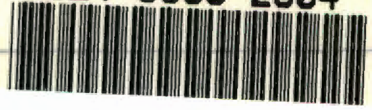
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Friend or Foe?

The invasive potential and aquacultural application of the sporophytic *Falkenbergia* stage of *Asparagopsis armata* in South Africa

(*Falkenbergia rufolanosa*, photograph courtesy of M.D. Guiry)

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2004 Honours

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ABSTRACT:

The presence of the sporophytic '*Falkenbergia rufolanosa*' phase of the invasive algal species *Asparagopsis armata* was first recorded in South Africa 57 years ago. The introduction of this highly invasive alga, of Australian/New Zealand origins, to Europe in the 1920's has since led to a number of recorded invasions by the gametophytic *Asparagopsis* phase in both the Mediterranean and Atlantic oceans. Recently however, a number of commercial uses for both phases of *A. armata* have been identified, which have given rise to industrial interest in the species as a candidate for commercial cultivation.

Previous studies on European strains of *F. rufolanosa* and *A. armata* have identified a number of life history traits, which not only increase *A. armata's* invasive ability, but also make it a useful species for commercial tank cultivation. However, different strains are known to have different environmental parameters which regulate their survival, growth and reproduction capabilities. This study investigated the growth of the South African strain of *F. rufolanosa* in culture, in response to different environmental variables, as well as the conditions necessary for tetrasporogenesis to occur, in order to assess its potential impacts, both as an invasive threat and as a species for commercial cultivation in South Africa.

A series of laboratory-based growth studies were conducted to determine the specific growth rate of *F. rufolanosa* under various conditions of temperature (10, 15, 20, 25 °C), irradiance (0.2-100 μmol photons. m^{-2}. s^{-1} and salinity (1, 5, 10, 15, 25, 35 ‰). A maximum specific growth rate of 8.41%. day^{-1} was found to occur at 15 °C, 20-25 μmol photons. m^{-2}. s^{-1} and 35 ‰. *F. rufolanosa* did not tolerate high temperatures (25 °C) nor low salinities (1, 5, 10 ‰) and died in these conditions, all other conditions were tolerated. Attempts to induce tetrasporogenesis in *F. rufolanosa* were unsuccessful.

These results indicate that *F. rufolanosa* can tolerate a wide range of environmental conditions, which suggests that it would be a useful species for commercial cultivation in South Africa, provided a suitable market for the product can be found. Its tolerance of a wide range of temperatures also means that *F. rufolanosa* may well increase its current distribution range in South Africa. However, at present, the occurrence of *F. rufolanosa* on its own, does not appear to be an invasive threat in South Africa, since the absence of the short days (<10 hrs daylight), prevents tetrasporogenesis from occurring and thus the subsequent development of the more invasive

Asparagopsis phase. However, further studies are needed to determine the exact triggers for tetrasporogenesis before commercial cultivation of *F. rufolanosa* in South Africa is permitted.

KEYWORDS:

Aquaculture; *Asparagopsis armata; Falkenbergia rufolanosa;* invasive traits; optimum growth conditions

INTRODUCTION:

Algal invasives

In recent decades, the introduction of non-native species to foreign ecosystems, both marine and terrestrial, has become a major topic of research and debate. While the majority of introduced species have little to no impact on the native community assemblages, it is the few species that become invasive which can pose a serious threat to the integrity of entire native ecosystems (Vitousek 1990). In marine habitats, nowhere is this more clear than the recent invasion of both the Mediterranean and Californian coastlines by the herbivory resistant green alga, *Caulerpa taxifolia,* which has brought the world's attention to the growing threats, both environmental and economic, that harmful invasive marine algae pose (Jousson *et al* 2000).

The invasion by *Caulerpa taxifolia* was an important starting point in invasive algal ecology. First introduced into the Mediterranean from a public aquarium in 1984, this seaweed has since spread to over more than 6000 hectares, outcompeting native species and severely reducing diversity in the affected areas (Meinesz *et al.* 1995). *Caulerpa taxifolia* may have been one of the first algal species to be recognised as invasive, but it is certainly not the last. Today, there are a number of seaweed species around the globe which have been identified as harmful alien invasives, and it is these species which are coming under increasing scientific scrutiny, as more and more countries begin to notice the effects of these alien invasives.

One such species is the subtidal red marine alga *Asparagopsis armata* (Harvey), also known as 'Harpoon weed' because of the barb-like hooks on its branching thalli. Classified as a member of the red algae (Rhodophyta), in the order Bonnemaisoniales, family Bonnemaisoniaceae, *A. armata* was first described by W. H. Harvey in 1855 from a specimen collected in Western Australia (Silva *et al.* 1996). Since this initial description, *A. armata* has been found in a number of countries' marine floras and today exhibits a widespread global distribution pattern throughout the oceans of the world. The first indications of its invasive nature appeared in the 1920's, when it was discovered at four separate European centers: once off the coast of Algeria, and three times in France (both Mediterranean and Atlantic) (Guiry & Dawes 1992). These almost simultaneous discoveries led to early speculations that they had been the result of four separate introductions. However its subsequent rapid spread throughout the rest of Europe suggests that *A. armata* is in fact a highly mobile invasive algal species capable of dispersing rapidly over large distances.

Although there are no hard and fast rules as to what makes a species likely to become invasive, studies on invasive seaweeds have found a number of common traits connected to a species' establishment and dispersal abilities, which have been suggested as important invasive characteristics (Boudouresque & Verlaque 2002, Nyberg and Wallentinus (In press)). One of the most common invasive traits found in these studies is the ability of the invasive species to tolerate a number of different environmental conditions and stresses, as these are generally regarded to be the limiting factors of a species biogeographic distribution.

A number of these studies have singled out *A. armata* as a highly invasive species, because of, among other things, its wide tolerance range (Maggs & Stegenga 1999, Boudouresque & Verlaque 2002). Recently, an unpublished study by Nyberg and Wallentinus (In press), which ranked introduced species in terms of invasive ability depending on its specific life history traits, suggested that *A. armata* is one of the top five invasive algal species found in Europe. However, while conservationists continue to search for methods with which to control invasive species like *A. armata*, there are a number people of who have taken an interest in this charismatic species for different reasons altogether.

Aquaculture potential

While the global aquaculture industry continues to grow rapidly (Tacon 2001), many farmers are realising that, along with the increased demand for fish, there is also a growing international market for seaweed products (Lüning & Pang 2003). In 2001, the seaweed aquaculture industry was already estimated to be worth $5-6 billion (Wikfors & Ohno 2001), with an annual global production of approximately ten million tons (fresh weight) (FAO 2001). At present, the main species under cultivation are used mostly for human consumption (*Porphyra, Laminaria, Undaria*) or the phycocolloid industry (*Kappaphycus, Eucheuma, Gracilaria*) (Wikfors & Ohno 2001), but there are a number of other species, and uses for seaweed, which are currently under investigation.

Previously, one of the major drawbacks to commercial seaweed cultivation was that the majority of seaweed products were low-value products, which either need to be extensively processed or grown in very large quantities in order to return any substantial profits (Jensen 1993). Heteromorphic life histories also complicate cultivation, as different phase often have different environmental requirements. However, as the aquaculture industry continues to expand, the ever-improving ability of farmers, with the aid of scientists, to cultivate seaweeds, both in open-water systems and in tank-

based cultures, in turn means that more and more seaweed species previously ignored for cultivation can now be cultivated successfully on a commercial basis (Lüning & Pang 2003).

Asparagopsis armata is one such species. Many of the species in the Bonnemaisoniaceae have well documented antibacterial and antifungal properties (Pesando & Caram 1984, Ballesteros *et al.* 1992), with *A. armata* being one of the best candidates for commercial cultivation. Not only does it have desirable chemical properties, but the very same traits which make this species a successful invader, also make it a useful species for rapid and simple commercial cultivation. Already, a number of European cosmetics companies have begun marketing a product known as ysaline, which is an extract of *A. armata* used for its antibacterial, fungicidal and food preserving properties (Haslin & Pellegrini 2001). Other recent studies have also shown that both the sporophyte and the gametophyte have an in vitro antiviral effect on the human immunodeficiency virus (HIV), inhibiting replication in the early stages of the virus (Haslin *et al.*2001). The increased interest in the integrated cultivation of seaweed, to remove excess nutrients from eutrophic fish monocultures has also earmarked *A. armata* as one of the better candidates for co-culture (SEAPURA 2001).

Ecology

The *Asparagopsis* genus comprises two morphologically similar species, the temperate *A. armata* and a tropical species, *A. taxiformis*, both of which are characterised by a free floating or epiphytic, sporophytic (*Falkenbergia*) phase and an attached, sometimes epiphytic, gametophytic (*Asparagopsis*) phase. These two species are in fact so similar that they can only be separated morphologically in their *Asparagopsis* phase, as the *Falkenbergia* stages are morphologically indistinguishable (Stegenga *et al.* 1997). While the temperate species, *A. armata,* is thought to be of Antipodean origin, its tropical counterpart *A. taxiformis* was described ten years before *A. armata* in 1845 by V. B. A. Trevisan from a specimen from Alexandria, Egypt (Silva *et al.* 1996).

The *Asparagopsis* and *Falkenbergia* phases are so morphologically different that it led to them initially being described as two different species, hence the different names for the two phases. It was only in 1939, after Feldmann and Feldmann managed to experimentally germinate carpospores from *A. armata* and in so doing produced *F. rufolanosa*, that it was first suggested that the *Falkenbergia* phase was in fact the sporophytic stage of the *Asparagopsis* growth form (Guiry & Dawes 1992). Both the *Asparagopsis* and the *Falkenbergia* phases have the ability to reproduce vegetatively, which would explain why it is not uncommon to find one phase without the other. An

example of this is *A. taxiformis's* distribution in North America, where the *Falkenbergia* phase has been commonly found in most of the southern states in the Gulf of Mexico, as well as along the Mexican coast, but not one collection of the *Asparagopsis* phase has yet been recorded (Guiry & Nic Dhonncha 2004).

Asparagopsis armata in South Africa

A. armata was first reported in South Africa waters in its *F. rufolanosa* phase by Stephenson in 1947, during his survey of South Africa's intertidal fauna and flora (Stephenson 1947). At the time of its discovery, it was still not clear that *F. rufolanosa* and *A. armata* were in fact two phases of the same species, and thus the name *A. armata* does not occur in South Africa's marine flora until 1984 when S. C. Seagrief published his catalogue of Southern African marine algae (Seagrief 1984). Since its initial discovery, it has since been reported at a number of sites along the Southern and Western coasts of the country. However, as yet, it has only been found as infertile plants, in its sporophytic *Falkenbergia* stage and there are no records of the gametophytic *Asparagopsis* stage being found in South Africa. Its tropical counterpart, *A. taxiformis*, has also been found in South Africa. It however, is frequently found in both phases, with its *Asparagopsis* phase occurring abundantly in the northern regions of Kwa-Zulu Natal (KZN) and southwards until Scottburgh in southern Natal.

Understanding the life history strategy of *A. armata* is not only integral to the prevention and identification of possible future invasions by this species along the South African coast but also in assessing its potential use in future aquaculture operations. Knowing its optimal environmental growth conditions will not only help us understand where this known invasive species may potentially become a threat, but will also help us assess its potential for cultivation within existing aquacultural facilities in South Africa. This study thus aims to:

1. Document *A. armata's* invasive history, including invasive traits and current and potential distribution patterns of the *Asparagopsis* genus, both globally and within South Africa

2. Determine the optimal growth conditions of the *F. rufolanosa* phase of the South African strain of *A. armata* in terms of temperature, light and salinity

3. Determine the conditions necessary for the induction of tetrasporogenesis in *F. rufolanosa*

4. Investigate the commercial applicability of *F. rufolanosa* for aquaculture in South Africa and its potential economic benefits

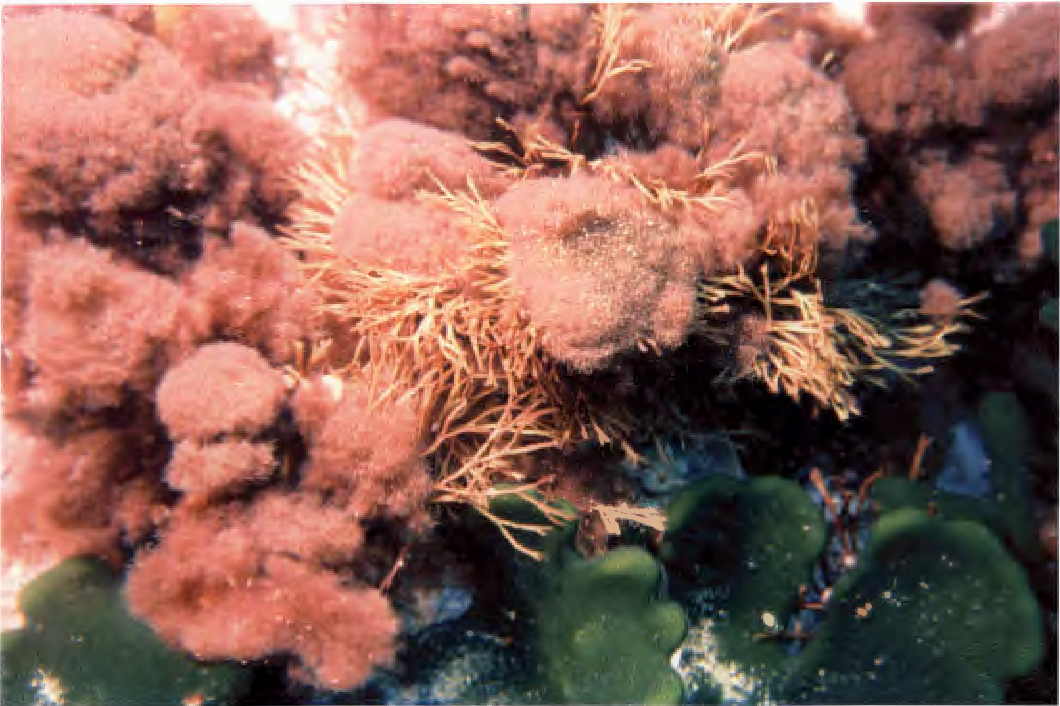

Figure 1: *Falkenbergia* (sporophyte) phase of *Asparagopsis armata*, taken at the collection site in Buffelsbay, South Africa. (Photograph courtesy of R. J. Anderson)

Figure 2: *Asparagopsis* (gametophyte) phase of *Asparagopsis armata*, taken in Scarborough, Moreton Bay, Queensland, Australia. (Photograph courtesy of M. D. Guiry)

METHODS:

Distribution data

Current global distribution patterns of both *Asparagopsis armata* and *Asparagopsis taxiformis* were determined using data from previous papers available at www.algaebase.org. (Guiry & Nic Dhonncha 2004). The South African distribution patterns of *A. armata* and *A. taxiformis* were gathered from a number of published and unpublished surveys undertaken between 1947 up until the present (Stephenson 1947, Stegenga *et al.* 1997, O. de Clerck & J. Bolton pers. comm.)

Collection

Fresh samples of *F. rufolanosa* were collected from Bordjiesrif in the Cape Point Nature Reserve, in the Western Cape province of South Africa. This is one of the few known sites in South Africa where *F. rufolanosa* is found growing abundantly throughout the year. Brick red in colour, the *Falkenbergia* (sporophyte) plants form dense clumps or pompoms up to 5 cm in diameter attaching epiphytically on other benthic seaweeds (see Figure 1) as well as floating freely on the substratum at depths between one and at least eight metres. Fresh samples were collected for each experiment using handheld nets (mesh size 2mm) by a group of divers with SCUBA equipment. Only unattached, free-floating specimens were collected in an effort to keep the samples as unialgal as possible.

Experimental Procedure

Measurement

The seaweed's growth form meant that it was difficult to accurately weigh or measure individual pompoms before the experiments without desiccating them to fatal levels. A method was thus devised whereby a standardised, concentrated inoculum of *F. rufolanosa* filaments, with a known mean filament length, was prepared for each experiment. The concentrated inoculum was prepared by trimming tiny filaments from pompoms of the *F. rufolanosa* floating in a petri dish of seawater, using a pair of dissecting scissors. This initial inoculum of seawater, with the trimmed filaments in it, was then strained through a net with a 1 mm mesh size, ensuring that only pieces ≤ 1 mm would fall through the sieve. The filaments which fell through the sieve were then collected in a single dish from which a subsample of sixty filaments was measured with an eyepiece micrometer on a compound microscope. The mean, standard deviation and variance were calculated from this sample, which was taken to be representative of the whole inoculum. The rest of the inoculum was

then distributed amongst the different replicates, which were then placed into the experimental treatments. The temperature, light and salinity experiments ran for a week, after which they were stopped and sixteen filaments from each replicate were re-measured, using the same eyepiece micrometer, and new means were calculated.

Growth conditions

All *F. rufolanosa* samples were grown in the same culture medium of Provasoli's Enriched Seawater (ES) solution. The inoculum of *F. rufolanosa* filaments was added to 200 ml of a one-third strength ES solution in 200 ml crystallising dishes (covered with petri dishes) which were then placed under the experimental conditions. The ES solution was prepared according to Starr and Zeikos (1987 (see appendix 1)). This is the concentration used for the laboratory culture of many red algae species, particularly the commercially valuable species *Gracilaria gracilis* (Engledow & Bolton 1992)

All growth experiments were carried out under a standard 16:8 light:dark photoperiod. This photoperiod was used to simulate typical summer daylengths in a temperate region, which is when *F. rufolanosa* occurs most abundantly (Guiry & Dawes 1992, Stegenga *et al.* 1997). The sexual reproduction experiment differed from the growth experiments in that it was done using a 8:16 light:dark photoperiod. In order to minimize any unevenness in the light source, the dishes were moved every second day during the experimental period.

Treatments

1. **Temperature**

Growth of *F. rufolanosa* was measured at 10, 15, 20 and 25 °C. Five replicates in 200 ml crystallising dishes were placed in each of the four temperature conditions. The crystallising dishes were kept at the desired temperature by immersing the dishes in regulated water baths to just below the lip of the dish. As this was the first experiment and the optimal light intensity was not yet known, a light intensity of 50-70 μmol photons. m^{-2}. s^{-1} was used. Ideally each temperature would have been tested at a number of different irradiances but unfortunately, due to the limited space in the different temperature treatments, only one light intensity was tested in this initial experiment.

2. **Light**

Growth of *Falkenbergia rufolanosa* was measured at irradiances of 0.2-0.4, 5-7, 20-25, 40-50, 70-80 and 100-110 μmol photons. m^{-2}. s^{-1}. Three replicates in 200 ml crystallising dishes with the *F. rufolanosa* inoculum were placed under the different irradiances at the temperature at which maximum growth occurred in the previous experiment (15 °C). Different irradiances were set up by varying the number of neon lights in the growth room and by shading certain areas with shade cloth. Irradiance levels were measured using a Skye SKD 200 light meter.

3. **Salinity**

F. rufolanosa's salinity tolerance was investigated at salinities of 1, 5, 10, 15, 25 and 35 parts per thousand (‰). Different salinity solutions were made up by producing two separate ES solutions (see Appendix 1) one using distilled water (0 ‰) and the other using ordinary seawater (35 ‰). These two solutions were then mixed together in differing quantities in 200 ml crystallizing dishes (see Table 1) to produce the required salinities. Three replicates of the *F. rufolanosa* inoculum at each salinity were then placed under the temperature and light conditions at which maximum growth occurred in the previous two experiments.

Salinity (‰)	Seawater (ml)	Distilled water (ml)
1	5.6	194.4
5	28.3	171.7
10	57	143
15	85.6	114.4
25	143	57
35	200	0

Table 1: Volumes of seawater and distilled water used to make up 200 ml of ES solution at the different salinities

4. **Sexual Reproduction**

Previous attempts to initiate tetrasporogenesis in *F. rufolanosa* in other studies have found that plants collected in different regions have a number of different requirements in terms of temperature, daylength and iodine and arsenic supplementation (see Guiry and Dawes 1992, Oza 1977). For the purpose of this study three replicates of the *F. rufolanosa* inoculum in ES solution were incubated for four weeks at 17 (± 1) °C with a 8:16 light:dark photoperiod under a light intensity of 20-25 μmol photons. m^{-2}. s^{-1}, as these were the conditions under which tetraspores had

been induced in most of the other strains found in the literature. Unfortunately, a lack of time and proper equipment meant that no other variations could be tried.

Statistical analysis

At the conclusion of each experiment, subsamples of filaments from each experimental replicate were measured and a new mean was calculated. Specific growth rates (SGR) were then calculated from the differences between the final and initial means using the following equation (from Wilson & Critchley 1997):

$$\mu = 100 \times \frac{\ln(L_t) - \ln(L_o)}{T_t - T_o}$$

Where:

μ = specific growth rate (%. day^{-1}): L_t = length at time t: L_o = initial length: $T_t - T_o$ = culture period

The means were then statistically compared within and between treatments using ANOVA, in order to determine whether the observed growth rates from the different experimental treatments were actually significantly different. Before using ANOVA, the data was first tested for normality and homoscedasticity (equal variances) with Levene's test to establish whether parametric statistical tests could be used. If significant differences were apparent in ANOVA, the data was tested with Fishers least significant difference (LSD) test at the 95% confidence level to establish where the differences were. All statistical analyses were done using the Statistica 6. program.

RESULTS:

Distributions

Figure 3: Global distribution pattern of *Asparagopsis armata* (data from Guiry & Nic Dhonncha 2004)

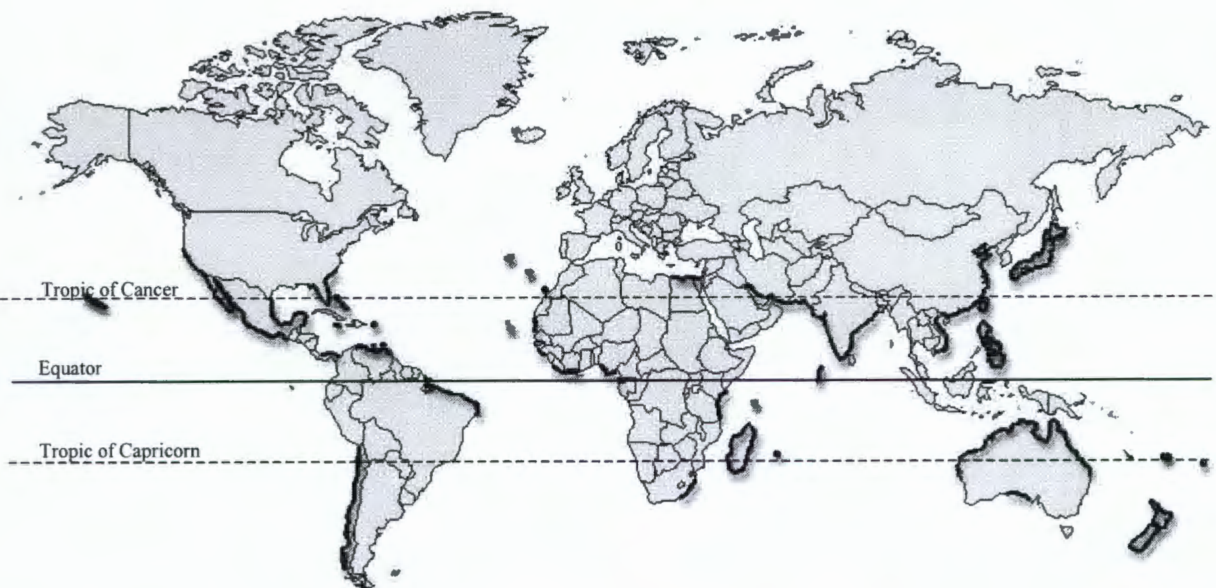

Figure 4: Global distribution pattern of *Asparagopsis taxiformis* (data from Guiry & Nic Dhonncha 2004)

A. armata (Figure 3) has a truly temperate distribution, although it occurs across most longitudes, it is only found above and below the tropics where the sea temperatures are considered warm to cold temperate. The only exceptions to this rule are its occurrence in India and Myanmar, which is tropical. *A. taxiformis's* distribution patterns (Figure 4) are not as clear as its temperate counterpart. It is found on all the major continents, but is mainly distributed between the tropics, and it is also found along considerably colder coastlines, such as New Zealand and Chile. When assessing these distributions, it is important to note that the literature from which they were taken does not always make it clear whether both phases, or just one phase, was found at each location. However, it is known that there are a number of cases, such as *A. armata's* distribution in South Africa and *A. taxiformis's* distribution in North and Central America, where only one phase, usually the *Falkenbergia* phase, was found without the presence of the *Asparagopsis* phase ever being recorded (Guiry & Nic Dhonncha 2004)

Figure 5: South African distribution patterns of *Asparagopsis armata* and *Asparagopsis taxiformis*.

There is a gap of approximately 600 km between *A. armata's* eastern distribution limit (Port Elizabeth) (Stephenson 1947) and *A. taxiformis's* western distribution limit (Scottburgh, southern KZN) (O. de Clerck pers. comm.) along the South African coastline (Figure 5). *A. taxiformis's* has been recorded in Kenya, Tanzania and Madagascar but has not yet been found in Mozambique

(Guiry & Nic Dhonncha 2004), which is not as well studied as Kenya and Tanzania. *A. armata's* western limit is situated at Platbank on the Cape peninsula (Stegenga 1997).

Growth experiments

Temperature

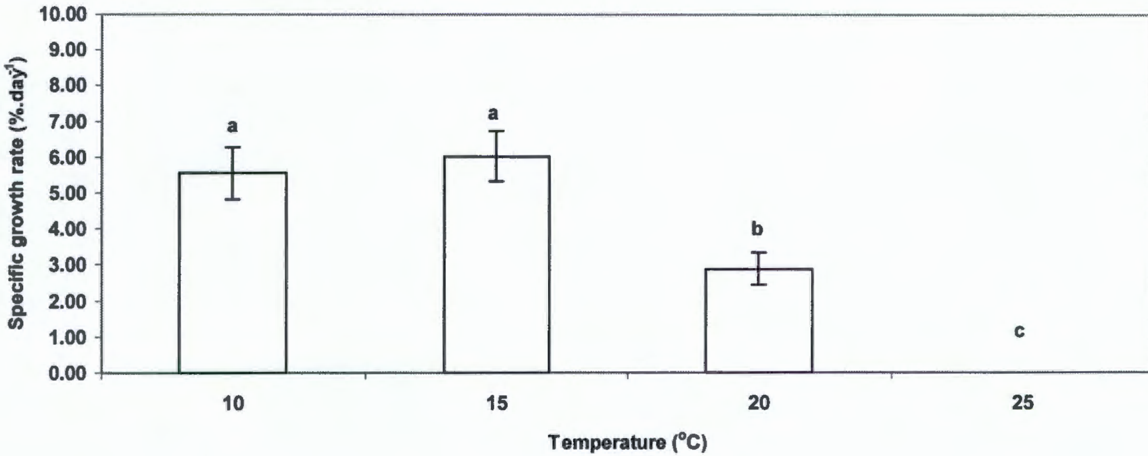

Figure 6: Specific growth rate of *F. rufolanosa* at different temperatures. Different letters indicate significant differences at the 5% level (one-way ANOVA and Fisher LSD post-hoc test). Data were log transformated in order for the assumptions of ANOVA to be met (vertical bars represent ± one standard error of the mean)

The maximum specific growth rate (SGR) of *F. rufolanosa* occurred at 15 °C (Figure 6), under a light intensity of 50-70 μmol photons. m^{-2}. s-1, however, this was not significantly different to the specific growth rate at 10 °C. *F. rufolanosa* did not survive well at higher temperatures, as it displayed a significantly lower growth rate at 20 °C and died and disintegrated in the 25 °C treatment (see Table 2). The 20 and 25 °C treatments were characterised by a general loss of pigment in the *F. rufolanosa*. It was also noted that in all experiments, probably due to the toxicity of *F. rufolanosa* (Sala & Boudouresque 1997), there was little to no epiphytic or diatomic growth in the crystallising dishes.

Table 2: Summary of specific growth rates (%. day^{-1}) for different temperature treatments

Temperature (°C)	No. of Replicates (N)	Average SGR	Min SGR	Max SGR	Std. Error
10	5	5.55	4.42	8.42	0.73
15	5	6.03	3.54	7.54	0.70
20	5	2.89	1.51	3.72	0.45
25	5	died	died	died	died

Light

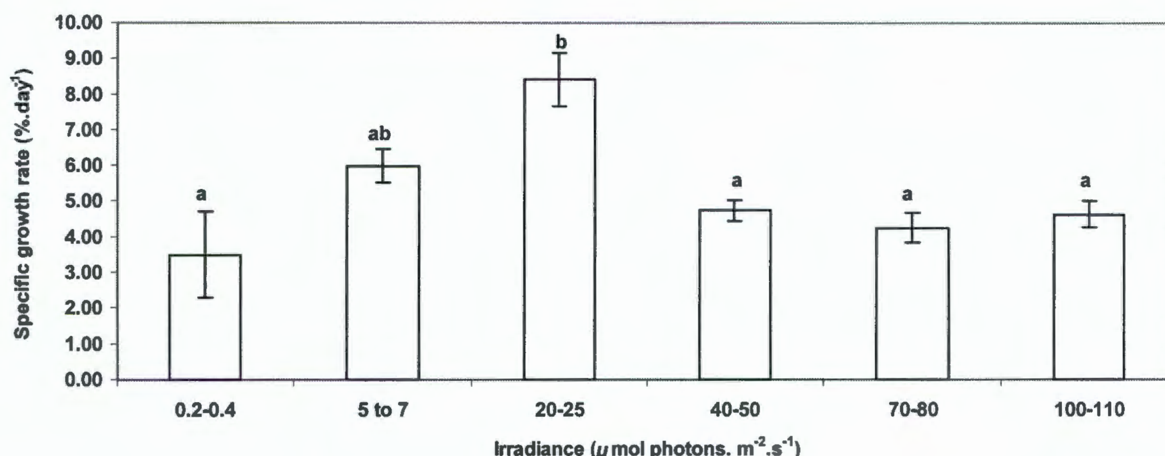

Figure 7: Specific growth rate of *F. rufolanosa* at different irradiances. Different letters indicate significant differences at the 5% level (unequal N one-way ANOVA and Fisher LSD post-hoc test) (vertical bars represent ± one standard error of the mean)

The maximum specific growth rate occurred at 20–25 μmol photons. m^{-2}. s^{-1} (Figure 7), under a temperature regime of 15 °C, although this was not significantly different to the growth recorded at 5-7 μmol photons. m^{-2}. s^{-1}. Growth rates steadily increased from the 0.2-0.4 to the 20-25 μmol photons. m^{-2}. s˙1 treatment, where they peaked, thereafter the higher irradiances resulted in decreased growth rates, which were similar for all the other treatments (see Table 3). The *F. rufolanosa* filaments in the 0.2-0.4 and the 100-110 μmol photons. m^{-2}. s^{-1} treatments all lost their pigmentation and were apparently dead.

Table 3: Summary of specific growth rates (%. day^{-1}) for different light intensity treatments

Light Intensity (μmol photons. m^{-2}. s^{-1}	No. of Replicates (N)	Average SGR	Min SGR	Max SGR	Std. Error
0.2 - 0.4	3	3.50	1.26	5.41	1.21
5 - 7	2	5.98	5.52	6.45	0.47
20 - 25	3	8.41	7.14	9.70	0.74
40 - 50	3	4.75	4.24	5.26	0.30
70 - 80	3	4.26	3.59	5.03	0.42
100 - 110	3	4.64	3.90	5.02	0.37

One replicate of the 5-7 μmol photons. m^{-2}. s^{-1} did not record any growth and the *F. rufolanosa* filaments lost colour and died. This replicate was thus excluded from the analysis as some error

probably occurred in the experimental procedure for this particular dish, for example, the dish may not have been properly cleaned before the experiment and still had toxic substances present in it, which would have killed the *F. rufolanosa.* The other two replicates in the 5-7 μmol photons.m^{-2}.s^{-1} treatment survived the experiment and grew normally which ensured that there was still enough data to make statistically significant observations.

Salinity

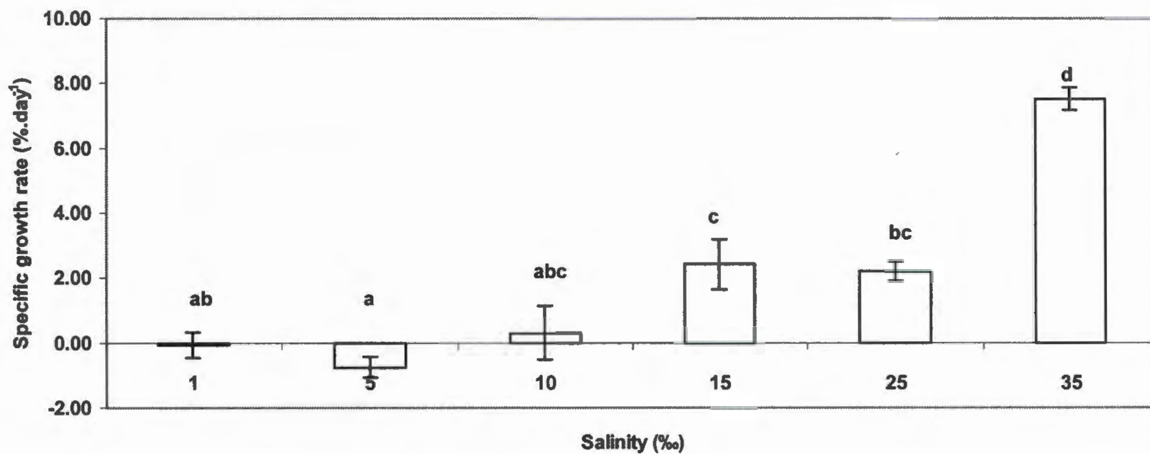

Figure 8: Specific growth rate of *F. rufolanosa* at different salinities. Different letters indicate significant differences at the 5% level (one-way ANOVA and Fisher LSD post-hoc test) (vertical bars represent ± one standard error of the mean)

Maximum growth of *F. rufolanosa* occurred at a salinity of 35 ‰ (Figure 8), at a temperature of 15 °C and under a light intensity of 20-25. μmol photons. m^{-2}. s^{-1}. This growth rate of 7.52%. day^{-1} is comparable to the growth rate obtained in the previous experiment under similar conditions. The other salinities all recorded significantly lower growth rates, with the 1, 5 and 10 ‰ treatments all dying, losing their pigmentation and not showing any significant growth at all.

Table 4: Summary of specific growth rates (%.day^{-1}) for different light intensity treatments

Salinity (‰)	No. of Replicates (N)	Average SGR	Min SGR	Max SGR	Std. Error
1	3	-0.07	-0.83	0.50	0.40
5	3	-0.75	-1.23	-0.17	0.31
10	3	0.31	-1.36	1.28	0.84
15	3	2.43	1.01	3.65	0.77
25	3	2.21	1.63	2.60	0.30
35	3	7.52	6.90	8.14	0.36

Sexual Reproduction

Attempts to initiate tetrasporogenesis in *F. rufolanosa* for this study were unsuccessful. After four weeks of incubation at 15 °C at short daylengths under a 8:16 light:dark photoperiod, although the *F. rufolanosa* had grown significantly, there were no signs of tetraspore formation.

DISCUSSION:

Asparagopsis armata invasions: past, present and future

A. armata was first described in Europe from a specimen found off the Atlantic coast of France in 1925 (Womersley 1996). By 1939 it had been identified by De Valéra from Galway Harbour in Ireland (Drew 1950) and subsequently spread to England, where it was first found in Devon (Harvey & Drew 1949), and has since spread further along the southeastern shores of both England and Ireland. At the same time, it has since spread to a number of locations within the Mediterranean basin, often forming dominant monospecific stands covering large sections of the coastline (Sala & Boudouresque 1997, Boudouresque & Verlaque 2002).

It is thought that *A. armata* was initially introduced to mainland Europe from either Australia or New Zealand, possibly in oysters, and then dispersed via rafting or shipping across to Ireland and than England. Interestingly, the majority of all European seaweed introductions are first observed in France, on both the Mediterranean and Atlantic coasts, which may be partly due to its long and diverse coastline, but is also probably related to the well-established aquaculture industry (Maggs &Stegenga 1999). *A. armata* is just one of over 85 seaweed species which are thought to have been introduced to Europe (Boudouresque & Verlaque 2002), however, *A. armata* differs from most of these other introduced species in that its invasive nature has made it one of the most dominant invasive species in the Mediterranean today (Sala & Boudouresque 1997, Boudouresque & Verlaque 2002).

There are a number of traits relating to a seaweed species' dispersal and establishment abilities, as well as its ecological impact that may increase its invasive ability (Boudouresque & Verlaque 2002, Nyberg and Wallentinus (In press). *A. armata*'s life-history incorporates a number of these invasive traits, making it one of the more invasive species in the Mediterranean (Nyberg and Wallentinus (In press)). The most successful invasive species tend to reproduce vegetatively, which both phases of *A. armata* are known to do, thus reducing the need for both gametophytic and sporophytic phases to

be present. This vegetative reproduction and growth also needs to be prolific in order for the species to outcompete other native species in the area; under optimal conditions in this study, *F. rufolanosa* had a fairly high maximum specific growth rate of over 8%. day-1. More importantly perhaps, the invasive species needs to have flexible habitat requirements and an ability to tolerate a number of different stresses and environmental extremes; *F. rufolanosa* tolerated and grew in a temperature range of 10 to 20 °C and potentially lower. The ability to avoid predators and disease in the invaded area is also likely to increase the species invasive ability (Boudouresque & Verlaque 2002). This last point is particularly valid in the case of *A. armata* as both phases of *A. armata* have chemical defences and thus toxic to most marine herbivores, as they known contain a number of polyhalogentaed compounds (Sala & Boudouresque 1997)

Of all the environmental factors that govern a seaweed's geographic distribution, it is commonly accepted that most species' distributions are primarily governed by their thermal requirements (Bolton 1986). This is why the ability of a seaweed species to tolerate a wide range of temperature conditions is considered to be one of the traits most likely to make it a successful invader. However, there are in fact a number of critical maximum and minimum temperatures for survival, growth and sexual reproduction, which determine a species' distribution. It is also known that these temperature limits may vary, not only between species as seen between *A. armata* and *A. taxiformis*, but also between different strains and heteromorphic life-history stages as well (Lobban & Harrison 1994).

Two previous studies on European strains of *F. rufolanosa* have independently found that it could survive temperatures of 5-25 °C and grew between 9-21 °C (Orfanidis 1991, Maggs & Stegenga 1999). This agrees with the results found in this study. However, these studies have all been done on the *Falkenbergia* phase of *A. armata*, the fact that the *Asparagopsis* phase has never been found in South Africa suggests that it might have different temperature limits, although it is unclear whether the *Asparagopsis* phase is intolerant of higher or lower temperatures. Its distribution patterns in Britain, where the *Falkenbergia* phase has been found as far north as the Shetland and Orkney Isles with the *Asparagopsis* phase restricted to the south-eastern coast (Farnham 1980), suggests that it is likely to be the latter. Distribution differences between heteromorphic life stages are not uncommon though, and there are a number of other countries in which the *Falkenbergia* phase of *A. armata* has a wider distribution range than the *Asparagopsis* phase and is also often found in areas where the *Asparagopsis* phase has never been recorded (Guiry & Nic Dhonncha 2004).

Many of the studies conducted on invasive marine organisms, have found that the estuarine and nearshore environments are often the first sites invaded after an introduction, as they are sheltered and therefore offer protection from environmental extremes (National Research Council 1995). In the case of *A. armata*, its intolerance of lower salinities indicates that it is unlikely to invade estuarine environments because of their decreased salinity levels. Studies have shown that subtidal species, like *A. armata*, are generally less tolerant of lower salinities than intertidal species, with most subtidal species unable to tolerate salinities of less than 18 ‰ (Lobban & Harrison 1994). It is therefore more likely that *A. armata* populations will be found further from the shore, where salinities are higher and more stable. Its ability to photosynthesize efficiently in low light conditions (see Figure 7) ensures that *A. armata* will be able to survive at considerable depths.

Another important factor limiting the distribution of the *Asparagopsis* phase is that, in order to become fertile and form tetraspores from which the gametophyte can grow, *F. rufolanosa* is known to require very specific conditions (Guiry & Dawes 1992). All published records of fertile plants of *F. rufolanosa* note that reproduction appears to be restricted to narrow window period in autumn, when there is a reduced photoperiod but sea temperatures are still high enough for tetrasporogenesis to occur (McLachlan 1967, Oza 1977, Guiry & Dawes 1992, Womersley 1996). Different strains also have different requirements; previous studies have found that the critical daylength for different *F. rufolanosa* plants varied from 8-10 hours, at temperatures ranging from 13 to 21 °C for periods of four weeks or longer (Guiry & Dawes 1992). A number of European strains studied also required additional iodine and arsenic supplementation in laboratory studies in order to induce tetrasporogenesis (Guiry & Dawes 1992).

Asparagopsis armata in South Africa

This study's attempts to induce tetrasporogenesis were unsuccessful. Although the study was far from comprehensive, it does indicate that the South African strain is similar to European strains, in that it also requires very specific conditions in order for reproduction to occur. The fact that *F. rufolanosa* has been found along South African coastlines for over half a century without the *Asparagopsis* phase ever being recorded indicates that reproductive conditions for *A. armata* probably do not exist in South Africa. It is interesting to note however, that Stegenga et al. (1997) have drawn tetrasporangia on *F. rufolanosa* in their illustration of the species in their book Seaweeds of the South African West Coast (1997). Nonetheless, the lack of daylengths shorter than 10 hours in South African waters is probably one of the most limiting factors to its reproduction.

The implications of *A. armata*'s limited reproductive potential for its distribution in South Africa are numerous. Although the *F. rufolanosa* stands in South Africa appear to be quite dominant in their environment, like the site at Bordjiesrif where the experimental samples were collected, these sites are fairly rare. In Europe, it is the *Asparagopsis* phase that is considered to be dangerously invasive rather than the *Falkenbergia* phase. This would explain why, as yet, *A. armata* is not considered an invasive species in South Africa.

However, the temperature experiments indicate that the South African strain can survive and grow in temperatures up to 20 °C but reaches its lethal limit somewhere between 20 and 25 °C. Its lower critical temperature is clearly below 10 °C, although how much lower is still unknown and needs to be studied further. These temperature limits suggest that *A. armata* should be able to expand it current distribution range quite substantially. At present, its eastward distribution limit is at Port Elizabeth (Stephenson 1947), while its western limit is at Platbank (Stegenga et al. 1997). If temperature is the only factor limiting *A. armata*'s distribution, then one would expect to see a much larger distribution range in Southern Africa, particularly along the west coast. Along the east coast, annual average sea temperatures only rise above 20 °C past Durban (Bolton 1986) but monthly means in summer probably reach lethal limits at a point closer to port Elizabeth, thus limiting their easterly distribution. However, temperatures on the west coast however never exceed the lethal limits (Bolton 1986) and it is thus surprising that *A. armata* is not found further up this coast. This would suggest that there might be some other limiting factor to its distribution in South Africa.

A possible explanation might that *A. armata*'s distribution is directly related to the amount of fish herbivory along the coastline. Sala and Boudouresque (1997) carried out a number of exclusion experiments in Spain, in areas dominated by *A. armata*. They found that when fish were excluded, *A. armata* gametophytes were rapidly overgrown by fleshy erect algal species. This suggests that *A. armata* can only outcompete other algal species when the levels of fish herbivory are high, since *A. armata*'s toxicity prevents it from being heavily grazed. In South Africa, perhaps within its temperature range, it is only in areas where herbivory plays a major role in algal communities, that *F. rufolanosa* manages to outcompete local algal species. The collection site at Bordjiesrif is known to have high rates of fish herbivory (R. Anderson pers. comm), which further strengthens this theory.

A. armata or *A. taxiformis*?

Although *A. armata* and *A. taxiformis* are considered to be warm/cold temperate and subtropical species respectively, their global distribution patterns suggest that this is not always the case. Although they appear to have different environmental requirements, particularly in terms of their thermal tolerance limits (Maggs & Stegenga 1999), there are a number of countries similar to South Africa, such as New Zealand, Chile and India, in which both species have been reported. Because the two species are very difficult to tell apart, these regions of mixed distributions make it difficult to know for certain, which species occurs where, they also led to earlier claims which suggested that *A. armata* and *A. taxiformis* may well be conspecific (Farnham 1980).

However, the diversity of coastlines and temperatures in countries like Chile and New Zealand means that, similar to their distribution in South Africa, they probably do not actually co-occur at any one site. Some of the occurrences which are reported in a countries marine flora, do not actually occur in that country but rather on colonies of that country. For example, although *A. taxiformis* is reported in New Zealand's marine flora, it is in fact only found on the Kermadec Islands, a New Zealand colony, which is closer to the tropics than New Zealand itself (Adams 1994). Genetic studies have also since been done which have found clear differences between the two species. All *A. armata* specimens were found to be genetically similar while *A. taxiformis* may be comprised of two or more cryptogenic species (Andreakis *et al.* 2004, Ní Chualáin (In press)). For the purpose of this study, the DNA of South African strain of *A. armata* was sequenced and was found to be genetically similar to other strains of *A. armata* from around the world (pers. comm. Andreakis Nikos).

Asparagopsis armata and its applications in aquaculture

A. armata may not be a popular species with conservationists but it certainly holds a large amount of appeal for commercial aquaculturalists. Not only does it have well documented chemical properties which make it a high-value product (Haslin & Pellegrini 2001), but its high growth rate and wide tolerance range of environmental conditions suggest that it would be easy to cultivate without highly technical, and therefore expensive, cultivation systems. The *Falkenbergia* phase of *A. armata* has a number of features which make it a very good candidate for commercial tank cultivation, both internationally and in South Africa. One of the most beneficial features of *F. rufolanosa* is that grows vegetatively, which simplifies any cultivation operations as there is no need to propagate seedstocks or to vary conditions in order to control *A. armata*'s life cycle, as they

do in the cultivation of *Porphyra* in Asia. Its free floating growth form is another positive feature for aquaculture, as this means that it will require little to no additional technology, other than the most basic aquaculture equipment such as tanks and aeration pipes.

In Europe, a number of organisations and universities have been collaborating on a European Union (EU) project under the name of SEAPURA, trying to identify worthwhile seaweed species to act as biofilters on integrated fish farms. *A. armata* has been identified as the leading species for cultivation, not only because of its highly efficient nutrient uptake capabilities but because there is also market for the seaweed once it has achieved its primary purpose of nutrient remediation (SEAPURA 2001). There are also reports of *A. armata* being cultivated by a number of other organisations in countries such as France and Ireland (Bord lascaigh Mhara 1999), however, it is difficult to verify these reports because of the closed nature of the aquaculture industry.

In South Africa, the majority of aquaculture operations involve the tank-based cultivation of abalone (Haliotis midae) (Sauer et al. 2003). Although abalone are herbivorous and thus produce far less nutrient pollution, the integration of seaweed cultivation in the abalone effluent water will improve farms' recirculation systems while the sale of the seaweed can also supplement their primary income. The most obvious positive feature of seaweed cultivation in South Africa is that most of the necessary facilities already exist because of the rapidly growing abalone industry. Fresh seawater is already being pumped ashore daily for abalone cultivation operations, this water could easily be further utilised, at no extra cost, if it was then transferred to seaweed tanks before being returned to the sea. The only added facilities needed would be specialised seaweed tanks or raceways, to maximise growth, which is a once-off cost. As long as a stable market can be found, the cultivation of a potentially high-value species like *A. armata*, even in its *Falkenbergia* phase, would not only improve the aquaculture facility's commercial viability, but it would diversify their interests at the same time.

All indications are that *A. armata* would be an ideal species for tank cultivation in South Africa. Its optimum growth temperature of 15 °C is the same, or close to, the temperature of the fresh seawater pumped ashore at most of the existing abalone farms in South Africa (L. Jansen pers.comm.). Its relatively low light optima of 20-25 µmol photons. m-2. s-1 means that no additional lighting equipment would be necessary and that the seaweed could be grown inside or outside in shaded tanks without much difficulty. Similarly, its toxicity, as noted in the experiments, may prevent epiphytic and diatomic growth from becoming problematic. Research is still needed however, to

ensure that this toxicity will not be passed on to the other aquacultured organisms. Its high growth rate, of at least 8.41%. day-1 if grown under optimal conditions, and known high nutrient uptake rates (SEAPURA 2001), means that farms could also reduce pumping costs by recirculating water which had passed through the seaweed biofilters, assuming that *A. armata* does not release any toxins into this water, rather than constantly pumping fresh water from the sea. Unfortunately, just how valuable *A. armata* is, is not known, as the foreign seaweed cultivation industry is reluctant to release any commercial information about their products and all attempts to communicate with both the cultivators and the pharmaceutical companies have proved fruitless at the time of this project going to print.

The South African strain of *A. armata* still requires a lot of research before local aquaculture operations can begin cultivating it commercially. One of the most important studies still needed would be an investigation of F. rufolanosa's nutrient requirements. Its high specific growth rate suggests that it will also have high nutrient requirements for optimal growth to be achieved. However, this should not pose a problem in South Africa, since it would not be difficult to utilize nutrient-rich effluent water from abalone farms to cultivate *F. rufolanosa* in. As a precursor to this study, *F. rufolanosa* was placed in tanks at an abalone farm, in order to investigate whether it could be grown within the existing aquaculture facilities. Although the seaweed's optimal growing conditions were unknown at the time, the seaweed survived for over four weeks without any additional inputs. However, there was minimal growth recorded, even though the water was at the optimum growth temperature (15 °C), which suggests that it might be necessary to increase the seaweed's nutrient supply, either by fertilisation or by placing the seaweed in the abalone effluent water.

The future of *Asparagopsis armata* in South Africa

The results of this study indicate that *F. rufolanosa* can tolerate a wide variety of environmental conditions. This in turn suggests that it would indeed be a good species for commercial cultivation in South Africa in terms of its minimal requirements for growth and wide tolerance ranges. However, securing a market for *F. rufolanosa* is likely to be much more difficult than growing it, as the major market at present is in Europe and, similar to many other aquacultural operations, appears to be a very closed industry. Then there is also the issue of ensuring that *A. armata* does not become an invasive threat to South Africa's marine biodiversity.

The inhibition of sexual reproduction in the South African strain of *A. armata* is probably crucial to the prevention of future invasions by this species in South Africa. At present, the absence of short enough daylengths to induce tetrasporogenesis, apparently ensures that only the *Falkenbergia* phase exists along South African coastlines. If this were to change, either because of genetic mutation within the species or because of accidental introductions of the *Asparagopsis* phase, there is a good chance that the more invasive *Asparagopsis* phase will be able to survive and flourish in South African waters. The only known difference between the two phases is that the *Falkenbergia* phase can tolerate lower temperatures, otherwise the *Asparagopsis* phase does not seem to have any additional requirements for growth compared to the *Falkenbergia* phase. Its ability to grow vegetatively means that only one successful introduction would be necessary for it to establish self-sustaining populations in South Africa.

It is already widely recognised that many of the invasive alien introductions that have occurred to date, have been as a result of aquacultural operations (Maggs & Stegenga 1999) and it is for this reason that it is important that further studies on triggers for tetrasporogenesis in the South African strain of *F. rufolanosa* are done before any commercial cultivation of the species is allowed to take place. A number of different conditions and supplements have been suggested in studies on European strains of *F. rufolanosa*, which would be a good starting point for further studies here in South Africa (see Guiry & Dawes 1992).

It is also suggested that further surveys along the South African coast are conducted in order to determine existing distribution limits of *F. rufolanosa* more accurately. *F. rufolanosa's* ability to survive and grow in low light conditions, as shown in the experiments, indicates that it is likely to survive at depths greater than 30 metres, which have not been previously studied in most of South Africa's seaweed surveys.

Understanding the environmental conditions under which *F. rufolanosa* can survive, grow and reproduce not only enables South African aquaculture operations to maximise their potential growth of *F. rufolanosa* and therefore profits, but it also ensures that conservationists will be better able to predict and control potential future invasions by *F. rufolanosa*. *A. armata* holds substantial potential for future cultivation in South Africa, however its dangerous invasive nature requires that it be closely monitored and further researched before any aquacultural cultivation of the species is authorized.

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APPENDIX 1:

Provasoli enriched seawater as in Starr and Zeikos (1987)

Step1 (Fe- solution)

Dissolve 351 mg of $Fe(NH_4)_2(SO_4)_2.6H_2O$ and 300 mg of Na_2EDTA in 500 ml of distilled water. Autoclave the solution

Step 2 (PII metal solution)

Dissolve the following chemicals in 100 ml of distilled water:

- 100 mg of Na_2EDTA
- 114 mg of H_3BO_3
- 4.9 mg of $FeCL_3.6H_2O$
- 16.4 mg of $MnSO_4.7H_2O$
- 2.2 mg of $ZnSO_4.7H_2O$
- 0.48 mg of $CoSO_4.7H_2O$

Adjust the pH to 7.8 and autoclave the solution

Step 3 (ES- enrichment concentrate)

Add 25 ml of each of the PII metal and Fe- solutions to 50 ml of distilled water. Dissolve the following chemicals in this solution:

- 350 mg of NaNO3
- 50 mg of Na2glycerophosphate.5H20
- 10 g of vitamin B12
- 0.5 mg of Thiamine
- 5 g of Biotin
- 500 mg of Tris buffer (Sigma)

Autoclave and store at 10 °C.

Step 4

To produce a one-third strength standard Provasoli ES solution, add 6 ml of ES-enriched concentrate to 1000 ml of autoclaved seawater/distilled water (depending on experiment)