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AN ABSTRACT OF THE THESIS OF David E. Pendleton for the Master of Science in Biology presented January 5, 1983.

Title: Potential impact of a proposed stack gas sulfur scrubber on *Gracilaria arcuata* (Rhodophyta).

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Gracilaria arcuata, a benthic macroalga, was selected as an indicator organism prior to a proposed pollution discharge. The relative abundance, distribution and productivity of this red alga in the outfall area were determined by field and laboratory studies. A bioassay was conducted to evaluate the potential impact of a proposed power plant stack gas sulfur scrubber on *Gracilaria*.

The total *Gracilaria* biomass in the proposed effluent outfall area was estimated to be 106,000 kg. Little change in abundance and distribution was observed over a 16-month period. Estimates of the energy contribution of *Gracilaria* productivity in the outfall area were 8.6 and 8.7×10^5 kcal per day, based on respirometry and growth studies, respectively.

A bioassay showed that pilot scrubber effluent concentrations of 25% or greater resulted in significantly lower *Gracilaria* growth. Concentrations of 50% or greater resulted in significantly lower photosynthetic rates. Most of the outfall area would be subjected to at

least 67% effluent if a full-scale scrubber is built. Therefore, the likelihood of complete decimation is high.

Similar results were found between field population productivity estimates made with an oxygen probe for large seaweed clumps in a large chamber, and productivity measurements made with a manometric, multiple chamber Gilson respirometer for small sprigs.

POTENTIAL IMPACT OF A PROPOSED STACK GAS SULFUR SCRUBBER ON *GRACILARIA ARCUATA* (RHODOPHYTA)

By

David E. Pendleton

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INTRODUCTION

The use of algal species as pollution indicators is widespread. Many freshwater and temperate marine unicellular algae have been used as pollution indicators and as standard bioassay test organisms. However, it is generally agreed (Blaker 1957, Lackey 1959, and Edwards 1972) that benthic organisms are best suited for use as indicator organisms because they reflect the dominant environmental conditions in an area over a long period. Hoyle (1975) completed an extensive list of benthic marine macrophyte species that have been used in pollution studies. Various growth bioassay methods (Skulberg 1966, Baalsrud 1967, Fitzgerald 1968, Fitzgerald and Nelson 1966) were described. However, only two of the pollution studies dealt with semitropical species (Doty 1968 and McNulty 1961) and none dealt with tropical species.

The use of benthic tropical marine macroalgae as pollution indicator organisms was proposed by Thorhaug (1976) because in tropical nearshore regions benthic macrophytes are the dominant producers and it is in these regions that man's impact is greatest.

In this study the benthic red seaweed Gracilaria arcuata was selected as a pollution indicator and bioassay test organism in an assessment of the potential impact of a proposed power plant stack gas scrubber located on Guam. Selection of G. arcuata was based on its relative abundance in the power plant outfall area, where its standing crop is greater than any other macrophyte species, and on the fact that

it is easily maintained in the laboratory. In addition, the biology of this species is relatively well known.

Gracilaria arcuata is one of the six species of this genus on Guam and one of about 100 Gracilaria species widely distributed throughout temperate and tropical waters of the world (Dawson 1966). This genus is the object of commercial harvest and culture in many areas of the world (Nelson et al. 1980). The most common species on Guam, G. arcuata, may prove to be a valuable mariculture candidate. Its potential uses include agar production, wastewater treatment and employment in standard bioassays. It grows relatively quickly, making it important as a primary producer in areas where a large standing crop exists. G. arcuata is found in a variety of habitats on Guam including the leeward, wide reef flats, where it sometimes washes ashore in great quantities; the Piti Channel tidal flats, where a somewhat stable population occurs; and even the Cabras Power Plant effluent canal, where temperatures over 37°C have been recorded and where the proposed scrubber effluent would be discharged.

In this study the potential impact of the proposed sulfur scrubber was estimated by field surveys which determined the relative abundance, biomass and seasonality of Gracilaria arcuata in the Piti Channel area and by a bioassay designed to show any difference in growth rates, respiration rates and productivity of Gracilaria grown under various concentrations of sulfur scrubber effluent provided by a scaled-down version of the proposed Cabras Power Plant stack gas scrubber. The field surveys were augmented by studies designed to characterize growth rates, productivity and respiration rates of Gracilaria and by an

analysis of the chemical and physical properties of seawater in the outfall area.

The scrubber systems of the pilot plant for the bioassay and the proposed full-scale plant use seawater as the stack gas sulfur dioxide absorbent. This process utilizes the natural alkalinity of seawater and also uses the dilution and aeration of scrubber effluent to minimize the decrease of pH and dissolved oxygen. Constituents which may be added to the seawater through use of this process include sulfate, sulfite, nickel, vanadium and petroleum hydrocarbons (R. W. Beck and Associates 1982).

MATERIALS AND METHODS

Field Surveys

Twelve monthly surveys with a point-quadrat method were conducted to quantify the abundance (% cover) of *Gracilaria* in the outfall lagoon, upper and lower Piti Channel and Tidal Flats B and C (see Figure 1). Fifty-four somewhat evenly spaced sampling sites were selected. A 1-m^2 quadrat was tossed haphazardly in the general area of each sampling site once during each monthly survey. The quadrat was divided into a grid of 25 squares, providing 16 interior points where the grid lines intersected. *Gracilaria* was recorded at every point at which it occurred. Values representing abundance were calculated from these data as percent of the total 864 points (16 interior points x 54 locations), i.e., n/864 x 100 = % cover (Tsuda 1972).

A 30-m transect was located within each of two sites where *Gracilaria* was most abundant. This allowed a more accurate estimate of standing crop in those areas. Sampling was accomplished with a 1-m^2 quadrat placed carefully on alternating sides of the transect at 1-m intervals. Relative abundance values were calculated in the same manner as those for the haphazard-toss quadrat survey. Transects were surveyed every 6 weeks for 14 months.

Total *Gracilaria* cover within the Piti Channel area was calculated by the formula, $T_{CP} = C \times A$, where C = average % cover determined by haphazard toss surveys and A = total sample area. Total *Gracilaria* cover within the outfall canal was calculated by the formula $T_{CO} = C \times$

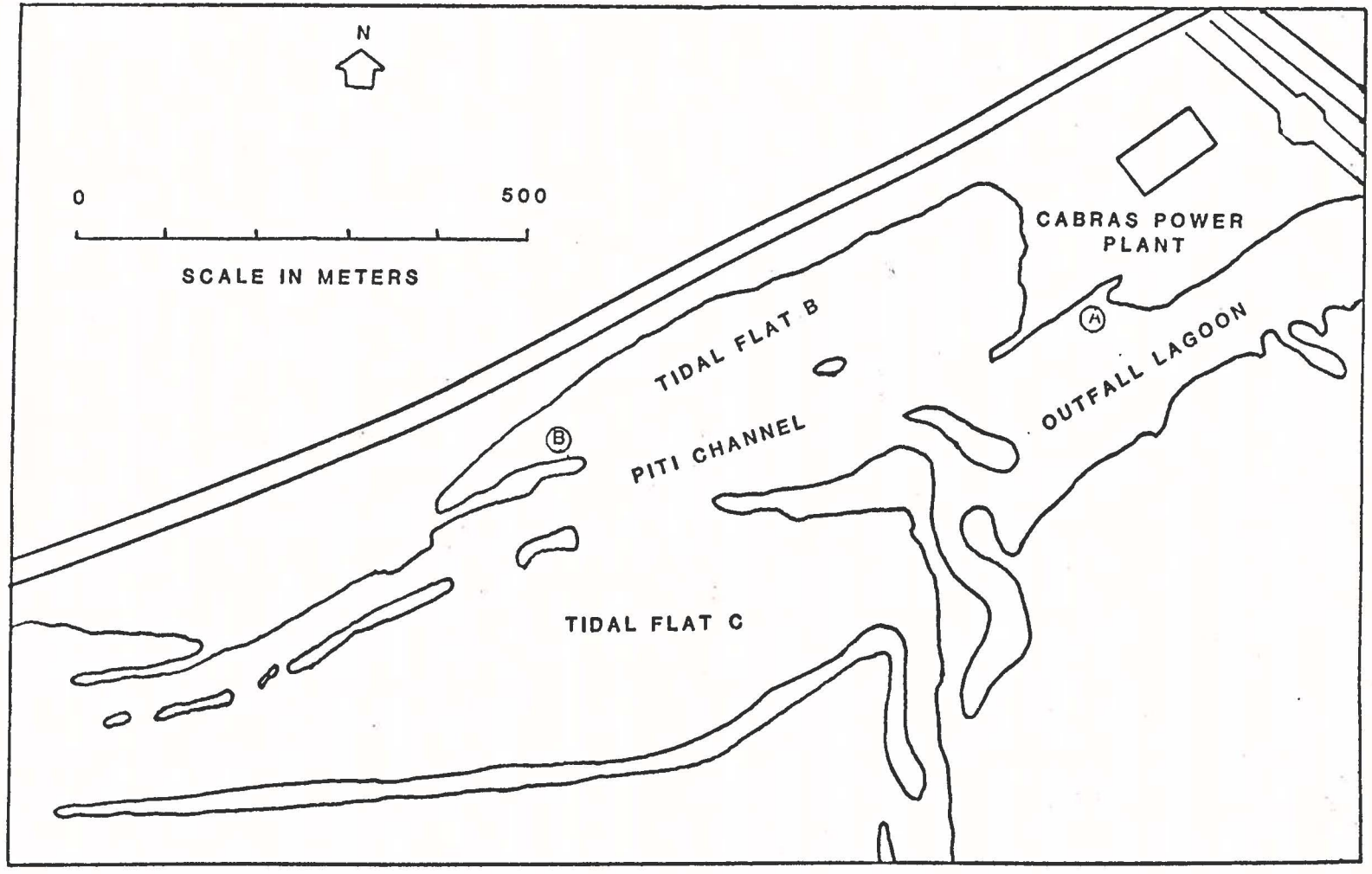

Figure 1. Cabras Power Plant, site of the proposed stack gas sulfur scrubber, and the effluent outfall area. Circled letters A and B indicate transect survey sites.

$A_T \times A_G/A_T$, where C = average % cover, A_T = representative transect sample area and A_G = total estimated area of the Gracilaria region in the outfall canal. Total Gracilaria cover (T_{CW}) within the westernmost portion of Tidal Flat B (dead-end channel) was estimated in the same manner. Total Gracilaria cover for the remainder of Tidal Flat B was estimated using the formula $T_{CB} = T_{CP} - T_{CO} - T_{CW}$, where T_{CP} = total cover of Piti Channel area, T_{CO} = total cover of outfall canal and T_{CW} = total cover of dead-end channel (since Gracilaria was found almost entirely in these regions).

Thickness of Gracilaria mats found below quadrat points was measured during two transect surveys and two haphazard-toss quadrat surveys. An average thickness for algal mats was then calculated. An estimate of Gracilaria mat volume was calculated by multiplying abundance (% cover) by average mat thickness.

Total biomass was calculated by using an estimated (biomass) x $(\text{clump volume})^{-1}$ ratio. Gracilaria mat volume estimates necessarily included water volume because of the form which G. arcuata assumes under natural conditions; the branched, cylindrical thalli are intertwined in such a way as to preclude accurate measurement of individual Gracilaria thalli. To determine the biomass/clump volume ratio, five small clumps were taken haphazardly from large mats in the outfall area. Each clump was covered with plastic wrap without altering clump volume significantly. Volume was determined for each clump by water displacement. Biomass for each clump was determined using a triple-beam balance. A mean of the five ratios thus generated was then calculated.

Growth Studies

For all growth studies Gracilaria arcuata was collected from the outfall area and trimmed to selected clump size categories. G. arcuata can "bud" from any part of its thallus, which makes it easy to manipulate in this manner. Each clump was loosely bundled in 3-cm nylon mesh and identified with an attached mylar tag. Each bundle was weighed to the nearest 0.1 g on a triple-beam balance at beginning and end of each test period. Prior to each measurement, the clump was blotted dry and visible epiphytes and commensal animals were removed.

Laboratory growth studies were conducted in both static and flow-through systems in order to separate system effects from effluent effects. In the static system, bundles were placed in 75-ℓ glass aquaria which had been filled with seawater. The tanks were placed so that they received direct sunlight and were shaded for about 6 hours each. Fresh water was added periodically to maintain a fairly constant salinity. Three flow-through systems were incorporated. One flow-through system used a large holding tank and small propylene baskets (30 x 45 x 10 cm). The bundles were attached to the bottom of the baskets, which were allowed to float in the large holding tank. The Gracilaria thus stayed about 10 cm below the water's surface. In the second flow-through system, each bundle was attached to a nylon line stretched across the top of a 600-ℓ polyurethane tank with a depth of about 1 m. Each bundle was suspended about 20 cm below the water's surface. The final flow-through system incorporated a relatively shallow tank with a bottom area of about 6 m^2. The water depth was maintained at about 20 cm. The tagged bundles or thalli were placed on

the bottom. Each flow-through system had a constant input of seawater pumped directly from the reef margin of Pago Bay.

Two field growth studies were conducted near transect B in the outfall area. Cages, designed to exclude herbivorous fish, were constructed of chicken wire and iron rebar. The cages, each 100 x 100 x 50 cm, were placed below low-tide level. Tagged Gracilaria bundles were attached to the monofilament nylon line within the cages and suspended in midwater. Because of tidal fluctuations, the thalli depth varied from about 20 to 100 cm.

The specific growth rate for each bundle within each test was calculated by the formula, $\mu = \frac{100 \cdot \ln(m_1/m_o)}{t}$, (Nelson et al. 1980) where m_o = initial mass in grams, m_1 = final mass in grams, ln = natural log, and t = time in days. The average mass of each bundle during the test period was calculated by the formula $\frac{m_o + m_1}{2}$. The mean specific growth rate ($\bar{\mu}$) for each test was determined by dividing the sum of the specific growth rates by the number of clumps tested.

Production and Respiration Studies

To determine estimates of Gracilaria productivity within the study site, it was necessary to test it in the tightly packed form that occurs in this area. A large respirometer chamber was built to accomodate 100-g Gracilaria clumps. The rectangular chamber was made of molded glass with rounded corners to prevent dead space. The interior volume was 9.56 ℓ. The rim of the chamber was covered by split plastic tubing permanently attached by silicone sealant. The tubing, like glass, is chemically inert and provided an effective seal between the container and lid. The glass lid contained a central opening made from

the top of a B.O.D. bottle. This provided a precise fit for a self-stirring Y.S.I. oxygen probe.

Three respirometer experiments were completed. Gracilaria clumps were collected and transported to the lab, where all observable commensals and epiphytes were carefully removed prior to placement in the chamber. Seawater placed in the chamber was first passed through a 0.45-μ filter. The chamber was then placed in a water bath and covered with aluminum foil for 1.5 h while O_2 consumption was monitored. The foil was next removed and the chamber was exposed to direct sunlight for 1.5 h while O_2 production was monitored. Stirring was constant and oxygen levels were monitored at 10-min intervals with the oxygen meter and probe. Linear changes were observed during all tests.

All data obtained by these investigations were expressed in μg $O_2 \cdot g^{-1} \cdot h^{-1}$. It was assumed that respiration remained constant during light and dark periods. Gross photosynthesis values were calculated as the sum of net photosynthesis and respiration.

Investigations with a Gilson respirometer were also conducted in order to have an independent check on the results from the previously described studies with large respirometers. Small Gracilaria sections were cut from growing tips 24 h before each experiment. Tips, weighing a total of about 1.2 g, were placed in each of 14 Gilson reaction flasks containing 15 mℓ of seawater. Light and dark periods lasted 40 to 50 minutes and resulted in linear responses. Temperature was maintained at 32°C and photon flux density, measured with a Licor model 185-A meter with an underwater sensor, during the light period, was 240 $\mu E \cdot m^{-2} \cdot s^{-1}$. Changes in micrometer readings are proportional to gas

volume changes according to the equation, $\mu\ell\ O_2 = \frac{273\ (P-PW)\ Vg}{760t}$ where P = barometric pressure, Pw = vapor pressure of seawater at temperature t in °K and Vg = $\mu\ell$ of gas exchanged (Umbriet et al. 1972). All volume changes were attributed to O_2 exchange (see Appendix I). Values for production and respiration are reported in terms of $\mu g\ O_2 \cdot g^{-1} \cdot h^{-1}$ where g = Gracilaria wet weight in grams. These values were derived from the relationship 1 $\mu g\ O_2$ = 0.7 $\mu\ell\ O_2$ at STP.

Physical and Chemical Properties of Outfall-Area Seawater

Two sites were selected for monitoring near transects A and B where Gracilaria was abundant. Dissolved oxygen, pH, salinity, nitrate and phosphate values were determined for duplicate grab samples every 3 h and 18 h. Dissolved oxygen, pH and salinity were also measured weekly during a 6-wk period. Dissolved oxygen measurements were accomplished using a Y.S.I. D.O. meter and probe calibrated daily. Measurements of pH were performed with an Orion 601 meter and probe calibrated daily with standard buffer solutions. Salinity determinations were made with a refractometer. Temperature ranges were measured weekly with maximum-minimum thermometers. Nitrate levels were determined with a Beckman B spectrophotometer in accordance with a cadmium reduction technique adapted from Stainton et al. (1977).

Bioassay

Gracilaria arcuata was collected from the outfall area and apportioned into bundles of about 20 g each with visible epiphytes and commensal animals carefully removed. Twelve bundles were placed into each of twelve, 30-ℓ aquaria containing a constant ambient seawater flow and located in a water bath. On the following day, scrubber

effluent was allowed to pass through dilution chambers and was thus properly diluted with various amounts of ambient seawater before it passed through the aquaria. The 12 aquaria containing the 6 dilutions (0, 25, 50, 75, 87.5 and 100% effluent) and replicates were positioned randomly in the water bath.

Two 40-watt fluorescent tubes and two 150-watt incandescent flood lamps were positioned 10 cm above each aquarium. Photon flux density was approximately 300 $\mu E \cdot m^{-2} \cdot s^{-1}$, which was above saturation and below photoinhibition levels (James 1982). Because of power limitations, six aquaria were lighted between the hours of 0015 and 1145 and the other six from 1215 to 2345 daily.

Seven days after Gracilaria collection, the clumps were removed from the aquaria, blotted dry, cleaned of all visible commensals and epiphytes, weighed, wrapped loosely in 3-cm nylon mesh, tagged and returned to the aquaria from which removed. The determination of initial weights was delayed for 7 days because initial growth after collection was probably influenced more by prior field conditions than by test conditions. Final weights were determined in the same manner 17 days after the initial weighing.

Gilson respirometer studies were completed before effluent was directed to aquaria and twice after completion of growth bioassay. For all studies, two small sprigs were removed from each Gracilaria bundle and placed in one of 12 Gilson reaction flasks corresponding to the aquarium from which the sprigs were removed. Each flask contaned 15 mℓ of seawater with the proper effluent concentration (in the initial test the effluent concentration of all flasks was 0). Analysis methods were

the same as in Gilson respirometry studies performed in conjunction with the field surveys.

Also at the end of the growth study, the respiration rates and productivity of selected Gracilaria clumps were analyzed using the 9.56-ℓ respirometry chamber described earlier. Five clumps from each of the four aquaria containing either 0% or 100% effluent were selected randomly. Each group of five seaweed clumps was placed separately in the respiration chamber. Test procedures followed those described previously.

Physical and chemical properties of 100% effluent and ambient seawater were determined from grab samples taken daily during the bioassay. Temperatures within each aquarium were also measured periodically with a mercury thermometer. Load was determined for each treatment by multiplying mean flow by effluent concentration. These data are summarized in Appendix II.

RESULTS

Field Surveys

Gracilaria abundance values (% cover) obtained from haphazard-toss and transect surveys are summarized in Figure 2. Little change in *Gracilaria* abundance occurred in the Piti Channel area during the 16-mo period, as determined by the haphazard-toss surveys. Transect surveys revealed that the outfall canal population varied between 18 and 55% cover and the dead-end channel population varied between 62 and 80% cover.

The average total *Gracilaria* cover for the Piti Channel area was estimated to be 9,700 m^2. The average total *Gracilaria* cover for the outfall canal and westernmost portion of Tidal Flat B were estimated to be 250 and 2,200 m^2, respectively. The remaining *Gracilaria* was located almost entirely within the remainder of the Tidal Flat B area.

Estimates of average *Gracilaria* biomass in the Piti Channel area, the outfall canal, the westernmost portion of Tidal Flat B and the remainder of Tidal Flat B are contained in Table 1. These are proportional to total *Gracilaria* cover values since mat thickness was similar between surveys and areas.

Growth Studies

Gracilaria growth study results are summarized in Table 2. Growth rates in static and flow-through systems were not markedly different. Growth rates in Tidal Flat B were obviously higher than average

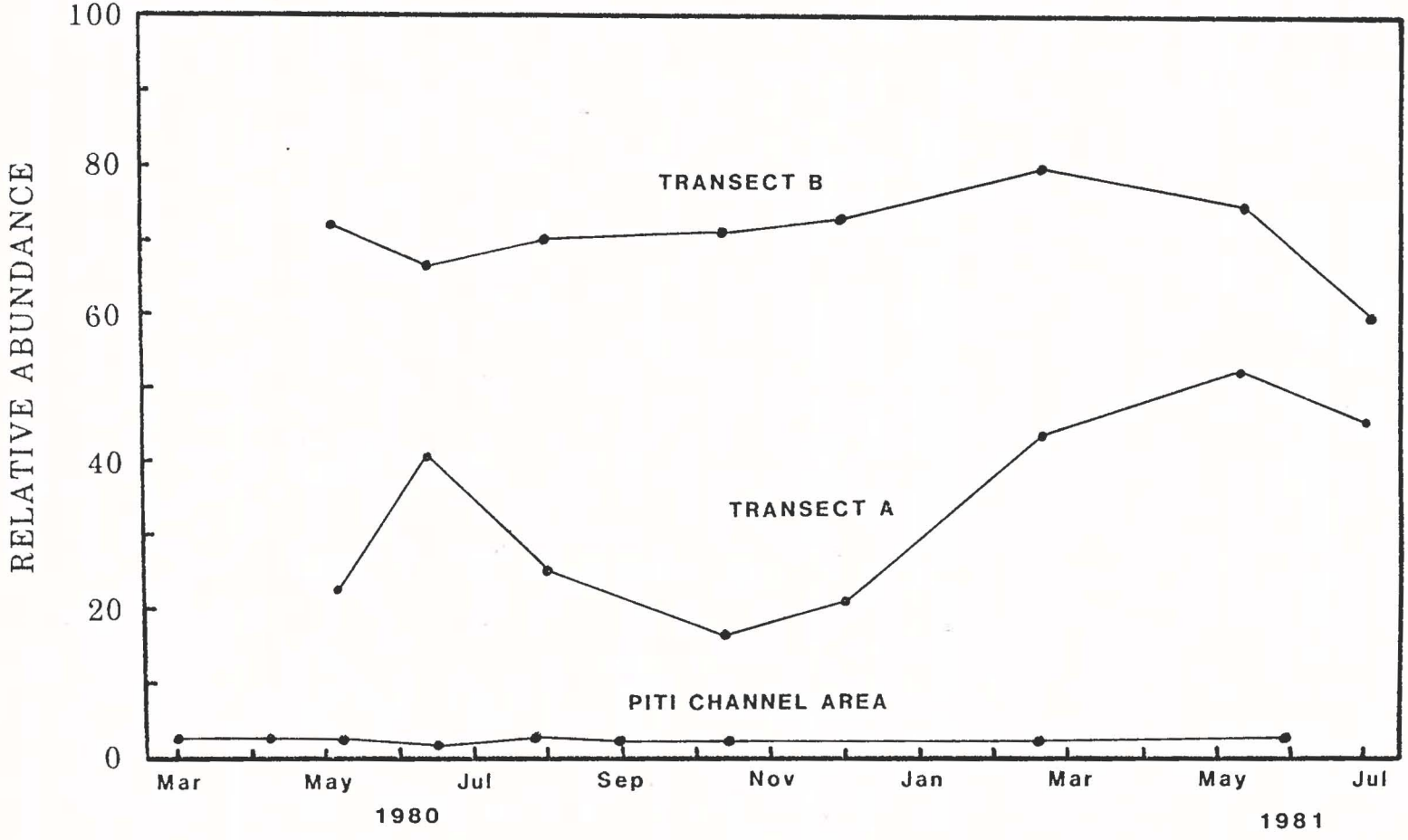

Figure 2. Gracilaria abundance in the Piti Channel area and in the outfall canal (transect A) and dead-end channel (transect B).

Table 1. Estimated averge *Gracilaria* biomass (kg) of the field population.

Piti Channel Area	106,000
Outfall Canal	2,900
Westermost Portion, Tidal Flat B	26,200
Remainder, Tidal Flat B	76,900

Table 2. *Gracilaria arcuata* growth study results; $\bar{\mu}$ is the mean specific growth rate as explained in the text.

Test System	Week	n	$\bar{\mu}$	SD
Static	1	20	1.814	1.5581
	2	20	1.649	0.9293
	3	14	0.123	0.9915
Initial flow-through	1	16	2.714	0.9522
	2	16	0.895	0.4174
	3-4	16	0.627	0.5754
Final flow-through	1	31	1.979	0.8393
	2	31	1.448	1.0471
	3	19	1.222	0.2717
	4	19	0.563	0.3739
Tidal Flat B	2	18	2.583	0.7903
	3	18	2.242	0.7182
	4-5	18	0.481	1.0159
	6-9	18	0.932	0.0470

laboratory growth rates for test periods up to 3 weeks. All laboratory growth studies resulted in declining growth rates with time. Gracilaria clumps began to fall apart after three weeks in the static test and after four weeks in the flow-through tests. Gracilaria clumps grown on Tidal Flat B remained intact after nine weeks. A decline in mean growth rate during the first five weeks was followed by an increase during the sixth through ninth weeks.

Production and Respiration Studies

Table 3 contains a summary of respirometer experiments with large Gracilaria clumps. Outfall Gracilaria exhibited slightly higher P/R ratios but gross photosynthesis for the final three tests was quite similar. Photosynthesis and respiration rates for Gracilaria sprigs tested in the Gilson respirometer, shown in Table 4, were markedly similar to Gracilaria clump photosynthesis and respiration rates. Respiration rates were generally higher and photosynthesis lower in the Gilson instrument.

Physical and Chemical Properties of Outfall Area Seawater

The results of physical and chemical analyses on weekly grab samples taken from the outfall canal and dead-end channel are contained in Table 5. Data indicate that relatively little change took place for all parameters tested within the outfall canal. Properties of the dead-end channel seawater varied much more during this period. Maximum and minimum temperatures were both higher and lower, respectively, in the dead-end channel. Dissolved oxygen and salinity of dead-end channel seawater samples were somewhat lower while pH for both areas varied little and remained nearly identical.

Table 3. Rates of respiration and gross photosynthesis (μg $O_2 \cdot g^{-1} \cdot h^{-1}$) of Gracilaria clumps in the large respirometer.

Sample Collected From	Respiration Rate	Photosynthesis Rate	P/R
Tidal Flat B	52	212	4.1
Tidal Flat B	57	367	6.4
Outfall	37	336	9.1
Outfall	42	330	8.3
Mean Values	47	316	6.7

Table 4. Mean rates of respiration (R) and gross photosynthesis (P) (μg $O_2 \cdot g^{-1} \cdot h^{-1}$) of Gracilaria growing tips in the Gilson respirometer.

Sample Collected From	n	R	SD	P	SD	P/R
Tidal Flat B	10	75	19	255	89	3.4
Tidal Flat B	11	60	9	295	22	5.9
Outfall	14	52	15	212	36	4.1
Outfall	12	42	16	174	59	4.2

Table 5. Mean values and standard deviations of weekly grab sample analyses of dissolved oxygen ($mg \cdot \ell^{-1}$), salinity (°/₀₀), pH and temperature (°C).

	n	D.O.	Salinity	pH	Temperature (Max.)	Temperature (Min.)
Dead-End Channel	5	7.11	29.8	8.34	38.2	30.3
		1.10	1.2	0.07	0.7	0.8
Outfall Canal	6	6.94	31.4	8.36	36.4	33.5
		0.48	0.4	0.05	1.0	0.8

The results of the 18-h grab sample analyses of outfall and dead-end channel seawater are shown in Figure 3. Dissolved oxygen in the dead-end channel showed a definite diurnal cycle. Salinity in the dead-end channel was quite variable, but appeared to be inversely related to nitrate levels. Salinity and nitrate levels in the outfall canal were each more or less constant. Seawater pH was slightly less in the dead-end channel with both sites exhibiting little pH change.

Bioassay

Mean specific growth rates for Gracilaria clumps within each treatment (% effluent) during the bioassay are shown in Figure 4. Since $F_{max\ .05(6,23)} = 2.54$ is not significant at $P>0.05$, the variances of the 6 treatments are considered homogeneous. Therefore, multiple comparisons among means were calcualted with the Student-Newman-Keuls test. Results of this test showed that the (25-100) % group means were not significantly different at $P>0.05$. The 0% group was shown to have grown significantly more than each category of the (25-100) % effluent group at $P<0.05$.

The mean results of Gilson respirometry studies completed after 24 and 27 days of bioassay are presented in Figure 5. Two-way anova paired comparisons of photosynthesis and respiration rates showed no significant changes between dates (F_s = 4.13 ns and 1.15 ns, respectively), at $P>0.05$. Therefore, these test results were combined. The Student-Newman-Keuls test was also calculated for these data since $F_{max\ .05(6,3)} = 60.2$ is not significant at $P>0.05$. Results of these tests showed that respiration rates did not differ significantly at $P>0.05$ between groups. The photosynthetic rates of the 0 and 25% effluent

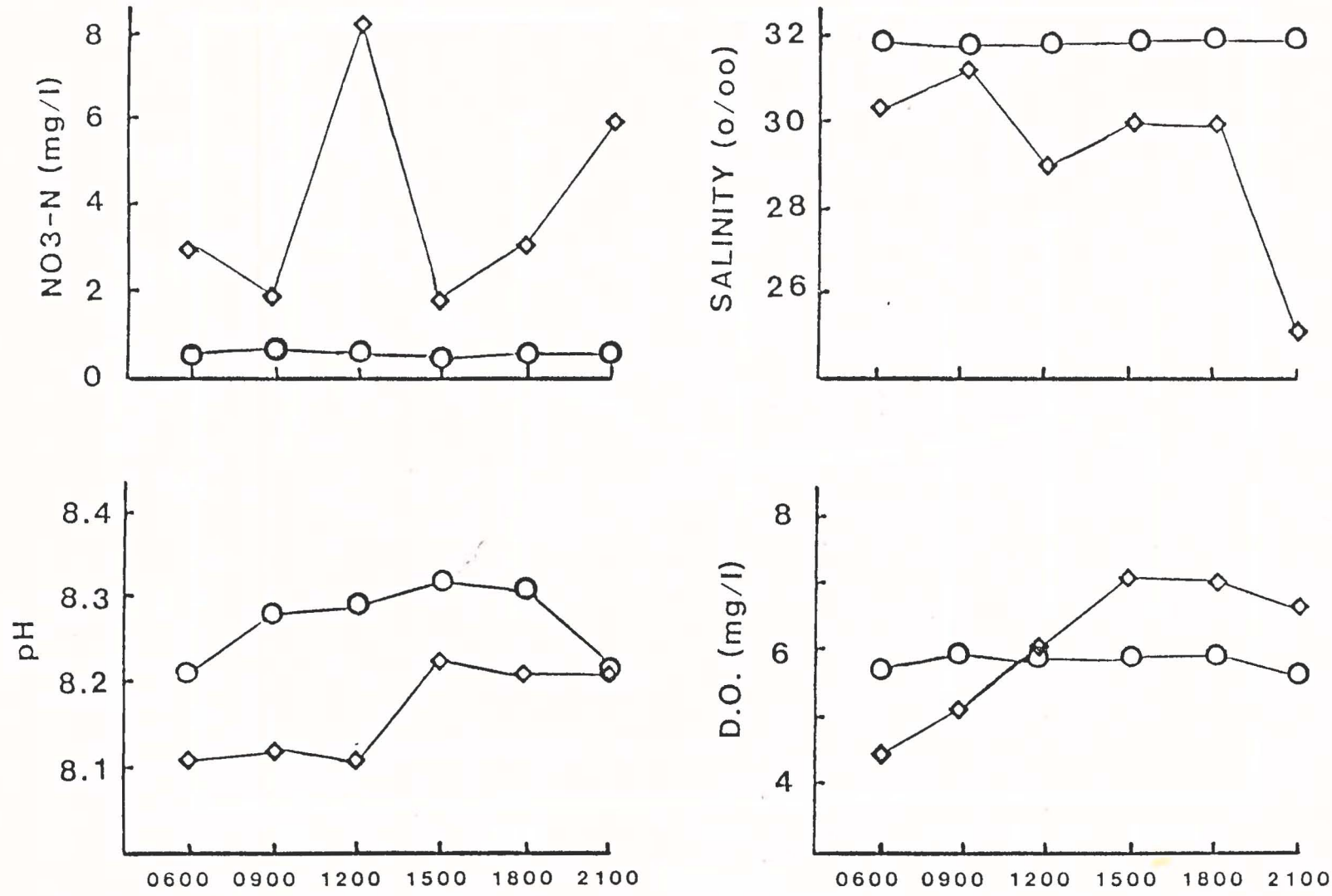

Figure 3. Outfall and dead-end channel seawater characteristics. Circles and diamonds indicate values obtained from analysis of samples taken from the effluent canal and dead-end channel, respectively.

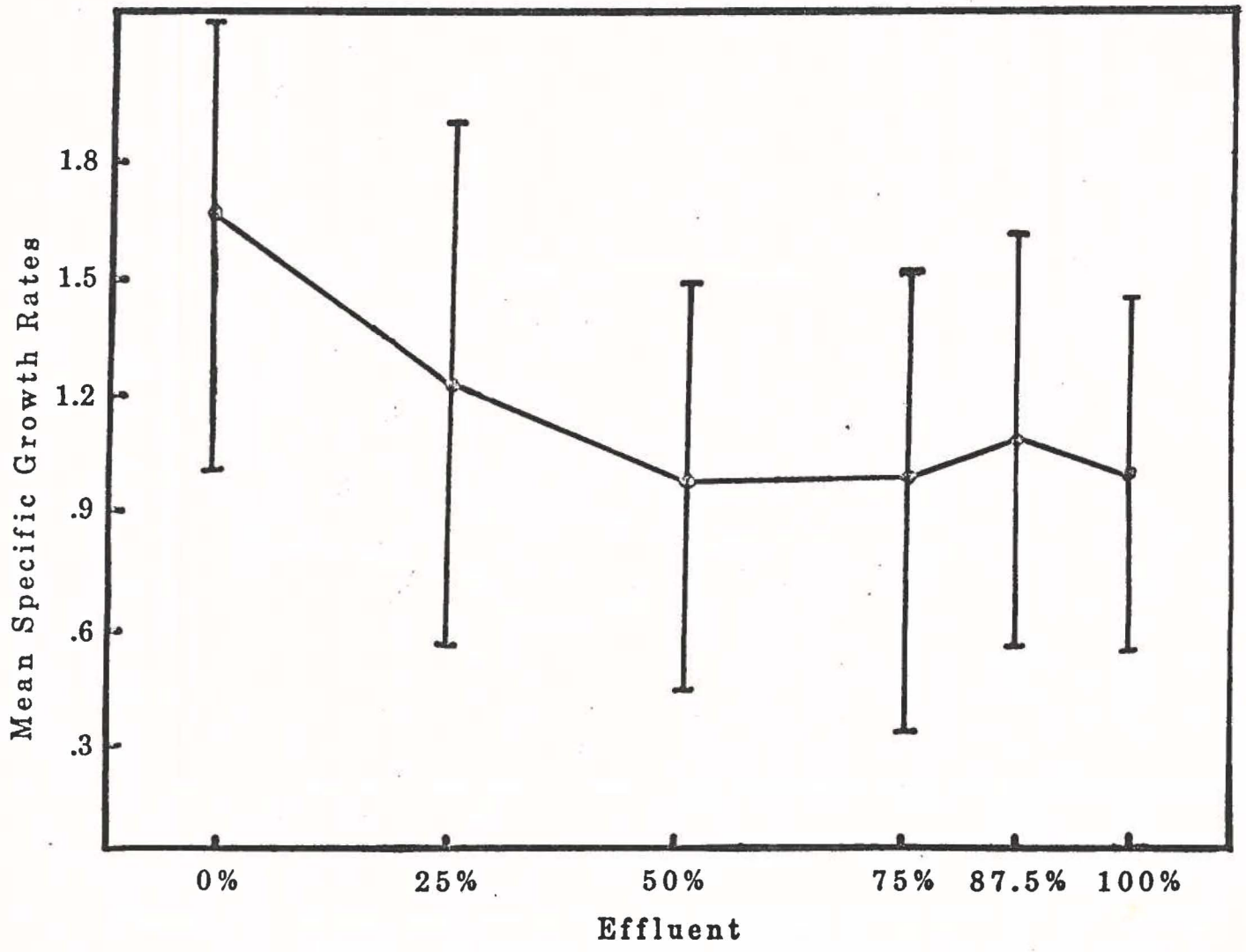

Figure 4. Mean specific growth rates ($\bar{\mu}$) for each treatment (% effluent), N = 24 for each treatment.

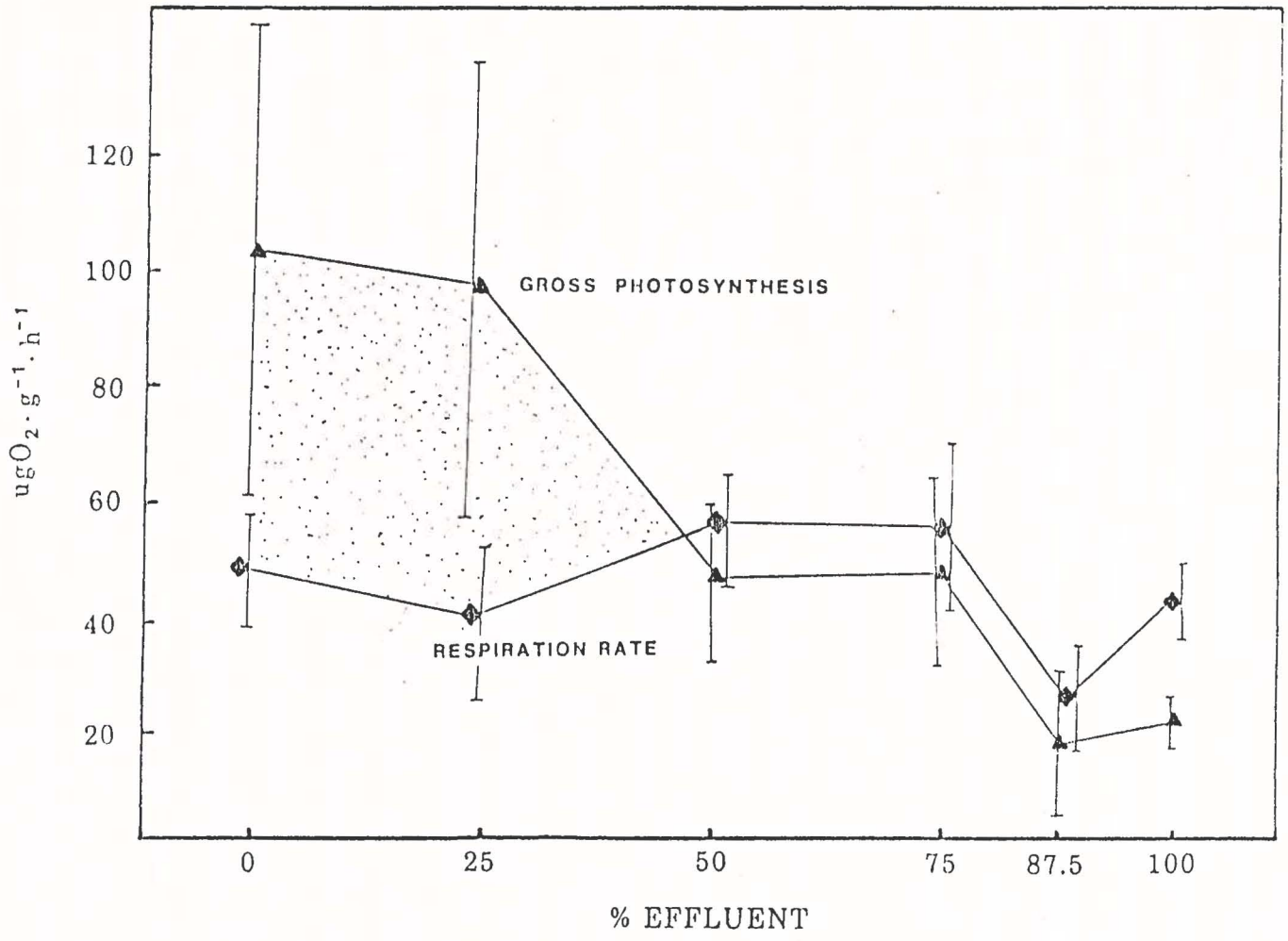

Figure 5. Combined mean respiration and production rates after 24 and 27 days of exposure to various effluent concentrations. N = 4 for each treatment, except for 100% effluent where N = 2.

groups were not significantly different at $P>0.05$. The photosynthetic rates of both the 0 and 25% groups were significantly greater than each of the 50 through 100% groups at $P<0.05$.

When respirometry tests on Gracilaria clumps were combined, mean respiration and production rates for the 0% effluent groups (n = 2) were 32.9 and 146 µg $O_2 \cdot g^{-1} \cdot h^{-1}$, respectively. The 100% effluent groups' (n = 2) respiration and production rates were 65.4 and 113 µg $O_2 \cdot g^{-1} \cdot h^{-1}$, respectively.

DISCUSSION

Indicator organisms are widely used in environmental impact studies surveying the effects of pollution. In this study, an indicator organism was selected prior to a proposed pollution discharge. The relative abundance, distribution and productivity of Gracilaria in the outfall area were determined by field and laboratory studies. A bioassay was conducted to determine the effect of pilot plant sulfur scrubber effluent on Gracilaria growth and on photosynthesis and respiration rates. The combination of studies provided a stronger statement of potential impact than would have been the case with only one approach.

A stable Gracilaria arcuata population exists in the outfall region. About 3% of this standing crop is located in the outfall canal and 25% in the dead-end channel, with most of the rest on the remainder of Tidal Flat B. This small variation in abundance is a desirable characteristic of an indicator species and will facilitate the assessment of any Gracilaria population change if a scrubber is placed on line. The existence of two areas of high Gracilaria abundance (one in the outfall canal and the other about 600 m from the outfall), which have now been extensively surveyed, can provide baseline data potentially valuable in comparing the effect of different concentrations of actual scrubber effluent.

The importance of Gracilaria as a primary producer was assessed in two ways: by growth studies and by respirometry experiments. This

allows an assessment of any scrubber effect on the ecosystem in terms of change in productivity.

The growth studies summarized in Table 2 show fairly high growth rates for the first week or two and then a rapid decline. The inital growth rates probably reflect conditions prior to collection. A compilation of all tests resulted in a mean specific growth rate of 1.39% per day. This is somewhat less than the 2.02% and 3.50% found by Nelson et al. (1980) for Gracilaria arcuata. However, if only the first weeks of my studies are considered, the mean growth rate was 2.27%.

An estimate of the energy contribution of Gracilaria to the Piti Channel area can be made on the basis of these growth studies. Again, the data of Table 2 indicate actual field growth rate as about 2.27% per day. The average caloric value of four Gigartinales species listed by Cummins and Wuycheck (1971) is 3.368 $kcal \cdot g^{-1}$ (dry weight). In this study, the wet weight of Gracilaria arcuata was determined to be 9.35 (n = 5, S.D. = 0.77) times greater than its dry weight. Therefore, each gram (wet weight) of standing crop contributed .0227 x 3.368 kcal x 9.35^{-1} or 8.1 $cal \cdot g^{-1} \cdot d^{-1}$. Since the estimated Piti Channel area standing crop is 1.06×10^5 kg, the total energy contribution of Gracilaria in this area is about 8.6×10^5 kcal per day.

The energy contribution of Gracilaria arcuata to this area can also be estimated using respirometry data. Respirometer studies using large clumps are more like actual field conditions than Gilson respirometry; therefore, data from Table 3 were used for these calculations. (Respiration and photosynthetic rates of Gracilaria clumps = 47 and 316 $\mu g\ O_2 \cdot g^{-1} \cdot h^{-1}$, respectively.) Odum (1971) lists the caloric value of

1 mg O_2 exchange to be equivalent to 3.5 calories. Assuming that respiration is a constant process, the number of calories used up by Gracilaria in 24 h is estimated to be 0.047 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ x 24 h x 3.5 $cal \cdot mg\ O_2^{-1}$ or 4.0 $cal \cdot g^{-1} \cdot d^{-1}$. If a light period of 12 h per day is used and it is assumed that saturation levels exist for 10 h, then the effective light period is about 11 h per day. The number of calories produced is, therefore, estimated to be 11 h x 3.5 $cal \cdot mg\ O_2^{-1}$ x 3.16 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ or 12.2 $cal \cdot g^{-1} \cdot d^{-1}$. The net energy production is thus estimated to be 8.2 $cal \cdot g^{-1} \cdot d^{-1}$. Using the standard crop estimate of 1.06×10^5 kg, the total Gracilaria arcuata production in the Piti Channel area is about 8.7×10^5 kcal per day. This estimate based on net productivity is remarkably close to the estimate based on growth.

Results of these studies on large Gracilaria clumps are similar to Gilson respirometry results. Generally, respiration was higher and production was lower in the Gilson instrument (see Tables 3 and 4). This general agreement in results between two widely different techniques, one designed specifically to reproduce as closely as possible natural conditions, suggests that other studies concerned with macroalgal productivity under natural conditions will benefit from the more "artificial" Gilson respirometry. The rapid accumulation of data with this instrument may offset any disadvantage in data interpretation. G. arcuata production and respiration rates as determined by Gilson respirometry in this study, at a temperature of 32°C and a salinity of 32°/$_{oo}$, are similar to results obtained by James (1982) under conditions of 30°C and a salinity of 30°/$_{oo}$. He found P_{net} = 180, and R = 67 µg $O_2 \cdot g^{-1}$ wet weight, respectively.

Monitoring of the seawater within the outfall canal and the westernmost portion of Tidal Flat B indicated some basic differences. The Gracilaria population within the outfall canal is subjected to seawater whose properties vary little; and, of course, no dilution of effluent discharge takes place. The Gracilaria within the dead-end channel experiences a much wider range of salinity, temperature and nitrate levels, apparently related to fresh water input and changes in current pattern. The potential impact of sulfur scrubber effluent on the Gracilaria population in the dead-end channel is not straightforward since there is less mixing of this water mass with the effluent than in other areas.

Bioassay data indicate that Gracilaria growth is significantly less when subjected to effluent concentrations of 25 to 100% than when subjected to 100% ambient seawater. Growth rates of control groups were 60% greater than those subjected to 50-100% effluent. It was readily apparent that most growth occurred during initial stages of the bioassay and little, if any, growth occurred in the treatment groups near the end of the test period. Gracilaria clumps subjected to effluent concentrations of 50% or greater were much more fragile than control clumps.

The Gilson respirometry tests on growing tips showed that those portions of the thalli subjected to high levels of effluent were significantly less productive than control-group growing tips. Growing tips subjected to effluent concentrations of 50% or greater were visibly paler and less abundant than of control clumps. The seaweed subjected to 25% effluent was not visibly different from the control group.

The difference in net productivity between control and treatment groups was not so marked when clumps were tested. This suggests that growing tips were more adversely affected by the effluent than the rest of the thalli. It also suggests that growing tips provide a more sensitive bioassay test material than the rest of the thalli. This provides another argument in favor of the Gilson instrument in seaweed bioassays in situations where growing tips can be tested.

Gracilaria arcuata thrives under a wide range of conditions. However, these experimental results indicate that sulfur scrubber concentrations of 50% or greater will result in the complete decimation of any G. arcuata population in the outfall area. This suggests that less widely tolerant macrophytes in this area will also be decimated. Figure 6 from R. W. Beck and Associates (1982) shows the predicted scrubber effluent concentrations in Piti Channel. If effluent characteristics of the full-scale scrubber match those of the pilot scrubber, it follows that implementation will result in the demise of the outfall canal Gracilaria population. Most of the Tidal Flat B population will be subjected to effluent concentrations of at least 67%. These areas would also most likely soon be devoid of any Gracilaria plants. The easternmost portion of Tidal Flat B contains only a few scattered Gracilaria plants. The possible effect of sulfur scrubber effluent here is not as clear-cut. The same is true of the westernmost portion of Tidal Flat B, where a large population exists. The scrubber effluent load in these two areas would be less than that for the rest of Tidal Flat B because of prevailing current patterns. Whether this would result in Gracilaria's continued survival in these areas is not

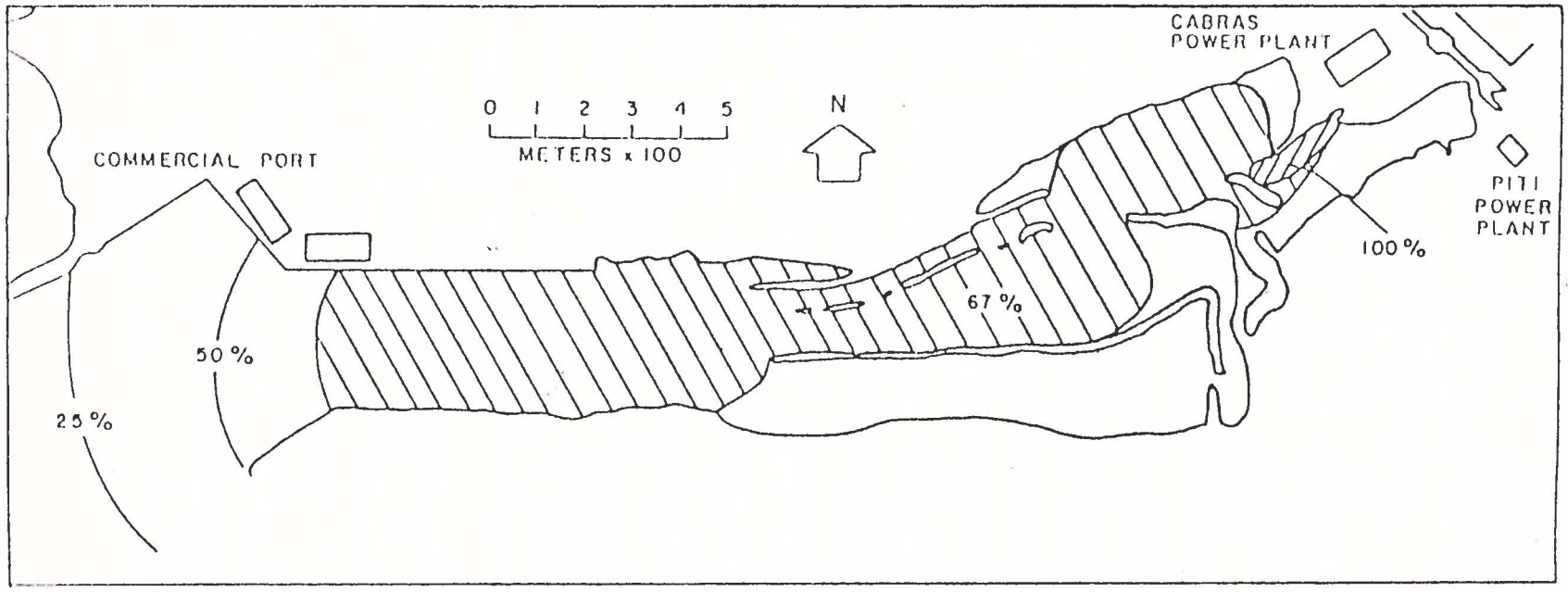

Figure 6. Projected effluent concentrations in the outfall area (from R. W. Beck and Associates 1982).

known. If any Gracilaria arcuata survive, it will likely be in these areas.

The complete elimination of G. arcuata in the outfall area and Tidal Flat B if a scrubber is built would result in the elimination of Gracilaria primary productivity in the area estimated to be about 8.6 x 10^5 kcal per day. If other macrophytes and planktonic algae are similarly affected, then, of course, primary productivity would be further decreased. However, replacement by other more tolerant species, such as blue-green algae, is possible. If only the Gracilaria in the dead-end channel remained intact and relatively unaffected, then Gracilaria primary productivity would be reduced to about 2.2 x 10^5 kcal per day.

The property or properties of scrubber effluent responsible for the demonstrated effect on Gracilaria were not determined; however, observations indicate some possibilities. These include temperature, soot accumulation, dissolved hydrocarbons or heavy metals and pH. Experimental design resulted in a direct correlation between percent effluent and temperature within test aquaria (see Appendix II). However, James (1982) demonstrated an increase in Gracilaria arcuata apparent photosynthesis between 30 and 40°C. This, of course, suggests that temperature was not possible for decreased productivity of treatment groups. It is assumed that soot accumulation and dissolved substances such as hydrocarbons and heavy metals were directly correlated with percent effluent and load. Therefore, these constituents, along with pH, which was inversely correlated with percent effluent, are likely candidates should further testing be deemed appropriate.

CONCLUSIONS

The abundance and distribution of Gracilaria arcuata in the Piti Channel area remained relatively constant during the 14 months of this study. The total biomass was estimated to be about 1.06×10^5 kg, with a net primary productivity of about 8.6×10^5 kcal per day.

In a bioassay, effluent concentrations of 25% or greater resulted in significantly lower Gracilaria growth. Productivity was significantly less in concentrations of 50% or greater. Field populations subjected to 50% effluent or greater will likely be decimated. Since almost the entire Piti Channel area would be subjected to 67% effluent or greater if a scrubber is operated, the likelihood of complete decimation is high. One possible exception is in the westernmost portion of Tidal Flat B, where about 25% of the Gracilaria population grows. Here the seawater characteristics are not determined wholly by effluent characteristics.

REFERENCES CITED

Baalsrud, K. 1967. Influence of nutrient concentrtions on primary production, p. 159-169. In T. A. Olson and F. J. Burgess (eds.), Pollution and Marine Ecology. Wiley, N.Y.

Blaker, R. W. 1957. Benthic animals as indicators of hydrographic conditions and climatic changes in Sualband waters. Ministry of Agr. Fish and Food, Fishery Invest. Ser II 20:1-49.

Cummins, K. W., and J. C. Wuycheck. 1971. Caloric equivalents for investigations in ecological energetics. Mitt Internat. Verein. Limnol. No. 18. 158 p.

Dawson, E. Y. 1966. Marine botany. Holt, Rinehart and Winston, Inc. xii + 371 p.

Doty, M. S. 1968. Biological and physical features of Kealakekua Bay, Hawaii. Univ. Hawaii, Hawaii Bot. Sci. Pap. No. 8. 210 p.

Edwards, P. 1972. Cultured red algae to measure pollution. Marine Pollution Bull. 3:184-188.

Fitzgerald, G. P. 1968. Detection of limiting or surplus nitrogen in algae and aquatic weeds. J. Phycol. 4:121-126.

Fitzgerald, G. P., and T. C. Nelson. 1966. Extractive and enzymatic analyses for limiting or surplus phosphorous algae. J. Phycol. 2:32-37.

Harvey, H. W. 1963. The chemistry and fertility of sea waters. Cambridge Univ. Press. viii + 240 p.

Horne, R. A. 1969. Marine chemistry. Wiley and Sons, Inc. xxii + 568 p.

Hoyle, M. D. 1975. The literature pertinent to the red algal genus Gracilaria in Hawaii. Marine Agronomy, U.S. Sea Grant Program, Hawaii. 340 p.

James, S. 1982. Photosynthesis and respiration of two species of red algae, Gracilaria arcuata and Gracilaria edulis, from Guam. Univ. Guam, M.S. Thesis in Biology. 17 p.

Lackey, J. B. 1959. The status of plankton determination in marine pollution analysis. p. 404-412. In E. P. Pearson (ed.), Proc. 1st Intern. Conf. Waste Disposal Marine Environment. Pergamon Press, N.Y.

McNulty, J. K. 1961. Ecological effects of sewage pollution in Biscayne Bay, Florida: Sediments and the distribution of benthic and fouling macro-organisms. Bull. Mar. Sci. Gulf and Caribbbean, 11:394-447.

Nelson, S. G., R. N. Tsutsui, and B. R. Best. 1980. Evaluation of seaweed mariculture potential on Guam: I. Ammonia uptake by, and growth of two species of Gracilaria (Rhodophyta). Univ. Guam Mar. Lab. Tech. Rept. No. 61. iv + 20 p.

Odum, E. P. 1971. Fundamentals of ecology. W. B. Saunders Co. xiv + 574 p.

R. W. Beck and Associates. 1982. Phase IV summary report. Seawater SO_2 scrubber environmental research program for Guam Power Authority.

Skulberg, O. M. 1966. Algal problems related to the eutrophication of European water supplies and a bioassay method to assess fertilizing influences of pollution on inland waters. p. 262-299. In D. F. Jackson (ed.), Algae and Man. Plenum Press, N.Y.

Stainton, M. P., M. J. Capel, and F. A. J. Armstrong. 1977. The chemical analysis of fresh water. Canada Fish. Mar. Serv. Spec. Publ. 180 p.

Thorhaug, A. 1976. Tropical macroalgae as pollution indicator organisms. Micronesica 12(1):49-65.

Tsuda, R. T. 1972. Morphological, zonational and seasonal studies of two species of Sargassum on the reefs of Guam. Proc. Seventh Inter. Seaweed Symp. p. 40-44.

Umbriet, W. W., R. H. Burris, and J. F. Stauffer. 1972. Manometric and biochemical techniques. Burgess Publ. Co. v + 387 p.

APPENDICES

APPENDIX I

There are some questions to the validity of volume change readings in the Gilson respirometer without the addition of a CO_2 buffer in the reaction flask sidearm. This buffer, hypothetically, would keep a constant CO_2 partial pressure and thus eliminate volume changes due to CO_2 uptake or release by Gracilaria. With constant CO_2 partial pressure, any volume change could then be attributed to O_2 exchange. However, it has been noted by Harvey (1963) that the diffusion of CO_2 across the seawater-air interface is infinitely slower than the diffusion of O_2. The rate of CO_2 exchange is decreased significantly by the hydration of that gas (Horne 1969). If this is the case, then manometer readings will reflect O_2 partial pressure changes and CO_2 partial pressure in air will remain virtually unchanged. To test this, an experiment was set up as follows: Seven Gilson respirometer flasks containing about 1.2 g each of Gracilaria in about 15 mℓ of seawater were subjected to light and dark periods. The sidearm contained 1 mℓ of 10% KOH with a paper wick to provide greater contact between air and KOH solution. Seven flasks were identical except without KOH solution. If the seawater-air interface was highly impermeable to CO_2, then the manometer readings of both groups would be similar. Results listed in the table below show respiration and photosynthesis

	n	R	SD	P	SD	P/R
w/KOH	7	.0503	.0141	.2069	.0438	4.113
w/o KOH	7	.0530	.0168	.2173	.0337	4.099

rates were not significantly different (F_s = 0.136 and 0.245, respectively) at P>0.05. This meant that following tests did not need KOH in the sidearm and that manometer readings could be attributed to the uptake and release of O_2. This greatly simplified testing procedures.

APPENDIX II

1. Characteristics of 100% effluent and 0% effluent (100% ambient seawater) used in bioassay (mean values and standard deviations).

% Effluent	n	D.O.	SD	pH	SD
0	27	6.81	0.83	8.34	0.08
100	27	5.65	0.32	7.65	0.17

2. Mean temperatures within each treatment (n = 8).

	0	25	50	75	87.5	100
Temp. °C	31.3	31.5	32.9	33.0	33.3	33.8
SD	1.3	1.3	1.2	0.8	0.9	1.0

3. Load (mean flow through each treatment aquarium x effluent concentration, n = 6).

	0	25	50	75	87.5	100
Load	0	95.2	134.8	340.0	300.9	325.8
SD	0	6.0	41.2	3.1	75.6	51.0