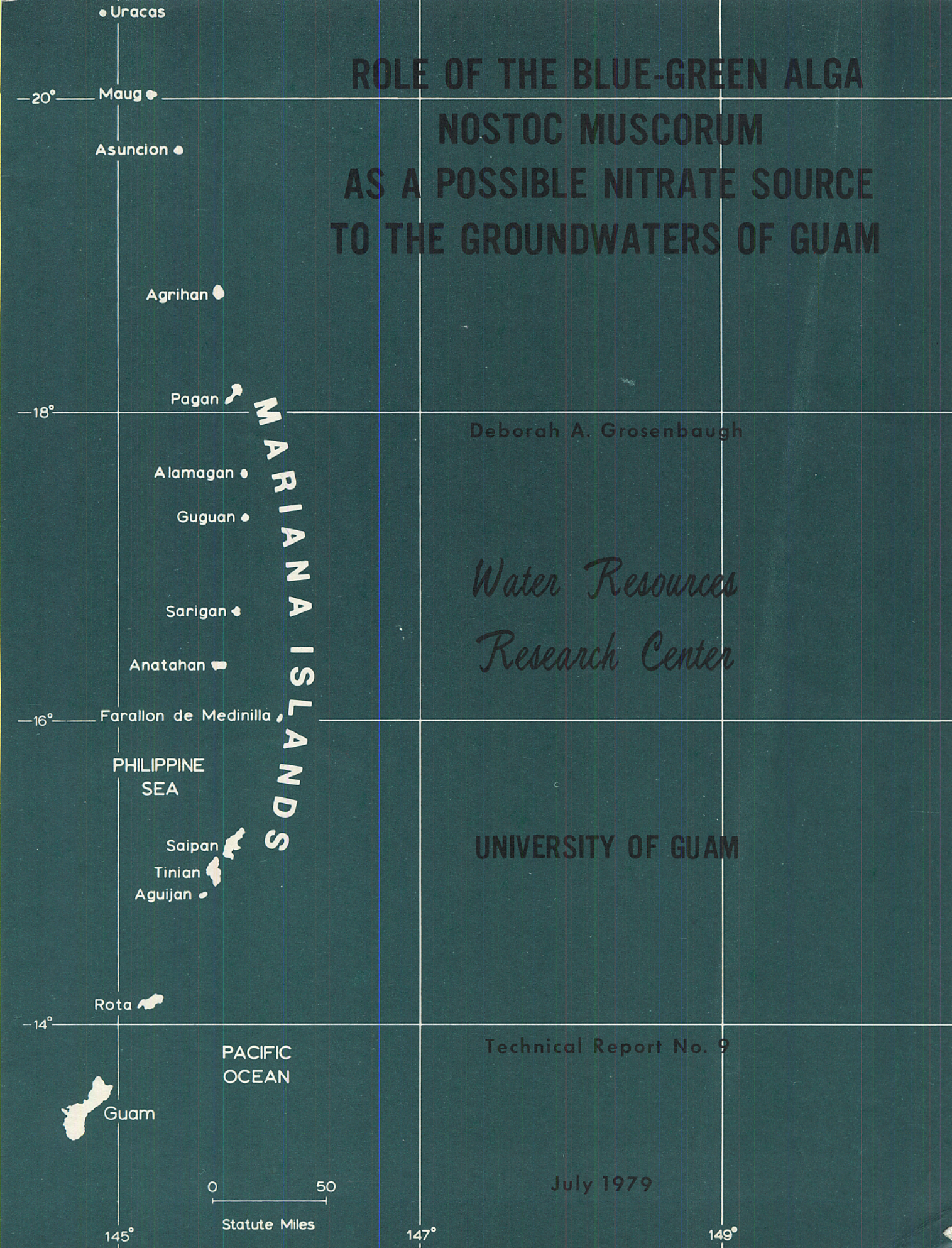

ROLE OF THE BLUE-GREEN ALGA NOSTOC MUSCORUM AS A POSSIBLE NITRATE SOURCE TO THE GROUNDWATERS OF GUAM

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Project Completion Report

for

ROLE OF THE BLUE-GREEN ALGA *NOSTOC MUSCORUM*
AS A POSSIBLE NITRATE SOURCE TO THE GROUNDWATERS OF GUAM

Project No. A-010-Guam, Grant Agreement No. 14-34-0001-8012

Principal Investigators: Roy T. Tsuda and Deborah A. Grosenbaugh

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ABSTRACT

An investigation of the nitrogen-fixing blue-green alga *Nostoc muscorum* Ag. as a possible contributor of nitrate to the groundwater of Guam was carried out from October 1977 to January 1978. Total potential nitrate contribution was determined by combining data obtained for ammonia excretion [illegible] of [illegible] per [illegible], percent nitrogen [illegible] and growth rates [illegible] with algal biomass estimates for Guam [illegible] to obtain a value of [illegible] µg NO_3-N/ml rainwater. Soil percolation studies were similarly extrapolated to produce a corresponding value of [illegible]$\times 10^{-4}$ µg NO_3-N/ml rainwater. Both values are well below those obtained from the groundwater, i.e., ca. 2 µg NO_3-N/ml. It is concluded that *Nostoc muscorum* does not contribute significantly to the high nitrate content of Guam's groundwater.

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INTRODUCTION

Ever since Mink (1976) noted that the nitrate (NO_3) concentration of the groundwater on Guam often exceeds 9 mg/ℓ NO_3 (2 mg/mℓ NO_3-N), there has been a concern over the high nitrate content of Guam's groundwater, its major source of potable water. Recent analyses from the Guam Environmental Protection Agency (GEPA) have shown that this value often exceeds 5 mg/ℓ NO_3-N. The United States Environmental Protection Agency (EPA) has set 10 mg/ℓ NO_3-N as the maximum acceptable standard for potable water.

Nitrate itself is not toxic, but nitrate is readily converted to nitrite (NO_2) in the human body. Upon conversion to nitrite, the molecule is then instrumental in the conversion of hemoglobin to methemoglobin. Methemoglobin is rendered incapable of carrying oxygen to the tissues, the resulting condition being referred to as methemoglobinanemia (Phillips and Todd 1978).

Serious and occasionally fatal poisonings in infants have been reported following the ingestion of well waters containing nitrates. The 1958 International Standards for Drinking Water (World Health Organization) states that ingestion of nitrates in excess of 50-100 ppm (11.3-22.6 mg/mℓ NO_3-N) by infants less than one year old may give rise to methemoglobinemia (Camp and Meserve 1974).

Nitrite has also been implicated as a possible cancer-causing agent. Nitrite, in the body, combines with other organic compounds to form nitrosoamines, some of which are potent carcinogens (Phillips and Todd 1978).

The source of groundwater nitrate has been attributed to biological surface phenomena (Mink 1976). The groundwater originates as rainwater, which percolates through surface soils to a Ghyben-Herzberg lens. Mink (1976) states that if the groundwaters were in equilibrium with the natural growth and decay cycle of biological matter, then one would expect a maximum of 1.0 mg/ℓ NO_3 (0.23 μg/mℓ NO_3-N) to be present in the water. Since this value is often exceeded, we must seek alternative sources for the cause of the high levels. One possible contributor is the heterocystous blue-green alga Nostoc muscorum Ag. The legume Leucaena leucocephala (Lam.) DeWit (Chamorro name: tangentangen), with its nitrogen-fixing root nodules, has also been suggested as contributing fixed nitrogen to the groundwater (Mink 1976).

The purpose of this study is to determine what possible effect, if any, Nostoc muscorum has on the nitrate content of Guam's groundwater. If the total estimated biomass of the alga is sufficient to account for the intrusion of large amounts of combined nitrogen into the lens systems, then it might be possible to control nitrate concentrations by keeping the biomass of N. muscorum to a minimum. On the other hand, if it is shown that N. muscorum plays no more than a minor role in nitrate contribution, alternative sources must be sought.

Nostoc muscorum is a heterocystous blue-green alga which is abundant on the limestone soils above Guam's northern aquifer. Nostoc muscorum has been shown capable of reducing atmospheric

dinitrogen to ammonia. It is among the most common of the nitrogen-fixing algae and is most abundant in tropical soil (Stewart 1973). Stewart et al. (1967), using the acetylene reduction technique, found that a species of Nostoc was capable of converting acetylene to ethylene at the rate of 1.62 Mu moles C_2H_2/mg protein/min. If the conversion ratio (3.2:1) for C_2H_2 reduced:N_2 fixed of Stewart et al. (1968) is used, a value of 0.51 Mu moles N_2 fixed/mg protein/min is obtained.

Nitrogenase, the enzyme responsible for the reduction of dinitrogen to ammonia, is common to all nitrogen-fixing organisms, differing only in minor chemical and physical characteristics (Stewart 1973). Nitrogenase consists of two metalloproteins that are inactive separately (Fogg 1974). Together they are about 275,000 MW and possess two molybdenum atoms at the active site. The presence of combined nitrogen inhibits the synthesis of nitrogenase, and various other compounds such as H_2, N_2O, NO and CO act as competitive inhibitors.

Nitrogenase requires ATP in order to reduce nitrogen. This requirement may be rate-limiting. The ATP requirement may also account for light dependency in nitrogen fixation. Other factors that have been shown to limit nitrogen fixation by nitrogenase are insufficient molybdenum, lack of phosphorus and low pH (Fogg 1974). Nitrogen fixation is also prevented by desiccation.

The overall equation for nitrogen fixation by nitrogenase is as follows:

$$3N_2 + 3H_2 + 12ATP \rightarrow 2NH_3 + 12ADP + 12P_i$$

Ammonia is thus the first stable product of nitrogen fixation. It is incorporated into the cellular metabolism as glutamine (Stewart 1973). Release of fixed nitrogen into the environment is accomplished primarily through decay of the plant tissue. Watanabe and Kiyohara (1960) reported that Bacillus subtilis Cohn was responsible for the release of 40 percent of the cell nitrogen as ammonia in ten days at 30°C in N. muscorum. It has also been shown that blue-green algae liberate combined nitrogen during the course of healthy growth (Fogg 1974).

Once the atmospheric nitrogen has been fixed in the form of ammonia, it then undergoes the process of nitrification to NO_2 and NO_3 by indigenous bacteria in the soil. Two different populations of soil bacteria are responsible for the different steps involved in this conversion (Alexander 1961). First, the Nitrosomonas population oxidizes ammonia to nitrite. This nitrite is then further oxidized to nitrate by the Nitrobacter population. The nitrate ions then percolate down through the permeable limestone into the fresh-water lens where they can be detected in the drinking water pumped up from wells.

MATERIALS AND METHODS

Both field and laboratory studies were conducted to derive information on the contribution of Nostoc muscorum to the nitrate content of Guam's groundwater. The field study consisted of harvesting the blue-green alga in selected areas above Guam's northern aquifer in order to derive values on biomass. Laboratory studies were designed to estimate the potential total nitrogen contributed by a given amount of the alga under partially controlled conditions and to estimate the total nitrogen contributed by a Nostoc-bacteria soil system under simulated in-situ conditions.

Estimates of Biomass

Estimates of the biomass of Nostoc muscorum on Guam were made on the basis of transects run in a selected area (Harmon) over the groundwater lens and on observations made in four ponding basins (Dededo, Barrigada, Latte Estates and Marianas Terrace) that discharge surface-storm runoff into the lens system.

A 1.0-km^2 area (Fig. 1) in Harmon was selected where N. muscorum was found in abundance and conditions for growth were favorable. Biomass studies made in this area would presumably give an upper limit estimate. The number of kilometers of roadway in the study area was determined from an aerial photograph obtained from the Guam Bureau of Planning. On the basis of these observations, the roads were divided into three types: type 1, well traveled, little to no N. muscorum; type 2, used only by local traffic, moderate

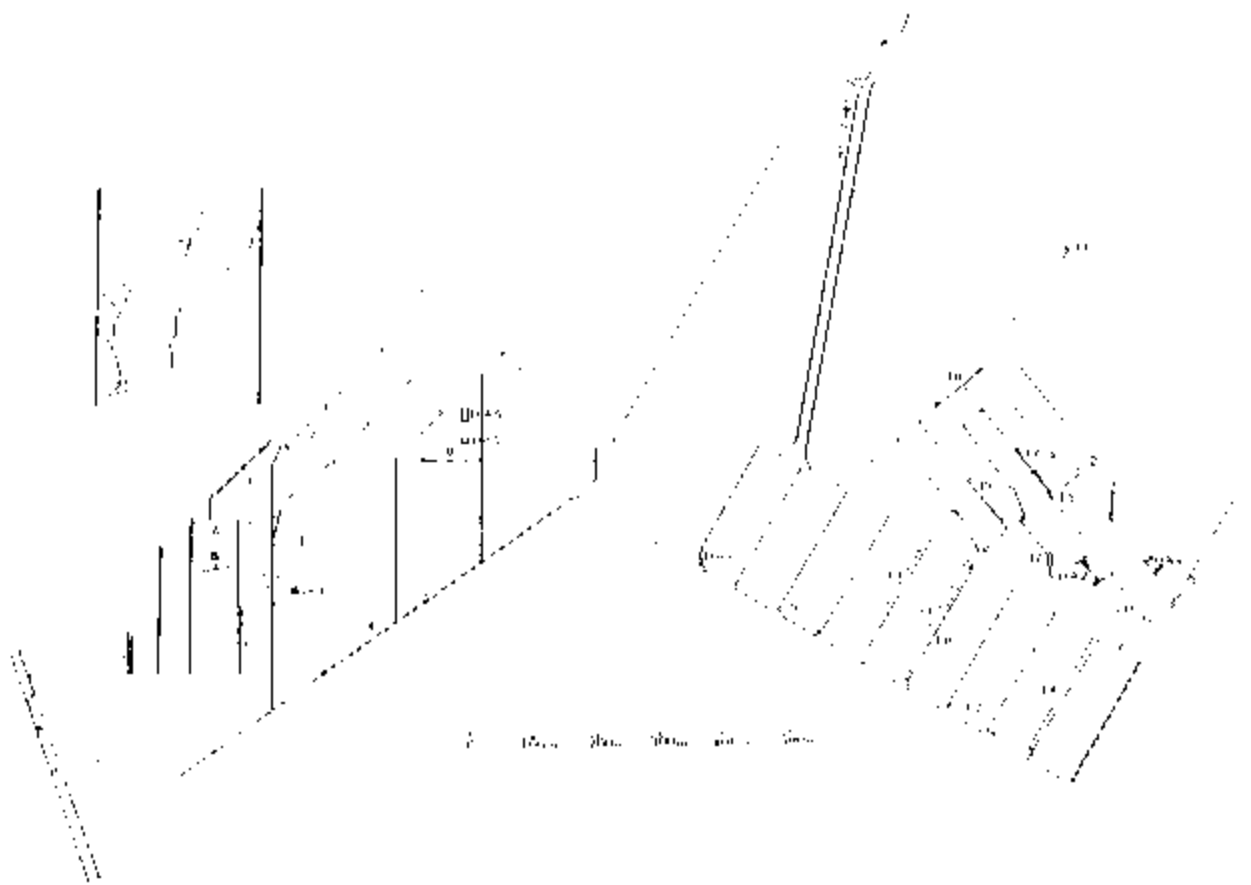

Figure 1. Barro Colorado study area showing the location of the transects and "disturbed areas" from which biomass estimates were obtained. Dashed lines indicate the boundary of the study area and the hatched areas (designated as DA) show the location of the "disturbed areas".

amounts of N. muscorum present along the sides; type 3, little-used, overgrown, N. muscorum abundant over the entire road surface.

Twenty-one 100-m transects were run along the various road types during the wet months of October through December, 1978. Nostoc muscorum cover was noted at 0.5 m intervals, referring to it as sparse (clumps at widely spaced intervals), medium (even, but not total cover), or heavy (even, total cover). Samples from various transects were obtained to determine an average dry weight for sparse, medium and heavy coverage. Average biomass for the three road types was then determined and extrapolated to the entire area, taking into consideration large abandoned foundations (referred to as disturbed areas, DA in Fig. 1) where algal growth was particularly abundant. The biomass value for the study area was then expanded to include the entire surface area over Guam's northern aquifer, estimated at 2.6×10^2 km^2 (ca. 100 square miles).

Since the ponding basin system plays a significant role in recharging the lens system, four ponding basins (Fig. 2) were investigated to determine if the N. muscorum inhabiting the basins was abundant enough to contribute a significant amount of fixed nitrogen. Since each ponding basin varies in configuration, drainage and vegetation, different transect methods were employed for each of three of the ponding basins, Marianas Terrace having no apparent N. muscorum biomass (Fig. 3). The ponding basins under study were selected on the basis of their location over the lens, their soil type and accessibility.

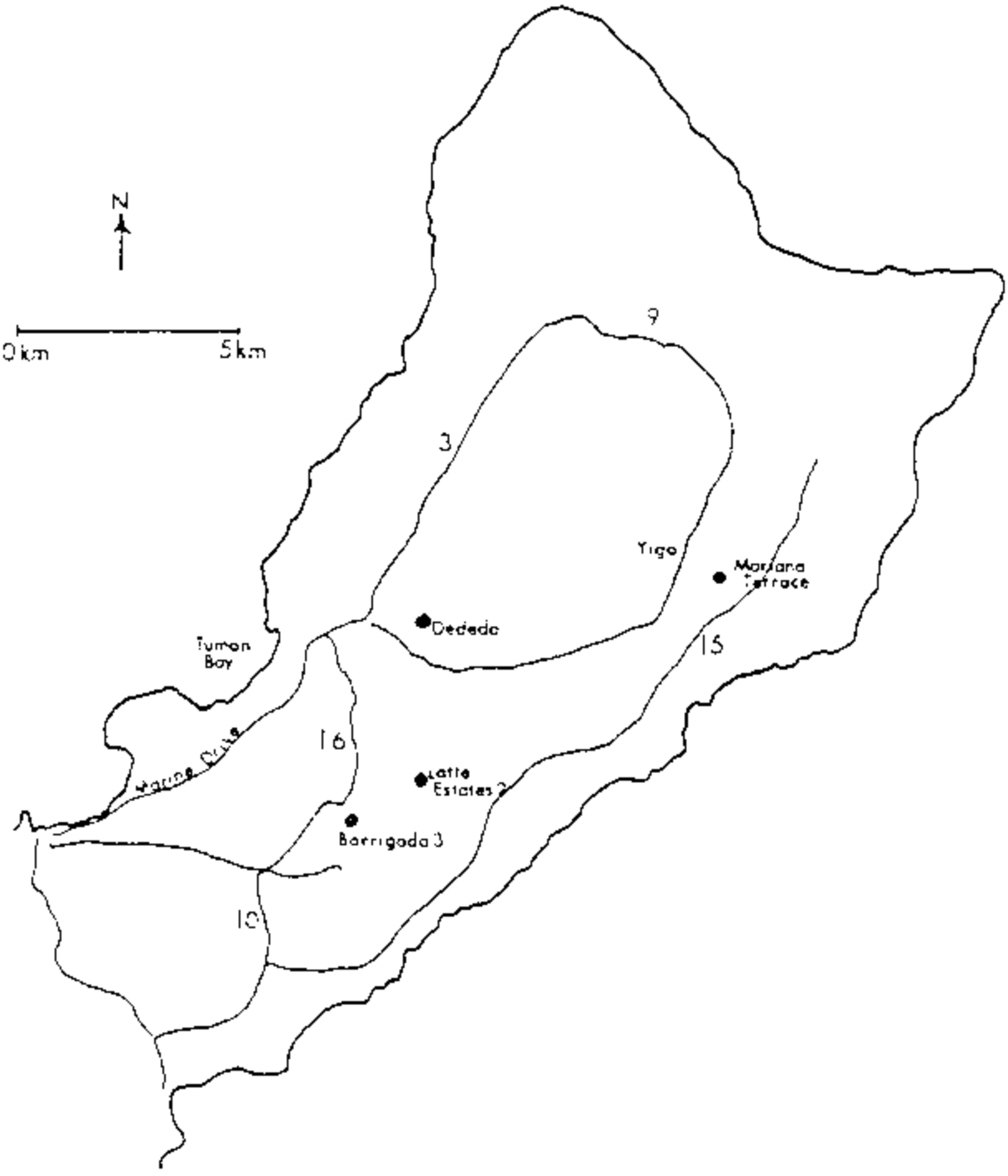

Figure 2. Location of ponding basins analyzed for Nasturtium microphyllum cover. Map from Zolan et al. (1978).

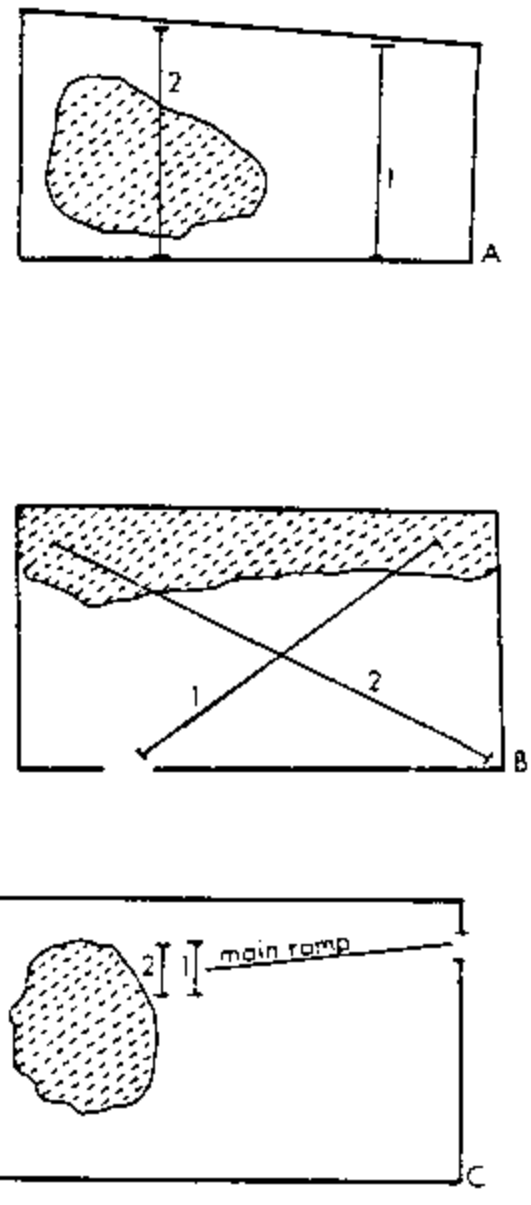

Figure 3. Location of transects at the three ponding basins. The ponding basins illustrated are A. Dededo, B. Barrigada No. 3, and C. Latte Estates No. 2. Hatched areas denote location of standing water.

Biomass estimates were derived primarily by running transects as shown in Fig. 3 and collecting all of the algae in a 0.25-m^2 quadrat on either side of the transect tape every 5m. The dry weight of the alga was then extrapolated to include a 0.5 m wide strip along the entire length of the transect. From this value algal biomass/m^2 was estimated for the entire 0.5 m wide strip and multiplied by the appropriate factor to account for the bottom surface area of the respective ponding basin.

A different method was employed in the case of Latte Estates No. 2. The occurrence of N. muscorum was restricted to an area approximately 100 m wide surrounding the main ponding area. Algal cover in the ramp area was determined by running two 10-m transects and calculating biomass as stated above. Nostoc muscorum surrounding the standing water was associated with low grass and thus difficult to sample. The area involved was estimated and the mean value for sparse coverage along the road transects was used to derive a biomass estimate. The summation of the estimates from the two areas provided a biomass value for the ponding basin. Transect lengths were as follows:

A. Dededo Ponding Basin - Transect 1 (70 m)
- Transect 2 (110 m)

B. Barrigada No. 3 - Transect 1 (80 m)
- Transect 2 (80 m)

C. Latte Estates No. 3 - Transect 1 (10 m)
- Transect 2 (10 m)

D. Marianas Terrace - No Nostoc muscorum