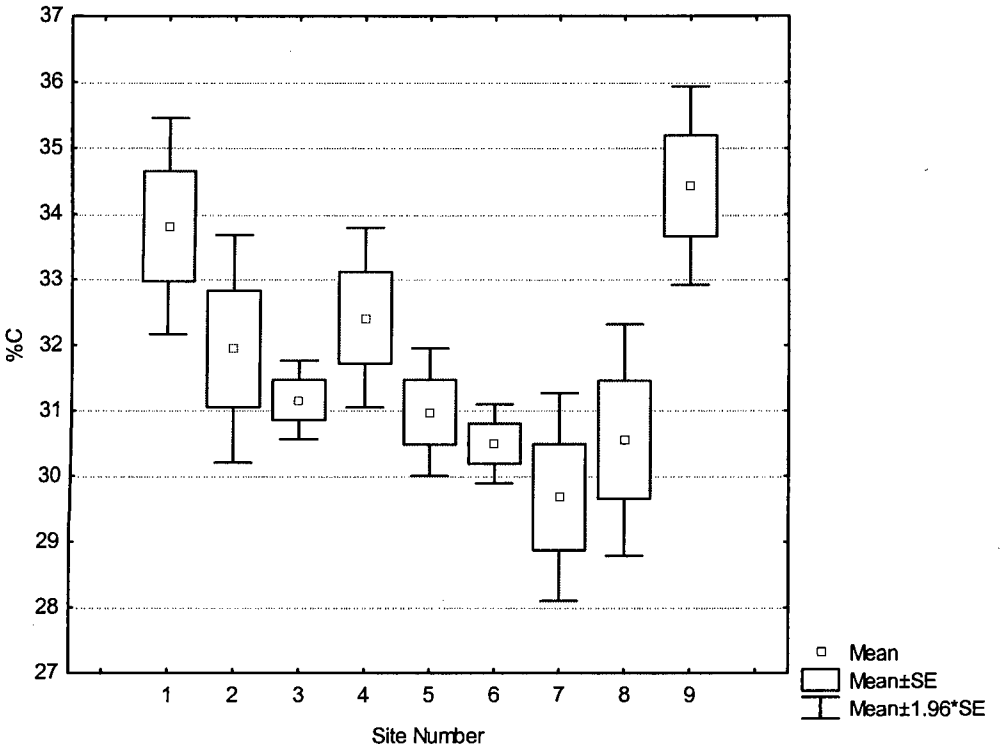

Figure 4: Carbon content (%) for kelp-*Ecklonia maxima* from Jacobsbaai (site1-8) and Mauritzbaai (site 9), South Africa.

4.2.5. C:N Ratio values

The C:N ratio (Fig 5) for kelp largely reflects the % N values as they have an inverse relationship, and N values vary proportionately more than C

values. Sites 1 and 2 had the lowest values while site 3 had the highest values. There was a significant difference between the sites at the 95% significances level (ANOVA, df=8, F=3.482, p=0.0034) with significance differences between site 1 and 3-5 (all comparisons, $p<0.05$).

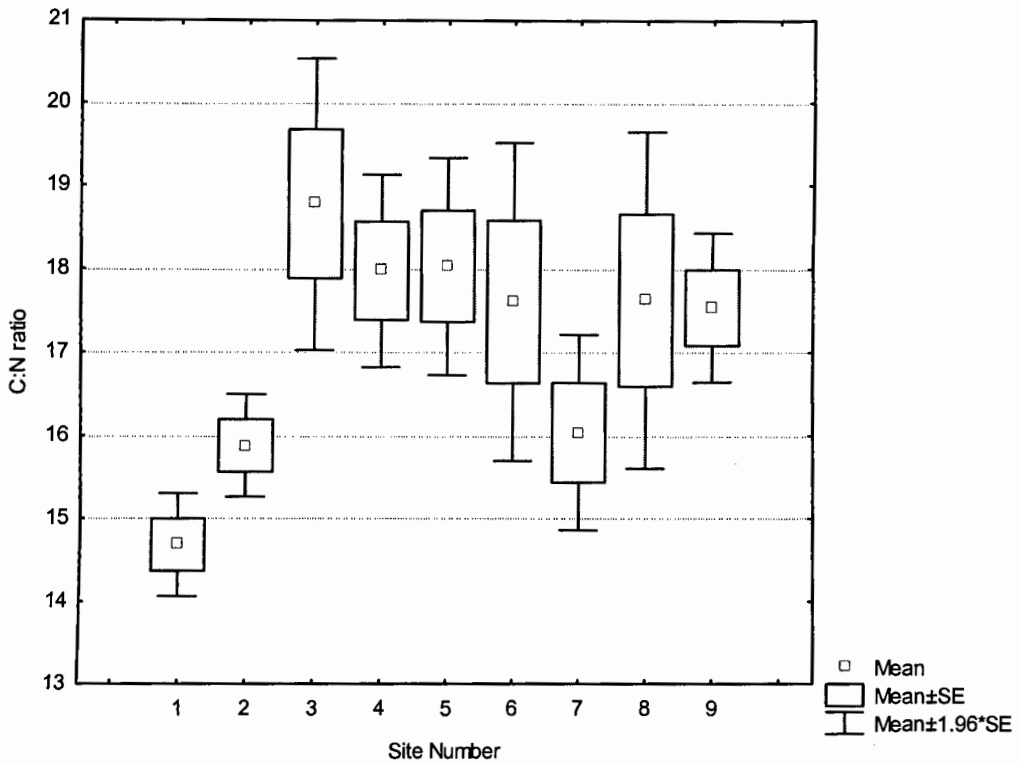

Figure 5: C:N Ratios of kelp-*Ecklonia maxima* from Jacobsbaai (site1-8) and Mauritzbaai (site 9), South Africa.

4.3. Mussel - *Mytilus galloprovincialis*

4.3.1. $\delta^{15}N$ values

Figure 6 shows the $\delta^{15}N$ values for the mussels at site 2-8. Site 1 was in the effluent flow and did not have any mussels present. Site 2 (15m from pipe, 7.96‰ to 8.46‰) which was right next to the flow was thus the closest to the outfall pipe. Site 9 (in another bay, 8.32‰ to 8.88‰) was

the control. Site 3 through 8 (7.52‰ to 8.35‰, 7.59‰ to 8.21‰, 7.72‰ to 8.12‰, 7.31‰ to 7.99‰, 7.30‰ to 8.88‰, 7.18‰ to 8.18‰, respectively) all have values more depleted than site 2 which suggests that the mussels are picking up particulate organic matter from the farm effluent. There appears to be two distinct groupings: site 3-5 and site 6-8. There was a significant difference between the sites (ANOVA, df=7, F=6.27, p=0.000054). Significant differences were found between site 9 and sites 3, 4, 6, 7 and 8 (all comparisons, $p<0.05$), at a 95% significance level. There was no significant difference between site 2 and site 9 (p=0.189).

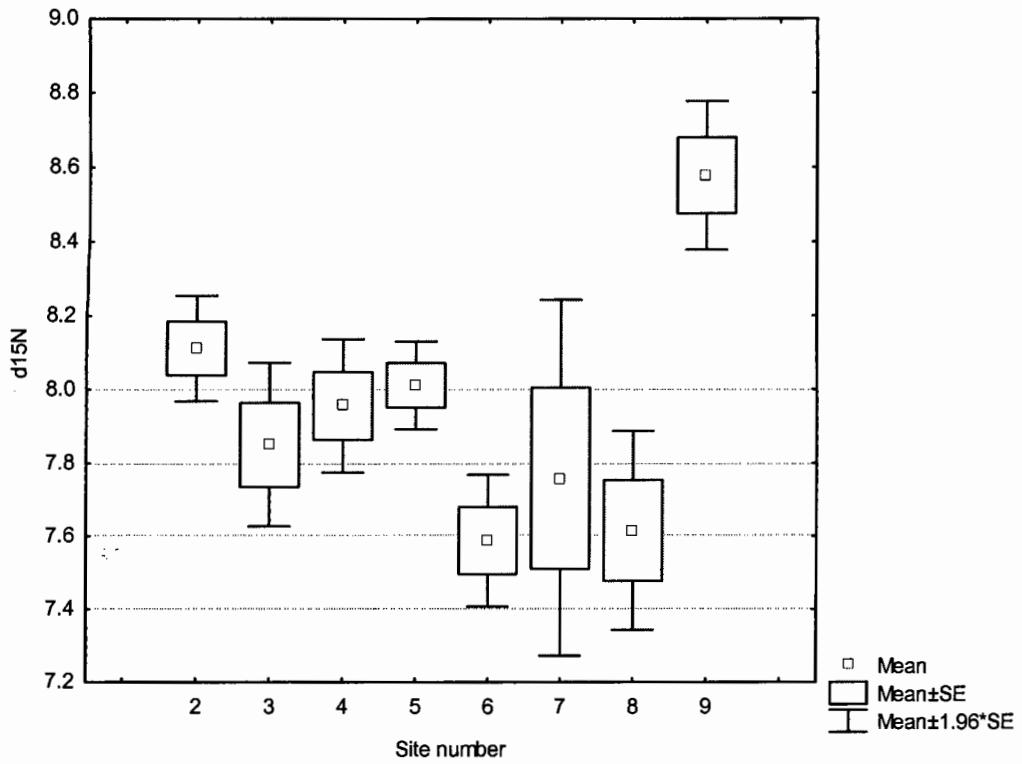

Figure 6: $\delta^{15}N$ values for the blue mussel *Mytilus galloprovincialis* from Jacobsbaai (sites 2-8) and Mauritzbaai (site 9), South Africa.

4.3.2. $\delta^{13}C$ values

Figure 7 shows the $\delta^{13}C$ values for mussels at each site sampled. Again site 1 was not sampled as no mussels were found there. Site 2 is more enriched (-14.03‰ to -14.80‰) relative to all the other sites. Site 9 (-15.07‰ to -15.43‰) which was the control is depleted relative to site 2 but more enriched than sites 3 (-16.44‰ to -17.24‰), 4 (-16.36‰ to .16.92‰), 5 (-16.46‰ to -16.75‰), 6 (-16.25‰ to .16.96‰), 7 (-15.32‰ to -16.81‰) and 8 (-16.12‰ to -16.72‰). Site 3 was the most depleted of all the sites. There was a significant difference between the sites at a 95% significance level (ANOVA, df=7, F=38.95, $p<0.0005$) with significant differences between site 2 and 3, 4, 5, 6, 7, 8 and 9; site 3 and 5, 7 and 9 and site 9 and all the other sites (all comparisons, $p<0.05$).

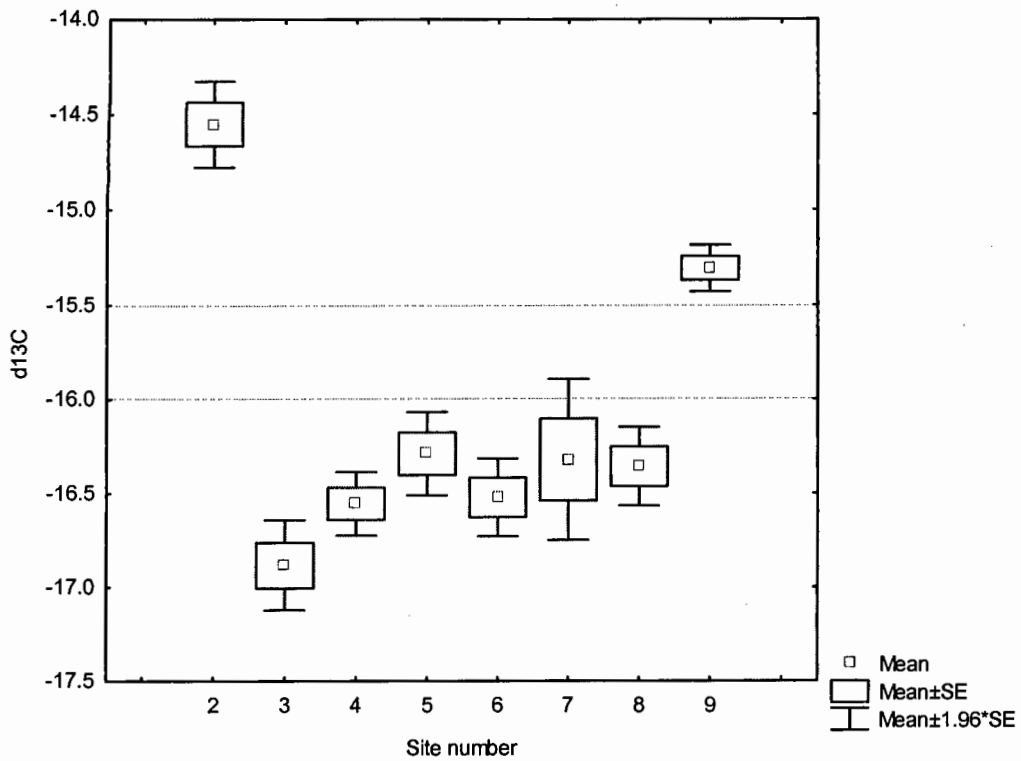

Figure 7: $\delta^{13}C$ values for the blue mussel *Mytilus galloprovincialis* from Jacobsbaai (sites 2-8) and Mauritzbaai (site 9), South Africa.

4.3.3. % N values

Percentage N in blue mussels (Fig8) increases from site 3 to site 8. Site 2 has a higher % N content than site 3 but lower than site 4-9. Site 9 (control) is lower than site 6-8. There was a significant difference between samples at the 95% significance level (ANOVA, df=7, F=6.14, p=0.000066) with significant differences between sites 2 and 6-8; site 3 and 6-8 and site 4 and 7 and 8 (all comparisons, $p<0.05$).

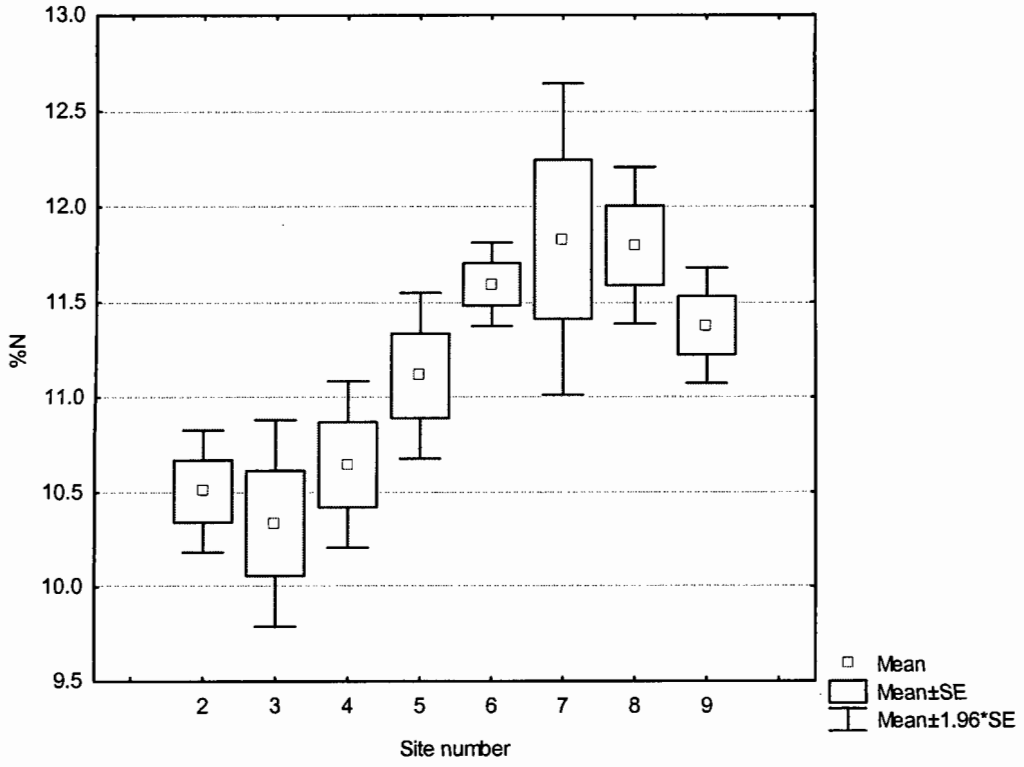

Figure 8: Percent N values for the blue mussel *Mytilus galloprovincialis* from Jacobsbaai (sites 2-8) and Mauritzbaai (site 9), South Africa.

4.3.4. % C values

There was no significant difference in the % C content between the 9 sites individually at a 95% significance level (all comparisons, $p>0.05$).

4.3.5. C:N Ratio values

The C:N ratio (Fig 9) for the mussels decreases between site 3 and 9 while site 2 is less than site 3 but higher than sites 5-9. Site 7 seems to have the lowest C:N ratio. There were significant differences between sites at the 95% significance level (ANOVA, df=7, F=5.24, p=0.000265) with significant differences between site 3 and site 6-9 and site 4 and 6, 7, 9 (all comparisons, $p<0.05$).

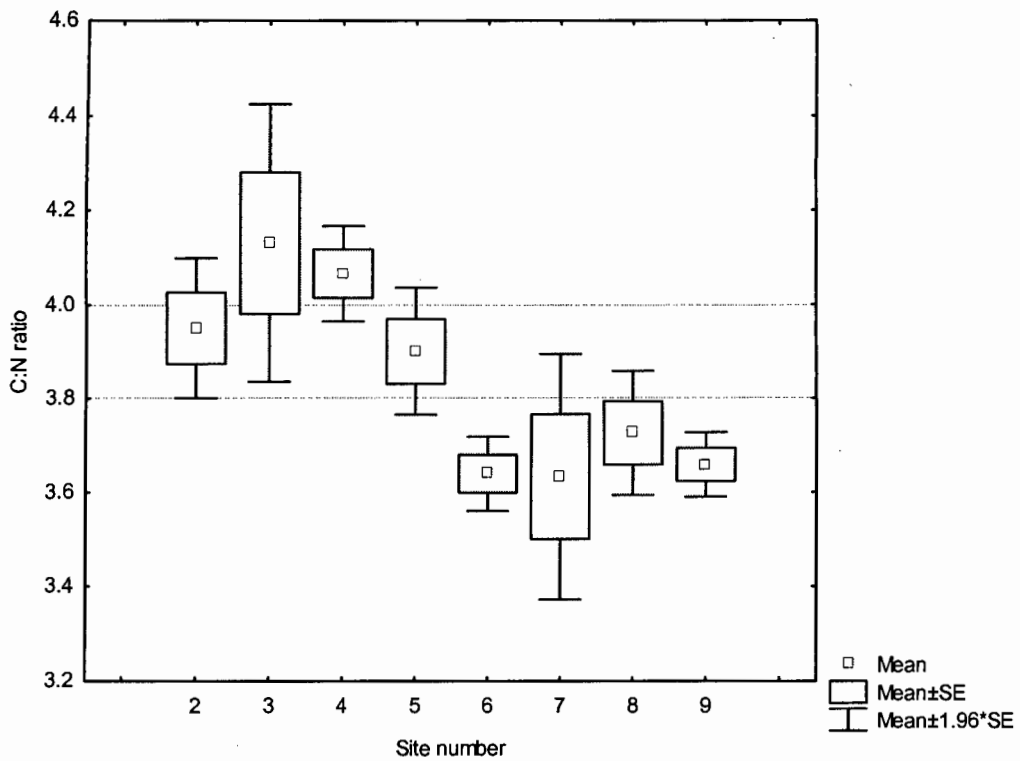

Figure 9: C:N ratio values for the blue mussel *Mytilus galloprovincialis* from Jacobsbaai (sites 2-8) and Mauritzbaai (site 9), South Africa.

Comparing the $\delta^{13}C$ and $\delta^{15}N$ of Kelp-*Ecklonia maxima* from the 9 sites sampled from Jacobsbaai with seawater from Moa Point in New Zealand (Rogers 2003), Farm sludge (FS), pipe sludge (PS) and Abfeed® (AF) from the Jacobsbaai Sea Products abalone farm (Fig 10) suggests that the farm sludge (FS) is the closest value to the sites. The Abfeed® and pipe sludge are also relatively close to the sites. The control is the furthest site from the sludge and Abfeed® samples.

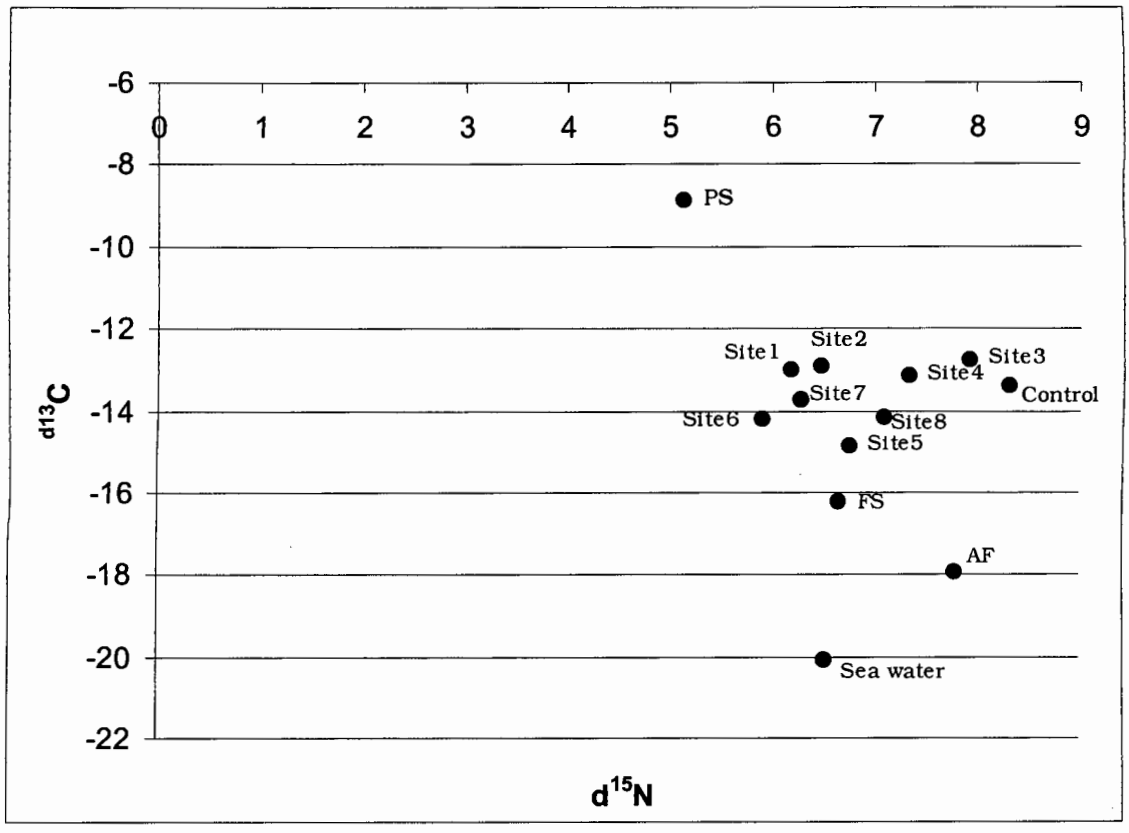

Figure 10: Comparison between $\delta^{13}C$ and $\delta^{15}N$ of Kelp-*Ecklonia maxima* from the 9 sites sampled from Jacobsbaai, seawater from Moa Point in New Zealand (Rogers 2003) and Farm sludge (FS), pipe sludge (PS) and Abfeed® (AF) from the Jacobsbaai Sea Products abalone farm, South Africa.

The values for the $\delta^{13}C$ and $\delta^{15}N$ of the blue mussel- *Mytilus galloprovincialis* from the 9 sites sampled from Jacobsbaai compared to seawater from Moa Point in New Zealand (Rogers 2003), phytoplankton (Bustamante and Branch 1996), kelp from our control site- site 9, kelp from site 1 directly in the effluent (5m from the pipe) farm sludge, pipe sludge and Abfeed® from the Jacobsbaai Sea Products abalone farm is shown in Figure 11. The kelp at the control site (site9), the Abfeed® and the farm sludge (FS) are the closest points to the cluster of sites sampled. The pipe sludge (PS), kelp from site1, seawater and phytoplankton are further away from the sites. Site 2 and the control are the closest to the kelp control site while sites 3-7 are closest to Abfeed® and farm sludge (FS).

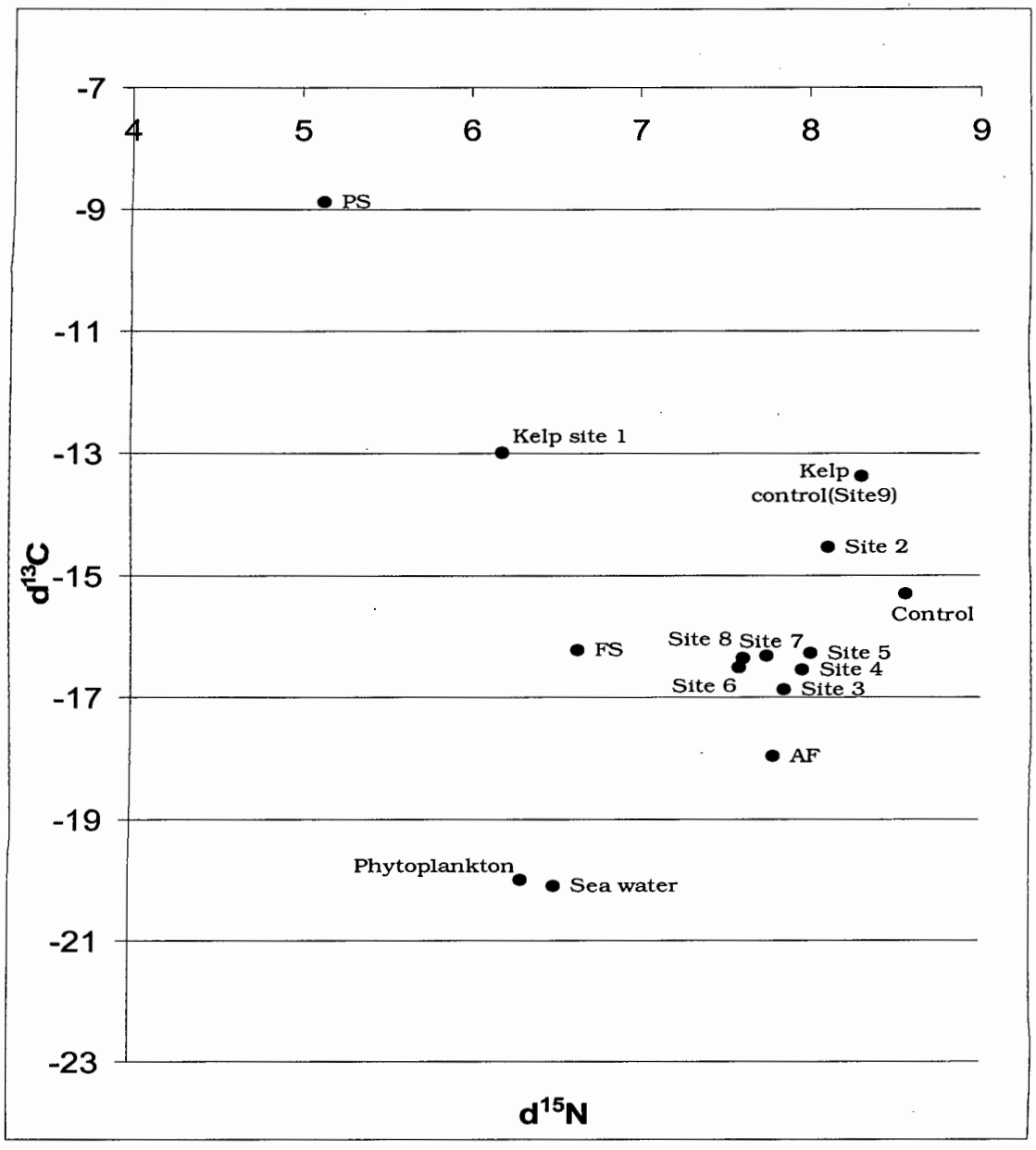

Figure 11: Comparison between $\delta^{13}C$ and $\delta^{15}N$ of the blue mussel- *Mytilus galloprovincialis* from the 9 sites sampled from Jacobsbaai, seawater from Moa Point in New Zealand (Rogers 2003), phytoplankton (Bustamante and Branch 1996), kelp from our control site- site 9, kelp from site 1 directly in the effluent (5m from the pipe) farm sludge (FS), pipe sludge (PS) and Abfeed® (AF) from the Jacobsbaai Sea Products abalone farm, South Africa.

5. Discussion

There is a distinct pattern emerging from the data for both the kelp and mussels sampled. Since we were unable to sample seawater and liquid effluent as end members for our study; values for sea water (Table 1) were taken from the literature and abalone sludge (Table 2) was measured inside the farm and outside the pipe to get a proxy for solid component of the effluent. Abfeed® (Table 2) was also measured to get an idea of initial input values into the aquaculture system. Abfeed® is mainly made up of fishmeal, soya bean meal, starch, vitamins and minerals (Troell *et al.* 2006). It contains around 35% protein, 43% carbohydrates, 5% fat, 1% crude fibre, 6% ash and ~ 10% moisture (Troell *et al.* 2006). Most of the nitrogen is thus fish-derived.

The data on the abalone sludge and the Abfeed® suggest that there are many processes taking place within the farm the most important of which (especially to the farm) is that the farm seems to be cleaning a lot of the remaining sediments before releasing the effluent into the sea *i.e.* the effluent is clean relative to the effluent leaving the abalone tanks. The percent N and C are higher (Table 2) in the farm sludge relative to the pipe sludge suggesting that the farm is cleaning their effluent before releasing it in to the sea.

5.1. Kelp- *Ecklonia maxims*

The $\delta^{15}N$ values (Fig. 2) for the kelp shows an almost cyclical pattern with lower values at the pipe and the highest value at site 9 the control. From the literature (Table1) the $\delta^{15}N$ values for the different seaweeds range from 7.5‰ to 8.9‰. Site 1 represents the effects of the effluent on the kelp as they are directly in the flow of the farms effluent. Site 2 and 3 are

more enriched than site 1 suggesting that they are exposed to less effluent from the farm than site 1. The values for site 4 and 5 suggest that they are exposed to more effluent than site 3. The effluent from the farm seems to be having the strongest effect on site 6 which has the lowest $\delta^{15}N$ values of all the sites. Site 8 and 9 are more enriched than site 6 suggesting that there is less of an effect of the effluent on these sites. Site 9 is by far the most enriched site which is what we would expect for the control site. The cause of this pattern is most likely the changing currents taking effluent from and bringing it back to the coast and the topography of the coast line (see Fig.1 (a) and (b)). The $\delta^{13}C$ values did not differ significantly between the sites which is what one might expect as kelp obtains their carbon from dissolved CO_2 during photosynthesis, which is presumably mostly atmosphere derived. The % N (Fig. 3) values for kelp indicate that there is more nitrogen available near the pipe decreasing with distance, which is what you would expect as the effluent would have higher dissolved nutrients than normal sea water. The % C (Fig. 4) almost mirrors the % N (Fig. 3) in that site 1 has *higher* values than sites 2-8 but site 9 has the highest % C. As carbon is assimilated during photosynthesis it is unlikely that this pattern is the result of the farms effluent and is most probable a product of the specimens' photosynthetic rate. It is possible that the difference in wave action at the different sites could be affecting the kelps ability to take up dissolved carbon from the water. There is a gradient of exposure from site 1 to site 8, with site 1 being sheltered while site 8 is the most exposed. The control site (site 9) was a large kelp bed, with a gently sloping shore, which could protect this site from wave action. The data suggests that the more sheltered sites (1 and 9) are able to take up more carbon than the sites that are exposed to wave action.

The C:N ratios (Fig. 5) indicate that the environment around the pipe is less nitrogen limited than further away as there seems to be more

nitrogen by the pipe which is what we would expect as the effluent discharged from the pipe has higher nutrient loads than the seawater. Comparing the $\delta^{15}N$ and $\delta^{13}C$ for the kelp from the 9 sites sampled from Jacobsbaai, seawater from Moa Point in New Zealand (Rogers 2003) and farm sludge, pipe sludge and Abfeed® from the Jacobsbaai Sea Products abalone farm is shown in Figure 10. This graph suggests that the kelp are mostly getting their nutrients from the Abfeed® and farm sludge (FS) which was expected as we predicted that they were taking up the nutrients in the effluent discharged from the farm. The unusual thing is that the kelp does not seem to be taking up the pipe sludge which we thought would be a closer approximation to what is available for the kelp to take up. It is possible that the sludge accumulating around the pipe was made up of particles that the kelp was unable to utilise effectively.

5.2. Mussels- *Mytilus galloprovincialis*

The $\delta^{13}N$ (Fig. 6) pattern for the mussels is less straightforward than the kelp as a pattern among the sites is less obvious. Table 1 gives some values from the literature for some bivalves with δ15N values ranging from 5.31 to 5.56. One big difference between the two study specimens is their method of nutrient acquisition. Kelp absorbs dissolved inorganic matter, while mussels (Dame 1996) filter particulate organic matter from the water. This difference could account for the different pattern observed in the data as the mussels are most likely filtering all kinds of particles from the water other than the ones coming from the farm such as phytoplankton or kelp bits. Bustamante and Branch (1996) found that filter-feeders use kelp detritus as their major source of organic nitrogen and carbon on the west coast of South Africa. The control, site 9, is more enriched which one would expect if it is unaffected by the farms effluent. Unfortunately site 1 which was in the effluent did not have any mussels,

which is interesting in itself. This could be a result of the fact that the farm pumps effluent out of it's pipes continuously, as *Mytilus galloprovincialis* only grow in the intertidal where they are only exposed to water for a certain amount of time as a result of the tides they are not permanently inundated with water and are probably unable to grow in the continuous flow of the pipes. Site 2 is only 15m from the pipe and right next to the flow of the effluent as it flows into the sea and it's possible that these individuals are less exposed to the effluent due to changing currents. Sites 3-8 are more depleted than site 2 and 9 which suggests that they are being exposed to the effluent. This pattern is again most likely the result of currents and the topography of the coastline.

Unlike the kelp the mussels are taking up their carbon from the particulate organic matter floating around in the sea water. The pattern that emerged from the $\delta^{13}C$ (Fig. 7) data is unusual as site 2 has a less negative value than site 9 (the control). All the other sites are more depleted relative to site 2 and 9. This suggests that site 2 is not coming into contact with the effluent but that it is being washed out and then brought back to the coast at site 3. The % N (Fig. 8) in the mussels, although higher than in the kelp, shows an opposite trend to the kelp with site 2 having the lowest % N value and site 7 the highest. This further suggests that the mussel sites next to the pipe are less affected by the effluent from the abalone farm than mussel sites further away. The C:N ratio (Fig. 9) for mussels suggests that the further from the pipe the environment is becoming more N depleted. Comparing the δ13C and δ15N of the blue mussel- *Mytilus galloprovincialis* from the 9 sites sampled from Jacobsbaai, seawater from Moa Point in New Zealand (Rogers 2003), phytoplankton (Bustamante and Branch 1996), kelp from our control site- site 9, kelp from site 1 directly in the effluent (5m from the pipe) farm sludge, pipe sludge and Abfeed® from the Jacobsbaai Sea Products abalone farm is represented in Figure 11. Mussels (Fig. 11)

seem to be taking up particles from un-polluted kelp, Abfeed® and farm sludge. They don't seem to be taking up significant amounts of phytoplankton, polluted kelp, pipe sludge and partials from "clean" seawater. The mussels did not seem to be taking up the pipe sludge as we had expected, which was also simulated in the kelp. This could be because the forms of particles accumulating around the pipes are not easily assimilated by the mussels. The mussels may not be taking up phytoplankton as it may be easier to assimilate the particulate matter from the farm than phytoplankton. Our results agree with the study by Bustamante and Branch (1996), mentioned above, in that the mussels seem to be taking up kelp-derived detritus. The control (site 9) and site 2 are close to the kelp from the control site further suggesting that these sites are not being influenced by the farms effluent.

6. Conclusions

From the data we can conclude that the effluent from the Jacobsbaai Sea Products abalone farm is having a localized effect along the coast where it is pumped out. Using stable carbon and nitrogen isotopes we were able to trace the effects of the effluent in both the kelp and mussels sampled. The patterns that emerged were by no means as straightforward as we might have expected. The kelp seems to show that the effluent from the farm is having a stronger effect on sites 1, 2, 4, 5 and 6 with less effect on sites 3, 7 and 8. There is an almost cyclical pattern suggesting that the currents and the topography may be affecting the distribution of the effluent. The mussels on the other hand are showing that site 3-8 are affected by the effluent from the farms and that site 2 and 9 are not. This suggests that the particulate matter from the farm is not reaching site 2 and site 9. The pattern observed in site 2 in unusual as it is right next to the flow of the effluent and yet the mussels do not seem to be picking up the particulate matter from the farm. We predicted that site 9 (control site) would be unaffected by the effluent from the farm and this is supported by the results. Our data suggests that the kelp are obtaining N from the farm sludge and Abfeed® while the mussels are taking up particles in the form of un-polluted kelp, Abfeed® and farm sludge. It is also possible that the size of the particles (from waste, kelp particles, phytoplankton *etc.*) could be affecting their relative uptake by the mussels, as different particles may have different uptake rates and or uptake amounts. To better understand the processes that are taking place in this ecosystem future study should look at (1) different parts of the kelp and mussels, (2) greater distances between the sites to include more of the coast line and (3) sample in different seasons.

The patterns could be an artefact of variations within individuals or variations between individuals of the same species (Smit 2001). The variation within individual organisms could be attributed to differences in growth rates between different parts (Smit 2001). In this study we tried to control for this by taking the middle of the middle blade but it is possible that in attempts to avoid waves that another part of the blade was clipped. Variability between individuals of the same species could be attributed to light intensity, salinity, temperature and age (Smit 2001). But as our methodology was sound we are confident in the patterns observed.

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9. Appendix

Table 3: Stable isotope values ($\delta^{15}N$ and $\delta^{13}C$ ± SD- standard deviation) and nitrogen (%) and carbon (%) for both kelp and mussels at each site (± SD= standard deviation).

	% N	$\delta^{15}N$	% C	$\delta^{13}C$	C:N Ratios
Ecklonia maximus					
Site 1- 5m from pipe	2.31	6.18 ±0.94	33.80	-13.01± 0.53	14.68
Site 2- 15m from pipe	2.01	6.48± 2.02	31.94	-12.94± 1.59	15.88
Site 3- 20m from pipe	1.67	7.93± 1.14	31.16	-12.76± 0.95	18.78
Site 4- 60m from pipe	1.81	7.34± 0.70	32.42	-13.16± 1.33	17.98
Site 5- 100m from pipe	1.73	6.76± 0.84	30.97	-14.87± 1.05	18.03
Site 6- 200m from pipe	1.76	5.91± 0.57	30.49	-14.21± 1.25	17.61
Site 7- 350m from pipe	1.87	6.28± 0.39	29.68	-13.73± 1.78	16.04
Site 8- 500m from pipe*	1.76	7.09± 1.21	30.55	-14.18± 0.95	17.63
Site 9- control	1.97	8.31± 1.06	34.43	-13.38± 0.56	17.54
Mytilus galloprovincialis					
Site 2- 15m from pipe	10.51	8.11± 0.18	41.52	-14.55± 0.28	3.9
Site 3- 20m from pipe	10.34	7.85± 0.28	42.49	-16.88± 0.30	4.13
Site 4- 60m from pipe	10.65	7.96± 0.23	43.23	-16.55± 0.21	4.07
Site 5- 100m from pipe	11.11	8.01± 0.15	43.29	-16.29± 0.28	3.90
Site 6- 200m from pipe	11.59	7.59± 0.23	42.19	-16.52± 0.26	3.64
Site 7 350m from pipe	11.83	7.76± 0.61	42.71	-16.32± 0.53	3.63
Site 8- 500m from pipe*	11.80	7.62± 0.34	43.90	-16.36± 0.26	3.73
Site 9 -control	11.38	8.58± 0.25	41.63	-15.31± 0.15	3.66

*Site 8 was not in a 'straight line with the pipe it was 150m from site 7 see figure 1(a).