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PRNC - 162

PUERTO RICO NUCLEAR CENTER

AGUIRRE POWER PROJECT

ENVIRONMENTAL STUDIES 1972

ANNUAL REPORT

Prepared for Puerto Rico Water Resources Authority

By the Staff of Puerto Rico Nuclear Center of the

University of Puerto Rico - March 1973

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APPENDIX A

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MACROZOOPLANKTON OF JOROS AND GUAYANILLA BAYS,  
SOUTHERN PUERTO RICO, AND ITS FLUCTUATIONS  
UNDER SPECIAL CONDITIONS

by

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Puerto

## INTRODUCTION

This paper deals with the species diversity, abundance and distribution of marine macrozooplankton in Jobos and Guayanilla Pays, southern Puerto Rico. It is part of the environmental studies undertaken by the Puerto Rico Nuclear Center at the proposed Jobos Bay thermoelectric power plant site and at the Guayanilla Ray power plant site where a fossil fuel power plant has been generating 300 mesawatts from 1957 to 1972 and 710 megawatts since 1972.

Information about the plankton of tropical estuaries and bays is limited and scattered, especially in the Caribbean area and adjacent waters. One of the first research of forts on zooplankton of Puerto Rico was conducted by Duran (1957), who worked on tintinnids of the island. later, Coker and Gonzalez (1960) and Gonzalez and Rowran (1965) studied copepods Fron Bahia Fosforescente, southern Puerto co.

In the Caribbean area and adjacent waters there have boon several zooplankton studies reported, including those of

Noore (1948), Suarez-Caabro (1959), Suarez-Caabro and Madruga (1964), Legare (1961), Zoppi (1961), Cuzen du est (1968), Reeve (1964, 1970), Suarer-Caabro and Gone: Aguirre (1965), Noryakova and Campos (1966), Cwre and Foy (1967) and Bowman and McCain (1971). An extensive bibliography on general plankton and the main groups of the area can be found in Rjdrnhers (1971).

?The main objectives of this study were to establish the main components of the macrozooplankton in Jobos and fuavanilla Rays, and their abundance and distribution over a relatively short period of tine as a baseline for understanding their future fluctuations with environmental changes.

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Since the number of sampling stations and the tire were limited, and the sampling procedures and the net sizes were variable, this research is restricted mainly to the identi. Fication?and distribution of the most abundant and common species of the macrozooplankton of Jobos and Cuayanilla Rays during November and Decenber 1971 and January,

February and March 1972. An extensive, scheduled study of the ecology of plankton in both bays and other areas around Muerto Rico is being continued by the Radioecology Division of the Puerto Rico Nuclear Center. Nevertheless, it is felt that the paucity of literature dealing with Planktonic communities in Puerto Rico justifies this preliminary paper.

## STUDY AREA

### Johes Kay

Jobos Bay is located in the southeastern part of Puerto Rico (Fig. 1) 20 nautical miles westward of Punta Tuna Light (approximately  $17^{\circ} 55' 00'' = 17^{\circ} 57' 3''$  and  $66^{\circ} 10' 0'' = 66^{\circ} 17' 29''$  WN). Johos Bay is one of the few natural harbors in the southern coast of Puerto Rico. It is formed by a complex of tropical marine communities: mangrove swamps, turtle grass (*Thalassia testudinum*) beds, muddy and silty bottoms, sandy beaches, and coral reefs. The total length of Jobos Bay, from the eastern tip of Cayos Ratones to the eastern-most part at Puerto Jobos is approximately

7 nautical miles (Fig. 2). The widest area is 2-2 nautical miles from Central Aguirre to Roca del Infierno. The bay is divided into three different zones: Inner bay, Mid bay and Aguirre Navigational Channel.

The Inner Bay is the eastern-most end of the bay separated from the Mid Bay by the line between Punta Rodeo and Central Aguirre Dock (Fig. 2). The Inner Bay is a shallow end of Jobos Bay surrounded by a narrow zone of mangroves on the shores. average depth is about 4m, but there are also a few shallow turtle grass beds at the north of the Inner Bay. A 6 m deep dredged channel is found near the southern shore. The Inner Bay has a silty bottom which gets stirred up during the normal 10-knot trade winds. the Surface currents move the turbid water westward to the Min Pay. There is an upwelling along the entire Inner Bay.

to trade winds. The deep current enters the Inner Bay from the Aguirre Navigational Channel and the Mid Bay (Puerto Rico Water Resources Authority, 1972).

The Mid Bay is bordered by Punta Rodeo on the east, Cayos Caribes on the south, and Punta Colchones on the west. The Mid Bay has mangroves growing on the eastern and western shores, while the southern side has mangrove-covered keys and a protecting fringing coral reef separating it from the Caribbean. There are turtle grass beds along the eastern and western shores. The central part of the Mid Bay is about 8m deep.

The Aguirre Navigational Channel is open to the Caribbean on the west between Cayos de Ratones, Cayo Vorrillo and Cayos de Pajaros, which channels are about 15m deep. On the east there is a 4-meter deep channel called Roca del Infierno. The northern side of the Aguirre Navigational Channel has extensive mangrove areas and on the southern side there are keys with mangroves and fringing reefs.

Along the shores of the keys and along the entire shoreline in the north there are extensive turtle grass beds. The depth in the central part of the Aguirre Navigational Channel is between 10 and 12m, The surface currents are



moving to the west generated by the trade winds, the North Equatorial current entering through Moca del Infierno and the hydrostatic head that pushes waves over the fringing reef and through the mangrove channels between the keys in Cayos de Rarea and Cayos Caribes. A deep current brings water through the entrances in the west into the Aguirre Navigational Channel during the flood tide. This water moves toward the east into the Mar Negro area, the Mid. bay and the Inner Bay. During the ebb tide water moves out of the bay in the entire water column (Puerto Rico Water Resources Authority, 1972).

Tides in Johos Bay are primarily diurnal with a complex pattern composed of two tidal waves, one with a daily cycle and another with a cycle of 13.3 days (Puerto Rico Water Resources Authority, 1972). The extreme tides of 30 cm occur when these two cycles are in phase.

Surface temperatures in January varied from 28° C. to 29° C. in the Inner Bay, from 29° C. to 30° C, in the Mid Bay and from 25° C, to 26° C. in the Aguirre Navigational Channel. In August the temperatures were from 30° C. to 31° C, in

the Inner Bay, from 31° C. to 32°C. in the Nid Tay and from 29° C. to 30° C. in the Aguirre Navigational Channel (Puerto Rico Water Resources Authority, 1972).

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### Guayanilla Bay

This bay (Fig. 1) is located 25 nautical miles eastward of Cabo Rojo Light (approximately 17° 57' 30" - 18° 00' 00" N, and 66° 45' 00" - 66° 48' 30" W). It is both the largest and one of the best hurricane harbors in Puerto Rico. The reefs and islands to the southeast break the sea, but not the wind. The harbor, between low and denuded Punta Guayanilla (Fig. 3) on the east and bluff-faced Punta Verraco on the west, is protected at its entrance by extensive reefs which extend a mile or more offshore. The entire bay is 4 nautical miles in length with a maximum width of 2 nautical miles.

This study is concerned with the area of the bay immediately adjacent to the power plant (Fig. 3). Cooling water is taken from an embayment to the west of the plant, and effluents are released into a cove to the southeast. The embayment from which water is taken is approximately a uniform 500 meters wide and 1050 meters long. Temperature ranges from 25.5° C. in winter to 30.5° C. in summer. The receiving cove is approximately 900 meters in length, the major part being about 389 meters wide and constricting to about 30 meters wide at its mouth. Cooling water was discharged into the cove at 10°C. above ambient, i.e. above the inlet bay temperatures. This temperature dropped to + 1° C. at within 109 m from the mouth of the discharge canal with a further decrease to + 5° C. at toward the mouth of the cove. There was a 0.1 - 0.5 knot steady surface current flowing out of the cove and a 0.02 knot deep current flowing into the cove at its mouth.

Depth in the embayment varied from 2 to 5 m, dropping to as much as 20 m at its mouth. An average depth was about 4.5. The discharge cove is more uniform, varying from

2-5 m, averaging about 4m deep.

## METHODS AND MATERIALS

Thirty-two surface stations were sampled in ten different areas during November and December 1971, and January, February and March 1972, at Johos and Guayanilia Bays (Fig. 1). All surface plankton samples taken with three mesh size 0.5 m nets that were provided with small flow meters. Most of the samples were collected with a

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macroplankton net of mesh opening size 380  $\mu$ m, ten stations were sampled using a net of mesh opening size of 60  $\mu$ m and three samples were obtained with a net of mesh opening size of 300  $\mu$ m.

The length of the tows ranged between 5 and 20 minutes.

Samples were preserved in 4% buffered formalin. Tempera-

ture, salinity and dissolved oxygen concentrations were also measured at the station during the towing.

For counting organisms, aliquots of each sample were removed from a well-shaken container by means of 5 cc, 10 cc or 20 cc spoons and diluted into a squared Petri dish.

All zooplankters in the subsamples were counted,

## COMPOSITION OF MACROZOOPLANKTON

### Jobos Bay

As in Guayanilla Bay, copepods were the most numerous planktonic forms in Jobos Bay. Among the calanoids

*Acartia tonsa* Dana was found in every area sampled (Fig. 2).

*E. Weltjeborgii* Giesbrecht occurred in areas 2, 3, 4, and

5. *Mirch* were detected in the mid-fay and close to shore in

the Aguirre Navigational Channel. *Acartia spinata* Festerly

was present in areas 2, 3, and 4, and *Acartia Tongiremis*

(Littjeborg) in area 5 only.

*Tenora turbinata* (Nana) was the second most common calanoid.

Te war recorded for all areas except number 3.

*Psoudodiaptonus cokeri* Gonzalez and Rowman occurred in areas S; Sami cin the western part of the bay.

Other calanoid species which appeared less common were

*Calanopia americana* F. Nahi, *Clausocalanus furcatus*

*irady*), *aracalanus crassifostris* Fe Dahl, *TaracaTanus*

*aculeatus* Gfesbrecht, *Paracalanus* spp., *Lahidocera scotti*

Glesbrecht and *Labidocera* spp.

The Cyclopoid copenous *Oithone hehes* Geshrecht, *O- nana*

Giesbrecht and the harpacticoid *Futerpina acutifrons* (Dana)

also occurred in the bay.

ALL those species of copepods except genus *Labidocera* men-

Honed above have been Feported in Puerto? Rice Aye enzales

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and Bowman (1965). In Puerto Rico *L. scotti* Giesbrecht is recorded by Coker and Gonzalez (1960) only in Bahia Montalva, southwestern Puerto Rico. This species also occurs in South Miseryne Bay, Florida (Reeve, 1970; Noodmansee, 1959; and Mavis, 1950). *A. longirenis* is not previously reported in Puerto Rico, but it is recorded in Cuban waters by Suarez-Caahro (1955).

*Lucifer faxoni* was present in all areas of Jobos Bay.

Juveniles of this species are reported in area #y\_tocated at the mouth of the bay. Among the larvaceans *Ofkopleura* (*exiliaria*) *dioica* Tol, *Oiteploura* (*Vexillaria*) *payee* Lohmann, *Ofkopleura* (*Cogearia*) *tonpicauda* (Vea) nant *Sikoptenra* (*Caecarta*) *fusiforais* Pe cormitopectea Chia) were present in Tohos Bay,

Immature specimens of chaetognaths as *Sagitta* spp. were present in all areas except in the Inner Tay (area 1) where *S. hispida* Conant was identified. *S. enflata* Grassi and *Krohnites meilis* (Grassi) were also found in Se ghee Bay.

A cladoceran, *Fredne* spp., was recorded in areas 4, 5 and 6, which were located in the entrances and in the midchannel of the bay. No identified species of pteropods and Siphonophores were present in those entrances.

Two species of mysids were identified. *Siriella chierchiai* (Cosca) was taken in areas 4 and 5 at Hee anes

area 6 during the day. *Mysidopsis* sp. (identified by Br.

Arattegard: a new species *Tetartopeus* found in Columbian coastal waters in 1973 and called *Mysidopsis* () was taken during a daytime tow in area 4. Among the Nyperiid amphipods the genus *Lirachyscelus* was identified in area 2.



WWso a garnarid Ss, was Fenorted In area 3.

Guayanijja bay

Copepeds were the most abundant holoplanktonic group found in the areas sampled at Cuayanilla May (Fig, ). *Acartia tonsa* was the most common species of copepod reported and appeared in all areas. *Acartia thomasi* and *Oithona* species were present in areas 1 and 2 and *Tenora* spp. were present in areas 3 and 4. Other less common species were *Juterpina acutifrons*, *Micrasetella norvegica* (Pocck) and juveniles of *Taetadiaptanus*: *Corer Seser tons ene Fhe* harpacticoid copepods were also present.

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All those species were previously reported for Bahia Fosforescente and adjacent waters, southern Puerto Rico, either by Gonzalez and Rowman (1965) or Coker and Gonzalez (1960). Most of the species mentioned above are recorded for similar areas by Davis (1950), Davis and Wiltians (1950), Noodmansee (1958), Suarez-Caabro (1959) and Peeve (1964, 197).

A sergesti?, Lucifer faxoni Torradsite, and insature

specimens of *Sagitea* spp. occurred in All-arcas. *Sagitta tenuis* Conant was identified in area 4. *Le faxoni* Tes

Been reported as abundant near the coast and specifically in Fiscaigne Bay, Florida (Rowman and Cain, 1967; and Noodmansee, 1968). *S. tenuis* is a typical neritic species (Suarez-Caabro, 1958; and Alvarifio, 1965).

Unidentified isopods were also found in different areas of the bay. Some gammarid amphipods, *Podocerus* spp. (probably *P. brasiliensis*) and *Corophium* spp., were also identified in the bay.

Among the larvaceans, *Dikopleura* (*Vexillaria*) *dieica*, 0. (*Coccaria*) *longicauda*, 0 (*extTiaria*) *rufescens* Tol, *Fritillaria* (*iryeera*) *petealis* T. *sargasst* (Cohnatn) and *F.* (*hurycereus*) *petiucids* (hisch) were recorded in the bay. All these species of larvaceans are reported for Puerto Rico for the first time, to the author's knowledge. They were found in Cuban waters by Tokioka and

Suarez-Castro (1956) and in Trinidad Island, Brazil, by  
Björnberg and Forneris (1955). Also, Flores (1965) re-  
ported these species of the genus *Oikepteura* for the  
coastal waters of Veracruz, Mexico.

The mysid *Mysidium columbiae* (Zimmer) was taken during  
May in area T. A large school was taken with a band net  
at the surface near a mangrove stand.

## DISTRIBUTION OF THE MACROZOOPLANKTON

### Jobos Kay

Jobos Bay has been divided arbitrarily into six sampling areas (Figs. 1 and 2). The Inner Bay, area 1, has been given little emphasis as the predicted current patterns caused by the proposed power plants should have little

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effect there. Most of the sampling effort has gone into area 2 (the intake area) and area 5 (the outfall area), Area 4, fioca del Infierno and the area sheltered by Cayos Carihe?and Cayos Rarca should be indicative of recruitment potential provided to the coastal areas directly adjacent to the power plant complex, Area 3 was sampled only in November 1971, and then only because it is a break point for surface waters coming through Boca del Infierno, This area is probably the most unstable environment, but at the same time the richest in the bay. Area 6 was sampled to determine a possible trend toward seaward drift from area 5 and to estimate recruitment problems associated with wetrial effects the heated effluent might have. Table 1 is a complete tabulation of plankton hauls analyzed to

gate. It shows the location, date, time and other parameters of the sampling. Tables 2 through 5 list plankton concentrations according to (A) number taken per cubic meter of water sampled ( $\#/\text{m}^3$ ) and (B) percentage of total catch that each plankton category constitutes (1 total catch).

As stated before, the Inner Bay (area 1) has been sampled scantily, and zooplanktonic forms caught in this area reached 30.4% in November 1971 (Table 2). The main groups were copepods (43.81), fish eggs (2.0% -- anchovy eggs 0.5%), siphonophores (0.43) and appendicularia (0.03%). *Lucifer faxoni* accounted for 4.0% in this area. Siphonophores were not common in these inshore waters. Specimens found in area 1 were probably taken there by deep water currents which enter from the open sea during flood tide and flow easterly along the bottom of the Aguirre Ship Channel in through the mouth of the bay and eastward along

the totality of the study (Puerto Rico Water Resources Authority, 1972). The percentage of zooplankton (49.64) in mid December was slightly lower than that of holoplankton. The major groups were brachyuran zoeas (28/02) cirripede nauplii (8.4) and penaeid larvae (4.19),

Copepods were the most abundant group among all plankton in study area 2 (Table 3). From mid December 1971 to the end of

nuaty and early February 1972, accounting for Meet,  
74-41 and 64.54 respectively. Permanent mantton ig wid  
hecenber incinded, in addition te conepats, appendicuracia  
(0.27) and 4. faxeni (1.24). chactopnathe (ee),  
aprendicularia (8.47), medusac (lait) and very foe

L faxoni (0.1%) were present at the ent of January.

Chaetopnaths decreased to 0.93; apnendicularia increased  
to F.1%; medusac decreased to 6.2%; and L. faxoni

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disappeared, but nemertineans (0.24) were reported for the  
first time in area 2. It is interesting to note that  
gorepods decreased, but cirripede nauplii increased to



Area 3 (Table 4) in early November was predominately holoplanktonic. Copepods, 86.4% during the night and 49.8%

In the day, were the most abundant group. Other permanent plankters were chaetognaths (1.8%, 1.9%); appendicularia (3.6%) 0.55); and *L. faxoni* (913%) 1143). Nauplius larvae (0.126), pluteus larvae (0.71) and porcellanid larvae (1.6%) were present only during the day station.

Area 4 (Table 5) in early November was also holoplanktonic (56.45), but appendicularia (19.24), chaetognaths (8.0%), cladocera (9.0%), medusae (1.0%) and siphonophores (2.08) Euphyas accounted for more than copepods (16.2%).

Medusae, pluteus larvae and polychaete larvae were not present in early December, but *L. faxoni* (0.4%) appeared at that time.

Area 5 (Table 6, station JR-7, JB-8 and JR-10) in early December was typically meroplanktonic. Rhabduran larvae (161), decapod larvae (163), fish eggs (20%), fish larvae (0.88), nauplius larvae (124), polychaete larvae (0.14) and porcellanid larvae (0.1%) accounted for a total of about 49%. Almost two months later (station JB-26, JB-27 and JB-29) the same area had become strongly holoplanktonic. Copepods, amphipods, appendicularia, chaetognaths,

and *L. faxoni* reached a total of 821 in area 5.

Area 6 (Table 7), same as areas 5 and 4, was predominately holoplanktonic in early November. Copepods (54.50) 5 chactognaths (29-24), appendicularia (3.3%), cladocers (118) and siphonophors. (0-8) reached a total of GA.9%

of the whole plankton community.. Meroplankton accounted for 41.1%, consisting of hrachyaran larvae (10.3%), decapod Larvae (12.34), fish engs (3.04) and others (4.64).

In early Deconber and February copepod peaks of 67.3% and 934 were found, respectively. In February larvae fad decreased to a? total of only 3.5%.

ayanilla Bay

The area of sampling in Cuayanilla Bay was located in the northeastern side of the bay (Fig. 3). General characteristics of stations are shown in Table 8. Tables 9 through 12 list plankton concentrations as: (A) number per cubic

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meter of water sampled (#/m<sup>3</sup>) and (R) percentage of total catch that each plankton category constituted (1 total catch).

Area 1 (Table 9) in early November was predominately holoplanktonic (99.5% of all plankton captured). Copepods and their nauplii accounted for 76.7% and 7.1% respectively, polychaetes for 6.4%, chaetognaths for 1.34. The only major meroplankton were cirripede nauplii (5.28),

In mid December (GR-A, GN-B) holoplankton had decreased to 691 of the plankton community (66.8% copepods, 2.1%

chactognaths, f.1% polychactes). Of the meroplankton 4% were brachyuran larvae (2/3 of which were porcellanid zoeas), caridean larvae accounted for 3.2%, penaeid larvae for 2.3%, and cirripede nauplii for 2%. It is interesting to note that copepod nauplii had disappeared and that fish eggs and larvae had remained relatively unimportant during this period. Crustaceans and bivalves were present in early December in minute quantities, but were absent later. Note that penaeid larvae appeared in mid December (2.2%).

In area 2 (Table 10), 90.2% of the community was planktonic during November, a figure that dropped to 68.9% in December, and further to 30.8% in March. The primary reason seemed to be the increasing numbers of amphipods? 10% in December, 30.8% in March; fish eggs: 7.6% in December, 19.3% in March; fish larvae: 0.54% in December, 5.4% in March; isopods: 1.8% in December, 6% in March; cirripede nauplii: 4.6% in December, 1% in March; and brachyurans: 2.8% in December, 4% in March. None of these latter groups occurred in significant numbers in November. (November samples were taken with 60 µm nets, later samples with 380 and 390 µm nets; therefore, the

decrease in the number of copepods may have been due to smaller copepods not being caught.)

In early March, bivalves occurred (St) as well as small bursts of foraminifers (0.3%), ostracods (0.2%), and tintinnids (1.4%), none of which were found at other times. Gastropod larvae constituted 3.8% of the early Moreh samples but were present in insignificant numbers (0.038) during November. Nematodes occurred in both November and March (0.03% and 9.2% respectively). It is interesting that brachyuran larvae were important only during December (2.81) and late March (4.1), and that polychaetes were abundant (2.71) only in March. Carideans appeared only in March (8.142). Copepod nauplii peaked in November (where they made up most of the 2.61), and early March (21.4%), and disappeared (for all practical purposes) in

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late March. Cirripede nauplii were also more abundant (3%) in early than late March (2%), but also peaked in December (4.68).

In area 3 (Table 11) holoplankton accounted for 61.6% of the community during November, 84.91 in December, and 60.5% in March. A high meroplankton percentage in November was primarily a function of fish egg production (16.2%) 5

Bastropod reproduction (1.8%), and appearance of tintinnids (5.78) and foraminifers (3.54). The latter two were absent in other periods. It should be noted

was also the only time appendicularia (0.6%) chaetes (0.3%) appeared in area 3.

in November

and poly=

The 59.58 nereplankton concentration in March was a function of a brachyuran breeding period (37c4t). a nurse oe  
Citripede nauplii (4.04), ant «healthy concentration of  
caridean larvae (4.55) and penacid Targas, (etd) dhe  
latter two appeared in areas only Inarehs Rate that  
the concentration of hrachyuran. zocas was greater. than  
the combined total for coneplat adults and hauphit foe 94) «  
Apparently eirrinede reproduction occurred noth in  
November (114) and Mlorch, preceding the eoepod. nauplii  
onthurst in March by a day? Ys Decenhers. Fish: Taevee  
Outnubered Tish ergs, the only tine this ceurred,  
chactognaths peaked durine Seecnher (4054) and Merch  
(7.18), L- faxonk, ahsent in Sovenbor, way found in smal]  
quantities tn Decenher and arche. hecenber was the. oniy  
Period plutcus larvae (9.6%) and hivalve larvae (0.6%)  
Were found in area 3: Shalt concentrations of mysids and  
Siphonophors oceurret solely in sisres

In area 4 (Table 12) holoplankton constituted 83.8% of the planktonic community. No copepod nauplii occurred while 77.7% of the community was copepod adults and in matures. Murchisonian larvae made up as much as 1.8% (6.7% porcellanid and as little as 3.64 within a 2-Period toward the end of the month). Cirripede nauplii constituted 5.8%, peracarid larvae 2.1%. Caridean larvae dropped from 3.44 to 1.24 within a matter of hours.

## Discussion

The plankton at Johos and Cuayanilla Pays was fundamentally neritic. However, it could be that some oceanic forms entered occasionally into the bays with tides



and oceanic surface currents.

The neritic plankton tended to have a far higher proportion of meroplanktonic forms. Crustaceans were easily predominant, both in numbers and species. The copepods represented the major group of crustacea in the zooplankton. Most of the species in this group belonged to Calanoida.

*Acartia tonsa* was the most abundant and common of Calanoida. It was present in all areas of both bays. *A. lilljeborgii* was also very common. *A. spinata* was limited to areas 4 and 5 in Johos bay. Another calanoid, *Tenora turhinata*, was relatively easy to identify in Johos and Guayanilla bays. *Psoulodiptomus cokeri*, a calanoid which goes to the surface during the night, was reported in night stations at some Johos and Guayanilla bays. Similarly to *A. tonsa*, a sergestid, *Lucifer faxon*

present in all sohos and Guayanilla Tay area?

Among the chaetognaths, immature specimens of the genus

*Gagea* were reported in most of the areas of hotbays.

Scipidids, *S. tenuis* and *S. enflata* were identified. When

more specimens appeared? 2° Few appendicularians® such

as *Oikoploura (Vexillaria) dioica* and *O. (Coccaris)*

*Jongicanda* were taken TWentifiettn Jobs and taayand La

Tays.

Diurnal distributions for the two study areas were repre-

sentative of zooplankton concentrations in general. The

larger copepods occurred in at least twice as great a

number in every area in Cuayanilla than at Jobose

Brachyuran larvae were comparable on the intake side and

the effluent bay at Guayanilla to the most productive

Jobos area, two and a half times greater at the mouth of the bay. Area 5 at Jobos had an appreciably greater fish density concentration than anywhere else in Guayanilla or Jobos, but again Guayanilla had a better overall production, Jobos' area 1 was comparable to Guayanilla area 1 for chaetocera, but Guayanilla 2 and 3 were much richer

than any other Jobos area, the mouth of Guayanilla (area

4) three and a half times better than Jobos 1. For total Picozooplankton, area 5 at Jobos (12.4/m<sup>3</sup>) had the richest concentration recorded, still only about 1/4 that of the Guayanilla input bay (area 1), The Guayanilla effluent

had almost twice as great a concentration and almost ten times as many plankters at its mouth as the most

Productive area in Jobos:

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The month of the effluent cove, even though the water temperature there is 5° C. above ambient, has three times as many copepods and fish eggs, two and a half times as many brachyuran larvae, three and a half times the chaetognath concentration and, in fact, approximately three times the total plankton than the embayment near the cooling water intake.

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?TABLE 2

SUI@ARY OF ZOOPLANKTON DATA FOR AREA I

JOROS BAY

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Appendicularia 0.2

Bivalve larvae

Brachyuran roe 13.0

caridean larvae 6.3

Chaetognathe

Cladoce

26.3 43.8

Deeapod larvae

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Fish eggs (total)

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Fish larvae

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Gastropod Larvae

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Nauplit (total)

?Copepods

Cirripedes

Ostracods

Penaeid larvae 2.0 3a

Pluteus larvae

Polychaete larvae

Porcellanide

Stphonophora

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Totals 55.8 100.0

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TABLE 4

SUMMARY OF ZOOPLANKTON DATA FOR AREA 3

JOROS BAY

Station Jp = 2 Station JB = 3

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Amphipods 2 0.3

JAppendicularia 3:3 36 os 0.5

Bivalve Larvae

Brachyuran Lary: 16 1s 60.6 366

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copepods 1 86.4 86.3 49:8

Decapod Larvae 37 4 146 a

Fish eggs (total) 07 08 16 09

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Totale 91.0 100.0 174.3 100.0

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## FORAMINIFERS OF GUAYANILLA BAY

Report No. 2

Introduction

The purpose of this study is to report the foraminiferal

assemblages of sediment samples taken in Guayanilla Bay and to indicate their possible relationships to pollution.

Samples of Stations G-3A to G-9A were taken on May 16, 1972 and samples of Stations G-22 to G-20 were taken on June 10, 1972.

Samples were taken with a Phieger corer of about 10 cm? inside diameter. The upper centimeter section of each core was taken from a11 samples. The sections from 1 to 2 cr and from 2 to 3 cm from the top of the core were taken from some of the samples. The sections were preserved in 30% ethyl alcohol in the field. The protoplasm of the foraminifers was stained in the laboratory with Bengal rose.

The foraminifers and other organisms of about the same size were counted per sample.

The map of Text - figure 1 shows the position of stations in Guayanilla Ray.

?Temperatures and Salinities

Table No. 2 shows temperatures and salinities. The highest temperature, 36.2°C., is at Station G-3k in the Eastern Central Lagoon and the closest to the outfall of hot water



of the electric plant.

Salinities are in the normal range in most of the bay.

Tables 3, 3A and 38 show the organisms comparable in size to foraminifers in Guayanilla, Mayaguez and Jobos Bay, respectively.

Nematodes are the most abundant organisms of Mayaguez and Jobos Bays, Generally they are several times more abundant than foraminifers. The largest number of nematodes per

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sample, 3580 individuals, is from Station 16, Jobos bay, and. they are about 30 times more abundant than foraminifers (119 individuals) .

The total number of foraminifers per sample is similar in the three bays. The total number of the other organisms is proportionally smaller in Guayanilla Bay than in Mayaguez

and Jobos Bays. The number of nematodes is not only small in Guayanilla Bay, but also the foraminifers are several times more abundant than nematodes.

The pollution of Guayanilla Bay is influenced mainly by industrial chemicals, while the pollution of Mayaguez and Jobos Bays is mostly organic. Marszaleck et al. (1969) suggested that the test of the foraminifers is a defense against environmental changes. The nematodes do not have any shell to protect their bodies, which are exposed to chemical pollution, while the test of foraminifers appears to constitute a protection against this type of pollution. This may be the explanation of their abundance in relation to other organisms. in Guayanilla Bay.

### Foraminiferal Biofacies

The most abundant foraminifer in the bay is *Ammonia catesbyana tepida*. This species, associated with two other groups of foraminifers, constitutes the two biofacies determined in Guayanilla Bay. The biofacies are:

Ammonia-Quingueloculina-Ammonbaculites and Ammonia-Fursenkoin

THE Te No "Shows the distribution of orantnt feral popetas  
tions per station on which the division in biofacies was  
nade.

The Ammonia populations of Guayanitla Bay are mostly consti-  
tuted by one subspecies, *A. catesbyana tepida* (Cushman) -  
Some specimens may be included-ss-t- "advena?(Cushman"y but  
the small individuals are difficult to distinguish from *A.*  
*catesbyana tepida*. Only a few individuals are large and  
Well developed and may be included as *A. catesbyans*  
*catesbyana*.

Ammonia-Quinqueloculina-Ammonbaculites Biofacies

?This biofacies covers the shallowest part of the study area  
(the stations range from 1m to 4.5 m of water depth).

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Most of the stations are in Eastern Central Lagoon:

Stations G-3A, G-SA, G-6A and G-9A. The Station G-7A is close to the entrance to the lagoon

The dominant species is *Ammonia catesbyana tepida*

large population was found in the Sample of CSE 4

temperature of 36.2°C. This value is above the maximum growth temperature (34°C.) and the maximum temperature

for reproduction (30°C.) found by Bradshaw (1961) for

specimens of this species from Southern California and

Baja California. The abundance of this species in Station

G-3A shows its high resistance to thermal pollution.

*Yingueloculina rhodiensis* is the most abundant species

Sp. *tietgens* in Station G9, ?The specimens of this

species in this bay are larger and with thicker costae

and

?are the ones reported for the polluted waters from Jobos

Bay (Seiglie).

## Ammonia Fursenkoina Biofacies

This biofacies includes the stations from 8.5 to 17.0 meters of water depth, Stations 6-24 to -29, The dominant species is *Ammonia catesbyana tepida* and the second in abundance is *Fursenkoina pontoni*.

Fursenkoina:

## Ammonia Biofacies

This biofacies corresponds to water depths from 17.5 to 18.5 meters (Stations G-22 to G-23).

*catesbyana tepida*

*F. pontoni* is the dominant species and *A*

?The?Second species in number,

nPyritized" Living Foraminifers

Table No. 5 shows the living specimens of foraminifers with pyrite inside the test, and the stations in which they have been found. *Ammonia catesbyana* forma *tepida* is the most common pyritized foraminifer and constitute the 10% as mean value of the total population of this species. in the stations in which it occurs. *Fursenkoina pontoni* is the second in number, but the percentage of "pyritized" specimens is only the 1%. It is the mean value of the total living populations of this species.

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### Species Diversity

Species diversity was determined per station by the formula of the information function, Its values are shown in Table No. 6 and in Text - figure 2. The formula of information function is used by the communication to predict the name of the next letter in a message. It is considered a good measure of species diversity (McArthur and McArthur, 1969, McArthur, 1965). It is expressed by?

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Where N is the total number of species in the sample and  $P_i$ , the proportion between the number of specimens of the  $i$ th species and the total number of specimens.

The values of Table No. 6 are not very different from the ones determined for Mayaguez Bay (Seiglie, 1972) and for Jobos Bay (Seiglie, Manuscript). The values are lower, generally, in the shallower stations than in the deeper ones. The lowest values are in the western central lagoon. Species diversity is lower in polluted water than in unpolluted waters, but the values determined in Guayanilla Bay cannot be directly related to pollution, because the values before pollution are not known. The species diversity was also determined for the top centimeter (0-1 cm), for the second (1-2 cm) and for the third centimeter (2-5 cm). Distortion of the surface may increase the num-

x of foraminifers and the species diversity in the second and third centimeters. However, despite this possibility, the species diversity is the lowest in the second and third centimeters (see Table No. 6).

## Conclusions and Summary

The low ratio between the number of nematodes and the number of foraminifers and the small number of nematodes per Sample are the most important relationships between the microfauna and the pollution. These relationships suggest that the foraminiferal test is an effective protection against pollution.

The *Ammonia* populations of Cuayanilla Bay and Mayaguez Bay are constituted mostly by *A. catesbyana tepida*, while most

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of Jobos Bay populations are constituted by *A. catesbyana tepida*. Some faunas of Jobos Bay are deformed and TE

is difficult to include them in a subspecies. The Factor

Central Lagoon. There contains large populations of *A. catesbyana tepida*

under conditions of the same pollution.



The most abundant foraminifers in most of Lagouez Bay are,  
in order of abundance, *Furcuncina pontona* and *Elorilus*

*rateloupii*, while in? Guayant F the most abundant species  
SP Raatht? cacectyara tesida and *Furcuncina pontoris* Phe  
occurrence of *Elorilus grevetoupi*: i Guayanilte Bay suggests  
that this species is not resistant to chemical pollutants

The dominance of *Amporia caterbyare tepida*, at depth in

Which *Furcuncina pontona* is dominant in Lagouez Bay, suggests

that this species is more resistant than *F. portoni* to chemical  
pollution.

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BAHIA DE

STATIONS

GUAYANILLA

Figure 1

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BAHIA DE

GUAYANILLA

SPECIES DIVERSITY

Figure 2

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[FORAMIFERAL POPULATIONS PER SAMPLE TH GUAYANELLA BAY

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mir fe yoo art a

Amobaculites dtrectus

seal 4 2 2

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?Ammovarginul ina fot ac % 2 2 4

fsmvosicatesbyian teptde (2) 83-248

?epalogertoa ef, anguton

olivine ef-infiata

Bs peudopticate

23! Ehowbolaiie

Bi vertapaade 1

Betzaline dowmank 1

Bs etetacule

Bilinina sarginges

Bullmineria eleganciseine 1

Ceibroephtdtu dace dale

Gs poeyam 2 2 1

Ch tee 2

Gjelogyra. snvorver

Discorbinelia floridenete 1

Eggerella ct, advena

Fissurina peituetaa 1

Florttan gesteloupit 1 5 2

urvenkotha posto

Globocassidelina nincta

Glomepire goreicelie 1

Gases 8

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Nonfoneila? fragile

Parvigenerioa tps

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Fettocalina sp.

Fy trigonal

?FFochoaaaina ef. advent

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---Page Break---

TARE & (Cont) a

FORAMINIFERAL POPULATIONS PER SAMPLE IN GUAYANILLA BAY

9 58 cate

or i on

?aewodtscus sp.

Aemauesinulina foliacea ?

Somats eatestytana tepida (1) 7

Bolivins cf, inélece

1 preadopl cave 1

2! thonbosaal te

Pe

3. variabitts

Brssetina Loving a

B. steiseula 1

Bitinine margioaea

Boldainetia elegantterion

Cetbroetphidtus discosdele

. poeyanen

Bee 1

Sjelogyes tavotve

Discorbinelia Aloridenets

igerella cf, advent

Pissurioa peiigedde

Elobecasstdut ine stnuca

Mielsenetie Labsoas

Wonionelia? fragilis

parvigeners tp.

woe eoeeda

Gingielocaling cfs ot

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shodiansis? (sapoth vee,

phan car oensie

Aoealina floctdana

Sageine cuban

Sign tlopsts arenaca

Frliscalina pe

4. eelgonuta?

Trochammina cf, advena

Ti stecorbte



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302

TARE 4 (COM) »

FORAMTNIFERAL POPULATIONS PER SAMPLE TX GUAYANILIA BAY

25 O26 G26 o27 oe one

or oor fe oi et ot

Aamodtscus 4

fAmomrpinul ia fol ace

Mrontalcaterbytane tepida 2) 361019535

?Angalogerina cf, angulosa aoa

olivina cfs indlace

3 Frowettis! ?

3. Strung

Be etristula

Bolimineria sleganetseion

Crtbroetphtatun discoldale 1

we 8

Sjelogyea tnvoens

Diacorbined in Cloridenets

Rggecelin cfs advent

Flocilut\_geateloupit 4 2 1

Pursenkotan pontont v2 ne

Clotecasetdulina miata 3 1

Gloasepire poedtaite 2

Ge tpe a 1

Nipkideina mtuteees 3

Tages tacvte 1 toa

Miltotiet ia: Labioze 1

elemiey fastte 5 1

Parvigenering sp. 2

fr brovoetpnidtinn

Poe, torviaa

Gisqueleculina ef. candesans roa

Qo ces pocyans a

9: shsatenete

Q. thodtensts? (swooth ver.2)

@ sep o 6 a 5 6

Bi ceotet i

Tellocetiee 4

Fe trigonal

Otro

eS tm

(2) Zour abnorent spectnens

?onfertant ef

omHlisie safe,

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Table No. 5, "Pyritized" Living foraminifera  
at the stations of Guayanilla Bay

303

6-23 Ge2e G26 GPS G27 G29

OL OI M2 OL OnI

*Amonia catesbyana* f. *tepida* 4 99 4&3

*Fursenkoina* *pontont* 12 1

Globocassidelina minuta 2

Nonfonelia cf. fragitis 1

Brizalina of, inflata 1

Table Wo. 6, Speckfie diversity by

Aoformition function, #.

Station Station

GA, Oe em, 1.26 6-23, O61 em. 2.07

GM, leew. 113 6-24, OL em. 1.86

GHA, 2-3 cm, 0.68, 6-26, 162 em. 1.37

GBA, Ont em, 1.26 25, Ont cm, 1.99

6A, Oot em. 1.31 6-26, Ont cm. 1.36

Goh, OL em. 1.56 6-26, 1-2 em, 0.76

GTA, 1-2 om, 0,93 0-27, Ort em. 1.58

GOA, OL em, 1.45 6-28, Onl em. 1.60

6-29, O-1 em, 2,03

n22, Olea, 1.72

1

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304

Study of the uffvet of Heated Water on Turtle Grass,

?Thalassia testudinum Xonig, in Guayanilla Bay

by Peter Schroeder

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Guayanilla Bay has a number of turtle grass beds, most of which appear to be dense, healthy and relatively unstressed by man's activities. Those beds of marine grasses found on either side of the mouth of the cove receiving heated effluence from the fossil fuel thermoelectric plant appear to be thinner, stressed and mixed with red-brown algal complexes.

Five stations have been established in the various turtle grass flats at Guayanilla, Station 1 is located immediately fo the south of the entrance to the thermally affected cove; Station 2 is located to the north of the cove's entrance and Station 3 is located some distance to the west of the entrance. Stations 4 and S are control stations located in a different part of Guayanilla Bay near the intake to the



power plant and are not subjected to thermal stress from the power plant.

Stations 1 and 2 show possible stress from current and/or the heated water leaving the cove. Station 3 is farther

from the entrance of the cove and is an extremely thick, large bed of *Thalassia* evidently nearly unaffected by the power plant. This bed of grass compares in biomass with the

control Stations 4 and 5.

Periodic samples have been taken with a specially constructed sampler (see diagram) from all five *Thalassia*

Stations and biomass figures for *Thalassia* from each sample have been ascertained, Dry weights of Various parts of the *Thalassia* plant (according to Tomlinson and Vargo, 1966)

Have been recorded as well as total weight of plant material.

After dry weight was recorded, the plant material was ground with mortar and pestle and stored in anticipation of chemical analyses, (In Florida elemental ratios go through annual fluctuation; see Walsh and Grow, 1972).

In order to determine the effect of the thermal discharge on *Thalassia* plants in Guayanilla Bay, a turtle grass transplant experiment is envisioned. Floating plant boxes have constructed from wood which are designed to hold the samples taken, from thick, healthy *Thalassia* flats in Guayanilla Bay. These boxes will hold the plants approximately fifteen inches below the surface and can be anchored wherever convenient. Once anchored, they can rise and fall with the tide and maintain the plants under nearly equivalent light conditions. In every sample certain blades will be marked (according to the technique of Zieman, 1968) in

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order to determine growth rates. At intervals of two to four weeks one sample will be replaced in each box and examined for chemical analyses. Concurrent examination of associated organisms can be made to determine the effects of thermal water on the species diversity within the turtle grass community.

The plant boxes will hold Taylor maximum-minimum thermometers and will be anchored at three points within the thermally affected cove. Two boxes will be located outside the cove in Guayanilla Bay to serve as controls, These will be located near existing apparently healthy grass beds.

It is hoped that this experiment will lead to a determination of maximum temperature at which Thalassia communities can exist in Puerto Rico. It may also indicate what form thermal stress first affects the plant itself,

---Page Break---

THALASSIA SAMPLER

0203 METER?

---Page Break---

PLANT BOX FOR GROWING

THALASSIA

( # waT6® Level

---Page Break---

Station Date collected

Br Ws/ie

Growing tips 3

ioote

shizomes

vertical (short) shoots

new leaves

old leaves

old sheathing leaves

Total

Grams per meter square

3a

Growing tape 8

soote

Rhizones:

vertical (short) shoots

new leaves

02d leaves

old sheathing leaves

Total

Gras per meter square

309

Wet weight Dry weight(grams)

5a +7596

8.8 2.9433

14 +3379

2.1901

342343

Te +7890

66.5 8.254

3,276 406.6

39.4952 3.8410

27.8235 5.5819

8.6453 2.0720

28.9616 346130

32.4598 3.5705

--- 2.8814

157.4558 21.5658

1,756 1062.4

---Page Break---

Station Date collected

4a Ws/te

Growing tips 19

Roots

?hizones

vertical (short) shoots

old sheathing leaves

?Total

Grams per meter square

BA 5/12

Growing tips 8

ieoots

Ahizpmes

vertical ( short) shoots

new Leavi

old leaves

old sheathing leaves

Total

Grams per meter square

Wet weight

52.5

25.6

2.5

11.6

49.7

30.0

172.9

8468.

310

Dry weight (grams)

6.3063

6.9540

2.2959

2.3040

4.9123

2.4976



25.2701

1244.8

4.3672

+4340

+3395

1.7307

546310

2.5356

25.0979

143.7

---Page Break---

an

Station Date collected wet weight Dry weight (grane)

38 Use

Growing tips 0

xoote 6.0252

snizone 346636

Vertical ~ Shoat +3313

Kew leaves 2.6558

01d leaves 4.7482

Sheathing 2.4386

Total 37.8423,

Grams per meter square 878.3

?3 sire

Growing tips 5

roots 92.1 9.7106

Hnizome no 10.2325

Vertical snoote 36 +4382

ew leaves 23.7 2.4897

014 leaves 70.6 6.7504

Sheathing 50.3 4.8225

Total 302.0 33.5038

Grams per meter square 14.832 2650.2

3B Use

Hoots 69.3 6.0076

shizone 27.8 3.9867

Vertical shoote ana 2.3280

New leeves 16.0 1.9849

old leaves 43.7 440320

Sheathing 35-4 2.1054

Total 203.3 20,5036

Grams per meter square 10310. 1009.8

---Page Break---

Station Date collected

aA Ts/re

Growing tips 3

Hoots

Rhizome

ertical shoots

New leaves

014 leaves

Sheathing

Total

Grams per meter square

2a Ws/T2

Growing tips 6

Roots

rhizome

Vertical shoots

New leaves

Old leaves

Sheathing

Total

Grams per meter square

3 Tas/Te

Growing tips 3

kote

ahizone

Verticel shoots

New lesves

Olé leaves

Sheathings

sotal

Grams per meter square

32

t weight Dry Weight (grams)

+5384

2.1910

=3226

1652

+8012

1.0447

420320

198.6

42.9 4.6713

26.0 4.7439

10.0 12.2880

we 2.2577

45.3 444390

14.2 2.3285,

150, 27.7284

7399. 873.4

3964 3.9966

47.4 7.6478

20.2 1.5865

21.3 2.6354

63.5 6.5108

22.2 2.2224

204.0 24.5999

10049 1222.8

---Page Break---

Station Date collected

8 1/s/Te

Growing tips 12

Hoots

Rhizome

Vertical shoots

New leaves

014 leaves

vheathing

Total

Grems per meter square

13 1/9/12

Growing tips ©

Hoots

Vertical Shoots

New leaves:

014 Leaves

Sheathing

cel

Grans per meter square

ea 1/3/12

Growing tips 4

voote

Verticn2 Shoote

Hew Leavee

014 Leaves

Sheathing,

Grems per meter square

Wet weight

30.0

17.3

63.9

23.9

46.7

127



206.2

10253

33.20

22.10

3.20

1.7

88

14.2

82.9

4084

33

Dry weight (grams)

4.8821

4.6692

7.6637

444300

4.7482

2.2363

28.3295

2395.5,

+9686

1.7347

1.0090

+4902,

6.6416

141680

212.0120

591.7

3.2841

1.9203

+3530

2.5469

+8289

2.4334

10. 3666

510.7

---Page Break---

Station Date collected

3B ans/ie

Growing tips 4

Hoots

Rhizome

Vertical Shoots

w leaves

Old leaves

Sheathing

?Total

Grams per meter square

4B 1as/T2

Growing tips 5

uoots

Hhizone

Vertical shoots

New leaves

014 leaves

Sheathing

Total

Grams per

ter square

5B 1ls/le

Growing tips 3

Roots

Rhizome

Vertical shoots

Wow leaves

014 leaves

Sheathing

Total

Grams per meter square

Wet weight

53.6

45.2

3.9

ws

50.5

40.2

210.8

10384

49.00

18.0

2.

21.3

63.5

21.3

178.2

e778.

219.4

33.9

15.0

32.0

79.8

66.0

34542

17043

314

Dry weight (grams)

5.0299

7.2897

1.4022

2.1856

8.8149

3.7725

27.4937

1396.4

5.6480

3.3848

+8900

2.6417

6.0376

1.9040

20.5662

1013.1

12.2036

5.3456

2.5230

3.9680

7.9486

6.2230

3362338

1637.1

---Page Break---

Station Date collected

a asta

Growing tipe 0

roots

izone

Vertical Shoots

New leaves

Olé leaves

Sheathing

?Total

Grams per meter square

2a, ea/Te

Gromng tips 6

xoote



Vertical shoots

Kew leaves

014 leaves

Shething

Total

Grams per meter square

3a 8/3/72

Roots

ahizone

Vertical shoots

New Leaves

02a leaves

Sheathing

Total

Grams per eter square

Wet weight

1.0

42

2.0

6.0

3.4

3.0

18.6

96

45.3

3064

25.8

9.6

22.2

26.0

199.2

7842

216

20.2

21.0

as

375

50.0

167.8

8266

35

Dry weight (grams)

+0572

+5827

+1329

+4680

+2597

+2165

1.7260

84.5

4.6880

547043

4.3285

2.0026

2.0432

2.3293

20.0959

983.9

2.8075

3.8406

2.0002

2.4467

542826

5.0420

22.4186

1055.1

---Page Break---

Station Date collected

4h 8/9/72

Growing tips 3

Hoots

Rhizome

Vertical shoots

New leaves

Old leaves

Sheathing

Total

Gren per meter square

1B 89/72

Growing tips 0

Roots

Rhizome

Vertical shoots

New leaves:

old leaves

Sheathing

?Total

Grans per meter square

2B 8/9/72

Growing tips 2

Hoots

Rhizome

Vertical shoote

New leaves

old leaves.

Sheathing

Total

Grams per meter square

Wet weight

59.0

25.0  
17.0  
23.0  
63.4  
35.7  
223.1  
10390

2.7  
5.0  
22  
2.0  
9.2.  
1.0  
21.9  
1374

21.3  
13.2  
15.7  
9.0  
21.7  
12  
210.2

5424

Dry weight (grams)

5.7621,

3.9234

2.5309

2.4296

6.5342

344082.

24.5782

1210.7

2780

+8426

+3334

+1638

+9355

No

2.5523

125.7

2.9340

243580



2.1389

+6617

2.1294

2.4950

22.7170

626.5

316

---Page Break---

Station Date collected

33 a/a/te

Growing tips 0

Roots

Hhizome

Vertical shoots

New leaves

018 leaves

Sheathing

Total

Grams per meter square

45 a/s/72

Growing tips 3

Hoots

ienizone

Vertical shoots

Now leaves

old leaves

Sheathing

Tota

Grams per meter square

5B ea/re

Growing tips 3

Hoots

Vertical shoots

New leaves

026 leaves

Sheathing

Total

Grane per meter square

Wet weight

35.2

28.4

10.0

12.6

36.0

22.6

143.8

7084

1563

28.1

16.5

a3

61.0

45.0

245.2

12079.

223.0

25.4

18.0

29.2

13.2

61.2

342.0

16847

Dry weight (grams)

3.2940

543607

1.5805

1.4264

4.2980

4.9496

19.3892

995-2

744103

4.8020

1.7390

2.4201

6.0315

4.1425

26.5456

1307.7

11.3560

4.5698

344536

3.0232

6.8974

5.7636

5.7636

2726.8

7

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Station Date collected Wet weight Dry weight (grams)

a 6/20/72

Growing tips 4

Roots 22.2 2.4209

xhizone 22.2 345903,

Vertical shoots 24 1.6339

New leaves 21.5 2.3455

014 leaves 40.5 3.6655,

Sheathing 16.5 1.7134

Total 131.3 15.3695

Grane per meter square 6468 111

MZAN WSIGHTS (GRAMS PER METER SQUARE)

Station Wet Weient bry Weight

1 2638 g/n® 360.7 g/n?

2 6187 @/n? 15061 @/n®

3 8440 e/a? 1086.2 @/n®

4 22277 @/a? 1285.3 @/a?

12565 ¢/n 2130246 g/m?

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Tomlinson, P.3. and 7. 2. Vargo (1966). On the Morphology and Anatomy of Turtle Grass *Thalassia Testudinum* (Hyérocharitaceae). Vegetative Morphology, Sul. lar, Sei, 16(4).

Grow (1972), Composition of

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Zieman, J.C. (1968

of the Sea-grass, *Thalassia Testudinum*, Thesis, Univ.

A Study of the Growth and Decomposition

Wiama. Coral Gabli Orids.

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MANGROVE. ROOT COMMUNITIES IN A  
THERMALLY ALTERED AREA IN GUAYANILLA BAY

by

Seppo Kolehmainen,

Thomas Morgan

and Roberto Castro

INTRONUCTIO!

Thermal tolerance of tropical ecosystems and marine organ-



isms have been studied very little and much of the available information in this respect comes from subtropics where the annual mean temperature is lower and the seasonal variation greater than in the tropics. Most of the thermal studies with tropical marine organisms in the Caribbean have been made in Biscayne Bay, Florida (Sader et al., 1970; Zieman, 1970; Thorhaug, 1970; Roessler and Zlenan, 1970; Singletary, 1971). Some old studies (Mayer, 1914, 1918) are still the only existing data for the Caribbean corals. Only some intertidal species have been studied in this respect in the West Indies (Southward, 1962).

To get applicable data on the thermal tolerance of the Species in Jobos Bay, studies were started in Guayanilla, fifty miles west of Jobos Bay, at the fossil fuel power plant site in October, 1971. The power plant has been operating on the eastern shore of Guayanilla Bay since 1987. In the spring of 1992 the output of the plant was 310 MW. Now the plant produces 710 MW. The ecosystems around the discharge area of the cooling water resembles those in the Aguirre Navigational channels viz; mangrove Swamps, turtle grass beds and mud Sotton communities,

?The effects of elevated temperatures upon plankton, benthic organisms, turtle grass beds, mangrove root communities

and fish are studied in Guayanilla Bay in their natural environment. ?The results of these studies show the effect of long-term exposures of living populations to elevated temperatures. This section presents the data on the mangrove root communities over a period of one year. Some preliminary data have been given earlier (Kolehmainen and Morgan, 1972). Data on plankton, turtle grass and fish are given elsewhere in this report.

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?The power plant in Guayanilla takes cooling water from an embayment on the west side of the plant and discharges the water 10° C, above the ambient through a 190 m long canal into a 900 m long semi-enclosed cove on the southeastern

side of the plant (Figure 1). Until March, 1972, the output of cooling water was 750 m<sup>3</sup>/min, (188,000 gal./min and after a new 400 MW unit was added the output was raised to 1510 m<sup>3</sup>/min, (398,000 gal./min.), the rate being the same as before.

In addition to the heated water, Guayanilla Bay receives chemical and carbohydrate pollutants from the nearby oil refineries and chemical plants. Oil slicks appear periodically and the concentration of heavy metals, including Hg, Cd, Cr, Ni, Pb and V, is high in sea water and the resident marine organisms. The power plant uses chlorine treatment against fouling organisms in the condensers and chromates as an anti-corrodant. Free chlorine disappears quite fast, however, in the discharge canal. Part of free chlorine could have been associated with dissolved organic compounds of which many have been known to be toxic in a chlorinated form.

Fish kills have been reported occasionally around and downwind from the oil docks of Commonwealth Oil-refining Company (CORCO) and near the effluent discharge of Pittsburg Plat Glass, Inc. (PPG). These fish kills are attributed to dumping of toxic chemicals. No fish kills have ever been reported in the cove that receives cooling water from the power plant. Since the cooling water is taken from the bay per se, it is polluted by chemicals and hydrocarbons. It

is therefore impossible to separate entirely the effects of elevated temperatures from other pollution. By using the intake area of the cooling water as a control area, the additional effect of elevated temperatures can be seen, however. The combination of chemical pollutants and heat may produce synergism, but if it exists, it only means that the effects of elevated temperatures are seen more pronounced. Therefore, it is safe to assume that whatever predictions on the effects of elevated temperatures are made with the data obtained in Guayanilla Bay, they will be conservative,

## METHODS

Temperatures were recorded at several mangrove stations and turtle grass beds with recording thermographs. In addition

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Figure 1. East side of Quayantlia Bay showing the location of the power plant and the sampling stations

---Page Break---

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to this continuous monitoring of water temperatures, temperatures were measured with a calibrated thermometer whenever samples were taken. The horizontal and vertical distribution of temperature in the heated area was measured three times -- 9 October 1971, 18 January 1972, and 20 October 1972, Currents were measured in the cove with recording current meters and with flow meters.

Mangrove roots with organisms were collected periodically at eight stations (Figure 1) and, in addition, spot checks were made in twelve other stations. The species present, their biomass and vertical zonation were determined with three to six randomly sampled roots at each of the eight stations. The randomness was achieved by selecting every third root of every third tree if the root was over 0.5 m long. These roots were cut at mean water level and surrounded with a 0.5 mm mesh net before lifting into the boat.

Crustaceans and fish were collected from the net and preserved with 70% alcohol while the roots with sessile organisms were put into polyethylene bags and kept in an icebox before they were taken to the laboratory. In the laboratory the roots were cut to 10 cm sections and the species present, the number of individuals (when applicable) and the biomass of each species as wet weight were determined in each section. The wet weight of species included the shells of mollusks and cirripedes, tubes of polychaetes and tests of ascidians, but only those of living specimens. Net weight - dry weight ratios of the species are being determined presently. Besides sampling of roots, collecting of species and visual observations were made at each station to establish a list of species present at each temperature zone.

### Temperature of Water

Ambient surface temperatures near the intake varied from 25°C. in winter to 31° C. in summer (Figure 2) and the temperature of the effluent varied from 35 to 40° C. When only the old generating units were operating two thermal

plumes could be seen, one at the mouth of the discharge  
?anal and one at the mouth of the cove to Guayanilla Bay  
(Figures 3, 4 and Plates I \ - £). At the mouth of the  
discharge Canal a rapid entrainment was observed. The  
temperature decreased over 2.5° C, within a distance of  
100m. From this distance toward the mouth of the cove

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\$3901.00 waenaia3s snow ane sen

MAS ARTO S Wort s SOTO AHO Ss RoZIAS BEES

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sce temperature on Jan '3, 1972. Soundings ae in feet.

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Power Plant

Z

Figure 4 . Surface temperatures on October 9, 1972

Distribution of mangroves also shown.

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Power Plont

S JN

oO \

Discharge Canal

\ \

PLATE IA, Water temperature on the surface - October 20, 1972

---Page Break---

PLATE IB, Water temperature at one meter!s depth.

October 20, 1972

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---Page Break---

PLATE IC. Water temperature at 2 meters depth ,

October 20, 1972

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---Page Break---

Po Power Plant

OB,

wis ?A\

\ Discharge Canal

y \

PLATE ID. Water temperature at 3 meters depth,  
October 20, 1972,

---Page Break---

PLATE IE.

Power Plant

? \

ischarge Canal

Water temperature at 4 meters depth,

October 20, 1972,

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---Page Break---

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the temperature decreased an additional 1.5° C. At the mouth of the cove the water was still 5.5° C. above the ambient. Inside the cove there was a thermocline at the depth of 2.5 - 3m. Below this depth the temperature of water was about 0.5° C. above the ambient (Figure 5). At the mouth of the cove the heated water rose to the surface and dissipated within 600 m (Figure 6). At the mouth of the cove there was a narrow deep channel coming toward the cove from the west. Outside this channel water here is very shallow (Figure 3) and, consequently, there is a limited supply of ontrainment water.

Testing of the cooling system for the new generating unit

was started in the middle of February by pumping water Occasionally at ambient temperature through the system. This lowered the temperature of the effluent about 5° C. This testing continued, on and off, until May, after which time test firing of the generating unit was started. While the new unit has been operating the temperature at the mouth of the cove has been 7.5° C. above the ambient. The daily mean temperature and the maximum and minimum at the mouth of the cove are given in Figure 7. The diurnal fluctuation of ambient temperature was about 1° C. while the diurnal fluctuation at the mouth of the cove was about 2c.

## Currents

The water was discharged from the old units at the mouth of the canal 0.75 m/sec. and the current at the nearest mangroves was 0.1 = 0.2 m/sec. At the mouth of the cove below the thermocline water was flowing into the cove at a velocity of 0.1 = 0.2 m/sec.. The water going in was 0.5°C. above the ambient, and thus 345 m<sup>3</sup>/min. was needed to entrain 750 m<sup>3</sup>/min. of heated water that came out of the discharge canal at 10° C. above the ambient to lower the temperature down to 5.5° C. above the ambient in the cove.

Subsequent to the operation of the new unit the currents have increased considerably. Earlier the current was laminal and flowed toward the mouth of the cove on the surface. Now there is a large eddy inside the cove circulating counterclockwise (Figure 8). Current velocities for the present situation are not yet studied, but according to the calculations 395 m<sup>3</sup>/min, entrainment water was needed to lower 1510 m<sup>3</sup>/min. from 1n to 7.5° C. above the ambient within the cove. This means that there was about the same volume of entrainment water going into the cove as before.

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Figure 8. Surface water currents in the cove in summer 1972.

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The turbulences in the discharge canal saturate the cooling water with oxygen and, consequently, oxygen readings in the thermal plume area were between 5 and 7 mg O<sub>2</sub>/l,

## Tides

Tides in this area resembled those in Jobos Bay. The difference between the high and low tide was about 30 cm.

## Mangroves

Guayanilla Bay was once surrounded by mangroves on all sides, but the construction of industrial plants, piers, jetties and landfills have decreased the area of mangroves to a small proportion of the original cover. The area of the mangroves adjacent to the power plant covered 19.6 hectares. At the waterline about 95% of the mangroves were red mangroves (*Rhizophora mangle*), the rest being



Black mangroves. *Sonneratia caseolaris* and white mangroves (*Laguncularia racemosa*). In the cove there were mangroves growing along the entire length of the east shore. The water temperature in the warmest place around the mangrove roots varied before from 33° C. in winter to 38° C. in summer. Now with the new unit in line the temperature in the summer of 1972 was 39.5° C.. The mangroves were reproducing in the cove even though the temperature was 9° C. above the ambient. Earlier there were a few dead trees at station 7 (Figure 1) and at the mouth of the cove at station 8, but this was not due to the temperature, but rather to the current which was eroding the sediment from around the roots of mangroves in such a degree that the trees were uprooted and fell down, Now with increased water currents more trees were failing and dying. The mangroves in other parts of the cove still appeared to be

### Mangrove Root Communities

Species composition: Mangrove root communities in the site show the effects of elevated temperatures. The number of species on the mangrove roots and the

temperature of water on 9 October 1971 at eight stations

(Figure 1) are given below:

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STATIONS.

Control \_1

Temp. °C. 30.0 31.2

Spp. Algae 6 5 5 Rk 7 4 5 5

Spp. Invertebrates 90 45 69 67 58 25 21 10

Total ¥ Spp. 96 «48 74 75 65 29 2815

The number of invertebrate species at control station are very high, This station had more species than any station in Jobos Bay, an area that does not have chemical pollution like Guayaniila Bay. There appeared to be some unknown ecological factors that made this station so favorable for Sessile organisms. The natural variation of the number of species is great in the mangrove root communities (see the section on mangrove root communities in Jobos Bay 1972 Annual

Report) and no two areas are exactly alike. A t-test was made between the different stations comparing the number of Species in each phylum. This showed that station 1 differed significantly from stations Control, 2, 3 and 4." Station 1 had only one row of poor-looking mangrove trees along the shoreline which may have been the reason for the snail number of species living on the roots. Stations Control, 2, 3 and 4 did not differ from each other significantly, but Stations 5, 6 and 7 did differ significantly from all the other stations ( $P = 0.05$ ). At 35°C. all the sensitive species were eliminated while above that temperature a few Species dominated the root communities. The importance of this temperature was seen clearly in the winter when the effluent was coming from the discharge canal at 35°- 36°C, Then many of the organisms that in summer were found only outside the cove immigrated into the cove and established fast-growing populations. This was especially noticeable among ascidians, polychaetes and crabs. They followed the reduction of the 35° C. isotherm into the cove all the way to station 7, when the water temperature increased in the spring these species gradually died and disappeared from the cove.

The number of species in different phyla is given in Table  
Gyrodactylidae were not as numerous in Guayanilia as in  
Jobos Bay. Molluscs had the most species at Control

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TABLE 1

san@ER OF SPECIES TH DIFFERENT PHYTA ON THE MANGROVE ROOTS On  
(OK OCTOBER 9, 1971

PRY

cyanophyta

entorophyta

Phaeophyta

Rhodophyta

Porifera

coctenterata

Annelida

Stpuncul ida

Yollusce

Arethropoda

aryoz0a

Eehinoderaata

Chordata

(Aseidiacea)

algae

# Invertebrates

Total

Control

27

25

45

48

203

46

1

13

14

aoa

w 7

,oa

wow

26

o3

12

te 46

5 8

7 67

BOS

10

18

38

65

25

29

2

a

26

10

1s

339

station and at station 3, but arthropods were the most numerous at most stations. "At station 1 ascidians had most species of all phyla. Annelids, molluscs, arthropods and ascidians were the most important groups at all stations. The list of species found at different stations is given in Table 2.

The species composition in the cove and at station 4 changed during the winter. When the temperature decreased larvae that were coming into the cove with the counter-current on the bottom settled on the mangrove roots and established colonies and populations. The number of species of macroorganisms in winter, 1972, at different stations is given below.

## STATIONS

Control 34

Temperature °C 25.5 26.0 26.4 27.2 28.9 31.5 33.0 34.5

# Species 94 45 74 7H NSH a8

The most pronounced increase in the number of species happened at stations 5, 6 and 7, Stations 4 and Control also showed a slight increase. The increase in the species diversity was due to the additional species of sponges polychaetes, crustaceans and ascidians. These changes all occurred in a four-month period which showed that a thermally altered area can recover rapidly. In this respect, thermal pollution is different from some other types of pollution. There are no residues left from thermal addition.

When the ambient temperature and, consequently, the temperature in the cove rose in the spring, the populations of the more sensitive species gradually disappeared. When the more sensitive species such as ascidians died, the surface area that became available was quickly utilized by the hardy species. Bluegreen algae, an encrusting sponge (*Halysarca* sp.), a calcareous tube dwelling polychaete



(*Tonafastepus jtelzatua*), etree oyster, ((tognanan seus),  
a periwinkte (*Littorina angul fers*), two faTanus. species  
and two crabs (*Kratus pisonii* and *Pachygrapsus t: ns rsus*)

were the more hardy species. Nacroalgae, coelenterates,  
echinoderms and ascidians were the most sensitive species.

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Stations

?TABLE 2 (cont 'd.)

Rhodophyta

Loventeria sp.

Polyeiphonta howe!

Polysiphonia #p.

[INVERTEBRATES

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Gastropoda

Bivalvia

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Stato

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Bionass,

?The biomass of organisms living on mangrove roots was also affected by the temperature and by water currents. The lowest biomass, in October 1971, was at the warmest area of the mangroves at station 7 (Figure 9). In this area the water current came from the discharge canal directly into the mangroves at high velocity. About 30 meters further behind a small point where the current velocity was very slow the biomass was more than an order of magnitude greater than at station 7, even though the temperature was only 0.1 - 0.2° C, lower than at station 7.

Station 6 is about 100 m from station 7. Temperature there was 0.4° C, lower than at station 7 and the current was very weak. The biomass at this station was the highest of all stations, but it was made up mainly of two species, the tree oyster and *Balanus amphitrite* var.

regene eeeon shad a lower FYomass than the station  
Biveach side of it. The reason for this is not known, but  
the low bionass may have been the result of the strong  
Current at this station. All the stations outside the  
cove had biomasses between 200 and 300 grams.

At stations Control, 5 and 6 molluscs were the  
Goninant group, while at station 2 ascidians, at station 4  
Qigae and-at station 7 cirripeds were the most important  
greups (Table 3). In general, algae, molluscs, cirripeds  
Gnd ascidians contributed most to the biomass.

In the winter the bionass of sponges, polychaetes,  
crustaceans and ascidians increased in the cove, but no  
Visible change was seen at the station outside the cove.

After the new power generating unit was fired the biomass  
for the Toots at station 5 increased because of the in

Increase in the number of tree oysters and *Balanus* spp. It appeared that tree oysters and cirripeds could compete better at higher temperatures. These species were also able to withstand stronger water currents than other species living on mangrove roots. The other stations inside the cove did not change their biomass, but bluegreen algae increased their proportion of the total biomass from the summer of 1971. Stations outside the cove exhibited only small changes in the biomass or species diversity except station 4 where the number of species of ascidian decreased. The biomass and the temperatures of five stations on 20 September 1972 are given below.

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Figure 9.. Biomass of organo

Figure 9. Biomass of organisms living on mangrove roots on October 9, 1971 at different stations as grams wet weight per root.? The isotherms at the same date are given too,

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ANNEDILIA

Polychaeta

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Teopode

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TABLE 4

Vertical Distribution of the Number of Species  
and the Biomass (Wet Weight) Calculated  
for All Stations on 9 October 1971

4 of Species Biomass

Depth, cm of Total of Total

0-9 3 0.8

10-19 10 8.6

20-29 20 22.2

30-39 39 22.5 21.9

40-49 20 16.5

50-59 12 12.2

60-69 7 10.5

70-79 3.5 5.6

80-89 1.6

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below the mean water level. In Jobos Bay most of the biomass was somewhat deeper because the biomass of ascidians in Jobos Bay was much higher than in Guayanilla Bay.

#### THERMAL EFFECTS IN MANGROVE ROOT ci

The mangrove trees in Guayanilla Bay were living in temperatures up to 40° C. until May 1972 the maximum temperature in the mangroves there was up to 38° C. and even at this high temperature the mangroves constituted a reproducing population. In the summer of 1972 the temperature increased to 40° C. for the first time due to a new generating unit that more than doubled the volume of cooling water, During Summer 1972 the mangroves in the heated cove appeared still to be surviving, but whether this high temperature will eventually have adverse effects on the mangroves will be seen in the future.

Mangrove root communities were much more sensitive to ele-



vated temperatures than mangrove trees. Species composition and biomass of organisms living on the mangrove roots in Guayanilla Bay reflected the long-term effects of the elevated temperatures. These effects showed in the population dynamics, growth rates, competition between species and in the seasonal migration of species.

The situation in Guayanilla was convenient for thermal effect studies because the cooling water was discharged into a semi-enclosed area where the surface area between isotherms was large enough and constant enough to illustrate the horizontal zonation of organisms according to the temperature. Since the elevated temperatures here were produced by a power plant, the data are directly applicable to other power plants. Species diversity with Simington populations of organisms: A low number of macroalgae in Guayanilla Bay compared to that of Jobos Bay may be an indication of stress by chemical pollution in Guayanilla Bay. However, more invertebrates were found in Guayanilla Bay than in Jobos Bay on mangrove roots. Nine species of algae and eighty-five species of invertebrates were found in common in both bays.

The most hardy sessile species were intertidal, cirripeds,

Balanus amphitrite var. pallidus and Balanus eburneus, but  
almost as tolerant was the tree oyster Tene Stee  
which lives in the lower part of the intertidal zone  
deeper, A polychaete, Pomatostegus stellatus, that lives

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#### TABLE 5

Maximum Daily Temperatures that the Following Species have  
been found in Coahuila Bay.

## PLANTS

cyanophyta woe

40

40

40

Chlorophyta Enterosorpha sp. »

Gaulerpa verticiliata 36

C. fastigiata 35

©. sertularioides 35

C.\_racemosa 35

Phacophyta Dictyota givarte 34

Rhodophyta Acanthophora sptetfera 35

Hucheusa acanthocladus 4

?Teacheophyte Rnizophors mangle 40

Avicennia nit 40

Laguncularia racenosa 40

## AMIMALS

Portfera 2»

36

35

35

34

3a

cue 33

Annelida Pomatostegus stellatus 38

Syllie 3

Phyllochaetopus claparedit %

?eodtce rub 36

Sabelia welanostigna 3

S. alge 35

Nereis dupertiit 35

Limbrinereie maculata 35

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below the mean water level. In Jobos Bay most of the biomass was somewhat deeper because the biomass of ascidians in Jobos Bay was much higher than in Guayanilla Bay.

## THERMAL EFFECTS IN MANGROVE ROOT COMMUNITIES

The mangrove trees in Guayanilla Bay were living in temperatures up to 40° C. tmtil May 1972/the maximum temperature

in the mangroves there was up to 38° C. and even at this, high temperature the mangroves constituted a reproducing population. In the summer of 1972 the temperature increased to 40° C. for the first time due to a new generating unit that more than doubled the volume of cooling water. During Summer 1972 the mangroves in the heated cove appeared still to be surviving, but whether this high temperature will eventually have? adverse effects on the mangroves will be seen in the future.

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Populations of organisms. A low number of macroalgae in Guayanilla may compared to that of Jobos Bay may be an indication of stress by chemical pollution in Guayanilla Bay. However, more invertebrates were found in Guayanilla Bay than in Jobos Bay on mangrove roots. Nine species of algae and eighty-five species of invertebrates were found common in both bays.

The most hardy sessile species were intertidal cirripeds, *Balanus amphitrite* var. *pallidus* and *Balanus eburneus*, but the most tolerant was the tree oyster *Teosnsee* status. Which lives in the lower part of the intertidal zone and deeper. A polychaete, *Ponostegus stellatus*, that lives

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Maximum Daily Temperatures that the Following Species have  
been found in Goayaniila Bay.

puants

cyanophyta Vlothetx sp. 40 º

?Tyngbye op. 40

Oscillatoria ap. 40

ius 6p. 40

Chlorophyta Enteromorpha sp. 39

Caulerpa verticillata 36

E. fastigiata 35

E. sertularioides 35

C. racemosa 33

Phaeophyta Dictyota divaricata %

Rhodophyta Acanthopora spectabilis 35

Eucheura acanthocladus Es

?Teuchophyta Rhizophora mangle 40

?Avicennia nitida 40

Laguncularia racemosa 40

## ANIMALS

Porifera Malinche ep. 2

Gallyspongia sp. 36

Mycale sp. 35

Halichondria 35

Tedania Spongia 34

Coelenterata Ethalia angulata 34

Erythropodium ceribae 3

?annelida Pomatostegus stellatus 28

Syllis sp. 37

Phylloporosoma claretti 36

[Eodice rubra 36



Sabella selanostigns 36

alga 35

35

35

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Annelida

mn

Arthropods

Bryozoa

Echinodermata

Chordata

?TexebelJa annulicilis

atulus sp.

?Sabellastarte nagnitice

Ltorine angulttere

Brachidontes ex

Nassarius vibex

Crassostrea rhizophorae

Great ise veppaceus

Ostres equestrian

Betalconchos seginty:

Hainoea elegans

Pododeamus rudis

Balanus snphitrite v. pallidus

rachygrapeus Cransversus

?Hratus plsonit

Gontopets cruentaca

CLibanarius cube

Hexapmopeur car ibbacus

Panopeus bermidensia

Eanopeus RarecAt

cross polite

Excorstians suedticornis

Ghthansiae Failte

Eorophiae sp.

Synuipheue Frtzmitterts

Wetopheys Sicornstue

crisia sp.

Qpbiocreza 1istoralie

Ophioehrie angulaca

Rotryllotées nigcun

Distaphia bermudenste

Polyclinus constellatug

Piplosons sicdonalat

Perophors virid

Seyela pare:

setéie aigrs

33

3

RORERRRESS

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Chordata

Clavelina picte

Polyeitor olive:

4

3%

3

33

33

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in the intertidal zone and below the intertidal zone was

associated with the former species in temperatures up to 38°C. (Table S). All these species have adapted for living in extreme conditions, not only in high temperatures, but also in varying salinities, low dissolved oxygen concentrations and polluted conditions. In these extreme conditions these species occupied all depths of the mangrove roots. These sessile species were poor competitors and in optimal conditions for mangrove roots Organisms in the middle and lower parts of the Toot these species were covered by macroalgae, encrusting sponges and (haliagsetsциdians until they died ?An encrusting sponge (liglisarea sp.) was also found in temperatures up to? 39°C,

is species grew as a thin layer around the roots in the heated cove, but outside the cove it was uncommon,

Free moving animals were in a better position to avoid too high temperatures. Once species of snail, periwinkle (*Littorina angulifera*) and two species of crabs (*Aratus pisonti* and *Pachygrapsus transversuay*) were found. Fao AE Station 7'as well as in the dishes they coped along with *Balanus amphitrite* var. *pallidus*. Periwinkle and the two crabs were capable of climbing above the surface to avoid water that was too hot. Bluegreen algae were found as a thin layer covering the bottom in shallow banks and also on the mangrove roots at station 7. In September 1972 when

the temperature in the cove was between 37.8 and 39.7° C. biomass of bluegreen algae was increased greatly from the situation in the previous summer. While bluegreen algae were found covering parts of mangrove roots only at's

in 1971, now bluegreen algae were seen on all mangrove roots in the cove, and there were large areas in the cove where the surface of water had flakes of bluegreen algae floating.

FRteromorpha and Ulothrix were the only algae besides bluegreens found in the Gove in October 1971. Three other polychaetes (*Syllis* sp., *Leodice* sp. and *Narphysa regalis*) besides *Pomatostegus* were found in the cove above 7. Two molluscs (*Erachidontes exustus* and *Succatvos vibex*) were found in the cove in summer, too. Two sponges (*Sphacrosoma walkeri* and *Cirolana parva*) and several crabs (*Libinia sinensis*, *Parva*, *Goniopsis* *guttata*, *Vanopeus bermudensis*, *Cailinectes savatensis*) were found in the cove in October 1971. Bryozoa (*Crisia* sp.) lived in temperatures up to 38° C. Five species of ascidians (*Distaplia bermudensis*,

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*Polyclinum constellatum*, *Didennum candidum*, *Diplosoma*  
*Bolye* in cons eae eaeay were found living in tempera-  
eee eo sort. pst of the other species did not

occur above 35° C, (Table 5).

about 3/4 of all species were found living in temperatures  
Shove 38° ?, The rest may very well be able to live at  
those high temperatures, but there may have been some other  
ological factors preventing their living in the area  
Ghere the temperature was this high in Guayanilla. The  
Tiversity of species on mangrove roots varies normally  
Greatly and the factors affecting this are still mostly  
Ehknows (see the section on the mangrove root communities  
in Jobos Bay 1972 Annual Report).

The results of this study show that both the species compo-  
Sition and biomass of mangrove root communities were not  
affected adversely in temperatures below 34° C. Between  
Sd and 35° C. the number of species dropped abruptly and



above this temperature the number of species was inversely

Related to the temperature of water

All the temperatures given in this paper are long-term mean

temperatures, thus there were short times, a few hours,

whereas while when the temperature at the stations col-

lected exceeded the mean temperature by one to two degrees.

The lack of information on the thermal tolerance of tropi-

cal marine organisms makes it impossible to compare the

thermal limits of the most important species found

in Cuayanilla Bay. A population of the snail, *Nassarius*

*tibexy* was found at 36° C. in Guayanilla Bay (see Tjørhaug

1959, p. 371-374) as the upper thermal

limit for this species in a 72-hour laboratory experiment.

The authors found the upper thermal limit of 36.1° C.

for *Poriclimenes americanus*, while in

Guayanilla the same species had a living population at

37° C. On the basis of these two limited examples it

appeared that the populations were living about two to

three degrees below their upper short-term thermal limits.

Actually, the populations in Cuayanilla were sometimes ex-

posed to temperatures up to 2° C, above the reported mean

temperatures. This means that even the most sensitive life

stages of these species were able to take these short-term

heat maxima. Fast immigration of larval forms that

depleted populations in the cove during the winter months  
Showed that the reproduction and the larval stages were not  
adversely affected by temperatures below 34°C.

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APPENDIX B

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## INVESTIGATIONS OF PRIMARY PRODUCTION

The few preliminary measurements made to date suggest that the biomass and production of phytoplankton in Jones Bay are substantial. Nutrient level measurements are consistent with high productivity where light intensity is adequate in the upper water column and on shallow bottom containing sea grasses and benthic algae. The magnitude of primary production in the major compartments of the system is of interest both for estimating the total primary production of the Bay and for establishing a baseline productivity data for comparison with later values in the altered system.

Major sources of primary production are (1) phytoplankton in the water column, (2) rooted sea grasses (primarily

Thalassia) and their algal epiphytes, (3) benthic algae,

allochthonous material entering the Bay waters, primarily from mangrove areas. The mangrove studies in Progress should supply information to estimate the input from the trees. The input of allochthonous organic material (other than sugar mill wastes) from shore run-off may be minor, since there are no permanent streams. There are inputs, especially to the Central Bay and ship Channel, from the ocean. Except for water entering the Bay through the Boca del Infierno and between the outer cayos, these inputs are at depth and may thus be less significant to the total productivity. Coastal marine values of biomass and productivity (e.g. Longgraves et al., 1970; Rurkholder et al., 1967) may be useful for estimating this component of input. If water transport into the Bay from the Southeast is quantitatively significant, measurement of productivity on the fringing reefs and shallow grass beds of the cayos may be indicated.

Studies in similar shallow marine situations (e.g. Pomeroy, 1960) suggest that where depths are greater than about 2 m, the bulk of primary production is by phytoplankton, even where ambient light levels at the bottom are higher than

those that occur in parts of the Bay. Although much of the Inner Bay is shallower than 2m (Tir. 1), the low ambient light level at the bottom (< 2% of surface intensity at 1 m depth in many places) and the scarcity of *Thalassia* deeper than 1m suggest that benthic productivity may be low

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much of the Inner Bay, in view of this and of the limited exchange with ocean water (probably lower in productivity), an estimate of production in the Inner Bay based on local phytoplankton alone may be sufficiently accurate.

The sampling program should undertake productivity measurements at a few locations within the Inner Bay near the surface and a set of measurements at one or more locations to get a vertical profile of productivity vs. light and depth (App. 1). The vertical profile data could be extrapolated to much of the Inner Bay, and the produc~

tivity vs. light data could be used to estimate sub-surface productivity in other areas, based on light measurements and surface productivity measurements. Productivity for the entire water column could be obtained by integrating the productivity vs. depth curves so obtained (e.g. Burkholder et al., 1967).

It seems unlikely that the small area of sea grasses in the Inner Bay (Fig. 2) could contribute significantly to the total production. These beds are not expected to experience heating due to power plant effluent. If grass distribution and abundance change significantly due to power plant operation (e.g. because of reduced turbidity in the Inner Bay), repeated surveys of the type already conducted (see "Ecology of Turtlegrass . . . ?" section, this Report) should detect the changes.

The distribution of significant quantities of macroalgae



in the Bay is poorly known. The benthic microalgae have not been studied, but they may be presumed to occur wherever light levels are adequate. Their productivity is significant in some similar shallow marine situations (e.g. Pomeroy, 1989, 1960; Jones, 1968). It is difficult to assess their importance in the extensive shallows of the Inner Bay (< 1m depth). Information about the compensation depth of phytoplankton from the vertical profile work discussed above should be helpful in estimating their distribution. In addition, some productivity measurements should be made of bare mud bottom in situ. Macroalgae, if present, would be included in the measurements (App. 2).

In both the Central Bay and the Ship Channel, shallows make a much smaller fraction of the total area than in the

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Inner Bay (Fig. 1). Although the water is less turbid, probably 1M or less of the area is shallow enough for significant benthic production (Fig. 2). There are, however, a number of acres of *Thalassia* present, which should

be unaffected by power plant operations and which might provide a useful control site. Moreover, much of shallow area occurring in the Central Bay does contain *Thalassia*.

A station should be sampled for primary productivity and leaf growth (App. 3). Probably either station 6 or 7

(see Fig. 2) should be used so as to permit correlation with existing and planned biomass data. A few measurements of bare bottom area at successively greater depths near

the same station should be made to estimate benthic algal productivity (App. 2).

In the Ship Channel, the bottom area divides rather abruptly

into a major area that is much too deep for significant

benthic production and a much smaller area of shallows,

most of which has a considerable cover of *Thalassia* (Fig. 1).

Only in parts of the Bahia de Cayo Puerea and Var Vepro are there extensive shallows without Thalassia. Much of the area will experience some temperature elevation from power plant operation, and major changes in currents will occur locally. Phytoplankton productivity should be measured in a few locations in the open Channel and in the Bahia de Cayo Puerca/Mar Negro area (App. 1). Productivity and leaf growth of Thalassia should be measured (App. 3) in Thalassia beds on both sides of the Channel, e.g., stations N and S (Fig. 2). In at least one case, an adjacent bare bottom area should be measured (App. 2). A bare bottom area in the Bahia de Cayo Puerca/Mar Negro area should also be measured.

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APPENDIX I

## MEASUREMENT OF BIOMASS

### AND PRODUCTIVITY OF PHYTOPLANKTON

Phytoplankton biomass would be measured directly by weighing the quantity of cells filtered from a large volume of water. It can be estimated indirectly from measurement of chlorophyll a extracted from cells filtered from the water. | The measurement of chlorophyll a also provides a measurement of "photosynthetic potential" from which, at known light intensity, productivity and efficiency can be estimated. Productivity would also be measured by changes in dissolved oxygen caused by photosynthesis and respiration. Light and dark bottles would be suspended in the Bay at various depths, and oxygen changes would be determined initially by Winkler analysis. After development of the polarographic "oxygen electrode" equipment (see Appendix 2), it may be feasible and preferable to adapt this equipment to plankton productivity measurements also.

The following steps would be involved in making the above measurements, using the Winkler analysis for oxygen. Initially, "step I-A" would be repeated successively at a Station throughout the daylight hours (e.g. once every

Zhours). From these results, a method would be derived for estimating daily production from a single measurement (e.g. Doty et al., 1967).

## 1. Sampling Plan

A. Take a sample of about 1 gallon from just below

the surface (avoiding the surface film) at each of the specified stations. Use a clean glass or

| For further details and discussion of analytical procedures, see Strickland and Parsons (1968),

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plastic container. Measure surface irradiance and irradiance at the depth of the center of the sample.

Measure temperature and oxygen concentration of the Bay water using the surface oxygen meter.

Measure salinity using the AO Refraction Salinometer.

Fill a clean 2-1/2 gallon cubitainer with water from just below the surface.?

At specified "Profile" stations, take a sample as in A? (above) and an identical sample at each 1/2 meter depth to within a few centimeters of the bottom.

Treat samples immediately in the boat as follows:

A. Pour the 1 gallon surface sample through a piece of 0.3 mm clean nylon netting. Deoxygenate this filtered sample per detailed instructions. Fill 4 clear 300 ml BOD bottles and 2 opaque 300 ml POD bottles completely full with the filtered sample water. (Insert stoppers if necessary while handling).



Label 2 clear bottles "IB", To each, add 1.0 ml of manganous sulphate by putting the tip of the automatic pipette just below the surface of the water in the bottle. Then add 1.0 ml of alkaline iodide solution in the same way, using its own automatic Pipette. Stopper the bottles tightly immediately without including air space, and shake the bottles

a

Initially an additional sample or two would be taken within a few tens of meters of the location to evaluate Patchiness of phytoplankton.

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thoroughly until the precipitate is evenly dispersed. Store the bottles out of the sun (do not Refrigerate). After a few minutes, shake then again and return then to storage.

Immediately stopper the other 2 clear bottles

after filling, without including air space, and label "LAY. fo the same with the 2 opaque bottles and label them "DR". Wire stoppers on tight using Stainless steel or chrome-nickel wire and put all LB and DR bottles back at the depth from which they were taken (use an anchor weight and a float just sufficient to keep the bottle up). Note the time the ROD hotties are put back in the Ray-

After the specified time, X, recover the LB and

DR bottles from the Ray, remove stoppers and add manganous sulphate and alkaline iodide in the same way as for the IB hotties. Shake well twice and store with the IB bottles. Record incubation time,

Ne

D. To the remainder of the 1 gal. surface sample add about § drops of well-shaken magnesium carbonat Suspension using an eye dropper, label the con= tainer "Pigments", shake well and store in the dark on ice.

III, At the shore base, within several hours, treat samples as follows:

A, Store IB, LB and DR hottles out of the sun (do not refrigerate).

B. Pour the water from the 2-1/2 gallon cubitainer through a piece of 0.3 mm clean nylon netting.

Measure and record the amount of water filtered.

Filter this water again through a 47 mm diameter,

0.45u Millipore filter. As soon as all water has

been filtered}, sprinkle a few drops of lit! on the

3

If phytoplankton are sufficiently numerous, an adequate sample for weighing may be obtained without filtering the full volume of water.

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filter paper.4 Drain the filter thoroughly dry under suction, Weigh and record the weight of the paper with phytoplankton.

C. Filter the "Pigments" sample through a 47 mm diameter, 0.45 (HA) Millipore filter. Record the quantity of water filtered,  $V_f$ . Shake vigorously in the hottle before filtering. Drain the filter paper thoroughly dry under suction, remove it from the filtration equipment, and trim away as much excess paper around the periphery as possible with clean scissors. keep only the part containing plankton.

Place the filter paper in a 50 ml stoppered graduated centrifuge tube. Add approximately 40 ml of 90% acetone using a polyethylene wash hottle, stopper the tube, and dissolve the Filterpaper by shaking the tube vigorously. Place the tube under refrigeration in complete darkness. ?shake the tube once more after about Tto 2 hours under refriperation.

IV, At the Nuclear Center laboratory, treat the samples as follows:

A. Within a few days after collection of the samples, perform the following analysis on all IR, DB and TR hoteles:

Take bottle from storage without shaking and remove stopper. Add 1.0 ml of concentrated (specific gravity 1.84) sulphuric acid to

the sample, placing the tip of the pipette just below the surface of the water. Re-

Stopper and shake thoroughly until the precipitate dissolves fully. Do not permit

air to become trapped in the bottle. Keep out of direct sunlight.

A few tests will be run initially weighing papers before and after adding HCl to get an estimate of Carbonate sediment in the samples. A tare weight for filter papers will also be established.

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Within a few hours, transfer 50.0 ml of the solution into a painted conical flask using a volumetric pipette. Titrate at once with standard 0.01 N thiosulphate solution until a very pale straw color remains. Add 5 ml of starch indicator and finish the titration, adding thiosulphate carefully until the blue solution just clears.

Use this first "end point"; do not wait for the solution to color again. Record the number of ml of thiosulphate solution used. If a blank value has been supplied when the reagents were prepared, subtract it from this number of ml. The result is called  $V_1$ , i.e.  $V_1 - V_{pg}$  of  $V_{ig}$ . Obtain

the value of  $x$  supplied with the reagents.

?

fs

Calculate the dissolved oxygen,  $O_2$ , in the bottle

and recor

$O = 1,6096 \times x_v$

where  $O$  is in mg  $O_2$ /liter

Calculate the productivity values for the sample

as follows, and record:

Gross photosynthesis, mg carbon/m<sup>2</sup> - hr =

$\frac{O - O_0}{t} \times V$

Net

Net photosynthesis, mg carbon/m<sup>2</sup> - hr =

$\frac{O - O_0}{t} \times V - \frac{O_2 - O_0}{t} \times V$

Nx 1.2

S Manganous sulfate and altatine iodide.

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Respiration, mg carbon/m<sup>3</sup> - hr =

$\frac{V_{O_2} \times N}{V_{O_2} - V_{O_2}}$

N

C. At least 20 hours but not more than 2 days after filtering from the water, analyze the "Pigments" samples as follows:

Remove centrifuge tubes from refrigeration and let them warm to room temperature. Add 90% acetone using a polyethylene wash bottle to make the liquid up to exactly 40.0 ml.

Replace the glass tube stoppers with plastic stoppers and centrifuge for about 10 min. at about 3000 - 4000 rpm.

Pour the clear liquid from the tube carefully to fill a Beckman DU spectrophotometer sample cell having a path length of 10 cm and a volume of about 30 ml. Immediately measure the extinction,  $T$ , of the solution in the



spectrophotometer against a reference cell  
 Filled with 90% acetone only. Measure at  
 wave lengths: 7500, 6650, 6450, 6300 and  
 4800 A. Record values to the nearest 0.001  
 unit in the range 0 - 9.4 and to the nearest  
 0.005 unit for extinctions greater than about  
 0.4. Then fill both cells with 90% acetone  
 (Sample cell and reference cell) and measure  
 extinction at each of the above wave lengths.  
 This gives a cell-to-cell blank correction,  
 $E_{6650}$ . Calculate the corrected extinctions at  
 each wave length as follows:

$$E_{6650} * B_{6650} (\text{as measured}) - E_{7500} (\text{as measured})$$

+k

$$= E_{6650}$$

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$$E_{6450} * F_{6450} (\text{as measured}) - E_{7500} (\text{as measured})$$

+ Bee gaso

$T6300 = F6300 \text{ (as measured)} - F759 \text{ (as measured)}$

+ Fecgsoo

$E4g00 * E4g00 \text{ (as measured)} - 3 * E590 \text{ (as measured)}$

+ Feegano

The sign for Ecc depends on the relative readings for the 2 cells when they were compared with both containing 90% acetone. Calculate the concentration ratios, C, of chlorophyll a as follows:

$1.6450 - 0.14 F6300$

$V_w$

where  $V_y$  is the volume, in liters, of water passed through the Millipore filter.  $\phi$  is in mg pigment/nd water.

Or get C value from nomograph supplied, using the corrected F values.

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## APPENDIX 2

### MEASUREMENT OF PRODUCTIVITY OF BENTHIC ALGAE

The details of this method are yet to be developed. It is planned to enclose an area of bottom mud and a small amount of overlying water in situ by placing the open side of a clear or opaque plastic chamber on the bottom. (A clear chamber would be used for measurement of net photosynthesis; an opaque chamber would measure respiration). It is desirable to provide stirring, e.g. by including a magnetic stirrer driven by battery power or compressed air. Plastic bags can be used as disposable test chambers to seal on very uneven bottoms.

An initial oxygen concentration measurement would be made immediately and one or more made subsequently after sufficient "incubation" time in situ to produce changes in oxygen sufficient for productivity computations. Initially the oxygen would be monitored frequently and tests run sequentially throughout most of the daylight hours. From these results, a method would be devised for estimating daily production from a single measurement (e.g. Doty et al., 1967). A light intensity measurement would be made with each oxygen measurement.

Oxygen could be measured by any method available, but the use of a polarographic oxygen electrode is much preferred for ease of frequent (or continuous) monitoring, minimum contamination (dilution) of samples and accuracy unaffected by oxygen saturation of water. The polarographic techniques and submersible equipment of Wells, (Cog, Welie and Weriss 1971) appear applicable almost unchanged, and equipment of this sort would be assembled for the purposes

The routine test procedure would involve (1) placing a clear and an opaque chamber on an appropriate bottom area with minimum disturbance of sediments, (2) connecting the stirrer power supply (battery or a/c), (3) taking an initial dissolved oxygen reading for each chamber, (4) taking a submerged and a surface irradiance reading, (5) marking the location, and (6) returning after the proper interval to take further readings. The oxygen electrode calibration should be checked occasionally (e.g. in water -

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saturated air). A calibration sheet would be used to convert current readings to oxygen concentrations.

Gross photosynthesis, net photosynthesis and respiration would be computed from oxygen concentration measurements in the same general manner as in Appendix 1, section IV.A. The productivity estimates so obtained would include the effects of the phytoplankton in the volume of water enclosed within the chamber. Values for phytoplankton productivity measured at the same light intensity either concurrently or at some similar station would be used, together with the known volume of water enclosed, to correct the chamber measurements so as to yield benthic productivity values.

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Doty, M. S., J. Newhouse and R. T. Tsuda, 1967. Daily Phytoplankton primary productivity relative to hourly rates. In: Improvement and Application of Benthic Algal <sup>14</sup>C Isotope Productivity Measuring Methods. Hawaii Botanical Science Paper No. 3. University of Hawaii.

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### APPENDIX 3

#### MEASUREMENT OF PRODUCTIVITY OF SEA GRASSES

The method for measurement of productivity of the entire plant is basically the same as that used for measurement

of benthic algal productivity (Appendix 2). The same or very similar equipment would be used. Bottom chambers would be sized so as to contain the height of sea grass leaves without excessive crowding and to cover an area containing a number of "short-shoots" (clumps of green leaves) in a moderately thick growth of *Thalassia*. Measurements of oxygen and light intensity would be taken in the same way. The measured oxygen concentrations would be converted to productivity and respiration values in the same way for the enclosed system. Phytoplankton productivity would be sub-

tracted out in the same way to give a total benthic productivity value, For sparse growths of *Thalassia*, benthic algal productivity and respiration should also be subtracted out to obtain estimates of values for *Thalassia* alone

(see Jones, 1968). Benthic algal productivity per unit area from an adjacent parcel of bare bottom, measured as

in Appendix 2, would be used, together with the area of

the chamber, to compute the correction. For dense growth of *Thalassia*, the shading of the bottom is probably such that the benthic algal correction would be negligible

(see Pomeroy, 1960). In any case, for estimation of primary productivity of the bay, it is the total benthic value-benthic algae plus sea grasses, as measured-that is of major interest, Although the productivity of algae epiphytic on *Thalassia* may be considerable (Jones, 1968), for purposes of this study, it seems unnecessary to separate this component from the productivity of the *Thalassia* plants.



Much of the photosynthetic production of *Thalassia* goes into growth of roots, rhizomes and the basal portion of short-shoots, which cannot be conveniently marked and subsequently harvested to measure growth. However, a substantial fraction of net photosynthesis results in growth of visible green leaf blades (Jones, 1968). Measurement

of growth rate of these leaves gives an estimate of productivity that is at least useful for comparative purposes. It provides some check on the estimate obtained by oxygen measurement. The leaves constitute the portion of the plant

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which has most rapid turnover and is passed along the food chain most quickly and conspicuously (Wood et al., 1967).

Measurement of green leaves gives information on the amount of plant material available to participate in photosynthesis.

Measurement of leaf growth would be included in future ecological *Thalassia* studies. The present sampling procedures include biomass measurements of leaves and other plant parts (see "Ecology of Turtle grass...?" section, this report). Additional information to be recorded would include number of green leaves, width of blade and length of blade, since the work of Thor haug and Stearns (unpublished manuscript) suggests that elevated water temperatures produce different effects on these different plant characteristics. AS a means of monitoring effects of elevated temperatures and seasonal effects, at some stations, semi-permanent quadrats should be defined, and a blade count should be made at each biomass sampling occasion.

In addition to the biomass measurements, leaf growth should be measured in the field by marking a group of leaves at the Station and subsequently harvesting the leaves to measure the elongation. A marking method similar to that of Zienan (1970) could be used (also see "Studies of the Effect of Heated Water on Turtle Grass ..." section, this report).

Leaves should be marked initially at the point of transition from sheath to blade. Feman's results (Feman, 1970, Fig.3) could be used to estimate total leaf elongation from elongation at the blade base. The sum of this new material plus the total length of any new leaves produced comprises the

growth for the time interval. It would be convenient to mark a group of leaves in this way at each Station sampling Occasion and harvest all or a portion of these leaves as a routine biomass sample at the next occasion. The length, width and weight of new leaf growth would then be taken Along with present biomass data. Leaves should be cleaned of epiphytes before weighing by washing in dilute acid in order to produce accurate and meaningful *Thalassia* biomass values.

The blade density and blade growth data would be especially effective for simple estimation of production in many areas having widely different grass coverage. The work of Thorhaug and Stearns (unpublished manuscript) indicates very similar growth per blade over a wide range of blade densities in Card Sound and good agreement between increase in blade area and increase in blade weight.

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marine turtle grass, *Thalassia testudinum* König, and  
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PHYSICAL OCEANOGRAPHIC

METHODS

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CURRENT STUDIES

by

E. D. woop

1, Meters - Hydro products Model 503

Calibrated in a swimming pool, the neters were

Suspended by floats and towed?the length of the pool at constant speed. Tows up and down the Length of the pool were averaged for velocity calculations. The pools circulating punps were off for several hours before and during calibra tion. Calibration was done at velocities of: +2, 15, and .8kt. The velocities shown by the ters'were a11 lower than measured and the corr: tion factors determined were

Meter # Factor

S

2

9

?The meters are checked periodically in the elec- tronics shop; and adjustments to the read-out circuitry made as required.

Current meter placenent.

The current meters have been installed by two different methods.

(4) Individually anchored meter installation is Sed where the meters are to be in different locations. The moter is attached to a weight (lead or cement) by a line measured to give the desired depth for the sensors, A Danforth anchor is attached to the weight by a 3m section of chain. The meter is suspended by a float which displaces about 20 liters. The float depth is adjusted so that it is about one meter below the surface. This puts the system out of sight of those who may molest, the equipment and deep enough so that it will not interfere with small craft operation. The meters are located by triangulation sites on shore or occassionally marked with a small bouy anchored separately.

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(ii) Group anchored meters are suspended from a path

(ii)

platform, either a raft, buoy, or ship. Surface meters are tethered and suspended by floats and actually measure currents at a depth of about 0.5 m rather than 0 m. The other meters are suspended from the platform in such a fashion as not to interfere with one another. Usual depths for our measurements have been 0 (0.18), 2, 4, 6 m measured from the sea surface to a point mid-way between the Savonius rotor and the direction vane of the meter.

The platform is usually secured with two anchors to reduce swinging caused by current meandering. Most measurements are for at

least 24 hours to take into account tidal and wind effects. The current meters are periodic-



ally checked by dives to insure that they are functioning properly.

« Interpretation of the data.

The current data is recorded by a Rustrak

recorder on pressure sensitive paper tape.

The settings are such that the paper moves approximately 2.5 cm hr<sup>-1</sup>. The exact rate is determined on individual recordings from the "in" and "out" times marked on the tapes.

The data are transcribed onto graph paper manually with meter error correcting and some smoothing.

The velocities and directions for each meter are averaged over convenient time periods and correlated usually with tide and wind data.

When calculating the flow through a channel, the cross-section is determined either from the charts or by direct measurement. The

cross-section is then divided into appropriate  
Sub-sections to correspond to the meter,  $\phi.8.$ ,  
if current meter depths are 0, 2, 4, & 6m  
then the depths of the sections are 0-1, 1-3,  
5-5, and S-bottom (in meters). From this  
information, flow can be determined by summing  
average vector velocity through the sub-section  
on a daily basis or on an hourly basis. Thus  
net flow can be determined as well as total  
flow in each direction. The data is usually  
reported as the average flow rate

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Drogue studies.

Drogues have been used to determine flow over @  
distance. The drogues were made of crossed sheets  
of plywood in some cases and parachutes in others.  
The main part of the drogue was placed at varying  
depths. A pole was mounted on the float of the  
drogue and equipped with a radar reflector for track-  
ing. The drogues were tested both with and without

structures above the water to determine the effect of wind drag. It was found to be on the order of 20% for a 12 mph wind and all drogue velocities were corrected accordingly.

## Dye Studies

Pe Spot Feieases of rhodamine-B dye are used to

etermine rates of flow over a distance and

degree of mixing. The dye is dissolved in acetic

acid and mixed with fresh water in the ratio of

about 75 g/l. The resultant mixture is nearly

the same density as sea water. A study usually

entails 8-12 releases of 10 liters each either

from a small boat or dropped from an airplane.

The dye spots are then photographed over a period

of several hours to delineate the surface current

patterns of a particular region. Releases are

Repeated for various sets of conditions. Since

the photography is limited to daylight hours,

a release is made early in the day to measure

the effects of low wind velocity usually exper-

enced during the night. Late afternoon releases

then give results for windy conditions. This

usually takes the tides into account as Puerto

Rico has essentially one tide a day.

Photography is often complicated by poor Light, low clouds, or lack of landmarks. These problems have been partially solved by using high speed film, taking angle shots normal to the shore-line?and placing float markers for reference points.

Dye studies are correlated with current meter measurements when practical.

Continuous Dye Releases.

Soluble rhodomine-B was released into the study region from an anchored raft at the rate of

7 kg/day for a three day period. The continuous dye release allowed visual and instrumental tracking of the surface currents of @ period of

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(3d) time. The dye plume was photographed from the air at three different times a day. Transects for the dye plume were made to map the extent surface spreading of the plume. Equipment was loaded in a 17' boat equipped with a portable generator. The concentration of dye in the plume was measured with a fluorometer equipped with a "flow-thru" cell. The water was pumped through a tube 3 in. long at a fast rate. The cell was fed through by-pass which gave nearly an instantaneous response to the water near the boat. The depth of the intake could be adjusted

from surface to 3m,

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## COLLECTION AND PREPARATIVE METHODS

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## COLLECTING METHODS FOR FISHES

F. D, MARTIN

fishes are collected for our studies by a number of methods including, gill nets, seines, cast nets, dip nets, hand Tines, £2h toxins, Spear guns and traps. The methods most commonly used were gill nets, seines and fish traps. Each method has its special uses and limitations and outlines of these three methods will be given here.

Gill nets are nets which depend on fish trying to force their way through the mesh of the net and becoming entangled therein by their gill coverings and/or fins. The nets we use are constructed of monofilament nylon which is virtually invisible in water. A lead line is used to hold the bottom down and a float line holds the net vertical, Normally we wish the net to remain stationary so that it does not tangle up or become lost while left in place. To accomplish this,

5 pound lead weights are attached to the lead lines at each end of the net. If currents in the area are strong,

additional weights are attached as needed. Marker buoys

are attached to the float lines at both ends of the

net by enough heavy cord (200 lb. test minimum) so that

the buoy floats with some slack in the line. Since the nets

we have are rigged to be set on the bottom, in the event that

floating nets are desired, empty plastic jugs (1/2 gallon or

larger) are attached every 8-10 meters along the float line

as the net is played out.

When setting nets near shorelines, we normally set the net

perpendicular to the shoreline because, on the average, fish

move more often parallel to the shoreline than to or from

it. Occasionally it is desirable to attach the net to an

object on shore. In this event, attach only the float line

go that the lead line can have enough slack to remain on the bottom.

The gill nets we use are normally stored in plastic garbage cans and when being set are fed directly from these over the side of the boat, In order to facilitate this, the net is usually fed over the bow or one of the sides well away from the motor and any cleats or other potential snags. As it is fed out, any tangles or kinks in the lines are

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straightened out as these are potential tearing or breaking points when pressure is put on the net to pull it in.

Normally the net is set in a straight line and with enough slack to keep the net from being pulled tight. Tight nets do not entangle fish as effectively as loose nets.

If wind direction allows it is generally easiest to feed the net out at about the speed the wind drifts the boat and to have the motor off, (Remember: gill nets do bad things



to outboard motors when wrapped around a prop and vice versa).

Otherwise one or two people play the net out while another backs the boat in the direction the gill net should run and at a very slow speed.

The net is then left for a period of time (never less than 30 minutes) and is then pulled back in starting from the down wind end or the end away from shore. All attachments are removed as the net is pulled over the side and the net is fed directly back into its garbage can. Fish are removed as they are pulled over the side and are placed in properly labeled containers. Techniques of removal depend on the fish, the mesh size of the net and degree of entanglement.

Experience is the only effective teacher in this area.

Gill nets are seldom left more than 2-4 hours because:

1.) of the danger of it drifting and becoming fouled on coral heads or other snags; 2.) crabs congregate and dine on the trapped fish thus eliminating much of your catch; and 3.) untended nets are temptations to persons who chance upon them.

Nets are rinsed with fresh water as soon as possible upon return to PRNC and then dried with as little exposure to direct sunlight as possible. Sunlight breaks down nylon and must be avoided as much as possible.

Gill nets are ineffective in catching eels of any kind and seldom catch territorial fish as they do not normally move enough to take them in the area of the net. They are quite selective in size, as fish below a certain size go through the mesh and do not become entangled.

Another method of fish capture is the seine. This is a flat net which is pulled through the water (some are not flat but have bags in the center, but these are not used here), The net is equipped with a lead line to keep the bottom down, a

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float line to keep the top on the surface and poles at each end to make pulling both lines at the same time possible.

The net is pulled by two or more people through the water and then either pulled up on the beach or lifted by getting the lead line well in advance of the float line and picking it up so that the fish are picked up on the surface of the net. The most common reasons for ineffectiveness are

1.) failure to keep the lead line on the bottom; 2.) snagging the net so that progress stops while the net is undone

3.) allowing the net to roll up while being pulled through grassy areas and, 4.) persons walking in front of the net and frightening the fish away before the net gets there.

Seines are ineffective in collecting: 1.) fish which are strong swimmers and can move faster than the seine; 2.) fish which bury in the bottom or hide among snags when

jumping fish which can go over the net and can force their way through the mesh (e.g. *herring* and needlefish)

The other common method used in collecting fish is fish toxins. The toxin we use is Pro-Noxfisi® which is a solution of rotenone in xylene with emulsifiers and synergists. The toxic effect is upon the gills of the fish so that they come to the surface for oxygen where they can be dipped up with long handled dip nets. When we do a poison station we are interested in establishing the biomass of fish for a given surface area. For this reason we fence off an area

th a fine meshed net before introducing the toxin. If the Station is to be near a convenient shore, we use the shore as one side of the fenced in area. We use a 100 foot net as the fence and if the station is using a shore as one Side, two poles are driven into the bottom 33 feet from the shore and 33 feet apart and the net is pulled around then to make a 33" by 33' square. Otherwise four poles are driven into the bottom so as to make a 25' x 25" square and the net is pulled around then. Also before introducing the poison, the lead line of the net is checked to make sure it is snug against the bottom, About 300 ml of the toxin are poured into a 2 gallon bucket containing 1 1/2 gallons of sea water.

This is introduced into the enclosure in the following manner.

About 1/2 is put in on the upcurrent side so that it will wash through the whole enclosure and the rest is spread over the surface of the enclosure as evenly as is feasible, Then the poison is mixed by swimming or wading through the area!

(Note: If you get any of the concentrated poison on your skin, rinse it off immediately; if you contact some of the diluted toxin while swimming in the area, it will not do any

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permanent damage but may irritate your eyes or produce  
nausea if ingested), Within 5-10 minutes, fish should  
Start surfacing and should be dipped up immediately as they  
will not stay on the surface once they die. If no fish  
Come up within 10 minutes, a second treatment using 500 -  
700 ml of concentrate is applied in the same manner as the  
first dose. In any case, when action slacks off (after 30-  
45 minutes) another dose similar to the first effective dose  
should be applied.

For obvious reasons fish from the enclosure must be kept  
separate from any fish captured outside the enclosure. Fish  
Seen to enter the enclosure from the outside are to be  
Counted as off station fish and the reverse situation will be  
Counted as on station fish. If you are not sure where it  
came from, it is an off station fish.

Collecting is terminated when fish are no longer coming up  
regularly and periods of 4 or 5 minutes occur between  
catches. The procedure at this point is to pull up all  
corner poles and to purse the fencing net by pulling lead  
Lines together so that the enclosure has a bottom of net.  
This is done by dragging the net along the bottom - the lead  
Line should never be lifted from the bottom. The easiest

way to do this is by pulling the net slowly onto the shore or into the boat by the lead line, The fishes which died and did not come to the surface will be picked up in this way.

Poison stations are less selective than most but, certain Species are not taken by this method. Strong winners Hequently eave the area during the setting up process and for'this feason the set up should be accomplished as quick) and with as Little wading? and splashing as possible and wid So wading in the area to'be enclosed until after the fencing Bperation ig complete. Many anali fishes which Live in crevices ani among the plants die in place and are not seen. The other group not. taken by toxins are the sharks and rays. They are net sensitive to this toxin and on occassion sharks cone, Into the general. area to feed on the incapacitated fish. Sarthe event that. this happens (and ehis partically never happens) get out of the waver with all due haste but with as Tittie spfasning a5 possible.

?The other methods of collecting are methods widely known and I will not discuss them here,

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?The one remaining point of discussion is the criteria for deciding on sampling areas. First priority is placed on establishing base lines for areas which should be directly effected by whatever is to happen in the areas. In the case of Jobs this is the intake area, the outfall area and the areas down current from the outfall which could receive effects directly from the thermal plume or which might be exposed to chemical effluents. A few areas which should receive no effects are sampled regularly as control stations.

Within each major area under consideration, subareas are established based on habitat type (mangrove root areas, turtle grass beds, open bottom areas, etc.) and each area is sampled by means appropriate for the habitat and at intervals so that seasonal variations can be established.



Normally the general area and the habitat types to be sampled are predetermined, but the suitability of a specific site for sampling on any given day is determined by the sampling technique to be used and conditions of tide, wind, etc. Seine stations cannot be deeper than 1 to 1 1/3 meters and must be relatively free of snags. Poison stations cannot be deeper than about 2 1/2 meters if they are to be quantitative and if they are deeper than about, 1 meter, the visibility should be such that the bottom can be seen easily, Fast currents or heavy wave action limit the effectiveness of poison stations. Beyond this, there are no simple guidelines and experience is the only teacher.

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## THE THALASSIA BEDS

Sampling Methods:

The sampling biomass of plants and benthic invertebrates was done with a special core sampler that was designed by ME Peter Schroeder. This sampler consists of a stainless steel pipe (16.1 cm diameter) with a crank. (Figure 1 in the Section on *Thalassia bede* in Guayanilla Bay). It samples an area of 20 cm<sup>2</sup>. The upper end of the pipe is covered with a screen of 0.8 mm mesh to prevent free moving animals from escaping. A rubber flap seals the upper end and prevents the core from dropping off when the sampler is lifted from the bottom. This sampler has proved to be very fast and quantitative both for the plants and the invertebrates. Two cores were sampled at six stations at a depth between 0.5 to 1 meter.

Plants and animals were separated from sediments in a standard set of geological sieves. *Thalassia* was separated to six different parts: old leaves, new leaves, sheathing, rhizomes, vertical shoots and roots (see Tomlinson and Vargo, 1966). *Thalassia* leaves were weighed after cleaning off all the sediments and filamentous algae. No effort was made to remove attached microorganisms from old leaves. These microorganisms include: diatoms, filamentous algae, foraminiferans and small polychaetes dwelling in calcareous tubes. Their biomass is only a fraction of one percent of the biomass of *Thalassia* and usually proportional to the biomass of old leaves. The

wet weight of plants and invertebrates and the number of specimens of different invertebrates were recorded. Dry weight was measured only for the biomass of plants.

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THALASSIA SAMPLER

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CORAL REEFS

erials and Methods

Sampling methods were geared to collect descriptive and quantitative data. Three methods were used, all involving the use of scuba diving:

**Transects:** A nylon line marked at 1 m intervals was used to make transects extending from as close to the reef flat as safe swimming allowed, to the beginning of the reef fore-slope. The transect usually covered a distance of 75 to 125 meters. Data from the first few transects were recorded on plexiglass plates, on which all corals and major cover organisms (such as sponges and gorgonians) along each meter interval were noted and recorded. Later transects were recorded photographically, but only every third meter was photographed. The transect data was used to describe the reef zonation and structure and to determine species dominance among the main cover organisms.

**Measured Quadrats:** Two meter by two meter areas were marked off with heavy stakes and nylon lines

or with a metal rod frame. The plots were then divided into 1/4 m<sup>2</sup> grids, The areas covered by each species of coral and other important organisms were recorded on a plexiglass plate or on film. The resulting diagrams or photographs were used to calculate percentages of bottom coverage, and dominance and species diversity among the major encrusting organisms.

Collected Quadrats: 1/4 m<sup>2</sup> areas of the above quadrats were collected in their entirety. Crow-bars and knives were used to remove sections of the reef rock which contained encrusting and boring organisms. The samples were immediately placed into large plastic bags, held next to the collecting site by a second diver, in order to

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ensure that none of the small free-living organisms such as crabs and brittle stars could escape. The Samples were stored in plastic buckets and refrigerated on their way back to the laboratory where they were sorted into phylogenetic groups, weighed and preserved for later identification. Corals were weighed and their surface area estimated. Pieces of pre-weighed and measured coral were dried at 110°C., reweighed, subjected to Clorox digestion, dried and weighed again to estimate the amount of living animal and plant material present. The pieces of coral rubble which remained after most of the encrusting and boring organisms had been removed were similarly treated to estimate the amount of non-removable material (some bryozoans, boring sponges, calcareous algae, and boring worms) still remaining. A factor was calculated from this and used to calculate the amount of organics in the total rubble sample. This total is listed in the biomass estimates as "miscellaneous."

In the case of station 10, three clumps of material were collected and labeled samples 10A, 10B and

10C. The narrowness and relief of the reef there made it very difficult to collect quadrat samples. for this reason, no transect data was collected for this area.

Total biomass estimates were derived by tracing the area covered by coral reefs from a standard WOSS map, cutting out and weighing the tracings and multiplying the estimated area by the average of the biomass estimates computed from the collected quadrats data.

Only the corals, gorgonians, mollusks, crustaceans and echinoderms were classified to genus and

species level. The classification of the sponges, annelids (present in very large numbers), sipunculids and other worm-like organisms was not attempted. I wish to acknowledge and thank Mr. Carlos Carrera, Department of Marine Sciences, University of

Puerto Rico for his help in classifying the brittle stars.

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## PREPARATION OF CUBITAINERS

Jaw #1 Label everything with date and station number.

1.

2

3.

4

Rinse with tap water.

Rinse with 4 N Nitric acid.

Rinse with distilled water.

Put on cap and put @ piece of masking tape labeled

"Prep." on the cubiteiner so it won't be confused

with dirty ones,

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COLLMOTION OF Sua Wa!

On station rinse the cubiteiner with @ little of the  
sea water you will be sampling.

F221 cubs teint

to avoid paint

current from the boat and motor

Bas, and oil.

Add 10 ml (cc) of concentrated HCL (hydrochloric acid).

Filter within 8 hours to get rid of bacteria and alges

If this can't be done add 1 ml chloroform and keep cool,

or freeze the whole thing. Save filter in plastic bag.

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## METHODS MANGROVE ROOTS

Sampling. and

?The mangrove root communities in Jobos Bay show enormous variations in the species composition and biomass. The reasons for this are by in large unknown, but it seems

like, salinity, temperature, food availability, wave

action, depth and pollution? are important factors controlling

?the mangrove root communities in Puerto Rico.

Eight sampling stations were selected to represent different types of mangrove root communities around the proposed

intake and discharge areas of the cooling water for the power plants. Station #1 was north of Cayo Puerca at the end of a cove that has received effluents from the

settling ponds of Central Aguirre (Figure 7). This area used to be badly polluted with dissolved organic compounds that utilized all oxygen from water. Part of the mangroves in this area were killed by the pollution and all of them showed signs of stress which could be seen from an unusually large number of aerial roots. Since spring 1972, this area has not been receiving waste from the sugar mill. Even months after the dumping of sugar mill effluents were stopped this area was anoxic on the bottom and producing HS and

was well saturated with oxygen and currents were moderate.

Sediments at this station were silt and fine sand. Water was shallow, between 0.3 and 0.5 meters. Temperatures up

to 31 °C were observed on the surface in the summer.

Station #7 was on the south side of Aguirre Navigational Channel at Cayos de Barca, This station was exposed to the wave action and the swells in the Navigational Channel. Water was well saturated with oxygen and clear. Summer temperatures up to 31 c were measured on the surface. Currents were noderate and flowing to the west. Water was shallow between 0.2 and 0.5 meters deep. Sedinents were

Station #3 was located on the east shore of Punta Colchionas inthe Midbay. This station was exposed to the wave action and swells, but it was somewhat protected by a wide shallow turtle grass bed in front of it. Water at this station

was light brown, well oxygenized. There was a weak southerly

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current passing by the station. At times of strong winds water was silty. Water temperatures rose up to 31-S-c in summer. Water at this station was 0.7 meters deep and the sediments mud.

Samples were collected twice. The first time in September, 1971 and the second time in July - August, 1972, The first collecting involved cutting a 30 cm section of ten roots

from the Roan sea level down- The species, genera or phyla  
were identified and the biomass of each of them measured.  
The results are given in the Aguirre Nuclear Plant Annual  
Report 1971 pp. 34-43. This study uncovered so many  
interesting aspects of the mangrove root communities that  
a more thorough investigation was undertaken.

In the second sampling whole mangrove roots were collected  
by cutting them at the mean high water level, which coincides  
with the uppermost sessile organisms on the roots. The  
roots were carefully surrounded with a 0.5 mm mesh net  
before lifting up. This way all the free swimming crusta-  
ceans and fish were also recovered. Three to six randomly  
chosen roots were collected at each station.

The roots were placed into large polyethylene bags and  
transported into the laboratory in an ice chest. In the  
laboratory the roots were cut to 10 cm sections from the  
upper tide level down. The organisms in each section were  
separated, identified and the wet weight of each species

was recorded. Because of the vertical zonation of organisms  
the biomass is given per root instead of per unit length

of root.

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## PREPARATION OF SAMPLES FOR ANALYSIS

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## PREPARATION OF BIOLOGICAL SAMPLES FOR AAS

Grind the dried sample to a fine powder.

Neigh out about 2 g sample in 125 ml erlennayer.

Add drop hy drop about 25 ml of aqui

regia.

Place on the hot plate, digest about 20 minutes at very low heat.

Bring to near dryness.

Dissolve with double distilled water (DDK).

Filter through pre-washed glass filters.

Dilute with MDW to 50 ml, Put in screw cap plastic bottles.

Pipette out three 17 ml aliquats into small plastic bottles.

Add proper standards of different concentrations of each element to each one. (Standard addition technique)

Run at AAS,

By plotting each sample on the graph, find out concentrations of the samples.

Report the result

milligram per gram,

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## BIOLOGICAL SAMPLE COLLECTION AND PREPARATION FOR TRACE ELEMENT, NUTRIENT AND CHLOROPHYLL ANALYSIS

Five or six organisms were collected at each sampling station. The species to be collected were selected: (1) to represent the various trophic levels (primary producers, herbivores and carnivores) and (2) because they made up a major part of the biomass.

collected from three habitats: the coral reef,



sia beds, and the mangrove root communities.

Immediately after Collecting, the samples were placed on ice until they could be frozen back in the laboratory. The following collecting procedure was used:

#### A. Coral Reef Samples:

Divers, using SCUBA equipment and, where possible, wearing plastic gloves, collected the chosen organisms and placed them in clean, unused plastic bags. Encrusting organisms were collected by hand, when possible, but more usually with the aid of a stainless steel knife or spatula. Care was taken to pour out all the sea water from the sample bags before the bags were closed and stored on ice.

#### B. Thalassia Bed Samples:

Organisms from this habitat were also collected and cleaned underwater. Cleaning involved removing sediment and extraneous encrusting material from the sample organisms. Once collected, samples were treated as

#### C. Mangrove Root Community Samples:

Organisms growing on the mangrove roots were collected

by hand or with the aid of a stainless steel knife in the case of the oysters, Sediment accumulated in the algae was washed out before the sample was collected. ?The mangrove and tree oyster were not cleaned of epiphytes since only the meat is used in the analysis. As before, sample bags were closed and stored on ice.

It was found that the chosen organisms were not equally present at all the sample stations. Substitutions were

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made where necessary, but emphasis was placed on trying to collect the same organisms at all stations of the same habitat type.

The Sample Preparation Procedure Is The Following:

The samples were defrosted, placed into acid-cleaned beakers, and weighed together with any fluids which may have been in the sample bag. Since all the sea water had been removed from the bags soon after collection, all fluids in the bags, after defrosting, were considered vital fluids which had been lost by the organisms during the freezing-thawing process. The beakers were then covered and dried to constant weight at 110° C. The dried samples were ground to a fine powder with a porcelain mortar and pestle and reweighed. Two grams subsamples were dissolved for trace element analysis and one gram subsamples were used for CIN and nutrient analysis:

Mollusks were removed from their shells before obtaining the wet weight. Sea urchins were ground shell and all. Coral samples were not ground, but the coral tissues were dissolved off the skeleton with a strong base instead of an acid.

Care was taken during all steps of the sample collection and preparation to try to avoid contamination from glassware, metal utensils, and human hands.

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## TREATMENT OF WATLP AND BIOLOGICAL SAMPLES FOR TRACE ELEMENT AND NUTRIENT ANALYSIS

Water:

Water samples should be filtered with 0.45 m filters as soon as possible after the collecting. After the filtering the samples should be acidified with concentrated HCl, 1 ml per liter of water.

For phosphate, nitrate and nitrite analysis 4 L of water is needed. Trace element analysis required 10 L.

Soluble orthophosphates are collected from 2 L of water by an anion exchange column and total soluble phosphates from 1 L of water, also with an anion exchange column, after all phosphates have been oxidized with a potassium persulfate digestion, The analysis of phosphates is carried out with an Autotechnicon autoanalyzer. Nitrate and nitrite are

also run by the autoanalyzer.

Eight liters of water are scavenged with Fe(0II)s to pre-concentrate Nn, Mg, Co, Cu, Ni, Pb, Zn, Cd, Cr, Ca, and Sr for analysis by atomic absorption spectroscopy.

Biologi ples

After the collecting all specimens are cleaned of silt and dirt, specimens of the same species put into a plastic bag, labeled, and frozen. In the laboratory the specimens are dissected, weighed, and dried at 105° C.. Part of the frozen sample 1s saved for pesticide and lig analysis.

Dried samples are cooled in a dessicator, weighed, and ground. Dried samples are analyzed for Carbon, hydrogen,

and nitrogen content with a gas chromatograph

For phosphate and trace element analysis 2 g of dried sample are digested by aqua regia wet digestion method.

Phosphates are analyzed with the autoanalyzer and trace elements (Fe, Mn, Mg, Co, Cu, Pb, Ni, Zn, Cd, Cr, Ca, and Sr) are analyzed with atomic absorption spectrophotometry

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## LABORATORY METHODS

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## CLEANING GLASSWARE

Rinse out all biological or chemical matter with tap water.

Wash using cleaner provided.

Rinse in tap water.

If biological material adheres to glassware, soak in NaOH solution. Rinse.

Rinse glassware with 6N. HCl.

Rinse two times with distilled water.

Dry glassware in low - heat oven.

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## LAB PREPARATION OF WATER SAMPLES

If the water has not been pre-filtered, do so. Save for counting.

Ferric Hydroxide Precipitation:

- a, Lower the pli of the sea water to 2.0.
- b, Add 10 mi of Ferric Chloride and mix well.
- ?. Add slowly, 6N NH<sub>4</sub>OH to raise the pH to 9.0.
- 4, Add 10 mi of separan after § minutes of precipitation.
- e. Allow sample to sit for 24 hours.
- £. Filter the water.

Put the precipitate in 250 mi plastic bottles.

Centrifuge

- i. Put the precipitate in 150 ml beakers and boil until nearly dry.



Put pre-cut filters into 1N HCl and leave for 20 minutes. Wash filters with distilled water and let sit for 15 minutes.

Filter what is left of precipitate and put into 25 ml plastic bottles for Atomic Absorption.

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#### NAA for TRACE METALS IN SUSPENDED PARTICLES IN SEA WATER

Cut to pieces a known weight of the filter and place the pieces in a 25 ml beaker,

Add 5 ml conc.  $\text{H}_2\text{SO}_4$  and approximately 3 drops  $\text{HNO}_3$ , cover beaker loosely.

Heat slowly to a boil. Evaporate to dryness

Wash beaker out 3 times with 3 ml aliquots of distilled

Water. Place washings in a 10 ml polyethylene vial,  
Place in a vacuum desiccator and evaporate to dryness.

Heat-seal vials under vacuum.

Irradiate for 5 minutes and allow 3 minutes for decay.

Count 400 seconds line-tine (Al, S, Ca, Ti, V, Cu) then

Count 1000 seconds line-tine starting {5 minutes after

(Na, Mg, Cl, Mn, Br, In, I)

?The same sample, or another portion of the sane air  
filter, processed as above, should be then irradiated  
for 2-3 hours in the reactor core as was suggested in  
Air Tape Analysis (please see Annual Report 1972)~

a.) Allow to cool 20-30 hours and count 2000 sec.

b.) Allow to cool 20-30 days and count 4000 seconds.

Run blanks using "clean" filters and standard reagents.

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# DETERMINATION OF REACTIVE PHOSPHORUS

## Introduction:

All methods for phosphate in sea water rely on the formation of a phosphomolybdate complex and its subsequent reduction to highly colored blue compounds. Methods using stannous chloride as a reductant at room temperature have been favoured as they are most sensitive and give less interference from easily hydrolysable organic compounds than

do other techniques. There are complexities in these

Methods due to interference from arsenic and to concealed blanks arising from the reduction of molybdate in sea water

in the absence of phosphate. An excellent program of

Comparative tests has been described by Jones and Spenser (J. Marine Biol. Assoc. U.K., 43:251, 1963).

The procedure given below is taken from the recent publication of Murphy and Riley (Anal. Chim. Acta, 27:31, 1962) and is so superior to other methods in terms of the rapidity and ease of analysis that it probably represents

the ultimate in sea-going techniques.

Method:

A. Capabilities

Range: 0.03-5 ug-at/liter

1, Precision at the 3 uG-AT/LITER LEVEL

The correct value lies in the range:

$\pm 0.03/n^{1/2}$  ug-at/liter.

Mean of n determinations +

2. Precision at the 0.3 uG-AT/LITER LEVEL

The correct value lies in the range:

Mean of n determinations  $\pm 0.02/n^{1/2}$  ug-at/liter.

3. Limit of Detection

The smallest amount of phosphate that can be detected with certainty is about 0.03 ug-at P/liter.

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Reject duplicate determinations if extinction values differ by more than 0.02 in the extinction range 0.5-1.0 or more than 0,01 in the extinction range 0.1-0.5,

If the duplicate extinction values differ by less than the above limits, take a mean value.

#### Outline of Method

The seawater sample is allowed to react with a composite reagent containing molybdic acid, ascorbic acid, and trivalent antimony. The resulting complex heteropoly acid is reduced in situ to give a blue solution the extinction of which is measured at 8850 Å-

## Special Apparatus and Equipment

130-1 capacity screw-capped polyethylene bottles  
marked on the side at 100ml (+2 ml) with a band  
of black tape

### Neutron Activation Techniques:

Handling of the samples was done in a clean room equipped  
with @ laminar-flow clean hood. Approximately 1g of the  
Gried material as sealed in either quartz ampules or in  
medical grade polyethylene tubing. (A number (10-20) of  
Samples With appropriate standards were packaged poly:  
ethylene container and irradiated in a TRIGA. TIT reactor.  
An integrated flux of about  $10^{18}$  n.cm<sup>-2</sup> was received by  
each package of samples. The sampics were cooled for 4  
Period of 2-4 weeks before counting.

A portion of each irradiated sample was placed in a tared  
Snap-top capsule, the weight determined and then couned? on  
4 <sup>31</sup>Ge (Li) detector.? The analyzer was calibrated using  
Radioactive standards. Three absorber's Cas Clana bet  
each about 1 an in thickness, were placed bétwees the  
Sample and the detector to reduce the low enereyCerpton

and Bremsstrahlung. The gamma-ray spectrum of each sample was analyzed and the concentrations calculated by a computer.

Standards of Ni-Co wire were placed in each package of samples to monitor the flux. The fast flux was calculated from the  $^{28}\text{Ni}(n,p)^{28}\text{Co}$  reaction and the thermal flux

from the  $^{59}\text{Co}(n,\gamma)^{60}\text{Co}$  reaction. Inorganic standards were

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included: Fe- $^{59}\text{Fe}$ , Co- $^{59}\text{Co}$  and Hg packaged both dried on plastic tape and as liquid in quartz vials.

The blanks run were of the plastic tape on which inorganic standards were irradiated,

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## PLANKTON: METHODS AND MATERIALS

### Methods and Materials

Plankton samples were obtained in several ways, The majority were taken using plankton nets of mesh sizes 380 $\mu$ , 300 $\mu$ , 202 $\mu$ , and 60 $\mu$ , pulled behind 13 to 17 foot dories for 5 to 30 minutes. For uniformity, the nets were pulled at speeds to keep them 1 to 3 feet below the water surface. The samples were then preserved in #4 buffered formalin and taken back to the laboratory for analysis.

A small flowmeter was suspended in the mouth of each 380 $\mu$  mesh net, volumes of water sampled estimated for the remaining nets used.

For counting organisms, aliquots of each sample were removed from a well-shaken container by means of Scc, 10cc, or 20cc spoons and diluted into a square Petri dish or a counting tray. All zooplankton in the sub-samples were counted under 10 to 30 power magnification. Phytoplankton and zooplankton were identified using 30 to 400 power magnification.



A few plankton samples were obtained using a 2-quart bucket to pour surface water through a 60 µm net sieve (primarily for phytoplankton and microzooplankton). One sample, JB-25, was taken utilizing a hand held concentrator over the side of a boat as it journeyed through a shallow canal between heavily mangroved cays

Biomass estimates were obtained in the following manner.

Zooplankton was sampled using a 200 µm mesh net, animals

(and incidental trash, phyto- and zooplankton) transferred to bottles that were placed, without preservative,

into an ice chest. Wet weights were determined as,

explained in the following. Phytoplankton and zooplankton

were sampled by using 2-quart buckets to fill

a 45 gallon cubitainer. This was then first filtered through

a 60 µm mesh sieve, with frequent washing off of captured

material to eliminate clogging, to separate phytoplankton

and then filtered through 0.45 µm membrane filters to separate

zooplankton. All samples were finally filtered through

membrane filters that had been HCL treated. Initially the

filters were wet with fresh water, a slight vacuum placed

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on them, then the sample poured through. Ten filters were weighed? wet (following 15 seconds of vacuum) and their average used as the weight of a standard filter. The filtered samples were subjugated to an additional 20 seconds of vacuum following apparent dryness, then were weighed on a Mettler balance accurate to 0.01 gram.

Future studies of zooplankton in Jobos Bay will emphasize seasonal and areal variability in the total standing stock and the more numerous species. Triplicate tows will be made at several stations with two 1/2 meter plankton nets (65u and 200y mesh size) to provide reliable estimates of the zooplankton community.

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PROCEDURE FOR COLLECTION OF PLANKTON AND PREPARATION  
(OF PLANKTON FOR NEUTRON ACTIVATION  
ANALYSIS AND ATOMIC ABSORPTION

1.) Sample should be collected off the front or sides of

the boat to avoid contamination from boat oil and gasoline.

2.) Avoid smoking, eating or coughing close to plankton sample.

3.) Collected plankton should be put into a large plastic container. Then add 2'L. fresh sea water (Take {Fesh?? Sea water from the front or sides of boat).

4.) Filter plankton through a funnel fixed with same mesh size filter as tow, using low vacuum suction. (To avoid breaking cells of plankton).

5.) After filtering, put plankton sample into small plastic bags and heat Seal? to avoid contamination.

Put plastic bag filled with plankton sample in plastic Jars. Opening of jars should be the same size as the rest of the container.

7.) Freeze sample immediately.

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## NITRATE AND NITRITE IN SEA WATER

The procedure used for the analysis of nitrate plus nitrite in sea water is described in Industrial Method 43-69W, Technicon Instruments Corporation, Tarrytown, New York 10591. Millipore filtered aged sea water, in place of synthetic sea water, was used in the preparation of

standard

were not

Complete

is to be

supplied

solutions and as a system wash. Air scrubbers used in the color reagent line.

instructions for the operation of the Auto Analyzer

found in the General Operating Instruction manual  
with the instrument.

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THERMAL NEUTRON ACTIVATION ANALYSIS  
OF AIRBORNE PARTICULATE MATTER  
IN THE SOUTH COASTAL AREA  
(OF PUERTO RICO

By

Tin Mo

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INTRODUCTION

In the past several years there has been mounting awareness and concern over the addition into our atmosphere and water bodies of waste byproducts by modern industry which might be injurious to the public's health. The presence of smoke, sulfates, and fallout in air is being routinely monitored by the U.S. Public Health Service, pollution control agencies of large cities, and many other scientific teams. The practice of burning coal, oil, and refuse, in addition to their combustion products: continually injecting many trace elements into the atmosphere:

At present, on the island of Puerto Rico, no large-scale program is conducted which is directed toward the detection of trace metals, even though air filter tapes are collected and are available for detection. Data and information on the level or concentrations of these pollutants in the air are necessary before they can be judged as hazardous:

In addition to this, a knowledge of these data, and the variation of the elemental distributions as a function of location and time is of importance in understanding the transport mechanisms for geochemical studies and for solving general problems of air pollution and pollution control. Correlations with public health problems can give an idea of the magnitude of potential problems.

The concentration of trace elements in air can be measured by many techniques. Thermal neutron activation analysis is relatively simple and is also nondestructive, if the study is limited to gamma-ray emitting nuclides and with the application of high resolution gamma-ray spectroscopy by means of lithium-drifted germanium [Ge(Li)] detectors.

The object of this study is to determine the conditions and procedures for ele

borne particulate matter by means of neutron activation analysis and Ge(Li) techniques (Dane et al, 1971, Dane et al, 1970, Tuttle, Vogt, Parkinson, 1971). Computer techniques for multi-nuclide gamma-ray spectrometry will be applied for data reduction (Dane et al, 1970). The measurement sensitivity for many induced nuclides should be more than adequate when the Puerto Rico Nuclear Center high flux TRIGA reactor (1-3' x 10m. area sect)



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is used in conjunction with the 40 cc Canberra [Ge(1i)] detector (of about 3.5 kev energy resolution) coupled to @ Packard 1024 channel analyzer.

Dust is usually collected on a filter by drawing air through a vacuum pump. This results in the filter becoming @ part of the sample. Hence, the choice of filter material is important and the proper filter should be free of high thermal neutron cross section elements. The commonly used asbestos and glass base filters which have a high collection efficiency for small particles, are not suitable for thermal neutron activation analysis as they contain large amounts of Na, Cl, and other elements. In this study, a cellulose ~ base filter paper (furnished by the Environ?

mental Science Division of Bendix Corporation, Baltimore, Maryland) which exhibits a low gamma-ray background after fan exposure to thermal neutrons will be used.

## EXPERIMENTAL PROCEDURES

pling

Air particulate samples are presently collected bi- and simultaneously at ten different locations along the south coast of the island of Puerto Rico. (The locations are described in the PRNC Aguirre Power Project Environmental Studies 1971 Annual Report and are shown in Figure 1.) These locations range from low population to high population density areas, areas which are in the immediate vicinity of industrial plants and those at varying distances from them.

Each air pump (manufactured by Environmental Science Division of Bendix Corporation) sampled approximately 12,260 cubic meters of air through 1.27  $\mu\text{m}$  area of filter paper during a period of two weeks. The exposed filter tape after removal from the pump is sealed inside a polyethylene bag. One centimeter square of the exposed tape is cut out to be irradiated each time. Each sample to be irradiated contains the dust from approximately 9,670 cubic meters of air.

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## PREPARATION FOR IRRADIATION

The procedure to be followed for development of a non-destructive thermal neutron activation analysis system for routine determination of heavy metals such as Cd, Ni, V,

Se and Hg in the paper tape samples are given below.

1

Using plastic gloves and a clean glass slide (or a sharp Teflon or polyethylene knife) tear off

4 piece of paper of 1 cm<sup>2</sup> area corresponding to every fourth spot of dust collected on the tape.

Place in a small Petri dish. Dry under an infrared lamp. Cool in a dessicator and weigh, Roll up and put inside a 1 cc polyethylene snaptop irradiation vial, Heat seal in vacuum with a Teflon coated soldering iron tip so as to minimize contamination of the sample. (A reduced line voltage for the soldering iron is to be used for a satisfactory heat seal.) This sample is ready for irradiation,

Cut off five 1 cm<sup>2</sup> pieces of clean paper tape.

Dry and weigh each one as done in 1.

3. To two of them, add known amounts of standard solutions of Ni, V, Hg, Zn and Se (1.0 pL of each with a concentration of 0.1 to 1.0 gm/L or 0.1 to 1.0 wg/spot). Dry under the infrared lamp. To the third piece of paper tape, add Cd carrier and repeat as above. Leave the remaining two paper pieces clean. Dry these under the infrared lamp too.

Put all in a dessicator.

4, Heat seal under vacuum each sample inside a 1 cc polyethylene snaptop irradiation vessel.

#### IRRADIATION AND COUNTING PROCEDURES

Since some of the induced nuclides like  $^{52}\text{V}$  have "short" half Lives, the samples would be irradiated for a period

of 1-2 minutes in  $1.5 \times 10^{13} \text{ cm}^{-2} \text{ sec}^{-1}$  Flux of the PRNC Teactor's fast transfer rabbit facility. Because

---Page Break---

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?the neutron flux from one irradiation to the next cannot .

be assumed to be constant, an accurately weighed Co or lin

standard wire will be used as a flux monitor. Th

will be taped to the side of the small polyethylene  
irradiation vials before they are encapsulated inside the  
outer irradiation rabbit.

?The short lived gamma-ray nuclide

about 3 minute

means of the Canberra (Ge(Li)] detector couple

ard 1024 channel analyzer by Counting for 400 si

time. ?The flux monitors will be counted for 20 seconds

each. ?Then the samples will be counted again for 1000

seconde live time at 15 minutes after the end of irradiation.

will be detected in the

Later, the samples will be reirradiated for a period of

2- 4 hours and counted at decay times of one and two days

for 2000 seconds live time and then for 4000 seconds live

time after 20 ~ 30 days of cooling to determine the contents

of long-lived nuclides. or the long irradiation all

samples in @ polyethy-

lene 4-cm in diameter, and lowered into the reactor

pool. Sample cooling during irradiation will be accomplished :

by @allowing the pool water to circulate through several

holes punched in the container bottle. The samples will be

confined to a single horizontal layer of vertically ori-

ented tubes at the bottom of the bottle and the bottle will.

be rotated 180° at half of the irradiation time to effect

normalization of flux. Fast neutron flux gradients are

usually about twice as large as thermal gradients, but the

only fast neutron reaction that will be used in this study

is in the determination of nickel,  $^{60}\text{Ni} (n, p) ^{60}\text{C}$

?The ratio of thermal to fast neutron flux will be determined at every irradiation site using the reactions



He ay) Sep and 99S (ny p) 2P. Entergerences by thresh-

old reactions will be checked experimentally.

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## AUTOMATED DATA REDUCTION

For the procedure of nondestructive neutron activation analysis to be practical and efficient when applied to large numbers of samples, such as in routine monitoring,

an automatic data reduction system is necessary (Dams et al 1970). An automatic instrumental data reduction system fast and accurate and many human errors are eliminated. However, human judgement should still be in the examination of the data and in devising procedures for checking the quality of the data.

In the present study a computer program will be developed to perform the following tasks:

1. qualitative determination of the presence of isotopes,

2. calculation of net peak areas

3. conversion of peak areas to weights of trace elements

subtraction of analytical blank due to filter materials

5. calculation of the concentrations of trace elements in the originally sampled air

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REFERENCES

Dams, R, et al 1971. Nondestructive multi-element neutron  
?activation analysis of air pollution particulates.

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42: pp. 861-867.

Tuttle, R. Fey J. Ry Vogt, and 7. P, Parkinson 1971.

Neutron activation analysis of trace elements in

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Environmental Pollution. pp. 119 - 137. Vienna,

TABA.

## ?SCOR/UNESCO PROCEDURE FOR CHLOROPHYLLS

### INTRODUCTION

?The following procedure was agreed to by a SCOR/UNESCO Working group and has been published by UNESCO in Monographs ?on Oceanographic Methodology.

### METHOD:

Concentration of Sample:

Use a volume (Note a) of sea water which contains about 1 ug chlorophyll a. Filter (Note b) through a filter (Note c) covered by a layer of  $MgCO_3$ ; (Note d).

Storage:

The filter can be stored in the dark over silica gel at 1C or less for 2 months but it is preferable to extract the damp filter immediately and make the spectrophotometric measurement without delay.

Extraction:

Fold the filter (plankton inside) and place it in a small (5-15 ml) glass, pestle-type homogenizer. Add 2-3 ml 90% acetone. Grind 1 min at about 500 rpm. Transfer to a centrifuge tube and wash the pestle and homogenizer 2 or

5 times with 90% acetone so that the total volume is 5-10 ml.

Keep 10 min in the dark at room temperature. Centrifuge (Note e) for 10 min at 4000-8000 g (Note f). Carefully,

pour into a graduated tube so the precipitate is not disturbed and if necessary dilute (Note g) to a convenient volume (Note h).

For Thi

sia Leaves

A weighed quantity of fresh *Thalassia* leaves ground in

ceramic mortar and pestle with some quantity of distilled water. Wash mortar and pestle with acetone so final solution 80% acetone. Keep 10 min in dark at room temperature then centrifuge for 10 min. Pour off supernate and read absorption on Beckman DU spectrophotometer. Re-extract precipitate with acetone to determine efficiency

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of first extraction. Maximum absorption for chlorophyll a in acetone reported at 663 nanometers. Chlorophyll b absorption maximum around 640 nanometers.

Measurement:

Use a spectrophotometer with a band-width of 30 Å or less,

and cells with a light path of 4-10 cm (Note i). Read

the extinction (optical density, absorbance) at 7500 (Note j), 6630, 6450, and 6300 Å against a 90% acetone blank.

Calculation

Subtract the extinction at 7500 from the extinctions at 6630, 6450 and 6300 Å. Divide the answers by the light path of the cell in centimeters, / TE these corrected extinctions are  $F_{6630}$  and  $F_{6450}$  the concentrations of chlorophylls. 189882 96?5 Acetone 50% extract as usual are given by the SCOR/UNESCO equations (refer to section IV.3.1). If the values are multiplied by the volume of extract in milliliters and divided by the volume of the seawater in Liters) the concentration of the chlorophylls in the seawater is obtained in  $\mu\text{g/liter}$  ( $\text{mg/m}^3$ )

## NOTES.

(a) The amount of chlorophyll a should be less than 10  $\mu\text{g}$ , otherwise a second extraction with 90% acetone might be necessary. With ocean water about 4-5 liters of sample should be used; with coastal and bay waters, sometimes one tenth of this amount is sufficient.

(6) Use no more than two thirds of full vacuum.

(c) Satisfactory filters include paper (Albet), cellulose (Cella "grob"), and cellulose ester (0.45-0.65  $\mu$  pore-size); the filter should be 30-60 mm in diameter. If these filters clog with inorganic detritus, use Schleicher & Schull 575.

(4) Add about 10 mg MgCO<sub>3</sub>/en? filter surface, either as 2 powder or 'as'a Suspension in filtered sea

---Page Break---

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(e) A swing-out centrifuge gives better separation than an angle centrifuge.

(8) If stoppered, graduated centrifuge tube is used,

the extract can be made up to volume and the Supernatant carefully poured or pipetted into the spectrophotometer cell:

(g) If turbid, try to clear by adding a little 100% acetone of distilled water or by centrifuging



gain.

(h) This depends on the spectrophotometer cell used,  
The volume should be read to 0.1 ml.

(4) Dilute with 904 acetone if the extinction is greater  
than 0.8,

(3) If the 7500 Å reading is greater than 0.005/cm  
Light path, reduce the turbidity as in Note g.

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## TRACE METALS AND MAJOR CATIONS IN BIOLOGICAL MARINE SAMPLES BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

In the last decade trace inorganics in the marine environment have been getting attention as much as organics such as pesticides, etc.

Neutron activation and atomic absorption spectrometry are two common and sensitive methods in this field. We have been using a Jarrel Ash AAS and a Perkin Elmer burner.

We receive our samples in coarsely-ground form, Sometimes out of necessity, due to the coarseness and resulting prolonged and possible incomplete digestion, we find it appropriate to pulverize the samples. This is preferable for All samples, but considering the length of time involved has not been performed on all samples up to now.

Using various weights of samples (0.25 - 1.0 g), we found the 1.0 g weight to be the most suitable since Some elements are not detectable using smaller weights.

To standardize techniques and to ascertain the most suitable one, i.e. giving the highest peaks, two sets of experiments were undertaken: the first to determine the best technique for dissolving and the second to determine the length of time for dissolving.

Table 1 shows the average readings for the various dissolving techniques. Methods using nitric acid, in general, were better than sulfuric acid methods. Loss of the sample with sulfuric acid methods may have contributed to the low readings because of the splattering when hydrogen peroxide was added. Aqua regia, by way of best and second best readings, was concluded to be the best method for getting the greatest peaks. Consequently, aqua regia is now the preparative method used in this lab.

In the second set of experiments samples were refluxed from time varying 15 minutes to 24 hours. No significant reading differences were noted. Therefore, we only reflux samples for approximately an hour, or possibly two, until dissolving appears to be complete:

Following is a step-by-step account of the method in use:

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1, Grind to a fine powder.

Weigh out a one gram sample, record weight to the nearest ten-thousandth place.

Dissolve in a 50 ml beaker containing aqua regia (11 ml/g), place on a hot plate.

4. Filter after two-hour refluxing.

5. Dilute with distilled water to 15 ml.

The following table shows the sensitivity of our readings

and the per cent standard deviation of identically prepared samples:

Element Detection Limit -d\_Deviation

Ca 0.026 ppm 5 ppm level

Cu 0.208 50 ppm level

Fe 0.4 300. ppm level

Ni 0.23 20 ppm level

Zn 0.02 300: ppm level

Co 0.118

10 ppm level

Mathematical Calculation:

Our calculations are reported in parts per thousand (ppt, mg/g). The samples are concurrently run with two blanks, treated in the same manner in the preparation steps as the samples. Sets of standards are run through AAS at least before and after samples. The graph heights are read and recorded. From the standards we construct a curve from which the sample and blank concentrations are read. Blank values are then subtracted from sample values to account for impurities which may occur during preparation.

?The final concentrations are calculated from the formula

given below:

$(eR = m/er. (ppt)$

P = Readings from standard curve

B = Blank

V = Volume of the dissolved

w<sub>e</sub> = Weight of sample

ple

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?The standards are currently made from inorganic salts.

a comparison it might be worthwhile to use organic trace metal standards, preparing them in the same manner as the samples since the biotic material we receive is organic.

For

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PROCEDURE. FOR ESTINATING DISSOLVED OXYGEN

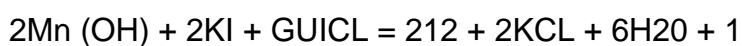
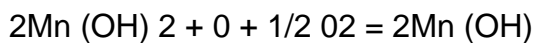
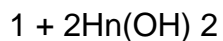
5 ML WINKLER METHOD

Introductios



The method to be described here is a modification of Fox and Wingfield (1938). It is convenient in that it allows one to measure the sample of water and run the Winkler reaction in the same syringe. It is also useful since small, but manageable samples may be analyzed. Finally, with sufficient precaution the method is accurate enough for many research purposes.

The Winkler reaction may be summarized as follow:



Thus the introduction of manganous chloride in the presence of alkali results in the formation of a manganous hydroxide

precipitate, ?The manganous hydroxide is oxidized by the oxygen of the sample to manganic hydroxide. Acidification of the sample then results in the oxidation of the  $KI$  to iodine by the manganic ions. Each molecule of oxygen liberates two molecules of iodine. The extent of the iodine released depends upon the amount of manganic ion formed, One then titrates the iodine with sodium thiosulfate using starch as the indicator. The reduction of the iodine to iodide by thiosulfate results in a loss of color following the disappearance of the starch-iodine complex.

In the following procedure there are two steps which must be done carefully. These include the volume measurement of the water sample and the standardization and titration with the sodium thiosulfate. The remainder of the reagents are generally added in excess of that required for the reaction.

#### Apparatus and Reagents

Reaction syringe - These are constructed from 10 ml syringes which have glass needle fittings. A capillary tube, which is drawn out to a fine tip, is attached to the

needle fitting with a piece of stiff plastic tubing. The capillary is fitted to the syringe in such a manner that there is a minimum of space between them. The fine capillary tip allows the reaction to proceed without diffusion of gases into or out of the syringe. For extremely accurate results the syringe volume should be calibrated by weighing with distilled water. For less accurate results, the volume of the sample can be measured using the syringe calibrations.

Burette - The burette is important, but need not be elaborate unless very accurate results are sought. For our purposes a 1 ml burette or a 1 ml tuberculin syringe with a fine capillary tip is adequate.

Titration Equipment - The most convenient arrangement is to make the determinations in a 25 ml beaker or Erlenmeyer flask which is stirred by a magnetic stirrer. The "flea" can be easily constructed by cutting a small piece of wire, such as a paper clip, into 1 cm lengths and cover with a piece of capillary glass. The titration should be done on

a white background with fluorescent lighting.

Reagents -

i

thiosulfate - Weigh out 24.82 g of sodium thio-  
te and dissolve in 1 liter of boiled and cooled  
distilled water. Dilute 1:9 for standardization,  
Weigh out exactly 0.8917 g K<sub>103</sub> and dissolve in 1 liter  
of distilled water to make 0.035N solution for stan-  
dardizing the thiosulfate. Fill a 10 ml burette with  
the diluted thiosulfate. Pipette accurately 2.0 ml of  
K<sub>103</sub> into the titration vessel and add 1.0 ml of 1% KI.  
Add 5 drops of concentrated H<sub>2</sub>SO<sub>4</sub> and 2 drops of  
starch and titrate. Repeat three times. Calculate  
the normality of the thiosulfate as follows:

xv = xv

KIO<sub>5</sub> Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>

0.05 = N x ¥

Note: N/100 Sodium thiosulfate is now commercially available as a standard.

2, MnCl<sub>2</sub> - 40 g/100 ml distilled water

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3. Alkaline Iodide = 32 g NaOH and 10 g KI to 100 ml distilled water,

4. KI - 1.0 g to 100 ml distilled water.

5. Starch indicator - 1g is added to 100 ml distilled water and heated slowly to 190° C. Cool and add 0-1 g of salicylic acid.

6. H<sub>3</sub>PO<sub>4</sub> - Concentrated reagent.

?Titration of Sample:

1, Using the 10 ml syringe with nozzle, fill the dead

space carefully with  $\text{MnCl}_2$ . Be sure to remove all air bubbles. {

2. Fill the syringe to the 5 mL mark with the water sample.

Again, be sure to exclude all bubbles.

Take in two times the dead space volume of alkaline iodide.

4. Rotate the syringe to mix the manganous hydroxide thoroughly.

5. Allow the syringe to set three minutes for oxygen absorption.

6. Draw in three to four times the dead space volume of concentrated  $\text{KI}$ . Rotate the syringe until the precipitate is thoroughly dissolved. The reaction has now stopped, and exposure of the solution to air does not result in error.

7. Eject the solution into a titration vessel, such as a

25 ml beaker, and wash the syringe twice with 2 ml aliquots of distilled water. Add the wash to the titration vessel.

8. Add two drops of starch indicator and titrate the sample with the standard thiosulfate. For this purpose, use a 1 ml tuberculin syringe or a 1 ml burette,

Rinse the 10 ml syringe thoroughly in tap water and distilled water, When the syringe is again rinsed with MnCl<sub>2</sub> it is ready to use.

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$$\text{O}_2(\text{STP}) = \frac{V \cdot n \cdot A}{1000}$$

2A, 5600

n = ml thiosulfate used in the titration

A = normality of thiosulfate

V = volume of the water sample titrated

A correction should be applied for the dissolved oxygen in

the reagents (MnCl and the Alkaline iodide). It is assumed to be about 3.4 ml/O<sub>2</sub>/l.

tice of the Technique

1, Place a sample of distilled water in a beaker and stir vigorously for about 1 hour using a small stirring motor. Take the temperature of the water and read the barometer. Remove three samples of the water and Determine the oxygen content, Compare your values with those obtained from the Handbook of Physics and Chemistry.

$\text{Bar.} \times 0.209 (1000 \llcorner) = \text{mlO}_2/\text{l}$

760

where Bar. is the barometric pressure and  $\llcorner$  is the ml Of oxygen which will dissolve in 1 ml of water at the Specified temperature if the sample is in equilibrium with an atmosphere of oxygen. Estimate the reagent

Yolume and make the correction for the reagent oxygen.



2. Try determining the oxygen content of a sample from  
Which most of the oxygen has been removed by nitrogen  
washing or boiling.

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Allen and Unwin Ltd. London. 1959. Chapter 14.

Fox, H.M., and Wingfield, C.A. 1938, A portable apparatus  
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volume of water. J. Exp. Biol., 15: 437.

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aay

STRONTIUM 89 AND 90 IN MILK

AND

TODINE - 151 IN MILK

by

Giiser G. Wood

IODINE - 131 in Milk

Apparatus:

1, 2,5 liter polyethylene annular counter containers.

1. 248 Trichloroacetic acid.
2. 124 Trichloroacetic acid,
3. 28 NaI Solution (20g AgNO<sub>3</sub>/1iter water).
4. 24 AgNO solution (20g AgNO<sub>3</sub>/Liter water).

Determinatio

1. Put 2 Liters of the whole milk sample to a 5 liter

beaker.

2, Add 2 ml of 40% formaldehyde solution.

3. Stir for § minutes and let stand at room temperature

for 1 hour.

4. Transfer 1 liter of sample into annular counter container, Add 1 liter water; mix and gamma count (C)).

5. To remaining sample, add with stirring, 900 ml of 24% trichloroacetic acid solution, and 1 ml of 24 Nal and then 1 ml of 2 AgNO<sub>3</sub> solution.

6. Stir for 30 minutes. Allow the precipitate to settle.

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4a

7. Filter by suction through a 15 cm WHATMAN #42 filter paper.

8. Wash with two 50 ml portions of 12% trichloroacetic acid solution. Discard the precipitate.

Transfer the liquid (filtrate and washings) to annular counter container. Mix well, Gamma count (C2)-

Calculation:

The net counting rate of 11 = c, -

The counter efficiency is determined by adding « known Standard to a  $^{131}\text{I}$  free milk. Decay constant is

0.086 day. Half life is 8.05 days.

Acct

A= Age!

Reference:

Harley, John H. 1967. Manual of Standard Procedures

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## STRONTIUM 89 AND 90 IN MILK:

### A, By Ion Exchange

Presery:

Immediately after the collection of a milk sample, a preservative, formaldehyde (about 3 ml 37% formaldehyde Solution per liter of milk) must be added. Sample must be refrigerated (at 32° - 34° F.) for at least two weeks to allow the Yttrium 90 daughter of Strontium 90 to reach into equilibrium with its parent.

### Reagents

Cation - Exchange resin: Dowex SOX-X8 (Na<sup>\*</sup> form, { 50-100 mesh), analytical grade (the chemical

grade can be used for this purpose after

Purified by a method given by Harley, G-04-01).

Carrier Solution: Sr<sup>o</sup>as Sr(NO<sub>5</sub>), : 20 mg Sr\*? per  
al.

Citrate Solution: 3N (pH 6.5).

Apparatus

Ton-exchange column:

Dimensions are shown at Fig.1.

Put § ml of distilled water in the 30 ml column,  
pour 170 m1 Dowex 50 W resin into it.

Procedure.

1, Put 1 liter of milk into the reservoir.

2, Put 1 ml strontium carrier into 10 ml of citrate

solution, mix until dissolved. Put this solution

into the milk with 5 ml distilled water, mix

well.

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443,

3. Open stopcock control flow rate at 1 ml/min.

4. Stop flow when just enough milk remains in column  
to cover resin.

Discard effluent milk.

6. Put 300 ml of warm distilled water through the  
column (about 10 ml/min).

7, Discard the effluent water.

Push the resin out of column in a very small

beaker or vial. Dry and gamma count.

aslen

Fritted

Glass disk

B. By Ashing:

Apparatu:

Analytical oven

Corningware dish

Muffle furnace

Procedure:

1, Put 2.5 kg evaporated on 5 kg fresh milk into  
corningware dish.



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2. Dry in oven at 100°C. for 48 hours.

Transfer the sample to a muffle furnace and raise the temperature slowly to 550°C. Ash at 550°C. for 24 hours.

Cool, weigh, and grind the ash to a fine powder.

5. Gamma count or

6, Dissolve in 1 ml of 6 N HNO<sub>3</sub>,

7. Dilute to a 25 ml in volumetric flask.

8. Run at Atomic Absorption.

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APPENDIX

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MATHEMATICAL SIMULATION OF THERVAL PLUMES

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Introductioi

The material in Appendix C, consisting of two parts,  
Tepresents a teoranination section 11{-0-1"b. (Phe Blane.  
EfEisent?SyScong-Heat environsentai influences, pp. 64-68)  
of the Aguirre Power Plant Complex Environmental? Report

?The first part is a detailed check (including data, equations used and references) of the values listed in Tables 111-2 and 111-3 of the Report, made at the request of the Puerto Rico Water Resources Authority, In addition, the distortion of the plume centerline by an ambient current of 0.5 ft/sec at 45° is computed by superposition of jet and ambient velocities.

The second part contains calculations, parametric studies and a modification of the basic Pritchard model intended to provide a quantitative basis for the evaluation of the applicability of the model to conditions existing in the Aguirre Ship Channel, and to examine the effect of re-entrainment on the predicted plume extent. Specifically, this part consists of an outline of the basic model equations, a comparison of required entrainment flow to ambient flow, the effect of entrainment of heated water on the size of the 4°F isotherm and the effect of deflection and subsequent reentrainment of heated water, impinging on the Cayos on its temperature. All calculations are for the case of two fossil plants.

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CALCULATIONS FOR TABLES ITT-2 AND III-3

of

AGUIRRE POWER PLANT COMPLEX ENVIRONMENTAL REPORT

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Basic Dat

ind Unit Conversio:

a) From p. 62, Ref. [1

Flow Condenser Excess Temp. Power

Fossil:  $Q_e = 226,000 \text{ gpm}$   $e_g = 18.74^\circ\text{F}$   $P_g = 60 \text{ Mwe}$

Nuclear:  $Q_y = 555,000 \text{ gpm}$   $e_n = 14.80^\circ\text{F}$   $P_y = 60 \text{ OHNE}$

b) From pp 3204 § 3209, Ref. [6]:

1 US gal. = 0.13368 Ft<sup>3</sup>

Tacre =  $4.3560 \times 10^4 \text{ ft}^2$

©) Converted Flow Data and Approximation

Fossil:

$Q = 226,000 \text{ gpm} = 503.528 \text{ Ft}^3/\text{sec} \sim 504 \text{ cfs}$

Nuclear:  $Q_n = 555,000 \text{ gpm} = 1256.54 \text{ ft}^3/\text{sec} \sim 1257 \text{ cfs}$

Equations for Computation of Table II

Let  $N_g = \text{Number of Fossil Plants}$

$N_y$  \* Sumber of Nuclear Plants

4) Power Production

Prot "Pe Ne # Py?

b) Flow Rate

$Q_{rot} = Q_e Ne + Q_n +$

©) Condenser Excess Temperature

Pctor? ( $ce = Of + NF * Cen = Qy +$

4) Discharge Nozzle Dimensions (Triangular)

$l_o$  \* exit velocity

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$h_g$  = exit depth

$b_g$  \* exit width

cc) "Normalized" Nozzle

For equal area and centroid, equivalent rectangle defined

by

Recomputed Values for Table I11-2:

a) Data

$Q_e = 50 \text{ Scfs}$   $P_p = 460 \text{ NNe}$   $c_g = 18.74^\circ\text{F}$

$Q_n = 1250 \text{ cfs}$   $P_y * G_{OON}$   $Q_e = O_{cg} = 14.80^\circ\text{F}$

$U_g = 6 \text{ ft/sec}$

b) Table Values:

Ne| xn] Peot | Qtr | Sector | Triangle | Normalized

uve} cfs] cr [bo [bo | bs | hg

i re fee foe | te

1 | 0 | 400 | sos | 18.74 | 16.83 | 10 | 12.62 | 6.67

920 | roto | 18.74 | 33.67 | 10 | 25.25 | 6.67

2 | 1 | 1s20 | 2260 | 16.56 | 37.67 | 20 | 28.25 | 13.33

|

(2 [2 fave [sie | 15.98 | sesso [20 | essa 13.33



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Equations for Computation of Table 111-3:

From Ref. (1), [2], [S]:

ley oe

Golty) = (0/0,)"2 by = 6 be

- 2

Ax 0.215 62

NOTE + Excess temperature at discharge orifice.

Recomputed Values for Table I11-3:

NOTE: Assumption of no cooling in discharge canal

9 = Octot, is made. For cooling in canal and  
resulting changes in Table III-3, see items 6,

and 8.

Proe| 460 Mite 920 Mie 1520 MWe 2120 Mie

0g | 18. 74°F 18.78°F 16.56°F 15.93°F

bs | 12.62  $\phi$ e 25.25 ft 28.25 ft 43.88 fe

e [co | ae fo | Ao co | Ae Re

ce | ee | acres

12] as [0.17] soo] 0.07 | 323] 0-31] eos] 1.06

to | 266 | 0.35] 532] 1.40] 465] 1.07] 668] 2.20

8 | ais | oes] ss] s.ar] 726] 2.60] 1944] 5.38

© | 739 | 2.69] 1478] 10.78 | 1291 | 8.23 | 1856] 17.00

4 | 1602 | 13.63 | 3325] s4.se | 2905 | 41,66 | 4176] 86.06

Equations and Parameters for Surface Cooling in

---Page Break---

a)

»)

o

a

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Equation for temperature change - Ref. [2]:

$n_o = u_a e P_o r O_e - 80$

canal Area A - Ref. [1], pp 62, 63:

$L = 7900 \text{ ft}$ ,  $w = 156.5 \text{ ft}$ ,  $A = 1.24 \times 10^8 \text{ ft}^2$

Surface Heat Loss Coefficient  $w$

$i$  Fron Ref. [4]

for wind = 12 mph

dew pt. = 70°F

water surface temp. = 80°F

3 \_\_\_ Btw.

vy 2 2.1991 x 1073 Eze See

ii From nef. [5]

for wind = 10 mph

water surface temp. = 80°F

excess temperature = 18°F

uz © 2.5361 x 1073 Btu

2° 2 Fer see

iii For computations use average value

elope ue x 1073 3 a Bt

we E(uy + ug) # 2.3676 x 10<sup>32</sup> 2.4 x 10S FEE GES

leat capacity of water - Ref. [4]:

» 6a BE

ccs 64 Fever

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## Surface Cooling in Car

Xen Prot Qtot = etoe =n 8

1 0 460 sos, t878@ tao

20 920101878 ot

2 1 1520 2260 16.56 0.38 16,22

2 2 21205510 t5.93 ott t5.72

Modified Values for Table 111-5:

Prot ?tne 460 920 1520 220

oF 1.01 17.88 16.22 1s.72

bg tt 12.62 25.25 28.25 43.08

le tortt |Agracres| co | Ao | to | Ae | to do

loa tsz\_ | 0.11 | 366] 0.56! st0| 0.47] 452) 1.01

| 0 219 | o.2¢ | ase] 1.16) eas | 0.98] 651] 2.09

5 sz | ose | 757] 2.83, 697] 2.40 1017] 5.10

6 609 1.83 1345 | 8.93 1239 | 7.57 | 1807 | 16.12

loa taco | 9.35 | sner [asses a7er | ae.se | anes | arse

i L

Equations for Plume Distortion - Ref. [3;

fect ge a4

Frere t Ben q

wet). BER

m-yORery

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## 10. Plume Distortion:

bata: Exit velocity  $u_e = 6 \text{ ft/sec}$

current:  $0.5 \text{ ft/sec}$  at  $45^\circ$ , consequently

$$b = 0.5 \cos 45^\circ = 0.354$$

$$U = 0.5 \cos 45^\circ = 0.354$$

$$V_v = 0.5 \sin 45^\circ = 0.354$$

. WE

Values of  $b_f$  and  $\epsilon_g$  correspond to those given in

items 5 and 8.

3) Values corresponding to item \$ (no canal cooling)

|Feoe| 460 xwe | 920 wwe | 1520 wwe} 2120 Me

fos | taser | 25.25 | e.2s | 43.88

|g | 1.74 18.78 16.56 | 15.93

o feel ael e [a] ela} ela

jaz! ae] 1s | sos] 26 | sea] 21] 493] 29

| 8) ase) an oro | 79] 788] 62] 1131] 87

92/1662 | 184 | 1434) 143 | 2055 | 199

so7 3040 | 615 | 5380! 476 | 4835 | 659

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b) Values corresponding to item 8 (with canal surface

cooling)

Prot| 460 Me 920 MWe | 1520 MWe | 2120 MWe

bs 12.62 25.25 28.25 43.88

& 17.01 17.88 16.56 15.72

2 ela & a = a ela

12} 162] 10} 359} 23] 330 | 20 | aso] 28

to | 235} 16] sar} sz] 47a | 32 | 696] 45

8) 372] 30} 826 | 69} 756 | so | i101) 84

6 | 678} 69] 1505 | 160| 1374] 135 | 1998 | 191

4 | 1599} 230} ssez | 535 | 3234 | 447 | 4699 | 633 |

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Harleman and Stolzenbach - "Preliminary Investigation  
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Jackson and Moreland (October 23, 1970)

Pritchard - "Design and Siting Criteria for Once-  
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Page No. 26¢ (1971)

Handbook of Chemistry and Physics (43rd Ed.)

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I. Basic Jet Model:

The basicy tNo-dimensional jet model is defined by Bq. (16  
ofrete hfe Y Fae Ge)

debs (Eley) »  $E_v = 6b, 3 B_{bey} a$

This variation of width leads to the velocity distribution

$u_m u_y (E_y/E)^{1/2} @$

If  $Q_0$  is the discharge rate from the orifice and  $Q(t)$  the

flow<sup>o</sup> across a section of the jet, then

$@ = a CEE)? ©$

and the entrainment flow is

$G_e = 2- =a [erp] r_0$

Finally, defining

$\theta_5$  = orifice excess temperature

$\theta_{@}$  = entrained water excess temperature

$\theta_{©}$  = excess temperature in the jet at  $\theta_9$

one obtains

Fol e9f 2 )

6.

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### 11, Rate of Entrainment - Two Fossil Plants

Assume that entrainment occurs over the entire length of the plume (approximately 500 ft) until it exits from the faves.. For two fossil plants with a discharge rate

$Q = 1010 \text{ ft}^3/\text{sec}$  and a triangular exit orifice of width

$W = 6 \text{ ft}$

we no)

$Q = (W^2/2) \sqrt{2gH} = 1010 \text{ ft}^3/\text{sec}$

$H = 200 \text{ ft}$  (geese

so  $Q = 1010 \text{ ft}^3/\text{sec}$ .

This compares well with the natural flow of approximately

0 fr8/sec across a section of the Aguirre Canal (Note that only one half of the entrained water comes from one side, so that the comparison is between 2538 ft<sup>3</sup>/sec and 3100).

More to the point, the momentum jet model does not depend on ambient cross flow for entrainment, The question is rather enough water (i.e, 2538 ft<sup>3</sup>/sec) can be withdrawn from the area east of the plume. If it is assumed that all Sherainment water must be drawn in between the cayos and rough the Boca del Infierno, then the induced velocity is

indicator of the availability of entrainment water. The stetional flow area of the cayos is approximately 1250 ni

ui af the Boca del Infierno 1100 m<sup>2</sup> giving a total area of

?proximately 25300 ft<sup>2</sup>, The induced velocity is therefore



$$u = 1/299 = 2538 \text{ }^{-0.1} \text{ ft/sec}$$

A 25300

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### III, Effect of Entrained Excess Temperatures:

If the entrained water is not at ambient temperature, but has an excess temperature of 9, the centerline isotherm distance is given by Eq. (5).

For the case of the two fossil plants and the 4°F. excess temperature isother

bys 151.5 ft, 6, = 17.9°F,, oe aR,

the effect of 6, is illustrated below:

ba Ay

ft acres

3034 45

3745, 68

4808 a4

6520 210

9576 453

15970 1258

35657 5585

125673 77953

NOTE: The values given above are computed on the basis of momentum jet only. Surface cooling and diffusion are neglected.

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#### IV, Modification for Reentrainment

To obtain a first estimate of the effect of reentrainment, the model shown schematically in Fig. 1 is assumed,

The portion of the plume  $E_{yct}$  only is of interest, At  
Peet the flow rate  $Q_0$  is partially reentrained,, the  
Reentrained portion being  $dz$ , Reentrainment is assumed  
to occur only over the section  $E_{icst}$  and complete  
St Rho" To entrained water with ambient air is also assumed

If the parameter  $\alpha$  is defined as

$B_6 y / by$  (6)

then  $Q_1$  and  $Q_2$  are obtained from Eq.(3).

$$Q_1 = \alpha C E M E y^{1/2} = Q_0 \alpha^{1/2} C E / E D^4 V_p = Q_0 (E Q / E, I!?)$$

a)

Since there is no reentrainment on  $\phi, sC_s$ , the excess temperature  $\theta$  becomes 1

$$\theta = (by/E)^{1/2} \quad (8)$$

$\theta^2$

For total mixing in the jet

$$\theta^2 = \theta_j^2 + e^2 \theta^2 \quad (9)$$

and for total mixing of reentrained water

$$\theta^2 \sim \theta_j^2 + 1 \quad F(\theta) = 0.092 \theta^2$$

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Substitution of Eq. (10) into (9) gives

an

and finally, use of (7) and (8) yields

$$\theta = 8 (eyW/2 az)^{1/2}$$

27 8

3)

Note that the temperature  $\theta$  is independent of the parameter  $\delta$ , that is of the ratio  $\delta/2$ . This is a natural consequence of the assumed total mixing.\* The temperature distribution in the plume is, however, dependent on the parameters  $a$  and  $\delta$  are not independent, since for steady state it is necessary that

$992 \delta \text{ oz} = 9 \cdot 0) \text{ as}$

which gives the restriction

as  $1 - \delta/2 \text{ aay}$

and I summarize results for the case of two fossil

at Aguirre with the following values of the parameters

by + 151.5 ft.

$\delta, = 5500 \text{ ee.}$

$\theta, = 17.90 \text{ } ^\circ\text{F,}$

Table I show the variation of  $f_j$ ,  $\theta$ , and  $a$  max with  $\theta$

Table IT gives the variation of  $\theta$  with  $a$ .

It should be noted that the present approximation does not

include surface cooling and will necessarily overestimate

a.

2

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Referenct

Pritchard, - "Design and

Cooling Systems."

26c (1971)

Siting Criteria for Once-Through

Chesapeake Bay Institute Paper No.

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TABLE -

1

TABLE - IT

= VSS v

2 \*Fl7.07 | iiss | s.94 | 3.96 | 3.30 | 2.97

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Notice

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