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BARD

FINAL REPORT

PROJECT NO. IS-1299-87

**Modeling of the Relationship Between the
Growth of Controlled Seaweed Sporelings and
their Acclimation in the Field Environment**

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Appendix VI

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Title

Modeling of the relationship between the growth of controlled seaweed
sporelings and their acclimation in the field environment.

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Abstract

The central hypothesis tested in this project claimed that suboptimal preconditioning of *Gracilaria* sporelings will better acclimate them under outdoor conditions. This hardening was expected to decrease the ratio of saturated versus unsaturated fatty acids. Different *Gracilaria* species have been tested experimentally by the American and Israeli investigators. Two of the four species tested confirmed this hypothesis. A comparison of the conclusions from the Israeli and American laboratories indicated that *G. verrucosa* and *G. tikvahiae* do not show the expected hardening process as seen in *G. domingensis* and *G. conferta*. It may be suggested that the difference between these two pairs of species is a result of their geographical distribution. The first pair has a very wide distribution, which shows a lower optimum temperature in indoor cultures, while the second pair is limited to a warmer environment. *G. domingensis* (Caribbean Sea) and *G. conferta* (eastern Mediterranean) have a higher temperature optimum in indoor cultures. The different source might impose a different response to suboptimal growth conditions.

Objectives of the original proposal

- 1) To support experimentally the hypothesis that suboptimally conditioned sporelings will more successfully acclimate (double their survival) under a field environment than will optimally conditioned ones. Suboptimal conditioning should be optimal for transplantation.
- 2) To establish this support at two geographically distinct areas using locally common species: *G. foliifera* and *G. conferta* (Israel) and *G. verrucosa* and *G. tikvahiae* (Florida).
- 3) To operate light intensity, temperature, nutrient concentration and salinity variables in the conditioning process of the sporelings, and to confirm in the laboratory the hypothesis of growth rate decrease and increase of lipid and unsaturated fatty acid concentration under suboptimal conditions.
- 4) To determine the field acclimation of the transplanted sporelings using survival and growth rate as target variables with season and pretreatment as independent variables.
- 5) To formulate an overall model of the preconditioned sporeling acclimation in a diverse environment.

1. Research Activities and Results (Body of Report)

1.1 Introduction

In the third year of the project, final experiments were conducted in both laboratories on the following subjects: Relationship of indoor sporeling growth rate (1) with their outdoor growth; (2) with their fatty acid concentration; (3) with their proline content. The initial hypothesis regarding the effect of sporeling stress conditions on their outdoor acclimation and their fatty acid content was evaluated.

1.2 The Israeli laboratory

1.2.1 Materials and methods

Indoor growth gradient table experiments and fatty acid determination were performed as reported previously. Outdoor acclimation of the preconditioned sporelings was also performed as reported previously; however, the sporelings were kept in small white net cylinders of 5 cm diameter and height, in order to protect them from being lost in the big tank section.

Proline was determined according to Weinberg, Lerner and Polyacoff-Mayber, 1982 (Physiol. Plant. 55: 5-10). One gram of fresh *Gracilaria* sporelings was rinsed with water. Extraction was performed using 0.4 ml toluene and 10 ml H₂O at 30°C by gentle shaking for 60 min. The liquid was decanted and re-extracted twice more. Five ml of the pooled supernatants were diluted to 5 ml NaCl 5 N and 5 ml H₂O, from which 1 ml was taken for the reaction. In the reaction, 1 ml of phosphate buffer and 3 ml of ninhydrin 2% were added to the 1 ml sample, and boiled for 100 min. Absorbance of the sample was determined in acetic acid glacial at a wavelength of 512 nm (Bergman and Loxley, 1970, Analyt. Chem. 42: 702-706). A proline calibration curve was prepared.

1.2.2 Results and discussion

Exp. 15. In this experiment, the fronds of *G. domingensis* sporelings were cultured on a growth gradient table at different levels of temperature, irradiance and ammonium concentrations. The salinity of the growth medium was 20 ppt, and fertilization was performed once a week during 16 hours. The experiments lasted for

5 weeks, 2-4 of which were considered in the ANOVA analysis. Highest growth rate of sporelings appeared at moderate irradiance ($105 \mu\text{E m}^{-2} \text{ s}^{-1}$), high temperature (25°C), at an ammonium concentration of 0.7-2.6 mM (Table 15.1). The significant main effects were temperature and ammonium concentration, while the irradiance was not a significant variable in the ANOVA. Only 20 out of 52 treatment combinations survived for 5 weeks, presumably because of the low salinity. The ln transformation did not significantly improve the model.

The outdoor acclimation experiment showed very diverse results, as it was still performed without net cylinders. Only 7 out of 11 sporelings that started the outdoor experiment survived one week without any weight loss, while only 2 did not lose weight after the second week. These survivors were preconditioned under different ammonium concentrations (0.7-2.6 mM), low to moderate irradiance ($25\text{-}105 \mu\text{E m}^{-2} \text{ s}^{-1}$) and moderate temperature ($20\text{-}25^\circ\text{C}$). No correlation was found between indoor growth conditions under salinity of 20 ppt; however, the few acclimated sporelings were preconditioned under optimal conditions.

Exp. 16. In this experiment, the fronds of *G. domingensis* sporelings were cultured on a growth gradient table at different levels of temperature, irradiance and ammonium conditions under a salinity of 40 ppt. Fertilization was also performed once a week during 16 hours. The experiment lasted for 6 weeks, five of which were considered in the ANOVA analysis. All three main effects were significant. Highest growth rate appeared at moderate irradiance ($100 \mu\text{E m}^{-2} \text{ s}^{-1}$), high temperature (25°C) and at medium to high ammonium concentrations (0.7-2.6 mM). Sporelings did not survive at low temperatures ($10\text{-}15^\circ\text{C}$) (Table 16.1, Figs. 1-3).

The outdoor acclimation experiment was performed with small net cylinders in which the sporelings were kept inside the bigger sectional tank. ANOVA showed that all three main effects were not significant. However, best acclimation was achieved under moderate irradiance ($100 \mu\text{E m}^{-2} \text{ s}^{-1}$), high temperature (30°C) and low to high additional ammonium (0.7-2.7 mM) (Table 16.2). These are definitely

suboptimal conditions, over the first two weeks, which confirms previous data. This species of *Gracilaria* showed better acclimation with suboptimal preconditioned sporelings. Sporelings which survived over 4 weeks of outdoor acclimation were all preconditioned at high irradiance ($225 \mu\text{E m}^{-2} \text{s}^{-1}$) at all temperatures and ammonium concentrations. No correlation was found between indoor and outdoor growth rate ($r = 0.1759$, $P = 0.2317$, Figs. 4-5).

Exp. 17. In this experiment, the fronds of *G. domingensis* sporelings were cultured on a growth gradient table at different levels of temperature, irradiance and ammonium concentration under a salinity of 40 ppt. The experiment lasted for five weeks, four of which were averaged, and it was fertilized as mentioned previously. Temperature and ammonium concentrations were the two significant main effects, while the irradiance was not significant. The ln transformation showed that all 3 main effects were significant. Highest growth rate appeared at high temperatures (30°C), all irradiance levels ($27\text{-}225 \mu\text{E m}^{-2} \text{s}^{-1}$) and high ammonium concentrations ($1.3\text{-}2.6 \text{ mM}$) (Table 17.1). In this experiment, the sporelings did serve as a source for fatty acid extraction. The dominant fatty acids were as before the 16:0 (23.4%) and 20:4 ($\omega 6$) (26.1%). The mean ratio of 16:0/20:4 equals 0.9 (Table 17.2, Figs. 6-8). A correlation analysis between indoor growth rate and fatty acid concentration showed no relationship between growth and 16:0 or 20:4 fatty acid concentration. However, an almost significant positive correlation ($P = 0.056$) was calculated between growth rate and the ratio of 16:0/20:4 fatty acid concentration. This indicates that growth shows a simultaneous positive relationship with saturated fatty acids and an inverse relationship with unsaturated fatty acids ($\text{growth} = 21.9 \times \text{ratio} + 25.8$). The ln transformed growth data showed significant positive correlation with saturated (16:0) ($G = 3.339 + 0.023 \text{ FA } 16:0$) and unsaturated fatty acid (20:4) ($G = 3.43 + 0.017 \text{ FA } 20:4$) (Figs. 9-11). This almost significant relationship supports the hypothesis that growth retardation due to any kind of stress increases the concentration of unsaturated fatty acids.

Exp. 18. Another experiment with *G. domingensis* under the same conditions was performed later in the year (March-May 1991). Only two main effects were significant: temperature and ammonium concentration, while the third one, irradiance, was not significant. Highest sporeling growth rate appeared at high irradiance ($225 \mu\text{E m}^{-2} \text{s}^{-1}$), high temperature (25°C) and ammonium fertilization (0.7-2.6 mM) (Table 18.1, Figs. 12-14). Sporelings did not survive at temperatures below 20°C .

The outdoor acclimation of the sporelings showed that the main effects of irradiance and temperature at the preconditioning experiment were significant, while the ammonium concentration variable was not significant (Table 18.2). Optimal preconditions for outdoor acclimation included moderate to high irradiance ($49\text{-}225 \mu\text{E m}^{-2} \text{s}^{-1}$), high temperature (25°C) and no ammonium concentration. These were suboptimal or stress conditions of indoor growth, which supported optimal outdoor acclimation, regarding irradiance and ammonium concentration. This relationship which appeared under favorable outdoor growth conditions again supports the hypothesis of this study.

In the correlation analysis between mean indoor and several outdoor growth rate means, a significant negative correlation was shown only between mean indoor growth rate and second week outdoor growth rate ($r = -0.515$, $P = 0.0002$) (Figs. 15-17). No correlation was found between mean indoor growth rate and outdoor dry weight percentage (Table 18.3, Fig. 18).

One may conclude that *G. domingensis* under early summer conditions shows better outdoor acclimation with stressed sporeling preconditioning or hardening. This is supported by a relative increase in unsaturated fatty acids.

Exp. 19. Another indoor experiment with *G. domingensis* under the same conditions of irradiance and temperature was performed later in the year (May-July 1991). Ammonium concentration did not serve as a variable. No variable showed a significant main effect in the mean of 9 weeks of growth. A separate calculation of 4 weeks average (2-5 weeks) showed no significant main effects, while the next five weeks (6-9 weeks) showed temperature and irradiance as significant main effects

(Table 19.1, Fig. 19). These sporelings were first tested for the concentration of the amino acid proline (Table 19.2). The only correlation found was between growth rate at weeks 2-5 and proline concentration ($G = 13.851 + 2.344 \text{ proline}$; $F = 5.497$, $P = 0.0410$) (Figs. 20-22).

The separation between the early and the late part of the growth gradient experiment was performed because of a decrease in growth rate after the fifth week of the experiment. However, the significant positive correlation found between growth rate (2-5 weeks) and proline concentration is irrelevant, because the proline was determined after nine weeks of the experiment. The insignificant inverse correlation between growth at weeks 6-9 and proline concentration suggests a more representative relationship.

A test showed a significant difference between weekly growth rate of fertilized and non fertilized sporelings (Table 19.2). The proline content of the treated and non treated sporelings showed a clear inverse relationship.

Exp. 20. The last indoor experiment was performed with the Israeli local *G. conferta* under the same irradiance, ammonium and temperature conditions (August-October 1991). All main effects and their interaction were significant after a $\ln$ transformation of the weekly growth rate ($\text{growth} + 10$). Without the transformation, the irradiance main effect was not significant. This was the result also in the *G. domingensis* experiments. Maximal weekly growth rate of *G. conferta* sporelings under indoor conditions appeared at low light, moderate temperature and moderate ammonium concentration (Table 20.1, Figs. 23-25).

The outdoor acclimation experiment lasted for 3 weeks. The last two weeks were statistically analyzed and showed that all the main effects were significant. Maximal weekly growth rate appeared under high irradiance, moderate temperature and no ammonium in the medium (Table 20.2). This growth rate is inversely related to the indoor growth rate, and therefore supports the basic hypothesis for the local Israeli *Gracilaria* species. The correlation between indoor growth rate and outdoor growth is negative, though not significant (Figs. 26-27).

Table 15.1 a. Effect of temperature (°C), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the indoor weekly growth rate (%) of *Gracilaria domingensis* sporelings. 1-3 sporelings per dish. The experiment lasted for 5 weeks (July-August 1990).

Light Temp		Ammonium concentration			
		0	0.66	1.32	2.64
		Mean ± STD	Mean ± STD	Mean ± STD	Mean ± STD
25	15	- -	jk 4.17 ± 5.89	jk 6.43 ± 14.57	jk 5.00 ± 8.66
	20	jk 12.50 ± 17.68	bcd ef 76.77 ± 18.97	bcd ef g 71.80 ± 20.79	abc 99.35 ± 38.24
	25	a 124.28 ± 78.73	ab 110.15 ± 19.84	bcd ef gh 66.40 ± 22.53	bcd ef 72.88 ± 19.94
	30	jk 8.30 ± 13.39	ijk 21.38 ± 19.82	- -	- -
48	15	jk 10.00 ± 14.14	k -3.87 ± 12.20	hijk 25.58 ± 1.90	jk 7.90 ± 11.17
	20	efghijk 41.15 ± 9.26	bcd ef gh 67.05 ± 30.90	bcd ef 73.91 ± 23.76	abc 95.26 ± 24.43
	25	abcde 83.93 ± 8.24	abc 104.90 ± 12.12	abcd 90.98 ± 21.68	abc 99.84 ± 16.02
105	15	- -	- -	jk 12.00 ± 16.97	jk 6.96 ± 14.55
	20	ghijk 28.05 ± 14.19	bcd ef 73.87 ± 12.21	bcd ef 73.19 ± 9.47	abcde 85.20 ± 17.33
	25	abcdef 81.73 ± 12.27	abcd 92.14 ± 17.20	abcdef 81.48 ± 28.75	abc 98.40 ± 9.04
180	15	- -	- -	k 1.19 ± 9.85	jk 3.93 ± 13.12
	20	fghijk 36.36 ± 3.05	defghij 49.16 ± 10.02	bcd ef 74.07 ± 10.19	abcdef 80.72 ± 17.22
	25	cdefghi 62.78 ± 22.90	bcd ef g 69.70 ± 20.76	abc 96.85 ± 0.85	abc 99.02 ± 4.21

R² = 0.8144 P = 0.0001

Different letters represent significant difference in the level of P < 0.05.

Table 15.1 b. Three-way ANOVA

Source	F	P
Light	1.63	0.188
Temperature	77.07	0.0001
Ammonium	4.93	0.0034
L x T	0.17	0.9844
L x A	1.11	0.3644
T x A	3.98	0.0009
L x T x A	1.38	0.1872

Table 15.2. Effect of preconditioning under different temperature ($^{\circ}\text{C}$), irradiance ($\mu\text{E m}^{-2}\text{s}^{-1}$) and ammonium concentration (mM) on the outdoor weekly growth rate (%) of *Gracilaria domingensis* sporelings (9-23 August 1990).

Light	Temp	Ammonium concentration							
		0		0.66		1.32		2.64	
		week 1	week 2	week 1	week 2	week 1	week 2	week 1	week 2
25	20			6.37	-			-1.14	-
	25	-15.07	-	-29.41	-9.52	36.75	-43.75	2.19	20.00
48	20					0.78	-80.50	15.07	-
	25			5.76	-				
105	20			55.32	-56.98	-41.24	15.29		

Temperature of water: $27.79 \pm 0.93^{\circ}\text{C}$.

Irradiance in the water: $185.0 \pm 17.3 \mu\text{E m}^{-2}\text{s}^{-1}$.

Table 16.1 a. Effect of temperature (°C), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the indoor weekly growth rate (%) of *Gracilaria domingensis* sporelings. 1-3 sporelings per dish. The experiment lasted for 6 weeks (October-December 1990).

Light	Temp	Ammonium concentration			
		0	0.66	1.32	2.64
		Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD
27	20	def 36.72 $\pm$ 23.24	abcdef 52.87 $\pm$ 20.83	abcdef 55.34 $\pm$ 5.12	abcdef 61.72 $\pm$ 29.79
	25	abcdef 47.09 $\pm$ 20.96	abcdef 57.28 $\pm$ 13.50	abcdef 58.58 $\pm$ 14.58	abcdef 58.69 $\pm$ 14.22
	30	abcdef 55.28 $\pm$ 25.70	abcdef 59.64 $\pm$ 13.35	abcdef 52.76 $\pm$ 18.90	bcddef 57.11 $\pm$ 20.57
49	20	def 36.47 $\pm$ 25.35	abcdef 61.11 $\pm$ 22.18	abcdef 60.55 $\pm$ 15.69	abcdef 69.36 $\pm$ 18.98
	25	abcdef 59.22 $\pm$ 38.44	abcdef 57.93 $\pm$ 10.10	abcdef 68.79 $\pm$ 22.09	abcde 73.24 $\pm$ 28.39
	30	bcddef 46.14 $\pm$ 28.89	abcdef 63.40 $\pm$ 22.21	abcdef 63.84 $\pm$ 35.11	abcdef 58.38 $\pm$ 47.20
100	20	ef 33.08 $\pm$ 25.35	abcdef 52.84 $\pm$ 23.67	abcdef 68.44 $\pm$ 13.42	a 91.04 $\pm$ 32.87
	25	abcdef 68.14 $\pm$ 35.97	abcde 74.57 $\pm$ 8.74	ab 87.61 $\pm$ 28.71	ab 87.15 $\pm$ 25.50
	30	abcdef 60.68 $\pm$ 42.67	abc 81.19 $\pm$ 30.10	abc 80.82 $\pm$ 36.29	ab 88.21 $\pm$ 50.35
225	20	f 27.17 $\pm$ 18.57	cdef 41.11 $\pm$ 22.79	abcdef 65.09 $\pm$ 14.70	abcdef 65.70 $\pm$ 14.53
	25	bcddef 46.05 $\pm$ 32.59	abcde 73.84 $\pm$ 14.06	abc 83.44 $\pm$ 20.22	abcde 72.08 $\pm$ 32.49
	30	abcdef 56.04 $\pm$ 36.25	abcde 74.97 $\pm$ 30.47	abcd 78.71 $\pm$ 45.47	abcde 72.53 $\pm$ 51.24

R² = 0.2589 P = 0.0503

Different letters represent significant difference in the level of P < 0.05.

Table 16.1 b. Three-way ANOVA

Source	F	P
Light	4.67	0.0036
Temperature	4.62	0.0110
Ammonium	8.73	0.0001
L x T	0.70	0.6529
L x A	0.55	0.8342
T x A	0.92	0.4796
L x T x A	0.16	1.0000

Table 16.2 a. Effect of preconditioning under different temperature ($^{\circ}\text{C}$), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the outdoor weekly growth rate (%) of *Gracilaria domingensis* sporelings. The experiment lasted for 4 weeks (November-December 1990). The first two weeks are averaged.

Light	Temp	Ammonium concentration			
		0	0.66	1.32	2.64
		Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD
27	20	abc 9.44 $\pm$ 1.12	abc 15.57 $\pm$ 28.42	abc 14.10 $\pm$ 0.25	abc 12.69 $\pm$ 5.18
	25	abc 7.61 $\pm$ 3.65	abc 2.46 $\pm$ 11.26	abc 13.03 $\pm$ 1.95	abc 7.94 $\pm$ 11.00
	30	abc 12.19 $\pm$ 2.79	abc 7.06 $\pm$ 13.23	abc 13.71 $\pm$ 19.39	abc -3.98 $\pm$ 23.07
49	20	abc 3.30 $\pm$ 9.19	abc -5.07 $\pm$ 19.68	abc 8.52 $\pm$ 9.43	abc 9.41 $\pm$ 9.68
	25	abc 15.59 $\pm$ 15.93	abc 5.64 $\pm$ 7.88	abc 5.20 $\pm$ 2.31	abc 9.10 $\pm$ 2.77
	30	abc -0.13 $\pm$ 2.03	abc 8.33 $\pm$ 3.69	abc -6.63 $\pm$ 16.49	abc 8.85 $\pm$ 4.36
100	20	bc -21.70 $\pm$ 24.35	abc 3.58 $\pm$ 2.91	abc 3.64 $\pm$ 2.82	abc -1.30 $\pm$ 8.39
	25	abc 4.59 $\pm$ 5.54	abc 6.92 $\pm$ 6.80	abc 6.64 $\pm$ 2.97	abc 7.03 $\pm$ 1.30
	30	abc 7.09 $\pm$ 6.99	abc 20.36 $\pm$ 4.62	abc 13.75 $\pm$ 12.40	a 24.77 $\pm$ 2.52
225	20	abc 6.94 $\pm$ 71.19	abc 11.46 $\pm$ 31.79	abc -12.94 $\pm$ 15.83	c -24.12 $\pm$ 55.44
	25	abc -9.45 $\pm$ 9.06	abc -2.19 $\pm$ 7.76	abc 6.18 $\pm$ 7.85	abc 5.04 $\pm$ 8.88
	30	abc 1.32 $\pm$ 7.35	abc 6.89 $\pm$ 1.63	abc -0.39 $\pm$ 2.78	abc 1.90 $\pm$ 19.53

$t = 19.7 \pm 1.6^{\circ}\text{C}$. Irradiance in the water: $30.5 \pm 13.5 \mu\text{E m}^{-2} \text{s}^{-1}$. $R^2 = 0.355$ $P = 0.974$

Different letters represent significant difference in the level of $P < 0.05$.

Table 16.2 b. Three-way ANOVA

Source	F	P
Light	1.41	0.2512
Temperature	0.72	0.4909
Ammonium	0.18	0.9066
L x T	0.97	0.4563
L x A	0.50	0.8690
T x A	0.22	0.9696
L x T x A	0.48	0.9540

Table 17.1 a. Effect of temperature (°C), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the indoor weekly growth rate (%) of *Gracilaria domingensis* sporelings. 1-3 sporelings per dish. The experiment lasted for 5 weeks (January-February 1991).

Light	Temp	Ammonium concentration			
		0	0.66	1.32	2.64
		Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD
27	20	jklmno 19.41 $\pm$ 11.84	jklmno 19.99 $\pm$ 6.38	ijklmn 32.11 $\pm$ 12.01	hijklm 32.98 $\pm$ 9.77
	25	efghijk 41.69 $\pm$ 9.12	bcd efghi 50.70 $\pm$ 12.23	abcde fghi 58.09 $\pm$ 18.67	bcd efghi 52.05 $\pm$ 17.09
	30	defghijk 43.05 $\pm$ 9.48	bcd efghi 48.64 $\pm$ 18.91	bcd efghi 50.10 $\pm$ 4.98	abcde fghi 59.04 $\pm$ 13.11
49	20	mno 10.34 $\pm$ 5.69	jklmno 17.98 $\pm$ 7.20	ghijklm 37.47 $\pm$ 12.62	cde fghijk 43.64 $\pm$ 23.90
	25	cde fghijk 44.04 $\pm$ 17.42	bcd efghi 54.48 $\pm$ 11.42	bcd efghi 49.30 $\pm$ 7.95	abcde fgh 61.49 $\pm$ 23.89
	30	bcd efghi 53.51 $\pm$ 21.59	abcde fghi 59.14 $\pm$ 9.88	abcde f 69.20 $\pm$ 20.71	ab 74.52 $\pm$ 24.54
100	20	lmno 12.07 $\pm$ 3.85	klmno 17.12 $\pm$ 4.77	ghijkl 38.62 $\pm$ 3.95	bcd efghij 46.39 $\pm$ 11.31
	25	hijklmn 32.41 $\pm$ 20.60	bcd efghi 49.04 $\pm$ 2.69	bcd efghi 56.11 $\pm$ 12.62	abcde 70.54 $\pm$ 27.88
	30	ghijklm 37.38 $\pm$ 14.00	abcd 71.18 $\pm$ 20.72	abcde 70.26 $\pm$ 23.94	a 84.68 $\pm$ 42.75
225	20	no 5.26 $\pm$ 3.58	o 0.13 $\pm$ 7.08	ghijkl 38.44 $\pm$ 6.91	ghijkl 38.42 $\pm$ 12.55
	25	ghijkl 39.54 $\pm$ 32.81	bcd efghi 50.73 $\pm$ 26.57	abc 72.30 $\pm$ 28.38	ab 73.18 $\pm$ 23.91
	30	fghijk 41.10 $\pm$ 21.55	abcde f 68.72 $\pm$ 10.99	abcde f 66.31 $\pm$ 21.15	abcde f 68.56 $\pm$ 35.47

Regular: $R^2 = 0.5821$ $P = 0.0001$. In transformed: $R^2 = 0.7204$ $P = 0.0001$ (not presented here)
 Different letters represent significant difference in the level of $P < 0.05$.

Table 17.1 b. Three-way ANOVA

Source	Regular		ln transformed	
	F	P	F	P
Light	1.51	0.2140	2.80	0.0414
Temperature	76.79	0.0001	127.00	0.0001
Ammonium	26.04	0.0001	47.86	0.0001
L x T	1.43	0.2050	3.09	0.0065
L x A	1.25	0.2660	2.62	0.0071
T x A	1.21	0.3048	9.63	0.0001
L x T x A	0.43	0.9803	1.15	0.303

Table 17.2. Fatty acid concentration

Growth conditions			Fatty acids		
Irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	Temp. ($^{\circ}\text{C}$)	Ammonium (mM)	16:0	20:4	16:0/ 20:4
27	20	0	17.3	17.2	1.006
		0.66	22.1	31.8	0.695
		1.32	9.2	19.9	0.462
		2.64	14.6	17.1	0.854
	25	0	17.0	13.4	1.269
		0.66	24.3	21.0	1.157
		1.32	21.8	24.5	0.890
		2.64	15.1	16.7	0.904
	30	0	20.6	22.8	0.904
		0.66	15.9	17.4	0.914
		1.32	21.8	23.7	0.920
		2.64	22.0	21.7	1.014
49	20	1.32	19.3	25.0	0.772
		2.64	27.2	29.4	0.925
	25	0	31.9	32.5	0.982
		0.66	21.1	20.9	1.010
		1.32	26.3	32.1	0.819
		2.64	29.1	42.3	0.688
	30	0	26.1	27.9	0.935
		0.66	25.5	28.8	0.885
		1.32	31.7	38.4	0.826
		2.64	28.4	33.6	0.845
100	20	1.32	27.1	32.8	0.826
		2.64	22.3	23.8	0.937
	25	0	27.9	25.0	1.116
		0.66	20.0	20.8	0.962
	30	0	28.2	27.7	1.018
		0.66	25.1	27.0	0.930
		1.32	28.1	30.5	0.920
		2.64	23.6	39.1	0.731

Table 17.2. Fatty acid concentration (page 2)

Growth conditions			Fatty acids		
Irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	Temp. ($^{\circ}\text{C}$)	Ammonium (mM)	16:0	20:4	16:0/ 20:4
225	20	1.32	21.9	22.5	0.973
		2.64	18.1	14.0	1.293
	25	0	27.4	24.1	1.137
		0.66	17.3	16.0	1.081
		1.32	19.6	24.2	0.810
		2.64	21.9	26.3	0.833
	30	0	30.4	32.8	0.927
		0.66	30.5	35.0	0.871
		1.32	24.3	27.3	0.890
		2.64	28.6	38.9	0.735

Table 18.1 a. Effect of temperature (°C), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the indoor weekly growth rate (%) of *Gracilaria domingensis* sporelings. The experiment lasted for 5 weeks (March-May 1991). The last four weeks were averaged.

Light	Temp	Ammonium concentration			
		0	0.66	1.32	2.64
		Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD
27	20	cdefghij 31.38 $\pm$ 27.47	abcde 62.08 $\pm$ 21.40	abcd 64.03 $\pm$ 9.07	abcdef 61.57 $\pm$ 18.62
	25	cdefghij 32.24 $\pm$ 17.35	abcd 63.55 $\pm$ 8.82	abc 64.86 $\pm$ 20.30	abcdefg 57.25 $\pm$ 17.80
	30	bcddefghij 34.10 $\pm$ 21.95	abcdefghi 53.03 $\pm$ 11.75	abcdefghi 50.65 $\pm$ 10.31	abcdefg 59.14 $\pm$ 21.35
49	20	efghij 28.22 $\pm$ 13.43	abcdefghi 50.78 $\pm$ 1.70	abcdefg 57.94 $\pm$ 27.63	ab 67.79 $\pm$ 29.02
	25	abcdefghij 38.08 $\pm$ 23.81	abc 64.72 $\pm$ 13.01	abcdefg 59.14 $\pm$ 20.34	ab 67.73 $\pm$ 24.01
	30	hij 21.81 $\pm$ 12.41	abcdefg 60.10 $\pm$ 10.14	defghij 29.92 $\pm$ 14.54	abcdefghij 41.74 $\pm$ 14.91
100	20	ij 20.80 $\pm$ 10.33	cdefghij 32.99 $\pm$ 11.05	abcdefghij 44.86 $\pm$ 16.66	abcdefgh 55.37 $\pm$ 17.25
	25	ghij 26.08 $\pm$ 15.66	abcde 62.45 $\pm$ 16.26	abcde 62.35 $\pm$ 17.29	a 69.52 $\pm$ 22.52
	30	abcdefghij 42.61 $\pm$ 34.18	a 71.28 $\pm$ 34.24	abcdefghi 50.29 $\pm$ 15.38	abcdefghi 47.67 $\pm$ 19.07
225	20	j 13.58 $\pm$ 9.48	hij 21.31 $\pm$ 21.98	abcdefghi 48.39 $\pm$ 22.57	abcdefghi 49.96 $\pm$ 27.52
	25	fghij 27.51 $\pm$ 18.00	a 69.39 $\pm$ 21.13	a 70.15 $\pm$ 18.10	a 68.58 $\pm$ 20.85
	30	cdefghij 32.56 $\pm$ 25.09	a 69.97 $\pm$ 23.83	abcdefghij 43.32 $\pm$ 17.18	efghij 28.70 $\pm$ 13.13

Regular: $R^2 = 0.484$ $P = 0.0001$. In transformed: $R^2 = 0.782$ $P = 0.0001$ (not presented here)

Different letters represent significant difference in the level of $P < 0.05$.

Table 18.1 b. Three-way ANOVA

Source	Regular		ln transformed*	
	F	P	F	P
Light	1.20	0.3127	5.52	0.0024
Temperature	7.19	0.0011	15.20	0.0001
Ammonium	22.47	0.0001	2.55	0.0663
L x T	2.53	0.0232	7.90	0.0001
L x A	0.26	0.9841	2.11	0.0474
T x A	3.49	0.0030	3.92	0.0029
L x T x A	0.62	0.8765	1.54	0.1163

*Last three weeks only.

Table 18.2 a. Effect of preconditioning under different temperature ($^{\circ}\text{C}$), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the outdoor weekly growth rate (%) of *Gracilaria domingensis* sporelings. The experiment lasted for 4 weeks (April-May 1991). The last two weeks are averaged and presented.

Light	Temp	Ammonium concentration			
		0	0.66	1.32	2.64
		Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD	Mean $\pm$ STD
27	20	lm 15.67 $\pm$ 11.03	ghijklm 30.22 $\pm$ 3.56	jklm 22.86 $\pm$ 2.15	m 11.66 $\pm$ 0.16
	25	abcdef 63.22 $\pm$ 4.26	abcd 67.47 $\pm$ 6.44	cdefghijk 50.22 $\pm$ 2.52	cdefghijk 46.70 $\pm$ 16.59
	30	abcdef 62.33 $\pm$ 11.34	cdefghijkl 42.57 $\pm$ 2.43	fghijklm 35.84 $\pm$ 9.37	defghijklm 39.29 $\pm$ 2.10
49	20	a 84.02 $\pm$ 27.92	abc 69.96 $\pm$ 1.78	cdefgh 52.62 $\pm$ 4.14	cdefghijk 43.94 $\pm$ 13.80
	25	cdefghijk 46.58 $\pm$ 21.46	abc 69.73 $\pm$ 7.36	bcddefgh 54.91 $\pm$ 11.21	cdefghi 51.63 $\pm$ 1.58
	30	cdefghijk 50.76 $\pm$ 5.75	hijklm 27.04 $\pm$ 4.08	ghijklm 31.98 $\pm$ 6.99	hijklm 29.75 $\pm$ 5.30
100	20	bcddefgh 55.45 $\pm$ 17.13	cdefgh 53.87 $\pm$ 12.82	bcddefgh 55.30 $\pm$ 1.94	ghijklm 30.52 $\pm$ 1.14
	25	abcdef 62.50 $\pm$ 0.43	cdefghijk 48.58 $\pm$ 13.15	cdefghij 51.32 $\pm$ 11.31	klm 22.35 $\pm$ 4.76
	30	cdefghijkl 42.03 $\pm$ 2.21	hijklm 27.10 $\pm$ 0.10	ijklm 23.17 $\pm$ 7.03	ghijklm 31.47 $\pm$ 2.28
225	20	ghijklm 33.36 $\pm$ 14.55	bcddefgh 54.24 $\pm$ 2.84	abcde 67.15 $\pm$ 0.74	abcde 65.06 $\pm$ 12.09
	25	cdefghijk 45.88 $\pm$ 5.21	abcdef 63.37 $\pm$ 0.52	-	abcdefg 58.42 $\pm$ 0.16
	30	cdefgh 52.72 $\pm$ 20.58	efghijklm 38.81 $\pm$ 19.34	cdefgh 53.17 $\pm$ 9.61	ab 82.13 $\pm$ 40.91

R² = 0.806 P = 0.0001. Temperature 21.7 $\pm$ 0.8 $^{\circ}\text{C}$. Irradiance in the water: 48.7 $\mu\text{E m}^{-2} \text{s}^{-1}$

Different letters represent significant difference in the level of P < 0.05.

Table 18.2 b. Three-way ANOVA

Source	F	P
Light	9.59	0.0001
Temperature	8.83	0.0006
Ammonium	2.44	0.0762
L x T	9.88	0.0001
L x A	4.35	0.0004
T x A	3.63	0.0048
L x T x A	1.21	2.297

Table 18.3 Effect of preconditioning under different temperature ($^{\circ}\text{C}$), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the dry weight (%) of outdoor *Gracilaria domingensis* sporelings (40 ppt)

Light	Temp	Ammonium concentration			
		0	0.66	1.32	2.64
27	20	14.03	13.10	13.35	13.71
	25	12.65	11.82	11.30	11.69
	30	11.28	11.63	11.82	12.01
49	20	11.72	12.40	11.10	11.52
	25	12.38	12.15	12.75	12.08
	30	11.91	12.81	12.96	13.40
100	20	12.44	11.92	11.69	12.56
	25	10.60	11.72	12.03	12.41
	30	12.80	13.08	14.72	14.04
225	20	12.79	12.11	11.16	12.49
	25	11.34	11.38	-	12.23
	30	11.48	11.61	11.87	12.55

Table 19.1 a. Effect of temperature ($^{\circ}\text{C}$) and Irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) on the indoor weekly growth rate (%) of *Gracilaria domingensis* sporelings. One sporeling per dish. The experiment lasted for 9 weeks (May-July 1991). The last five weeks are averaged.

Light	Temp	Growth Mean $\pm$ STD
27	20	21.12 $\pm$ 7.45
	25	24.82 $\pm$ 5.11
	30	22.62 $\pm$ 7.46
49	20	30.88 $\pm$ 8.40
	25	27.79 $\pm$ 5.72
	30	33.34 $\pm$ 16.30
100	20	34.70 $\pm$ 8.47
	25	29.74 $\pm$ 7.36
	30	39.48 $\pm$ 16.82
225	20	34.41 $\pm$ 9.53
	25	29.70 $\pm$ 8.97
	30	56.62 $\pm$ 18.29

$R^2 = 0.4589$ $P = 0.0007$

Table 19.1 b. Two-way ANOVA

Source	F	P
Light	6.77	0.0007
Temperature	4.65	0.0143
L X T	1.85	0.1088

Table 19.2. Proline concentration (mg/g FW) in the sporelings of *Gracilaria domingensis* presented after 9 weeks of growth

Irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	Temperature ($^{\circ}\text{C}$)			Mean weekly growth rate
	20	25	30	
27	527.3	1671.5	1408.4	-
49	1168.5	2878.1	372.0	-
100	376.9	336.0	<315.8	-
225	2401.7	2381.8	<363.4	-
100 #	-	2439.6	-	50.34 $\pm$ 25.53
100 # *	-	3645.7	-	24.11 $\pm$ 15.07

* Not fertilized.

Cultured in growth chamber for 2 weeks

Table 20.1 a. Effect of temperature ($^{\circ}\text{C}$), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the indoor weekly growth rate (%) of *Gracilaria conferta* sporelings. 1-3 sporelings per dish. The experiment lasted for 5 weeks (August-September 1991).

Light	Temp	Ammonium concentration			
		0		0.66	
		Mean $\pm$ STD		Mean $\pm$ STD	
27	10	fghi	1.58 $\pm$ 1.62	ghi	1.53 $\pm$ 1.64
	15	def	12.01 $\pm$ 2.88	defg	10.43 $\pm$ 6.59
	20	d	13.42 $\pm$ 1.93	ab	34.93 $\pm$ 15.64
	25	defg	10.84 $\pm$ 3.52	bc	25.74 $\pm$ 8.43
	30	defghi	4.94 $\pm$ 2.20	de	12.73 $\pm$ 3.44
49	10	ghi	1.42 $\pm$ 1.56	ghi	1.27 $\pm$ 1.86
	15	defgh	9.34 $\pm$ 1.24	defgh	8.88 $\pm$ 4.10
	20	defg	10.22 $\pm$ 6.80	a	37.55 $\pm$ 16.10
	25	defg	11.16 $\pm$ 7.19	abc	29.90 $\pm$ 13.98
	30	defghi	7.00 $\pm$ 3.83	defghi	5.37 $\pm$ 3.35
100	10	ghi	1.35 $\pm$ 2.40	efghi	2.33 $\pm$ 1.62
	15	defghi	7.02 $\pm$ 2.57	bc	27.46 $\pm$ 10.42
	20	defg	10.51 $\pm$ 6.65	abc	29.81 $\pm$ 9.20
	25	defg	10.02 $\pm$ 5.98	abc	29.55 $\pm$ 8.93
	30	defgh	8.51 $\pm$ 3.54	c	23.28 $\pm$ 10.37
225	10	i	-2.12 $\pm$ 3.80	hi	-0.39 $\pm$ 1.30
	15	defghi	4.39 $\pm$ 0.52	defg	10.55 $\pm$ 4.70
	20	defg	10.72 $\pm$ 5.12	c	24.16 $\pm$ 5.05
	25	de	12.69 $\pm$ 3.46	bc	27.87 $\pm$ 10.19
	30	defghi	7.84 $\pm$ 4.74	c	22.48 $\pm$ 7.26

$R^2 = 0.754$ $P = 0.0001$

Different letters represent significant difference in the level of $P < 0.05$.

Table 20.1 b. Three-way ANOVA

Source	F	P
Light	2.20	0.0905
Temperature	57.83	0.0001
Ammonium	124.26	0.0001
L x T	2.47	0.0055
L x A	2.53	0.0593
T x A	14.16	0.0001
L x T x A	2.17	0.0153

Table 20.2 a. Effect of preconditioning under different temperature ($^{\circ}\text{C}$), irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) and ammonium concentration (mM) on the outdoor weekly growth rate (%) of *Gracilaria conferta* sporelings. The experiment lasted for 3 weeks (October 1991). The last two weeks were averaged.

Light	Temp	Ammonium concentration			
		0		0.66	
		Mean $\pm$ STD		Mean $\pm$ STD	
27	10	defghi	23.47 $\pm$ 4.71	efghi	20.70 $\pm$ 2.16
	15	hij	4.68 $\pm$ 4.05	ghi	12.20 $\pm$ 3.85
	20	-	-	hij	6.13 $\pm$ 0.42
	25	-	-	j	-19.41 $\pm$ 0.63
	30	-	-	hij	6.59 $\pm$ 4.94
49	10	ij	2.19 $\pm$ 2.01	hij	8.59 $\pm$ 5.17
	25	cdefg	41.51 $\pm$ 10.13	fghi	19.74 $\pm$ 7.26
	30	cd	50.60 $\pm$ 17.86	hij	5.00 $\pm$ 2.97
100	10	defgh	34.05 $\pm$ 7.48	bc	65.97 $\pm$ 31.56
	15	cde	49.55 $\pm$ 20.15	ghi	15.04 $\pm$ 7.27
	20	bc	65.57 $\pm$ 23.10	ghi	11.49 $\pm$ 6.59
	25	cdef	45.88 $\pm$ 19.55	defgh	33.37 $\pm$ 10.12
	30	defghi	22.37 $\pm$ 3.92	ghi	14.78 $\pm$ 12.77
225	15	hij	4.33 $\pm$ 3.64	hij	7.82 $\pm$ 2.10
	25	a	109.16 $\pm$ 27.28	b	83.50 $\pm$ 25.74
	30	bc	63.88 $\pm$ 28.66	bc	64.30 $\pm$ 27.62

$R^2 = 0.842$ $P = 0.0001$. Temperature 24.3 ± 0.8 $^{\circ}\text{C}$

Irradiance in the water $34.2 \mu\text{E m}^{-2} \text{s}^{-1}$

Different letters represent significant difference in the level of $P < 0.05$.

Table 20.2 b. Three-way ANOVA

Source	F	P
Light	26.32	0.0001
Temperature	9.59	0.0001
Ammonium	15.00	0.0003
L x T	10.78	0.0001
L x A	1.47	0.2318
T x A	5.89	0.0005
L x T x A	2.91	0.0212

List of Figures

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- Fig. 2. Effect of temperature ($^{\circ}\text{C}$) and ammonium concentration (mM) on the square transformed weekly growth rate (%) of *G. domingensis* sporelings on the growth gradient table (Exp. 16).
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- Fig. 11. Correlation between $\ln$ transformed weekly indoor growth rate of *G. domingensis* sporelings and the ratio of 16:0/20:4 fatty acid content (Exp. 17). Almost significant ($P = 0.056$).
- Fig. 12. Effect of temperature ($^{\circ}\text{C}$) and irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) on the $\ln$ transformed weekly growth rate (%) of *G. domingensis* sporelings on the growth gradient table (Exp. 18). First and second weeks are not included.
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- Fig. 17. Correlation analysis between weekly indoor growth rate of *G. domingensis* sporelings and first two weeks outdoor growth rate (Exp. 18). Not significant.
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- Fig. 19. Effect of temperature ($^{\circ}\text{C}$) and irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) on the growth rate (%) of *G. domingensis* sporelings on the growth gradient table (Exp. 19). Average of 4 last weeks. Significant.
- Fig. 20. Correlation analysis between weekly indoor growth rate of *G. domingensis* sporelings and proline concentration (Exp. 19). Duration: 2-9 weeks. Not significant.
- Fig. 21. Correlation analysis between weekly indoor growth rate of *G. domingensis* sporelings and proline concentration (Exp. 19). Duration: 2-5 weeks. Significant.
- Fig. 22. Correlation analysis between weekly indoor growth rate of *G. domingensis* sporelings and proline concentration (Exp. 19). Duration: 6-9 weeks. Not significant.
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- Fig. 27. Correlation analysis between weekly indoor growth rate of *G. conferta* sporelings and weekly outdoor growth rate in the first week (Exp. 20). Not significant.

EXPERIMENT B16 - INSIDE

TEMPERATURE

LIGHT *** 27 o-o-o 49 □-□-□ 100 ◇-◇-◇ 225

EXPERIMENT B16 - INSIDE

AMMONIA

*** 0.00

o-o-o 0.66

□-□-□ 1.32

◇-◇-◇ 2.64

Fig 3

EXPERIMENT B16 - INSIDE

AMMONIA *** 0.00 o-o-o 0.66 □-□-□ 1.32 ◇-◇-◇ 2.64

Fig 4

36

EXPERIMENT B16

MEAN GROWTH OUTSIDE

MEAN GROWTH INSIDE

Fig 5

EXPERIMENT B16

SECOND WEEK IS NOT INCLUDED IN EXPERIMENT OUTSIDE

EXPERIMENT B17 - INSIDE

LIGHT *** 27 o-o-o 49 □-□-□ 100 ◇-◇-◇ 225

Fig 7

EXPERIMENT B17 - INSIDE

AMMONIA *** 0.00 o-o-o 0.66 □-□-□ 1.32 ◇-◇-◇ 2.64

Fig 8

EXPERIMENT B17 - INSIDE

AMMONIA *** 0.00 e-e-e 0.66 □-□-□ 1.32 ◇-◇-◇ 2.64

EXPERIMENT B17

EXPERIMENT B17

FATTY ACID 20:00

EXPERIMENT B17

EXPERIMENT B18 - INSIDE

FIRST AND SECOND WEEKS ARE NOT INCLUDED

TEMPERATURE

LIGHT *** 27 o-o-o-o 49 □-□-□-□ 100 ◇-◇-◇-◇ 225

EXPERIMENT B18 - INSIDE

FIRST AND SECOND WEEKS ARE NOT INCLUDED

TEMPERATURE

LN(GROWTH)

AMMONIA *** 0.00 --- 0.66 --- 1.32 --- 2.64

EXPERIMENT B18 - INSIDE

FIRST AND SECOND WEEKS ARE NOT INCLUDED

AMMONIA

*** 0.00

--- 0.66

--- 1.32

--- 2.64

Fig 15

EXPERIMENT B18

EXPERIMENT B18

EXPERIMENT B18

Fig 18

EXPERIMENT B18

EXPERIMENT B19 -- INSIDE

INCLUDING WEEKS 6 10 & 14

TEMPERATURE

MEAN GROWTH

LIGHT *** 27 o-o-o 49 □-□-□ 100 ◇-◇-◇ 225

EXPERIMENT B19

Fig 21

53

EXPERIMENT B19 (2-5 WEEKS)

EXPERIMENT B19 (6-10 WEEKS)

EXPERIMENT B20 - INSIDE

LIGHT *** 27 o-o-o 49 □-□-□ 100 ◇-◇-◇ 225

EXPERIMENT B20 - INSIDE

AMMONIA *** 0.00 ◇◇◇ 0.66

Fig 25

EXPERIMENT B20 - INSIDE

AMMONIA *** 0.00 ◇◇◇ 0.66

EXPERIMENT B20

FIRST WEEK IS NOT INCLUDED IN EXPERIMENT OUTSIDE

Fig 27

59

EXPERIMENT B20

MEAN GROWTH INSIDE

FIRST WEEK OF GROWTH OUTSIDE

Third Year Report to BARD

1.3

U.S. Lab.

2.2.1 Materials and Methods.

The third and final year (1 August, 1990 to 30 September, 1991) included a 2 month extension to complete a series of experiments. This final year concentrated on the use of sporelings produced from carpospores of Gracilaria tikvahiae collected in January, 1990 from the Skyway jetties at the mouth of Tampa Bay as originally proposed. The second year report described the techniques to obtain sporelings, the procedures developed to carry out analyses on fatty acid composition, and statistical analyses.

2.2.2 Results and Discussion

Fourteen experiments were carried out during the third year of which two utilized branches only (Expts 50, 51) and the remainder used sporelings in culture. Based on the data obtained during the second year, detailed experiments were designed to determine if hardening of the sporelings in a variety of temperature, salinity, and irradiance conditions would effect growth in the common garden phase. Fatty acid composition was also analyzed after growth in the hardening and common garden phases of four experiments. Freshly collected plants from the original population at the Skyway jetties and two other populations of Gracilaria tikvahiae from Tampa Bay were used in a final set of

experiments (Expts 59, 60) to compare acclimation ability and health of the 18 month old cultured sporelings.

Expt. 48. Sporelings were cultured in a factorial hardening experiment using 10 and 20 °C, and 15 and 30 ppt salinity. After 4 weeks the sporelings were moved to common garden conditions of 20 °C and 30 ppt salinity for a second four week period. Growth rates were significantly lower for plants held in the lower temperature during hardening but growth did not differ after both populations were transferred to the common garden temperature of 20 °C. Salinity did not show any affect on growth at either phase. The data does not support the hypothesis of a hardening effect.

Expt. 49. Sporelings were cultured for 4 weeks in a 3-way factorial hardening experiment (16, 26 °C; 15, 30 ppt; 5, 55 uE m⁻²s⁻¹) using a gradient table and then transferred to a common garden conditions in a growth chamber (10 °C, 33 ppt, 10 uE m⁻²s⁻¹) for a second four weeks. Relative growth rates (ratio of hardening to common garden rates) all declined when moved to the low temperature common garden conditions (Fig 49-1). There was no effect of any combination during hardening on growth during the common garden.

Expt. 50. Branches of G. tikvahiae cultured in Expt. 47 were grown on the gradient table to test the effect of salinity (15, 30 ppt) and light (5, 35 uE m⁻²s⁻¹) for four weeks and then transferred to a common

garden conditions (10°C , 33 ppt, $50 \text{ uE m}^{-2}\text{s}^{-1}$) and cultured for another 4 weeks. Growth for all 4 treatments decreased and was similar after transfer to common garden conditions (Fig. 50-1). There was no suggestion of hardening effect after transfer.

Expt. 51. Experiment 50 was repeated as described using the

Expt. 53. Sporelings were grown in two culture chambers at 10 and 20 °C using 5 and 50 $\mu\text{E m}^{-2}\text{s}^{-1}$ and 15 and 35 ppt during a 4 week hardening period and then placed in common garden conditions of 10 °C, 10 $\mu\text{E m}^{-2}\text{s}^{-1}$, and 15 ppt. Although there were large variations in percent growth rates, no pattern suggesting hardening was evident (Fig. 53-1).

Expt. 54. Sporelings were again grown in chambers during hardening (10, 20 °C; 15, 30 ppt; 5 $\mu\text{E m}^{-2}\text{s}^{-1}$) and during the common garden conditions (10 °C, 30 or 15 ppt) for 4 and 5 weeks respectively. Plants cultured at 20 °C showed significantly higher relative growth rates than those at the lower temperature during hardening and transfer to common garden conditions. Salinity did not affect growth in either the hardening or common garden phases. Fatty acid composition did not differ between treatments after hardening. Thus hardening in this case suggests that the lower temperature had a negative impact on sporeling survival when transferred to the common garden conditions.

Expt. 55. Sporelings were hardened on the gradient table using a factorial experiment (22-26, 18-20 °C; 5-8, 35 $\mu\text{E m}^{-2}\text{s}^{-1}$; 15, 35 ppt) for 4 weeks. Common garden conditions were 10 °C, 2-5 $\mu\text{E m}^{-2}\text{s}^{-1}$, 15 ppt) for another 4 weeks. Fatty acid composition did not differ between all treatments cultured under high light at the end of hardening (Fig. 55-1). Sporeling growth decreased during the common garden phase for

all treatments cultured under high irradiance. Hardening did not appear to result in a change in growth.

Expt. 56. Sporelings were first exposed to a 2 week hardening (10, 20 °C, 15 and 30 ppt, 10-15 $\mu\text{E m}^{-2}\text{s}^{-1}$) and then 2 weeks of common garden conditions (10 °C, 15 ppt, 10-15 $\mu\text{E m}^{-2}\text{s}^{-1}$). Plants hardened at the low temperature showed little decrease in growth regardless of salinity and plants hardened at the higher temperature showed similar growth in the common garden low temperature.

Expt. 57. Sporelings were allowed to acclimate to their hardening conditions (15, 33 ppt; 17.5, 25.5 °C; 38-57 $\mu\text{E m}^{-2}\text{s}^{-1}$) for 2 weeks and their photosynthetic and respiratory rates were monitored after 5, then 14 days. Measurements were again made after 2 weeks in common garden conditions (10°C, 15 ppt). Growth responses were lower during hardening but the same for all treatments during common garden conditions. Photosynthetic responses indicated acclimation during exposure to the two temperatures but no change to salinity.

Expt. 58 and 59. Sporelings were cultured along with freshly collected branches of Gracilaria tikvahiae from the original site in Tampa Bay (Skyway jetties) and growth, pigment levels and photosynthetic responses to irradiances (P-I curves), salinity, and temperature were measured before, during and after hardening and common garden conditions. Statistical analyses have been done but the data has not yet been analyzed. These experiments were designed to determine if the

sporelings, in culture for over 18 months were as healthy and responsive as field material and if acclimation was more evident in fresh branches. Growth data in both experiments indicate that the sporelings were more responsive and showed acclimation to the hardening common garden conditions.

Expt. 60. Sporelings and freshly collected branches of Gracilaria tikvahiae from two sites in Tampa Bay, an estuary and open coast site, were grown under 15 and 25 °C, 10 and 35 ppt, and 50 $\mu\text{E m}^{-2}\text{s}^{-1}$ for 3 weeks and then cultured under common garden conditions of 22 °C, 20 ppt, and 75 $\mu\text{E m}^{-2}\text{s}^{-1}$ for 2 weeks. Growth, pigment levels, photosynthesis rates were monitored at the beginning, during and after culture in both phases. Statistical analyses have been completed but the data has not yet been studied to determine effect of acclimation.

Expt. 61. In the final experiment, the effect of stress (lack of nutrients) was tested in a factorial hardening experiment (15, 24 °C; 10, 30 ppt, 27-39 $\mu\text{E m}^{-2}\text{s}^{-1}$) using sporelings cultured for 8 weeks on the gradient table. Growth, pigment level, and photosynthetic responses were measured. The data has not yet been analyzed.

2.2.3 Summary of Year 3. research. The experiments conducted during this final year emphasized the effect of common garden conditions and in all cases hardening did not show any significant effect on sporeling response. Also, fatty acid composition was not effected except for

short periods following hardening and did not correlate with any pattern relating growth to composition. Thus, we must conclude that hardening, as described for vascular plants is not evident in sporelings of Gracilaria tikvahiae.

Experiments 57 through 61 were designed to study acclimation in sporelings and compare them to freshly collected populations of the same species, either from the original site or other sites in Tampa Bay. These studies are being analyzed and copies of any publications will be sent to BARD. These experiments have already demonstrated that the sporelings are able to acclimate to new conditions and have retained broad tolerances to salinity, temperature and irradiances similar to those observed with freshly collected branches.

Figure 4s-1. Relative growth rates at hardening (first point) and common garden (second point) for 12 treatments. All show decrease in growth.

Figure 5U-1. Relative growth rates at hardening (first point) and after growth in common conditions (second point show uniform drop due to low temperature of common garden.

Figure 52-1. Fatty acid composition differed after culturing in low and high temperature during hardening.

Figure 53-1. Relative growth rates of hardened plants (first point) and after common garden culturing (second point).

FATTY ACID

Figure 55-1. Fatty acid composition for 7 treatments cultured under 35 uE irradiance.

Figure 55-2. Relative growth rates after hardening (first point) and after common garden conditions (second point) show decrease in growth for all treatments.

1.4. General discussion

G. verrucosa sporelings on the growth gradient table showed that the temperature variable was significant while the irradiance variable was not significant. Optimal irradiance had a wide range, and optimal temperature fluctuated between 10-20°C as a function of sporeling size. Best outdoor acclimation occurred with the optimal preconditioning of the sporelings on the growth gradient table. Therefore, the results for this species did not support the main hypothesis that stressed sporelings will acclimate better under outdoor conditions. The ratio of 16:0/20:4 fatty acids was inversely related to the growth rate of these sporelings. This relationship did not support the hypothesis that in stressed sporelings, this ratio will decrease, or that the concentration of unsaturated fatty acids will increase. The same relationship in *G. tikvahiae* sporelings also did not support this hypothesis, both having lower temperature optimal growth conditions.

Optimal conditions for sporeling growth in *G. domingensis* are high temperature (20-30°C), low irradiance, which is already saturating growth ($> 27 \mu\text{E m}^{-2} \text{ s}^{-1}$), a salinity of 30-40 ppt, and low ammonium concentration ($> 0.7 \text{ mM}$). In the summer experiments, there is a significant inverse correlation between indoor and outdoor growth rate. There is also a positive correlation between indoor growth rate and the ratio between saturated and unsaturated fatty acids. Those two relationships indicate that suboptimal indoor growth rate, under certain conditions, supports maximal outdoor growth rate and the increase of unsaturated fatty acids in the sporelings. An inverse relationship was also found between growth rate and proline concentration.

G. conferta tips showed best acclimation with suboptimal preconditioning. No relationship was found between growth rate of the tips and the 16:0/20:4 ratio of their fatty acids. Sporelings of this species, which were obtained after a continuous effort, showed an insignificant inverse relationship between indoor and outdoor growth. Therefore, the results for *G. conferta* tips and sporelings support the main hypothesis. They are similar in this sense to *G. domingensis*, and both also have the same optimal indoor growth conditions.

The major problem which was approached in this project was the relationship between indoor sporeling growth and their outdoor acclimation. This problem was studied with all the programmed objectives.

The acclimation of suboptimal preconditioned sporelings was tested, in different seasons, and showed that summer acclimation was best with suboptimal preconditioned sporelings of two species only. The successful species were *G. domingensis* and *G. conferta*, while *G. verrucosa* and *G. tikvahiae* were not successful. Only *G. domingensis* showed the inverse relationship between indoor growth rate and unsaturated fatty acids. It may be suggested that the difference between these two pairs of species is a result of their geographical distribution. The first pair has a very wide distribution, which shows a lower optimum temperature in indoor cultures, while the second pair is limited to a warmer environment. *G. domingensis* (Caribbean Sea) and *G. conferta* (eastern Mediterranean) have a higher temperature optimum in indoor cultures. The different source might impose a different response to suboptimal growth conditions.

2. Evaluation of research achievement

The partial support of the major hypothesis is an innovative contribution to the ecophysiology of seaweeds, which had been known from terrestrial plants. It also suggests a technology for the routine preconditioning of sporelings in commercial mariculture ponds. According to the above mentioned results, the sporelings of each species at each season will be hardened differently before transplantation into ponds.

3. Description of cooperation

Simultaneous indoor and outdoor experiments were performed using local species of *Gracilaria* in Florida and Israel. The same methods were used in both laboratories. The conclusions from the two regions were compared and interpreted. Correspondence and bilateral visits of the scientists and a student permitted the comparison of the experimental systems and direct discussions of the final steps.