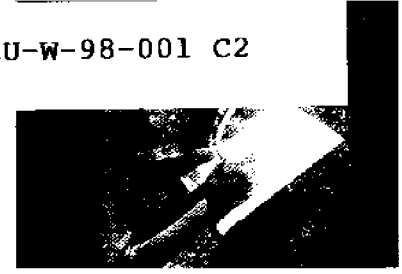

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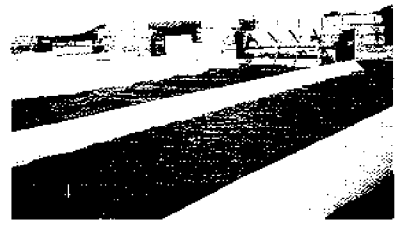
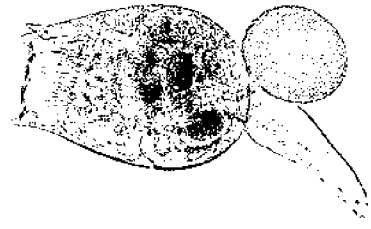
Summer 1998





Sea Grant College Program
School of Ocean and Earth Science and Technology
University of Hawai'i

Proceedings of the 2nd Annual Undergraduate Research Program

Summer 1998

June 2000

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University of Hawai'i Sea Grant College Program
School of Ocean and Earth Science and Technology

Department of Land and Natural Resources
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University of Hawai'i Marine Option Program
School of Ocean and Earth Science and Technology

Aquasearch Incorporated
Natural Energy Laboratory of Hawai"i Authority

Cover (from top to bottom): Student observing the propellar on the SS Kaua'i*; Lena Asano growing her rotifers at the Anuenue Fisheries Research Center; Raceways at Aquasearch Inc. Laboratory, NELHA, Kailua-Kona; Sara Peck and William Young in the culturing laboratory at Aquasearch Inc.; A basic rotifer that has eaten algae with eggs; and Lena Asano counting rotifers or algae at Anuenue Fisheries Research Center. Photos of Lena Asano and the rotifers are courtesy of Harry Ako. All other photos are courtesy of Marine Option Program.*

Publication of these research papers in a compiled volume is intended as an introduction to the scientific publication process, including informal colleague review, review by mentors, and review for technical style and format. Scheduling and distance constraints and software and file inconsistencies precluded some authors from the opportunity to review their "galley proofs" or make changes and corrections. Please use this as a draft volume; herein you will see examples of how text and figures work (or don't work) on the printed page.

This publication is funded by a grant from the National Oceanic and Atmospheric Administration, project #P/PM-2, which is sponsored by the University of Hawaii Sea Grant College Program, SOEST, under Institutional Grant No. NA36RG0507 from NOAA Office of Sea Grant, Department of Commerce. The views expressed herein are those of the author(s) and do not necessarily reflect the views of NOAA or any of its subagencies. UNIHI-SEAGRANT-MR-98-02.

Table of Contents

Abstract

Five undergraduate student research projects were jointly funded by the University of Hawaii Sea Grant College Program, Hawaii Department of Land and Natural Resources and Aquasearch Inc. during the summer of 1998. Project topics were selected to study the optimal salinity, light intensity and incubation period for astanxanthin production in the green alga *Haematococcus pluvialis*, fishing and non-fishing activities in the Waikiki Marine Life Reserve, a restoration project transplanting black coral, and the population of *Octopus cyanea* in Kaneohe Bay. Each project produced a final written report, and all students presented their results at a concluding symposium.

The Effect of Light on *Haematococcus pluvialis* and the Production of Astaxanthin

Principal Investigator:
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Funding by:
University of Hawai'i Sea Grant College Program Summer Internship
State of Hawai'i Department of Land and Natural Resources
Aquasearch Inc.

Final Report
January 30, 1999

Abstract

The accumulation of the ketocarotenoid (3,3'-dihydroxy-4,4'-diketo beta carotene) astaxanthin and the subsequent reddening of the green alga *Haematococcus pluvialis* are affected by light intensity and exposure time (Becker, 1994). Under unfavorably high light intensities, significant amounts of astaxanthin are produced. The threshold point at which this accumulation is activated was examined by reducing an irradiation level of 800 µE with four levels of shade (0, 30, 51, and 73%). Four trials were conducted for periods up to 1 week each, with varying degrees of success. Statistical analysis indicated that, at the light levels used in these experiments, incubation time was the primary determinant of green absorbance (an indicator of astaxanthin concentration) and that light intensity was of minor importance.

Acknowledgments

I wish to thank the following institutions for supporting the internship under which this research was conducted: Aquasearch, Inc.; the State of Hawaii Department of Land and Natural Resources; the College of Agriculture, Forestry and Natural Resource Management at the University of Hawaii at Hilo; and the University of Hawaii Marine Option Program; and the University of Hawaii Sea Grant College Program. I particularly wish to thank Drs. Miguel Olaizola, Sherwood Maynard and Kevin Hopkins, Ms. Sharon Ziegler-Chong, and the crew at Aquasearch, Inc. for their patience and encouragement. But most importantly, I must thank my wife for allowing me to spend a summer away from my family responsibilities following my dreams.

Introduction

The microalga, *Haematococcus pluvialis*, is cultured for its ability to accumulate large quantities of natural astaxanthin (Fan *et al.*, 1994). This red carotenoid pigment is used by the feeds industry in the production of specialized color-enhancing feeds for salmon, trout, shrimp and poultry (Johnson et al., 1980 as cited in Fan *et al.*, 1994).

Astaxanthin production by microalgae is believed to be a chemical response to photic saturation, by synthesizing a sunscreen for photo-protection of internal organs from excessive lighting conditions (Elliot, 1934 and Kobayashi *et al*, 1992). Although astaxanthin production is highest under light conditions, astaxanthin production can be induced in darkness by limiting growth conditions (Kobayashi *et al.*, 1992 as cited by Fan *et al.*, 1994).

H. pluvialis is a motile biflagellated green alga (Chlorophyta) that displays both phototaxic and photophobic characteristics (Cecconi *et al.*, 1996) depending upon light intensity. In shallow culture units, the alga may be unable to descend deep enough to reduce light intensity to non-deleterious levels. When this occurs, astaxanthin is produced. Simultaneously with astaxanthin production, *H. pluvialis* may also encyst forming a red aplanospore. Encystment is typically a last-resort method used to survive adverse conditions. Temperature, salinity, limiting nutrients, and pH balance have all been implicated as factors affecting encystment (Zlotnik *et al.*, 1993). The cysts are negatively buoyant and sink to the bottom of the tanks from where they can be readily harvested.

Aquasearch Inc. cultivates *H. pluvialis* at the Natural Energy Laboratory of Hawaii Authority located on the Keahole Pt., Kailua-Kona, Hawaii (latitude N 19° 43.5' longitude W 156° 3.75'). In an effort to maximize production, research was conducted, under laboratory conditions, to determine the optimum light intensity and duration for astaxanthin synthesis. As part of this research, four experiments were conducted during an undergraduate internship sponsored by the Hawaii Department of Land and Natural Resources, the University of Hawaii Sea Grant College Program, and Aquasearch, Inc.

Materials and Methods

An environmentally-controlled test chamber was constructed at Aquasearch facilities in Kailua-Kona, Hawaii to provide a range of light intensities (about 100 to 800 µE) and a constant temperature of 25 ± 1° C . A shaker table with a capacity of 40 petri dishes was installed inside a reflective semi-enclosed light chamber. The table was powered by a direct-drive 50 RPM, 1 3 amp RH motor bolted to the exterior of the chamber. At the top of the chamber, three halogen lamps rated at 500 watts ea., provided a constant light intensity of 800+, LE. A two inch deep plexiglass water tray supplied with 17° C seawater with a translucent diffuser installed between the lights and the shaker table. This water tray/diffuser distributed the light evenly, eliminating harmful ultra-violet light, and reduced test chamber

temperatures to 28°C. Addition of an external fan further reduced chamber temperature to the desired 25° C ± 1 °C (Figure 1).

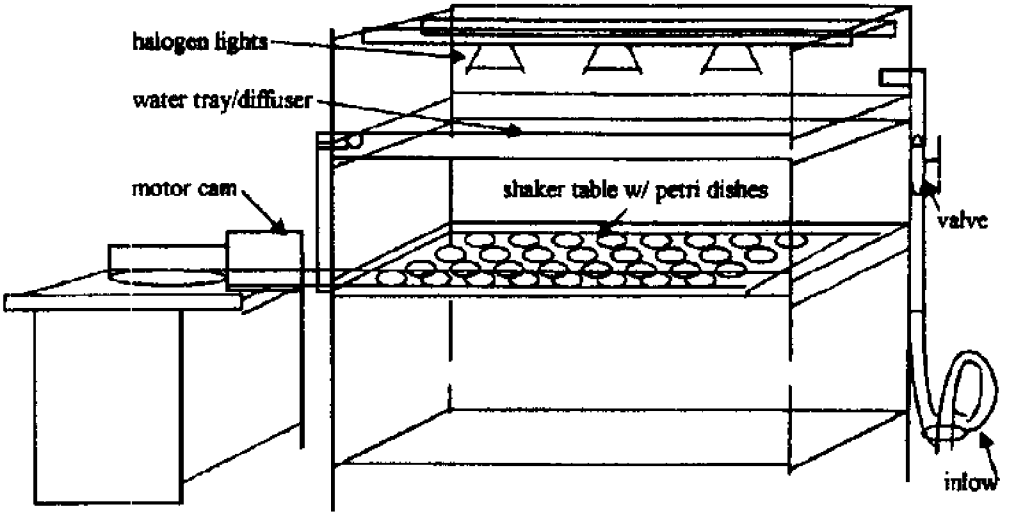

Figure 1. Simplified Schematic of Test Chamber

Although the apparatus had a capacity of forty 30-ml petri dishes, only 32 were used for each experiment. They were arranged in 4 rows of 8 dishes each (Figure 2). Columns 3 and 4 were covered with 30% shade cloth, columns 5 and 6 with 51%, columns 7 and 8 with 73%, and columns 9 and 10 had no shade. In this way, there were 8 dishes at each light intensity.

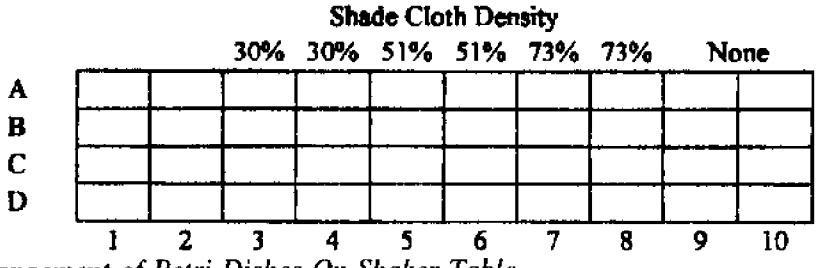

Figure 2. Arrangement of Petri Dishes On Shaker Table

At the start of each of the 4 experiments, algae samples were collected before daylight from outdoor bioreactors (experiments 1, 2 and 4) or an indoor chemostat (only experiment 3). The samples were then diluted with freshwater at a 2:1 algae freshwater ratio. Sodium bicarbonate was added to the diluted samples at the rate of 0.02 g/ml freshwater. Each dish was filled with 30 ml of diluted sample. Two holes of different sizes were drilled into each cover to allow air exchange. The shaker table's constant agitation circulated available nutrients within each dish. Cell counts of both the undiluted and diluted samples were made using a Coulter counter. Other activities at the start of each experiment included red and green light absorbency measurements using the AQS-R/G meter (i.e., a specialized spectrophotometer); samples were collected and frozen for later astaxanthin analysis; light intensity was measured with a Li-Cor 185 photometer, and fluorescence was measured with a Walz mini PAM (pulse amplitude modulator) fluorometer.

Although the basic procedures described above were the same for the four experiments, other parameters changed as the system operations evolved. Exact times for sample collection are contained in the Appendix tables. Major changes in the experiments are as follows:

Experiment 1 - This experiment started on 15 July at 10 AM and ended 72 hours later on 18 July. Even though an automatic voltage regulator (UPS) had been installed to minimize

power surges, the motion of the table was still rather violent causing much of the media and algae to spill. Coagulation was observed to clog the Coulter counter so a vortex mixer was used to separate particles with only partial success.

Experiment 2 - This experiment was conducted from 25 to 30 July. As efforts to control the power surges which caused violent shaker table movements had been unsuccessful, wide rubber bands were used as a gasket between the petri dish bottoms and tops. Further, these bands minimized sliding by acting as anti-skid surfaces. The last set of samples during this experiment may have been mixed up when violent power surges caused the dishes to scatter (even though the bands minimized movement under normal conditions.

Experiment 3 - This experiment was inoculated on July 30 at 17:30 and ended on 2 August. Unfortunately, problems arose when the fluorometer became defective and unreliable. Also, sampling was disrupted by staff absences.

Experiment 4 - This experiment started on 6 August and lasted until 11 August. Operationally, it was essentially the same as Experiment 3 except that the fluorescence measurements were not taken.

Data were tabulated in Microsoft Excel. Graphical and regression analyses were conducted using Statmost. These regression analyses included simple and multiple regressions. Light dosage during each exposure period was computed by multiplying light intensity by the length of the period in seconds. As Experiment 4 appeared to be most successful, its results were analyzed first. Data from the other experiments were then examined.

Results and Discussion

Once the problems with spillage were solved with by sealing the petri dishes with rubber bands, the experimental apparatus worked fairly well except for fluctuations in light intensity caused by an inadequate electrical source Average light intensities ranged from 180 to 637 µE with standard errors ranging from 4 to 8 µE (Table 1) However, the occasional wide variance in light intensity clearly evident in the maximum and minimum values is a cause of concern.

Table 1. Effect of cover on light intensity (µE)

	Shade Net Density			
	None	**30%**	**51%**	**73%**
Mean	637	349	237	180
Standard Error	8	6	5	4
Sample Size	93	93	93	93
Minimum	454	256	160	128
Maximum	765	507	377	345

The effect of incubation time on cell density, green absorbance, red absorbance and R/G ratio during Experiment 4 are presented in Figures 3 to 6. The data summaries for this and the other experiments are contained in Appendix Tables 1 to 4. Although no effect on cell density was apparent, it was clear that incubation time was positively correlated to green and red absorbance and negatively correlated to RtG ratio. The best relationship was between incubation time and green absorbance (an indicator of astaxanthin level) with an R^2 of 0.69.

Light dosage effects on cell density, green absorbance, red absorbance and R/G ratio were also examined (Figures 7 to 10). Both green absorbance and RJG ratio were related to dosage but incubation time by itself had higher significance. This indicates that light intensity had little effect during this experiment. This was further verified when multiple regressions of the form:

Green absorbance = f incubation time, average light
R/G ratio = f incubation time, average light

were computed. In both cases, the coefficients associated with light intensity were not significant.

The data from the earlier experiments were then examined to determine if they could be combined with Experiment 4 data to form a more generalized model relating incubation time to green absorbance and R/G ratio. Experiment 1 data were immediately rejected because of negative absorbance values indicating problems in either the measuring equipment or the operation of the equipment. The effect of incubation time on green absorbance was then evaluated using data from both Experiments 3 and 4 (Figure 11). The results were very similar to that attained with Experiment 4 alone, although the combined data did not show an initial rapid increase. Thus, a linear relationship was fitted to the combined data instead of the quasi-linear (square root transformation) relationship used for Experiment 4 alone. Experiment 2 data was then included (Figure 12). The Experiment 2 data showed a wide degree of scatter and extend well outside the ranges of the combined Experiment 3 & 4 data. Thus, it was decided to reject the Experiment 2 data. The last analysis conducted was the relationship of incubation time on the R/G ratio for combined Experiments 3 and 4. A negative correlation is apparent but a functional form which adequately describes the relationship has yet to be determined (Figure 13).

These preliminary analyses indicate that, under the light intensities of 100 to 800 µE, incubation time alone can be used to predict the relative degree of astaxanthin production. Higher light intensities and longer experimental periods than those used in these experiments might well produced different results. It is suggested that the experiments be continued at greater light intensities and for longer durations. Further, to minimize nonexperimental effects, it is essential that the test apparatus be connected to a reliable power supply.

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Figures

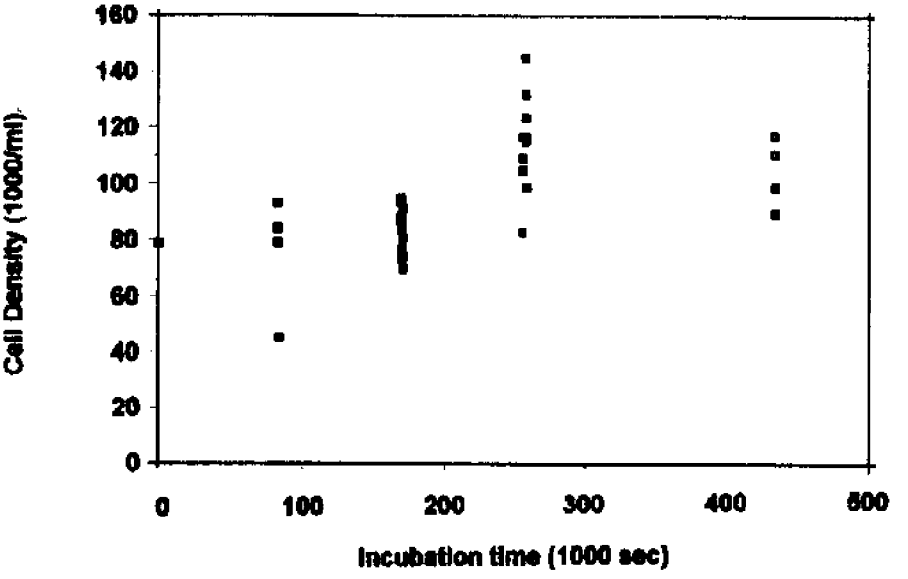

Figure 3. Effects of incubation time on cell density in Experiment 4.

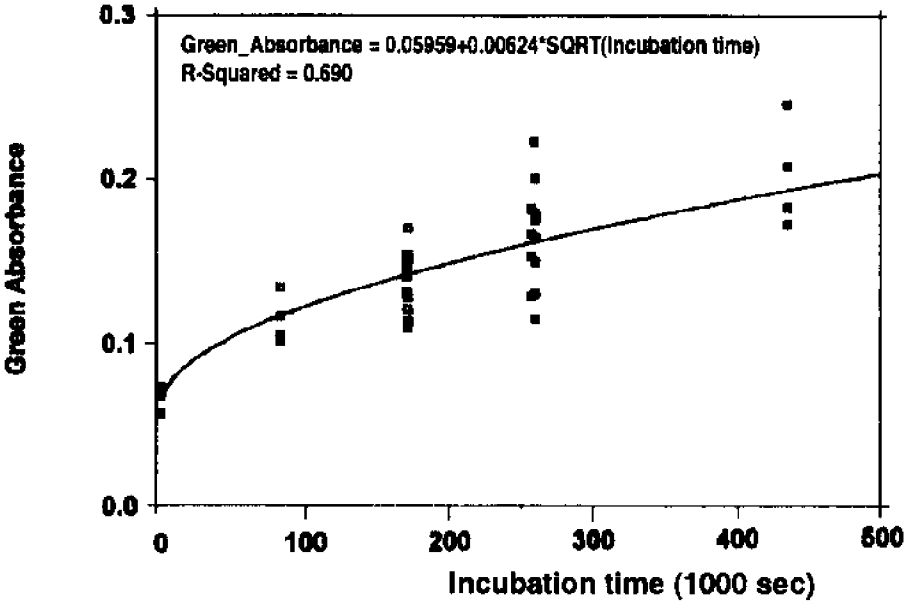

Figure 4. Effect of incubation time on green absorbance in Experiment 4.

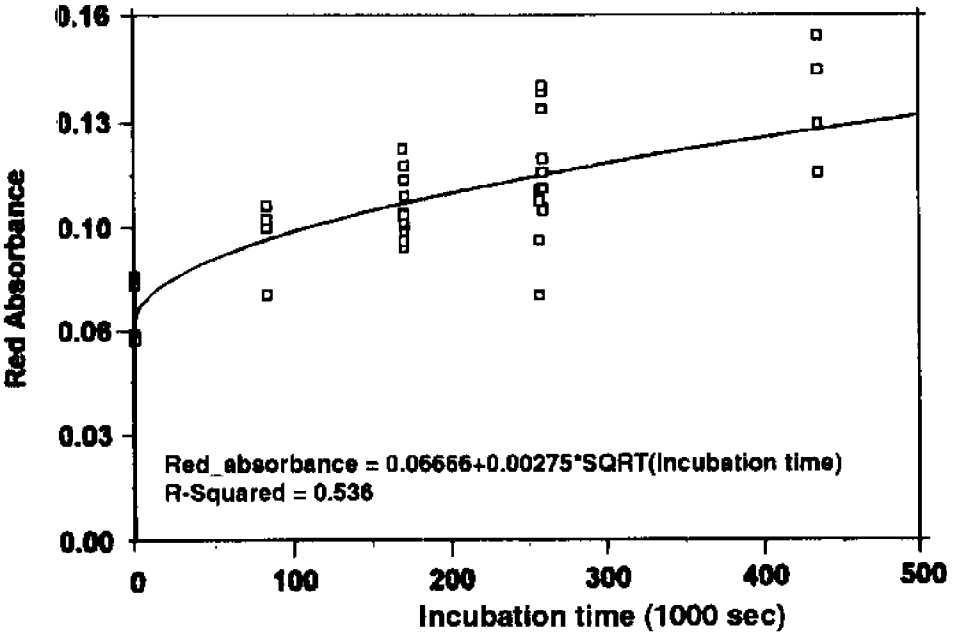

Figure 5. Effects of incubation on red absorbance in Experiment 4.

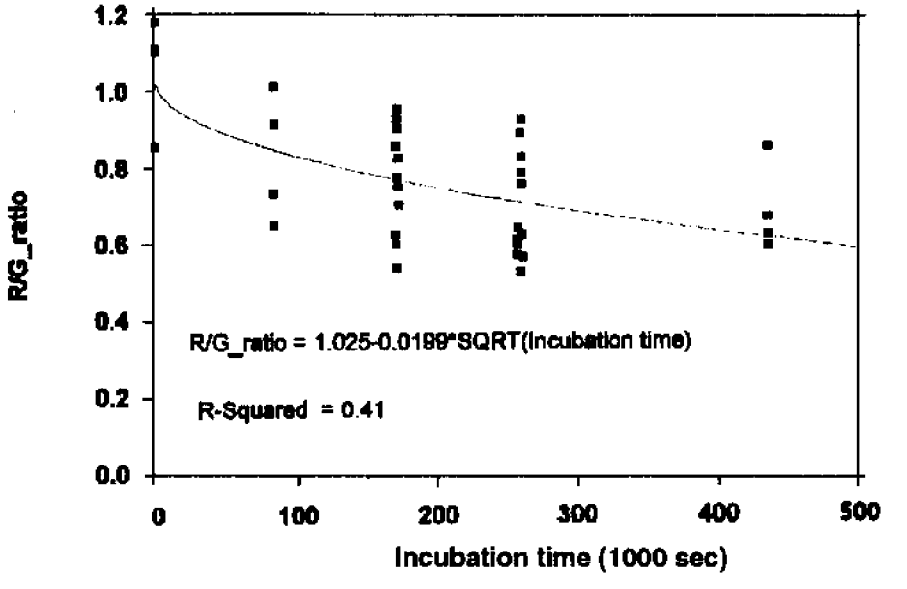

Figure 6. Effect of incubation time on R/G ratio in Experiment 4.

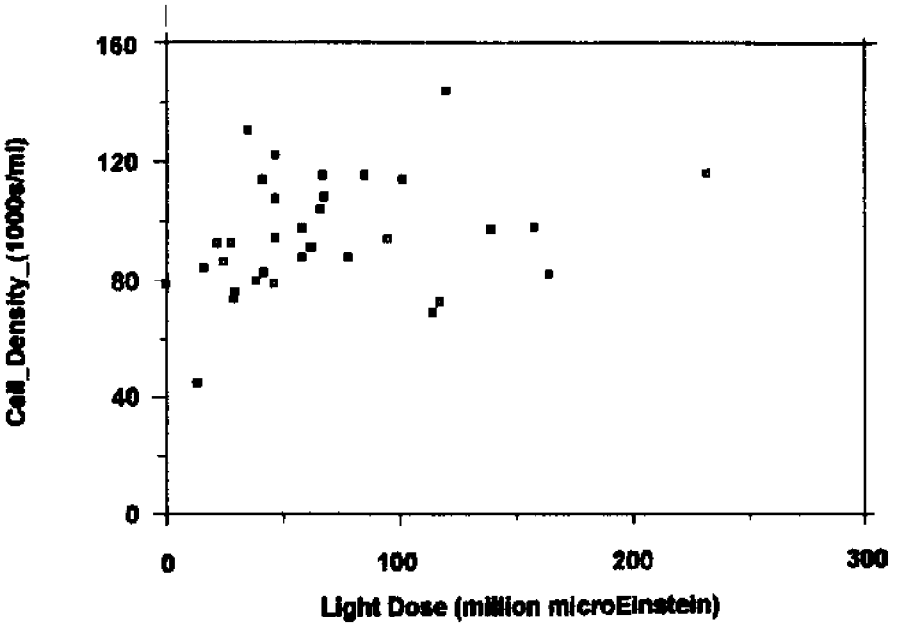

Figure 7. Effects of light dosage on cell density in Experiment 4.

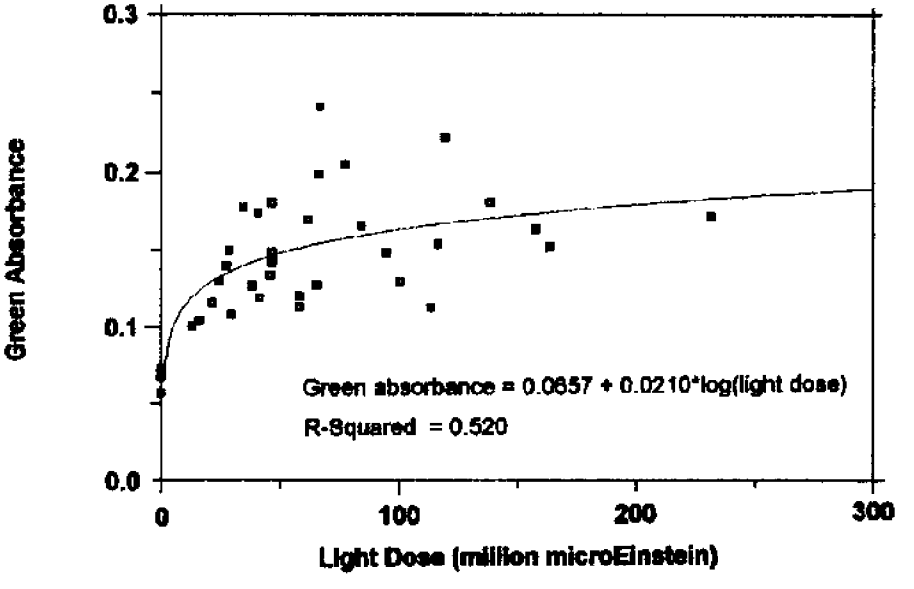

Figure 8. Effect of light dosage on green absorbance in Experiment 4.

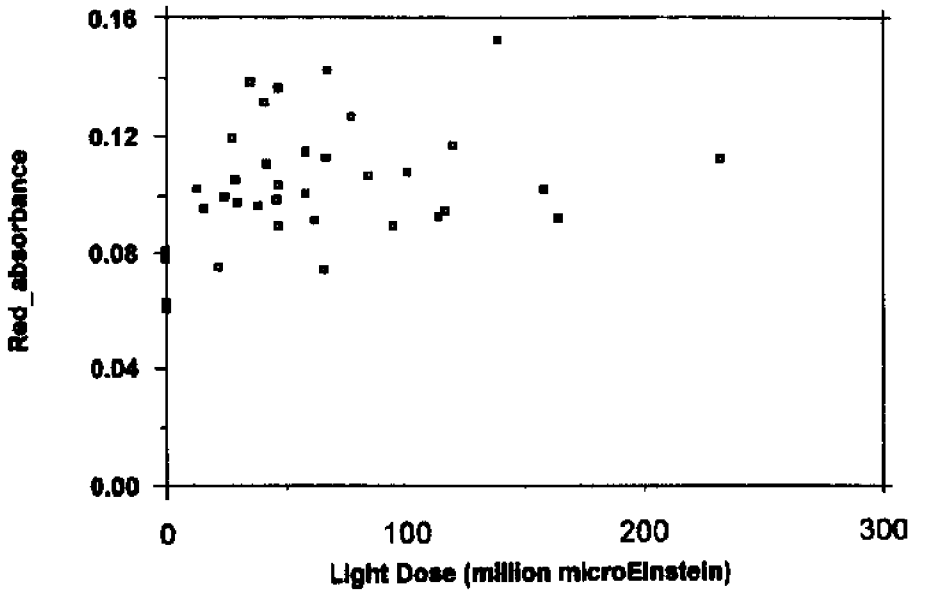

Figure 9. Effect of light dosage on red absorbance in Experiment 4.

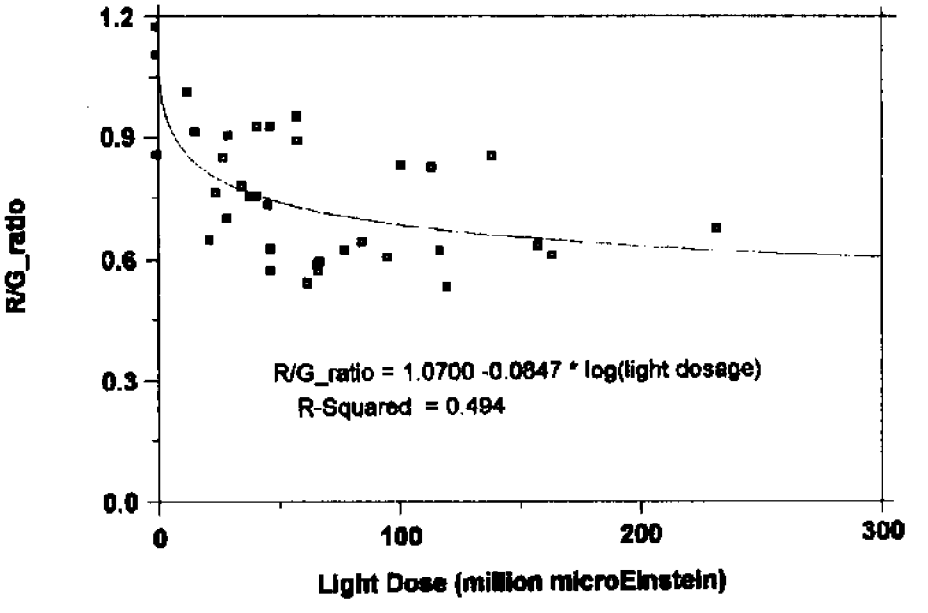

Figure 10. Effect of light dosage on R/G ratio in Experiment 4.

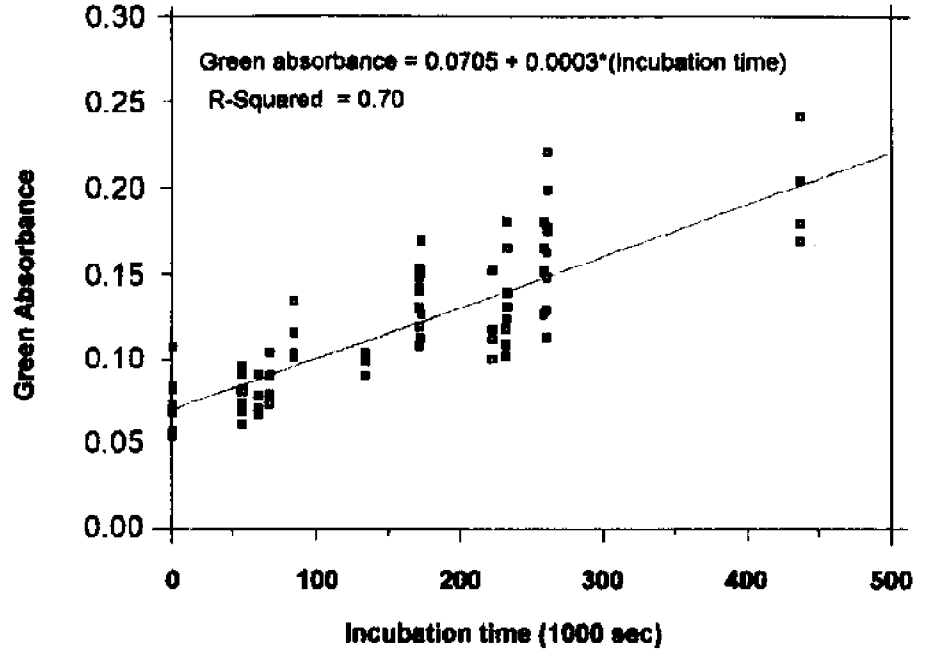

Figure 11. Effect of incubation time on green absorbance in Experiments 3 & 4.

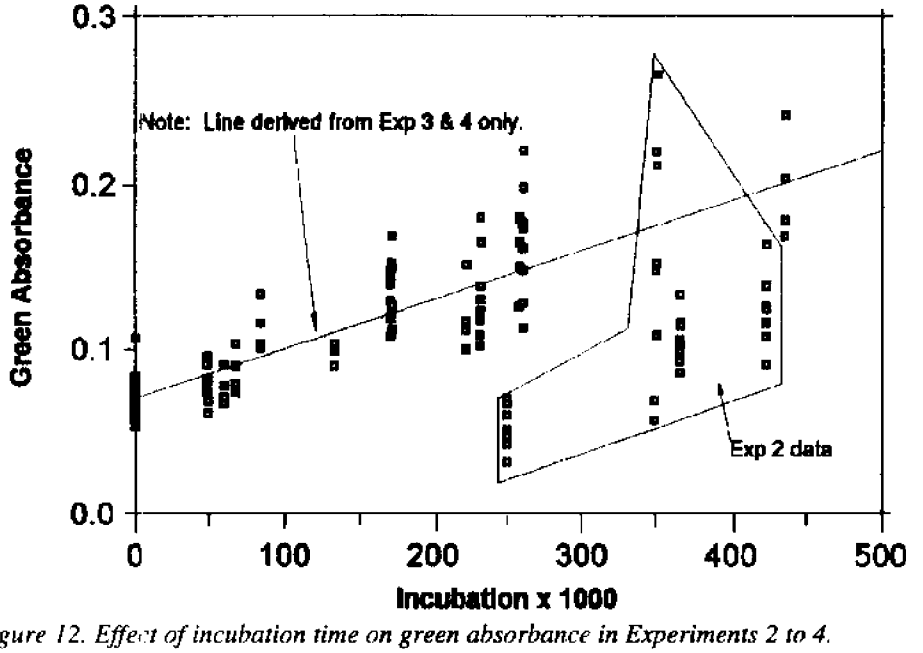

Figure 12. Effect of incubation time on green absorbance in Experiments 2 to 4.

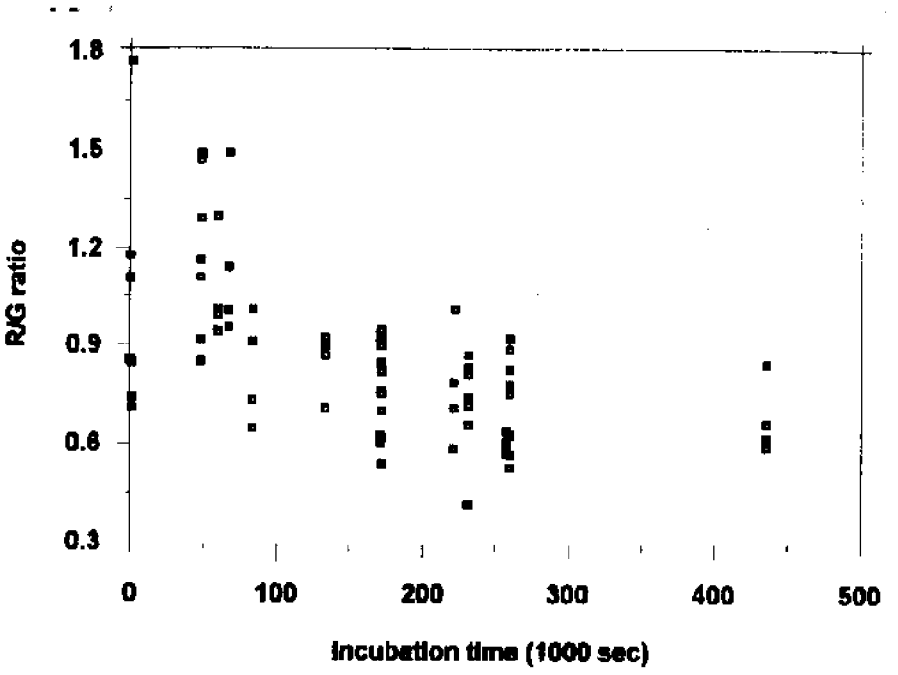

Figure 13. Effect of incubation time on R/G ratio in Experiments 3 & 4.

APPENDIX

Summary Data Tables

&

Regression Output

Experiment 1

Start date 7/15/98
End date 7/18/98
T0 7/15/98

Sample position	Sampling date	Sampling time	Incubation period(s)	average cell count	Light	Light dose	red absorb	green absorb	R/G	Fo	Fm	Fv/Fm
D10 To	7/15/98	10:00	0	161214	0	0.00E+00	-0.043	-0.046	0.936			
D8 To	7/15/98	10:00	0	161214	0	0.00E+00	-0.038	-0.034	1.091			
D6 To	7/15/98	10:00	0	161214	0	0.00E+00	-0.026	-0.036	0.725			
D4 To	7/15/98	10:00	0	161214	0	0.00E+00	-0.041	-0.030	1.365			
D10	7/15/98	12:05	7500	162772	545	4.09E+06	0.046	0.053	0.863	66	245	0.731
D8	7/15/98	12:05	7500	158711	128	9.61E+05	0.032	0.047	0.672	70	280	0.751
D6	7/15/98	12:05	7500	163477	173	1.30E+06	0.048	0.043	1.097	59	238	0.749
D4	7/15/98	12:05	7500	177277	267	2.00E+06	0.044	0.034	1.291	61	256	0.760
D9	7/15/98	15:05	18300	163574	570	1.04E+07	-0.048	-0.043	1.111	270	720	0.625
D7	7/15/98	15:05	18300	162773	191	3.49E+06	-0.051	-0.045	0.325	299	929	0.677
D5	7/15/98	15:05	18300	162779	258	4.73E+06	-0.015	-0.045	0.325	355	1016	0.650
D3	7/15/98	15:05	18300	152936	382	6.99E+06	-0.049	-0.044	1.123	267	767	0.652
C10	7/16/98	8:10	79800	258149	717	5.72E+07	0.056	0.021	2.636	343	825	0.584
C8	7/16/98	8:10	79800	191226	176	1.41E+07	0.008	0.020	0.427	347	880	0.604
C6	7/16/98	8:10	79800	189324	238	1.90E+07	0.015	0.018	0.861	350	911	0.609
C4	7/16/98	8:10	79800	227976	341	2.72E+07	-0.028	0.019	-1.441	285	752	0.619
C9	7/16/98	13:25	98700	250868	731	7.22E+07	0.047	0.048	0.980	372	689	0.461
C7	7/16/98	13:25	98700	205045	239	2.36E+07	0 042	0.056	0.754	373	956	0.608
C5	7/16/98	13:25	98700	224794	331	3.27E+07	0.054	0.063	0.849	321	818	0.607
C3	7/16/98	13:25	98700	220022	466	4.60E+07	0.056	0.1W	0.562	381	925	0.585
B10	7/16/98	16:00	108000	257373	762	8.22E+07	-0.041	-0.052	0.793	390	705	0.459
B8	7/16/98	16:00	108000	242021	195	2.11E+07	-0.045	-0.054	0.837	409	1194	0.655
B6	7/16/98	16:00	108000	316401	280	3.03E+07	-0.066	-0.123	0.533	554	1545	0.641
B4	7/16/98	16:00	108000	257975	364	3.93E+07	-0.050	-0.080	0.623	287	696	0.584
B9	7/17/98	10:10	173400	188982	738	1.28E+08	0.051	0.074	0.697	356	805	0.557
B7	7/17/98	10:10	173400	185606	252	4.37E+07	0.042	0.049	0.852	248	585	0.571
B5	7/17/98	10:10	173400	167189	336	5.83E+07	0.058	0.081	0.712	391	953	0.588
B3	7/17/98	10:10	173400	181274	488	8.47E+07	0.039	0 055	0 705	274	600	0.541
A10	7/18/98	7:35	250500	343561	574	1.44E+08	-0.053	-0.101	0.522	212	493	0.566
A8	7/18/98	7:35	250500	288129	163	4.07E+07	-0.085	-0.143	0.598	285	891	0.677
A6	7/18/98	7:35	250500	274678	205	5.13E+07	-0.040	-0.072	0.549	140	360	0.610
A4	7/18/98	7:35	250500	280780	283	7.10E+07	-0.049	0.186	-0.262	413	1014	0.592
A9	7/18/98	7:55	251700	357862	585	1.47E+08	-0.044	-0.049	0.901	149	303	0.506
A7	7/18/98	7:55	251700	342339	203	5.10E+07	-0.0B6	-0.097	0.884	330	1236	0.729
A5	7/18/98	7:55	251700	171496	247	6.21E+07	-0.051	-0.094	0.545	216	719	0.701
A3	7/18/98	7:55	251700	306520	3B4	9.66E+07	-0.074	-0.100	0.740	298	640	0.698

Experiment 2

Start date 7/25/98
End date 7/30/98
T0 7/25/98 10:15

Sample position	Sampling date	Sampling time	Incubation period(s)	average cell count	Light	Light dose	red absorb	green absorb	R/G	Fo	Fm	Fv/Fm
D10 To	7/25/98	10:15	0	70209	0	0.00E+00	0.092	0.061	1.510	155	288	0.460333
D8 To	7/25/98	10:15	0	70209	0	0.00E+00	0.057	0.057	0.993	154	290	0.467667
D6 To	7/25/98	10:15	0	70209	0	0.00E+00	0.054	0.061	0.886	137	288	0.521667
D4 To	7/25/98	10:15	0	70209	0	0.00E+00	0.048	0.078	0.614	153	288	0.469333
D10	7/28/98	7:30	249300	67525	614	1.53E+08	0.031	0.070	0.447	267	545	0.512427
D8	7/28/98	7:30	249300	52225	148	3.68E+07	0.024	0.067	0.358	207	514	0.561541
D6	7/28/98	7:30	249300	61634	196	4.89E+07	0.017	0.060	0.291	212	508	0.572761
D4	7/28/98	7:30	249300	57767	280	6.98E+07	0.023	0.051	0.449	251	531	0.529528
D9	7/28/98	7:35	249600	52438	624	1.56E+08	0.022	0.031	0.704	241	576	0.630666
D7	7/28/98	7:35	249600	45267	135	3.38E+07	0.084	0.045	1.857	222	526	0.560458
D5	7/28/98	7:35	249600	46250	178	4.45E+07	0.060	0.047	1.278	195	439	0.556105
D3	7/28/98	7:35	249600	53634	313	7.81E+07	0.042	0.042	0.998	249	593	0.579846
C10	7/29/98	11 10	348900	104171	726	2.53E+08	-0.034	0.057	-0.607	90	155	0.419
C8	7/29/98	11:10	348900	214557	176	6.16E+07	0.091	0.069	1.329	100	182	0.449667
C6	7/29/98	11:10	348900	184099	258	9.02E+07	0.135	0.148	0.918	99	187	0.462
C4	7/29/98	11:10	348900	120998	341	1.19E+08	0.072	0.219	0.331	230	511	0.547333
C9	7/29/98	11:20	349500	214557	731	2.55E+08	0.131	0.153	0.858	164	375	0.561
C7	7/29/98	11:20	349500	106538	177	6.18E+07	0.183	0.211	0.867	150	293	0.488
C5	7/29/98	11:20	349500	116148	242	8.44E+07	0.146	0.109	1.339	203	472	0.569
C3	7/29/98	11:20	349500	115217	401	1.40E+08	0.143	0.265	0.541	177	350	0.492667
B10	7/29/98	15:40	365100	106907	715	2.61E+08	0.064	0.106	0.599	232	443	0.473333
B8	7/29/98	15:40	365100	86854	196	7.14E+07	0.066	0.093	0.714	303	848	0.641333
B6	7/29/98	15:40	365100	94420	262	9.55E+07	0.063	0.134	0.468	192	440	0.560333
B4	7/29/98	15:40	365100	103712	358	1.31E+08	0.076	0.099	0.760	200	375	0.463
B9	7/29/98	15:50	365700	125337	668	2.44E+08	0.048	0.117	0.406	204	362	0.435667
B7	7/29/98	15:50	365700	160263	184	6.74E+07	0.077	0.115	0.674	237	587	0.591667
B5	7/29/98	15:50	365700	78990	238	8.71E+07	0.069	0.086	0.802	117	206	0.428667
B3	7/29/98	15:50	365700	108847	405	1.48E+08	0.068	0.103	0.654	224	470	0.519333
A10	7/30/98	7:35	422400	107651	587	2.48E+08	0.076	0.127	0.599	158	379	0.582333
A8	7/30/98	7:35	422400	82043	163	6.89E+07	0.093	0.140	0.662	226	700	0.675
A6	7/30/98	7:35	422400	113322	224	9.44E+07	0.091	0.117	0.781	200	675	0.701
A4	7/30/98	7:35	422400	80187	317	1.34E+08	0.075	0.108	0.702	182	460	0.601
A9	7/30/98	7:45	423000	123494	549	2.32E+08	0.083	0.164	0.506	156	361	0.565333
A7	7/30/98	7:45	423000	111253	165	6.96E+07	0.103	0.091	1.134	221	799	0.723667
A5	7/30/98	7:45	423000	100466	208	8.80E+07	0.087	0.139	0.626	209	774	0.729
A3	7/30/98	7:45	423000	108265	357	1.51E+08	0.078	0.125	0.620	194	555	0.650333

Experiment 3

Start date 7/25/98
End date 7/30/98
T0 7/25/98 10:15

Sample position	Sampling date	Sampling time	Incubation period(s)	average cell count	Light	Light dose	red absorb	green absorb	R/G	Fo	Fm	Fv/Fm
D10 To	7/30/98	18:30	0	87798	0	0.00E+00	0.072	0.084	0.847	214	796	0.731
D8 To	7/30/98	18:30	0	87798	0	0.00E+00	0.075	0.107	0.707	213	790	0.729
D6 To	7/30/98	18:30	0	87798	0	0.00E+00	0.093	0.053	1.766	192	709	0.727
D4 To	7/30/98	18:30	0	87798	0	0.00E+00	0.060	0.082	0.739	190	705	0.730
D10	7/31/98	7:45	47700	82347	637	3.04E+07	0.106	0.096	1.106	166	447	0.628
D8	7/31/98	7:45	47700	84771	166	7.92E+06	0.108	0.074	1.465	208	685	0.696
D6	7/31/98	7:45	47700	79637	209	9.99E+06	0.074	0.081	0.916	197	613	0.577
D4	7/31/98	7:45	47700	72912	301	1.43E+07	0.136	0.091	1.485	190	560	0.661
D9	7/31/98	7:55	48300	68114	644	3.11+07	0.079	0.061	1.288	172	439	0.610
D7	7/28/98	7:55	48300	49703	142	6.87+06	0.058	0.069	0.850	185	657	0.718
D5	7/28/98	7:55	48300	69187	192	9.26E+06	0.096	0.082	1.161	189	617	0.693
D3	7/28/98	7:55	48300	68883	346	1.67E+07	0.120	0.081	1.486	185	549	0.663
C10	7/31/98	11 00	59400	54475	748	4.44E+07	0.067	0.071	0.942	203	446	0.546
C8	7/31/98	11 00	59400	75615	186	1.10E+07	0.079	0.078	1.010	223	656	0.659
C6	7/31/98	11 00	59400	74166	261	1.55E+07	0.086	0.067	1.295	228	648	0.648
C4	7/31/98	11 00	59400	78021	326	1.94E+07	0.090	0.091	0.994	245	637	0.616
C9	7/31/98	13:10	67200	70242	725	4.87E+07	0.118	0.079	1.488	178	415	0.570
C7	7/31/98	13:10	67200	55833	204	1.37E+07	0.084	0.074	1.139	217	683	0.682
C5	7/31/98	13:10	67200	69782	272	1.83E_07	0.086	0.009	9.783	208	620	0.665
C3	7/31/98	13:10	67200	78169	370	2.48E+07	0.104	0.104	1.007	227	610	0.627
B10	8/1/98	7:30	133200	84319	737	9.81E+07	0.073	0.104	0.706	261	660	0.604
B8	8/1/98	7:30	133200	99146	195	2.60E+07	0.089	0.099	0.901	307	892	0.656
B6	8/1/98	7:30	133200	93450	267	3.55E+07	0.079	0.090	0.869	249	538	0.629
B4	8/1/98	7:30	133200	96890	348	4.63E+07	0.083	0.090	0.925	369	958	0.620
B9	8/2/98	8:00	221400	83181	729	1.61E+08	0.068	0.117	0.584	104	270	0.611
B7	8/2/98	8:00	221400	85599	233	5.17E+07	0.108	0.152	0.707	218	640	0.660
B5	8/2/98	8:00	221400	72105	375	8.30E+07	0.088	0.112	0.786	331	893	0.631
B3	8/2/98	8:00	221400	85664	489	1.08E+08	0.101	0.100	1.011	348	951	0.629
A10	8/2/98	10:45	231300	50821	542	1.25E+08	0.076	0.102	0.741	60	112	0.425
A8	8/2/98	10:45	231300	62636	331	7.65E+07	0.075	0.180	0.416	50	132	0.624
A6	8/2/98	10:45	231300	94100	291	6.74E+07	0.098	0.118	0.827	259	653	0.605
A4	8/2/98	10:45	231300	75589	381	8.81E+07	0.089	0.109	0.810	43	133	0.675
A9	8/2/98	10:50	231600	82832	542	1.25E+08	0.115	0.131	0.873	89	293	0.686
A7	8/2/98	10:50	231600	60923	185	4.28E+07	0.103	0.124	0.833	73	237	0.679
A5	8/2/98	10:50	231600	70707	234	5.43E+07	0.092	0.139	0.658	121	338	0.643
A3	8/2/98	10:50	231600	74659	381	8.82E+07	0.118	0.165	0.716	122	459	0.738

Experiment 4

Start date 8/6/98
End date 8/11/98
T0 8/6/98 10:30

Sample position	Sampling date	Sampling time	Incubation period(s)	average cell count	Light	Light dose	red absorb	green absorb	R/G	Fo	Fm	Fv/Fm
D10 To	8/6/98	10:30	0	79023	0	0.00E+00	0.081	0.073	1.107	732	680	0.698
D8 To	8/6/98	10:30	0	70923	0	0.00E+00	0.061	0.071	0.855	732	680	0.698
D6 To	8/6/98	10:30	0	70923	0	0.00E+00	0.078	0.067	1.177	732	680	0.698
D4 To	8/6/98	10:30	0	70923	0	0.00E+00	0.063	0.057	1.103	732	680	0.698
D10	8/7/98	9:45	83700	78809	551	4.61E+07	0.098	0.134	0.730			0.602
D8	8/7/98	9:45	83700	44830	155	1.30E+07	0.102	0.101	1.011			0.698
D6	8/7/98	9:45	83700	83899	191	1.60E+07	0.095	0.104	0.912			0.531
D4	8/7/98	9:45	83700	92454	259	2.17E+07	0.075	0.116	0.647			0.637
D9	8/8/98	10:00	171000	93327	556	9.50E+07	0.089	0.148	0.601			0.666
D7	8/8/98	10:00	171000	86097	143	2.44E+07	0.099	0.130	0.762			0.692
D5	8/8/98	10:00	171000	92577	161	2.76E+07	0.119	0.140	0.847			0.642
D3	8/8/98	10:00	171000	93844	274	4.69E+07	0.089	0.142	0.624			0.662
C10	8/8/98	10:05	171300	72265	682	1.17E+08	0.094	0.153	0.615			0.655
C8	8/8/98	10:05	171300	76229	173	2.96E+07	0.097	0.108	0.900			0.627
C6	8/8/98	10:05	171300	82657	243	4.17E+07	0.110	0.119	0.924			0.635
C4	8/8/98	10:05	171300	87449	339	5.80E+07	0.114	0.120	0.947			0.657
C9	8/8/98	10:15	171900	68980	662	1.14E+08	0.092	0.112	0.820			0.613
C7	8/8/98	10:15	171900	73526	169	2.90E+07	0.105	0.150	0.700			0.571
C5	8/8/98	10:15	171900	79689	224	3.85E+07	0.096	0.127	0.752			0.686
C3	8/8/98	10:15	171900	90462	362	6.22E+07	0.091	0.169	0.538			0.708
B10	8/9/98	10:00	257400	81582	638	1.64E+08	0.091	0.151	0.602			0.758
B8	8/9/98	10:00	257400	107612	182	4.69E+07	0.103	0.180	0.570			0.716
B6	8/9/98	10:00	257400	103441	256	6.59E+07	0.074	0.127	0.583			0.713
V4	8/9/98	10:00	257400	115242	329	847E+07	0.106	0.165	0.638			0.715
B9	8/9/98	10:40	259800	97252	609	1.58E+08	0.101	0.162	0.625			0.739
B7	8/9/98	10:40	259800	113587	158	4.11E+07	0.131	0.174	0.752			0.723
B5	8/9/98	10:40	259800	97252	224	5.81E+07	0.100	0.113	0.889			0.706
B3	8/9/98	10:40	259800	113587	389	1.01E+08	0.107	0.129	0.825			0.701
A10	8/9/98	10:40	259800	143430	261	1.20E+08	0.116	0.220	0.527			0.763
A8	8/9/98	10:40	259800	30361	136	3.52E+07	0.138	0.177	0.778			0.746
A6	8/9/98	10:40	259800	121903	181	4.70E+07	0.136	0.148	0.921			0.743
A4	8/9/98	10:40	259800	115061	257	6.68E+07	0.112	0.198	0.566			0.716
A9	8/11/98	11:20	435000	114589	533	2.32E+08	0.111	0.169	0.661			0.605
A7	8/11/98	11:20	435000	107993	155	6.75E+07	0.142	0.241	0.590			0.592
A5	8/11/98	11:20	435000	87100	179	7.76E+07	0.126	0.204	0.618			0.569
A3	8/11/98	11:20	435000	96321	320	1.39E+08	0.152	0.179	0.846			0.589

Quasi-Linear Regression Analysis Results

EXPERIMENT 4 – FIGURE 4

Variables: X = "Incubation_time_(1000_sec)", Y = "Green_Absorbance"
Transform: X+SQRT (Incubation_time_(1000_sec))

Line Equation (Y=a+bX) : Y = 0.0595931+0.00623641*X
Fitted Equation: Green Absorbance = 0.0595931+0.00623641*SQRT (Incubation_time_(1000_sec))
Equation: Green_Absorbance = 0.0595931 + 0.006263641*Incubation_time_(1000_sec)

Variable	N	Mean	Variance
Incubation_time	36	13.0624	31.3442
Green_Absorbanc	36	0.1411	0.0018

Regression Coefficient	= 0.006236
Standard Error of B	= 0.000716
Y-Intercept	= 0.059593
R-Squared	= 0.690252
Adjusted R-Squared	= 0.681142
Standard Error of Estimate	= 0.023731

XBAR = 13.0624 YBAR = 0.141056 Total N = 36
Var. X = 31.3442 Var. Y = 0.00176611
The 95.0% confidence limits for the slope are: [0.00478037, 0.00769244]

Analysis of Variance Table

Source	DF	SS	MS	F	P
Linear	1	0.0427	0.0427	75.7667	1.916E-024
Deviation	34	0.0191	0.0006		
Total	35	0.0618	0.0018		

Goodness of Fit Statistics ...
Coefficient of Determination: 0.690252275
Correlation Coefficient: 0.830814224
Model Selection Criterion: 1.060885992

Parameter Statistics
95.00% Confidence Intervals
Parameter a:0.059593084
StdDev: 0.010160173
Univariate ...
LOW: 0.038945129
HIGH: 0.080241039
Supporting Plane:
LOW: 0.033586625
HIGH: 0.085599544
Parameter b: 0.006236406
StdDev: 0.000716465
Univariate ...
LOW: 0.004780374
HIGH: 0.007692438
Supporting Plane:
LOW: 0.004402508
HIGH: 0.008070304

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Quasi-Linear Regression Analysis Results

EXPERIMENT 4 - FIGURE 5

Variables: X = "Incubation_time_(1000_sec)", Y = "Red_absorbance"
Transform: X=SQRT (Incubation_time_(1000_sec))

Line Equation (Y=a+bX): Y = 0.0666562+0.00275255*X
Fitted Equation: Red_absorbance = 0.0666562+0.00275255*SQRT(Incubation_time_(1000_sec)
Equation: Red_absorbance = 0.0666562 + 0.00275255*Incubation_time_(1000_sec)

Variable	N	Mean	Variance
Incubation_time	36	13.0624	31.3442
Red absorbance	36	0.1026	0.0004

Regression Coefficient	= 0.002753
Standard Error of B	= 0.000439
Y-Intercept	= 0.066656
R-Squared	= 0.536365
Adjusted R-Squared	= 0.522729
Standard Error of Estimate	= 0.014537

XBAR = 13.0624 YBAR = 0.102611 Total N = 36
Var. X = 31.3442 Var. Y = 0.000442759
The 95.0% confidence limits for the slope are: [0.00186062, 0.00364448]

Analysis of Variance Table

Source	DF	SS	MS	F	P
Linear	1	0.0083	0.0083	39.3336	1.238E-019
Deviation	34	0.0072	0.0002		
Total	35	0.0155	0.0004		

Goodness of Fit Statistics ...
Coefficient of Determination: 0.536365249
Correlation Coefficient: 0.732369613
Model Selection Criterion: 0.657547101

Parameter Statistics
95.00% Confidence Intervals
Parameter a: 0.066656174
StdDev: 0.006223856
Univariate ...
LOW: 0.054007776
HIGH: Q.079304572
Supporting Plane:
LOW: 0.050725296
HIGH: 0.082587052
Parameter b: 0.002752551
StdDev: 0.000438888
Univariate ...
LOW: 0.001860623
HIGH: 0.003644g78
Supporting Plane:
LOW: 0.001629153
HIGH: 0.003875949

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Quasi-Linear Regression Analysis Results

EXPERIMENT 4 - FIGURE 6

Variables: X = "Incubation_time_(1000_sec)", Y = "R/G_ratio"
Transform: X=SQRT (Incubation_time_(1000_sec))

Line Equation (Y=a+bX): Y = 1.02506-0.0198604*X
Fitted Equation: R/G_ratio = 1.02506-0.0198604*SQRT (Incubation_time_(1000_sec)
Equation: R/G_ratio = 1.02506 - 0.0198604*Incubation_time_(1000_sec)

Variable	N	Mean	Variance
Incubation_time	36	13.0624	31.3442
R/G_ratio	36	0.7656	0.0303

Regression Coefficient	= -0.019860
Standard Error of B	= 0.004104
Y-Intercept	= 1.025064
R-Squared	= 0.407894
Adjusted R-Squared	= 0.390479
Standard Error of Estimate	= 0.135921

XBAR = 13.0624 YBAR = 0.102611 Total N = 36
Var. X = 31.3442 Var. Y = 0.0303101
The 95.0% confidence limits for the slope are: [-0.0282001, -0.0115207]

Analysis of Variance Table

Source	DF	SS	MS	F	P
Linear	1	0.4327	0.4327	23.4221	6.206E-016
Deviation	34	0.6281	0.0185		
Total	35	1.0609	0.0303		

Goodness of Fit Statistics ...
Coefficient of Determination: 0.407893984
Correlation Coefficient: 0.638665784
Model Selection Criterion: 0.412958468

Parameter Statistics
95.00% Confidence Interval~
Parameter a:1.025063836
StdDev: 0.058194399
Univariate . .
LOW: 0.906798588
HIGH: 1.143329084
Supporting Plane:
LOW: 0.876106693
HIGH: 1.174020979
Parameter b: -0.01986042g
StdDev: 0.004103696
Univariate . .
LOW: -0.028200138
HIGH: -0.011520711
Supporting Plane:
LOW: -0.030369439
HIGH: -0.009356410

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Linear Regression Analysis Results

EXPERIMENT 4 - FIGURE 8

Variables: X = "NEWCOL", Y = "Green_Absorbance"
Equation: Green_Absorbance = 0.0657 + 0.0210* 1 oz (light dosage)

Variable	N	Mean	Variance
NEWCOL	36	3.5934	2.0890
Green_Absorbanc	36	0.1411	0.0018

Regression Coefficient	= 0.020976
Standard Error of B	= 0.003453
Y-Intercept	= 0.065679
R-Squared	= 0.520455
Adjusted R-Squared	= 0.506350
Standard Error of Estimate	= 0.029527

The 95.0% confidence limits for the slope are: [0.0139588, 0.0279941]

Analysis of Variance Table

Source	DF	SS	MS	F	P
Linear	1	0.0322	0.0322	36.9005	6.893E-007
Deviation	34	0.0296	0.0009		
Total	35	0.0618	0.0018		

Goodness of Fit Statistics ...
Coefficient of Determination: 0.520954509
Correlation Coefficient: 0.721425331
Model Selection Criterion: 0.623805406

Parameter Statistics
95.00% Confidence Intervals
Parameter a: 0.065679249
StdDev: 0.013348723
Univariate ...
LOW: 0.038551381
HIGH: 0.092807117
Supportinq Plane:
LOW: 0.031511226
HIGH: 0.099847272
Parameter b: 0.020976436
StdDev: 0.003453153
Univariate ...
LOW: 0.013958786
HIGH: 0.027994087
Supporting Plane:
LOW: 0.012137583
HIGH: 0.029815289

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Linear Regression Analysis Results

EXPERIMENT 4 - FIGURE 10

Variables: X = "NEWCOL", Y = "R/G_ratio"
Equation: R/G_ratio = 1.0700 -0.0847*log (light dosage)

Variable	N	Mean	Variance
NEWCOL	36	3.5934	2.0890
R/G_ratio	36	0.7656	0.0303

Regression Coefficient	= -0.084700
Standard Error of B	= 0.014688
Y-Intercept	= 1.069997
R-Squared	= 0.494442
Adjusted R-Squared	= 0.479573
Standard Error of Estimate	= 0.125595

The 95.0% confidence limits for the slope are: [-0.11455, -0.0548496]

Analysis of Variance Table

Source	DF	SS	MS	F	P
Linear	1	0.5245	0.5245	33.2524	1.732E-006
Deviation	34	0.5363	0.0158		
Total	35	1.0609	0.0303		

Goodness of Fit Statistics ...
Coefficient of Determination: 0.494442003
Correlation Coefficient: 0.703165701
Model Selection Criterion: 0.570981404

Parameter Statistics
95.00% Confidence Interval.
Parameter a: 1.069997486
StdDev: 0.056779913
Univariate ...
LOW: 0.954606819
HIGH: 1.185388152
Supporting Plane:
LOW: 0.924660929
HIGH: 1.215334043
Parameter b: -0.084699809
StdDev: 0.01g688275
Univariate ...
LOW: -0.1145g9974
HIGH: -0.054849643
Supporting Plane:
LOW: -0.122296613
HIGH: -0.047103005

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Linear Regression Analysis Results

EXPERIMENTS 3 & 4 - FIGURE 11

Variables: X = "Incubation_x_1000", Y = "Green_Absorbance"
Equation: Green Absorbance = 0.0705 + 0.0003*Incubation_x_1000

Variable	N	Mean	Variance
Incubation_x_10	72	158.333	12445.784
Green_Absorbanc	72	0.120	0.002

Regression Coefficient = 0.000311

Standard Error of B	= 0.000024
Y-Intercept	= 0.070513
R-Squared	= 0.698164
Adjusted R-Squared	= 0.693852
Standard Error of Estimate	= 0.022993

The 95.0% confidence limits for the slope are: [0.000262453, 0.000360019]

Analysis of Variance Table

Source	DF	SS	MS	F	P
Linear	1	0.086	0.086	161.914	7.003E-20
Deviation	70	0.037	0.001		
Total	71	0.123	0.002		

Goodness of Fit Statistics ...
Coefficient of Determination: 0.698163826
Correlation Coefficient: 0.835561982
Model Selection Criterion: 1.142315324

Parameter Statistics
95.00% Confidence Intervals
Parameter a: 0.070512560
StdDev: 0.004726606
Univariate ...
LOW: 0.061085642
HIGH: 0.079939478
Supporting Plane:
LOW: 0.058690987
HIGH: 0.082334133
Parameter b: 0.000311236
StdDev: 0.000024460
Univariate ...
LOW: 0.000262453
HIGH: 0.000360019
Supporting Plane:
LOW: 0.000250061
HIGH: 0.000372412

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The Types, Distribution, and Intensity of Fishing and Nonfishing Activities Within the Waikiki Marine Life Conservation District and Waikiki-Diamond Head Shoreline Fisheries Management Area

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Abstract

The aim of this investigation was to determine the types, distributions, and intensities of fishing and non-fishing activities in the Waikiki Marine Reserve on Oahu, Hawai'i. The marine reserve consists of two adjacent areas: a marine life conservation district (MLCD) that has been permanently closed to fishing since 1987 and a fisheries management area (FMA) that is open to limited fishing in even number years. The area was surveyed from June 24 through August 10, 1998. Fishing was most prevalent in the FMA, while non-fishing activities were more evenly distributed in both areas. Fishing activities continued throughout all hours of the day, while non-fishing activities were minimal from dusk to dawn. More fishing and non-fishing activities were observed on weekends than weekdays.

Introduction

Coral reefs have remarkable productivity when compared to other parts of the tropical ocean. The majority of coral reefs are exploited in a variety of ways, ranging from "low impact" tourism, such as recreational diving, to "high impact" extractive uses such as fishing for food fish and collecting animals for aquarium trade (Stewart, 1993). Hawai'i's reefs are not an exception. In 1994, Richard Grigg analyzed reef fish decline in Hawai'i possibly associated with sewage outfalls (Grigg, 1994). Grigg concluded that overfishing was the cause of the decline rather than the sewage. Fishing is recognized as the largest threat to coastal biodiversity and targeted species through reductions of fishery populations and disruption of multi-species assemblages (Russ and Alcala, 1989; Safina, 1997).

Exploitive fishing techniques add selective pressures upon fish populations, including local extirpation (Man *et al.*, 1995; Russ and Alcala, 1989). Extractive fishing may result in strong artificial selection gradients, which may lead to decreases in growth and minimum size at first maturity. For example, the reproductive age of the Arctic cod (*Gadus morhua)* has dropped by one to four years in the last 80 years, while the average size of Chinook salmon (*Onchorhynchus gorbuscha)* has declined more than 50 percent in the last 60 years (Law, 1991; Bohnsack, 1993). Hypothetically, reef fish may be subject to similar artificial selection.

Fishery management includes establishment and enforcement of size limits, closed seasons, restricted areas, limited entry, and quotas or bag limits. Some of these management techniques have been ineffective or too expensive to monitor (Bohnsack, 1993). Marine reserves act as restricted areas and offer several advantages. Marine reserves are easier to manage due to reduced needs for funding and personnel (Roberts and Polunin, 1991).

In theory, marine refuges should protect a population of adults so they can grow to large, highly fecund sizes. Protecting breeding stocks of large adults from fishing should aid in recovery of exploited species, because egg production increases exponentially with fish size (Roberts, 1993). Therefore, a few large fish are as productive as many small fish (Bohnsack, 1993; Roberts, 1993). Marine life conservation areas may help support fisheries in adjacent areas via export of fish eggs, larvae, juveniles, and adults (Alcala 1988; Carr and Reed, 1993). Fish export through larval recruitment is nearly impossible to quantify (Carr and Reed, 1993; Man *et al.*, 1995; Dugan and Davis, 1993; Polacheck, 1990). Refuges adjacent to fishing grounds may provide positive benefits to the fish and fishers, though the mechanisms are essentially unknown (Carr and Reed, 1993; Polacheck, 1993; Roberts and Polunin, 1991). Reserves have the potential to enhance biomass but factors like the magnitude of fishing effort in the non-closed areas are important to know, to determine how effective the reserve is (DeMartini,1993). Export of reef fish biomass is best recorded on Apo Island in the Philippines. Local fishers reported that their catch, including Lutjanids, Carangids, and Serranids, had increased by fifty percent or more after ten percent of the fishing grounds was set aside as a refuge for a decade (Russ and Alcala, 1996).

The Waikiki Reserve consists of two adjacent zones, each subject to different management regulations (Figure 1). Removal of marine life has been permanently prohibited for ten years in the Marine Life Conservation District (MLCD), whereas the neighboring area is a Fisheries Management Area (FMA) that undergoes alternate years of harvest (even-numbered years) and closure (odd-numbered years). Any fishers observed in the MLCD, and any fishers observed in the FMA, using a spear between 1800 and 0600 hours, or possessing any trap or net except for throw nets, were considered unlawful. Signs posted in the reserve show the boundaries, the regulations, and a number to call, for the public to report any unlawful activities. Both areas are also used year round for non-extractive ocean recreational activities. The FMA adjacent to the MLCD provides a perfect study site because, Marine Life Conservation Districts in Hawai'i may represent an undisturbed reef fish community and have standing crops of reef fish higher than areas open to fishing, which may export biomass to adjacent fishing areas (Grigg, 1994).

The aim of this investigation was to determine the types, distributions, and intensities of fishing and non-fishing activities in the Waikiki MLCD and FMA. This study was designed to complement research currently underway in the reserves utilizing visual census, tag and release methods, and ultrasonic telemetry to determine population structures and movements of fish populations.

Methods and Materials

Data collection was facilitated by use of prepared data sheets provided by the Division of Aquatic Resources (Appendix A). The area of the MLCD and FMA (approximately 1,200,000 m^2) was surveyed in two-hour time periods. The survey route was designed around a paved path and easily accessible viewing areas of the study site (Figure 2).

Seventy-five surveys were completed totaling 132 hours, covering all days of the week, from June 24 until August 10 (Table 1). Materials used were Bushnell binoculars (8 x 32) and bicycles. The outer reserve boundary is 500 yards seaward from the high water mark or to the edge of the first reef, which ever is further. When the reef was not visible, this boundary was difficult to determine; therefore the color change of the ocean from light blue to dark blue was used. The inner reserve boundary was defined by coastline from the Kapahulu Groin to the Diamond Head Lighthouse (Figure 1). As many fishers as possible were approached and asked about their catch. Any catch was identified by species or genus, and the total length of each fish was visually estimated.

Fishing and non-fishing activities occurring within the two-hour time periods were recorded (Appendix B). Each person or vessel was counted as one activity. For example, two fishers observed in the same area between 1700 and 2000 hours, were recorded as two activities for both the 1600 to 1800 hours survey, and the 1800 to 2000 hour survey (Appendix A).

Statistical analysis included chi-square distributions, correlation coefficients, means, and percentages. The chi-square analysis was used to determine if fishing or non-fishing activities were more significant in the FMA or MLCD, or more significant on weekdays or weekends. Correlation coefficient was used to see if there was any correlation between fishing and non-fishing activities. Means were used to determine the mean number of fish captured by successful fishers. Percentages were used to determine the prominent fish families caught by spear and rod and reel methods.

Results

Seven different fishing methods were observed in the Waikiki Reserve (Figure 3, Appendix B). Chi -square analysis determined a 97.5% significance level of fishing within the FMA versus the entire Waikiki Reserve. Fishing activities peaked between 1600 and 1800 hours in the FMA (Figure 4). There was no correlation between fishing and non-fishing activities per time interval. Chi-square analysis determined that fishing activities were independent of weekends and weekdays. Thursday and Friday were the days that fishing activities were most common, while negligible amounts of fishing occurred on Mondays (Figure 5).

1. **Rod and Reel**: The primary fishing method observed was rod and reel (Figures 3, 4). A total of 340 individual rod and reel fishers were observed, and four percent were unlawfully fishing within the MLCD. Of the total 422 fishing events observed in the FMA, 73 percent were rod and reel fishers. Twenty rod and reel fishers were interviewed and 65 percent had no catch. Approximately six percent of the rod and reel fishers observed were unexpectedly interviewed. The mean number of fish caught by the seven successful fishers was 1.1.

Mullidae was the family most commonly captured by the interviewed rod and reel fishers, accounting for 38 percent of all fish captured (Table 2).

2. **Spear**: The next most frequent fishing activity was spearfishing (Figures 3, 4). Over the study period 107 spearfishing events were observed. Five percent of spearfishing was observed within the MLCD during weekdays only. Of the total fishing activities observed in the FMA, 23 percent were spearfishing. Eleven percent of spearfishing was observed outside of the permitted time period. Seventeen percent of the spearfishermen observed were interviewed. A total of 18 spearfishers were asked about their catch, and 22 percent

had none. The mean number of fish caught by the 14 successful fishers was 1.4. Acanthuridae and Mullidae were the most important fish families in spearfishing catches, accounting for 53 percent and 16 percent of the total fish catch respectively. Six octopi were also observed as spearfishing catches.

3. **Handpole**: Handpoles are also known as liftpoles or *o'ama* poles. Nine individuals using handpoles were observed. Seven of the handpole events were observed on weekends and one handpole was used illegally within the MLCD. No catch was observed.
4. **Other**: Throwmets, scoopnets, gillnets, and handlines were also observed, all within the FMA. No catch was observed.

Six categories of non-fishing activities were observed (Figure 3, Appendix B). In contrast to fishing activities, non-fishing activities occurred independently of region (FMA and MLCD). Eighty-two percent of all the activities observed were non-fishing activities. The peak hours of non-fishing activities were between 1000 and 1200 hours (Figure 4). Chi-square analysis determined that non-fishing activities were independent of weekends versus weekdays. Non-fishing activities were most common on Sundays and least common on Fridays (Figure 5).

1. **Surfboard**: A total of 1312 surfboard activities were observed. Surfboard activities accounted for 60 percent of observed non-fishing activities and 33 percent were within the MLCD. The large number of surfers skewed comparison between fishing and non-fishing activities, so surfboard activities were excluded from other data analysis.
2. **Sailboat**: A total of 268 sailboats accounted for 12 percent of the non-fishing activities. Forty-four percent of sailboats were observed within the MLCD.
3. **Motorboat**: A total of 214 were observed, comprising ten percent of non-fishing activities. Thirty-seven percent of motorboats were observed within the MLCD.
4. **Other**: A total of 203 kayaks comprised nine percent of the non-fishing activities. A total of 178 canoes accounted for eight percent of the non-fishing activities. Jetskis and windsurfers made up less than one percent of all the observed non-fishing activities.

Discussion

Extraction of fish species from twelve families and one of invertebrate species was observed from the FMA. According to the observations, the MLCD appeared to offer reef fish protection from many types of fishing activities. No fish were observed to be extracted from the MLCD, although there is a possibility that the fishers were discreetly performing this illegal activity. There was significantly less fishing within the MLCD than in the FMA, which may be due to the difference in shoreline accessibility, the time that the reserve has been in place, or the local community abiding the laws of the reserve.

Enforcement of fishing regulations in the Waikiki Reserve is primarily based upon public reports of illegal fishing activities. Significantly, few illegal fishing activities were observed other than spearfishing outside of the permitted time periods. If increased enforcement of fishing regulations is desired, it is recommended that enforcement focus on night spearfishing. Additionally, if fish capture within the FMA is to be reduced; rod and reel fishing would have to be limited to the daytime. However, the actual efficiency of nighttime rod and reel fishing is not currently known.

Although the study was brief, the activity data identified the most common types and temporal distribution of effort. Peak hours of fishing and non-fishing activities within the MLCD and the FMA during the study period were also identified. The following suggestions would improve future surveys of this type. A control site outside of the FMA and MLCD could have been included in the study to compare the Waikiki Reserve to adjacent non-restricted fishing areas. But, the two zones adjacent to the Waikiki Marine Reserve include Diamond Head Point, which is prone to high winds and large surf, or Waikiki Beach, which contains different habitat than the FMA and MLCD. A control site was not surveyed because the adjacent zones are not comparable to the Waikiki Reserve for the reasons mentioned above. However, the alternate fishing years of the FMA may act as a control site for annual fishing activities surveys.

The survey should have included counts of observed swimmers, snorkelers, and scuba divers. These data points would have been useful to determine if the MLCD is preferred for

these activities. A possible advantage of a MLCD, is that it provides a chance to see natural assemblages within the Waikiki area (Stewart, 1993).

In addition, a grid-square analysis of the study area would have been helpful, with the marked location of all activities, indicating where most of the activities occurred. For example, the movements of spearfishers could be observed and documented from a kayak by using the Global Positioning System and a grid square map. Variation in data could be a result of the following: seasonal water conditions on the South shore, a holiday on July 4th occurred during the study period, the daily effort of data sampling, and data collection during the evening.

One of the many objectives of marine reserves is protection of a critical spawning biomass to ensure recruitment supply to adjacent fish areas via larval dispersal or by adult movements (Russ and Alcala, 1996). If juvenile fish are found within fished areas, it can be assumed there is recruitment, though it is unknown where the parents reside without genetic analysis. Future studies should be made to determine if artificial selection is changing reef fish populations, and what types of management techniques may be employed to maintain genetic diversity. If a current catch per unit effort study was conducted within the FMA, it could be compared with current visual census, tag and recapture, and ultrasonic telemetry data to gauge the effects of the fishing, and provide insight into basic types of fish extracted from the FMA. Studies relative to these will determine if Hawai'i reef fisheries can be maintained through reserves.

Acknowledgments

Our thanks to: Carl Meyer, Kim Holland, Kimberly Lowe, Sherwood Maynard, Rose Pfund, the Marine Option Program, Sea Grant, and DLNR. Kimberly Lowe provided us with observation equipment and helped us compile our data concisely. We received several ideas for approaching fishers and data analysis from Carl Meyer and Kim Holland. Sherwood Maynard offered support and feedback after our bi-weekly reports, which kept the project organized and directed.

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Figures

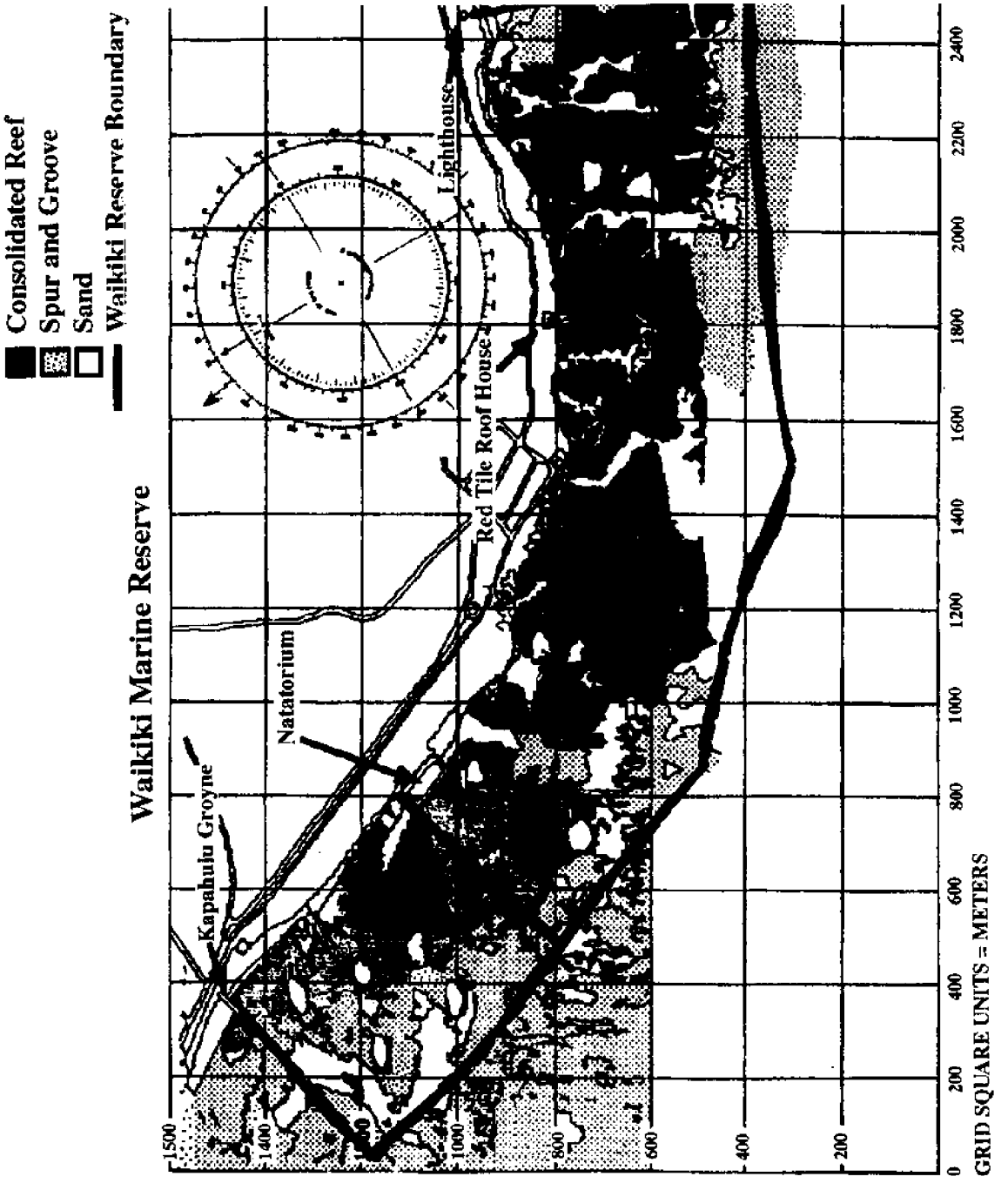


Figure 1. Marine Life Conservation District and Fisheries Management Area within Waikiki Diamond Head Fisheries Area. Oahu, Hawai'i (Carl Meyer, pers. Comm.).

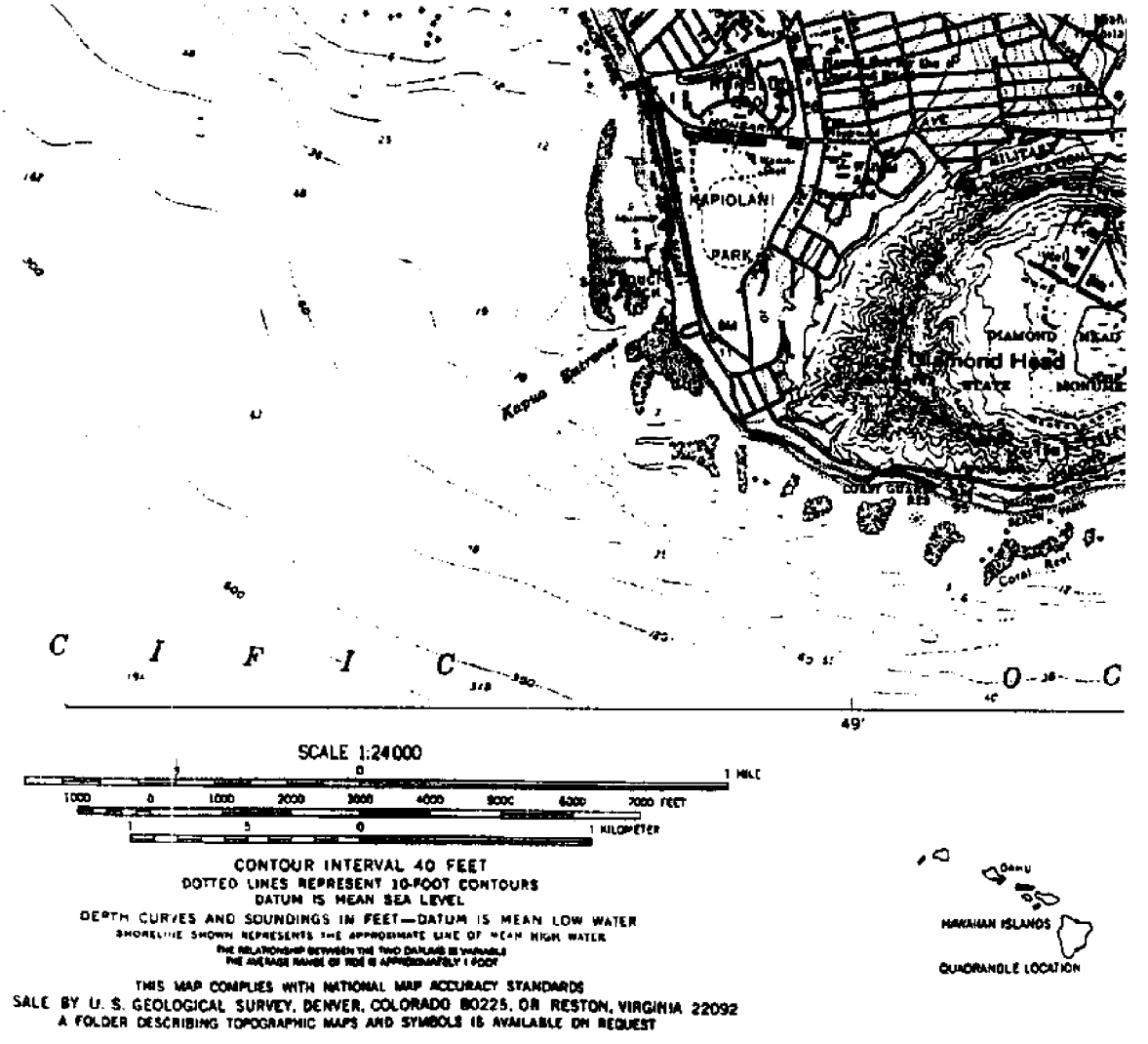


Figure 2. Survey route.

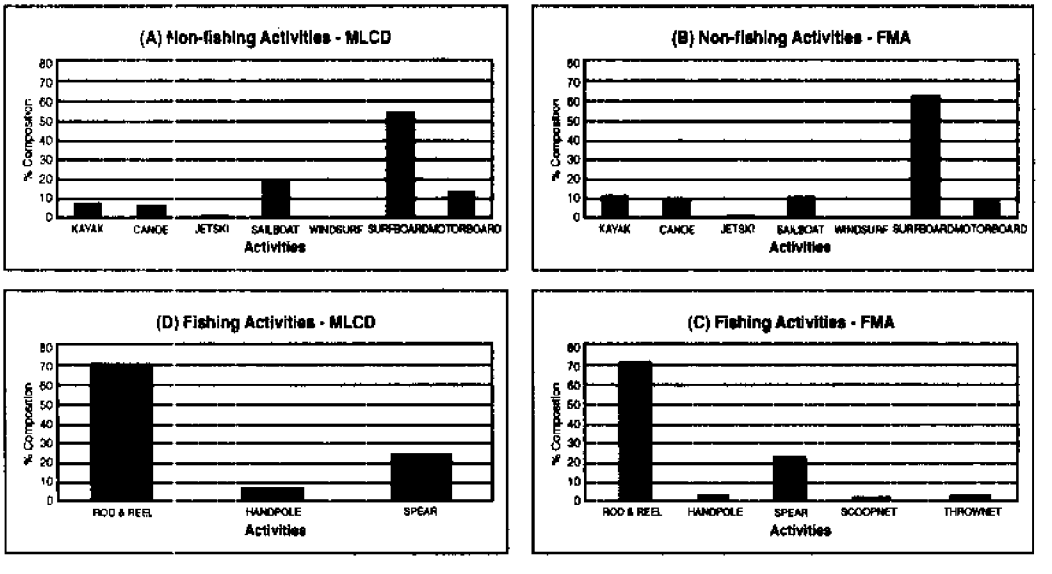
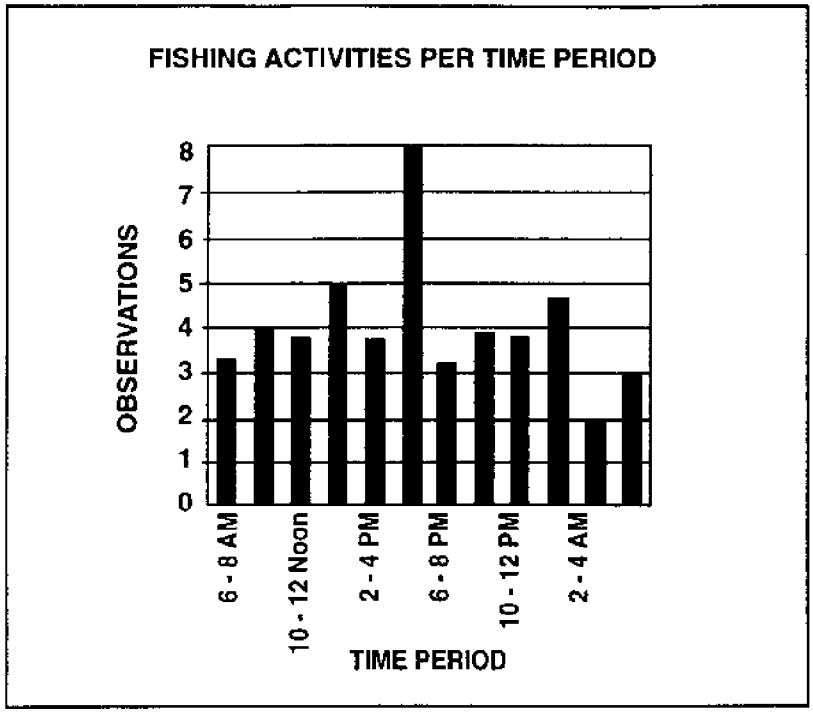


Figure 3. Percent composition of non-fishing (a, b) and fishing activities (c, d) in the FMA and MLCD.



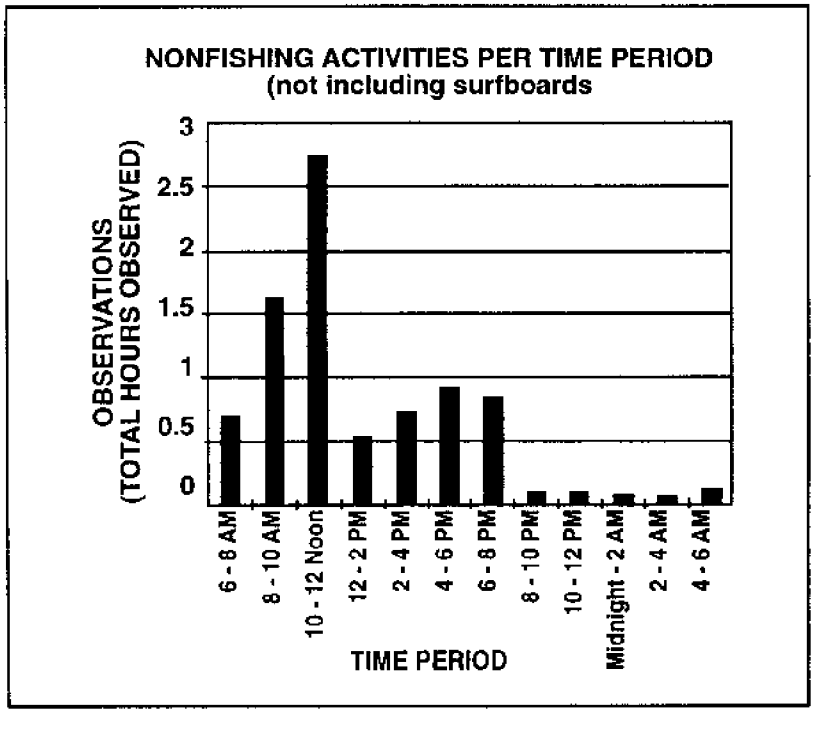


Figure 4. Peak hours of fishing (a) and non-fishing (b) activities per time period.

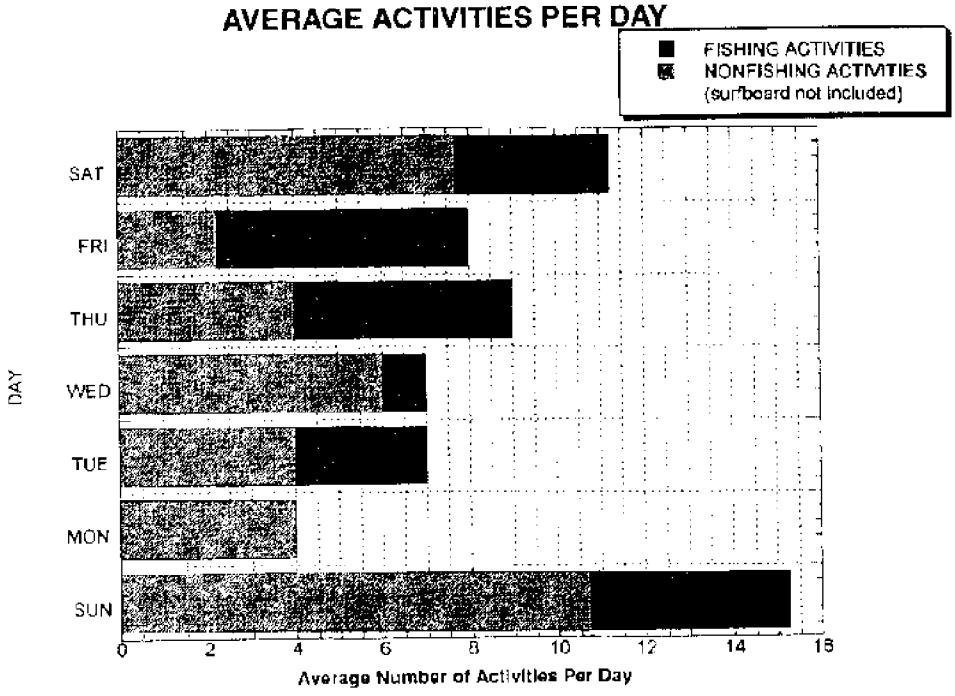


Figure 5. Average activities per day.

Tables

Table 1. Sampling effort per time interval

12-2AM(A)	2-4AM(B)	4-6AM(C)	6-8AM(D)	8-10AM(E)	10-12PM(F)	12-2PM(G)	2-4PM(H)	4-6PM(I)	6-8PM(K)	8-10PM(M)	10-12AM(N)
7/10(fri)	7/10(fri)	7/10(fri)	7/7 (tue)	7/7 (tue)	11-12/6/739(mon)	7/7 (tue)	7/2 (thur)	7/8 (wed)	7/1 (wed)	7/5 (sun)	7/8 (wed)
7/20(mon)	7/20(mon)	7/20(mun)	7/3 (fri)	6/28(sun)	11-30-12-6/27 (sat)	6/27(sun)	7/7 (tue)	7/4 (sat)	7/8 (wed)	7/19(sun)	7/19(sun)
7/25(sun)	7/25(sun)	7/25(sun)	7/20(mon)	7/3 (fri)	10-25-12-6/28 (sun)	6/29(mon)	7/8 (wed)	7/4 (sat)	7/19(sun)	7/23(thu)	7/27(mon)
7/7 (fri)	7/7 (fri)	7/7 (fri)	7/7 (fri)			7/3 (mon	7/30(thu)	7/9 (thu)	7/21(tue)	7/24(fri)	7/25(sun)
7/9 (sun)	7/9 (sun)	7/9 (sun)	7/9 (sun)			7/16(thu)	2/3-6/25 (thu)	7/9 (thu)	7/2 (sun)	7/25(sat)	10-4-D-11-30/7/3(fri)
						7/25(sat)	3-4-6/24 (wed)	4056/7/5(sun)	6058/7/25(sat)	7/3(fri)	10-05-12-7/9 (thu)
						12-1-30-7/21 (tue)	3-4-6/24 (wed)	4056/7/5(sun)	6058/7/25(sat)	7/2 (thu)	
						1-30-2/7/4(sat)		4-5-6/25 (thu)	5-50/7/40/29(mon)		8/9-3/5/7/4(sat)
								5-6-6/27(sat)	7/1-18-6/27 (sat)		

Table 2. The types, size, location, and type of fishing activity observed.

FAMILY NAME	LOCAL NAME	NUMBER	SIZE(cm)	LOCATION OF CATCH	METHOD
Mullidae	moana	1	16	FMA-Makalei Beach Park	rod and reel
Mullidae	moana	1	20	FMA-San Souci Beach Park	rod and reel
Mullidae	weke-'ula	1	30	FMA-Outrigger Canoe Club beach	rod and reel
Carangidae	'omilu or papio	1	30	FMA-Outrigger Canoe Club beach	rod and reel
Labridae	'awela	1	18	FMA-Makalei Beach Park	rod and reel
Balistidae	humuhumu...	1	25	FMA-Makalei Beach Park	rod and reel
Priacanthidae	'aweoweo	1	N/A	FMA-location unknown	rod and reel
**Kuhliidae	aholehole	8	5	MLCD margin, possible poaching	rod and reel
**Holocentridae	'ala'ihi	1	10	MLCD margin, possible poaching	rod and real
TOTAL	**8 species**	**16**			
Acanthuridae	palani	1	18	FMA-San Souci Beach Park	spear
Acanthuridae	palani	2	15	FMA-San Souci Beach Park	spear
Acanthuridae	ma'i'i'i	3	15	FMA-San Souci Beach Park	spear
Acanthuridae	manini	1	15	FMA-San Souci Beach Park	spear
Acanthuridae	kala	1	35	FMA-San Souci Beach Park	spear
Acanthuridae	kala	1	55	FMA-San Souci Beach Park	spear
Mullidae	moana	2	28	FMA-San Souci Beach Park	spear
Mullidae	moana	1	20	FMA-Diamond Head Beach Park (DHBP)	spear
Mullidae	moana kea	1	22	FMA-San Souci Beach Park	spear
Mullidae	kumu	1	25	FMA-San Souci Beach Park	spear
Scaridae	'ele'ele	1	50	FMA-San Souci Beach Park	spear
Pomacentridae	mamo	1	17	FMA-San Souci Beach Park	spear
Aulostomidae	nunu	1	50	FMA-San Souci Beach Park	spear
Holocentridae	'u'u	2	20	FMA-San Souci Beach Park	spear
Octopodidae	tako	6	N/A	FMA-San Souci Beach Park & DHBP	spear
TOTAL	**12 species**	**25**			

Appendices

Appendix A. Creel Survey Form Used For The Observations

Date ____/___/9___ DAY: S M T W Th F Sa Holiday Sea State: 0 1 2 3

Wind direction: mph 1) Kona (SW)____ 2) Trades ____ 3) Other (specify)_____

Rainfall: 1) Dry 2) Rainy 3) Variable

Cloud Cover: 1) Clear 2) Partly Cloudy 3) Mostly Cloudy

Time Period (circle the correct time period:

A)12-2 am	B)2-4 am	C)4-6 am	D)6-8 am	E)8-10 am	F)10-12	G)12-2pm	H)2-4pm	I)2-4pm	J)4-6pm	K)6-8pm	M)8-10pm	N)10p-12a

AREA	PLEASURE BOATS						
	Kayak	Canoe	Jetski	Sailboat	Windsurf	Surf	Motorized
MLCD							
FMA I							
FMA II							

Geartype	Gear Code	Tally of Fishers by Geartype		
		Beginning of Period	Additional Arrivals (End)	Final
Rod & Reel	1			
Handpole (bamboo)	2			
Spear	3			
Scoopnet	4			
Gillnet	5			
Surroundnet	6			
Thrownet	7			
Handpick	8			
Handline	9			
Bow & Arrow	10			
Crabnet	11			
Crossnet	12			
Other (specify)				

Time started tally _____ Time completed tally _____

Comments _____

_____

APPENDIX B: RAW DATA

NONFISHING ACTIVITIES

			KAYAK		CANOE		JETSKI		SAILBOAT		WINDSURF		SUREBOARD		MOTORBOAT		
Date	Time Start	Time End	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	Observer
29-Jun	11:00AM	12:00PM	1	4	0	0	0	0	0	2	0	0	4	14	0	3	SABRINA
29-Jun	12:00PM	2:00PM	1	2	0	0	0	0	3	9	0	0	7	34	1	0	SABRINA
29-Jun	5:40PM	7:40PM	7	19	5	9	0	0	2	4	0	0	12	22	2	4	SABRINA
30-Jul	12:10AM	1:30AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
30-Jul	3:00AM	4:00AM	0	0	0	0	0	0	0	0	0	0	0	0	1	1	YHAZI
30-Jul	4:00AM	6:55AM	0	0	0	0	0	0	0	0	0	0	0	0	1	5	SABRINA
30-Jul	6:00AM	8:00AM	1	3	0	1	0	0	0	1	0	0	0	7	1	0	YHAZI
27-Jul	10:00PM	12:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
28-Aug	12:00PM	2:00PM	0	7	0	1	0	0	0	0	0	0	12	32	0	0	YHAZI
30-Jun	4:15PM	6:00PM	0	2	0	1	0	0	0	2	0	0	0	0	1	0	SABRINA
30-Jun	6:00PM	8:00PM	4	2	6	1	0	0	2	1	0	0	2	18	3	1	SABRINA
02-Jul	2:00PM	4:00PM	0	3	0	2	0	1	0	3	0	0	4	29	0	0	YHAZI
02-Jul	8:00PM	10:00PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
07-Jul	6:20AM	8:00AM	0	1	0	1	0	0	1	0	0	0	1	3	0	0	SABRINA
07-Jul	8:00AM	10:00AM	0	0	0	5	0	0	0	1	0	0	0	14	0	1	SABRINA
07-Jul	12:00PM	2:00PM	0	2	0	2	0	0	0	5	0	0	3	8	0	2	YHAZI
07-Jul	2:00PM	4:00PM	0	1	0	2	0	0	0	5	0	0	4	22	0	2	YHAZI
21-Jul	12:00PM	1:30PM	0	2	0	1	0	0	0	2	0	0	7	16	0	0	YHAZI
21-Jul	6:00PM	8:00PM	0	0	0	14	0	0	1	2	0	0	6	32	2	3	SABRINA
24-Jun	3:00PM	4:00PM	0	0	0	1	0	0	1	1	0	0	3	5	0	0	YHAZI
01-Jul	6:00PM	8:00PM	6	8	11	16	0	0	5	4	0	0	14	16	1	4	SABRINA
08-Jul	4:00PM	6:00PM	0	6	0	0	0	0	0	1	0	0	10	36	0	0	YHAZI
08-Jul	2:00PM	4:00PM	0	1	0	1	0	0	0	4	0	0	0	23	0	0	YHAZI
08-Jul	6:00PM	7:30PM	0	0	3	4	0	0	4	3	0	0	0	18	1	3	SABRINA
08-Jul	10:00PM	12:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
25-Jun	2:00PM	3:00PM	0	1	0	1	0	0	0	3	0	3	9	12	0	4	YHAZI
25-Jun	4:00PM	5:00PM	0	3	0	2	0	0	5	5	0	0	4	0	0	0	SABRINA
16-Jul	12:00PM	2:00PM	1	0	0	0	0	1	2	3	0	0	1	19	0	1	YHAZI
09-Jul	4:00PM	6:00PM	0	10	0	3	0	0	2	5	0	0	16	37	0	2	YHAZI
09-Jul	10:05PM	12:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
24-Jul	8:00PM	10:00PM	0	0	0	0	0	0	0	1	0	0	0	0	1	1	SABRINA
03-Jul	6:00AM	8:00AM	2	5	2	4	0	0	0	0	0	0	3	15	0	0	SABRINA
03-Jul	8:00AM	10:00AM	4	6	0	11	0	0	2	14	0	0	11	36	2	3	SABRINA
03-Jul	10:40PM	11:10PM	0	0	0	0	0	0	1	0	0	0	0	0	0	0	SABRINA
10-Jul	4:00AM	6:00AM	1	0	0	0	0	0	0	0	0	0	0	2	0	2	SABRINA
10-Jul	2:00AM	4:00AM	0	0	0	0	0	0	0	0	0	0	2	0	0	0	YHAZI
10-Jul	12:00PM	2:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
24-Jul	8:00PM	10:00PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
24-Jul	10:00PM	12:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
27-Jun	11:30PM	12:00PM	0	3	1	3	1	0	2	1	0	0	4	9	2	0	SABRINA
27-Jun	12:00PM	2:00PM	0	1	0	0	0	0	0	4	0	0	0	31	0	2	SABRINA
27-Jun	5:00PM	6:00PM	0	2	0	1	0	0	3	1	0	0	15	5	1	0	SABRINA
27-Jun	7:15PM	8:00PM	0	1	0	0	0	0	1	0	0	0	7	2	0	0	YHAZI
25-Jul	12:00AM	2:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	1	YHAZI
25-Jul	2:00AM	4:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
25-Jul	4:00AM	6:00AM	0	0	0	0	0	0	0	0	0	0	0	2	0	1	SABRINA
25-Jul	12:00PM	2:00PM	0	5	0	2	1	0	8	10	0	0	12	49	2	4	SABRINA
25-Jul	6:05PM	8:00PM	0	0	0	0	0	0	5	1	0	0	8	20	3	2	SABRINA
25-Jul	8:00PM	10:00PM	0	0	0	0	0	0	5	0	0	0	0	0	0	0	SABRINA
26-Jul	10:00PM	12:00AM	0	0	0	0	0	0	5	0	0	0	0	0	0	0	SABRINA
28-Jun	8:00AM	10:00AM	0	7	0	0	0	0	0	0	0	0	12	8	0	0	YHAZI
28-Jun	10:25AM	12:00PM	2	9	0	11	1	1	4	4	0	0	10	52	3	3	SABRINA
05-Jul	4:05PM	6:00PM	0	3	0	3	0	0	8	9	0	0	15	37	2	2	SABRINA
05-Jul	8:00PM	10:00PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
19-Jul	6:00PM	8:00PM	3	1	2	8	0	0	8	8	0	0	9	17	4	4	SABRINA
19-Jul	8:00PM	9:45PM	0	0	0	0	0	0	0	0	0	0	0	0	2	2	SABRINA
19-Jul	10:35PM	12:00AM	0	0	0	0	0	0	0	0	0	0	0	0	2	2	YHAZI
2-Aug	6:00PM	8:00PM	0	4	0	3	0	0	0	0	0	0	25	40	1	2	YHAZI
7-Aug	12:00AM	2:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
7-Aug	2:00AM	4:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
7-Aug	4:00AM	6:00AM	0	0	0	0	0	0	0	0	0	0	0	3	0	0	SABRINA
7-Aug	6:00AM	8:00AM	1	1	0	3	0	0	0	0	0	0	0	4	0	2	SABRINA
9-Aug	12:00AM	2:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
9-Aug	2:00AM	4:00AM	0	0	0	0	0	0	0	0	0	0	0	0	1	0	YHAZI
9-Aug	4:00AM	6:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
9-Aug	6:00AM	8:00AM	0	4	2	9	0	0	0	0	0	0	3	20	1	10	SABRINA
10-Aug	10:00AM	12:00PM	2	4	0	0	0	0	0	0	0	0	5	15	0	3	YHAZI
30-Jul	2:00PM	4:00PM	0	2	0	1	0	0	2	2	0	0	11	33	0	0	YHAZI
04-Jul	1:30PM	2:00PM	0	5	0	1	0	2	0	3	0	1	0	29	0	2	SABRINA
04-Jul	3:00PM	4:00PM	2	6	1	4	1	0	18	5	0	0	15	33	15	3	SABRINA
04-Jul	4:00PM	6:00PM	3	4	0	0	0	0	0	1	0	0	13	30	5	1	YHAZI
04-Jul	4:00PM	6:40PM	3	6	2	12	1	2	14	10	0	0	26	34	14	4	SABRINA
04-Jul	8:00PM	9:35PM	0	0	1	0	0	0	2	2	0	0	0	0	2	0	SABRINA
10-Aug	2:00PM	4:00PM	0	5	0	0	0	0	0	3	0	0	6	19	1	7	YHAZI
TOTAL			43	161	35	143	6	7	118	144	0	4	331	986	80	122	
Total number of hours		131.75															

note: Activities not observed are not listed (Appendix A).

FISHING ACTIVITIES

			ROD AND REEL		HANDPOLE		SPEAR		SCOOPNET		GILLNET		THROWNET		HANDLINE		
Date	Time Start	Time End	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	MLCD	FMA	Observer
29-Jun	11:00AM	12:00PM	0	1	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
29-Jun	12:04PM	2:00PM	0	5	0	0	0	3	0	0	0	0	0	0	0	1	SABRINA
29-Jun	5:50PM	7:40PM	0	1	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
29-Jul	12:10AM	1:50AM	0	0	0	0	0	2	0	0	0	0	0	0	0	0	SABRINA
29-Jul	3:00AM	4:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
29-Jul	4:00AM	5:55AM	0	2	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
29-Jul	6:00AM	8:00AM	0	0	0	1	0	0	0	0	0	0	0	0	0	0	YHAZI
27-Jul	10:00PM	12:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
01-Aug	12:00PM	2:00PM	0	3	0	0	0	2	0	0	0	1	0	0	0	0	YHAZI
30-Jun	4:15PM	6:00PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
30-Jun	6:--PM	8:00PM	0	15	0	0	0	6	0	0	0	0	0	0	0	0	SABRINA
02-Jul	2:00PM	4:00PM	0	0	0	0	0	1	0	0	0	0	0	0	0	0	YHAZI
27-Jul	8:00PM	10:00PM	0	18	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
27-Jul	6:20AM	8:00AM	0	5	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
03-Jul	8:00AM	10:00AM	0	5	0	0	4	0	0	0	0	0	0	0	0	0	SABRINA
27-Jul	12:00PM	2:00PM	0	2	0	0	0	7	0	0	0	0	0	0	0	0	YHAZI
27-Jul	2:00PM	4:00PM	0	1	0	0	0	4	0	0	0	0	0	0	0	0	YHAZI
21-Jul	12:10PM	1:30PM	0	2	0	0	1	6	0	0	0	0	0	0	0	0	YHAZI
21-Jul	6:00PM	8:00PM	2	2	0	0	0	7	0	0	0	0	0	0	0	0	SABRINA
24-Jun	3:00PM	4:00PM	0	0	0	0	0	4	0	0	0	0	0	0	0	0	YHAZI
31-Jul	6:00PM	8:00PM	1	4	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
28-Jul	3:00PM	6:00PM	0	0	0	0	0	4	0	0	0	0	0	0	0	0	YHAZI
08-Jul	2:00PM	4:00PM	0	5	0	0	0	2	0	0	0	0	0	0	0	0	YHAZI
08-Jul	6:00PM	7:50PM	0	2	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
08-Jul	10:00PM	12:00AM	0	7	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
25-Jun	2:00PM	4:00PM	0	0	0	0	0	9	0	0	0	0	0	0	0	0	YHAZI
25-Jun	4:00PM	5:00PM	0	0	0	0	0	2	0	0	0	0	0	0	0	0	SABRINA
16-Jul	12:00PM	2:00PM	0	9	0	0	0	15	0	0	0	0	0	0	0	0	YHAZI
29-Jul	4:00PM	6:00PM	0	10	0	0	0	2	0	0	0	0	0	0	0	0	YHAZI
09-Jul	10:05PM	12:00AM	0	13	0	0	0	1	0	0	0	0	0	0	0	0	SABRINA
23-Jul	8:00PM	10:00PM	0	13	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
13-Jul	6:00AM	8:00AM	0	12	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
13-Jul	8:00AM	10:00AM	2	2	0	0	0	5	0	1	0	0	0	0	0	0	SABRINA
13-Jul	10:40PM	11:10PM	0	2	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
10-Jul	4:00AM	6:00AM	0	2	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
10-Jul	2:00AM	4:00AM	0	2	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
10-Jul	12:00PM	2:00AM	0	9	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
24-Jul	6:00PM	10:00PM	0	6	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
24-Jul	10:00PM	12:00AM	0	9	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
27-Jun	11:30PM	12:00PM	0	0	0	0	0	4	0	0	0	0	0	0	0	0	SABRINA
27-Jun	12:00PM	2:00PM	0	1	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
27-Jun	5:00PM	6:00PM	3	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
27-Jun	7:11PM	8:00PM	0	5	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
25-Jul	12:03AM	2:00AM	0	8	0	0	0	2	0	0	0	0	0	0	0	0	YHAZI
25-Jul	2:00AM	4:00AM	0	7	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
25-Jul	4:00AM	6:10AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
25-Jul	12:00PM	2:10PM	0	2	0	0	0	4	0	0	0	0	0	0	0	0	SABRINA
25-Jul	6:05PM	8:00PM	0	8	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
25-Jul	8:00PM	10:00PM	0	13	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
25-Jul	10:00PM	12:00AM	2	7	0	0	0	5	0	0	0	0	0	0	0	0	SABRINA
28-Jun	8:00AM	10:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
28-Jun	10:25AM	12:00PM	3	2	0	0	0	5	0	0	0	0	0	0	1	0	SABRINA
05-Jul	4:05PM	6:03PM	0	4	0	0	0	1	0	0	0	0	0	0	0	0	SABRINA
05-Jul	8:09PM	10:00PM	0	1	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
18-Jul	6:00PM	8:00PM	0	6	0	0	0	0	0	2	0	0	0	0	0	0	SABRINA
19-Jul	8:00PM	9:43PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
19-Jul	10:25PM	12:00AM	0	2	0	1	0	0	0	0	0	0	0	0	1	0	YHAZI
2-Aug	6:00PM	8:00PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
2-Aug	12:00AM	2:00AM	0	5	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
2-Aug	2:00AM	4:00AM	0	6	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
2-Aug	4:00AM	6:00AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
2-Aug	6:00AM	8:00AM	0	4	0	0	0	2	0	0	0	0	0	0	0	0	SABRINA
9-Aug	12:00AM	2:00AM	0	18	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
9-Aug	2:00AM	4:00AM	0	3	0	0	0	0	0	0	0	0	0	0	0	0	YHAZI
9-Aug	4:00AM	6:00AM	0	0	0	3	0	0	0	0	0	0	0	0	0	0	SABRINA
9-Aug	6:00AM	8:00AM	1	7	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
10-Aug	10:00AM	12:00PM	0	0	0	0	0	0	0	0	0	0	0	0	1	0	YHAZI
30-Jul	2:00PM	4:00PM	0	6	1	0	0	2	0	0	0	0	0	2	0	0	YHAZI
28-Jul	1:30PM	2:00PM	0	0	0	0	0	2	0	0	0	0	0	0	0	0	SABRINA
24-Jul	3:00PM	4:00PM	0	3	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
24-Jul	5:00PM	6:00PM	0	0	0	2	0	0	0	0	0	0	0	0	0	0	YHAZI
24-Jul	4:00PM	6:00PM	0	7	0	1	0	0	0	0	0	0	0	0	0	0	SABRINA
24-Jul	8:00PM	9:35PM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SABRINA
10-Aug	2:00PM	4:00PM	0	1	0	0	0	3	0	0	0	0	0	0	0	0	YHAZI
TOTAL			14	307	1	8	5	97	0	3	0	1	0	5	0	1	
Total number of hours			131.75														

note: Activities not observed are not listed (Appendix A)

Transplanting Black Coral as a Restoration Project

Principal Investigator:
Anthony Montgomery
Assistant Aquarium Biologist
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Advisors:
Dr. Bruce Carlson, Director
Dr. Cindy Hunter, Curator
Waikiki Aquarium

Final Report:
November 1998

Abstract

Black coral (*Antipathes dichotoma* and *A. grandis*) has been collected from Hawaiian waters since 1958 for the purpose of jewelry production. Since then many divers have collected black coral for commercial and personal reasons. This has raised concerns over the potential sustainability of this fishery. Pioneering work in 1976 by Dr. Richard Grigg at the University of Hawai'i predicted that a harvest size limit of greater than 1.2 meters (48 inches) would allow the fishery to be sustainable. After more than 20 years of voluntary compliance with this harvesting limit, the black coral bed in the Auau Channel on Maui is shown to be sustainable. However, there remain areas where black coral is rare. This project has focused on the feasibility of transplanting in order to "re-seed" areas with small black coral populations. Forty black coral fragments were transplanted in a total of four different locations on three islands. Analyses of growth and transplanting success are pending subsequent surveys. Surveys of symbiotic organisms on individual colonies along with surveys of natural fragmentation were conducted. The number of symbionts on a colony may be an indicator for general health of the colony. Natural fragmentation does not appear to have a significant contribution to the population structure. Recommendations are made to limit black coral harvesting to areas for which population structure is known and monitored.

Introduction

Black corals are ahermatypic corals found in all oceans and usually found in deeper water (below 30 meters) (Grigg, 1965). This group of corals (Order: Antipatharia) is poorly studied due to the deeper habitat and their rareness. These corals acquired their name due to their skeletal composition (protein and chitin) which is black. Their outer tissue color varies depending on species; it may be yellow, green, red, orange, white, or brown. The growth forms found among black corals range from whips to fans to bottle brushes to trees, and the height of these corals can be up to 3 meters or more. The growth rates of black corals range from about 3 cm/year (Grange, 1985) to just over 6 cm/year (Grigg, 1976). Very little information exists on the reproduction of these corals, but one species found in Hawai'i, *A. dichotoma*, matures at about 12 years of age or 1 meter in height (Oishi, 1990). The critical habitat is not fully understood; however, there are certain environmental parameters that may be essential. These parameters include low light levels, low surge motion, substantial current, and low levels of sedimentation (Grigg, 1964).

Black coral has been commercially harvested in many localities around the world. Some of these locations include Hawai'i (Grigg, 1964), Tonga (Tonga Department of Lands, Survey and Natural Resources, 1987), Ecuador (Romero, 1997), and throughout the Caribbean (Wells *et al.*, 1983). Some of these localities have been depleted of black coral because of over-harvesting. The life history characteristics (slow growing, long life span, older age of maturity) of these corals increase the potential for over-harvesting without regulation at some level. This has caused black corals to be listed as commercially threatened by the International Union for Conservation of Nature and Natural Resources (IUCN). They have also been listed as Appendix 2 Animals by Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Appendix 2 animals are not endangered, but they may become endangered if action is not taken. The actual extinction of these animals seems unlikely due to their depth distributions. Most species that are commercially sought can be found at depths beyond most sport divers' limits. However, complete populations can be and have been severely reduced by divers collecting them for curios or jewelry production.

The commercial harvest of black coral in Hawai'i began in 1958 (Grigg, 1998). This fishery is unique when compared to other parts of the world in that it is potentially sustainable. If divers abide by recommendations to harvest trees greater than 1.2 meters (48 inches) in height and the current demand does not exceed the amount collected, Hawai'i can support commercial black coral harvesting (Grigg, personal communication). The current demand is determined by the retailer's need for raw black coral. Factors involved in the demand are product competition, improved manufacturing techniques for the jewelry produced, stock piling, and importation of foreign black coral products. Presently, there are no regulations on the collection of black coral in Hawai'i. The species harvested are *A. dichotoma*, *A. grandis*, and *A.ulex*. However, *A. ulex* only accounts for 1% of the harvest while *A. dichotoma* and *A. grandis* make-up 99% of the harvest (Oishi, 1990). Dr. Richard Grigg of the University of Hawai'i made the 1.2-meter (48 inches) recommendation in 1976 after surveying black coral (*A. dichotoma* and *A. grandis*) in the Au'au Channel and Kauai. This recommendation was again made in 1998 after surveying the same area on Maui. After years of harvesting, Grigg found that this bed is sustainable, providing economic demand and harvesting do not change. Grigg again recommended that this size limit be passed as an official state regulation (Grigg, 1998).

This study will attempt to test the feasibility of transplanting black coral. If black coral can be successfully transplanted, then "reforestation" actions can be conducted. It is believed that small fragments can be taken form large parents and transplanted back onto the reef. These fragments will then continue to grow and create new colonies eventually becoming reproductive and contributing to the diversity of these corals. Monitoring techniques such as symbiont surveys conducted on individual parent colonies can be used to monitor the health of the colonies.

Methods

The transplanting procedure first required fragments to be collected (6 to 20 cm) from large parent colonies (greater than 0.75 meters). Fragments were placed in a small plastic container and taken to the surface. The fragments were prepared on the surface by placing a small cable tie around the base of the fragment. A two-part epoxy (Z-Spar Splash Zone) was mixed on the surface. The fragments were taken down to the site of planting. A hole is located in which the fragment is to be placed. Next, the hole was filled with epoxy, and epoxy was placed around the cable tie at the base of the coral. Finally, the fragment was firmly pushed into the hole and secured by the epoxy.

Fragments were transplanted in two types of locations. They were transplanted next to the parent colony and away from the parent colony. The fragments transplanted away from the parent colonies were transplanted in an area where the habitat seemed suitable based on environmental requirements and surrounding antipatharians. These transplants may serve as a type of control to the transplanting process.

Coral was transplanted in 4 locations. These locations include Makua, Oahu (approximately 21° 30.404' N, 158° 14.287' W); Kahuku, Hawaii (18° 58.666' N, 155° 45.719' W); Honokohau, Hawaii (19° 40.932' N, 156° 02.229' W); and 2 reefs around Makena, Maui (20° 38.46' N, 156° 27.42' W) (Figure 1). One fragment *(A. ulex)* was transplanted at Makua on July 11, 1997 at a depth of 45.7 m (150'). Ten fragments *(A. dichotoma)* were transplanted at Kahuku on June 30, 1998 at a depth of 27.4 m (90') and 9 fragments (*A. dichotoma*) were transplanted at Honokohau on July 2, 1998 at a depth of 25 m (82'). Ten fragments (*A. dichotoma*) were transplanted at two different reefs at Makena on July 4 and July 5, 1998 at depths of 34.4 m (113') and 25.9 m (85'), respectively. After the transplants were completed, a photographic record was taken of each fragment in order to obtain total height measurements at a later time. This record was taken with a Nikonos V camera and an underwater slate with centimeter marks as a background. This produced a permanent record of the height of each fragment. All sites except the Makua site were marked with a small buoy.

Two types of surveys were completed. A survey of the substrate around the parent colonies counted fragmented branches (fragmentation survey). A survey of the flora and fauna living among the branches of the parent colonies was also conducted (symbiont survey). The fragmentation survey consisted of a visual survey of a circular area around each parent colony within a 1 meter radius. The symbiont survey consisted of a visual as well as a video survey of all branches on the parent. The number of each species was recorded on an underwater slate.

Video was taken of several individual colonies and the habitat around these black coral colonies. From this video, observations were made in order to characterize critical habitat or verify observations taken by previous experience.

Results

The photographs of the transplanted corals revealed total height measurements for all corals (Table 1). The Kahuku, Honokohau, Makena #1, and Makena #2 sites had average heights of 10.9 cm, 12.2 cm, 14.2 cm, and 9.1 cm, respectively (Table 1). The size ranges for the Kahuku site are 7 to 19 cm while the size ranges for the Honokohau site are 10 to 17 cm. The size ranges for the Makena #1 and Makena #2 sites are 7 to 20 cm and 5 to 14 cm, respectively. The fragment at Makua is 20 cm.

The one fragment (*A. ulex*) transplanted at Makua was transplanted on July 11, 1997. As of October 12, 1997 there was no apparent growth. As of August 3, 1998 there was again no apparent growth. The survey on August 3, 1998 can not be verified due to equipment failure although it was apparent that pinnulation (breaking of the small branches) was occurring.

The symbiont surveys were completed for both parent colonies at Kahuku, Makena, and Makua (Table 2). The Kahuku parent colony had a total of 2 species although there were also some unidentified encrusting species growing on the parent. The Makena parent colony had a total of at least 6 species on the coral. The Makua colony had two species living among the branches. Another colony surveyed near the Kahuku colony also had only two species in association.

The fragmentation survey has shown small fragments around the bases of all parent colonies (Table 3). The Kahuku colony had a total of 12 fragments around the base of the colony with another separate colony attached to the substrate. It is uncertain if this colony originated from the larger colony above it. The Makena colony did not show any fragments around the base, but it did have a separate colony growing next to it. It is also uncertain if this colony originated from the larger colony next to it.

Video and personal observations among black coral habitats and individual colonies showed some qualitative characteristics. Black corals at the Kahuku site seemed to look healthier because of the increased number of branches and healthier tissue when compared to the Makena colonies. Healthier tissue is obvious because of the increased number of polyps per area and the color of the tissue. Healthy tissue for *A. dichotoma* is bright red, while unhealthy tissue is pale. The Kahuku site also had a much larger density of colonies than Makena. The shallowest colony at Kahuku appeared not to be as healthy as deeper colonies due a large portion of dead skeleton. The Kahuku site had a slight amount of surge while the Makena site did not appear to have any surge. The Kahuku site seemed to have more current than the Makena site. These observations are only qualitative during a short period of time and should be interpreted for their limited value.

Discussion

The corals transplanted to date create a baseline study to follow the feasibility of a larger restoration project in the future. In order to determine the feasibility of transplanting, growth data over a long period of time must be measured. Follow-up measurements have not been taken on the coral transplants, so no conclusions can be made on the feasibility of black coral transplanting at this time. Even if measurements were taken one month after transplanting, the feasibility may not have been able to be determined. The growth rate of *A. dichotoma* is 6.4 cm per year (Grigg, 1976). The maximum expected growth over a month would be 0.5 cm. This would be difficult to measure and show any significant growth taking experimental error into consideration.

It is the recommendation of this study to follow-up on fragments on a six-month basis. This may show an expected growth of 3.2 cm after six months, which should start to demonstrate significant growth over experimental error. However, it may require one to two years to show statistically significant growth. After a data set of one to two years can be analyzed, the feasibility of transplanting black coral may be able to be determined.

The intention of the symbiont surveys was to develop a baseline condition of the parent before fragments were collected from the parent. Changes over time in the organisms on the parent colonies may be indicators of impact from collecting fragments. However, the organisms on the parent may also represent the existing health of the colony. This would also support the hypothesis that they can serve as a monitoring system. Monitoring should be conducted semi-annually.

The symbiont surveys showed a larger diversity on the colonies at Makena when compared to Kahuku. The Makena colonies did not look as healthy due to decreased number of branches and condition of the tissue. These are qualitative observations that can not be quantified easily. Increased number of symbionts may indicate a decreased health condition because most of the symbiotic organisms require space on the coral. In order for the symbiont to live on the coral, it must take up space that would otherwise be covered with coral tissue. This creates an opportunity for other encrusting organisms such as sponges and hydroids to start growing. However, it is suggested that indicators of good health may include: increased number of branches, more polyps per branch area, decreased number of symbiotic animals, and decreased amounts of dead branches. These indicators can be

quantified and possibly used to classify the health of individual colonies. Environmental conditions may also be a factor in the reduced health of individual colonies. If an area does not have sufficient current or is prone to surge throughout the year, then this may diminish the health of colonies. This would make the colony more prone to acquiring epizoic organisms.

The fragmentation survey shows that large colonies fragment small branches. The reason for this phenomenon is unknown. The fragments that were surveyed were mostly lying loose on the bottom, usually down current and down slope. Fragments were seen away from the parent colony as well, but these were not represented by the survey. The Kahuku colony had 12 fragments while the Makena colony did not have any. This can be explained by the bottom substrate. The Kahuku site is a gentle slope with reef bottom while the Makena site is a short wall about 2 meters in height with a sandy bottom underneath the colony. The colony at Kahuku is growing straight up from the reef while the Makena colony is growing straight out of the wall. If the Makena colony release fragments, then most likely the fragments fall into the sand. In the future, the fragmented branches could be collected and transplanted instead of collecting fragments from the parent colony. Each site had one small colony attached to the substratum next to the parent colony. The source(s) of these colonies are unknown, but they did not appear to be from fragmentation of the parent based on their location. Natural fragmentation does not appear to be significantly contributing to the population due to their lack of establishment. However, more work is needed to understand the role of natural fragmentation.

Black coral has been harvested for more reasons than jewelry production. There have been a few accounts of black coral being collected for the aquarium trade. There are three confirmed instances during the summer of 1998 where black coral was collected alive for the aquarium trade. All three of these accounts involved collecting small whole trees. The Waikiki Aquarium has encouraged the collectors to only harvest fragments rather than whole trees in order to avoid any pressure on the population. These cases were special cases and will be avoided in the future if the size limit of 1.2 meters (48 inches) is passed as an official regulation. This regulation should also address the possibility of people collecting fragments for the aquarium trade. The collection of fragments is not as much of a concern as collecting complete colonies, but it may become a concern with the expansion of the aquarium trade. The public has shown an interest in any attempt to restore the black coral populations around the Hawaiian Islands. There is one report of an individual transplanting black coral along the North Shore of Oahu. So far, he has reported short-term success although there are no data to support this success. Mike Severns of Mike Severns Diving has expressed a great interest in restoring the black coral population around Molokini Island, although the history of the black coral population around Molokini is unknown. This correspondence shows an interest among the community in protecting this resource. This may open opportunities for community involvement in restoration projects similar to this in the future.

Grigg surveyed the Auau Channel on Maui in 1975 and 1998. These surveys were intended to obtain data on the recruitment of the population. If harvesting is adversely impacting the population, then the recruitment should reflect the impact. The study found no decrease in recruitment between 1975 and 1998. The two surveys did show a difference in the abundance of large colonies (greater than 48 inches or 19 years old). The 1998 survey showed fewer large colonies than the 1975 survey. These data indicate that divers are complying with the recommended size limit of 1.2 meters (48 inches) proposed in the 70's, and the population is sustainable at this level of harvesting. If divers were not complying with the recommended size limit, then they would be affecting the reproductive potential of the population by removing the smaller immature colonies. This would in turn reduce the amount of recruitment back into the population. Also, the reduction in only large colonies during the 23 years shows divers have mainly collected the large colonies (Grigg, 1998).

Fear of changes in the future economics has caused the State of Hawai'i to consider passing the 1.2 meter (48 inch) size limit as an official regulation. The state also needs to consider whether such a regulation alone can completely protect black coral from over-harvesting. The data collected by Grigg show the characteristics only for the black coral population in the Auau Channel. The populations of other smaller beds across the state also need to be investigated and protected from any future harvesting until they can be shown to

be sustainable. This should not affect present harvest levels as only a small portion of black coral comes from outside the Auau Channel.

During the course of this study, a relationship between black coral and the Tinker's Butterflyfish, *Chaetodon tinkerii*, was noted. This relationship can also be seen in video of black coral habitats recorded in the past. Whenever several large colonies of black coral are seen, the Tinker's Butterflyfish are usually present nearby. It is uncertain whether a relationship truly exists between these animals. However, it is possible that black coral may provide specific food or habitat for the Tinker's Butterflyfish. However, more work needs to be done before any conclusions can be made about this relationship.

Recommendations

The recommendations put forth in this report are to limit black coral harvesting to populations that have been shown to be sustainable. The populations would include the Auau Channel on Maui and possibly the beds on Kauai and southwest Hawai'i (Big Island). If the Auau Channel on Maui can supply Hawai'i's market without fears of over-harvesting, then there is no reason for black coral to be harvested in areas of unknown population structure. Harvesting areas of unknown population structure may not only hinder future scientific research on this group of poorly understood corals, but it may also reduce the black coral diversity in less populated areas.

Conclusions

Conclusions on the feasibility of transplanting black coral can not be determined until further surveys are completed. The transplanted fragments should be monitored on a semi-annual basis for 1 to 2 years before any recommendations can be made on the methods used in this study to propagate black coral. Natural fragmentation is not believed to be a significant contribution to the population based on observations of their establishment. Colony health may be monitored by factors such as: increased number of branches, more polyps per area, color of tissue, decreased number of symbiotic animals, and decreased amounts of dead areas. This report also recommends that the State of Hawai'i consider limiting black coral harvesting to areas of known population structure.

Acknowledgements

I would like to thank Sea Grant, the Department of Land and Natural Resources (DLNR), and the Waikiki Aquarium for supplying the funding for this study. A special thanks goes to Dr. Bruce Carlson, Dr. Cindy Hunter, and Dr. Richard Grigg for their continued support of this project and tremendous contributions. I would also like to thank Pete Basabe and Mike Severns for their help with surface support while in the field. Also, a special thanks goes to Mike Connolly for his dedicated, hard work in the field.

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Figures

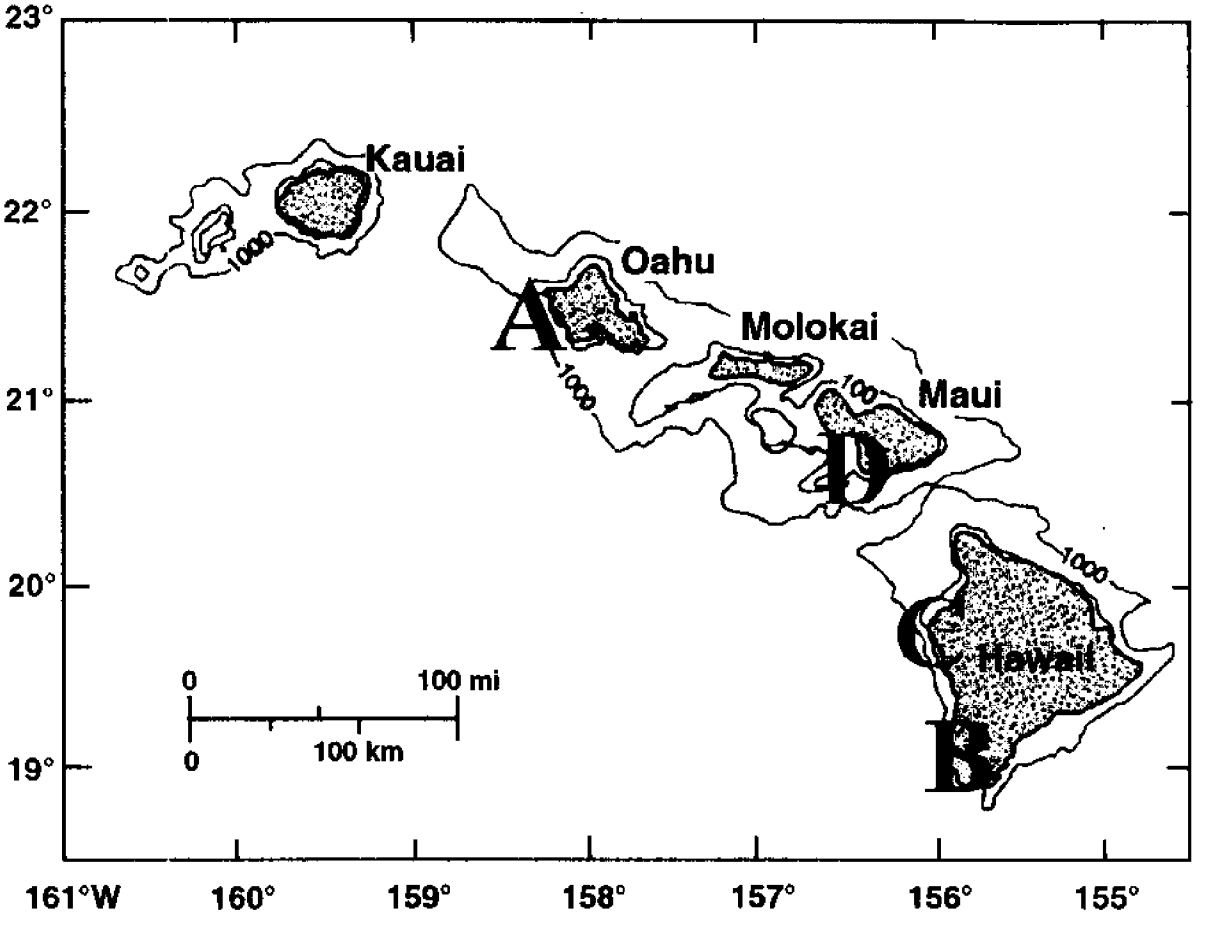
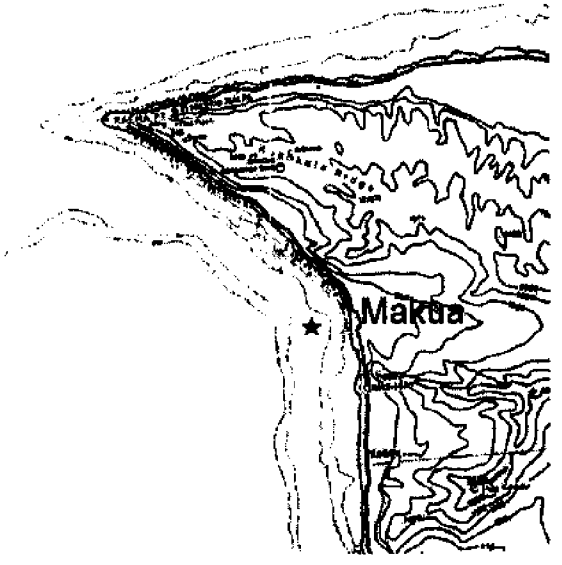
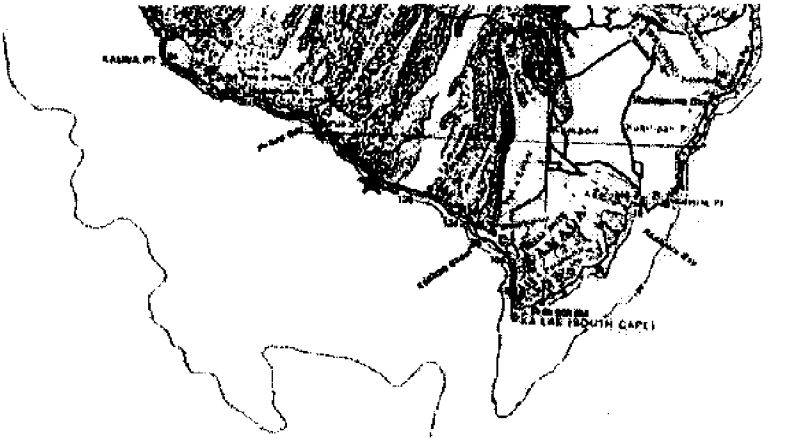


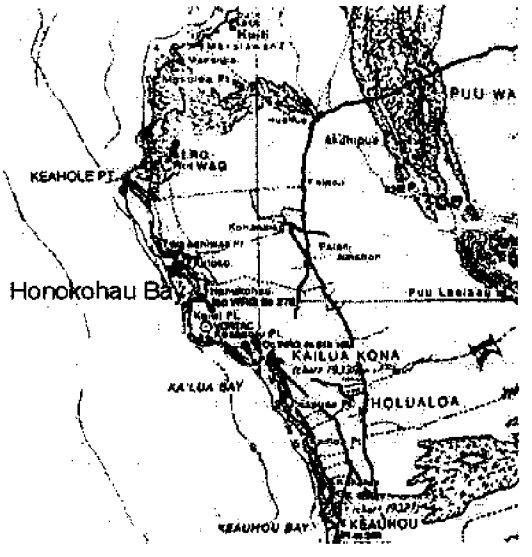
Figure 1. Map of Hawaiian Islands A) Makua, Oahu, B) Kahuku, Hawai'i, C) Honokohau, Hawai'i; D) Makena, Maui (Grigg, 1998).



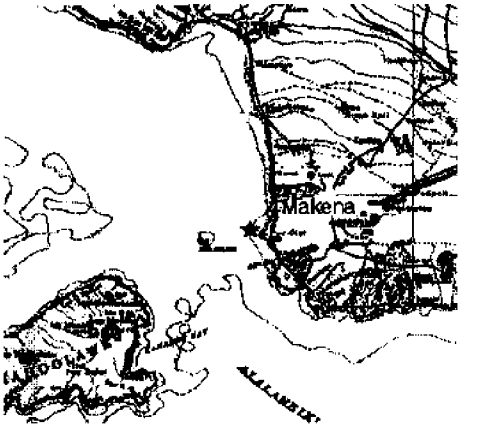
A) Makua, Oahu
(approximately 21° 30.404' N, 158° 14.287'W)



B) Kahuku, Hawaii
(18° 58.666' N, 155° 45.719' W)



C) Honokohau, Hawaii
(19°40.932'N, 156°02.229 W)



D) Makena, Maui
(20° 38.46' N, 156° 27.42' N0

Figure 2. Maps showing four locations of transplanting.

Tables

Table 1. Total measurements of all transplanted corals.

Makua	Kahuku #1	Honokohau	Makena #1	Makena #2
20 cm	11 cm	13 cm	20 cm	12 cm
	19 cm	15 cm	15 cm	7 cm
	10cm	11 cm	18 cm	7 cm
	7 cm	17 cm	16 cm	10 cm
	12 cm	10 cm	11 cm	13 cm
	11 cm	12 cm	14 cm	7 cm
	9 cm	10 cm	15 cm	14 cm
	10 cm	11 cm	12 cm	8 cm
	9 cm	11 cm	14 cm	8 cm
	11 cm		7 cm	5 cm
20 cm	**10.9 cm**	**12.2 cm**	**14.2 cm**	**9.1 cm**

Table 2. Symbiont survey results showing all species found living among the branches of black coral.

Makua Site		
# of individuals	Common name	Scientific name
1	Long-nosed Hawkfish	*Oxycirrhites typus*
20	Oysters	*Pteria brunnea*
Kahuku #1 Site*		
# of individuals	Common name	Scientific name
36	Oysters	*Pteria brunnea*
1	Long-nosed Hawkfish	*Oxycirrhites typus*
2	Tinkerís Butterflyfish	*Cheatodon tinkerii*
Kahuku #2 Site		
# of individuals	Common name	Scientific name
24	Oysters	*Pteria brunnea*
1	Stony coral	*Pocillipora* sp.

* Did not transplant from this colony.

Table 3. Results of fragmentation survey.

Makua		
Compass Bearing	Number of fragments	Size of fragments
0	0	-
90	0	-
180	0	-
270	0	-

Kahuku #1 *		
Compass Bearing	Number of fragments	Size of fragments
0[1, 2]	2	3", 1"
90[2]	2	1", 3"
180	0	-
270	0	-

Kahuku #2		
Compass Bearing	Number of fragments	Size of fragments
0[1]	2	1", 8" (attached)
90	9	1", 8", 6", 2", 4", 2", 1", 5", 3"
180[2]	2	3", 2"
270[2]	0	-

Makena		
Compass Bearing	Number of fragments	Size of fragments
0	0	-
90[1]	1	8" (attached)
180[2]	0	-
270	0	-

* Did not transplant from this colony.
[1] Down-current.
[2] Down-slope.

Population Density of *Octopus cyanea* in Kaneohe Bay

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Final Report:
August 31, 1998

Abstract

According to fish harvest data, *Octopus cyanea* comprised 44.7% (25851.8 lbs.) of the estimated total annual harvest of fishes and invertebrate species in Kaneohe Bay during the period of March 1991 to February 1992 (Everson, 1994). Most of the *O. cyanea* catch reported was not sold for commercial use. Despite its dominance in annual fishing yield, estimates on population density and distribution across Kaneohe Bay have never been done. Results from searches of approximately 26,800 square meters of area throughout Kaneohe Bay during the months of July and August of 1998 showed patterns of distribution to be related to substratum type. Substratum was grouped into four categories: coral, loose coral rubble, sand, and pavement. The majority of active octopus dens were found in loose coral rubble. Despite lack of optimum substrate, densities on Coconut Island Refuge proved to be higher than densities found throughout Kaneohe Bay.

Introduction

According to fish harvest data, *Octopus cyanea* comprised 44.7% (25851.8 lbs.) of the estimated total annual harvest of fishes and invertebrate species in Kaneohe Bay during the period of March 1991 to February 1992 (Everson, 1994). When compared to all other species, *O. cyanea* was by far the most productive fishery. The second was goatfish at just 8.6% of annual harvest. Throughout all four seasons, *O. cyanea* dominated the catch (Everson, 1994). Despite its dominance in annual fish harvest, no study has ever been done to determine the abundance and distribution of *O. cyanea* in Kaneohe Bay.

O. cyanea have a lifespan of only twelve to fifteen months (Van Heukelem, 1976). They have single matings and die soon after. In addition, reproduction is programmed. Females, upon reaching sexual maturity, if they have not found a mate, will lay an unfertilized clutch of eggs and die. A female *O. cyanea* can lay up to 700,000 eggs. *O. cyanea* are direct developers. Therefore, upon hatching, at a size of approximately only 3mm total length, they can ink, change color, and swim with a rapid forward motion at 1 to 3cm for prey. The hatchlings have a planktonic stage until they are approximately 1 cm in length.

Because they have a planktonic stage, and are well-developed motile individuals during this state, one could predict that juvenile *O. cyanea* hatched on a protected reef could easily maintain populations on nearby nonprotected reefs in the Bay. The Coconut Island Marine Laboratory Refuge has had refuge status for over 35 years. The refuge consists of the reefs and bay waters surrounding Coconut Island from the high-water mark on the island seaward to twenty-five feet beyond the outer edges of the reefs. It is unlawful to take any aquatic life from within the boundaries of the refuge (Division of Aquatic Resources, July 1997). Hawai'i Institute of Marine Biology now has its own guards whose duties include the monitoring of the refuge reefs. As a result, resources have been better protected than in the past and, therefore, may be more abundant on Coconut Island reefs than on other nearby non-protected reefs in Kaneohe Bay. A common question of fisheries managers is: Can refuges increase the stocks of fishes within refuges, and can they increase fishing yields on adjacent non-protected reefs?

A study done at Hol Chan Marine Reserve in Ambergris Cay in Belize showed a higher biomass of fishes per unit area of protected reef compared to other non-protected reefs in the area (Roberts and Polunin, 1994). Data from a marine reserve at Goat Island, northern New Zealand suggests that marine reserves may affect the local abundances of certain species of marine animals, but not others (Cole, *et al.*, 1990).

Little is documented about the distribution and population density of *O. cyanea* throughout the reef flats and reef slopes of Kaneohe Bay. In spite of extensive literature on octopuses, reliable data concerning their behavior in nature is difficult to find (Yarnall, 1969). Studies actually performed in Kaneohe Bay are even more scarce (Forsythe, 1997).

The purposes of this study are: (1) to estimate the population density of *O. cyanea* throughout Kaneohe Bay, (2) to determine which substratum types are the optimal habitats for *O. cyanea*, (3) to determine the patterns of distribution and abundance of *O. cyanea* throughout the bay, and (4) to compare densities on the reefs within Coconut Island Refuge to densities of *O.*

cyanea on other non-protected reefs in the bay to determine the effect of refuge status on this stock.

One hypothesis with regard to densities and patterns of distribution is that one should be able to find an optimal habitat. Once an optimal habitat is known, future surveys of areas of the Bay where densities have been and are currently highest, can be used to determine whether the population of *O. cyanea* is in trouble due to factors such as overexploitation by fishermen, or changes and/or reduction of available habitat. The high fishing effort and yield shows that *O. cyanea* is an important local resource. This study will provide initial estimated densities and distribution patterns that may be used as a basis for more in-depth studies of *O. cyanea* populations and potential management in the future.

Methods and Materials

Development of Investigator's Search Image

Several weeks were spent interviewing octopus fisherman about what patterns in substrate to look for in finding active octopus dens, and approximately 60 hours were spent snorkeling over reef flats of various types to develop a search image for octopus dens and the octopus itself. Approximately 9 hours were spent actually snorkeling with octopus fishermen who went out with me to show me what an active octopus den looks like on various substrata.

Selection of sampling areas

Patch reefs were selected at random throughout Kaneohe Bay, as were sampling areas in the backreef area of the barrier reef between Kapapa Island and the sandbar, in order to get a representation of areas in the North, Mid, and South sections of the Bay (Figure 1). A reef numbering system for patch reefs in the bay is already in use (Roy, 1970). According to octopus fishermen, *O. cyanea* can be found on reef flats as well as on the reef slopes. Fishing efforts seemed to be more prevalent on the reef flats possibly because of the sparser coral cover, so it was decided that for the short duration of this study, transects on patch reefs would be done on the reef flats.

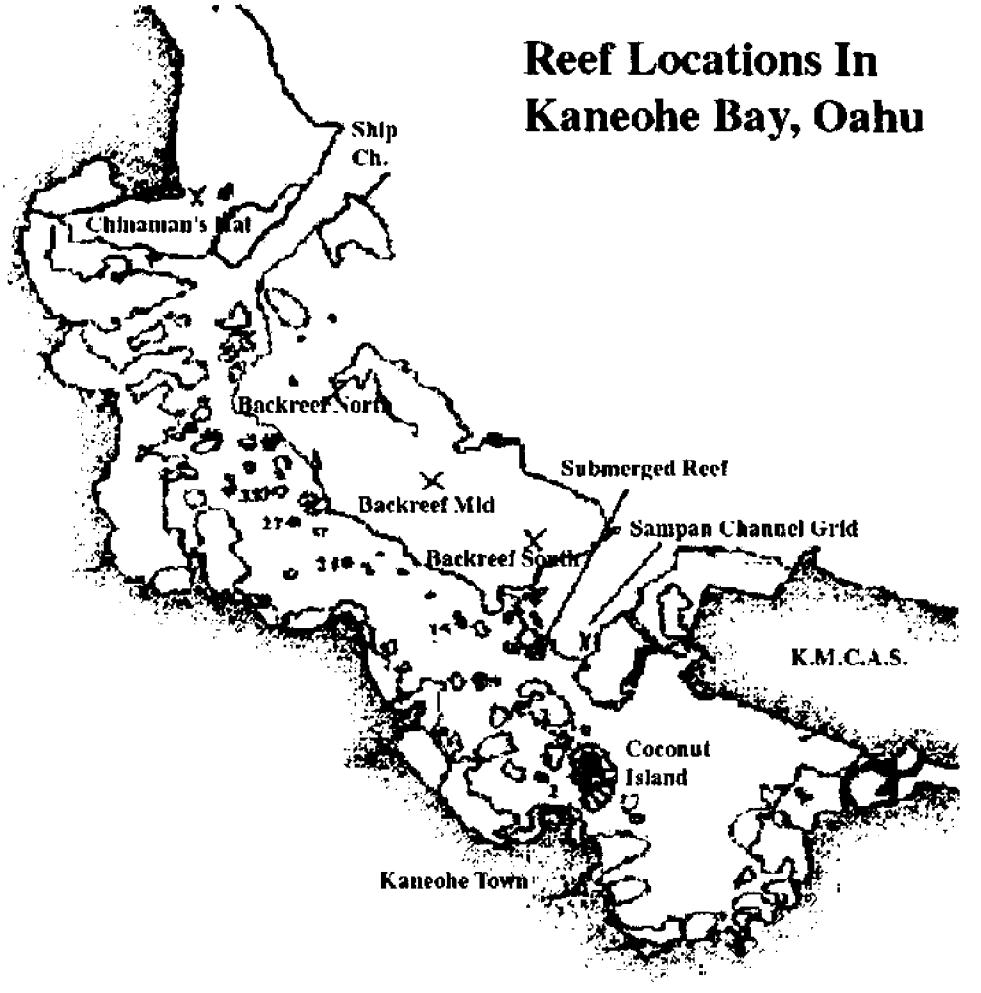

Figure 1. Map of sites searched throughout Kaneohe Bay.

Sampling methods and materials

Sampling of reef flats was done during the hours of 8:00am -- 5:00pm, during the months of July and August of 1998. Records on prevailing winds were obtained from weather data sheets located at Coconut Island. Wind and currents may have an effect on the distribution of corals, macroalgae, and substrate types on each patch reef. Data showed prevailing winds to be out of the east and east/northeast a majority of the time so we decided to run east/west transects to ensure a representation of substratum types across each reef flat.

A temporary North to South transect was run down the center of each patch reef from the most northern point of the reef crest to the opposite reef crest using a compass heading of 180°. The position of the north/south transect was recorded using GPS coordinates for resurveying of the reefs in the future. The length of the north/south transect was then divided by 6 to calculate 5

evenly spaced points at which east/west transects would be run. Each east/west transect was run using a compass heading of 90 degrees east from the center line to the east reef crest and a compass heading of 270 degrees west from the center line to the west reef crest (Figure 2). Each east/west transect was searched by swimming the length of the transect tape and searching 2 meters on either side of the tape.

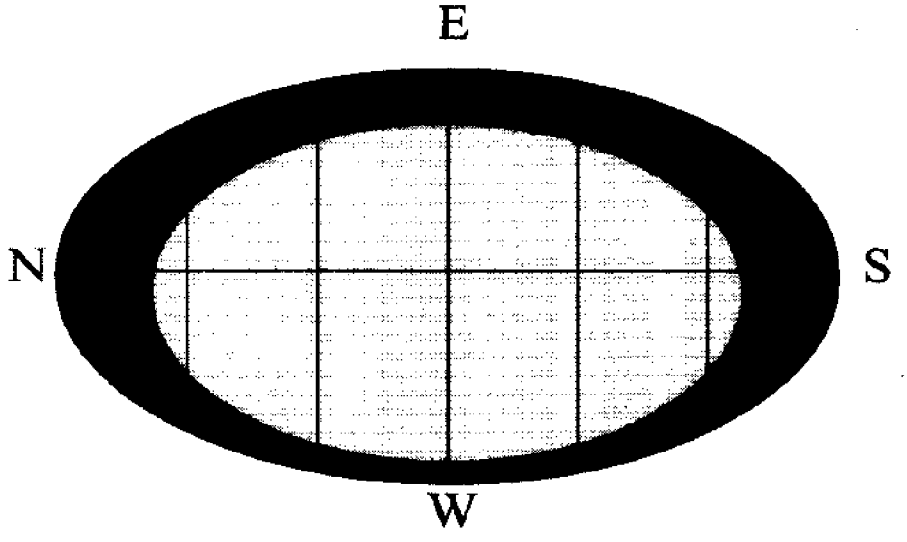

Figure 2. Patch Reef Transects

In the Backreef area and Chinaman's Hat, 100 x 100 meter grids were searched in a similar fashion as was done on the path reefs. A southern point was selected and its coordinates, determined by GPS, were recorded. A temporary North/South transect line was run using a compass heading to 360°. Five east/west transects were run using a compass heading to 90° east at 0, 25, 50, 75, and 100 meters along the north/south line (Figure 3). Each east/west transect was searched by swimming the length of the transect tape and searching 2 meters on either side of the tape.

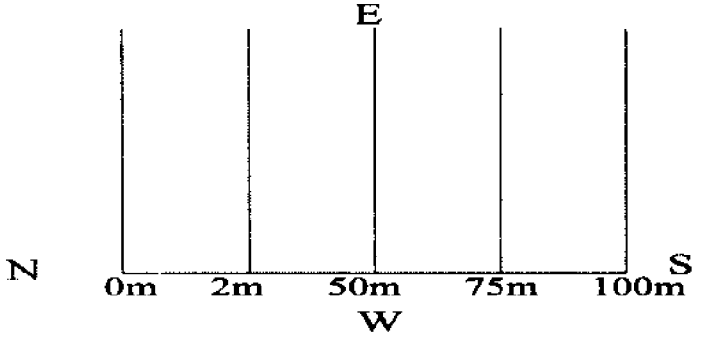

Figure 3. Transect Grids in front of Chinaman's Hat and Backreef areas

A grid to the right of the Sampan channel, which was already in place for a *Dascylis albicella* spawning study, was also searched. A compass heading was used to determine the direction of these lines. Lines at 4-meter intervals from each other were searched by swimming along the length of the line and searching 2 meters on either side of the line.

Transects around Coconut Island were run using the 50 meter markers already in place at the reef crest around the island. Nine (9) markers were selected around the entire island and temporary 50 meter transects were run using a compass heading from each marker straight into the shoreline. Each line was searched by swimming the length of the line and was searched to 2 meters on either side of the line.

Data recorded along each east/west transect included: the total length of the transect, the number of active dens, the number of old dens, the number of octopuses and the proportion of each line in various substratum classes. The extent of each substratum class was estimated by recording the beginning and ending points of each substratum type along the transect.

The cryptic nature and camouflage capabilities of *O. cyanea* make detection of live animals along the reef difficult. For this reason, active octopus dens were used rather than live animals to estimate population density. Behavior and foraging studies done by Yarnall, Van Heukelem, Mather, and Forsythe indicated that an *O. cyanea* occupies one den for up to four weeks. None of these papers indicated that an octopus occupied more than one den at one time. In fact, video recordings of foraging efforts were done on the same animals which left and returned to the same den on foraging trips (Forsythe, 1997).

For any octopus den sighted the following data were recorded: the distance of the den from the west reef crest, the substratum type in which it was found, whether it was occupied or not, and whether the den was being actively used or was inactive. Because dens are shallow, an octopus occupying a den was easily seen by simply looking into the entrance.

Octopuses choose potential home sites and modify them rather than selecting appropriate unmodified sites for homes (Mather, 1997). Dens are excavated most often in coral rubble but may sometimes be found in living coral (Van Heukelem, 1983). We defined a den located in rubble or sand as a burrow usually no deeper than approx. 20-30 cm deep with an opening approximately 10 cm in diameter. A den in a coral area was either a round hole in which the octopus had literally broken live pieces of coral right out of the middle of the top of the coral head and burrowed inside, or an area at the base of the coral head where the octopus had broken out pieces of live coral and burrowed in the sand under the coral head. Dens in the coral heads were also fairly shallow like those found in the rubble and sand. There were loose piles of broken coral rubble in front of the dens that are used by the animals to cover themselves up when they are occupying the den. In some cases dens would have small, empty crab carapaces around the entrance, but in most cases discarded food items could not be used as indicators because there were none. One occupied den was actually an unmodified hole in the pavement. There is a possibility that this animal may simply have been hiding from us as it saw us swim by. However, it appeared to be in a sleeping position with an arm wrapped around its head and mantle similar to Houck's observations of inactivity periods of *O. ornatus* (Houck, 1982) as well as several of our sightings of *O. cyanea* in active rubble dens. For this reason, the den found in the pavement was

included in the data of active dens. Figure 4 shows two examples of octopus dens that were found in a rubble area.

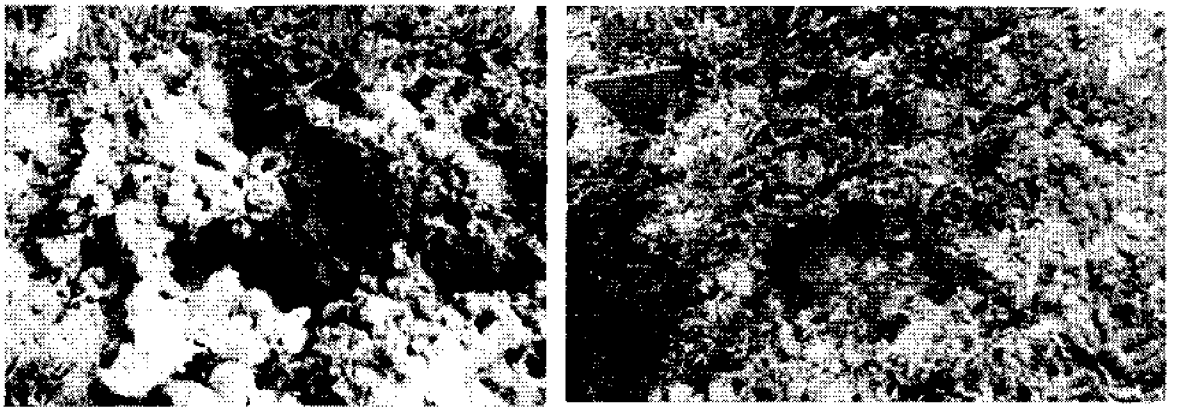

Figure 4. Examples of octopus dens in rubble area.

Whether a den was identified as active or inactive was occasionally very subjective. Inactive dens were either filled in with sand and rubble because of heavy wave action in that area, or the loose pile of broken coral rubble in front of the den had a thick layer of algal growth indicating that the rubble had not been turned or disturbed by an octopus actively using the den. An active den's pile of broken coral rubble was free of algal growth because an octopus was regularly using the rubble. We had the opportunity to go back to some areas where we had marked active dens. Within four weeks time, the dens we had originally marked active were either completely filled in by sand and rubble or were so overgrown with algal cover that they were obviously no longer is use. For this reason, I feel comfortable that dens that were identified as active, actually were active.

Substratum types were recorded into sixteen different categories based on primary substrate and secondary substrates in the same area. For example R-C/S was an area primarily consisting of loose rubble with occasional patches of sand and/or isolated coral heads. These sixteen types were grouped into four main categories for data analysis, coral, broken coral rubble, and sand (Mather, 1997), as well as a fourth category of pavement (solid limestone). Coral is defined as live coral cover. Rubble is defined as loose rocks and broken, dead coral covering a hard sandy bottom. Sand is defined as primarily sand cover. Pavement is defined as flat, hard, dead coral areas, which are covered with algal growth.

Results

Optimal Habitat

An analysis of substrates found in our search areas showed a large majority of the substratum found to be rubble at 47% of the total area as seen in Figure 5.

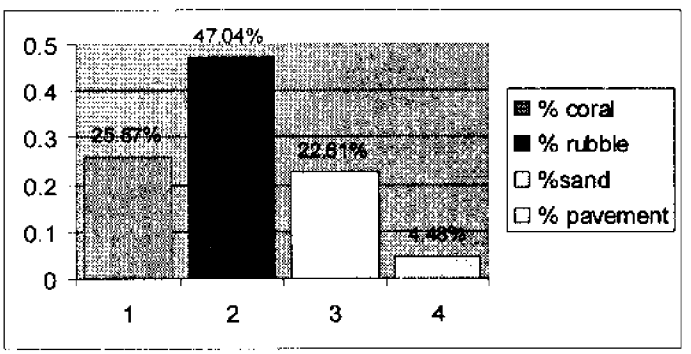

Figure 5. Percent of substratum in each category in search areas across Kaneohe Bay.

Thirty active dens were found during our searches. Based on the percentage of substrate in each category, if the 30 active dens were evenly distributed across all four substratum types, one would expect to find 8 in coral, 14 in rubble, 7 in sand, and 1 in pavement. Instead, we found 2 in coral, 22 in rubble, 1 in pavement, and 5 in sand (Table 1).

Table 1. Number of active dens. random expected vs. per substratum type

	# active dens per substrate				
	C	R	S	P	Total
Proportion of each substrate searched	0.26	0.47	0.23	0.04	
expected # active dens per substrate	8	14	7	1	30
observed # active dens per substrate	2	22	5	1	30

(C=coral, R=rubble, S=sand, and P=pavement)

A much higher than expected number of dens was found in rubble and much lower frequency was found in coral (Goodness of fit test, $G=10.98$, $p<0.025$). This is probably due to the fact that the rubble areas are easily excavated and there is lots of loose broken coral which can be used around the outside of the den.

An analysis of each individual search area shows the distribution of substratum types (Fig 6).

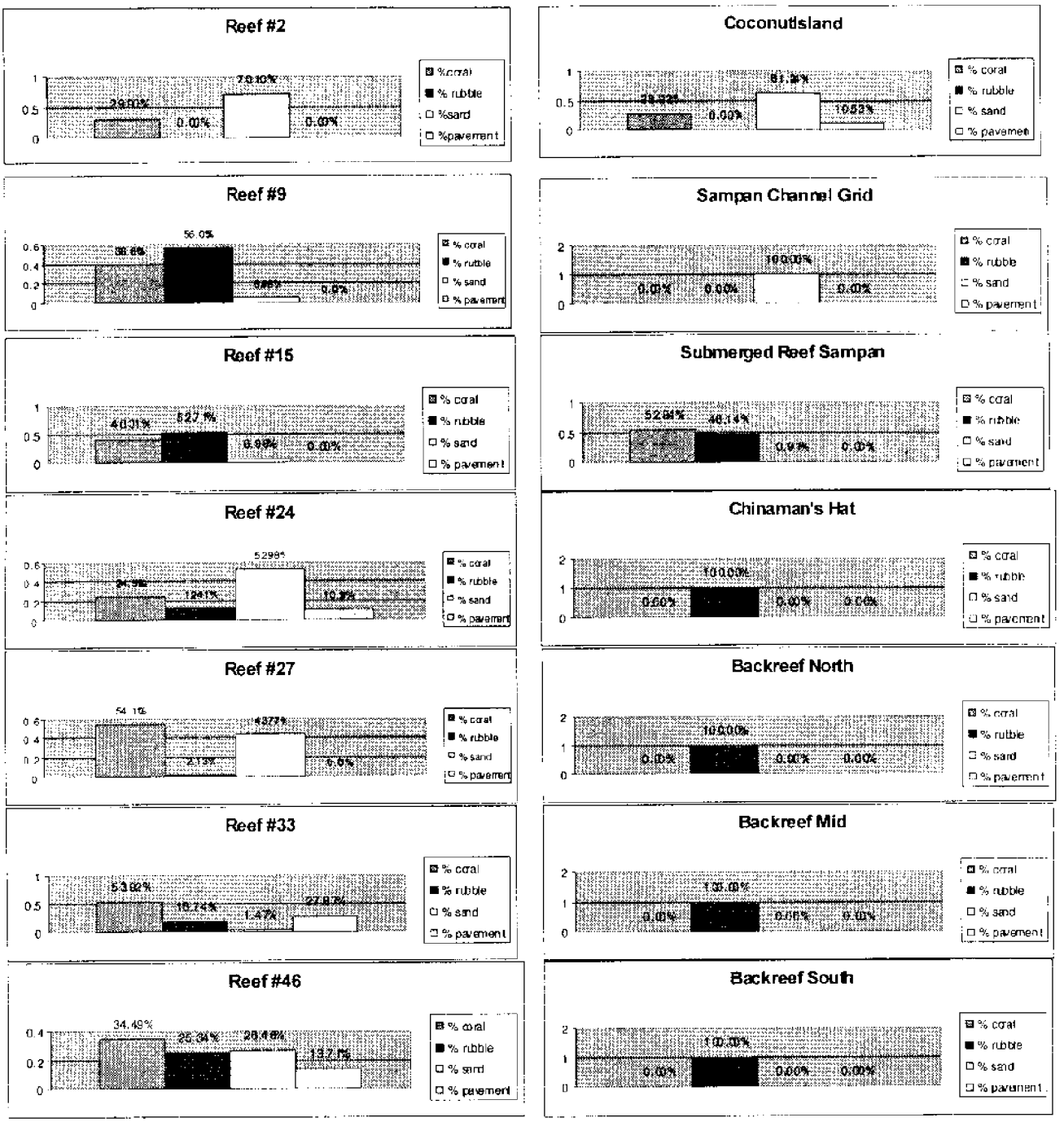

Figure 6. Comparison of percentage of substratum types in each search area.

Patterns of density & distribution

Chinaman's Hat, Sampan Channel, the Backreef and one submerged reef area near Sampan channel were all primarily rubble in the outer part of the Bay. For comparisons, these outer bay areas were grouped together, and patch reefs in the inner part of the Bay were

categorized as another group. The patch reefs were oval in shape with the longest length being oriented North to South. These usually had a ring of live coral growth around the perimeter of the oval and a mix of rubble, sand, and pavement inside the ring. The flats of patch reefs in the north part of the bay had more relief than those of patch reefs in the mid or south parts of the Bay. They were also covered by deeper water and were subject to heavier wave action than other patch reefs in the Bay. This was probably due to their proximity to the large boat channel at the north end of the bay.

The average density of active dens throughout Kaneohe Bay was 0.12 per 100 m^2 of search area. A comparison of dens per 100 m^2 per search area did not indicate any pattern of distribution from north to south in the bay (Figure 7). Outer bay sites had higher densities than most patch reefs (Figure 7). This may be due to the higher percentage of loose coral rubble at the outer bay sites as seen in figure 6. Also, densities at Coconut Island Refuge were higher than all but one of the patch reefs, reef #15 (Figure 7).

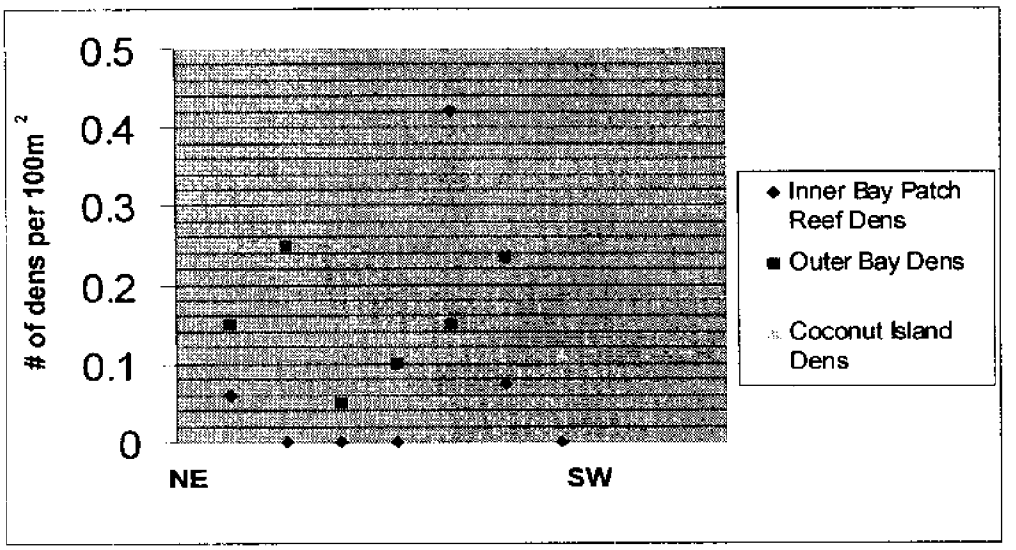

Figure 7. Number of active dens per 100m^2 per search area with areas sequenced from Northwest to Southeast in the Bay. (Inner Bay Patch reefs from left to right: #46, #33, #27, #24, #15, #9, and #2. Outer Bay areas from left to right: Chinaman's Hat, Backreef North, Backreef Mid, Backreef South, Submerged reef near Sampan Channel, Grid in Sampan Channel.)

If distance from the reef crest were a function of predator avoidance, one would expect octopus dens to be found as far in from the reef crest as possible. Our data show ten out of fourteen dens on patch reefs were between 25% and 50% in from nearest reef crest (Figure 8). This distance appears to be more a function of substratum type than of predator avoidance since most dens were found in rubble areas and patch reefs had a perimeter ring of dense coral cover. This perimeter ring of live coral could account for the lack of dens in the first 25% of area from

the reef crest, thus supporting the hypothesis that *O. cyanea* prefer loose coral rubble for den locations.

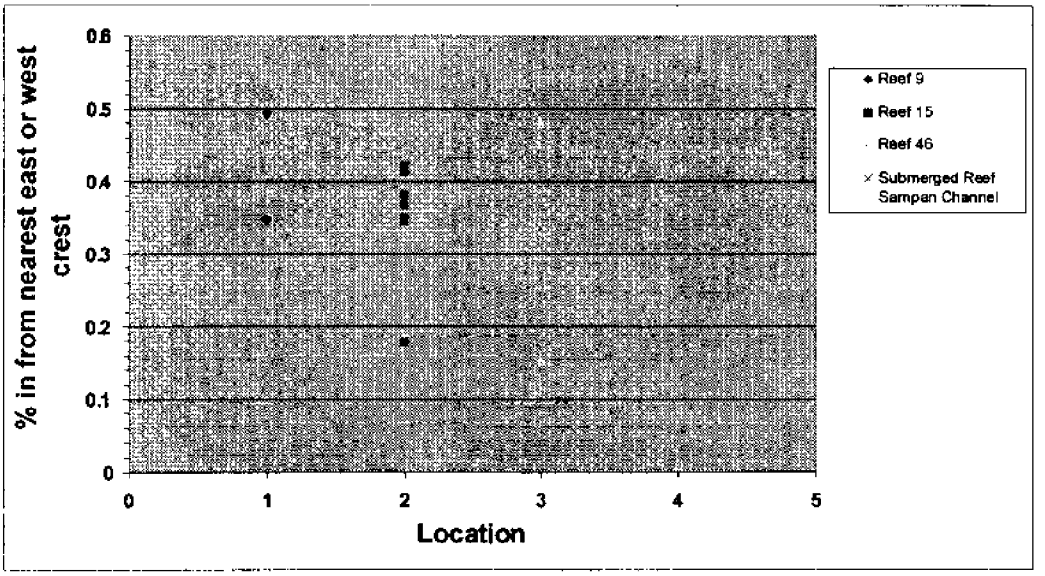

Figure 8. Percent distance of active dens from nearest east or west reef crest as a function of transect length.

Discussion

A total of 26,800 square meters were recorded and only 30 active dens and 5 live octopuses occupying dens were identified. The densities acquired from this study were obtained during what is regarded as a season of low density. Although, throughout all four seasons, *O. cyanea* dominates the fishing catch in Kaneohe Bay there is seasonality to the number of *O. cyanea* caught. According to catch data in 1994, there were 2,266 lbs. of *O. cyanea* caught in the spring, 6,107 lbs. in the summer, 11,027 lbs. in the fall, and 6452 lbs. in the winter (Everson, 1994). Also, conversations with several octopus fishermen indicated, this has been a relatively low year for *O. cyanea* in Kaneohe Bay. Personal observation of the octopus fishing effort while we were out in the bay also confirms this. We saw very few fishermen looking for octopus while doing censuses in the Bay. The low densities we obtained may have been in part, due to the season considering our searches extended from July to August. The densities may also be due, in part, to the fact that we had to develop a search image for identifying dens and animals. However, although our search image improved over the course of this study, we did spend approximately five weeks developing our search image. We are, therefore confidant that our searches were consistent enough to provide accurate data throughout the study.

The high frequency of searched areas with 0 dens makes it difficult to detect and support patterns of distribution and abundance. However, some patterns can be suggested. Certainly,

density of dens was greatest on rubble areas. Densities appear to be higher in outer bay areas, even after correcting for gross differences in substratum classes.

Coconut Island had a higher density than inner bay patch reefs despite its composition of mostly sand and live coral. Therefore, as a protected area, Coconut Island may very well be contributing to the success of the octopus population in Kaneohe Bay. A more in-depth study of Coconut Island and its surrounding reefs would have to be done to determine conclusively if this is true.

O. cyanea seems most common in the shallow reefs in the autumn and winter months (Van Heukelem, 1976 & Everson, 1994). However a systematic study of changes in distribution with season has never been conducted. This census should be repeated during the fall, winter, and spring to obtain an accurate picture of the population density of *O. cyanea* throughout the year. A multi-year monitoring program would be needed to produce accurate population data.

Acknowledgements

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Determination of the Optimal Salinity for Astaxanthin Synthesis in the Unicellular Green Alga, *Haematococcus pluvialis*

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Abstract

The freshwater alga *Haematococcus pluvialis* Flowtow (Chlorophyceae), which produces the ketocarotenoid astaxanthin, was cultivated at Aquasearch Inc. at the Natural Energy Laboratory of Hawai'i Authority at Keähole Point, Kona during the Summer of 1998. It was grown under salt concentrations of 0, 1, 2, and 3 parts per thousand (ppt) in small scale, and 0 and 2 ppt in large scale to determine the optimal salt concentration for astaxanthin synthesis. The results have shown that 0 ppt was the optimal salt concentration for the maximum production of astaxanthin in both small and large scale experiments.

Introduction

The unicellular green alga, *Haematococcus pluvialis* Flowtow (Chlorophyceae, Order Volvocales), is well known for its ability to produce large amounts of the ketocarotenoid astaxanthin (3, 3- dihydroxy-β, β-carotene — 4, 4 — dione) under extreme environmental conditions (Goodwin, 1986; Fan et al., 1994). Astaxanthin is being used as an additive to crustacean and fish aquaculture feeds to enhance pigmentation of their flesh and skin. It's also being used in poultry feed to give skin and eggs an attractive color (Davies, 1976; No and Storebakken, 1991; Sommer *et al.*, 1992; Choubert and Heinrich, 1993). The economic value of this pigment has been on the rise because of the doing away of synthetic pigments by governmental policies due to health issues. Besides astaxanthin, *Haematococcus pluvialis* produces many other pigments. Under optimal growth conditions, vegetative cells reproduce by mitosis in which primary carotenoids are produced, namely β-carotene, lutein, violaxanthin, neoxanthin, and zeaxanthin (Harder *et al.*, 1995). When the algae are exposed to undesirable conditions, the vegetative cells undergo changes in photosynthesis, physiology, and morphology, including astaxanthin synthesis and formation of large red aplanospores (Harker *et al.*, 1995). Other changes include the loss of two posterior flagella, formation of immobile aplanospores (Elliot, 1934), accumulation of large lipid bodies in the protoplast which contain the astaxanthin (Lang, 1968), formation of a thick sporopollenin cell wall resistant to oxidative degradation (Burczyk, 1987), and lowered photosynthetic rates (Hagen *et al.*, 1992).

Experiments for this study were done at Aquasearch Inc. at the Natural Energy Laboratory of Hawai' Authority (NELHA) Keähole Point, Kona, on the Big Island of Hawai'i. The aim of this study was to determine the optimal salinity for astaxanthin production by the Chlorophyte, *Haematococcus pluvialis*. My hypothesis is that this freshwater alga maximizes the production of the ketocarotenoid astaxanthin under a 0ppt salt concentration. A study done by Cordero *et al.* (1996) showed that the optimal salinity for astaxanthin synthesis was 2ppt (34mM NaCl). Two studies done by Harker *et al.* (1995) displayed 2.3ppt (40mM NaCl) as the optimal salt concentration. Boussiba and Vonshak (1991) determined that exposing cells to a salt stress of 8ppt (136mM NaCl) caused complete cessation of growth. These preceding experiments were all done on a relatively small scale in 5000-ml, 250-ml, and 500-ml Erlenmeyer flasks, respectively. I was able to compare my small-scale experiments to these studies. However, a large-scale experiment was my ultimate goal.

In this study five laboratory experiments were conducted in order to determine the optimal salinity for the production of astaxanthin. Upon completion of these, one pond

experiment was carried out in order to determine the salt concentration for the maximum production of astaxanthin on a large scale.

This project was also a means for me to learn various scientific methods, including the use of numerous tools and equipment necessary for biological research. During this project I also learned, in detail, the growth parameters, life history, morphology, photosynthesis, and aplanospore formation of the green alga, *Haematococcus pluvialis*.

Life history

The life history of *Haematococcus pluvialis* includes four different stages where no sexual stage intervenes (Figure 1). The macrozooid stage consists of flagellated cells in the range of 8-50 μm in diameter. In the resting stage, or hematocyst stage, cells are non-motile and possess thick resistant cellulose walls. In the microzooid stage, cells are cylindrical in shape, relatively small at 20 μm or less, and swim much more actively than macrozooids.The palmella stage consists of non-motile cells. The palmella generation alternates with the macrozooids and microzooids, and the hematocysts are developed from any stage in the life cycle (Elliot, 1934). Figure 2 shows a large red encysted cell in the hematocyst stage and green dividing macrozooids.

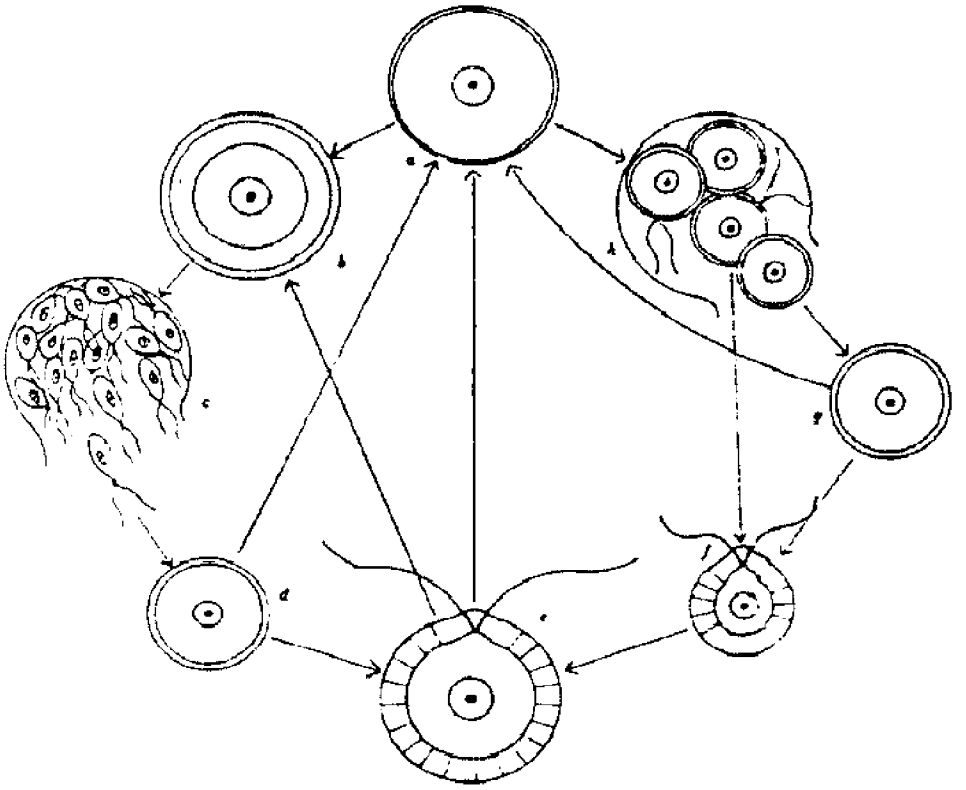

Figure 1. Life cycle of Haematococcus pluvialis: a, h, g, d palmella stage; e, f macrozooid stage; b hematocyst stage; c microzooid stage (Elliot, 1934).

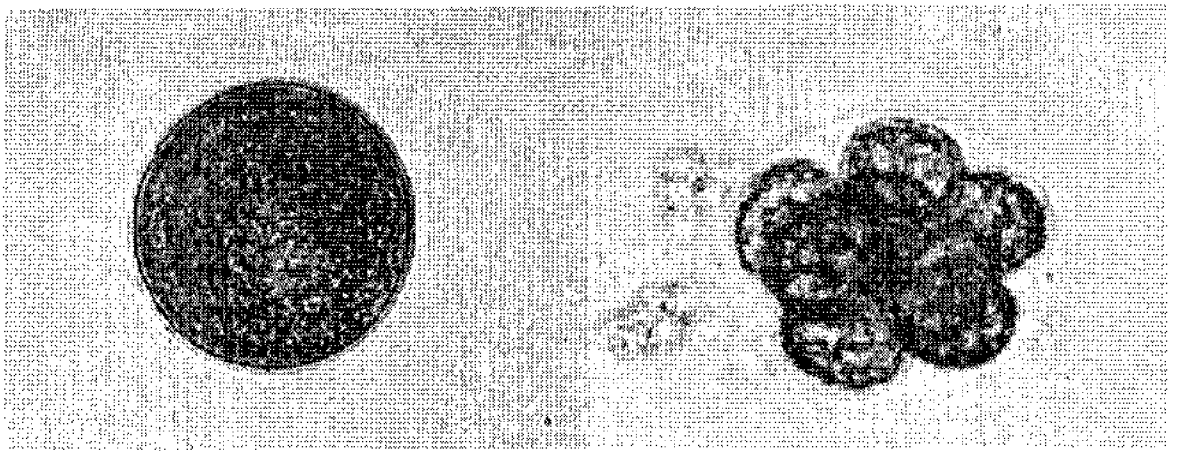

Figure 2. Hematocyst (left) and dividing macrozooids (right).

Salt experiment # 1

Objective

The objective of this experiment was to determine the optimal salt concentration for the production of astaxanthin.

Materials and Methods

Organism- Haematococcus pluvialis Flowtow (used for all experiments) was obtained from the culture collection maintained at Aquasearch Inc. at Ke'ahole Point, Kona.

Growth conditions- The alga was cultivated in eight 500-ml Erlenmeyer flasks containing 50-ml of culture and 450-ml of water of various salinities. The final salt concentrations were 0, 2, 4, and 6 ppt, run in duplicate. 100 mg of sodium bicarbonate was added to the media prior to inoculation. The cultures were maintained at 29 °C, aerated by bubbling air, and continuously illuminated by three grow- and three white-fluorescent lights supplying a light intensity of 160 μmol photon m^{-2} s^{-1} as measured by a Li-Cor 185 quantum sensor.

Measurements and Calculations- Cell number was determined with a Coulter counter and a hemacytometer. The hemacytometer was also utilized to count red, encysted, and dead cells, in which all values were standardized to represent one ml of culture. The red cell counts were used to calculate the reddening percentages, encysted cell counts were compared with red cell counts in determining whether they occurred simultaneously and dead cell counts were used to monitor the health of the cultures on a daily basis.

Results

The experiment was run for seven days (June 21-28, 1998). Coulter counts displayed a distinct relationship between culture growth and salt content (Figure 1), which were useful data in designing the next experiment. The trend showed that the cultures with less salt grew substantially more. However, the Hemacytometer counts were unreliable because of the low cell concentration used (which was corrected in the following experiment).

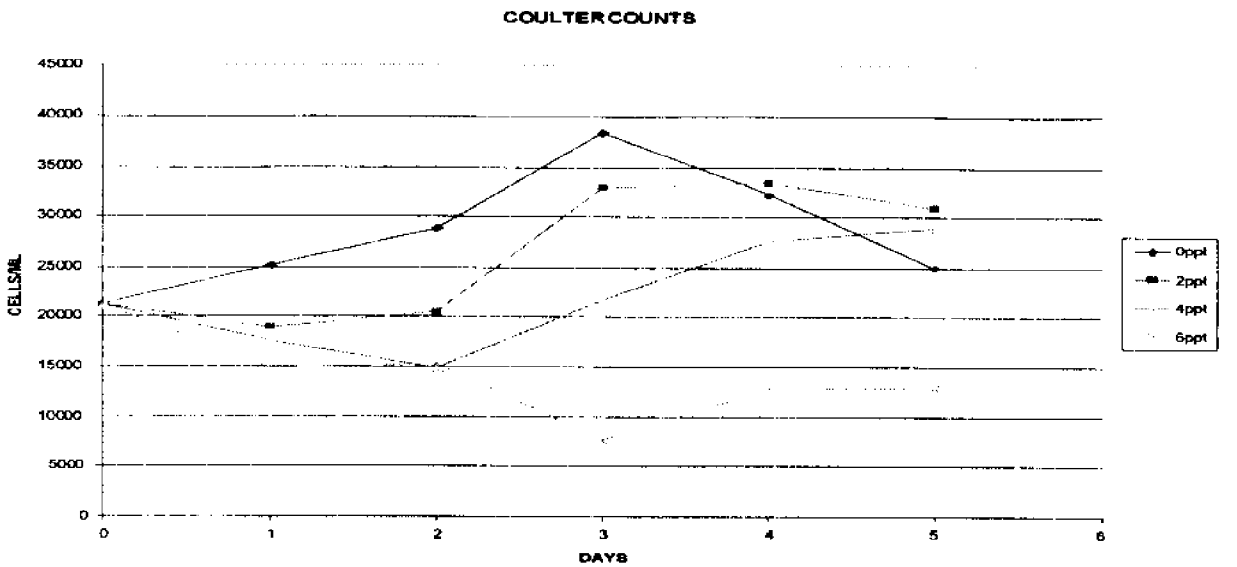

Figure 3. Haematococcus pluvialis mean cell numbers under various salt treatments using Coulter counts for Salt experiment # 1.

Salt experiment # 2

Objective

The objective of this experiment was to determine the optimal salt concentration for the production of astaxanthin.

Materials and methods

Growth conditions- The conditions were similar to experiment # 1 with a few changes, including a smaller range of salinities (0, 1, 2, and 3 ppt), a larger inoculum (initial cell count of 9 x 10^4 cells/ml), and a higher light intensity of 280 µmol photon $m^{-2} s^{-1}$.

Measurements and Calculations- pH and fluorescence measurements were made in addition to the measurements described in experiment # 1.

Results

Experiment # 2 was run for only four days (July 2-6, 1998) because of high pH levels found on the third day. The results were not analyzed because of this factor. The high pH levels were due to heavy photosynthesizing and insufficient CO_2. The high alkalinity caused a decrease in cell growth of the microalgae. At this point the pH level needed to be stabilized to study the salinity effects independently.

Co_2 implementation- In determining the CO_2 flow rate needed to run salt experiment # 3, a trial culture was set up with similar growth conditions as exp. # 2, with no salt added. The CO_2 was fed into the airline at 5% (140cc/min). The initial pH was recorded as well as hourly readings with the purpose of stabilizing the pH level. After the first hour, the pH level had dropped to 7.3 from 7.9, at which time I lowered the flow to 3 % (80cc/min). The pH level remained at 7.3 for the next 24 hrs (at 80cc/min). This was the flow rate for exp. # 3.

Salt experiment # 3

Objective

The objective of this experiment was to determine the optimal salt concentration for the production of astaxanthin.

Materials and Methods

Growth conditions- Conditions were similar to exp. # 2 with a few exceptions. The initial cell count was 1.1×10^4 cells/ml instead of 9×10^3 cells/ml, and CO_2 was fed through the airline at 3 % (80cc/min). The pH remained stable throughout the experiment at 7.0-7.5.

Measurements and Calculations- Cell counts and microscope observations were done daily. Microscope observations consisted of estimating reddening and encysted percentages, counting live and dead *Haematococcus* cells, and the monitoring of contamination such as grazers. Growth rates were calculated by the following equation:

$$\text{Growth rate (per day)} = \text{Ln}(C_2/C_1) \qquad [1]$$

Ln is the natural logarithm, C_1 is the cell counts on day one and C_2 is the counts on the next day.

Astaxanthin analysis- 1-ml samples were filtered (through a micro filtration apparatus), placed in 15-ml sample tubes with 10 ml of DMSO (Dimethyl sulfoxide), sonicated (with an ultrasonic cell disrupter), placed in freezer for three hours and left out to defrost until the next day. Once centrifuged, samples of the supernatant were placed into the spectrophotometer, and absorbance readings were taken. The supernatant is the solution in the upper half of a sample vessel (once it has been centrifuged) that is free of any solid particles. The solid material on the bottom of the vessel is called the pellet. The

absorbance value of the supernatant was used to calculate the percentage of astaxanthin in the cultures using the equation:

$$\% \text{ Astaxanthin} = [\text{Abs (10 ml)}]/[10(0.5)(\text{Dry wt.})(190.8)] \qquad [2]$$

Abs stands for the absorbance of light at a wavelength of 489 nm (reading from spectrophotometer), 10 ml is the extraction volume (amount of DMSO used), the 10 used is a unit conversion factor, the 0.5 represents the sample volume (in ml), the Dry weight corresponds to the total % dry weight per 1 ml sample and 190.8 is the extinction coefficient in DMSO.

Dry weights- 0.5 ml samples were filtered through pre-weighted glass fiber filters (GFF) and then dried in an oven. The filters had nominal pore sizes of 0.7 µm. The weights were used to calculate the total biomass and total astaxanthin (%astax x dry weight) of each culture.

Fluorescence- The fluorescence of the *Haematococcus* cells was measured with a PAM fluorometer. The pH was also measured to monitor the daily acidity of the cultures.

Results

This experiment was run for six days (July 12-18, 1998). The Coulter and hemaecytometer counts were very similar. Both methods showed 0ppt salt cultures with the highest cell counts, followed by 1ppt and so on (Table 1). The growth rates had the same correlation. The 0ppt culture also had the highest total astaxanthin, astaxanthin percentage, and total biomass (Figures 4 and 5). The 1ppt salt culture had the highest reddening percentages, including the most total red cells (Coulter counts x % Red) and no significant relationship was found between various salinities and encystment of cells (Table 2). The fluorescence readings showed that the 0ppt culture was the healthiest.

Table 1. Coulter counts and hemacytometer counts for salt experiment 3, including calculated growth rates (Figures represent day 2 totals).

Coulter counts				
Salinity (ppt)	0	1	2	3
Cell counts ($x10^3$ cells/ml)	250	180	150	130
Growth rate (per day)	0.7	0.5	0.3	0.2
Hemacytometer counts				
Salinity (ppt)	0	1	2	3
Cell counts ($x10^3$ cells/ml)	170	120	100	90
Growth rate (per day)	0.5	0.3	0.2	0.1

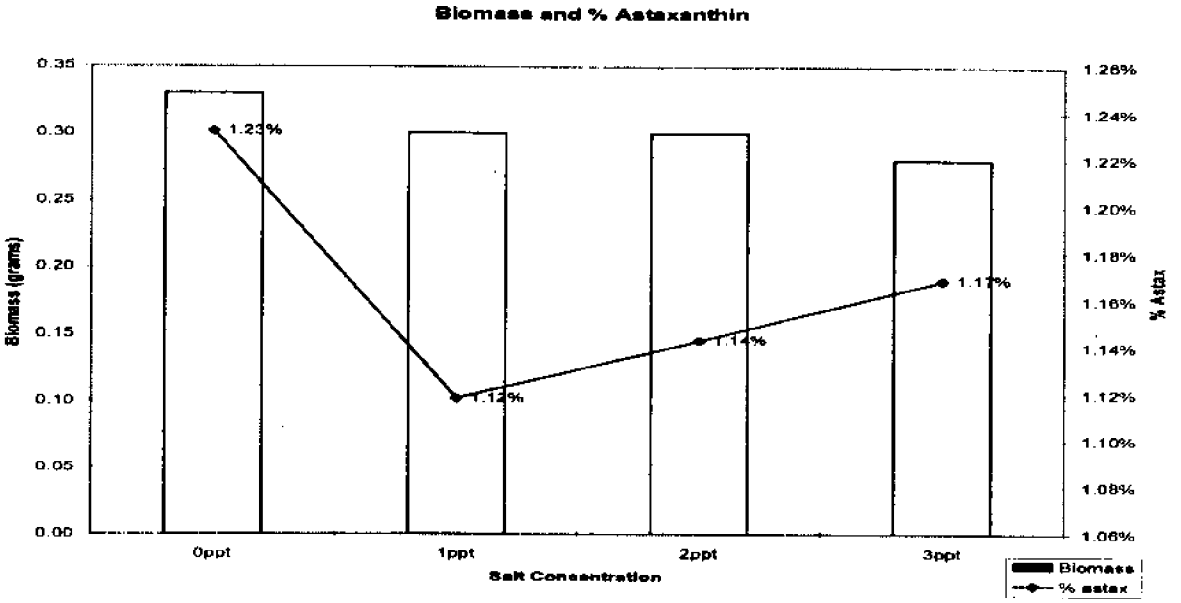

Figure 4. Mean total biomass and mean astaxanthin percentage for Haematococcus pluvialis cultures grown in experiment # 3.

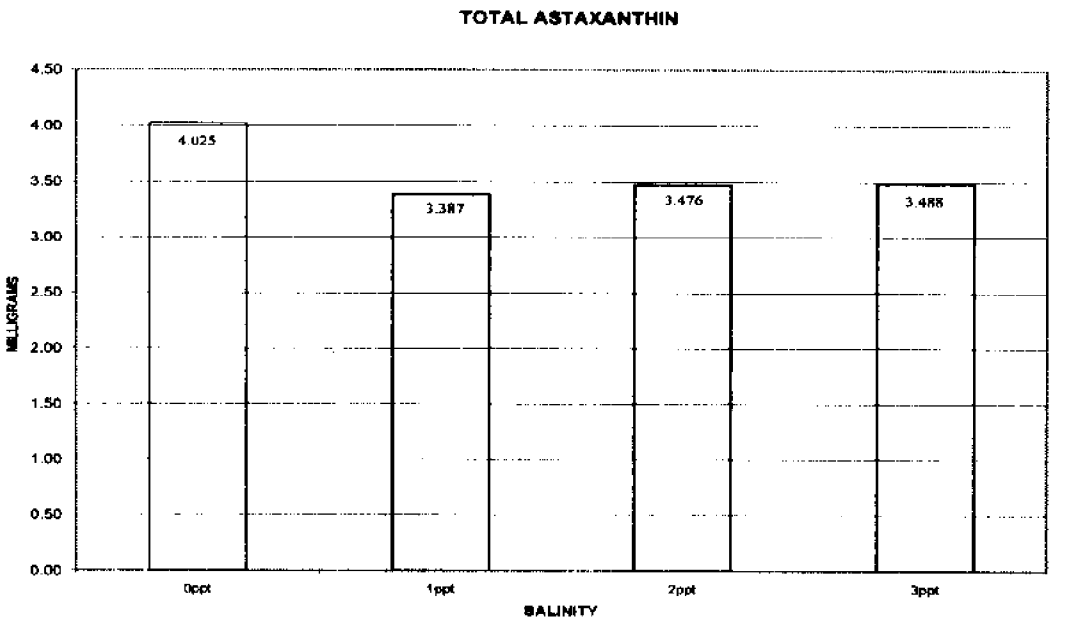

Figure 5. Mean total astaxanthin concentration of Haematococcus pluvialis for experiment # 3.

Table 2. % red, total red cells, % encysted, and fluorescence readings for salt experiment # 3 (Figures represent final day (6) totals).

Salinity (ppt)	0	1	2	3
% red	50	92	62	70
Total red cells(x10^3 cells/ml)	42	59	49	49
% encysted	100	100	100	100
Fluorescence (Fv/Fm)	0.595	0.537	0.53	0.525

Conclusion

The 0 and 1ppt salt cultures were found to be optimal salt concentrations for the maximum production of the ketocarotenoid astaxanthin, followed by 2 and 3ppt. This information was useful in the next several experiments

Salt experiment # 4

Objective

The purpose of this experiment was to determine whether the timing of the salt addition had a significant effect on the total production of astaxanthin.

Materials and Methods

Growth conditions- The growth conditions were similar to experiment # 3, with some exceptions. Ten cultures were grown instead of eight; the addition of salt occurred on the second and third days instead of at the beginning (all started at 0ppt). On the second day, cultures 1a and 1b were converted to 1ppt salt and 2a and 2b were changed to 2ppt salt. On the third day, four more cultures were changed to 1ppt and 2ppt. This left two cultures with no salt added to act as the control. Measurements and calculations were the same as experiment # 3.

Results

This experiment was run for seven days (July 23-30, 1998). The 0 ppt culture had the highest total and percent astaxanthin (Figures 6 and 7). No significant relationship was found with the timing of the salt addition. However, the cultures with salt added on the third day had the highest reddening percentages (Figure 8).

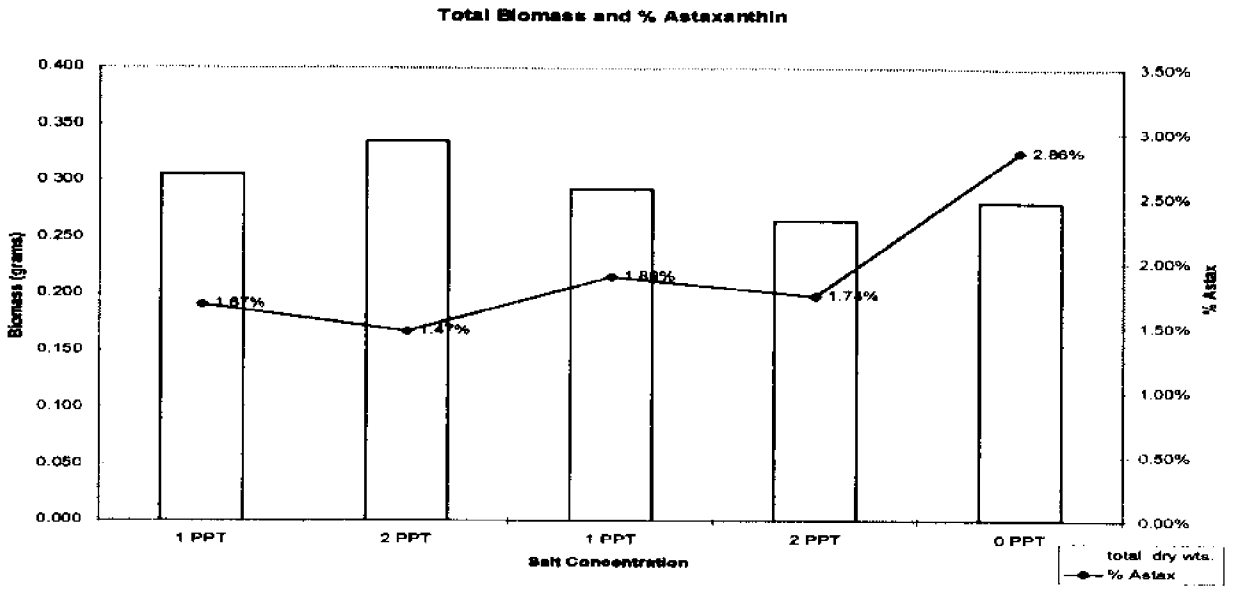

Figure 6. Mean total biomass and mean % Astaxanthin of Haematococcus pluvialis for experiment # 4. The first two columns represent the cultures with the salt addition on Day two. The next two are Day three salt additions.

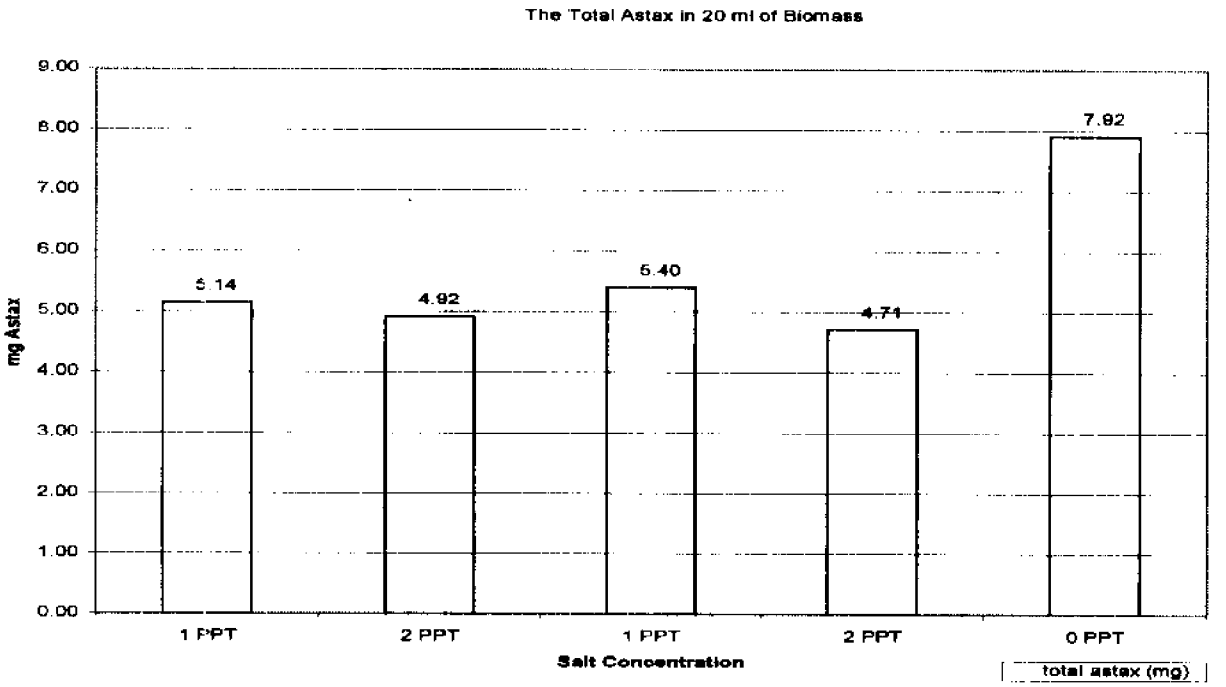

Figure 7. Mean total astaxanthin (in mg) per 20 ml of culture in experiment # 4. The first two columns represent the cultures with the salt addition on day two. The next two day three salt additions.

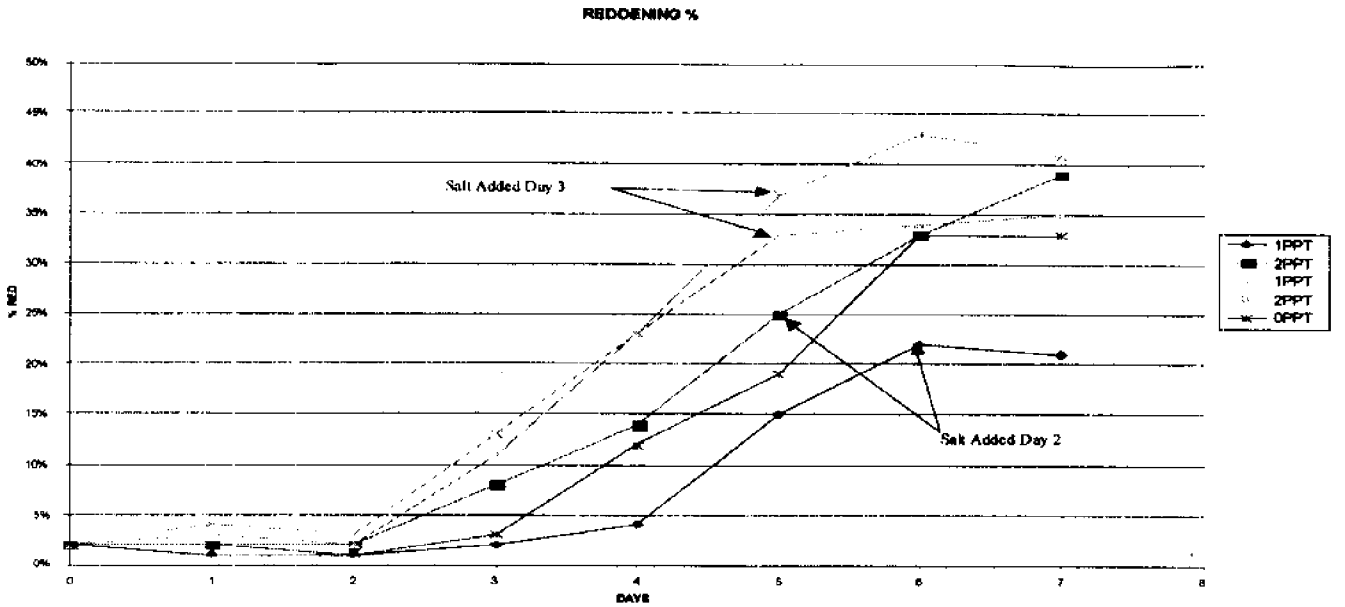

Figure 8. Reddening percentage of Haematococcus cells in experiment # 4.

Conclusion

The cultures with no salt added produced the most astaxanthin; therefore it was a good parameter to work with in the ponds. Adding salt to the cultures was inhibiting growth and producing less biomass of product. A series of large-scale pond experiments were run based on these results.

Salt experiment # 5

Objective

The purpose of this experiment was to determine whether the timing of the salt addition had a significant effect on the total production of astaxanthin.

Results

This experiment had the same parameters as experiment # 4. The results were questionable because of grazers and ciliates in the cultures. The grazers were much larger than the *Haematococcus pluvialis* alga (Figure 9). The grazer in this picture is approximately 150 μ in length; the *Haematococcus* cells are between 30 and 40 μ in diameter. Total and percent astaxanthin are shown in figure 10.

Figure 9. Grazer unicell and Haematococcus cells.

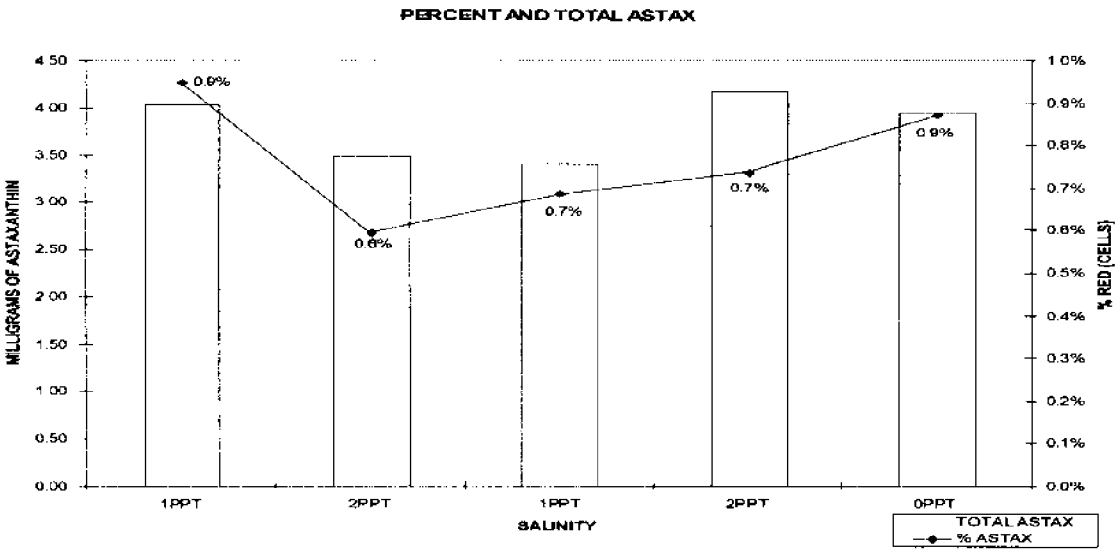

Figure 10. Mean percent and total astaxanthin for experiment # 5.

Pond experiment # 1

Objective

The objective of this experiment was to test the results of experiments 3 and 4 on a larger scale. Since the cultures with no salt added produced the most astaxanthin, no salt was used in this experiment.

Materials and Methods

Growth conditions- This experiment consisted of two pond cultures (grown outdoors in sunlight), one with 2ppt salt and the other with no salt added. The cultures were agitated by large paddle wheels. Pond 1 (P01-2ppt) was run for 6 days (July 28-Aug. 3, 1998) and Pond 2 (P02-0ppt) was run for 5 days (July 30-Aug 4, 1998). Both ponds were inoculated by a continuous harvest system, which is a slow culture feeding system. P01 was inoculated with 4000 liters (at 313 k cells/ml) over two days and P02 with 2000 liters (at 246 k cells/ml) over one day. Thus, P01 was inoculated with more than two and a half times the number of cells compared to P02. Two kg of sodium bicarbonate were added to each pond. The mean photo flux density for the ponds per day was 54.3 mol photon m^{-2} compared to 24.2 mol photon m^{-2} for small scale experiments three through five.

Measurements and calculations- Daily cell counts and microscope observations were made, as well as an astaxanthin analysis. The total biomass (in kg) going into each pond was calculated by the equation:

$$\text{Total biomass} = (\text{cells/ml})(\text{liters inoculated})/10^3$$

where cells/ml was the cell count for the module at the time it was harvested into the pond, liters inoculated were the volume of inoculum used (in thousands of liters). The total biomass coming out of each pond was dried and weighed (table 3). A fattening factor was quantified by these data (total out/total in) also shown in table 3.

Results

P02 had much higher cell counts by day 5 even though it was inoculated with much less culture (Figure 11). P01 began reddening much quicker than P02. However, by day 5, both ponds were 80 percent red (Figure 12). The total dry weight that came out of P01 was 2.48 Kg; P02 had 2.54 Kg. The astaxanthin percentage for P01 was 2.21% and 3.34% for P02. The total astaxanthin for P02 was 84.84 grams; P01 had only 54.81 grams (table 3).

Conclusion

The pond culture with no salt added (P02) produced more biomass and astaxanthin pigment than the 2ppt salt culture. P02 had a much higher fattening factor than P01 (5 compared to 2, respectively). The reddening in the pond with no salt occurred due to intense sunlight and a lack of nutrients. Although the light intensity was much

higher in the pond experiment than in small scale, the results were quite similar in the sense that no salt produced more astaxanthin.

Table 3. Pond experiment 1data.

Pond:	P01	P02
Salt concentration	2ppt	0ppt
Inoc.volume (L)	4000	2000
10^3 cells/ml (inoc)	313	246
Biomass in (kg)	1.252	0.492
Biomass out (kg)	2.48	2.54
Fattening factor	2	5
Days reddening	6	4
Astaxanthin %	2.21%	3.34%

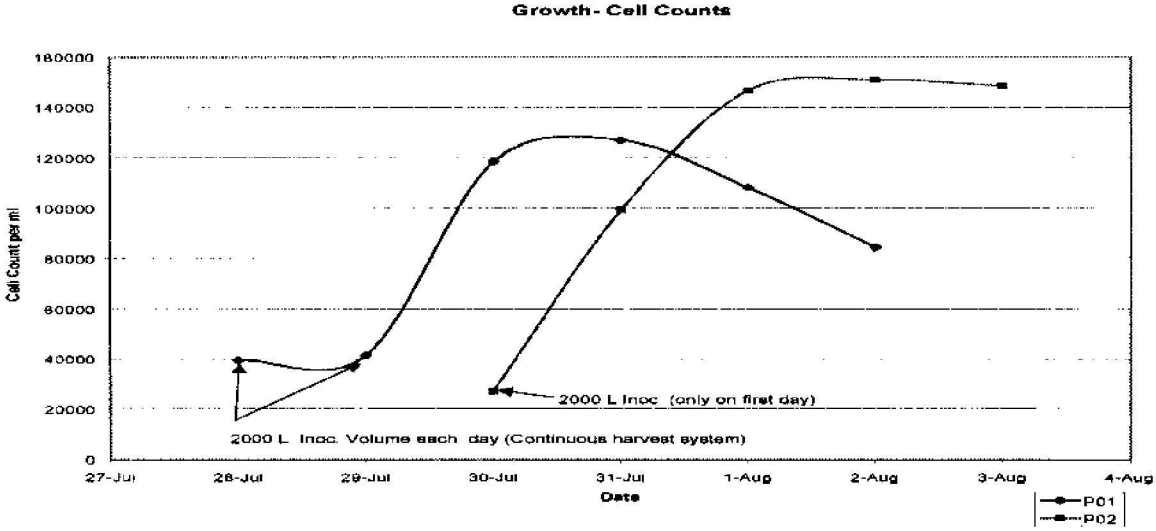

Figure 11. Haematococcus pluvialis *Coulter counts for Pond experiment # 1.*

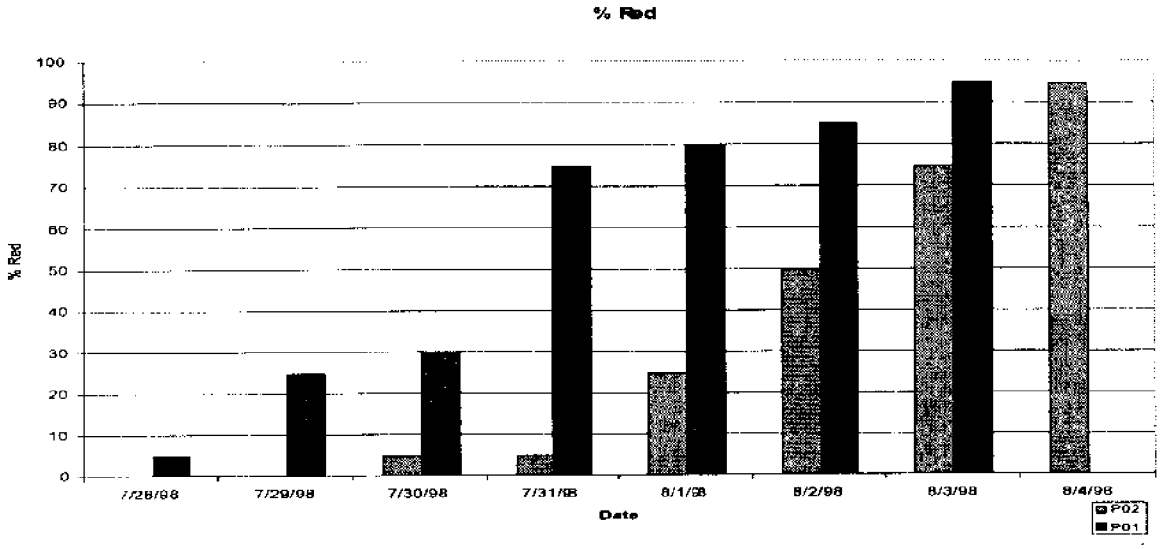

Figure 12. Daily reddening percentages for Pond experiment # 1.

Pond experiment # 2

Objective

The objective of this experiment was to continue testing the reddening of *Haematococcus pluvialis* cells under 0ppt and 2ppt salt concentrations (2ppt being the control culture).

Materials and Methods

Growth conditions- Growth conditions were similar to pond experiment 1. Again it consisted of two pond cultures, one with a 2ppt salinity and the other with 0ppt (P06 and P08, respectively, shown in table 4). Both ponds were inoculated by a batch system (all at once), and they were run for four days (Aug. 8-11). The mean photon flux density per day was 51.2 mol photon m^{-2}.

Results

P08 (0ppt culture) had a higher cell count than P06 (2ppt culture) after two days, which has been the trend for the no salt cultures (Figure 13). P06 had a higher fattening factor and astaxanthin percentage than P08 (table 4).

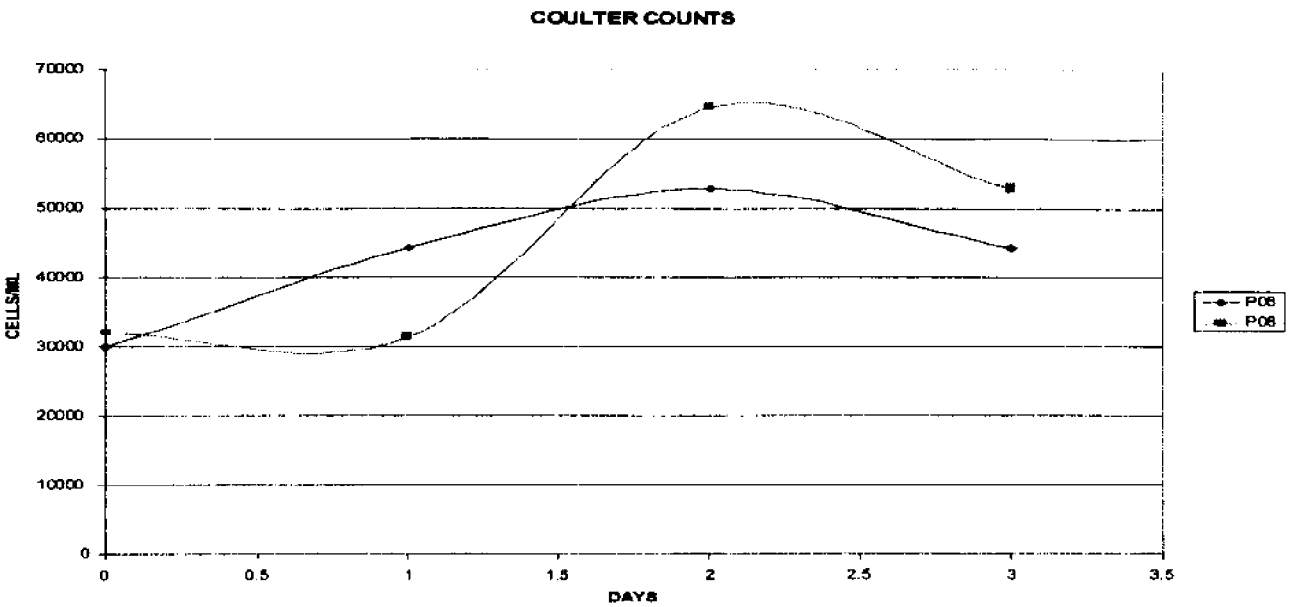

Figure 13. Haematococcus pluvialis Coulter counts for Pond experiment # 2.

Table 4. Pond experiment 2 data.

Pond:	P06	P08
Salt concentration	2ppt	0ppt
Inoc.volume (L)	2000	2000
10^3 cells/ml (inoc)	268	208
Biomass in (kg)	.536	.536
Biomass out (kg)	2.18	1.08
Fattening factor	4	2
Days reddening	4	4
Astaxanthin %	2.24%	1.82%

Pond experiment # 3

Objective

The objective of this experiment was to continue the ongoing testing for the reddening of *Haematococcus pluvialis* cells, under 0ppt and 2ppt salt concentrations (2ppt being the control culture), to obtain the optimal salinity for astaxanthin production.

Materials and Methods

Growth conditions- Growth conditions were similar to pond experiment 1 and 2. Again it consisted of two pond cultures, one with a 0ppt salinity and the other with 2ppt (P01 and P02, respectively, shown in table 5). These ponds were inoculated by a continuous harvest and the mean photon flux density per day was 49.7 mol photon m^{-2} for P01and 53.1 mol photon m^{-2} for P02.

Results

P01 (0ppt culture) had higher cell counts than P02 (2ppt culture)(Figure 13). P01 also had a higher fattening factor than P02 even though it got inoculated with half as much culture as the 2ppt salinity culture (Table 5). The 2ppt culture did produce a higher astaxanthin percentage (Table 5).

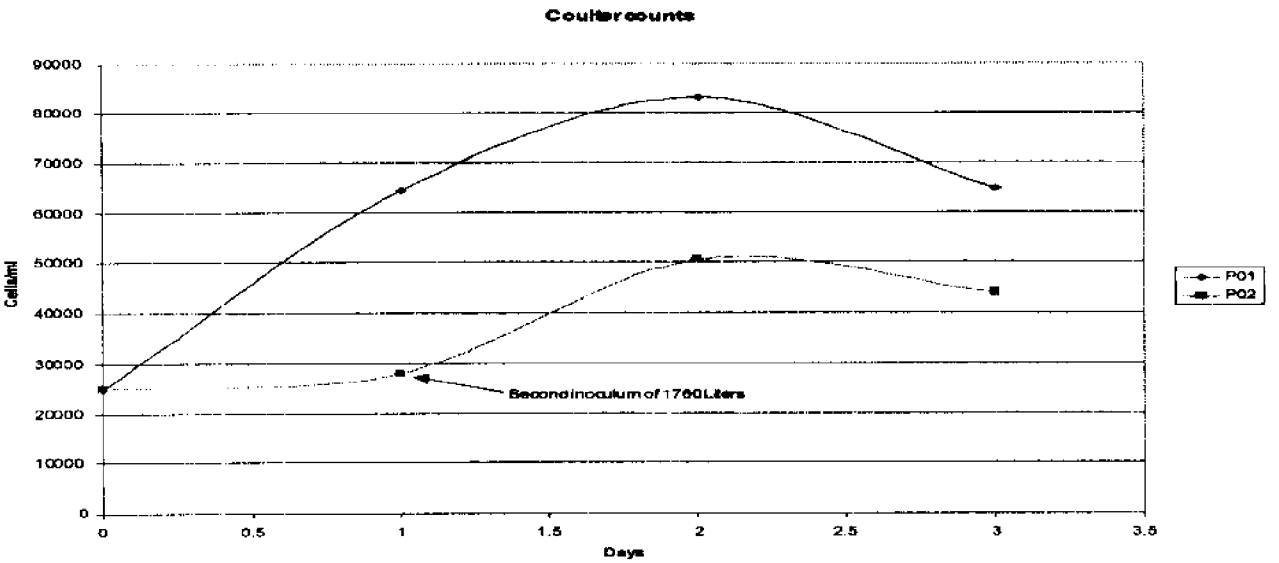

Figure 14. Haematococcus pluvialis Coulter counts for Pond experiment # 3.

Table 5. Pond experiment 3 data.

Pond:	PO1	PO2
Salt concentration	0ppt	2ppt
Inoc.volume (L)	1760	3520
10^3 cells/ml (inoc)	235	269
Biomass in (kg)	.414	0.947
Biomass out (kg)	2.05	2.3
Fattening factor	5	2.5
Days reddening	4	4
Astaxanthin %	1.98%	2.59%

Discussion

Most of the salt experiments, carried out at Aquasearch, found that cultures with no salt added produced the most of the ketocarotenoid astaxanthin. The cultures with salt added had much higher mortality rates, largely due to the toxicity of the salt. This explains the higher cell counts, growth rates, and fattening factors for the 0ppt cultures. However, not all of the experiments resulted in the 0ppt culture having the most astaxanthin produced or fattening factor and so on.

Other unknown factors may be involved in contributing to a variety of results. One possible factor, which may be significantly important, is the interaction between the salt and intense sunlight. There are many factors which may intensify another factor's effect when present; therefore, many more tests, including significance tests, should be carried out.

Literature on this topic all came to the conclusion that adding salt to the *Haematococcus* cultures in concentrations of 2-3 ppt (35-50 mM) produced the maximum yield of the ketocarotenoid astaxanthin. One possible explanation for 0 ppt not being found as optimal for astaxanthin accumulation is that the experiments run had relatively low light intensities. This would mean that salt stress was the overwhelming factor, not light intensity, and the cultures with no salt added would produce astaxanthin much slower than the cultures with salt added. The pond cultures were exposed to a photon flux density of 600-2500 µmol photon m^{-2} s^{-1} during the day. The highest light intensity used reported in the literature with the addition of salt was 89 µmol photon m^{-2} s^{-1} (Harker et al., 1995).

In a study by Boussiba and Vonshak (1991), continuous aeration was supplied with 1.5 % CO_2 in which the pH was maintained between 6.8 and 7.0. The light used had a photon flux density of 85 µmol photon $m^{-2} s^{-1}$ as opposed to 280 µmol photon $m^{-2} s^{-1}$ used in my small scale experiments. Since there was more light for photosynthesis in my experiment, the CO_2 flow rate was set proportionally higher.

I recommend that ongoing experiments with 0ppt salinity (2ppt as the control) be done in the cultivation of *Haematococcus pluvialis.* Varying light levels and cell densities may also show significant interactions with certain saline levels in the process of astaxanthin formation and encystment. There may also be other factors that are affecting the factors already mentioned, such as nutrient levels or circulation. Studying these interactions may supply us with results, which are economically, biologically, and economically beneficial.

Evaluation of Learning

Taking the Fellowship program at Aquasearch Inc. was a worthwhile educational experience. I was trained to use numerous instruments and equipment, including the spectrophotometer, centrifuge, autoclave, vortex, microson ultrasonic homogenizer (sonicator), impulse sealer, photo sensor, Coulter counter, temperature sensor, Hemacytometer, pH meter, R/G-98 photometer, and Pam fluorometer. I have learned to manage my time well because I always seemed to be pressed for time working at Aquasearch. I have gained a better understanding of how to write proposals and reports (scientific format).

My computer skills improved significantly since working at Aquasearch Inc. I learned how to make graphs and format data sheets (in MS Excel). Analyzing my data was difficult until I learned how to present my data where it could be useful (with the use of graphs).

I also learned to work individually on my own work schedule and also as a team. Much of my time was spent on individual work where I learned to manage my time according to the task at hand.

Acknowledgements

I would like to thank the Department of Land and Natural Resources, the University of Hawai'i Sea Grant College Program, and Aquasearch for giving me the opportunity to participate in the third annual Undergraduate Summer Research Fellowships. It has been a wonderful experience for me and I am truly grateful. In

conducting research in microalgae cultures at the Aquasearch at the Natural Energy Laboratory of Hawaii Authority at Ke'ahole Point on the Big Island I have gained new interests in the field of microalgae, and possibly a career opportunity.

I would also like to thank mentors Miguel Olaizola, Karla McDermid, and Sherwood Maynard for their time and effort put into the project; and especially to Miguel for the incredible patience he displayed for the entire Summer.

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